Biochemistry and Biophysics Reports 12 (2017) 166–171

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Alleviation of lipopolysaccharide/D-galactosamine-induced liver injury in leukocyte cell-derived chemotaxin 2 deficient mice

Akinori Okumura[a,1], Takeshi Saito[b], Minoru Tobiume[c], Yuki Hashimoto[e], Yuko Sato[c], Takashi Umeyama[e], Minoru Nagi[e], Koichi Tanabe[d], Hiroyuki Unoki-Kubota[a], Yasushi Kaburagi[a], Hideki Hasegawa[c], Yoshitsugu Miyazaki[e], Satoshi Yamagoe[e,*]

[a] Department of Diabetic Complications, Diabetes Research Center, Research Institute, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan
[b] NARO Western Region Agricultural Research Center, 1-3-1 Senyu-cho, Zentsuji, Kagawa 765-8508, Japan
[c] Department of Pathology, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan
[d] Department of Food Science and Human Nutrition, Faculty of Agriculture, Ryukoku University, 1-5 Yokotani, Seta Oe-cho, Otsu, Shiga 520-2194, Japan
[e] Department of Chemotherapy and Mycosis, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

ARTICLE INFO

Keywords:
Leukocyte cell-derived chemotaxin 2
LPS/d-GalN-induced liver injury
Interferon-γ

ABSTRACT

Leukocyte cell-derived chemotaxin 2 (LECT2) is a secreted pleiotropic protein that is mainly produced by the liver. We have previously shown that LECT2 plays an important role in the pathogenesis of inflammatory liver diseases. Lipopolysaccharide/D-galactosamine (LPS/D-GalN)-induced acute liver injury is a known animal model of fulminant hepatic failure. Here we found that this hepatic injury was alleviated in LECT2-deficient mice. The levels of TNF-α and IFN-γ, which mediate this hepatitis, had significantly decreased in these mice, with the decrease in IFN-γ production notably greater than that in TNF-α. We therefore analyzed IFN-γ-producing cells in liver mononuclear cells. Flow cytometric analysis showed significantly reduced IFN-γ production in hepatic NK and NKT cells in LECT2-deficient mice compared with in wild-type mice. We also demonstrated a decrease in IFN-γ production in LECT2-deficient mice after systemic administration of recombinant IL-12, which is known to induce IFN-γ in NK and NKT cells. These results indicate that a decrease of IFN-γ production in NK and NKT cells was involved in the alleviation of LPS/D-GalN-induced liver injury in LECT2-deficient mice.

1. Introduction

Leukocyte cell-derived chemotaxin 2 (LECT2) was originally identified as a human neutrophil chemotactic protein [1]. It is a secreted hepatic protein with a molecular mass of approximately 16 kDa [2], and the amino acid sequence has been well conserved in vertebrates. Accumulating evidence indicates that LECT2 is a multi-functional protein closely associated with several diseases [3–9]. However, its functional mechanisms are still poorly understood. LECT2 belongs structurally to the peptidase family M23 (PF01551), whose members typically exhibit zinc-dependent metalloprotease activity. This protein family is widespread in bacteria, but LECT2 is the only M23 family protein found in vertebrates. The crystal structure of human LECT2 has recently been reported; interestingly, this suggests that LECT2 lacks endopeptidase activity [10]. Three distinct receptors, each with an original ligand, have been reported as LECT2 receptors [9,11,12]. It is likely that LECT2 interacts with these receptors to regulate multiple cellular functions through intracellular signaling cascades.

It has previously been reported using LECT2-deficient mice that LECT2 plays an anti-inflammatory role in two recognized models of T cell-dependent inflammation: a concanavalin A-induced hepatitis model and a Staphylococcal enterotoxin A/D-galactosamine-induced toxic shock syndrome model [3,13]. In contrast, lipopolysaccharide/D-galactosamine (LPS/D-GalN)-induced acute liver injury has often been used as an animal model of fulminant hepatic failure. This is primarily mediated by macrophage/Kupffer cells [14,15], and several pro-inflammatory cytokines participate in this model [16]. In particular, TNF-α and IFN-γ are known to be crucial mediators that induce hepatocyte apoptosis and liver injury [17–20].

In this study, we demonstrated that LECT2-deficient mice are less sensitive than wild-type mice to LPS/D-GalN-induced hepatitis. We analyzed the production of several pro-inflammatory cytokines,

Abbreviations: LECT2, leukocyte cell-derived chemotaxin 2; LPS, lipopolysaccharide; d-GalN, d-galactosamine; GPT, glutamic pyruvic transaminase; MNC, mononuclear cell

* Corresponding author.
E-mail address: syamagoe@nih.go.jp (S. Yamagoe).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrep.2017.09.011
Received 27 October 2016; Received in revised form 22 August 2017; Accepted 27 September 2017
Available online 13 October 2017

revealing a marked decrease in IFN-γ production derived from hepatic NKT and NK cells. The reduction of IFN-γ in LECT2-deficient mice may be involved in the alleviation of this hepatitis.

2. Materials and methods

2.1. Mice

LECT2-deficient mice on a BALB/cA background were created as previously described [3,4]. Age-matched wild-type BALB/cA mice purchased from Clea Japan (Tokyo, Japan) were housed in the National Institute of Infectious Diseases (NIID). The mice used in this study were maintained under specific pathogen-free conditions and were used at 7–14 weeks of age in accordance with the guidelines of the NIID Animal Care and Use Committee.

2.2. Administration of LPS/D-GalN or recombinant IL-12

Wild-type and LECT2-deficient mice were injected intraperitoneally with 700 mg/kg D-GalN (Nacalai Tesque, Kyoto, Japan) plus 50 μg/kg LPS (from *Escherichia coli* O111:B4; Sigma, St. Louis, MO). Sera were collected at 1.5 and 5 h after LPS/D-GalN injection to measure the levels of cytokines and glutamic pyruvic transaminase (GPT) activity. To analyze the effect of IL-12, mice were injected intraperitoneally with recombinant mouse IL-12 (GT; Minneapolis, MN) at a dose of 1 μg/20 g body weight. Sera were collected at 7 h after treatment to measure the level of IFN-γ.

2.3. Cytokine and transaminase measurement

The enzyme-linked immunosorbent assay method was used to determine levels of IFN-γ (mouse IFN-γ Quantikine ELISA kit; R & D Systems, Minneapolis, MN), IL-lβ (mouse IL-1 beta ELISA kit; Thermo Fisher Scientific, Rockford, IL), and IL-18 (mouse IL-18 ELISA kit; Molecular Biology Laboratory, Nagoya, Japan), according to the manufacturers' instructions. The levels of IL-6, IL-10, IL-12 p70, and TNF-α were quantified using OptEIA ELISA sets with assay diluent and a TMB substrate reagent set (BD Bioscience, San Diego, CA). Serum GPT activities were measured with a transaminase CII-test Wako kit (Wako, Osaka, Japan).

2.4. Preparation of lymphocytes from the liver

Mice anesthetized with ether were killed by exsanguination via the axillary artery and their livers removed. Hepatic mononuclear cells (MNCs) were prepared as described previously [3]. Briefly, the liver was pressed through a 200-gauge stainless steel mesh, and suspended in Eagle's MEM (Sigma) supplemented with 5 mM HEPES (pH 7.5) and 2% FBS. After washing, the pellet was resuspended in 35% Percoll solution (GE Healthcare Bio-Sciences Ab, Uppsala, Sweden) containing heparin (100 U/ml), and centrifuged at 2000 rpm for 15 min. The pellet was resuspended in RBC lysis solution (155 mM NH_4Cl, 10 mM $KHCO_3$, 1 mM EDTA, 17 mM Tris-HCl, pH 7.3) and washed twice with MEM medium.

2.5. Histopathological analysis

The formalin-fixed, paraffin-embedded livers were cut into 3-μm thick sections and mounted on silane-coated glass slides. Histopathological studies were performed using hematoxylin–eosin (H & E) staining.

2.6. Flow cytometric analysis

Single-cell suspensions from the hepatic MNCs were incubated with monoclonal antibodies against cell surface markers (BD Biosciences Pharmingen, San Diego, CA). PE-labeled CD1d-α-galactosylceramide tetramer [3] and FITC-, PE-, or APC-conjugated Abs specific for mouse CD3 (145-2C11), CD49b (DX5), Mac-1 (M1/70), and Gr-1 (RB6-8C5) were used for flow cytometric analyses. For the detection of intracellular IFN-γ in hepatic MNCs, the cells were prepared from wild-type and LECT2-deficient mice at 3 h after LPS/D-GalN injection. Hepatic MNCs were cultured in RPMI-1640 supplemented with 10% FBS and 10 μg/ml of brefeldin A (Sigma) for 2 h. The cells were then stained with FITC-conjugated anti-CD3 and PE-conjugated anti-DX5 antibodies, fixed, permeabilized, and again stained with APC-conjugated anti-IFN-γ (XMG1.2) antibody by using a BD cytofix/cytoperm kit (BD Biosciences Pharmingen) according to the manufacturer's instructions.

2.7. Real-time RT-PCR

Quantitative real-time RT-PCR was performed using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. Primers and TaqMan probes specific to TNF-α and IFN-γ were obtained from Assay-on-Demand Gene Expression Products (Applied Biosystems), and from TaqMan Pre-Developed Assay Reagents (Applied Biosystems), respectively. 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 using ABI PRISM 7000 SDS software version 1.0 (Applied Biosystems).

2.8. Statistical analysis

Statistical significance for the survival study was determined via Kaplan–Meier analysis using the log-rank test. Other data were statistically analyzed based on Student's *t*-test, and the differences were considered significant when p values were less than 0.05. Data are expressed as mean ± standard error of the mean.

3. Results

3.1. Alleviation of LPS/D-GalN-induced liver injury in LECT2-deficient mice

We investigated whether LECT2 deficiency affected LPS/D-GalN-induced liver injury. As shown in Fig. 1A, the survival rate of LECT2-deficient mice after the injection of LPS/D-GalN was significantly higher than that of wild-type mice ($p < 0.001$). Serum GPT is an important indicator of hepatic injury. Consistent with the survival curve result, the serum GPT level in the LECT2-deficient mice was about seven times lower than that in the wild-type mice at 5 h after the LPS/D-GalN injection (Fig. 1B). These results were further confirmed by H & E staining (Fig. 1C). In wild-type mice, the histologic features showed moderate inflammation around the interlobular duct. The inflammatory cells consisted mainly of lymphocytes and plasma cells, with small numbers of neutrophils. In addition, there was congestion, sinusoidal extension, hemorrhage, and focal necrosis around the central vein. In contrast, pathological changes in the LECT2-deficient mice were far less severe or not detected. Hepatic histologies in non-treated controls of wild-type and LECT2-deficient mice are shown in supplemental Fig. 1S. We did not observe any histological differences in these two strains. These results indicate that LPS/D-GalN-induced acute liver injury was alleviated in the LECT2-deficient mice.

3.2. Cytokine levels after LPS/D-GalN-induced acute liver injury

Several proinflammatory cytokines were induced by the LPS/D-GalN-induced acute liver injury [16]. We therefore compared the serum levels and hepatic transcriptional levels of several cytokines between the wild-type and LECT2-deficient mice treated with LPS/D-GalN. At 1.5 h after the administration of LPS/D-GalN, the serum concentrations of TNF-α and IFN-γ in the LECT2-deficient mice were significantly

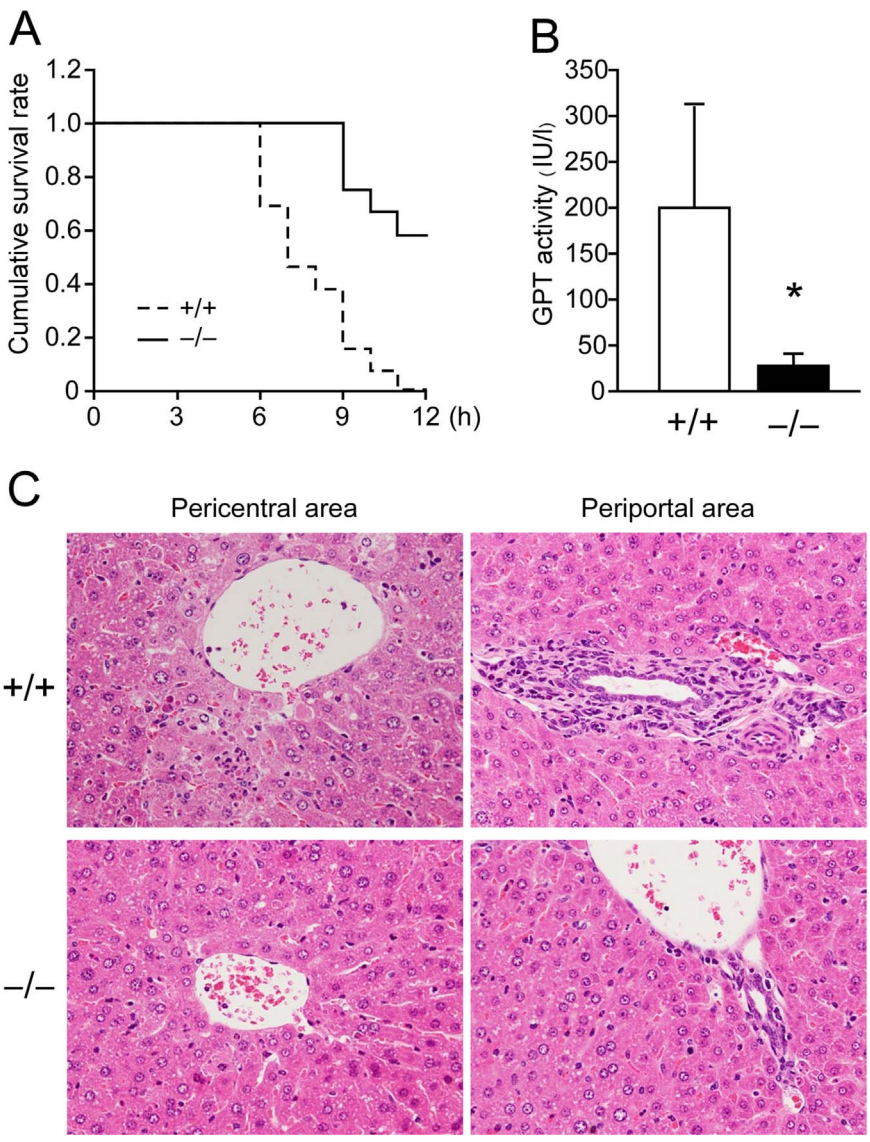

Fig. 1. The lower susceptibility of LECT2-deficient mice to LPS/D-GalN acute liver injury. A mixture of D-GalN (700 mg/kg) and LPS (50 μg/kg) was intraperitoneally injected into wild-type (+/+) and LECT2-deficient (−/−) mice. **(A)** Comparison of survival rates between the wild-type (n = 13) and LECT2-deficient (n = 12) mice after injection with LPS/D-GalN. The survival curves were created following the Kaplan–Meier method and compared by the log-rank (Mantel–Cox) test. The LECT2-deficient mice showed a significantly higher survival rate than the wild-type mice ($p < 0.001$). **(B)** Serum GPT levels of wild-type and LECT2-deficient mice at 5 h after the injection of LPS/D-GalN. Values are the mean and SEM of the results from six female mice. * $p < 0.05$. **(C)** Histopathological characterization of wild-type and LECT2-deficient mice at 5 h after the injection of LPS/D-GalN. H & E stain at 200× magnification.

decreased compared with those in wild-type mice (Fig. 2A). The serum IFN-γ induced was about four times lower in the LECT2-deficient mice than in the wild-type mice. This difference was considerably greater than the difference in TNF-α. The serum level of IFN-γ remained lower in the LECT2-deficient mice at 5 h after the administration (Fig. 2B). We did not observe significant differences in the production of other cytokines at 1.5 and 5 h, but some cytokines (IL-1β, IL-6, IL-10, and IL-12) tended, reproducibly, to be slightly lower in the LECT2-deficient mice (Fig. 2B). In addition to systemic level of cytokines, we also analyzed the levels of TNF-α and IFN-γ expression in the liver (Fig. 2C). We did not observe any difference between the wild-type and LECT2-deficient mice in the transcriptional level of TNF-α. However, IFN-γ expression was significantly lower in the LECT2-deficient mice than in the wild-type mice, in accordance with the serum results.

3.3. IFN-γ production in hepatic NK and NKT cells

LPS/D-GalN-induced acute liver injury depends not only on TNF-α but also on IFN-γ [17,18,20]. As shown in Fig. 2, the reduction of IFN-γ level in the LECT2-deficient mice was considerably greater than that of TNF-α. We also detected a decrease in IFN-γ expression in the livers of the LECT2-deficient mice. We therefore focused on hepatic NK and NKT cells, which are the major producers of IFN-γ in livers treated with LPS [21]. First, we evaluated the amount of hepatic NK and NKT cells in LECT2-deficient mice on a BALB/c background; previously, we have shown an increased population of NKT cells in LECT2-deficient mice on a C57BL/6 background [3]. The total numbers of MNCs obtained from the livers of the wild-type and LECT2-deficient mice were comparable ($2.3 \pm 0.8 \times 10^5$ cells in the wild-type vs. $2.7 \pm 0.7 \times 10^5$ cells in the LECT2-deficient mice; $n = 6$; $p = 0.355$). The MNCs were analyzed by flow cytometry using the anti-DX5 and anti-CD3 antibodies in order to detect NK and NKT cells [22]. There were no significant differences in the proportion of $DX5^+CD3^-$ cells and $DX5^+CD3^+$ cells between the wild-type and LECT2-deficient mice, indicating the proportions of NK and NKT cells were not significantly different in BALB/c background mice (Fig. 3A). The proportion of NKT cells was confirmed by using CD1d-tetramers and anti-CD3 antibodies [3]. The content of the CD1d-tetramer$^+$CD3$^+$ cells was also comparable between the wild-type and LECT2-deficient mice. Finally, flow cytometric analysis using anti-DX5, anti-CD3, anti-Gr-1, and anti-Mac-1 antibodies and CD1d-tetramers did not reveal any significant differences in hepatic MNCs between the wild-type and LECT2-deficient mice (Fig. 3A).

Next, we analyzed the IFN-γ production of hepatic NK and NKT cells. We prepared hepatic MNCs 3 h after LPS/D-GalN injection. The total amounts of hepatic MNCs obtained from the livers of wild-type and LECT2-deficient mice were comparable ($6.7 \pm 2.3 \times 10^5$ cells in

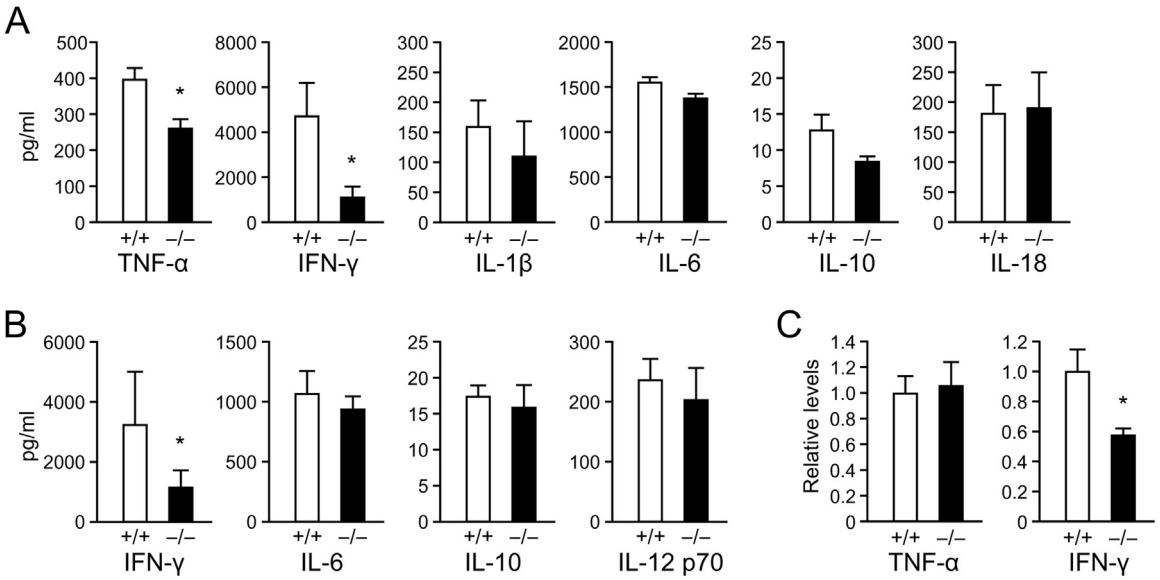

Fig. 2. Serum levels and transcriptional levels of proinflammatory cytokines after LPS/D-GalN administration. The serum levels of cytokines in wild-type (+/+) and LECT2-deficient (−/−) mice at 1.5 h **(A)** and 5 h **(B)** after the LPS/D-GalN injection. Values are the mean and SEM of the results from 7 to 10 female mice. **(C)** Liver RNAs were prepared from mice at 1.5 h after LPS/D-GalN administration. Quantitative real-time PCR was performed. The relative ratios of the PCR products in the wild-type (n = 10) vs. the LECT2-deficient (n = 8) mice are shown. * $p < 0.05$.

wild-type vs. 5.0 ± 1.3 × 10^5 cells in LECT2-deficient mice; n = 3; p = 0.324). These cells were incubated in a culture medium containing brefeldin A for 2 h and were then subjected to flow cytometric analysis. As shown in Fig. 3D, DX5-positive cells in the LECT2-deficient mice significantly reduced the production of IFN-γ compared with that in wild-type mice (p = 0.001), but there was no significant difference in IFN-γ-producing DX5-negative cells derived from the two mice types (Fig. 3B). The proportion of IFN-γ-producing $DX5^+CD3^-$ cells was clearly lower in the LECT2-deficient mice than in the wild-type mice (Fig. 3C, p = 0.05). In addition, the proportion of IFN-γ-producing $DX5^+CD3^+$ cells in the LECT2-deficient mice also tended to be lower than that in wild-type mice (Fig. 3D, p = 0.06). These results indicate that the IFN-γ production derived from hepatic $DX5^+CD3^-$ NK and $DX5^+CD3^+$ NKT cells in the LECT2-deficient mice decreased in LPS/D-GalN-induced acute liver injury.

3.4. IFN-γ production following treatment with recombinant IL-12

IL-12 derived from macrophages is a major cytokine in LPS stimulation [23]. It has been reported that NK and NKT cells predominantly produce IFN-γ through stimulation by IL-12 from macrophage/Kupffer cells in hepatic injuries [21,23]. We therefore examined whether IFN-γ production by IL-12 stimulation was affected by LECT2 deficiency. As shown in Fig. 4, the serum level of IFN-γ in the LECT2-deficient mice was significantly lower than that in the wild-type mice ($p < 0.05$).

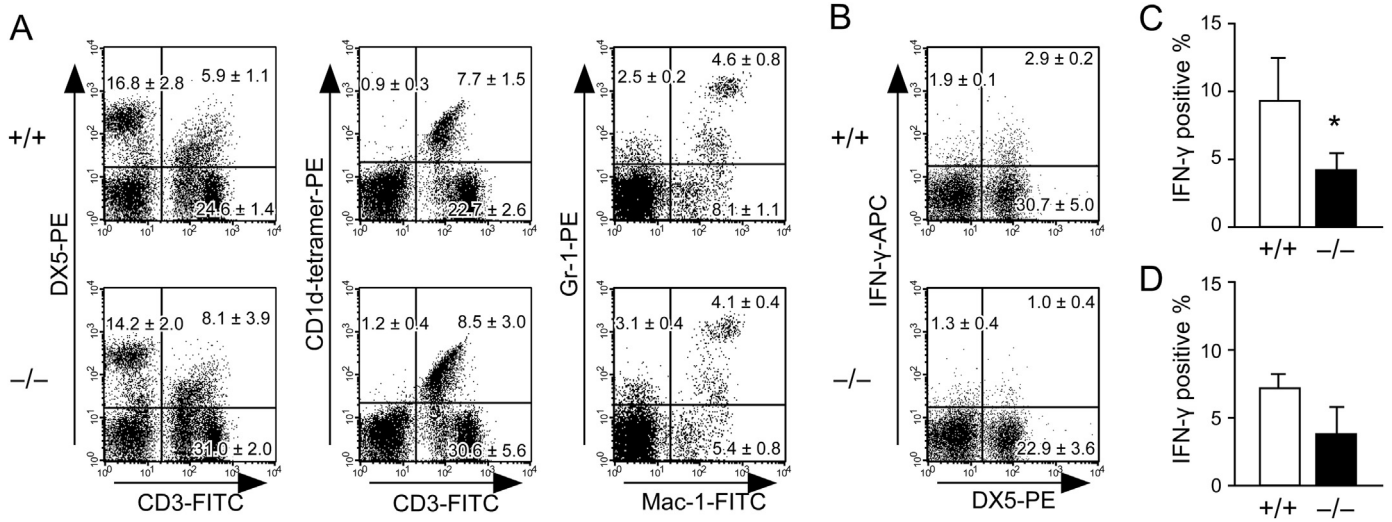

Fig. 3. The reduction of IFN-γ-producing hepatic NK and NKT cells in the LECT2-deficient mice. (A) The numbers of NKT and NK cells in the livers of the wild-type (+/+) and the LECT2-deficient (−/−) mice on a BALB/c background were comparable. Two-color staining was performed for CD3 and CD49b (DX5), CD3 and CD1d-tetramer, and Mac-1 and Gr-1 against hepatic mononuclear cells. Representative flow cytometric plots are shown. **(B–D)** The IFN-γ-producing hepatic NK and NKT cells were reduced in the LECT2-deficient mice treated with LPS/D-GalN. **(B)** Two-color staining for DX5 and IFN-γ against hepatic mononuclear cells in the wild-type (+/+) and LECT2-deficient (−/−) mice. **(C)** Detection of IFN-γ in $DX5^+CD3^-$ NK cells by three-color staining for DX5, CD3, and IFN-γ. * $p < 0.05$. **(D)** Detection of IFN-γ in $DX5^+CD3^+$ NKT cells. The numbers in the small panels of flow cytometric plots indicate the mean percentages and SEM of fluorescence-positive cells in the corresponding areas. Values are the mean and SEM of the results from 3 to 4 female mice.

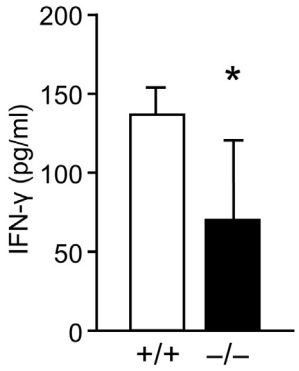

Fig. 4. The serum IFN-γ level was reduced in LECT2-deficient mice treated with recombinant IL-12. The wild-type (+/+) and LECT2-deficient (−/−) mice (n = 5, each) were injected intraperitoneally with recombinant mouse IL-12 at 1 μg/20 g body weight. After 7 h, sera were prepared and subjected to IFN-γ ELISA measurement. * $p < 0.05$.

4. Discussion

In this study, we have shown that LECT2-deficient mice are less sensitive than wild-type mice to LPS/D-GalN-induced hepatitis. LPS/D-GalN-induced acute liver injury is a well-established animal model primarily mediated by TNF-α derived from macrophage/Kupffer cells [14,15,17,18]. We found the induced level of TNF-α in the LECT2-deficient mice to be significantly lower than that in the wild-type mice. We also found significantly lower IFN-γ production in the LECT2-deficient mice. IFN-γ is also known to contribute to LPS/D-GalN-induced liver apoptosis and injury [20], as in other liver diseases [24]. The reduction in serum IFN-γ in the LECT2-deficient mice was notably greater than that of TNF-α. Furthermore, the expression level of hepatic TNF-α mRNA was comparable between the wild-type and LECT2-deficient mice. In contrast, hepatic IFN-γ mRNA was significantly reduced in the LECT2-deficient mice. We therefore focused attention on the hepatic cells producing IFN-γ.

IFN-γ is mainly produced from hepatic NK and NKT cells through stimulation by LPS [21,23]. We started by checking the number of hepatic NKT cells in LECT2-deficient mice, because we previously reported increased NKT cells in LECT2-deficient mice compared with the number in wild-type mice on a C57BL/6 background. We showed the number of hepatic NKT cells was comparable between the wild-type and LECT2-deficient mice, suggesting the number of hepatic NKT cells varies according to the genetic background of mouse strain. We also found no significant difference in the number of hepatic NK cells between the wild-type and LECT2-deficient mice. Next, we analyzed the production of IFN-γ from hepatic NK and NKT cells in this hepatitis. The proportion of IFN-γ-producing $DX5^+$ cells (NK and NKT cells) was significantly lower in the LECT2-deficient mice than in the wild-type mice. However, we found no difference in IFN-γ-producing $DX5^-$ cells between these mice types. In agreement with these results, there were fewer IFN-γ-producing $CD3^-DX5^+$ NK and $CD3^+DX5^+$ NKT cells in the LECT2-deficient mice than in the wild-type mice. These results indicated that the decrease of IFN-γ production by hepatic NK and NKT cells was one of the causes of the reduced IFN-γ production in LECT2-deficient mice treated with LPS/D-GalN. It is known that IFN-γ production by NK and NKT cells is regulated by IL-12 induced by LPS challenge [21,25]. We found that the level of IFN-γ induced by recombinant IL-12 administration was also reduced in the LECT2-deficient mice.

This reduction of IFN-γ in LECT2-deficient mice may be explained as follows. We previously suggested that NKT cells show Th-2 biased cytokine polarization in LECT2-deficient mice in the C57BL/6 background [3,5]. As shown in supplemental Fig. S2, concanavalin A-induced hepatitis in LECT2-deficient mice in the BALB/c background was also severe and the key cytokine, IL-4, was increased similar to mice in the C57BL/6 background. LECT2 may also affect the development of NKT cells in the BALB/c background, driving them to a Th2-bias. Moreover, it has been reported that the IFN-γ production of NK cells is affected by IFN-γ secreted by NKT cells [26–28]. It is therefore possible that the reduction of IFN-γ in NKT cells led to a total reduction of IFN-γ production in the LECT2-deficient mice. However, following α-GalCer treatment, IL-4 levels were comparable between LECT2-KO and wild-type mice in the BALB/c background, as shown in supplemental Fig. S3. This differs from a previous study showing that α-GarCer treatment enhanced the production of IL-4 in LECT2-KO mice in the C57BL/6 background [3], suggesting alternatives to Th2-bias. For example, LECT2 may directly regulate the function of NK/NKT cells to enhance IFN-γ production by IL-12. Investigating the regulation of this process may lead to the identification of new LECT2 signaling pathways.

The decreased level of TNF-α in LECT2-deficient mice is also important in LPS/D-GalN-induced acute liver injury. It is known that IFN-γ activates macrophages to enhance the production of several cytokines, such as TNF-α [25,29]. Thus, the reduction of IFN-γ may affect the production of TNF-α. In addition, it has recently been reported that LECT2 modulates macrophage functions via the CD209a receptor [9]. LECT2 may directly regulate the expression of TNF-α in this hepatitis. Further studies about LECT2's role related to macrophages are needed not only for this hepatitis model but also for other pathological conditions.

Generally, inflammation is regulated by several cytokines. Pro-inflammatory cytokines (such as TNF-α, IL-1b, IL-6, IL-12, and IFN-γ in Fig. 2) promote inflammation. Conversely, IL-10 is an anti-inflammatory cytokine. The balance of pro- and anti-inflammatory cytokines influences the severity of inflammation. Decreased levels of IFN-γ and TNF-α in LECT2-KO mice could lead to tilt this balance toward anti-inflammation.

In summary, LPS/D-GalN-induced acute liver injury is an experimental model of fulminant hepatitis. We have shown that this hepatitis is alleviated in LECT2-deficient mice. Our results indicate that decreased production of IFN-γ in hepatic NK and NKT cells contributes to this alleviated hepatitis in these mice. The pathological function of LECT2 in hepatitis may depend on the initiation of immune response cells such as T cells or macrophages.

Acknowledgements

This work was supported by JSPS KAKENHI Grant Number JP23590275 and JP26670229. The authors would like to thank Enago (www.enago.jp) for the English language review.

Appendix A. Transparency document

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2017.09.011.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2017.09.011.

References

[1] S. Yamagoe, Y. Yamakawa, Y. Matsuo, J. Minowada, S. Mizuno, K. Suzuki, Purification and primary amino acid sequence of a novel neutrophil chemotactic factor LECT2, Immunol. Lett. 52 (1996) 9–13.

[2] S. Yamagoe, T. Akasaka, T. Uchida, T. Hachiya, T. Okabe, Y. Yamakawa, T. Arai, S. Mizuno, K. Suzuki, Expression of a neutrophil chemotactic protein LECT2 in human hepatocytes revealed by immunochemical studies using polyclonal and monoclonal antibodies to a recombinant LECT2, Biochem. Biophys. Res. Commun. 237 (1997) 116–120.

[3] T. Saito, A. Okumura, H. Watanabe, M. Asano, A. Ishida-Okawara, J. Sakagami, K. Sudo, Y. Hatano-Yokoe, J.S. Bezbradica, S. Joyce, T. Abo, Y. Iwakura, K. Suzuki, S. Yamagoe, Increase in hepatic NKT cells in leukocyte cell-derived chemotaxin 2-

deficient mice contributes to severe concanavalin A-induced hepatitis, J. Immunol. 173 (2004) 579–585.
[4] A. Okumura, T. Saito, I. Otani, K. Kojima, Y. Yamada, A. Ishida-Okawara, K. Nakazato, M. Asano, K. Kanayama, Y. Iwakura, K. Suzuki, S. Yamagoe, Suppressive role of leukocyte cell-derived chemotaxin 2 in mouse anti-type II collagen antibody-induced arthritis, Arthritis Rheum. 58 (2008) 413–421.
[5] M. Anson, A.M. Crain-Denoyelle, V. Baud, F. Chereau, A. Gougelet, B. Terris, S. Yamagoe, S. Colnot, M. Viguier, C. Perret, J.P. Couty, Oncogenic β-catenin triggers an inflammatory response that determines the aggressiveness of hepatocellular carcinoma in mice, J. Clin. Invest. 122 (2012) 586–599.
[6] H.T. Ong, P.K. Tan, S.M. Wang, D.T. Hian Low, L.L. Ooi, K.M. Hui, The tumor suppressor function of LECT2 in human hepatocellular carcinoma makes it a potential therapeutic target, Cancer Gene Ther. 18 (2011) 399–406.
[7] F. Lan, H. Misu, K. Chikamoto, H. Takayama, A. Kikuchi, K. Mohri, N. Takata, H. Hayashi, N. Matsuzawa-Nagata, Y. Takeshita, H. Noda, Y. Matsumoto, T. Ota, T. Nagano, M. Nakagen, K.I. Miyamoto, K. Takatsuki, T. Seo, K. Iwayama, K. Tokuyama, S. Matsugo, H. Tang, Y. Saito, S. Yamagoe, S. Kaneko, T. Takamura, LECT2 functions as a hepatokine that links obesity to skeletal muscle insulin resistance, Diabetes 63 (2014) 1649–1664.
[8] M.D. Benson, LECT2 amyloidosis, Kidney Int. 77 (2010) 757–759.
[9] X.J. Lu, J. Chen, C.H. Yu, Y.H. Shi, Y.Q. He, R.C. Zhang, Z.A. Huang, J.N. Lv, S. Zhang, L. Xu, LECT2 protects mice against bacterial sepsis by activating macrophages via the CD209a receptor, J. Exp. Med. 210 (2013) 5–13.
[10] H. Zheng, T. Miyakawa, Y. Sawano, A. Asano, A. Okumura, S. Yamagoe, M. Tanokura, Crystal structure of human leukocyte cell-derived chemotaxin 2 (LECT2) reveals a mechanistic basis of functional evolution in a mammalian protein with an M23 metalloendopeptidase fold, J. Biol. Chem. 291 (2016) 17133–17142.
[11] C.K. Chen, C.Y. Yang, K.T. Hua, M.C. Ho, G. Johansson, Y.M. Jeng, C.N. Chen, M.W. Chen, W.J. Lee, J.L. Su, T.C. Lai, C.C. Chou, B.C. Ho, C.F. Chang, P.H. Lee, K.J. Chang, M. Hsiao, M.T. Lin, M.L. Kuo, Leukocyte cell-derived chemotaxin 2 antagonizes MET receptor activation to suppress hepatocellular carcinoma vascular invasion by protein tyrosine phosphatase 1B recruitment, Hepatology 59 (2014) 974–985.
[12] C.K. Chen, W.H. Yu, T.Y. Cheng, M.W. Chen, C.Y. Su, Y.C. Yang, T.C. Kuo, M.T. Lin, Y.C. Huang, M. Hsiao, K.T. Hua, M.C. Hung, M.L. Kuo, Inhibition of $VEGF_{165}$/VEGFR2-dependent signaling by LECT2 suppresses hepatocellular carcinoma angiogenesis, Sci. Rep. 6 (2016) e31398.
[13] M.H. Dang, H. Kato, H. Ueshiba, M. Omori-Miyake, S. Yamagoe, K. Ando, K. Imanishi, Y. Arimura, I. Haruta, T. Kotani, M. Ozaki, K. Suzuki, T. Uchiyama, J. Yagi, Possible role of LECT2 as an intrinsic regulatory factor in SEA-induced toxicity in d-galactosamine-sensitized mice, Clin. Immunol. 137 (2010) 311–321.
[14] C. Galanos, M.A. Freudenberg, W. Reutter, Galactosamine-induced sensitization to the lethal effects of endotoxin, Proc. Natl. Acad. Sci. USA 76 (1979) 5939–5943.
[15] M.A. Freudenberg, D. Keppler, C. Galanos, Requirement for lipopolysaccharide-responsive macrophages in galactosamine-induced sensitization to endotoxin, Infect. Immun. 51 (1986) 891–895.
[16] G. Sass, S. Heinlein, A. Agli, R. Bang, J. Schumann, G. Tiegs, Cytokine expression in three mouse models of experimental hepatitis, Cytokine 19 (2002) 115–120.
[17] K. Pfeffer, T. Matsuyama, T.M. Kundig, A. Wakeham, K. Kishihara, A. Shahinian, K. Wiegmann, P.S. Ohashi, M. Kronke, T.W. Mak, Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to L. monocytogenes infection, Cell 73 (1993) 457–467.
[18] J. Rothe, W. Lesslauer, H. Lotscher, Y. Lang, P. Koebel, F. Kontgen, A. Althage, R. Zinkernagel, M. Steinmetz, H. Bluethmann, Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by Listeria monocytogenes, Nature 364 (1993) 798–802.
[19] M. Leist, F. Gantner, I. Bohlinger, G. Tiegs, P.G. Germann, A. Wendel, Tumor necrosis factor-induced hepatocyte apoptosis precedes liver failure in experimental murine shock models, Am. J. Pathol. 146 (1995) 1220–1234.
[20] W.H. Kim, F. Hong, S. Radaeva, B. Jaruga, S. Fan, B. Gao, STAT1 plays an essential role in LPS/d-galactosamine-induced liver apoptosis and injury, Am. J. Physiol. Gastrointest. Liver Physiol. 285 (2003) G761–G768.
[21] S. Seki, Y. Habu, T. Kawamura, K. Takeda, H. Dobashi, T. Ohkawa, H. Hiraide, The liver as a crucial organ in the first line of host defense: the roles of Kupffer cells, natural killer (NK) cells and NK1. 1 Ag+ T cells in T helper 1 immune responses, Immunol. Rev 174 (2000) 35–46.
[22] H. Arase, T. Saito, J.H. Phillips, L.L. Lanier, Cutting edge: the mouse NK cell-associated antigen recognized by DX5 monoclonal antibody is CD49b (α_2 integrin, very late antigen-2), J. Immunol. 167 (2001) 1141–1144.
[23] G. Trinchieri, Interleukin-12: a cytokine at the interface of inflammation and immunity, Adv. Immunol. 70 (1998) 83–243.
[24] C.J. Horras, C.L. Lamb, K.A. Mitchell, Regulation of hepatocyte fate by interferon-γ, Cytokine Growth Factor Rev. 22 (2011) 35–43.
[25] J.R. Schoenborn, C.B. Wilson, Regulation of interferon-γ during innate and adaptive immune responses, Adv. Immunol. 96 (2007) 41–101.
[26] M. Emoto, M. Miyamoto, I. Yoshizawa, Y. Emoto, U.E. Schaible, E. Kita, S.H. Kaufmann, Critical role of NK cells rather than $V\alpha14^+$NKT cells in lipopolysaccharide-induced lethal shock in mice, J. Immunol. 169 (2002) 1426–1432.
[27] C. Carnaud, D. Lee, O. Donnars, S.H. Park, A. Beavis, Y. Koezuka, A. Bendelac, Cutting edge: Cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells, J. Immunol. 163 (1999) 4647–4650.
[28] G. Eberl, H.R. MacDonald, Selective induction of NK cell proliferation and cytotoxicity by activated NKT cells, Eur. J. Immunol. 30 (2000) 985–992.
[29] K. Schroder, P.J. Hertzog, T. Ravasi, D.A. Hume, Interferon-γ: an overview of signals, mechanisms and functions, J. Leukoc. Biol. 75 (2004) 163–189.