Major Histocompatibility Complex Class I Related Molecules Control the Development of CD4+8- and CD4-8- Subsets of Natural Killer 1.1+ T Cell Receptor-α/β+ Cells in the Liver of Mice

By Toshiaki Ohteki and H. Robson MacDonald

Summary

Normal mouse liver contains prominent subsets of CD4+8- and CD4-8- T cell receptor (TCR)-α/β+ cells with intermediate TCR levels. We show here that these cells express the natural killer (NK)1.1 surface antigen and have a restricted TCRVβ repertoire that is highly skewed to Vβ7 and Vβ8. Surprisingly, both CD4+8- and CD4-8- subsets of NK1.1+ TCR-α/β+ cells are absent in the liver of β2-microglobulin deficient mice, which do not express major histocompatibility complex (MHC) class I or "class I-like" molecules. Analysis of reciprocal radiation bone marrow chimeras established with β2-microglobulin deficient and wild-type mice demonstrates that MHC class I expression on radiosensitive (presumably hematopoietic) cells is required for the development of NK1.1+ TCR-α/β+ cells in the liver. In the liver of MHC class II deficient mice, the CD4+8- and CD4-8- subsets of NK1.1+ TCR-α/β+ cells develop normally. Collectively our data suggest that NK1.1+ TCR-α/β+ cells require interaction with a MHC class I-related ligand on hematopoietic cells for their development. This unusual property of liver T cells is shared by a subset of CD4-8-NK1.1+ TCR-α/β+ thymocytes, suggesting a common lineage independent of the mainstream of T cell development.

Materials and Methods

Mice. C57BL/6 mice were purchased from Harlan Olac Ltd (Bicester, UK). βm deficient (-/-) mice (1, 2) and wild-type (+/-) littermates were bred and maintained in the animal facilities of the Swiss Institute for Experimental Cancer Research (Epalinges, Switzerland). In most experiments, βm -/- mice were used originally crossed with C57BL/6 mice and the C57BL/6 background was confirmed by staining with an anti-NK1.1 mAb. MHC class II deficient Aα -/- mice (6) and wild-type (+/-) littermates on a C57BL/6 background were kindly provided by Dr. H.
between Bzm deficient and wild-type mice, recipients were lethally irradiated (950 rad, 117 rad/min, 137Cs source) and reconstituted 1 d later with 15-20 × 10^6 T cell-depleted bone marrow cells. For the following 8 wk, teramycin (1.5 g/liter) was added to the drinking water. Chimeras were killed 10 wk after reconstitution and chimerism was monitored by flow microfluorometry analysis using B8-24-3 (anti-Kb) mAb.

**Antibodies and Flow Cytometric Analysis.** The following mAb conjugates were used in this study: H57-597-PE (anti-TCRβ; Caltag Laboratories, San Francisco, CA); PK136-biotin (anti-NK1.1; PharMingen, San Diego, CA); GK1.5-PE and GK1.5-FITC (anti-CD4; Becton Dickinson & Co., Mountain View, CA); 53-6.7-FITC (anti-CD8). Unconjugated rat, mouse, or hamster mAbs against the following TCR Vβ domains were prepared in our laboratory: B20.6.5 (anti-Vβ2); KJ25 (anti-Vβ3); KT4-10 (anti-Vβ4); MR9-4 (anti-Vβ5); 44-22.1 (anti-Vβ6); TR310 (anti-Vβ7); F23.1 (anti-Vβ8.1,8.2,8.3); F23.2 (anti-Vβ8.2); MR10-2 (anti-Vβ9); B21.5 (anti-Vβ10); and RR3-15 (anti-Vβ11). FITC-conjugated goat anti-rat Ig (Caltag Laboratories) or goat anti-mouse IgG1 or IgG2a (Southern Biotechnology Associates, Inc., Birmingham, AL) were used with unconjugated mAbs. Rat or mouse Ig was used to block free Ig sites before addition of anti-CD4-PE and anti-NK1.1-biotin. All samples were further stained with streptavidin Tri-color and analyzed by FACScan® and the Lysis II program (Becton Dickinson & Co.).

**Results**

**Liver Is a Major Source of NK1.1* TCR-α/β* Cells.** 40-50% of total MNC in the liver of 8-10-wk-old C57BL/6 mice are TCR-α/β+ (12-14). As shown in Fig. 1, about half of these cells express NK1.1+. The NK1.1* TCR-α/β+ subset in liver had approximately threefold lower surface TCR-β intensity than NK1.1* TCR-α/β+ cells in liver, LN, and spleen (Fig. 1). This phenotype clearly corresponds to the so-called "intermediate" T cells in liver which express TCR-β and CD3 at levels higher than those in spleen, LN, and bone marrow. Since the total number of MNC recovered per liver was 5.0 ± 1.2 × 10^6, the absolute number of NK1.1* TCR-α/β+ cells was 10^6. NK1.1* TCR-α/β+ cells were rather rare in other organs, i.e., 0.5% in thymus, LN, and bone marrow, 1.5% in spleen, and 0.3% in IEL (Fig. 1). Thus, NK1.1* TCR-α/β+ cells were most frequently seen in liver. However, 15-20% of HSN-/- thymocytes in adult C57BL/6 mice were NK1.1* TCR-α/β+ (data not shown) as reported elsewhere (18, 19). NK1.1* TCR-α/β+ cells or "intermediate" TCR cells in thymus and liver appear shortly after birth and increase with age (14, 18, 20). As shown in Fig. 2, neonatal liver contains 10-fold more NK1.1* TCR-α/β+ cells than thymus; however the absolute number of NK1.1* TCR-α/β+ cells in these organs is approximately equal in the adult.

We further analyzed the CD4/CD8 phenotype of liver NK1.1* TCR-α/β+ cells. As shown in Table 1, they were comprised mainly of CD4+8* cells (65%) and CD4+8cells (30%). A small subset of CD4+8* NK1.1* TCR-α/β+ cells was also detectable (5%).

**TCR-β Usage of NK1.1* TCR-α/β+ Cells in Liver.** A previous study (13) has shown that Vβ8 is overrepresented among TCR intermediate cells in liver. TCR-β expression on the surface of NK1.1* TCR-α/β+ liver cells and control CD4+8* thymocytes but lower than mature T cells (12-14). Since the total number of MNC recovered per liver was 5.0 ± 1.2 × 10^6, the absolute number of NK1.1* TCR-α/β+ cells was 10^6. NK1.1* TCR-α/β+ cells were rather rare in other organs, i.e., 0.5% in thymus, LN, and bone marrow, 1.5% in spleen, and 0.3% in IEL (Fig. 1). Thus, NK1.1* TCR-α/β+ cells were most frequently seen in liver. However, 15-20% of HSN-/- thymocytes in adult C57BL/6 mice were NK1.1* TCR-α/β+ (data not shown) as reported elsewhere (18, 19). NK1.1* TCR-α/β+ cells or "intermediate" TCR cells in thymus and liver appear shortly after birth and increase with age (14, 18, 20). As shown in Fig. 2, neonatal liver contains 10-fold more NK1.1* TCR-α/β+ cells than thymus; however the absolute number of NK1.1* TCR-α/β+ cells in these organs is approximately equal in the adult.

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Figure 2. Ontogeny of NK1.1⁺ TCR-α/β⁺ cells in the liver and thymus of C57BL/6 mice. At indicated time points, liver MNC and HA⁺ thymocytes (prepared as described in Materials and Methods) were stained with H57-597-PE and PK136-biotin plus streptavidin Tri-color. The absolute number of NK1.1⁺ TCR-α/β⁺ cells per organ is shown.

Figure 3. TCR-Vβ repertoire of CD4⁺ LN cells, CD4⁺ NK1.1⁺ and CD4⁻8⁻ NKI.1⁺ liver T cells. Cells were pooled from three to five C57BL/6 mice aged 8 to 10 wk and stained with a panel of anti-Vβ mAbs. Each bar represents the average of two to three experiments.

LN cells was therefore investigated using a panel of mAbs (Fig. 3). CD4⁺ NK1.1⁺ T cells in liver use Vβ8 (69.8 ± 0.9%), Vβ7 (16.0 ± 1.3%), and Vβ2 (8.2 ± 0.3%) at much higher frequency when compared with CD4⁺ LN T cells (21.4 ± 1.5, 14 ± 0.2, and 6.1 ± 0.3%, respectively). Among the Vβ8 family, Vβ8.2 (55.0 ± 1.4%) is dominant. Other Vβ such as Vβ4, Vβ6, Vβ10, and Vβ11 were virtually absent in CD4⁺ NK1.1⁺ T cells. The Vβ usage of CD4⁺8⁻ NK1.1⁺ T cells was similar to CD4⁺ NK1.1⁺ T cells, i.e., 67.5 ± 8.5% Vβ8 and 22.0 ± 4.1% Vβ7. It is noteworthy that the intensities of Vβ8, 7, and 2 staining of CD4⁺ NK1.1⁺ T cells were somewhat lower than those of CD4⁺ LN T cells (data not shown). It is interesting to note that both CD4⁺8⁻ and CD4⁺8⁻ subsets of NK1.1⁺ TCR-α/β⁺ cells in thymus also overexpress Vβ8, mainly Vβ8.2 (9-11, 18, 21). The similarity of phenotype and Vβ usage suggest that CD4⁺ NK1.1⁺ T cells in liver and thymus are more closely related to CD4⁺8⁻ NK1.1⁺ T cells than to conventional CD4⁺ NK1.1⁺ T cells (18, 21).

Table 1. CD4/CD8 Phenotype of NK1.1⁺ TCR-α/β⁺ Cells in the Liver

<table>
<thead>
<tr>
<th>Subset</th>
<th>Percent positive among</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK1.1⁺ TCR-α/β⁺ cells</td>
<td>NK1.1⁺ TCR-α/β⁺ cells</td>
</tr>
<tr>
<td>CD4⁺8⁻</td>
<td>65.0, 68.3</td>
</tr>
<tr>
<td>CD4⁺8⁺</td>
<td>6.5, 4.9</td>
</tr>
<tr>
<td>CD4⁻8⁻</td>
<td>30.1, 29.5</td>
</tr>
</tbody>
</table>

The numbers indicate results from separate experiments. Pools of two to four C57BL/6 mice were used in each experiment, and liver cells were stained with CD4-FITC and/or CD8-FITC, H57-597-PE, and NK1.1-biotin plus avidin Tri-color.

Impaired Development of NK1.1⁺ TCR-α/β⁺ Cells in the Liver of B2m Deficient Mice. To investigate the possible role of MHC or MHC-related molecules in the development of NK1.1⁺ TCR-α/β⁺ cells in liver, we compared the frequency of these cells in B2m⁺ and B2m⁻ deficient mice on a C57BL/6 (NK1.1⁺) background. The former mice (1, 2) failed to express conventional MHC class I molecules (H-2K, H-2D, and H-2L) as well as other β2m-associated proteins such as Qa, Tla, Hmt, and CD1, whereas the latter (6) lacked conventional MHC class II molecules. Surprisingly, NK1.1⁺ TCR-α/β⁺ cells were decreased by >90% in the liver of B2m⁻ mice as compared with their littermate B2m⁺/+ controls (Fig. 4 and Table 2). This dramatic reduction was apparent in both CD4⁺8⁻ and CD4⁺8⁻ subsets of NK1.1⁺ TCR-α/β⁺ cells. In the liver of MHC class II deficient (B2m⁻) mice, the development of NK1.1⁺ TCR-α/β⁺ cells was essentially normal for both CD4⁺8⁻ and CD4⁺8⁻ subsets (Fig. 4 and Table 2). Thus, the behavior of NK1.1⁺ TCR-α/β⁺ cells in the liver of MHC deficient mice is strikingly similar to that reported recently for CD4⁺8⁻ NK1.1⁺ thymocytes, which also appear to require MHC class I-related molecules for their development (11).

MHC Class I-related Molecules on Hematopoietic Cells Control the Development of NK1.1⁺ TCR-α/β⁺ Cells in Liver. The mechanism underlying the failure of NK1.1⁺ TCR-α/β⁺ cells to develop in the liver of B2m⁻ mice was further investigated in radiation bone marrow chimeras. Conventional thymus-derived CD4⁺ or CD8⁺ T cells primarily dependent upon interactions with MHC molecules on radioreistant (presumably epithelial) cells in the thymus of such chimeras in order to be positively selected during development (22). In contrast, the development of the CD4⁺8⁻ NK1.1⁺ TCR-α/β⁺ subset in the liver of reciprocal radia-
Table 2. Analysis of NK1.1+ TCR-α/β+ Cells in the Liver of β2m or Aα Deficient Mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>Liver (Percent NK1.1+ TCR-α/β+)</th>
<th>LN (Percent NK1.1+ TCR-α/β+)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>CD4-8-</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>β2m+/−</td>
<td>26.8 ± 4.3</td>
<td>17.2 ± 2.9</td>
</tr>
<tr>
<td>β2m−/−</td>
<td>1.6 ± 0.5</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Aα+/−</td>
<td>29.5, 34.2</td>
<td>19.8, 23.4</td>
</tr>
<tr>
<td>Aα−/−</td>
<td>35.2, 32.7</td>
<td>23.5, 22.1</td>
</tr>
</tbody>
</table>

Liver MNC from control or mutant mice were analyzed for NK1.1+ TCR-α/β+ cells as in Fig. 4. Data are mean ± SD of three to five mice unless otherwise indicated (values for individual mice). LN cells are included for comparison.

Figure 4. Analysis of NK1.1+ TCR-α/β+ cells in the liver of MHC deficient mice. For the detection of NK1.1+ TCR-α/β+ cells or CD4+ NK1.1+ cells, liver MNC were stained with H57-597-PE or CD4-PE and PK136-biotin plus streptavidin Tri-color. For the detection of CD4-8- TCR-α/β+ cells, liver MNC were stained with CD4-FITC, CD8-FITC, and H57-597-PE.

Discussion

Our data indicate that a ligand expressed on hematopoietic cells and necessary for the development of NK1.1+ TCR-α/β+ cells in liver is lacking in β2m deficient mice. As discussed earlier, this putative selecting ligand could be...
### Table 3. Analysis of CD4+NK1.1+ Liver MNC in Reciprocal Radiation Bone Marrow Chimeras Established between β2m Deficient (−) and Wild-type (+) Mice

<table>
<thead>
<tr>
<th>Donor Host</th>
<th>Percent NK1.1+</th>
<th>Percent Vβ8+ in CD4+</th>
<th>Percent NK1.1+CD4+</th>
<th>Percent NK1.1−CD4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>7.4, 8.9</td>
<td>61.2, 63.6</td>
<td>27.7, 29.8</td>
<td></td>
</tr>
<tr>
<td>−</td>
<td>7.6, 4.4</td>
<td>53.8, 53.4</td>
<td>27.9, 22.1</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>1.3, 1.0</td>
<td>ND</td>
<td>22.9, 24.1</td>
<td></td>
</tr>
<tr>
<td>−</td>
<td>1.1, 0.9</td>
<td>ND</td>
<td>25.1, 27.6</td>
<td></td>
</tr>
</tbody>
</table>

Liver MNC from two individual mice were analyzed in each group. Chimerism was checked by staining with B8-24-3 (anti-Kb) mAb and cells were analyzed after excluding host-derived cells in (+ to −) and (− to +) cases. The proportions of donor-derived cells were 92%, 73% in (+ to −) chimeras and 74%, 76% in (− to +) chimeras. ND, not detectable because of the small percentage of NK1.1+CD4+ cells.

Either a classical MHC class I molecule or some other β2m-associated protein such as the product of the Qa, Tla, Hmt, or CD1 locus. Since NK1.1+ TCR-α/β+ cells in liver are primarily of the CD4-8− or CD4-8+ (but not CD4-8+−) phenotype, selection by a classical MHC class I molecule seems rather unlikely. Rather, we would propose that the development of NK1.1+ TCR-α/β+ cells in liver depends upon interaction of the TCR with a nonclassical class I molecule. Since the latter class of molecules is relatively nonpolymorphic (23) and (at least in some cases) appears to present a limited number of peptides (24, 25), such an interaction could account for the highly restricted TCR Vβ usage of NK1.1+ TCR-α/β+ cells. In the context of this model, the fact that several antigens of microbial origin can be presented by nonclassical class I molecules (26, 27) is of particular interest, since “TCR-α/β intermediate” (presumably NK1.1+) cells in liver have been shown to expand dramatically during bacterial infections (12).

The origin of NK1.1+ TCR-α/β+ cells in liver is controversial (28). In this respect, the striking similarities in TCR Vβ phenotype and MHC class I dependence observed for NK1.1+ TCR-α/β+ cells in thymus and liver raise obvious questions concerning a possible common lineage independent of the mainstream of T cell development. Indeed, it has been shown that some NK1.1+ TCR-α/β+ cells in the spleen of young athymic mice can originate from a grafted neonatal thymus (8). On the other hand NK1.1+ TCR-α/β− cells (or cells with a similar phenotype) clearly develop to some extent in several organs (including spleen and liver) of aged nude mice (14, 20, 29), arguing against an obligatory thymic origin of these cells. Given the apparent requirement for hematopoietic (rather than epithelial) cells for their generation, it remains possible that NK1.1+ TCR-α/β+ cells develop independently at multiple sites of hematopoiesis.

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References
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