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Critical Role of Leukocyte Function-Associated Antigen-1 in Liver Accumulation of CD4⁺NKT Cells

Masashi Emoto,* Hans-Willi Mittrücker,* Rudolf Schmits,† Tak W. Mak,‡ and Stefan H. E. Kaufmann*‡

In contrast to peripheral lymphoid organs, a high percentage of T cells in the liver are CD4⁺NKT cells. We asked whether adhesion molecules play any role in the accumulation of CD4⁺NKT cells in the liver. Liver CD4⁺NKT cells expressed ICAM-1 and high levels of LFA-1. In the livers of LFA-1-deficient mice, the number of CD4⁺NKT cells was markedly decreased. This reduction was restricted to the liver, and no reduction was found in the other organs analyzed. In contrast, the number of liver CD4⁺NKT cells in ICAM-1-deficient mice was only marginally reduced. In a reciprocal radiation thymocyte reconstitution system with LFA-1-deficient and wild-type mice, LFA-1 expressed on liver cells other than CD4⁺NKT cells was required for an accumulation of CD4⁺NKT cells in the liver. These results demonstrate a crucial role for LFA-1 in the accumulation of CD4⁺NKT cells in the liver. The Journal of Immunology, 1999, 162: 5094–5098.

NKT cells have been identified in the thymus and peripheral lymphoid organs of mice and show a wide variety of biological functions such as cytokine production and cytotoxicity (1). The proportion of NKT cells is markedly higher in the liver compared with other organs (2, 3), raising the possibility that these cells develop in the liver directly. Although there is still controversy over whether NKT cell development is thymus-dependent or -independent (1), it has been shown that the thymus is essential for the development of these cells (1, 3–5). NKT cells are controlled by β₂-microglobulin (β₂m)³-associated nonpolymorphic CD1 molecules, which are expressed on double-positive thymocytes at high density (2–4, 6–10). NKT cells mature in the absence of thymic epithelial cells (2, 6–8).

LFA-1 (CD11a/CD18) is a member of the β₂ integrin family of cell adhesion molecules, which is expressed exclusively on leukocytes, albeit at different levels, and is involved in numerous immunological functions, including cell migration into tissues (11). ICAM-1 (CD54) was initially identified as a ligand for LFA-1 (11). Evidence has been presented that NKT cells in the thymus and lymph nodes express ICAM-1 and high levels of LFA-1 (10, 12, 13). Liver NKT cells have also been shown to express ICAM-1 and high levels of LFA-1 by indirect means (3, 14, 15). These findings raise the question of whether these cell adhesion molecules are involved in the accumulation of NKT cells in the liver.

In the present study, we analyzed the proportions of CD4⁺NKT cells in the livers of different mouse strains deficient in the expression of adhesion molecules to clarify the role of these molecules in the accumulation of CD4⁺NKT cells to the liver. The proportion of CD4⁺NKT cells in the liver was markedly reduced in LFA-1-deficient mice, whereas marginal reduction was observed in ICAM-1-deficient mice. Migration of CD4⁺NKT cells to the liver was observed in wild-type (wt) mice that had been irradiated and reconstituted with thymocytes from LFA-1-deficient mice. In contrast, liver migration was not observed in LFA-1-deficient mice that had been irradiated and reconstituted with thymocytes from wt mice. Our results demonstrate that the LFA-1 expressed on liver cells other than CD4⁺NKT cells plays a pivotal role in the accumulation of CD4⁺NKT cells in the liver and suggest that ICAM-1 is not essential for the residence of CD4⁺NKT cells in the liver.

Materials and Methods

Mice

Breeding pairs of H-2I-Aβ (Aβ⁻⁻) mice were kindly provided by Dr. D. Mathis (Institut de Génétique et de Biologie Moleculaire et Cellulaire, Strasbourg, France) (16). C57BL/6 scid/scid (SCID), β₂m⁻⁻, and ICAM-1⁻⁻ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). LFA-1⁻⁻ mice were generated by homologous recombination using 129/J-derived ES cell lines as described previously (17). Mutant mice were backcrossed to C57BL/6 (β₂m⁻⁻, Aβ⁻⁻, and ICAM-1⁻⁻; >8 generations; LFA-1⁻⁻ and LFA-1⁻⁻: 3 generations). These mutants, as well as Thy1.1 congenic C57BL/6 (Thy1 congenic) and C57BL/6 wt mice, were maintained under specific pathogen-free conditions at our animal facilities at the Federal Institute for Health Protection of Consumers and Veterinary Medicine (Berlin, Germany) and were used at 2–6 mo of age.

Monoclonal Abs

The following mAbs were purified from hybridoma culture supernatants: anti-NK1.1 mAb (PK136), anti-FcγRI mAb (2.4G2), anti-TCRαβ mAb (H57-597), and anti-CD4 mAb (YTS191.1). Anti-NK-1.1 mAb was biotinylated; anti-NK1.1 mAb, anti-TCRαβ mAb, and anti-CD4 mAb were conjugated with FITC. Phycoerythrin (PE)-conjugated anti-CD4 mAb (H129.19) and streptavidin (SA)-conjugated Red 670 were purchased from Life Technologies (Gaithersburg, MD). Biotinylated anti-CD11a (LFA-1α) mAb (M17/4), FITC-conjugated anti-CD54 (ICAM-1) mAb (H9.2B8), FITC-conjugated anti-CD90.1 (Thy1.1) mAb (HS151), and PE-conjugated anti-CD90.2 (Thy1.2) mAb (53-2.1) were obtained from PharMingen (Hamburg, Germany).

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3 Abbreviations used in this paper: β₂m, β₂-microglobulin; Aβ, H-2I-Aβ; LMNC, liver mononuclear cell; PE, phycoerythrin; SA, streptavidin; wt, wild type.
Cell preparation and flow cytometry

Liver mononuclear cells (LMNCs) and intestinal intraepithelial lymphocytes were prepared as described previously (3). Cells from the thymus, spleen, lymph nodes, and bone marrow were prepared by conventional methods. Peritoneal nonadherent cells were obtained as described previously (18). Cells were stained with appropriate dilutions of mAb. Biotinylated mAbs were visualized by SA-conjugated Red 670. Before staining, cells were incubated with 50 μg/ml anti-FcγR mAb. Each staining step was performed at 4°C for 30 min; each washing step was performed with PBS containing 0.1% BSA and 0.1% sodium azide. After staining, cells were washed and subsequently fixed with 1% paraformaldehyde. Stained cells were acquired by FACScan (Becton Dickinson, Mountain View, CA), and lymphoid cells were analyzed with CellQuest software.

Radiation and thymocyte transfer

Thy1 congenic (Thy1.1+, LFA-1+) or LFA-1+/− (Thy1.2+, LFA-1−) mice were irradiated at 600 rad and reconstituted on day 2 with 3 × 10⁷ thymocytes from LFA-1+/− or Thy1 congenic mice, respectively. Mice were killed 2 days after reconstitution, and the proportions of CD4⁺NK1⁺ T cells that migrated to the liver were monitored with anti-Thy1.1 or anti-Thy1.2 mAb by flow cytometry. SCID mice received 3 × 10⁷ thymocytes i.v. from LFA-1+/− or LFA-1−/− mice, and the proportions of CD4⁺NK1⁺ T cells that migrated to the liver were analyzed by flow cytometry 2 days after injection.

Results

Expression of NK1.1, ICAM-1, or LFA-1 on CD4⁺ liver T cells

We first assessed the expression of NK1.1, ICAM-1, and LFA-1 molecules on CD4⁺ liver T cells. Consistent with previous findings (2, 3), a high proportion of NK1-expressing cells was identified within CD4⁺ liver T cells in C57BL/6 mice. This cell population was markedly reduced in β₂m−/− mice but remained virtually unaffected in Aβ−/− mice (Fig. 1). CD4⁺ liver T cells segregated into two main populations on the basis of ICAM-1 and LFA-1 expression: one population expressed ICAM-1 and/or high levels of LFA-1; the other did not. The relative proportion of ICAM-1-expressing CD4⁺ T cells and/or high levels of LFA-1-expressing CD4⁺ T cells was markedly reduced in β₂m−/− mice, but not in Aβ−/− mice (Fig. 1). Virtually all CD4⁺NK1⁺ T cells in the liver expressed ICAM-1 and high levels of LFA-1, whereas the majority of CD4⁺NK1− liver T cells were devoid of ICAM-1 and expressed low levels of LFA-1 (Fig. 2). Consistent with previous findings (2, 3), virtually all CD4⁺NK1⁺ liver T cells expressed TCRαβ at an intermediate intensity (data not shown).

Influence of ICAM-1 or LFA-1 deficiency on CD4⁺ NKT cells in the liver

Because CD4⁺NKT cells in the liver expressed ICAM-1 and high levels of LFA-1, we wondered whether these molecules were involved in the accumulation of this cell population to the liver. To address this issue, we used ICAM-1−/− or LFA-1−/− mice. Representative results are shown in Fig. 3, and the results from four to six mice are summarized in Table I. Although the relative proportion of CD4⁺NK1⁺ T cells in the liver was only slightly reduced in ICAM-1−/− mice, it was markedly diminished in LFA-1−/− mice. The relative proportion of CD4⁺NK1− T cells was not affected by LFA-1 deficiency (see Fig. 3). These results reveal that LFA-1 rather than ICAM-1 is crucial for the accumulation of CD4⁺NKT cells to the liver. The number of CD4⁺NK1⁺ T cells was slightly reduced in the livers of LFA-1−/− mice compared with C57BL/6 mice. Because LFA-1 surface expression is slightly reduced in LFA-1−/− mice compared with LFA-1−/+ mice (17), we assume that the marginal reduction of CD4⁺NKT cells in LFA-1−/− mice is caused by reduced LFA-1 surface expression.

Lack of influence of LFA-1 deficiency on the presence of CD4⁺NKT cells in lymphoid organs

To determine whether LFA-1 is involved in the development of CD4⁺NKT cells, we assessed the presence of this cell population.
in lymphoid organs. The relative proportion of CD4+NK1+ T cells was virtually unaffected in all lymphoid organs of LFA-1+/− mice compared with their heterozygous littermates (Fig. 4). We even observed a slight increase in the lymph nodes, spleen, and bone marrow of LFA-1+/− mice. These results suggest that LFA-1 is not involved in the development of CD4+NKT cells but does play a central role in the recruitment of these cells to the liver.

Migration of thymic CD4+NKT cells to the livers of irradiated or SCID mice and the influence of LFA-1

We wondered which cell type expressing LFA-1 (CD4+NKT cells or some other cells such as Kupffer cells, which have been found to express LFA-1 (19)) regulates the accumulation of CD4+NKT cells in the liver. Because virtually all CD4+NKT cells have been shown to express Thy1 (1, 3), we employed a reciprocal radiation thymocyte-reconstitution system with Thy1 congenic and LFA-1−/− mice to address this issue. As shown in Fig. 5, a considerable population of CD4+NK1+ T cells derived from donor thymocytes (Thy1.2+) appeared in the livers of Thy1 congenic recipients that had been irradiated and reconstituted with thymocytes from LFA-1−/− mice. Comparable results were obtained using C57BL/6 or Thy1 congenic mice that had been irradiated and reconstituted with thymocytes from Thy1 congenic or C57BL/6 mice, respectively (data not shown). In contrast, donor thymocyte-derived CD4+NK1+ T cells (Thy1.1+) were rare in the livers of LFA-1−/− mice that had been irradiated and reconstituted with thymocytes from Thy1 congenic mice. Note that the migration efficiency of donor thymocytes to livers was similar in the two groups (Fig. 5). Consistent with these results, the proportions of donor thymocyte-derived CD4+NK1+ T cells in the livers of SCID mice that had received thymocytes from LFA-1+/− or LFA-1−/− mice were comparable (data not shown). These results suggest that the accumulation of CD4+NK1+ T cells in the liver is regulated by LFA-1 expressed on liver cells other than CD4+NKT cells, although CD4+NKT cells express high levels of LFA-1 themselves.

Discussion

This paper reveals a fundamental role for LFA-1 in the accumulation of CD4+NKT cells in the liver. Reduction of this cell population was restricted to the liver only, and no alterations were observed in other organs. In contrast, the accumulation of CD4+NKT cells in the liver was only marginally reduced in ICAM-1−/− mice. These results demonstrate that LFA-1, but not ICAM-1, is crucial for the accumulation of CD4+NKT cells in the liver. The reciprocal radiation thymocyte-reconstitution experiment with LFA-1-deficient and wt mice revealed that LFA-1 expressed on liver cells other than CD4+NKT cells was required for an accumulation of CD4+NKT cells in the liver. We assume that

Table I. Proportions and absolute numbers of CD4+NK1+ T cells in livers of C57BL/6, ICAM-1+/−, and LFA-1+/−, and LFA-1−/− mice.

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>% of CD4+NK1+ T Cells</th>
<th>Absolute Number of CD4+NK1+ T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>19.5 ± 1.8</td>
<td>(4.2 ± 0.4) × 10^6</td>
</tr>
<tr>
<td>ICAM-1+/−</td>
<td>15.4 ± 1.5</td>
<td>(3.6 ± 0.3) × 10^6</td>
</tr>
<tr>
<td>LFA-1+/−</td>
<td>16.3 ± 2.6</td>
<td>(3.5 ± 0.5) × 10^6</td>
</tr>
<tr>
<td>LFA-1−/−</td>
<td>1.5 ± 0.3</td>
<td>(3.0 ± 0.5) × 10^6</td>
</tr>
</tbody>
</table>

*LMNCs were stained as described in Fig. 3. The percentages of CD4+NK1+ T cells within small lymphoid cells were determined. Absolute numbers of CD4+NK1+ T cells were calculated as follows: recovery numbers of LMNCs × percentages of CD4+NK1+ T cells) × 10^7. Data are expressed as the mean ± SD of four to six mice.
interactions of ligands thus far unknown on CD4+ NKT cells with LFA-1 on partner cells participate in the accumulation of CD4+ NKT cells in the liver. In addition to ICAM-1, ICAM-2 and ICAM-3 can serve as ligands for LFA-1 (11). One candidate ligand is ICAM-1, which was found on CD4+ NKT cells (see Fig. 2). However, our data with ICAM-1−/− mice argue against an essential role for this molecule in the accumulation of CD4+ NKT cells in the liver. Yet, our data do not exclude a role for the ICAM-1 expressed on CD4+ NKT cells under normal conditions, because it is possible that the absence of ICAM-1 is compensated by other adhesion molecules. Further studies are aimed at identifying the ligands for LFA-1 expressed on CD4+ NKT cells and the LFA-1-expressing cell type, which is crucial for the liver accumulation. Although we assume that LFA-1 regulates the migration of CD4+ NKT cells to the liver, we do not exclude the alternative possibilities that LFA-1 plays a role in either the retention of CD4+ NKT cells or the local proliferation of a limited number of this cell population in the liver.

There is still controversy regarding whether NKT cells develop in a thymus-dependent or -independent manner (1). Because a markedly higher proportion of NKT cells has been identified in the liver compared with other organs (2, 3), it is possible that the liver is the major site for NKT cell development. Our data show that the accumulation of CD4+ NKT cells in the liver is regulated by LFA-1 expression, suggesting accumulation to the liver of these cells or their precursors. Although one group has claimed higher numbers of NKT cells in the livers of nu/nu mice (20–22), other investigators including us were not able to identify NKT cells in nu/nu mice (1, 3, 23). Moreover, one study shows the development of NKT cells in fetal thymic organ cultures, and another report shows the requirement of CD1-expressing double-positive cortical thymocytes for the generation of NKT cells, suggesting thymus-dependent development (4, 6). While this work was under review, a paper was published in which the rapid regeneration of peripheral NKT cells was achieved via proliferation in the bone marrow after in vivo administration of anti-CD3 mAb or IL-12 even in the absence of the thymus (24). It is possible that bone marrow NKT cells proliferate and maintain homeostasis of peripheral NKT cells even in the absence of the thymus. However, it remains unclear whether NKT cell development in bone marrow is thymus-dependent under normal conditions. Our data show that thymic NKT cells migrate to the liver, suggesting that the thymus provides a reservoir for NKT cells in the periphery, at least in the liver. A comparison of CD1 expression on double-positive thymocytes from LFA-1−/− and LFA-1+/− mice revealed no difference (M.E., unpublished observation). Moreover, in thymus and peripheral lymphoid organs, the numbers of CD4+ NKT cells were not reduced. These results not only exclude the possibility that the reduction of CD4+ NKT cells in LFA-1−/− mice involves CD1, but also suggest that CD4+ NKT cells develop normally in the absence of LFA-1.

In summary, our data indicate a crucial role for LFA-1 in the accumulation of CD4+ NKT cells in the liver. Although CD4+ NKT cells express high levels of LFA-1, we find that LFA-1 on CD4+ NKT cells is not required for liver accumulation. Rather, we demonstrate that LFA-1 on liver cells other than CD4+ NKT cells is crucial for accumulation of the latter in the liver. Because Kupffer cells express LFA-1, they represent candidates for these cells (19).

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