Interleukin-15 Prevents Concanavalin A-Induced Liver Injury in Mice via NKT Cell-Dependent Mechanism

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Administration of concanavalin A (Con A) induces a rapid and severe liver injury in mice. Natural killer T (NKT) cells are recognized to be the key effector cells, and a variety of cytokines [e.g., interleukin 4 (IL-4), IL-5, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α)] have been shown to play vital roles in Con A–induced liver injury, whereas the role of IL-15, a critical cytokine in the development and homeostasis of NKT cells, remains obscure. In this study, pretreatment with IL-15 prevented mice from Con A–induced mortality, elevation of serum transaminase, liver necrosis, and hepatocyte apoptosis. Depletion of NKT cells abolished Con A–induced liver injury, which could be restored by adoptive transfer of purified NKT cells but not by that of in vivo or in vitro IL-15–treated hepatic NKT cells. Furthermore, transfer of wild-type NKT cells to CD1d−/− mice restored liver injury, whereas transfer of IL-15–treated NKT cells did not. IL-15 pretreatment decreased the NKT-derived IL-4, IL-5, and TNF-α production, thereby resulting in less infiltration of eosinophils, which play a critical role in Con A–induced liver injury. In conclusion, IL-15 protects against Con A–induced liver injury via an NKT cell–dependent mechanism by reducing their production of IL-4, IL-5, and infiltration of eosinophils. These findings suggest that IL-15 may be of therapeutic relevance in human autoimmune-related hepatitis. Supplementary materials for this article can be found on the HEPATOLOGY website (http://interscience.wiley.com/jpages/0270-9139/index.html). (HEPATOLOGY 2006;43:1211-1219.)

Hepatitis is a worldwide health care problem. Concanavalin A (Con A)–induced hepatitis is a well-established experimental murine model,1 being characterized by markedly increased serum levels of transaminase and simultaneous infiltration of T cells, eosinophils,2-3 and Kupffer cells4 into the liver. The hepatic natural killer T (NKT) cells play essential roles in the Con A–induced liver injury by releasing a variety of cytokines, including interleukin 4 (IL-4), IL-5, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α).5-11 Interestingly, many other cytokines are also involved in Con A–induced liver injury; for example, IL-6, IL-10, IL-11, and IL-22 show hepatoprotective effects,12-16 whereas IL-12 and IL-18 aggravate Con A–induced hepatitis.17,18 IL-15 is a member of the 4-helix bundle family of cytokines that interacts with a heterotrimeric receptor consisting of the β and γ subunits of the IL-2 receptor and a specific, high-affinity IL-15–binding subunit, IL-15Rα. IL-15 is produced by multiple cells such as macrophages, epithelial, and fibroblast cells.19,20 More importantly, IL-15 is an indispensable cytokine for the proliferation, survival, and homeostasis of NKT cells.20,21 However, the role of IL-15 in this NKT cell–mediated liver injury remains unclear. We now report that IL-15 pretreatment leads to an IL-15-dose–dependent protection of liver injury. The protective effect of IL-15 is via NKT cell–dependent manner by directly attenuating IL-4 and IL-5 production, resulting in less eosinophils infiltration into the liver.

Abbreviations: Con A, concanavalin A; NKT, natural killer T; IL, interleukin; INF-γ, interferon gamma; TNF-α, tumor necrosis factor alpha; PBS, phosphate-buffered saline; mAb, monoclonal antibody; FITC, fluorescein isothiocyanate; RT-PCR, reverse transcription polymerase chain reaction; ALT, alanine aminotransferase; TUNEL, terminal deoxynucleotidyl transferase-mediated nick-end labeling; MNC, mononuclear cell; EPO, eosinophil peroxidase.

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Materials and Methods

Reagents. Con A was purchased from Sigma Chemical Co. (St. Louis, MO) and dissolved in phosphate-buffered saline (PBS) at a concentration of 2 mg/mL. Human recombinant IL-15 (Peprotech, London, UK) has a specific activity of \(2 \times 10^6\) U/mg. Human IL-15 shares approximately 73% sequence identity with murine IL-15 and can be used by mice. The monoclonal antibodies (mAb) used in this study include fluorescein isothiocyanate (FITC)-conjugated anti-NK1.1 (eBioscience, San Diego, CA), PE-anti-IL-4, PE-anti-IL-5, PE-anti-IFN-\(\gamma\), PE-anti-TNF-\(\alpha\) (CALTAG, Burlingame, CA), PE-anti-NK1.1, PE-anti-CD122, PE-anti-CD132, and Cyanochrome-anti-CD3 (BD PharMingen, San Diego, CA), Biotin-anti-IL-15R\(\alpha\) (R&D Systems, Minneapolis, MN).

Animals. Eight- to ten-week-old male C57BL/6 mice were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) mice were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science. CD1d\(^{+/+}\) were purchased from the Shanghai Experimental Center, Chinese Academy of Science.

Preparation of Mouse Hepatocytes. Primary mouse hepatocytes were isolated by collagenase perfusion. Briefly, mouse liver was perfused with HEPES buffer followed by perfusion of the liver with collagenase solution. The isolated hepatocytes were washed with cold PBS. The number and viability of isolated cells were determined by trypan blue exclusion. Trypan blue-negative hepatocytes were then stained extracellularly with Annexin-V as recommended by the manufacturer (BD Pharmingen) to determine the apoptosis cells or stained with IL-15R antibodies for flow cytometric analysis.

Cell Depletion. The anti-mouse-CD3, -CD4, -CD8, -NK1.1, and -anti-IL-4 antibodies were obtained from partially purified hybridoma culture supernatants using ammonium sulfate precipitation. The hybridoma cell lines were purchased from American Type Culture Collection (Rockville, MD). Anti-mouse-IL-5 (clone TRFK-5) was purchased from R&D systems. Mice were given two injections of the indicated mAb 48 and 36 hours before Con A injection to deplete specific immune cells and to block specific cytokines in vivo. To deplete NK cells, a dose of 50 \(\mu\)L of anti-ASGM1 antiserum (Wako Pure Chemical Industries, Ltd., Japan) diluted in 250 \(\mu\)L of pyrogen-free PBS was intravenously injected into mice 1 day before treatment. To deplete NK cells and NKT cells, mice were injected intraperitoneally with 0.5 mL PBS containing 250 \(\mu\)g anti-NK1.1 (mAb PK136, ATCC). Depletion of immune cells was confirmed by flow cytometry, and this protocol produced a >90% decrease in the number of the indicated cells.

Liver Mononuclear Cell Preparation. Mouse livers were removed and pressed through a 200-gauge stainless steel mesh and then suspended in RPMI 1640 medium (Gibco, BRL) and centrifuged at 50g for 5 minutes. Supernatants containing hepatic mononuclear cells (MNCs) were collected, washed in PBS, and re-suspended in 40% Percoll (Sigma) in RPMI 1640 medium. The cell suspension was gently overlaid onto 70% Percoll and centrifuged for 20 minutes at 750g. MNCs were collected from the interphase, and washed twice in PBS. The degree of contamination by Kupffer cells and hepatocytes was minimal.

Purification of NK1.1\(^+\)CD3\(^+\) T (NKT) Cells. For purification of NKT cells, mice were injected with anti-ASGM1 to deplete NK cells (NK1.1\(^+\)CD3\(^-\)). Twenty-four hours later, hepatic lymphocytes were isolated from...
these NK-depleted mice and stained with FITC-NK1.1 mAb and then incubated with anti-FITC Microbeads (Miltenyi Biotec, Auburn, CA) for 15 minutes at 4°C. NK1.1 cells were enriched by positive magnetic cell sorting (MACS) according to the manufacturer’s protocol. Approximately 90% of the magnetic cell sorting–purified cells were NK1.1 and CD3 positive.

Adoptive Transfer of Hepatic NKT Cells. Anti-NK1.1 antibody was injected into mice 48 and 36 hours before adoptive transfer, the time when antibody had depleted recipients’ NK and NKT cells and did not affect the transferred NKT cells. Adoptive hepatic NKT cells were fixed, permeabilized, and stained with intracellular antibodies diluted in PBS containing 2% fetal calf serum. Cells are analyzed by FACS Calibur flow cytometer with CellQuest software (BD PharMingen). Cells are analyzed by FACSCalibur flow cytometer with CellQuest software (Becton Dickinson, Franklin Lakes, NJ).

Flow Cytometric Analysis. MNCs were stained with antibodies diluted in PBS containing 2% fetal calf serum and 0.1% NaN3. For the intracellular cytokine assay, cells were fixed, permeabilized, and stained with intracellular IL-4, IL-5, IFN-γ, and TNF-α using a Cytofix/Cytoperm plus kit (BD PharMingen). Cells are analyzed by FACSCalibur flow cytometer with CellQuest software (Becton Dickinson, Franklin Lakes, NJ).

Reverse Transcription Polymerase Chain Reaction Analysis. RNA was extracted from liver tissue using Trizol Reagent (Invitrogen, Carlsbad, CA). One microgram RNA was reverse transcribed. The primer sequences used in polymerase chain reaction (PCR) were as follows: β-actin: sense, 5′-GGA CTC CTA TGT GGG TGG CGA GG-3′; antisense, 5′-GGG AGA GCA TGC CCT CGT AGA T-3′. IL-4: sense, 5′-ACA AAA ACT TGA GAG AGA TCA T-3′; antisense, 5′-AGT AAT CCA TTT GCA TGA TGC TCT TAT-3′. IL-5: sense, 5′-GCC ATG GAG ATT CCC ATG AGC ACA-3′; antisense, 5′-GCC TTC CAT TGC CAA CTC TGT AC-3′. Eotaxin-1 (CCL1): sense, 5′-GGC AGAGC ACA CCA-3′; antisense, 5′-CTA TGG CTT TCA GGG TGC AT-3′. Eotaxin-2 (CCL24): sense, 5′-ACC CCA GCT TTT AAT CTT GA-3′; antisense, 5′-AAG GAC GTG CAG CAA GAT G-3′. RNAmic (CCL5): sense, 5′-ACC CAT GTG CTT GGC AGC ACA CCA-3′; antisense, 5′-CTA GCT CAT CTC CAA ATA GTT GAT-3′.

Assay of Hepatic EPO Activity. Hepatic EPO (eosinophil peroxidase) activity was measured as described previously.23,24 The enzyme activities of the hepatic tissues were calculated by subtracting the mean background optical density (OD) and expressed as change of OD490 per minute.

Statistical Analysis. The statistical comparison between experimental and control groups was conducted by Student t test, and P < .05 was considered significant.

Results

IL-15 Prevents Mice From Con A–Induced Liver Injury. IL-15 is a necessary cytokine for the survival and homeostasis of NKT cells. To know the role of IL-15 in Con A–induced liver injury, exogenous IL-15 was administered subcutaneously to mice. Fig. 1A illustrates that IL-15 pretreatment exerted a dose-dependent protection of liver injury. Pretreatment with 20 or 40 μg/mouse of IL-15 markedly inhibited Con A–induced elevation of serum ALT levels, whereas 10 μg/mouse of IL-15 only partly alleviated ALT elevations, and 2 μg/mouse IL-15 had no hepatoprotective effect. Next, serum ALT levels were measured at different times after Con A injection in IL-15– and PBS-treated groups. As shown in Fig. 1B, in the Con A–treated group, serum ALT started to increase as early as 6 hours after Con A injection, peaked at 24 hours, and declined thereafter. In the IL-15–treated group, significantly lower serum ALT levels were detected, particularly at 12 and 24 hours after Con A injection. Furthermore, we examined whether IL-15 protected mice from a lethal dose of Con A. As shown in Fig. 1C, 80% of IL-15–treated mice survived, whereas 100% of PBS-treated mice died within 12 hours after injection with a lethal dose of Con A.

Liver histological studies were also used to determine the protective effect of IL-15 on Con A–induced liver injury. As shown in Fig. 2A, light microscopy examination showed massive necrosis in the livers of mice 24 hours after treatment with Con A alone, whereas mice pretreated with IL-15 showed minor damage. TUNEL assay showed significant hepatocyte apoptosis in the livers of mice treated with Con A, which was markedly prevented by IL-15 pretreatment (Fig. 2B). Similarly, hepatocytes isolated from IL-15–pretreated mice showed lower Annexin-V expression than that without IL-15 pretreatment, indicating that IL-15 can protect hepatocyte from apoptosis (Fig. 2C).

IL-15 Protects Against Con A–Induced Hepatitis via NKT Cell–Dependent Mechanism. Because IL-15 receptors did not express on hepatocytes (Supplemental Fig. 1), IL-15 did not possibly exert any direct protective
effects on hepatocytes. Therefore, we observed the change of liver lymphocytes and found that both absolute numbers and ratios of hepatic T, NKT, and NK cells did not significantly change after IL-15 pretreatment (Fig. 3A). We then explored the possible functional variation of lymphocyte populations in IL-15–treated mice. Various antibodies including anti-mouse-CD3, -CD4, -CD8, and -ASGM1, were used to eliminate the corresponding murine lymphocyte subpopulation. As already known and also shown in this study (data not shown), anti-mouse-CD3 or -CD4 antibodies abolished Con A–induced liver injury, mainly because of NKT cell depletion. Depletion of murine CD8\(^+\) T cells and ASGM1\(^+\) NK cells did not influence Con A–induced liver injury in either IL-15- and PBS-treated mice (Fig. 3B), indicating that the protective effect of IL-15 on Con A–induced hepatitis is not mediated via NK- or CD8\(^+\) T cell–dependent mechanisms.

Next, to investigate whether IL-15 protects against Con A–induced liver injury via modulation of NKT cells, adoptive transfers of \(\text{in vivo}\) or \(\text{in vitro}\) IL-15–treated hepatic NKT cells were performed in NK and NKT cell–depleted mice. Treatment with anti-NK1.1 (PK136) antibody completely depleted NK and NKT cells (Fig. 3C) and abolished Con A–induced elevation of serum ALT levels (Fig. 3D), but did not influence the transferred NKT cells thereafter (Supplemental Fig. 2). Adoptive transfer of hepatic NKT cells isolated from PBS-treated mice restored Con A–induced hepatitis, whereas adoptive transfer of hepatic NK T cells from IL-15–treated mice failed to restore Con A–induced liver injury, indicating that IL-15 inhibits the ability of NKT cells to restore Con A–induced liver injury. Furthermore, adoptive transfer of \(\text{in vitro}\) IL-15–treated hepatic NKT cells also prevented NKT-depleted mice from Con A–induced liver injury, suggesting that IL-15 directly acts on NKT cells (Fig. 3D). To further confirm these results, we used NKT cell-deficient CD1d\(^{-/-}\) mice as recipients. Adoptive transfer of wild-type NKT cells treated with IL-15 \(\text{in vitro}\) or from \(\text{in vivo}\) IL-15–treated mice into CD1d\(^{-/-}\) mice also suppressed Con A–induced hepatitis (Fig. 3E). Taken together, these results indicated that IL-15 protected against Con A–induced hepatitis via an NKT cell–dependent mechanism.

**IL-15 Inhibits Cytokine Productions of NKT Cells in Con A–Induced Hepatitis.** NKT cells rapidly secrete a large amount of IL-4, IL-5, IFN-\(\gamma\), and TNF-\(\alpha\), all of which have been shown to play essential roles in Con A–induced liver injury.\(^3\)\(^-\)\(^12\) We next wanted to know whether IL-15 influences these cytokines’ production. As shown in Fig. 4A-D, IL-15 pretreatment reduced serum levels of IL-4 by 30% and 50% at 2 and 3 hours after Con A injection and reduced serum IL-5 at 3, 6, and 12 hours after Con A injection. Serum TNF-\(\alpha\) production also decreased in IL-15–treated mice although the serum levels of IFN-\(\gamma\) did not alter. We then used flow cytometry to analyze intracellular cytokines produced by hepatic NKT, T, and NK cells. As shown in Fig. 4E, IL-4, IL-5, IFN-\(\gamma\), and TNF-\(\alpha\) were mainly produced by NKT cells, and partially produced by T and NK cells. IL-15 pretreatment greatly suppressed NKT-derived IL-4, IL-5, and TNF-\(\alpha\) production, whereas IFN-\(\gamma\) production of NKT cells was not obviously influenced.

**IL-15 Pretreatment Reduces Infiltration of Eosinophils in Con A–Induced Liver Injury.** Recently, Louis et al.\(^3\) reported that depletion of eosinophils completely
prevented Con A–induced liver damage,3 and IL-4 and IL-5 played an essential role in recruiting eosinophils into liver.2,3 Here, we observed that when anti-IL-4 or anti-IL-5 Ab was used to deplete corresponding cytokines, the ALT levels and infiltration of eosinophils into the liver were greatly decreased (Fig. 5A). As already shown in Fig. 4E, IL-4 and IL-5 were mainly secreted by hepatic NKT cells and IL-15 pretreatment completely suppressed IL-5 production and significantly decreased IL-4 production. We further demonstrated that IL-15 pretreatment inhibited the IL-4 and IL-5 productions of purified NKT cells by reverse transcription polymerase chain reaction (RT-PCR) analysis (Fig. 5B). We then measured the EPO activity to confirm the eosinophils infiltration. As shown in Fig. 5C, Con A injection significantly elevated EPO activity in the livers of PBS-treated mice but not in that of IL-15–treated mice. We also observed that IL-15 pretreatment reduced the expression of eotaxin-1 (CCL11) and eotaxin-2 (CCL24) in liver, two important chemokines playing an important role in eosinophils recruitment (Fig. 5B). Because eotaxin is the ligand of CCR3, the main receptor expressed on eosinophils’ surface,25-27 we then used immunohistochemical staining to detect eosinophils in liver. As shown in Fig. 5D, Con A–treated mice had massive liver necrosis, which was surrounded by brown CCR3+/H11001 cells (eosinophils). IL-15 pretreatment greatly reduced eosinophil infiltration into the liver. Additionally, the numbers of eosinophils per field in Fig. 5D were almost parallel to those of Fig. 5C. Therefore, down-regulating NKT-derived-IL-4 and IL-5 production in liver by IL-15 treatment is an important mechanism responsible for IL-15 to attenuate eosinophil infiltration.

Discussion

Traditionally, IL-15 is thought to be a proinflammatory factor in autoimmune diseases such as rheumatoid arthritis (RA), ulcerative colitis (UC) and psoriasis.28,29 IL-15 contributes to the pathogenesis of these autoimmune diseases through (1) recruiting and activating T cells to the inflammatory tissue; (2) leading to TNF-α and IFN-γ secretion by autoreactive T cells; and (3) protecting autoreactive T cells from apoptosis.30,31 On the contrary, in Con A model, IL-15 administration neither recruited more lymphocytes into liver nor protected NKT cells from apoptosis (Fig. 3A). Moreover, IL-15 down-regulated serum TNF-α production (Fig. 4D) and did not influence IFN-γ production (Fig. 4C). Meanwhile, although IL-15 can induce autoreactive T cells to attack self organ, it is usually a long-term inflammation process. In Con A–induced fulminant hepatitis, the process was too short because ALT level peaked at 24 hours and declined to normal level at 48 hours. Why IL-15 plays a
different role in chronic autoimmune diseases, such as RA, UV and psoriasis, and fulminant hepatitis, such as Con A model, needs further investigation.

Recently, Bulfone-Paul et al. reported that human IL-15-murine IgG2b fusion protein completely prevented mice from anti-Fas antibody-induced serious liver injury, the first report about IL-15 protective effect on liver injury. Six hours later, mice were treated with Con A (15 μg/g). Sera were collected 24 hours after Con A injection for measuring ALT levels. They focused on that IL-15 exerted direct anti-apoptotic effects on activated T cells from many types of tissues, including liver, spleen, and thymus. However, in their study, they did not survey the inflammatory process (e.g., the injury interaction between leukocytes and hepatocytes) and how IL-15 protected mice from the fulminant hepatitis. We consider that the underlying mechanisms of Fas-induced murine hepatitis are totally different from Con A-induced hepatitis. The former model is through a clear target molecule, a death receptor that may directly induce the apoptosis of a variety of cells (including hepatocytes) in many organs (such as liver); the latter is through an extremely complicated and unclear cellular immunologic process. Because IL-15Rα, IL-15Rβ (CD122) and IL-15Rγ (CD132) were not expressed on hepatocytes from our study (Supplemental Fig. 1), we concluded that IL-15 would not directly act on hepatocytes. Because NKT cells are recognized to play vital roles in Con A-induced liver injury.
jury and IL-15 is an indispensable cytokine in the development and homeostasis of NKT cells, we thought IL-15 might directly influence NKT cells’ function. Now, we demonstrated the hepatoprotective effect of IL-15 was via NKT cell-dependent mechanism. Because Fas-induced hepatitis is not possibly mediated by inflammatory leukocytes such as NKT cells and eosinophils, the actual protective mechanisms of IL-15 on apoptosis of hepatocyte and liver injury in this model are not known.

In fact, except for IL-15, many other cytokines such as IL-6, IL-10, and IL-22 were shown to have a protective effect in Con A–induced liver injury through different mechanisms.13,14,16 IL-10 reduced the Con A–induced hepatotoxicity by diminishing the production of proinflammatory cytokines IL-12, IFN-γ, and TNF-α13; IL-22 was a survival factor for hepatocytes and protected mice from Con A–induced liver injury via STAT3 activation; IL-6, similarly to IL-15, alleviated the Con A–induced liver injury through an

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**Fig. 4.** IL-15 pretreatment decreases NKT-derived cytokine production in Con A–induced hepatitis. Mice were treated subcutaneously with PBS or IL-15 for 6 hours, followed by administration with Con A. Serum samples were collected at various times post-Con A injection, and serum levels of IL-4 (A), IL-5 (B), IFN-γ (C), and TNF-α (D) were measured by ELISA. Values in panels A-D are shown as means ± SEM (n = 5). **P < .01, *P < .05 as comparison with Con A group. (E) For flow cytometric analysis, 1 hour after Con A injection, liver MNCs were prepared and examined for various intracellular cytokines production. T (CD3+NK1.1−), NKT (CD3+NK1.1+) and NK (CD3−NK1.1+) cells were then examined for intracellular IL-4, IL-5, IFN-γ and TNF-α by flow cytometry after triple staining with anti-NK1.1, anti-CD3, and the indicated intracellular antibodies. Con A, concanavalin A; PBS, phosphate-buffered saline; NK, natural killer.
**Fig. 5.** IL-15 pretreatment reduces infiltration of eosinophils in Con A-induced liver injury. (A) Mice were injected with anti-mouse-IL-4 and -IL-5 Abs or control-IgG. Sera and livers were collected 16 hours after Con A injection. ALT levels and EPO activities were measured. Values are shown as means ± SEM (n = 3). **P < .01. (B) Mice were killed 1 hour after Con A injection. RNA samples from Purified NKT cells or liver tissue were used to test expressions of IL-4, IL-5, eotaxin-1, eotaxin-2, and RANTES. RT-PCR analysis was used with corresponding primers. (C) Mice were treated with PBS or IL-15 6 hours before Con A injection. Livers were collected 16 hours after Con A injection. EPO activities were measured. (D) Liver samples were collected 16 hours after Con A injection for anti-CCR3 staining (original magnification ×200). N, necrosis area. Ten fields were randomly selected and the numbers of eosinophil were counted. Con-A, concanavalin A; ALT, alanine aminotransferase; RT-PCR, reverse transcription polymerase chain reaction; PBS, phosphate-buffered saline.

NKT-dependent manner, as reported in our previous publication. However, adoptive transfer of *in vitro* IL-15–treated NKT cells attenuated liver injury (Fig. 3D-E), whereas transfer of *in vitro* IL-6-treated–NKT cells did not. The reason is that IL-6 influenced NKT cells in CD4+ T cell-dependent manners, whereas IL-15 directly acted on NKT cells, as indicated in this article.

NKT cells have the unique potential to rapidly secrete large amounts of cytokines, which might be important for the initiation and regulation of some immune responses and autoimmune diseases. In the Con A model, NKT–derived IL-5 might improve eosinophil maturation and infiltration, and NKT-derived IL-4 might upregulate expression of eotaxin-1 (CCL11), eotaxin-2 (CCL24), and RANTES (CCL5), which are ligands of eosinophil receptor CCR3. Fig. 5A showed liver hepatitis was completely inhibited in anti-IL-4 Ab-treated mice while partly inhibited in IL-5–depleted mice because IL-4 also induced an increase in the expression of granzyme B and FasL on NKT cells. Moreover, we and others found NKT cells treated with IL-15 failed to produce IL-5. These best explain why IL-15–treated NKT cells may exert negative regulatory effects on eosinophils. The negative regulatory effects of IL-15 on eosinophils were also observed in asthma, which is related to decreased production of IL-5. However, the molecular mechanism regarding how IL-15 reduces the production of IL-4 and IL-5 is not clear.

Previous studies reported that TNF-α played an important role in the Con A model by neutralizing TNF-α or using TNFR knockout mice. We also found IL-15 pretreatment could not only reduce serum TNF-α level but also suppress the hepatic NKT-derived TNF-α production (Fig. 4D-E). However, NKT cells are not the only main source for secreting TNF-α; Kupffer cells are possibly another important source of TNF-α in liver. We still do not know whether IL-15 directly inhibits TNF-α production by Kupffer cells.

In summary, we report that IL-15 pretreatment prevented Con A–induced mortality, elevation of serum transaminase, and liver necrosis, clearly indicating that IL-15 is able to protect mice from Con A–induced liver injury. The protective effects of IL-15 on Con A–induced liver injury were mediated via an NKT cell–dependent mechanism by inhibiting their IL-4, IL-5, and TNF-α productions and therefore reducing eosinophil infiltration into the liver. IL-15 is potentially of therapeutic relevance in treating human autoimmune-related hepatitis.

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