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*J Immunol* published online 26 September 2012
http://www.jimmunol.org/content/early/2012/09/26/jimmunol.1201181
Role of Hepatocyte-Derived IL-7 in Maintenance of Intrahepatic NKT Cells and T Cells and Development of B Cells in Fetal Liver

Bingfei Liang,* Takahiro Hara,* Keisuke Wagatsuma,* Jia Zhang,† Kazushige Maki,*‡ Hitoshi Miyachi,‡ Satsuki Kitano,‡ Chihiro Yabe-Nishimura,‡ Shizue Tani-ichi,* and Koichi Ikuta*

The liver contains a variety of resident immune cells, such as NK cells, NKT cells, T cells, macrophages, and dendritic cells. However, little is known about how IL-7, which is produced by hepatocytes, functions locally in development and maintenance of liver immune cells. To address this question, we established IL-7–floxed mice and crossed them with albumin promoter-driven Cre (Alb-Cre) transgenic mice to establish conditional knockout of IL-7 in hepatocytes. The levels of IL-7 transcripts were reduced 10-fold in hepatocyte fraction. We found that the absolute numbers of NKT and T cells were significantly decreased in adult liver of IL-7f/f Alb-Cre mice compared with IL-7f/f control mice. In contrast, NK cells, dendritic cells, and B cells were unchanged in the IL-7f/f Alb-Cre liver. The number of Vα14+ invariant NKT cells was significantly reduced in liver, but not in thymus and spleen, of IL-7f/f Alb-Cre mice. Furthermore, B cell development was impaired in perinatal liver of IL-7f/f Alb-Cre mice. This study demonstrates that hepatocyte-derived IL-7 plays an indispensable role in maintenance of NKT and T cells in adult liver and development of B cells in fetal liver, and suggests that hepatocytes provide a unique IL-7 niche for intrahepatic lymphocytes. The Journal of Immunology, 2012, 189: 000–000.

Interleukin-7 is a cytokine essential for lymphocyte development and survival. The IL-7R consists of the common cytokine receptor γ-chain and the unique IL-7R α-chain (IL-7Rα). Mice deficient in either IL-7 or IL-7Rα show markedly reduced numbers of T and B cells (1, 2). Early T and B cells require IL-7 for proliferation and survival in the thymus and bone marrow, respectively. In addition, IL-7 plays important roles in differentiation of positively selected CD8 T cells in the thymus (3). Furthermore, IL-7R and the transcription factor STAT5 control V(D)J recombination in the TCRγ and IgH loci (4–6). In the periphery, IL-7 regulates T cell homeostasis by enhancing survival and proliferation of naive and memory T cells (7). IL-7 is produced by mesenchymal and epithelial cells in lymphoid organs, as such thymic epithelial cells, bone marrow stromal cells, fibroblastic reticellular cells in lymph nodes, epidermal keratinocytes, intestinal epithelial cells, and hepatocytes (8–13).

The liver is a distinctive organ with unique immunological features (14). It contains numerous resident blood cells, such as NK cells, NKT cells, conventional T cells, macrophages, and dendritic cells (DCs), and is enriched in innate immune cells. Intrahepatic lymphocytes (IHLs) are influenced by hormones, cytokines, and pathogens. Notably, NK cells are present at high frequency in the liver even in other organ. Invariant NKT (iNKT) cells express a form of the TCRβ chain with specificity for the MHC class I-like molecule CD1d (15). In response to Ag stimulation, iNKT cells rapidly produce IL-4 and IFN-γ, modulating the nature and magnitude of immune responses. On the other hand, during late embryogenesis, fetal liver is the major site of B cell development.

Several cytokines function to maintain innate immune cells. NK cells reportedly depend more on IL-15 than on IL-7 for maintenance in liver (16). In addition, NK cells require IL-15 for differentiation and survival (17). Furthermore, yō T cells require IL-7 and IL-15 for both differentiation and survival (18–20). TLR stimulation reportedly induces IL-7 from the liver and enhances T cell survival in the periphery (21). However, little is known about local function of IL-7 produced by hepatocytes in maintaining innate immune cells in the liver.

To address these questions, we established IL-7–floxed mice and crossed them with albumin promoter-driven Cre (Alb-Cre) transgenic (Tg) mice to establish conditional knockout of IL-7 in hepatocytes. We found that the absolute number of NKT and T cells in adult liver significantly decreased, and that B cell development was impaired in fetal liver of IL-7f/f Alb-Cre mice. These observations indicate that hepatocyte-derived IL-7 plays an essential role in development and maintenance of IHLs and implies that hepatocytes provide a unique IL-7 niche for IHLs.
Materials and Methods

Generation of IL-7–floxed mice

The targeting vector was constructed by Red recombination technology with a murine bacterial artificial chromosome clone containing the IL-7 locus (RP23-446B12), as described previously (22). One loxP sequence was inserted 58 bp upstream of IL-7 exon 4, and the neomycin resistance gene cassette flanked by FRT sequences on both sides and one loxP sequence at the 3′ end was inserted 52 bp downstream of exon 4 (see Fig. 1A). The targeting vector was retrieved from a modified bacterial artificial chromosome clone with the 8.7-kb 5′-homologous fragment, a neomycin resistance gene cassette, and a 1.9-kb 3′-homologous fragment, flanked by diptheria toxin A subunit CDN4A. The linearized targeting vector was introduced into the KY1.1 embryonic stem (ES) cell line derived from C57BL/6 X 129S6/SVEvTac mouse embryos (a kind gift of Dr. Junji Takada, Osaka University) (23). Homologous recombinants were screened by PCR and confirmed by Southern blot analysis with 5′ and 3′ probes (see Fig. 1B). Targeted ES clones were injected into blastocysts, and chimeric mice were bred with F1pe Tg mice to remove the FRT-flanked neomycin resistance gene cassette (a kind gift of Dr. Masayoshi Mishina, University of Tokyo, and obtained from Dr. Kenji Sakamizu, Niigata University) (24). IL-7–floxed mice obtained were backcrossed into C57BL/6 mice six times and bred with Alb-Cre Tg mice (a kind gift of Dr. Mark A. Magnuson, Vanderbilt University School of Medicine, and obtained from Dr. Hideaki Kaneto, Osaka University) (25) to remove the loxP-flanked exon 4. Mice were maintained under specific pathogen-free conditions in the Experimental Research Center for Infectious Diseases at the Institute for Virus Research, Kyoto University. All mouse protocols were approved by Kyoto University.

Cell preparation

IHLs were isolated as described previously (26, 27). In brief, mice were perfused with PBS via the left ventricle until the liver became opaque, and isolated liver was passed through a 100-μm cell strainer (BD Biosciences). The total number of cells was then resuspended in a 33% Percoll solution and centrifuged at 2000 rpm for 10 min at room temperature. After lysing RBCs, cells were then washed with PBS supplemented with 0.2% BSA and 0.02% Na$_2$SO$_4$. Hepatocytes were isolated as described previously (28, 29). In brief, mice were perfused with collagenase via portal vein, and isolated liver was minced and digested with collagenase, pronase E, and DNase for 30 min. The cell suspension was centrifuged at 20 × g for 5 min, and the cell pellet was washed once with PBS.

Ahs and flow cytometry

The following Abs were used: anti-NK1.1 (PE or PE/Cy7, clone PK136), anti-CD3ε (FITC, clone 145-2C11), anti-CD4 (allophycocyanin-eFluor780, clone RM4-5), anti-CD8a (eFluor50, clone 53-6.7), anti-CD11c (PE, clone N418), anti-CD11b (FITC, clone M1/70), anti-B220 (allophycocyanin or allophycocyanin-eFluor780, clone RA3-6B2), anti-CD19 (allophycocyanin or eFluor780, clone M41), anti-NK1.1 (allophycocyanin, clone 2B8), anti-γδTCR (PE, clone GL5), and CD44 (PE-Cy7, clone IM7). Allophycocyanin-conjugated CD1d-tetramer loaded with α-galactosylceramide was purchased from ProMimune (Oxford, U.K.). Viable cells were analyzed by a FACSCanto II flow cytometer (Becton Dickinson, Franklin Lakes, NJ) using FlowJo software (Tree Star, Ashland, OR).

Real-time RT-PCR

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) and reverse transcribed using ReverTra Ace (TOYOBO, Osaka, Japan) with oligo(dT)$_{12-18}$ primer. cDNA was amplified with IL-7 and CD3e primers as real-time RT-PCR. Amount of IL-7 gene relative to CD3e gene was compared. Primer sequences were: IL-7, 5′-TTTCCTCGATGGTCTTTCT-3′ and 5′-CCCTAATCTGGCGACGACAC-3′; thymic stromal lymphopoietin (TSLP), 5′-TGAGAGAAGCCAGCTAGTCTC-3′ and 5′-GGCATTGTTCTGAGCTGC-3′; IL-15, 5′-GTGACATTTATCCAGTGTCTG-3′ and 5′-TTGTTGCCAGACAGTGGC-3′; HPRT, 5′-GGTTGGATACAGCCCGACTTGTGTTG-3′ and 5′-GATTCA-CTTGGCCCTACCTTGAGC-3′.

Intracellular staining

For staining of Bcl-2, a PE-labeled hamster anti-mouse Bcl-2 set was used according to the manufacturer’s protocol (BD Biosciences, San Jose, CA). Cells were washed with PBS, resuspended in fixation buffer (eBioscience, San Diego, CA), and incubated at room temperature for 20 min. Cells were then washed, suspended in permeabilization buffer with PE–anti-Bcl-2 Ab, and incubated at room temperature for 20 min. Samples were washed and then suspended in flow staining buffer (eBioscience). PE hamster IgG isotype control served as control (BD Biosciences).

Results

Generation of mice conditionally deficient in IL-7

To investigate organ-specific functions of IL-7 in vivo, we established IL-7–floxed mice and crossed with Alb-Cre Tg mice (25) to obtain mice deficient in IL-7 in hepatocytes (Fig. 1A, 1B). We isolated hepatocytes by collagenase perfusion and compared IL-7 DNA levels between IL-7f/f and IL-7f/f Alb-Cre mice by real-time PCR. Hepatocyte IL-7 DNA was 9-fold reduced in IL-7f/f Alb-Cre mice compared with IL-7f/f control mice (Fig. 1C). Considering the contaminating cells in hepatocyte fraction, the IL-7 gene is efficiently deleted in hepatocytes of IL-7f/f Alb-Cre mice. We next compared IL-7 mRNA levels between IL-7f/f and IL-7f/f Alb-Cre mice by real-time RT-PCR. Hepatocyte IL-7 mRNA was 11-fold decreased in IL-7f/f Alb-Cre compared with IL-7f/f control mice, whereas whole liver IL-7 transcripts were reduced only 3-fold (Fig. 1D). In contrast, IL-7 mRNA levels were unchanged in thymus, suggesting that IL-7 deletion was hepatocyte specific (Fig. 1E). We then compared differences in transcript levels of TSLP and IL-15 between IL-7f/f and IL-7f/f Alb-Cre livers and found that they were comparable, suggesting that expression of other cytokines did not change to compensate for decreased IL-7.

Hepatocyte-derived IL-7 functions to maintain NKT and T cells in liver

To determine the function of hepatocyte-derived IL-7 in the liver, we first compared IHLs between IL-7f/f and IL-7f/f Alb-Cre mice. Notably, the number of IHLs was significantly decreased in IL-7f/f Alb-Cre mice compared with IL-7f/f control mice (Fig. 2A). We then stained IHLs with anti-CD3 and anti-NK1.1 Abs, and analyzed them by flow cytometry. Absolute numbers and percentages of NKT (NK1.1$^+$CD3$^+$) and T (NK1.1$^-$CD3$^+$) cells relative to total IHLs were significantly reduced in IL-7f/f Alb-Cre compared with IL-7f/f control mice, whereas numbers of NK cells remained unchanged (Fig. 2B). To test whether IL-7 production from hepatocytes has distinct effects on NKT and T cell subpopulations, we analyzed CD4 and CD8 expression. Percentages and cell numbers of each subpopulation were similarly reduced in both NKT and T cells of IL-7f/f Alb-Cre mice (Fig. 2C), indicating that hepatocyte-derived IL-7 functions to maintain NKT and T cells in the liver. The data also indicate that lack of IL-7 production in the liver was not compensated for by IL-7 produced from other organs or by other cytokines produced in liver.

Maintenance of DCs and B cells is not impaired in the liver of IL-7–floxed Alb-Cre mice

We next compared DCs between IL-7f/f and IL-7f/f Alb-Cre liver. IHLs were stained with anti-B220, anti-CD11b, anti-CD11c, and anti-CD19 Abs and flow cytometry
Hepatocyte-derived IL-7 is required for maintenance of NKT and T cells in liver. (A) IHLs were isolated from IL-7f/f and IL-7f/f Alb-Cre mice. The mean ± SE of total cell numbers was calculated from three to four mice at 8–12 wk old. (B) IHLs were stained with anti-CD3 and anti-NK1.1 Abs (left panels). The percentages of cells for a given phenotype relative to whole IHLs are shown. The absolute numbers of NK (NK1.1⁺ CD3⁺), NKT (NK1.1⁺ CD3⁺), and T (NK1.1⁺ CD3⁺) cells were calculated from the total cell numbers and the percentage of each cell population for each mouse (right graph). (C) IHLs were stained with anti-CD4, anti-CD8, anti-CD3, and anti-NK1.1 Abs (left panels). The absolute numbers of CD4 (CD4⁺), CD8 (CD8⁺), and DN (CD4⁺ CD8⁺) cells in NKT (upper right graph) and T (lower right graph) cell populations were calculated as in (B). Student t test for unpaired data were used to compare values between two groups (*p < 0.05, **p < 0.01, ***p < 0.005).

### FIGURE 1

Generation of IL-7 conditional knockout mice. (A) Targeting strategy for the IL-7–floxed allele. Boxes indicate exons and cDNAs. Open and closed triangles indicate FRT and loxP sequences, respectively. Horizontal bars indicate 5’ and 3’ probes. The targeting allele gave rise to a 12.8- and a 5.8-kb BamHI fragment with the 5’ and 3’ probes, respectively. (B) Homologous recombination in ES cells, the targeted allele with the neomycin cassette was obtained. The neomycin cassette was removed by crossing with Flpe Tg mice. Finally, the IL-7 conditional knockout allele was generated by crossing resultant animals with Alb-Cre Tg mice. (C) Homologous recombination was confirmed by Southern blot analysis with 5’ and 3’ probes. The targeting allele gave rise to a 12.8- and a 5.8-kb BamHI fragment with the 5’ and 3’ probes, respectively. (D) IL-7 gene in hepatocytes was measured by real-time PCR and normalized to that of CD3e gene. Values are the means ± SE from two experiments. (E) IL-7 transcripts in hepatocytes (left) or whole liver (right) were measured by real-time RT-PCR and normalized to those of HPRT. Values are the means ± SE from two to three experiments. (F) IL-7, TSLP, and IL-15 transcripts were measured by real-time RT-PCR and normalized to those of HPRT. Values are the means ± SE from two to three experiments. Student t tests for unpaired data were used to compare values between two groups (***p < 0.005). B, BamHI.

anti-CD8α Abs, and analyzed by flow cytometry. There was no significant difference in plasmacytoid DC (CD11c⁺ B220⁺), myeloid DC (CD11c⁺ CD11b⁺ CD8α⁺), and lymphoid DC (CD11c⁺ CD11b⁻ CD8α⁺) subpopulations in IL-7f/f compared with IL-7f/f Alb-Cre mice (Fig. 3A). We next analyzed IHLs using anti-CD19 and anti-CD3 Abs. The percentage of hepatic B cells (CD19⁺ CD3⁻) relative to total IHLs was slightly increased in IL-7f/f Alb-Cre compared with IL-7f/f control mice (Fig. 3B). However, the number of B cells was unchanged in IL-7f/f Alb-Cre mice (Fig. 3C). These results suggest that maintenance of hepatic DCs and B cells is independent of IL-7 produced by hepatocytes.

Hepatocyte-derived IL-7 is required for iNKT cell maintenance

Vα14⁺ iNKT cells reportedly depend primarily on IL-15 for maintenance, but only slightly on IL-7 (16). To test whether iNKT cells are impaired in the liver of IL-7f/f Alb-Cre mice, we stained IHLs with CD1d tetramer, which identifies iNKT cells expressing the canonical Vα14 TCR. In agreement with results of NK1.1 and CD3 staining (Fig. 2B), the percentages of iNKT cells relative to total IHLs were ~2-fold lower in IL-7f/f Alb-Cre than in IL-7f/f control mice (Fig. 4A, 4B). Furthermore, numbers of iNKT cells were reduced ~5-fold in IL-7f/f Alb-Cre mice (Fig. 4B), suggesting that hepatocyte-derived IL-7 is crucial to maintain iNKT cells in liver. By contrast, the numbers of iNKT cells in thymus and spleen were unchanged between IL-7f/f and IL-7f/f Alb-Cre mice.

It is known that both immature and mature stages of NKT cells colonize peripheral organs, and that NKT cells at immature stages can further differentiate into more mature stages in the periphery (30). To determine whether IL-7 produced by hepatocytes alters NKT cell differentiation in the periphery, we assessed iNKT cells by CD44 and NK1.1 expression, and divided them into stage 1 (CD44⁺ NK1.1⁺), stage 2 (CD44⁺ NK1.1⁺), and stage 3 (CD44⁺ NK1.1⁺). Percentages of iNKT cells did not differ significantly at any stage between IL-7f/f Alb-Cre and IL-7f/f mice (Fig. 4C). Approximately 80% of iNKT cells were at stage 3 in liver, th-

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mus, and spleen. The absolute numbers of iNKT cells were overall decreased at all stages in the liver of IL-7f/f Alb-Cre mice (Fig. 4D). These results suggest that hepatocyte-derived IL-7 plays a role in maintaining hepatic iNKT cells at different developmental stages.

Development and maintenance of NKT and T cells were unchanged in the lymphoid organs of IL-7f/f Alb-Cre mice

To determine whether hepatic IL-7 has any effect on NKT and T cells in other lymphoid organs, we analyzed lymphocytes from thymus, spleen, lymph node, bone marrow, and peripheral blood of IL-7f/f Alb-Cre and IL-7f/f mice. Both percentages and absolute numbers of total, CD4 T, and CD8 T cells were unchanged in these organs (Fig. 5A–C). In addition, the number of NKT cells also remained unchanged in these organs (Fig. 5D, 5E). Furthermore, the absolute numbers of iNKT cells at the three developmental stages were unchanged in thymus and spleen (Fig. 4C, 4D). These results suggest that hepatocyte-derived IL-7 functions in maintenance of NKT and T cells only within the liver and imply that hepatic IL-7 functions locally.

Hepatic IL-7 regulates maintenance of NKT and T cells in a Bcl-2-independent manner

IL-7 promotes lymphocyte survival by inducing antiapoptotic proteins, such as Bcl-2 and Bcl-XL (31). Forced expression of Bcl-2 partially rescues T cell development in IL-7Rα−deficient mice (32, 33). To determine whether hepatocyte-derived IL-7 alters expression of Bcl-2 in NKT and T cells, we analyzed expression of Bcl-2 protein. Hepatic NKT and T cells, as well as thymic T cells, displayed comparable levels of Bcl-2 in IL-7f/f Alb-Cre and IL-7f/f mice (Fig. 6). These results suggest that hepatocyte-derived IL-7 may control maintenance of hepatic NKT and T cells independent of Bcl-2. An alternative possibility is that the cells with lower Bcl-2 levels may have died.

Fetal B cell development is impaired in IL-7f/f Alb-Cre mice

In mouse, the liver is the major site of B cell development in late embryonic stages. Thus, we asked whether hepatocyte-derived IL-7 functions in development of fetal B cells. First, we compared levels of IL-7, TSLP, and IL-15 mRNAs between fetal liver of IL-7f/f Alb-Cre and IL-7f/f mice at embryonic day 18.5. Levels of IL-7 transcripts were greatly reduced in IL-7f/f Alb-Cre fetal liver, whereas TSLP and IL-15 transcript levels remained unchanged (Fig. 7A). Next, we analyzed fetal liver cells by flow cytometry. We found that percentages and absolute numbers of B220+ cells were moderately reduced (by 40%) in IL-7f/f Alb-Cre mice compared with control mice (Fig. 7B, 7C). The percentages of both early c-Kit+B220+ and late c-Kit−B220+ B lymphoid cells were reduced in IL-7f/f Alb-Cre fetal liver. In contrast, the number and percentage of c-Kit+B220+ hematopoietic progenitors remained unchanged. We also investigated neonatal liver of IL-7f/f Alb-Cre and IL-7f/f mice, and found that numbers of B220+ cells were reduced in IL-7f/f Alb-Cre neonatal liver (Fig. 8). These results suggest that hepatocyte-derived IL-7 functions in B cell development between late fetal and neonatal stages.
in IL-7f/f Alb-Cre mice (Figs. 7, 8). Therefore, our study demonstrated that hepatic NKT and T cells are severely reduced in the thymus and periphery of IL-7f/f Alb-Cre mice, and stained with anti-CD3, anti-NK1.1, and anti-Bcl-2 Abs. Representative histograms show intracellular Bcl-2 expression of NK (NK1.1^CD3^), NKT (NK1.1^CD3^), and T (NK1.1^CD3^) cells. Data represent one of two independent experiments with similar results.

The liver consists of various cell types such as hepatocytes, sinusoidal, biliary, and vascular endothelial cells, hepatic stellate cells, Kupffer cells, and IHLs (14). Hepatocytes constitute only about two thirds of the total population in the liver. The levels of IL-7 transcripts in hepatocytes of IL-7f/f Alb-Cre mice were reduced by 11-fold compared with control mice (Fig. 1D). Considering the possible contamination of other cells in isolated hepatocyte fraction, this reduction suggests that the IL-7 gene was almost completely deleted in hepatocytes. On the other hand, the levels of IL-7 transcripts in whole liver were reduced by 3-fold compared with control mice, suggesting that one third of the IL-7 transcripts in the liver are derived from the nonhepatocyte population. Sinusoidal and vascular endothelial cells are the candidate cells expressing IL-7.

It is still unknown how hepatic NKT and T cells receive IL-7 from hepatocytes. The majority of NKT and T cells probably reside in sinusoidal space, whereas a part of them may be present in perisinusoidal space (the space of Disse). Because sinusoidal endothelial cells have many small holes and gaps in and between the cells, NKT and T cells in sinusoidal space may extrude their cytoplasm and make direct contact with hepatocytes. This is one possibility that NKT and T cells might receive IL-7 from hepatocytes. The other possibility is that hepatocyte-derived IL-7 may be immobilized on the extracellular matrix of sinusoidal endothelial cells, and that the IL-7 produced by other liver cells does not contribute to the local IL-7 concentration in sinusoid.

NKT cell homeostasis is still under investigation. IL-15 reportedly plays a major role in proliferation and/or survival of iNKT cells, with IL-7 playing a minor role (16). In that report, CD8^+ thymocytes including iNKT cells were transferred into wild type, IL-15^−/−, IL-7^−/−, and IL-15^−/− IL-7^−/− recipient mice, and their cell division was compared by CFSE dye dilution. However, the contribution of IL-7 was unclear in this study, because NKT and T cells are severely reduced in the thymus and periphery of IL-7^−/− and IL-15^−/− IL-7^−/− mice, and cannot be compared with those in IL-15^−/− mice. In contrast, our study unequivocally dem-

**Data Table**

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**Discussion**

In this study, we determined local functions of hepatocyte-derived IL-7 in development and maintenance of IHLs using IL-7–floxed and Alb-Cre Tg mice (Fig. 1). We found that NKT and T cells were significantly reduced in adult liver of IL-7f/f Alb-Cre mice, whereas the number of NK cells, B cells, and DCs remained unchanged (Figs. 2, 3). The number of iNKT cells was decreased in liver, but not in thymus and spleen, suggesting that hepatic IL-7 functions primarily in liver (Figs. 4, 5). Although intrahepatic NKT and T cells were decreased in number, Bcl-2 expression was unchanged (Fig. 6). Finally, we observed that B cell development was partially impaired in liver between fetal and neonatal stages in IL-7f/f Alb-Cre mice (Figs. 7, 8). Therefore, our study demonstrates that hepatocyte-derived IL-7 plays an indispensable role in maintaining NKT and T cells in adult liver and in development of B cells from late fetal to neonatal liver. This study suggests that hepatocytes provide a unique IL-7 niche for IHLs.

- FIGURE 6. Bcl-2 expression is unchanged in NKT and T cells of adult IL-7f/f Alb-Cre liver. IHLs and thymocytes were isolated from IL-7f/f and IL-7f/f Alb-Cre mice, and stained with anti-CD3, anti-NK1.1, and anti-Bcl-2 Abs. Representative histograms show intracellular Bcl-2 expression of NK (NK1.1^CD3^), NKT (NK1.1^CD3^), and T (NK1.1^CD3^) cells.
onstrates that IL-7 produced by hepatocytes plays an important role in homeostasis of intrahepatic NKT cells.

The physiological function of hepatic IL-7 is largely unknown. It was reported that LPS stimulation induces IL-7 from the liver and enhances expansion and survival of T cells in the periphery (21). When IL-7 expression was depleted in hepatocytes in vivo via short hairpin RNA, homeostatic expansion of naive CD8 T cells and clonal expansion of Ag-specific CD8 T cells were partially impaired. In contrast, our study demonstrated a physiological role of hepatocyte-derived IL-7 in maintaining naive NKT and T cells in liver. Further studies with IL-7f/f Alb-Cre mice should elucidate unknown functions of hepatic IL-7 in immune and autoimmune responses.

IL-7 plays a major role in B cell development in adult bone marrow. Mesenchymal stromal cells in bone marrow produce IL-7, as well as SCF and CXCL12, to support early B progenitors. In contrast, during fetal and perinatal life, B cell development occurs in liver. Fetal B lymphopoiesis may be less dependent on IL-7 than that occurring in adults (34). The identity of IL-7–producing cells supporting fetal B cell development has been elusive. Fetal liver contains mesenchymal stromal cells similar to those seen in bone marrow (35). These cells produce IL-7, which likely supports fetal B cell development. On the other hand, hepatocytes reportedly produce multiple cytokines including IL-7 (36). Therefore, both mesenchymal stromal cells and hepatocytes may support B lymphopoiesis in fetal liver. In our study, the IL-7 transcripts were reduced 10-fold in IL-7f/f Alb-Cre fetal liver, suggesting that hepatocytes are the major source of IL-7 in fetal liver, whereas adult liver might contain considerable proportion of IL-7–producing nonhepatocytes. Furthermore, IL-7f/f Alb-Cre mice showed partially impaired B cell development in fetal liver (Fig. 7). Because B lymphoid cell numbers were reduced 5-fold in IL-7−/− fetal liver (data not shown), our study suggests that hepatocytes and other stromal components support B lymphopoiesis in fetal liver and implies that fetal hepatocytes constitute an IL-7 niche for B precursors.

Hepatocyte-derived IL-7 may have some roles in immune responses. TLR stimulation reportedly induces IL-7 from the liver and enhances T cell survival, CD8 T cell cytotoxic activity, and development of experimental autoimmune encephalitis (21). In this report, they took gene knockdown approach by transfecting short hairpin RNA vector into the liver in vivo. Because transfection of DNA may induce undesirable cellular responses and IL-7 depletion is more efficiently achieved by knockout approach, the question on the functions of liver IL-7 in immune responses will be better addressed by using the IL-7f/f Alb-Cre mice in the future.

In conclusion, we defined the role of IL-7 produced by hepatocytes in maintenance and development of IHLs using hepatocyte-
specific IL-7 conditional knockout mice. We show that hepatocytes are a unique IL-7 niche for NKT cells, T cells, and early B cells. These findings should accelerate the understanding of hepatocyte function in the development and maintenance of the immune system. IL-7 conditional knockout mice could also serve as a powerful tool to dissect precise roles of IL-7 in the immune response.

Acknowledgments

We thank Drs. J. Takeda, G. Kondoh, and K. Yusa for the KY1.1 ES line and targeting system, Dr. N.G. Copeland for the red recombination system, Drs. M.A. Magnuson and H. Kaneto for the Alb-Cre mouse, T. Sakabe, H. Hayashi, and S. Kamioka for excellent technical assistance, and members of the Ikuta laboratory for discussion.

Disclosures

The authors have no financial conflicts of interest.

References


