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Increase in Hepatic NKT Cells in Leukocyte Cell-Derived Chemotaxin 2-Deficient Mice Contributes to Severe Concanavalin A-Induced Hepatitis

Takeshi Saito,* Akinori Okumura,* Hisami Watanabe,† Masahide Asano,‡ Akiko Ishida-Oka|ura,*, Junko Sakagami,‡ Katsuko Sudo,‡ Yoshimi Hatano-Yokoe,§ Jelena S. Bezbradica,¶ Sebastian Joyce,¶ Toru Abo,‖ Yoichiro Iwakura,‡ Kazuo Suzuki,* and Satoshi Yamagoe*

Leukocyte cell-derived chemotaxin 2 (LECT2) was originally identified for its possible chemotactic activity against human neutrophils in vitro. It is a 16-kDa protein that is preferentially expressed in the liver. Its homologues have been widely identified in many vertebrates. Current evidence suggests that LECT2 may be a multifunctional protein like cytokines. However, the function of LECT2 in vivo remains unclear. To elucidate the role of this protein in vivo, we have generated LECT2-deficient (LECT2−/−) mice. We found that the proportion of NKT cells in the liver increased significantly in LECT2−/− mice, although those of conventional T cells, NK cells, and other cell types were comparable with those in wild-type mice. Consistent with increased hepatic NKT cell number, the production of IL-4 and IFN-γ was augmented in LECT2−/− mice upon stimulation with α-galactosylceramide, which specifically activates Vα14 NKT cells. In addition, NKT cell-mediated cytotoxic activity against syngeneic thymocytes increased in hepatic mononuclear cells obtained from LECT2−/− mice in vitro. Interestingly, the hepatic injury was exacerbated in LECT2−/− mice upon treatment with Con A, possibly because of the significantly higher expression of IL-4 and Fas ligand. These results suggest that LECT2 might regulate the homeostasis of NKT cells in the liver and might be involved in the pathogenesis of hepatitis. The Journal of Immunology, 2004, 173: 579–585.

Leukocyte cell-derived chemotaxin 2 (LECT2) was originally identified from the culture fluid of the human T cell line SKW-3 in the process of screening for a novel neutrophil chemotactic protein (1). LECT2 is expressed preferentially in the liver in a constitutive manner. LECT2 protein is secreted into the bloodstream (2, 3). Proteins homologous to LECT2 have been isolated in many vertebrates (4, 5). LECT2 is identical with chondromodulin II, which was identified as a growth stimulator for chondrocytes and osteoblasts (6). The polymorphism of human LECT2 at Val58Ile is associated with the severity of rheumatoid arthritis in the Japanese population (7). We recently reported that the expression of mouse LECT2 was transiently decreased during Con A-induced hepatitis, an experimental model for human autoimmune hepatitis that is induced by the expression of cytokines and cytotoxic molecules associated with effects from other immune cells, such as CD4+ T lymphocytes and macrophages (8). Thus, LECT2 seems to be a multifunctional protein like cytokines. However, the function of LECT2 in vivo remains unclear.

NKT cells form a distinctive T cell subpopulation that has some of the characteristics of NK cells. NKT cells are present in various lymphoid organs and are especially abundant in the liver (9–11). In mice, NKT cells commonly express a semi-invariant TCR and NK1.1 Ag, and their development and functions are regulated by CD1d (9, 12). There is growing evidence that NKT cells play an important role in immune responses (9–13). It is well established that NKT cells express large amounts of cytokines, especially IFN-γ and IL-4, and their functional disorders are characteristic of various diseases (9, 12, 13). Recently, some groups reported that NKT cells played an essential role in Con A-induced hepatitis (14–16).

In this study we generated LECT2−/− mice with the aim of clearly identifying the role of LECT2 in vivo. We found that these mice showed an increased number of hepatic NKT cells, which appeared to function as they do in wild-type mice. To examine the biological effect of this phenotype, we used a Con A-induced hepatitis model. The deficiency of LECT2 led to severe liver injury, possibly because of excessive expression of IL-4 and Fas ligand (FasL) by the increase in the number of hepatic NKT cells.

Materials and Methods

Mice

Wild-type C57BL/6J (B6) mice purchased from CLEA Japan were housed at the National Institute of Infectious Diseases. Mice used in this study...
were maintained under specific pathogen-free conditions and were usually used at 8–12 wk of age according to the guidelines of the National Institute of Infectious Diseases animal care and use committee.

**Generation of LECT2−/− mice**

To construct the targeting vector (pKO-9), two fragments (5’ end, 7.7 kb; 3’ end, 1.4 kb) of the genomic DNA flanking the coding region of the 129-derived lect2 gene (17) were subcloned between the BamHI and SpeI sites and between the AarI and Scal sites of the pBluescript II KS+ vector (Stratagene, La Jolla, CA), respectively. The pGKneobpA cassette (18) was inserted between SpeI and AarI for positive selection (Fig. 1A). The DT-A cassette (19) was ligated at the 5’ end of the targeting vector for negative selection. E14.1 ES cells (1 × 107 cells) were transferred with the linearized targeting vector by electroporation and were selected with G418 (Invitrogen, Carlsbad, CA). Homologous recombinants were screened by PCR and confirmed by Southern blot hybridization (Fig. 1A; data not shown). The forward primer (P2) in the pGKneobA cassette was 5’-GGTGGAATGTTGAATGTGTCATGG-3’ , and the reverse primer (P5) outside the targeting vector was 5’-ACCATCTACTAGCTCTTGTAG-3’. Chimeric mice were generated by the agarose separation method (19) with some modifications. The chimeras were mated with B6 mice, and homozygous mutant mice were generated by the intercrossing of heterozygotes. LECT2−/− mice and littersmates were genotyped by PCR (Fig. 1B). The primer sequences used were as follows: LECT2-common, 5’-CCACCCCCACCTAAGATGTATGCTGC-3’, LECT2-wild-type, 5’-CCAGAGTTCATATTGCTGCTGTGGG-3’, and LECT2-knockout, 5’-CTCTCTGAGGATTCCTGAGGGG-3’.

**Immunoblot analysis**

Two micrograms of serum prepared from LECT2−/− mice and their littersmates was resolved by SDS-PAGE (4–20%) and transferred to Immobilon-P (Millipore, Bedford, MA). The blots were incubated with rabbit anti-mouse LECT2 against a recombinant mouse LECT2 produced stably in B95-A (Millipore, Bedford, MA). The blots were incubated with rabbit anti-mouse LECT2 against a recombinant mouse LECT2 produced stably in CHO cells. The immunoreactive protein was visualized using an ECL detection kit (Amersham Pharmacia Biotech, Piscataway, NJ).

**Cytotoxicity assays**

Male B6 (wild-type controls) and LECT2−/− B6 mice were injected i.v. with 25 mg/kg Con A (type IV; Sigma-Aldrich). Two, 5, and 8 h after Con A injection, sera were collected to measure the level of cytokines and glutamic pyruvic transaminase (GPT) activity, and liver tissue was reverse transcribed and subjected to PCR analysis. The primers used are described in Materials and Methods. D, Serum from mice was analyzed by Western blot analysis using rabbit anti-LECT2 polyclonal Ab (2).

**Histology and TUNEL staining**

Liver tissues were fixed in 4% neutralized formalin and embedded in paraffin, then sliced to a thickness of 3 μm. For histological examination, sections were stained with H&E. Apoptotic cells were detected with an In Situ Cell Death Detection Kit, POD (Roche, Mannheim, Germany).

**Semiquantitative RT-PCR**

The total liver RNA for each mouse was isolated using Isogen reagent (Wako Pure Chemical). One microgram of RNA was reverse transcribed with a ReverTra Ace (Toyobo, Osaka, Japan) to obtain cDNA. The primer sequences used in PCR were as follows: LECT2, 5’-ACGTTGCTGACGCTATTGGC-3’ and 5’-AGGTTATGCTTGCTGGGTCACTGGAG-3’. The primers were confirmed by PCR and confirmed by Southern blot hybridization (Fig. 1A; data not shown). The forward primer (P2) in the pGKneobA cassette was 5’-GGTGGAATGTTGAATGTGTCATGG-3’, and the reverse primer (P5) outside the targeting vector was 5’-ACCATCTACTAGCTCTTGTAG-3’.

**Lymphocyte preparations**

Mice anesthetized with ether were killed by exsanguination via the axillary artery. The liver and spleen were then removed. Hepatic mononuclear cells (MNCs) were prepared as described previously (20). Briefly, the liver lobes were minced to small pieces, pressed through 200-gauge stainless steel mesh, and suspended in Eagle’s MEM (Sigma-Aldrich, St. Louis, MO) with 5% HEPES (pH 7.5) and 2% FBS. After washing, the pellet was resuspended in 5% Percoll solution (Amershams Pharmacia Biotech, Uppsala, Sweden) containing heparin (100 U/ml) and centrifuged at 2000 rpm for 15 min. The pellet was then resuspended in RBC lysis solution (155 mM NH4Cl, 10 mM KHCO3, 1 mM EDTA, and 17 mM Tris, pH 7.3) and washed twice with MEM. Splenocytes were prepared by forcing minced spleens through stainless steel mesh and were used after RBC lysis.

**Flow cytometric analysis**

Single-cell suspensions from the liver and spleen were incubated with mAbs against cell surface markers (BD Pharmingen, San Diego, CA). PE-labeled CD14-α-galactosylceramide (CD14-α-GalCer) tetramer (21) and FITC-, PE-, allopurinol-, or PerCP-conjugated Abs specific for murine CD3 (145-2C11), CD4 (RM4-5), NK1.1 (PK136), Gr-1 (RB6-8C5), Mac-1 (M1/70), and FasL (MFL-3) were used for flow cytometric analyses. Apoptotic cells were stained with an Annexin V-FITC Apoptosis Detection I kit (BD Pharmingen) and then analyzed with FACSCalibur using CellQuest software (BD Biosciences, San Jose, CA).

**Administration of α-GalCer**

α-GalCer was kindly provided by Kirin Brewery (Gunma, Japan). This reagent was dissolved in 0.5% polysorbate 20 (Nikkol Chemical, Tokyo, Japan) at a concentration of 200 μg/ml, then further diluted with physiological saline. For in vivo administration, α-GalCer was injected i.p. at a dose of 100 μg/kg body weight. For in vitro stimulation of MNCs, α-GalCer was added to the culture medium (RPMI 1640 supplemented with 2% FBS) at a concentration of 100 ng/ml.

**Cytokine and transaminase measurement**

The levels of serum IFN-γ, TNF-α, and IL-4 were quantified using an OptEIA ELISA set (BD Pharmingen). Serum GPT activity was measured with a Transaminase CII-test Wako kit (Wako Pure Chemical, Osaka, Japan).

**Histology and TUNEL staining**

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TC-3' and GAPDH, 5'-GGCCATGAGGTCCACCACCCTGTTGCTGTA-3' and 5'-GCCCTGCCCACTCCACCACCAACTTT-3'. Reactions were conducted under the following conditions: pre-cycling at 94°C for 1 min, then 25 cycles consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and polymerization at 72°C for 1 min.

**Quantitative real-time RT-PCR**

Quantitative real-time RT-PCR was performed using ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. Primers and TaqMan probes specific for TNF-α, IL-4, and FasL were obtained from Assay-on-Demand Gene Expression Products (Applied Biosystems), and IFN-γ was derived from TaqMan Pre-Developed Assay Reagents (Applied Biosystems). For endogenous control, the level of GAPDH in each sample was assayed using TaqMan Rodent GAPDH Control Reagents VIC (Applied Biosystems). Data analyses were performed on ABI PRISM 7000 SDS software version 1.0 (Applied Biosystems).

**Statistical analysis**

Results were expressed as the mean ± SD. Statistical analyses were conducted using Student's t test or the Mann-Whitney U test. A value of $p < 0.05$ was considered significant.

**Results**

**Generation of LECT2−/− mice**

To delete the lect2 gene, a targeting construct carrying a neomycin cassette was placed within the lect2 locus in such a way that all exons for this gene were deleted (Fig. 1A). Correct targeting of the lect2 locus was confirmed by PCR and genomic Southern blot analysis (Fig. 1B; data not shown). LECT2−/− mice were backcrossed for 10 generations to B6 and intercrossed to generate LECT2−/− mice. Complete loss of LECT2 expression in the liver and serum of LECT2−/− mice was confirmed by RT-PCR and immunoblot analysis, respectively (Fig. 1, C and D). LECT2−/− mice were born at the Mendelian ratio and were indistinguishable in appearance from wild-type mice. Both male and female LECT2−/− mice were fertile.

**Flow cytometric analysis of MNCs from LECT2−/− and wild-type mice**

LECT2 is preferentially expressed in the liver (2). LECT2−/− mice exhibited no obvious abnormality in the size or histology of the liver or in serum GPT activity. In addition, the sizes of other tissues, such as spleen and thymus, were normal. We recently reported a possible additional role of LECT2 in Con A-induced hepatic injury (3). Con A-induced hepatitis results from injuries inflicted by various immune cells such as CD4+ T cells and NK1.1+ T cells (8, 14–16). Therefore, we measured the proportions of immune cells in the liver by flow cytometric analysis. Interestingly, we observed that the percentage of hepatic CD3+ NK1.1+ cells in LECT2−/− mice was significantly higher than that in wild-

![FIGURE 2. Flow cytometric analysis of mononuclear cells in LECT2−/− mice. Two-color staining for CD3 and NK1.1, CD4 and NK1.1, and Mac-1 and Gr-1 against MNCs from the liver (A) or the spleen (B) was performed. C, Hepatic MNCs were stained with CD1d-α-GalCer tetramer and anti-CD3 Ab, and CD1d-α-GalCer tetramer and anti-CD4 Ab. The numbers in the small panels indicate the representative percentages of fluorescence-positive cells in corresponding areas in six mice.](http://www.jimmunol.org/.../2017)
type mice (16.0 ± 4.6% in wild-type vs 26.1 ± 6.2% in LECT2−/−; p < 0.01; n = 6; Fig. 2A). The proportion of CD4+ NK1.1+ cells in the livers of LECT2−/− mice also increased compared with that in wild-type mice (11.1 ± 3.5% in wild-type vs 19.3 ± 4.8% in LECT2−/−; p < 0.01; n = 6; Fig. 2A). Furthermore, the proportion of CD4+ among the CD3+NK1.1+ cells of LECT2−/− mice was higher than that in wild-type mice (70.1 ± 6.9% in wild-type vs 79.4 ± 1.2% in LECT2−/−; p < 0.01; n = 6). In contrast, there were no differences in the contents of other cell types such as CD3−NK1.1− cells (i.e., conventional T cells), CD3−NK1.1+ cells (i.e., NK cells), or granulocytes (Fig. 2A). The total amounts of MNCs obtained from the livers of wild-type and LECT2−/− mice were also comparable (2.3 ± 0.4 × 10⁶ cells in wild-type vs 2.2 ± 0.7 × 10⁶ cells in LECT2−/−; n = 6; Fig. 2A). We also examined the proportions of immune cells in spleen, thymus, and bone marrow, but could find no significant differences (Fig. 2B; data not shown). These results suggest that LECT2−/− mice contain an increase in the proportion of hepatic NK cells. The majority of NK cells express invariant Vα14-Jα18 for TCR that bind to a glycolipid Ag α-GalCer, presented by CD1d. Invariant Vα14 NK cells can be detected by using CD1d tetramer loaded with α-GalCer (21, 23, 24). LECT2−/− mice contained approximately twice the proportion of CD3+CD1d-α-GalCer tetramer+ (12.2 ± 3.5% in wild-type vs 21.7 ± 3.6% in LECT2−/−; p < 0.01; n = 6) and CD4+CD1d-α-GalCer tetramer+ cells (9.7 ± 3.0% in wild-type vs 17.7 ± 3.0% in LECT2−/−; p < 0.01; n = 6) in the liver (Fig. 2C). We also confirmed by semiquantitative RT-PCR analysis that the expression of Vα14-Jα18 transcripts was definitely high in the livers of LECT2−/− mice (data not shown).

**FIGURE 3.** Effect of α-GalCer treatment. A, IFN-γ and IL-4 expression of mice administered i.p. with α-GalCer (100 μg/kg; n = 6 at each time point). *p < 0.05; **p < 0.01. The data are expressed as the mean ± SD. B, IFN-γ and IL-4 production by MNCs. MNCs from the liver (2 × 10⁶ cells/ml) were cultured in RPMI 1640 supplemented with 2% FBS. Cytokines released into the culture supernatants were measured 24 h after treatment with α-GalCer (100 ng/ml). The mean values of the results obtained in triplicate are shown with the SD. The data shown are representative of four experiments.

**Responsiveness to α-GalCer treatment**

NKT cells express high levels of cytokines, especially IFN-γ and IL-4, in specific response to α-GalCer treatment both in vivo and in vitro (25). As previously stated, LECT2−/− mice contain approximately twice the proportion of Vα14 NKT cells that are found in wild-type mice. To address the specific reactivity of NKT cells, we next measured cytokine production after the i.p. administration of α-GalCer to LECT2−/− mice and wild-type mice. Two hours after this treatment, LECT2−/− mice produced a significantly higher level of IL-4 and a slightly increased IFN-γ level compared with wild-type mice (Fig. 3A). We also measured the release of IFN-γ and IL-4 from cultured hepatic MNCs after stimulation with α-GalCer. As Fig. 3B indicates, higher amounts of IFN-γ and IL-4 were produced from the MNCs of LECT2−/− mice.

**Cytotoxicity assay**

We next examined two types of cytotoxicity of MNCs prepared from the liver and spleen of wild-type and LECT2−/− mice. NK cell-sensitive cytotoxicity primarily mediated by the perforin-granzyme system was assayed against YAC-1 cells, whereas NKT cell-sensitive cytotoxicity, primarily mediated by the Fas/FasL system, was assayed against syngeneic thymocytes (13, 22, 26–28). Hepatic MNCs from LECT2−/− mice showed substantially greater cytotoxicity against syngeneic thymocytes than did those from wild-type mice (Fig. 4), indicating that the Fas/FasL-mediated cytotoxicity of hepatic MNCs in LECT2−/− mice was much higher than that in wild-type mice. We also observed a slight increase in cytotoxicity in hepatic MNCs from LECT2−/− mice against YAC-1 cells (Fig. 4). The cytotoxicity of splenocytes against syngeneic thymocytes and YAC-1 cells was comparable between wild-type and LECT2−/− mice (Fig. 4).

**Con A-induced hepatitis**

Growing evidence indicates that NKT cells contribute significantly to the onset of Con A-induced hepatitis by expression of IL-4 and activation of cytotoxic systems (14–16). To examine whether the increase in NK cells in LECT2−/− mice affects susceptibility to Con A-induced hepatitis, LECT2−/− and wild-type mice were injected i.v. with Con A. Serum GPT activity in LECT2−/− mice was elevated within 5 h after Con A administration compared with that in wild-type mice (Fig. 5A). In LECT2−/− mice only, histological examination showed a focal degenerative change, and cell clusters consisting of apoptotic cells could be detected in the liver 5 h after Con A injection (Fig. 5B). Con A-induced hepatitis requires the activation of immune cells accompanied by the secretion of various cytokines (13–16, 29). We therefore measured serum

**FIGURE 4.** Cytotoxicity assays. MNCs were prepared from the liver and spleen of wild-type and LECT2−/− mice. NKT cell-sensitive cytotoxicity was determined using syngeneic B6 thymocytes as target cells. NK cell-sensitive cytotoxicity was determined using YAC-1 cells. Triplicate cultures lasted 4 h at the indicated E:T cell ratio. *p < 0.05; **p < 0.01.
cytokine levels during the course of the liver injury. Significant elevation of IL-4 in the serum was observed in LECT2−/− mice (Fig. 5C). In contrast, the levels of TNF-α and IFN-γ were not significantly different between wild-type and LECT2−/− mice (Fig. 5C). Furthermore, the levels of IL-6 and IL-10 in these mouse types were also comparable (data not shown). To compare the local expression of cytokines, quantitative real-time RT-PCR analysis of liver tissue RNA at 2 h after Con A injection was performed. The results revealed that IL-4 and FasL expression in the liver of LECT2−/− mice was significantly higher than that in wild-type liver (Fig. 5D). FasL is known as an effector molecule in Con A-induced hepatic injury, and NKT cells primarily express it (16). Thus, the increased hepatic LECT2−/− NKT cells contribute to the severity of Con A-induced hepatitis.

FasL expression and increase in annexin V-positive CD3int NK1.1+ cells during Con A-induced hepatitis

To examine whether the NKT cells of LECT2−/− mice indeed expressed large amounts of FasL upon stimulation with Con A, the proportion of CD3int NK1.1+ cells expressing FasL was determined by flow cytometric analysis. Three hours after Con A injection, the CD3int NK1.1+ cells in LECT2−/− mice expressed approximately twice the amount of FasL that was found in wild-type mice (Fig. 6A). Conventional T cells of both wild-type and LECT2−/− mice expressed scarcely any FasL (Fig. 6A).

Next, we analyzed the proportion of NKT cells that become apoptotic upon stimulation with Con A, because current evidence suggests that hepatic NKT cells are eliminated by apoptosis after Con A injection (16). The three-color staining of CD3int NK1.1+ and annexin-V demonstrated that hepatic CD3int NK1.1+ cells of both wild-type and LECT2−/− mice decreased 3 h after Con A injection, and the proportion of annexin V-positive cells was higher in LECT2−/− mice. In contrast, conventional T cells were not stained with annexin V (Fig. 6B). These results suggest that hepatic NKT cells in LECT2−/− mice showed increased activation-dependent apoptosis, as is the case in wild-type mice.

Discussion

LECT2 was originally noted for its possible neutrophil chemotactic activity (1). In addition, it was independently reported to be a growth-stimulating factor for chondrocytes and osteoblasts and was named chondromodulin II (6). To determine the function of LECT2 in vivo, we generated LECT2−/− mice. In some preliminary experiments we could not easily assess any clear differences related to the above two activities. Therefore, based on the possible roles of LECT2 in liver injury and its tissue-specific expression (2, 3), we focused on the liver.
In the present study we found an increased proportion of hepatic CD^3^+^CD^4^+ NKT cells in LECT2^−/−^ mice compared with that in wild-type mice (Fig. 2A). Moreover, we observed that the proportion of hepatic CD^3^+^CD^4^+^CD1d−/− NKT cells in LECT2^−/−^ mice was about double that in wild-type mice (Fig. 2C), indicating that LECT2^−/−^ mice have an increased proportion of hepatic Vα14 NKT cells. The production of IL-4 and IFN-γ 2 h after administration of α-GalCer in mice would be primarily derived from NKT cells (30). Furthermore, the higher production of both cytokines from the hepatic MNCs treated with α-GalCer is consistent with this result. Therefore, the differences in the levels of IL-4 and IFN-γ after stimulation with α-GalCer in vivo and in vitro could be explained by the increased number of NKT cells in LECT2^−/−^ mice. In addition, the Fas/ FasL-sensitive cytotoxicity of hepatic MNCs in LECT2^−/−^ mice was much higher than that in wild-type mice. In contrast, this cytotoxicity of spleen cells from both wild-type and LECT2^−/−^ mice was comparable. We also could find no significant differences in the percentage of CD^3^+^NK1.1^+^ cells in the spleen cells of LECT2^−/−^ and wild-type mice. Therefore, augmentation of the Fas/FasL-sensitive cytotoxicity shown in hepatic MNCs was possibly due to the increased percentage of NKT cells in LECT2^−/−^ mice. In addition, the NK-sensitive cytotoxicity in hepatic MNCs from LECT2^−/−^ mice was slightly higher than that in MNCs from wild-type mice. At present, although the reason for this slight enhancement is not clear, the cytotoxicity of hepatic NK cells also might be enhanced in LECT2^−/−^ mice. Thus, all these results indicate that LECT2^−/−^ mice show an increase in hepatic NKT cells, which appear to function as they do in wild-type mice.

To explore the biological effects of the increased number of hepatic NKT cells in LECT2^−/−^ mice, we compared wild-type and LECT2^−/−^ mice using the Con A-induced hepatitis model. The onset of Con A-induced hepatitis requires a complicated process of activation of cytokines from immune cells. Both IFN-γ and TNF-α play crucial roles in Con A-induced hepatitis (29, 31). Recent reports have pointed out that activated NKT cells expressing IL-4 also play an essential role in Con A-induced hepatitis and mediate subsequent activation of the cytotoxic pathways (14–16). We showed that LECT2^−/−^ mice were clearly sensitive to Con A-induced hepatitis (Fig. 5, A and B), and that the levels of IL-4 and FasL in LECT2^−/−^ mice were higher than those in wild-type mice (Fig. 5, C and D). These findings strongly suggest that a higher level of IL-4 expression induces excessive FasL and also probably granzyme B (15), resulting in the production of a number of apoptotic hepatocytes (Fig. 5B) and a larger proportion of annexin V-positive hepatic CD^3^+^NK1.1^+^ cells (Fig. 6). Recently, several reports showed that the NK1.1 marker of CD^3^+^NK1.1^+^ cells is down-modulated on activation (32–35). Therefore, the decrease in CD^3^+^NK1.1^+^ cells after Con A challenge in both wild-type and LECT2^−/−^ mice (Fig. 6) might be due not only to apoptosis of NKT cells, but also to down-regulation of the NK1.1 marker.

The reason for the increase in hepatic NKT cells in LECT2^−/−^ mice is an important issue. LECT2 might participate in the differentiation, development, or both of NKT cell lineages. There is considerable evidence for genes that positively regulate the development of NK or NKT lineages (36). In contrast, LECT2^−/−^ mice have an increase in NKT cells in the liver, suggesting that LECT2 might negatively regulate the development of NKT cell lineages. However, considering that there were no obvious differences in CD^3^+^NK1.1^+^ cells of spleen, thymus, and bone marrow (Fig. 2C; data not shown), a role for LECT2 might be related to the homeostasis of NKT cells in the liver. For example, LECT2 might control an immune state in the liver by regulating the selection and/or expansion of hepatic NKT cells or the homing activity of NKT cells toward the liver. Moreover, a distinctive feature of NKT cells in LECT2^−/−^ mice is that the difference in IL-4 production between wild-type and LECT2^−/−^ mice is greater than that in IFN-γ production upon stimulation with α-GalCer. The possible IL-4 dominance might be related to the observation that mice containing an increased number of NKT cells tend to be IL-4 dominant (37–40), or immature NKT cell lineages tend to exhibit the
Th2-type-dominant phenotype (41, 42). It is possible that an imbalance in the proportion of NKT cells in LECT2−/− mice affects an immune state, which is associated with the pathogenesis of certain immune diseases.

In summary, our results revealed that the number of hepatic NKT cells was increased in LECT2−/− mice and suggested that LECT2 may play an important role in the homeostasis of NKT cells in the liver. Although a deficiency of LECT2 does not cause any significant abnormality in mice under physiological conditions, they become susceptible to Con A-induced hepatitis, probably due to excessive production of IL-4 and FasL from NKT cells. Thus, it is possible that LECT2 might be involved in the pathogenesis of hepatitis or other inflammatory diseases in humans through modulation of NKT cell activity.

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