



# Deficiency of interleukin-19 exacerbates lipopolysaccharide/D-galactosamine-induced acute liver failure

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**ABSTRACT.** Interleukin (IL)-19 is a cytokine clustered in the IL-20 cytokine superfamily with both anti-inflammatory and pro-inflammatory aspects depending on the etiology of inflammatory disease. The function of IL-19 has been evaluated in cutaneous and inflammatory bowel diseases, but has not been studied in liver diseases. Here, we examined the effect of IL-19 on acute liver failure (ALF) using two mouse models of ALF: lipopolysaccharide and D-galactosamine (LPS/GalN)-induced model and concanavalin A (ConA)-induced model. In the LPS/GalN-induced ALF model, which is mainly caused by the innate immune response of liver macrophages, IL-19 knockout (KO) mice showed increased plasma level of liver deviation enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) compared with wild-type (WT) mice. In histopathology of liver sections, IL-19 KO mice exacerbated liver injury with marked hemorrhagic lesions and hepatocellular death in the liver compared with WT mice. In this model, mRNA expressions of pro-inflammatory chemokines, CCL2 and CCL5 were increased in liver tissue from IL-19 KO mice compared with WT mice. Moreover, the mRNA expressions of IL-19 and its receptor subunit were induced in liver tissue by LPS/GalN administration. However, there is no difference in liver injury between WT and IL-19KO in the ConA-induced ALF model induced by CD4<sup>+</sup> T cell activation. These data suggest that IL-19 has a protective effect against inflammation-mediated liver injury, which is dependent on the etiology.

**KEY WORDS:** acute liver injury, chemokines, interleukin-19, liver

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The liver is an important organ responsible for the metabolism of toxins and drugs, and liver dysfunction caused by massive cell death of hepatocyte can be life-threatening. Acute liver failure (ALF) is a rare syndrome characterized by impaired liver function with acute abnormality of liver blood tests in patients without underlying chronic liver disease, and is associated with clinical symptoms developing coagulopathy and hepatic encephalopathy [16, 17]. This disease is primarily caused by viral infection such as hepatitis virus, drug/toxin overdose, and hypoxic liver injury associated with circulatory failure. The mechanisms that cause hepatocyte dysfunction include direct induction of hepatocyte death and indirect hepatocyte death associated with abnormal activation of immune function. In particular, cytokine-mediated signaling cascades are thought to play an important role in both exacerbating cell injury and protecting hepatocytes in immune system-mediated hepatocyte death.

Interleukin-19 (IL-19) is a cytokine clustered in the IL-20 subfamily, which together with itself includes IL-20, IL-22, IL-24, and IL-26, based on the similarity of their conformations and the types of receptors it shares [6]. It is accepted that IL-20 subfamily cytokines, including IL-19, play an important role in the homeostasis of epithelial functions [10, 12]. The mechanism by which these cytokines regulate cellular function is exerted through activation of the Jak-stat signaling pathway, which is activated after binding to its receptors, particularly through phosphorylation of STAT3 [4]. As a result, these cytokines induce STAT3-dependent gene expression, cause cell proliferation and anti-microbial production by epithelial cells in organs such as the skin and gastrointestinal tract, and act as a defense against invasion of pathogenic microorganisms. IL-19 has been reported to have an anti-inflammatory effect on immune cells, which can suppress the pathogenesis of inflammatory diseases such as inflammatory bowel disease [1]. Although several IL-20 subfamily cytokines have been reported to be involved in the pathogenesis of acute and chronic liver diseases [2, 3], the pathological role of IL-19 in liver disease has not been verified.

To investigate the role of IL-19 in liver diseases, we performed experiments using two mouse ALF models with different

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pathological mechanisms, lipopolysaccharide/D-galactosamine (LPS/GalN) and concanavalin A (ConA)-induced model. The LPS/GalN-induced model is used to study the inflammatory response of innate immune cells and TNF $\alpha$ -dependent hepatocellular apoptosis, whereas the ConA-induced model reflects the pathogenesis of auto-immune and viral hepatitis mediated by CD4<sup>+</sup> T cell activation [7].

## MATERIALS AND METHODS

### Animals

IL-19 knockout (IL-19 KO) mice were obtained as described previously [5]. IL-19KO mice with C57BL/6 background were backcrossed onto BALB/c background for at least 10 generations. BALB/c heterozygous mice were intercrossed to generate mutant and control mice. All mice used were 8–15 weeks old. All animal experiments were approved by institutional policies of Osaka Prefecture University Animal Care and Committee.

### Mice model of acute liver failure

To induce acute liver injury, mice were i.p. injected with LPS (*E. coli* 0111:B4) (Sigma-Aldrich, St. Louis, MO, USA) (5  $\mu$ g/kg) and GalN (Tokyo Chemical Industry, Tokyo, Japan) (700 mg/kg) diluted in saline solution. Mice were injected with ConA (Sigma-Aldrich) (20 mg/kg, i.v.). After indicated time, mice were euthanized, and blood and liver tissue were collected.

### Measurement of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels

Liver injury was estimated by biochemical blood markers AST and ALT. Blood samples were collected from heart with heparin-treated needle and syringe, and then centrifuged at  $\times 10,000$  g for 10 min at 4°C to separate plasma fraction. Measurement of AST and ALT in plasma sample was performed using Transaminase C-II-test WAKO (Wako Pure Chemical, Osaka, Japan) according to the manufacturer's instructions with brief modifications.

### Histology

Liver tissue was fixed with 10% neutral buffered formalin, and then processed for routine paraffin embedding. Sections (3- $\mu$ m thick) of the specimens were stained with hematoxylin and eosin (HE).

### RNA isolation and reverse transcription-polymerase chain reaction (RT-qPCR)

Total RNA was isolated from liver tissue by using TRIzol<sup>TM</sup> Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and reverse transcribed using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). Equal amounts of cDNA template were used in quantitative real-time PCR (RT-qPCR) reactions with specific primers and KAPA SYBR<sup>®</sup> FAST qPCR Master Mix (KAPA Biosystems, Inc., Wilmington, MA, USA). The specific primers used for qPCR reactions were 5'-CTCCTGGGCATGACGTTGATT-3' (sense) and 5'-GCATGGCTCTCTTGATCTCGT-3' (antisense) for IL-19, 5'-CAGGTGCTTCCAGTCCGCT-3' (sense) and 5'-CTCTCCTGGAATCCCCAAAGT-3' (antisense) for IL-20RB, 5'-TTAAAAACCTGGATCGGAACCAA-3' (sense) and 5'-GCATTAGCTTCAGATTTACGGGT-3' (antisense) for CCL2, 5'-GCTGCTTTGCCTACCTCTCC-3' (sense) and 5'-TCGAGTGACAAACACGACTGC-3' (antisense) for CCL5, 5'-GTTGGATACAGGCCAGACTTTGTTG-3' (sense) and 5'-GAGGGTAGGCTGGCCTATAGGCT-3' (antisense) for hypoxanthine phosphoribosyltransferase (HPRT). Data were collected and analyzed by Step one plus Real-Time PCR-system (Applied Biosystems, Foster City, CA, USA) and expression of target gene was normalized to the level of HPRT expression in each sample according to the  $\Delta\Delta$ Ct method.

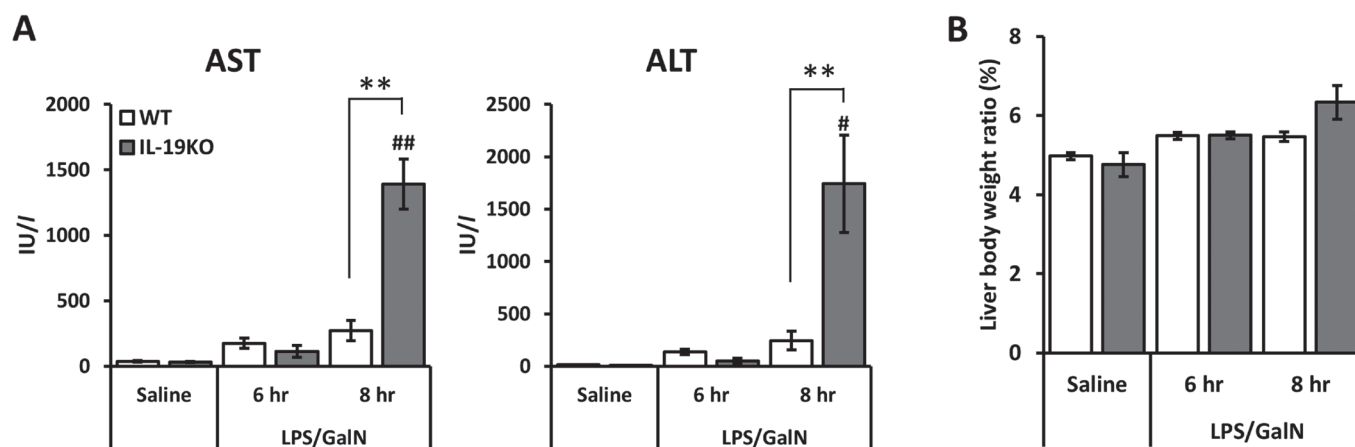
### Statistical analysis

All data are presented as means  $\pm$  standard error of the mean (SEM). Statistical significances in parametric data were analyzed using Student's *t*-tests to detect differences between 2 groups. Statistical significance was determined by one-way ANOVA for nonrepeated measures to detect differences among 6 groups. The differences between groups were determined using the Tukey test. A value of  $P < 0.05$  was considered statistically significance.

## RESULTS

To examine whether IL-19 deficiency contributes liver injury in LPS/GalN-induced ALF model, we compared the extent of liver injury in IL-19 KO mice and that in WT mice. To estimate liver injury, we measured plasma levels of AST and ALT, liver deviated enzyme activity (Fig. 1A). Plasma levels of AST and ALT in WT mice were increased by injection of LPS/GalN at 6 and 8 hr after treatment compared with saline-injected control. Plasma levels of AST and ALT in LPS/GalN-treated IL-19 KO mice were significantly increased at 8 hr after treatment compared with WT mice, while ALT was slightly but decreased in IL-19 KO at 6 hr after treatment. Similarly, liver weight ratio associated with hemorrhagic lesions in the liver, tended to increase in LPS/GalN-treated IL-19 KO mice after 8 hr (Fig. 1B).

We next examined histopathological changes in mice treated with LPS/GalN (Fig. 2). Mild hepatocellular death and inflammatory cell infiltration were observed in WT mice after LPS/GalN treatment compared with saline-treated control mice. However, no clear difference was observed between WT and IL-19 KO mice at 6 hr after treatment. IL-19 KO mice exacerbated



**Fig. 1.** Interleukin (IL)-19 knockout (KO) mice showed exacerbation in lipopolysaccharide/D-galactosamine (LPS/GalN)-induced liver injury. Wild-type (WT) and IL-19KO mice were injected with saline or LPS/GalN. (A) Levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma sample were determined at 6 and 8 hr after treatment. (B) Liver tissue was removed and weighed at the indicated time after saline or LPS/GalN injection and liver body weight ratio was calculated. Data are mean  $\pm$  SEM.  $n=3-6$  per group; \*\* $P<0.01$  (WT vs. IL-19 KO), # $P<0.05$ , ## $P<0.01$  (Saline vs. LPS/GalN)

liver injury and showed marked hemorrhagic lesions, hepatocellular death, and neutrophil infiltration in the liver at 8 hr after treatment.

We next investigated the inflammatory chemokine expressions in liver tissue at 8 hr after LPS/GalN treatment by using RT-qPCR (Fig. 3A). Under the condition of saline treatment, the expression of CCL2 and CCL5 in WT and IL-19KO was very low or below the detection (data not shown). CCL2 and CCL5 expressions were significantly increased in liver tissue of IL-19 KO mice compared with WT mice.

To confirm whether IL-19 expression is increased during a pathological process, we performed RT-qPCR for IL-19 using liver tissue from LPS/GalN-administrated mice (Fig. 3B). IL-19 expression was induced in liver tissue of WT mice at 8 hr after LPS/GalN treatment. Similarly, IL-20RB, a component of IL-19 receptor, was significantly increased in LPS/GalN-treated liver tissue.

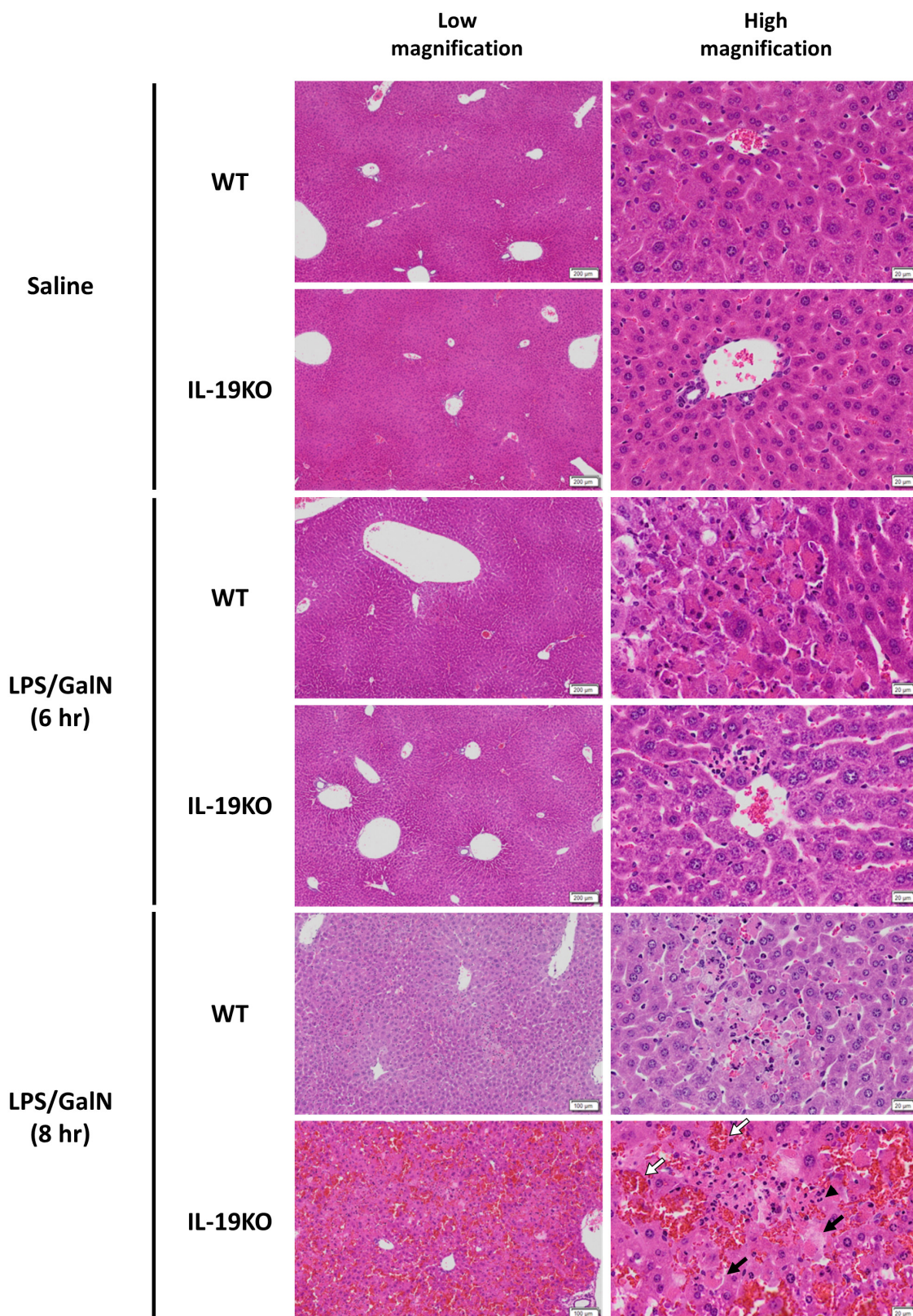
Finally, to determine whether deficiency of IL-19 contributes liver injury in other model, we investigated ConA-induced ALF model in which T cell activation causes liver injury. Elevated plasma liver deviated enzyme levels were observed with ConA-treatment compared with saline-injected control, but there was no difference between WT and IL-19 KO mice (Fig. 4). Moreover, histopathology of liver sections showed that ConA administration induced focal hepatic necrosis, but IL-19 deficiency had no effect on the extent of liver injury compared with WT mice (Fig. 5).

## DISCUSSION

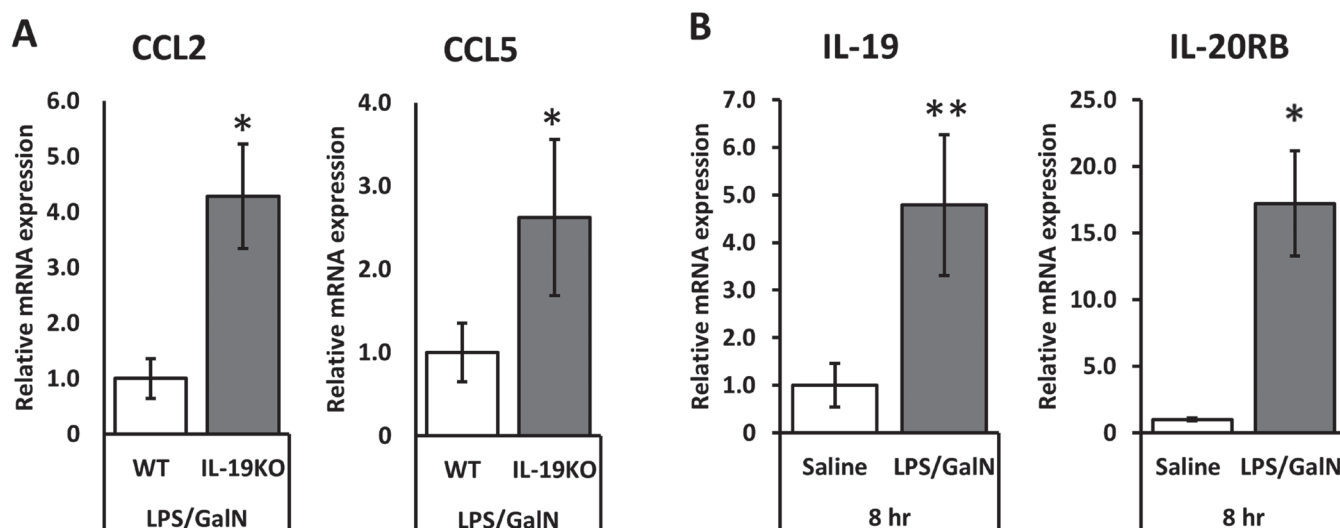
IL-19 has been reported to be a cytokine with both pro- and anti-inflammatory aspects, depending on the type of disease. In this study, we reported that IL-19 deficiency caused more severe liver injury and increased inflammatory chemokine expression in the LPS/GalN-induced ALF model. Moreover, IL-19 and its receptor subunit were induced in liver tissue in this model. Therefore, it is possible that IL-19 exerts a protective effect on LPS/GalN-induced liver injury through suppression of inflammatory chemokine production. In this model, GalN reduces the intracellular pool of uracil nucleotides and inhibits the synthesis of RNA and protein making hepatocyte susceptible to apoptotic cell death induced by TNF $\alpha$  produced by Kupffer cells [7]. In mouse studies, LPS-activated Kupffer cells induce the production of inflammatory mediators such as TNF $\alpha$  and CCL2 and subsequent hepatocyte death, and CCL2-mediated infiltration of monocytes into liver tissue has been shown to be an exacerbating factor for liver injury in ALF [18]. Taken into account that IL-19 has an anti-inflammatory effect on monocytes/macrophages [1], it is possible that IL-19 regulates the activation of Kupffer cells and monocytes in liver treated with LPS/GalN. Therefore, future studies are needed to determine whether IL-19 inhibits inflammatory chemokine production in Kupffer cells.

Some types of cytokine can directly induce anti-apoptotic effect on hepatocytes. For example, monoclonal antibody treatment against IL-22, IL-20 superfamily cytokine, partially suppressed STAT3 phosphorylation and exacerbated liver injury in ConA-induced liver injury [11]. Furthermore, IL-22 overexpression may induce STAT3 phosphorylation, and antiapoptotic and mitogenic protein expression. It has been reported that LPS treatment has a protective effect against liver injury caused by Fas activation. This protective effect is mediated through STAT3 activation in experiments with ruxonitinib, a STAT3 inhibitor [8]. Moreover, it has been reported that IL-20, which shares the receptor with IL-19, may induce STAT3 phosphorylation in mouse primary hepatocyte [15]. In light of these reports, we used primary cultured mouse hepatocytes to test whether IL-19 could directly induce phosphorylation of STAT3 in hepatocytes. However, no induction of STAT3 phosphorylation was observed (data not shown).

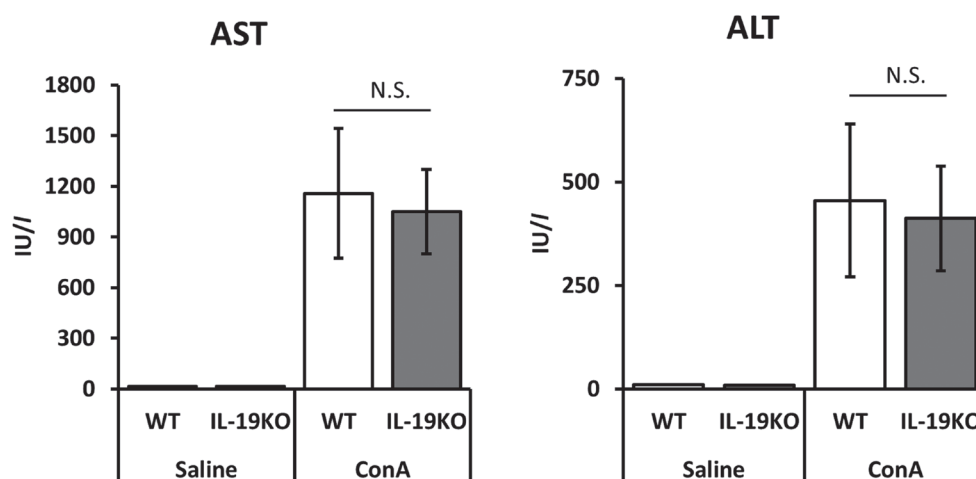
In this study, we could not observe the difference in liver injury caused by ConA injection in WT and IL-19 KO mice. ConA is



**Fig. 2.** Hematoxylin and eosin (HE) sections in lipopolysaccharide/D-galactosamine (LPS/GaIN)-induced liver injury. Liver sections of representative sections were stained with HE staining. Scale bar, 200  $\mu\text{m}$  or 100  $\mu\text{m}$  (left panels) and 20  $\mu\text{m}$  (right panels). Hemorrhage lesion, hepatocellular death and neutrophil infiltration are indicated by white arrows, black arrow arrows and black head respectively.  $n=3-6$  per group.



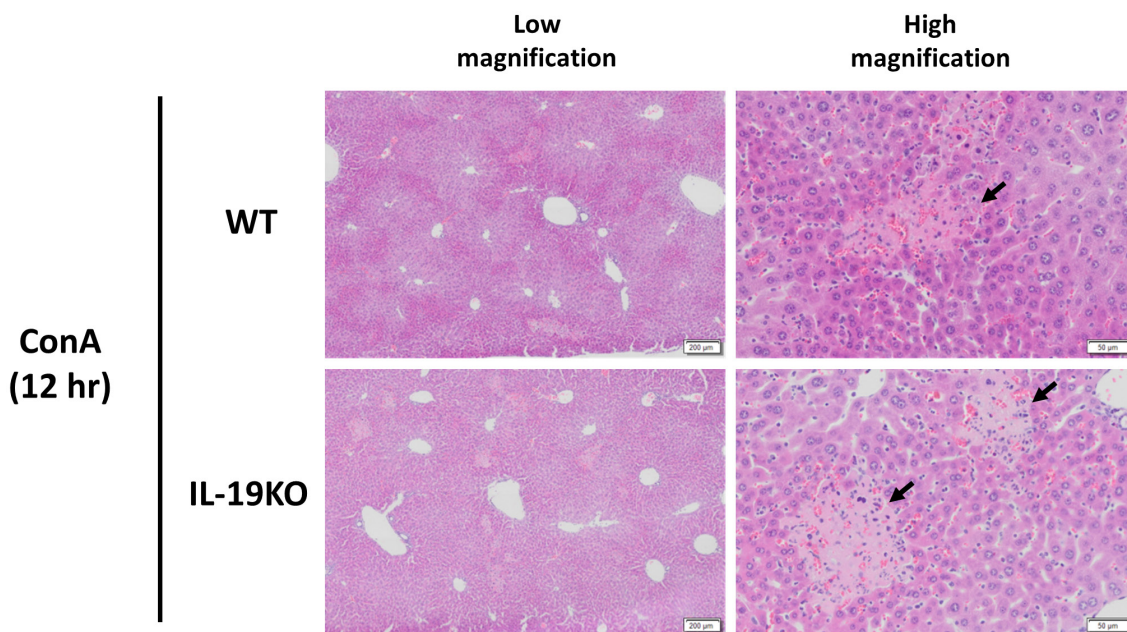
**Fig. 3.** mRNA expressions in lipopolysaccharide/D-galactosamine (LPS/GalN)-induced liver injury. (A) CCL2 and CCL5 mRNA expression of liver tissue from wild-type (WT) and interleukin (IL)-19 knockout (KO) mice injected with lipopolysaccharide/D-galactosamine (LPS/GalN) at 8 hr after treatment. (B) IL-19 and IL-20RB mRNA expression of liver tissue from WT injected with saline or LPS/GalN at 8 hr after treatment. Data are mean  $\pm$  SEM.  $n=5$  per group; \* $P<0.05$  (WT vs. IL-19KO). \* $P<0.05$ , \*\* $P<0.01$  (Saline vs. LPS/GalN).



**Fig. 4.** No difference was observed in concanavalin A (ConA)-induced liver injury between wild-type (WT) and interleukin (IL)-19 knockout (KO) mice. WT and IL-19KO mice were administrated with saline or ConA. Levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma sample was determined at 12 hr after treatment. Data are mean  $\pm$  SEM. Saline-WT:  $n=2$ , Saline-IL-19KO:  $n=2$ , ConA-WT:  $n=7$ , ConA-IL-19KO:  $n=9$  per group.

a plant-derived lectin extracted from *Canavalia ensiformis* that induces hepatocyte necrosis by activating mitogenic CD4<sup>+</sup> T cells and hepatic TNF $\alpha$  expression [9]. Several previous reports have suggested that IL-19 regulates T cell response [14]. In addition, in ConA-induced model, Kupffer cells are involved in the development of liver injury. Depletion of Kupffer cells by clodronate liposomes has been reported to reduce TNF $\alpha$  expression and liver injury induced by ConA injection [13]. The contribution of IL-19 may depend on the mechanism of the liver pathology. Therefore, we need to verify whether hepatic IL-19 expression is induced by ConA injection.

There are few reports of the effects of IL-19 on the pathogenesis of acute liver disease. This report indicate that IL-19 may contribute to the pathogenesis of liver disease. The two mouse ALF models are frequently used as experimental models of ALF to investigate mechanism of inflammation-mediated liver injury, but neither fully cover the clinical symptoms and mechanisms of ALF. It is unclear whether IL-19 also has a protective effect against liver injury in clinical ALF, and we should investigate the kinetics of IL-19 expression in human and other animal ALFs.



**Fig. 5.** Hematoxylin and eosin (HE) sections in concanavalin A (ConA)-induced liver injury. Liver sections of representative sections were stained by HE staining. Scale bar, 200  $\mu\text{m}$  (left panels) and 50  $\mu\text{m}$  (right panels). Necrotic lesion is indicated by black arrows.  $n=7-9$  per group.

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