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Cutting Edge: Contribution of NK Cells to the Homing of Thymic CD4\(^+\)NKT Cells to the Liver\(^1\)

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In contrast to peripheral lymphoid organs, in the liver a high proportion of T cells are CD4\(^+\)NKT cells. We have previously reported that LFA-1 plays a pivotal role in the homing of thymic CD4\(^+\)NKT cells to the liver. In the present study, we further assessed which cell type participates in the homing of thymic CD4\(^+\)NKT cells to the liver. The accumulation of donor thymocyte-derived CD4\(^+\)NKT cells in the liver of SCID mice that had been reconstituted with thymocytes from C57BL/6 mice was severely impaired by in vivo depletion of NK cells, but not Kupffer cells in recipients. These results suggest that NK cells participate in the homing of thymic CD4\(^+\)NKT cells to the liver. We assume that LFA-1 expressed on NK cells is involved in this mechanism. The Journal of Immunology, 2000, 165: 1729–1732.

NKT cells represent a peculiar T cell population that is distinct from mainstream T cells. NKT cells express NKR-P1 (NK1.1), and the majority express an invariant TCR encoded by V\(α\)14 and Jo281 gene segments preferentially associated with TRC\(\beta\)8.2. The development of V\(α\)14 NKT cells depends on CD1d expressed on CD4\(^+\)8\(^+\) cortical thymocytes (1, 2–4). V\(α\)14 NKT cells encompass CD4\(^+\) and CD4\(^-\)8\(^-\) cell populations and are prominent in the liver compared with other organs (5, 6). Evidence has accumulated that the thymus is essential for the development of V\(α\)14 NKT cells (1, 6–12).

LFA-1 (CD11a/CD18) is a cell adhesion molecule that mediates adhesion to various cells expressing their ligands, ICAM-1 (CD54) and ICAM-2 (CD102) (13). LFA-1/ICAM interactions play a crucial role in leukocyte recirculation and migration (13). LFA-1 is expressed on most leukocytes including NK cells and NKT cells (1, 13–15). In the liver, LFA-1 is also expressed on sinusoidal lining cells such as Kupffer cells (16, 17).

We have previously shown that LFA-1 plays a crucial role in the homing of thymic CD4\(^+\)NKT cells to the liver (17). However, it remained elusive which cell type expressing LFA-1 participates in the homing of thymic CD4\(^+\)NKT cells to the liver. The data presented here point to NK cells in the liver as a major cell population that directs thymic CD4\(^+\)NKT cells to the liver.

Materials and Methods

Mice

C57BL/6 scid/scid (SCID) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). LFA-1\(^-/-\) mice were generated as described previously (18). LFA-1\(^-/-\) mice backcrossed onto C57BL/6 mice (more than three generations), SCID mice, and C57BL/6 mice were maintained under specific pathogen-free conditions, and female mice were used at 8–10 wk of age.

Antibodies

Anti-Fcy\(R\) mAb (2.4G2), anti-NK1.1 mAb (PK136), anti-CD4 mAb (YTS191.1), anti-CD3e mAb (145-2C11), and anti-F4/80 mAb (CI A3–1) were purified from hybridoma culture supernatants. Anti-NK1.1 mAb was biotinylated, and anti-CD4 mAb and anti-CD3 mAb were conjugated with FITC by standard methods. PE-anti-CD4 mAb (H129.19) and streptavidin (SA)\(^3\)-Red 670 were purchased from Life Technologies (Gaithersburg, MD). FITC-anti-ICAM-2 mAb (3C4), biotin-anti-NK1.1 mAb (PK136), FITC-anti-TCR\(\beta\) mAb (H57-597), and FITC-anti-rabbit IgG were obtained from PharMingen (Hamburg, Germany). Anti-asialo GM1 Ab and rabbit IgG were purchased from Wako Chemicals (Neuss, Germany) and Sigma-Aldrich (Schnelldorf, Germany), respectively. Cy2-anti-rat IgG was obtained from Jackson ImmunoResearch (West Grove, PA).

Cell preparation and flow cytometry

Liver mononuclear cells (LMNC) and intestinal intraepithelial lymphocytes were prepared as described previously (6). Lymphoid cells from other organs were prepared by conventional methods. After blocking with anti-Fcy\(R\) mAb, cells were stained with conjugated mAbs, and biotin-mAbs were visualized with SA-Red 670. After staining, cells were washed and subsequently fixed with 1% paraformaldehyde. Stained cells were acquired by FACScan (Becton Dickinson, Mountain View, CA), and small lymphoid cells were analyzed with CellQuest software (Becton Dickinson).

\(^{4}\) Abbreviations used in this paper: SA, streptavidin; LMNC, liver mononuclear cells; Cl\(_2\)MBP-L, multilamellar liposome-encapsulated dichloromethylene bisphosphonate; PBS-L, liposome-encapsulated PBS.
In vivo depletion of Kupffer cells or NK cells

Multilamellar liposome-encapsulated dichloromethylene bisphosphonate (Cl1-MBP-L) was prepared as described previously (19). Cl1-MBP was a gift from Boehringer Mannheim (Mannheim, Germany). To deplete Kupffer cells, mice were injected i.v. with 200 μl of Cl1-MBP-L (containing 1 mg of Cl1-MBP) suspended in PBS as described previously (19). As a control, mice were injected i.v. with 200 μl of liposome-encapsulated PBS (PBS-L). To deplete NK cells, mice were injected i.p. with 5 mg of anti-asialo GM1 Ab. Rabbit IgG was used as a control. Kupffer cell- or NK cell-depleted SCID mice, as well as control SCID mice, were fed water containing antibiotics throughout the experiment.

Detection of Kupffer cells

SCID mice were treated with Cl1-MBP-L or PBS-L as described above, and 3 days later livers were collected. Liver cryosections were stained with anti-F4/80 mAb followed by Cy2-anti-rat IgG. In Cl1-MBP-L-treated mice, Kupffer cells were depleted by 90–100%.

Results

Thymic CD4⁺ NKT cells preferentially home to the liver

SCID mice were reconstituted with thymocytes from C57BL/6 mice, and the appearance of donor CD4⁺ NKT cells in the liver of recipients was monitored by microfluorometry. A small population of CD4⁺NK1.1⁺ cells was identified in the liver of SCID mice before reconstitution (Fig. 1A), although these cells did not express TCR (data not shown). A distinct population of CD4⁺NK1.1⁺ cells was identified in the liver of recipients on day 3 after reconstitution, and the proportion remained virtually unchanged until day 7 (Fig. 1A). Recovery numbers of LMNC were comparable on days 3, 5, and 7 after reconstitution. A vast majority of CD4⁺NK1.1⁺ cells in the liver of recipients expressed TCRαβ, but not TCRγδ (data not shown). Thus, donor thymocyte-derived CD4⁺NKT cells accumulated in the liver of recipients by day 3 after reconstitution. We next examined the tissue distribution of donor thymocyte-derived CD4⁺NKT cells in recipients on day 3 after reconstitution. The accumulation of donor CD4⁺NKT cells in recipients was prominent in the liver, scarce in the spleen, and no accumulation was found in other organs analyzed (Fig. 1B). These results indicate that thymic CD4⁺NKT cells preferentially home to the liver.

LFA-1 expressed on liver cells other than CD4⁺NKT cells participates in the homing of thymic CD4⁺NKT cells to the liver

SCID mice were reconstituted with thymocytes from LFA-1⁻/⁻ or C57BL/6 mice, and the presence of donor CD4⁺NKT cells in the liver of recipients was assessed on day 3 after reconstitution. Numbers of donor CD4⁺NKT cells in the liver of recipients were increased regardless of the origin of thymocytes used for reconstitution, although the accumulation efficiency of CD4⁺NKT cells from LFA-1⁻/⁻ donors was slightly higher compared with that from C57BL/6 donors (Fig. 2). This was probably due to a dilution effect because CD4⁺8⁺ thymocytes from C57BL/6 mice accumulated more efficiently in the liver of recipients than those from LFA-1⁻/⁻ mice (data not shown). These results indicate that homing of thymic CD4⁺NKT cells to the liver occurs independently of LFA-1 expression on these cells. It appears more likely that LFA-1 on liver cells directs CD4⁺NKT cells to this organ.

Kupffer cells do not participate in the homing of thymic CD4⁺NKT cells to the liver

Because Kupffer cells express LFA-1 (16, 17), we asked whether LFA-1 on Kupffer cells participates in the homing of thymic CD4⁺NKT cells to the liver. To address this issue, Cl1-MBP-L was employed to deplete Kupffer cells in vivo (19). SCID mice were injected with Cl1-MBP-L, and 3 days later the depletion of Kupffer cells was assessed by immunohistochemical procedure using anti-F4/80 mAb. Consistent with previous findings (19), Kupffer cells became undetectable on day 3 after Cl1-MBP-L treatment (data not shown). No measurable alterations were found in LMNC by Cl1-MBP-L treatment (data not shown). We then assessed the consequences of Kupffer cell depletion on homing of thymic CD4⁺NKT cells to the liver. SCID mice were injected with Cl1-MBP-L, reconstituted with thymocytes from C57BL/6 mice, and the proportion of donor CD4⁺NKT cells in the liver of recipients was assessed on day 3 after reconstitution. The proportion of CD4⁺NKT cells in the liver of recipients was comparable among Cl1-MBP-L-, PBS-L-, or PBS-treated groups (Fig. 3). These results suggest that Kupffer cells are not essential for the homing of thymic CD4⁺NKT cells to the liver.
NK cells participate in the homing of thymic CD4+ NKT cells to the liver

NK cells are abundant in the liver and express high levels of LFA-1 (14). Therefore, we wondered whether LFA-1 on NK cells plays a role in the homing of thymic CD4+ NKT cells to the liver. Consistent with previous findings (20, 21), a vast majority of liver NK cells expressed high levels of asialo GM1 on their surface, whereas asialo GM1 was not expressed on conventional CD4+ T cells (Fig. 4). Asialo GM1 was marginally expressed on CD4+ NKT cells (Fig. 4). Accordingly, most liver NK cells were depleted on day 3 after anti-asialo GM1 Ab treatment (Fig. 5), whereas the frequency of CD4+ NKT cells remained virtually unaffected (Fig. 5). Selective depletion of NK cells, but not NKT cells, by anti-asialo GM1 Ab treatment is consistent with findings by others (22). Administration of rabbit IgG did not affect any of these cell populations, thus excluding nonspecific effects (Fig. 5). These results suggest that NK cells are not essential for the persistence of CD4+ NKT cells in the liver.

To directly assess the role of NK cells in the homing of thymic CD4+ NKT cells to the liver, SCID mice were treated with anti-asialo GM1 Ab to deplete NK cells, reconstituted with thymocytes from C57BL/6 mice, and the proportion of donor CD4+ NKT cells to the liver of recipients was severely impaired by NK cell depletion (Fig. 6). These results suggest that NK cells participate in the homing of thymic CD4+ NKT cells to the liver.

Discussion

The present study shows that the thymus serves as a source for liver CD4+ NKT cells, and it provides evidence that NK cells contribute to the homing of CD4+ NKT cells to the liver. Donor thymocyte-derived CD4+ NKT cells accumulated in the liver, but not in other organs, of recipients, suggesting preferential homing of thymic CD4+ NKT cells to the liver. However, the proportion of donor thymocyte-derived CD4+ NKT cells in the liver of recipients was low (4–5%) after reconstitution, as compared with the proportion of CD4+ NKT cells in the liver of normal C57BL/6 mice (5, 6). We consider it likely that intact thymus, which can constantly supply CD4+ NKT cells to the periphery, is necessary for further accumulation of this cell population in the liver. The accumulation of donor thymocyte-derived CD4+ NKT cells in the liver of recipients was impaired by in vivo depletion of NK cells, but not Kupffer cells. These results suggest that NK cells rather than Kupffer cells participate in the homing of thymic CD4+ NKT cells to the liver. Consistent with this, after birth NK cells numerically increased in the liver before CD4+ NKT cells (23).
Kupffer cells influence the differentiation of liver-specific NK cells (24), and we cannot exclude the possibility that Kupffer cells participate in the homing of CD4+ NKT cells to the liver. Apparently, CD4+ NKT cells expressed low levels of asialo GM1 on their surface, and, hence, CD4+ NKT cells in the liver of C57BL/6 mice were slightly affected by anti-asialo GM1 Ab treatment. Therefore, we cannot exclude the possibility that the residual anti-asialo GM1 Ab in recipients impaired the accumulation of donor thymocyte-derived CD4+ NKT cells in the liver. Nevertheless, we consider it more likely that the effects of anti-asialo GM1 Ab are best explained by assuming that NK cells participate in the homing of CD4+ NKT cells to the liver.

The number of NKT cells in the liver was markedly reduced in LFA-1−/− mice compared with C57BL/6 mice (15, 25). This reduction was restricted to the liver only, and no measurable alterations were found in other organs. A small, but distinct population of NKT cells was detectable in the liver of LFA-1−/− mice, and the proportion in the liver was comparable to that in the spleen (15, 25). These findings not only imply that the homing of a vast majority of NKT cells to the liver is LFA-1 dependent, but also suggest that the homing of a small population of NKT cells to the liver, and of virtually all NKT cells to other peripheral lymphoid organs such as spleen, is LFA-1 independent. Recent studies have indicated control of NK cells by NKT cells (26, 27). Taken together with our findings, the picture of a bidirectional cross-talk between NK cells and NKT cells emerges.

The number of CD4+ NKT cells was slightly reduced in the liver of ICAM-1−/− mice compared with C57BL/6 mice (15). In addition to ICAM-1, ICAM-2 is also a ligand for LFA-1 in the mouse (13). Because CD4+ NKT cells also expressed ICAM-2 (M. Miyamoto and M. Emoto, unpublished observation), LFA-1/ICAM-2 interactions could also participate in homing. Further studies are aimed at clarifying the role of ICAM-2 in the homing of CD4+ NKT cells to the liver.

In summary, our data reveal a possible contribution of NK cells to the homing of thymic CD4+ NKT cells to the liver. Because NK cells express high levels of LFA-1, we consider LFA-1 expression on NK cells critical.

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References

FIGURE 6. Proportions of donor thymocyte-derived CD4+ NKT cells in the liver of NK cell-depleted thymocyte-reconstituted SCID mice. SCID mice were treated with 500 μl of anti-asialo GM1 Ab (5 mg) or rabbit IgG (5 mg), and 3 days later reconstituted i.v. with 3 × 107 thymocytes from C57BL/6 mice. LMNCs were prepared on day 3 after reconstitution and stained and analyzed as described in Fig. 1. Representative results from four mice per group are shown.