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Interleukin 10 deficiency exacerbates halothane induced liver injury by increasing interleukin 8 expression and neutrophil infiltration

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ABSTRACT

The prediction and prevention of drug-induced liver injury (DILI) have been hindered by limited knowledge of the underlying mechanisms, in part the result of a lack of animal models. Using a newly established DILI model induced by halothane, we found increased liver damage susceptibility in interleukin 10 (IL-10) knockout (KO) mice. Extensive neutrophil infiltration and chemoattractant factor interleukin 8 (IL-8) expression in IL-10 KO mice were observed after halothane administration. The elevation of IL-8 expression was NF-κB- and P38 MAPK-dependent. In addition, increased signal transducer and activator of transcription factors (STAT) 1 and STAT3 were observed in halothane treated IL-10 KO mice. Exogenous IL-10 treatment protected susceptible mice from halothane induced liver injury (HILI). In conclusion, IL-10 deficiency increases susceptibility to HILI and increased IL-8 expression as well as neutrophil infiltration may be responsible for this phenomenon.

1. Introduction

Drug-induced liver injury (DILI) is a major safety concern in drug development and disease treatment. In the United States, DILI accounts for approximately 25% of liver failure cases seen in intensive care units [1]. Due to its association with significant patient morbidity and mortality, DILI is currently the most common cause for the withdrawal of drugs from the pharmaceutical market [2].

The prediction and prevention of DILI have been hindered by limited knowledge of the underlying mechanisms, in part the result of a lack of animal models. The only widely studied model of DILI is the acetaminophen (APAP)-induced liver injury model, particularly in mice. Recently, You et al. described a new DILI model induced by halothane, a widely used inhalation anesthetic agent [3]. Halothane is known to cause both mild and severe forms of hepatotoxicity. Mild liver injury, with transient increases in serum aminotransferases, occurs in approximately 20% of patients treated with halothane [4]. In a much smaller percentage of patients, subsequent re-exposure to halothane could cause massive hepatocyte necrosis, frequently leading to fulminant liver failure (halothane hepatitis). Interestingly, common strains of mice vary in their susceptibility to halothane toxicity. This model resembles the mild form of halothane induced liver injury (HILI) observed in approximately 20% of patients treated with halothane.

In a study by You et al. [3], the importance of the role of neutrophils in the pathogenesis of HILI was confirmed. It has

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been demonstrated that the metabolism of halothane to the reactive metabolite trifluoroacetylchloride (TFA) is essential in causing hepatotoxicity. However, the inherent ability of metabolizing halothane to TFA cannot explain the interstrain and intrastrain dependent susceptibility of guinea pigs to HILI [5,6], suggesting that other mechanisms are also important. Pro-inflammatory mediators such as inducible nitric oxide synthase (iNOS), tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6 and IL-8 were significantly upregulated in susceptible strains. These data emphasized the importance of immune malfunction in the development of HILI. However, the role of anti-inflammatory cytokines, which have been implicated as critical in the APAP-induced liver injury model [7,8], remains unknown.

In this study, we found increased susceptibility to HILI in IL-10 knockout (KO) mice over that in wildtype (WT) mice. Increased neutrophil infiltration and neutrophil chemoattractant factor interleukin 8 (IL-8) expression in IL-10 KO mice was observed after halothane administration. The protective role of IL-10 in HILI was further investigated.

2. Materials and methods

2.1. Animals and treatments

Male C57BL/6 and IL-10 KO mice (22~24 g, C57BL/6 background) were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA), and maintained in the Center for Laboratory Animal Care at the Shanghai Institutes for Biological Sciences.

To establish the HILI model, animals were injected intraperitoneally (i.p.) with halothane (30 mmol/kg, Sigma, USA) dissolved in 2 mL of olive oil.

For exogenous IL-10 treatment, recombinant mouse IL-10 (R&D, MN, USA) was given at a dosage of 10 μg/kg by intravenous injection, 30 min before and 1 h after halothane treatment.

Mice were anesthetized with pentobarbital sodium and exsanguinated by cardiac puncture, then perfused thoroughly with PBS before the livers were removed for subsequent experiments.

2.2. ALT measurement

Blood was collected by cardiac puncture after the mice were anesthetized. Blood samples were allowed to clot at 4 °C overnight before sera were prepared by centrifugation at 10,000× g for 20 min. Serum alanine aminotransferase (ALT) levels were measured using a diagnostic assay kit (Shanghai Yihua Medical Science & Technology, Shanghai, China) according to manufacturer instructions.

2.3. TUNEL labeling

Paraffin-embedded liver tissues were assayed for DNA fragmentation using a terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) reaction according to the manufacturer's instructions (Roche Molecular Biochemicals, Indianapolis, USA). Sections were then examined by light microscopy. A total of five tissue sections were analyzed for each animal.

2.4. Immunofluorescence labeling of neutrophils

Livers were dissected from mice and small pieces snap frozen in Tissue-Tek Oct Compound (Sakura, Tokyo, Japan). Cryostat sections (8 μm) were fixed in 96% ethanol for 20 min at room temperature. After washing and blocking with 5% (v/v) normal goat serum (Sigma) in PBS for 20 min, the slides were incubated overnight at 4 °C with primary PE-labeled anti-mouse Gr-1 and FITC-labeled anti-mouse Mac-1 and monoclonal antibody (MAb) (BD Bioscience, CA, USA). After washing with PBS, slides were then mounted. The numbers of Mac-1+ Gr-1+ cells were counted using a Nikon Eclipse80i microscope.

2.5. RNA extraction and reverse transcription

Total RNA was isolated from cell pellets and liver tissues using RNeasy Mini Kit (Qiagen, Hilden, Germany). Genomic DNA was removed from total RNA prior to cDNA synthesis using the RNase-free DNase Set for DNase digestion during RNA purification (Qiagen). RNA was stored at −80 °C. First-strand cDNA synthesis was performed for each RNA sample using

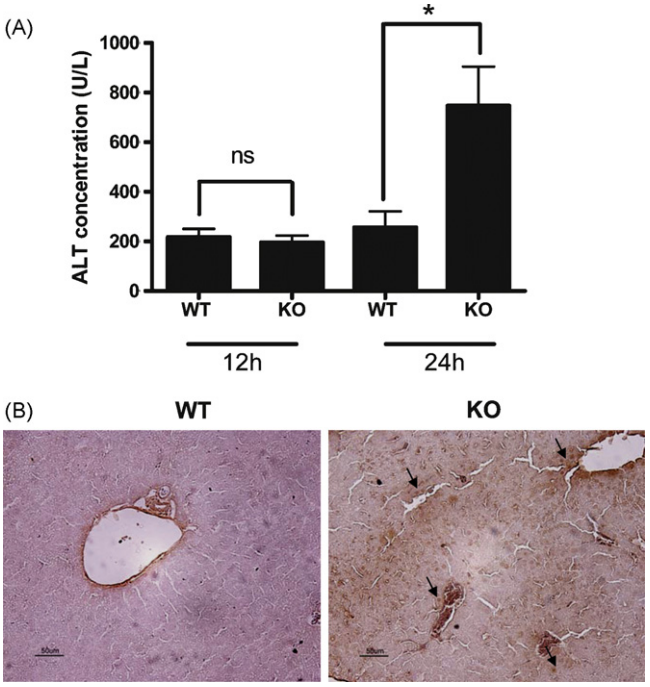

Fig. 1 – Halothane induces more severe liver injury in IL-10 KO mice. Wild type C57BL/6 mice and IL-10 KO mice (8~10 weeks old, male) were injected i.p. with 30 mmol/kg body weight of halothane. (A) Serum ALT levels were determined at various time points after halothane treatment. Results were shown as mean ± S.D. of 8–10 mice per group, and individual samples were assayed in triplicate. (B) TUNEL labeling in mouse liver tissues 24 h after halothane treatment. Arrows indicate TUNEL positive nuclei (200×, final magnification). Results are representative of five experiments. * $P < 0.05$.

Sensiscript RT Kit (Qiagen). Random hexamers were used to prime cDNA synthesis.

2.6. Real-time PCR

Gene expression of IL-8, IL-8Ra and IL-8Rb mRNA was performed by real-time PCR using SYBR Green master mix (Applied Biosystems, Foster City, USA). Thermocycler conditions included an initial holding at 50 °C for 2 min, then 95 °C for 10 min. This was followed by a two-step PCR program consisting of 95 °C for 15 s, and 60 °C for 60 s for 40 cycles. Data was collected and quantitatively analyzed on an ABI Prism 7900 sequence detection system (Applied Biosystems). The β-actin gene was used as an endogenous control to normalize for differences in the amount of total RNA in each sample. All quantities were expressed as number of fold relative to the expression of β-actin.

β-Actin: sense 5′TGTCCACCTTCCAGCAGATGT 3′,
anti-sense 5′AGCTCAGTAACAGTCCGCCTAGA 3′.
IL-8: sense 5′CACCCTCTGTCACCTGCTCAA 3′,
anti-sense 5′ATGGCGCTGAGAAGACTTGGT 3′.
IL-8Ra: sense 5′GCCGTCATTTTCTGCCTC 3′,
anti-sense 5′ATGGCGCTGAGAAGACTTGGT 3′.
IL-8Rb: sense 5′ CTGCTACTAGCCTGCATCAGCA 3′,
anti-sense 5′ CCACATGGCTATGCACACAAAC 3′.

2.7. Electrophoretic mobility shift assay (EMSA)

Nuclear extracts of liver tissues were prepared as described previously [9]. The NF-κB–binding consensus single-strand oligonucleotide (5′-AGTTGAGGGGACTTTCCCAGGC-3′) was first annealed with the complement oligonucleotide. The annealed DNA fragment was labeled with [γ-^{32}P] dATP (Amersham, Piscataway, USA) using T4 polynucleotide kinase (Promega, Madison, USA). Nuclear proteins (15 μg) were incubated with 2.5 ng of ^{32}P-labeled double-stranded oligonucleotide probes for 30 min at room temperature. The mixture was electrophoresed on 4% polyacrylamide gels with 0.5 × Tris-borate-ethylenediaminetetraacetic acid buffer at 4 °C.

2.8. Western blot analysis

Immunoblot assays were performed using whole liver extracts resolved by electrophoresis on a 10% SDS-polyacrylamide gel and transferred to PVDF membrane. β-actin, signal transducer and activator of transcription factors (STAT) 3, phospho-

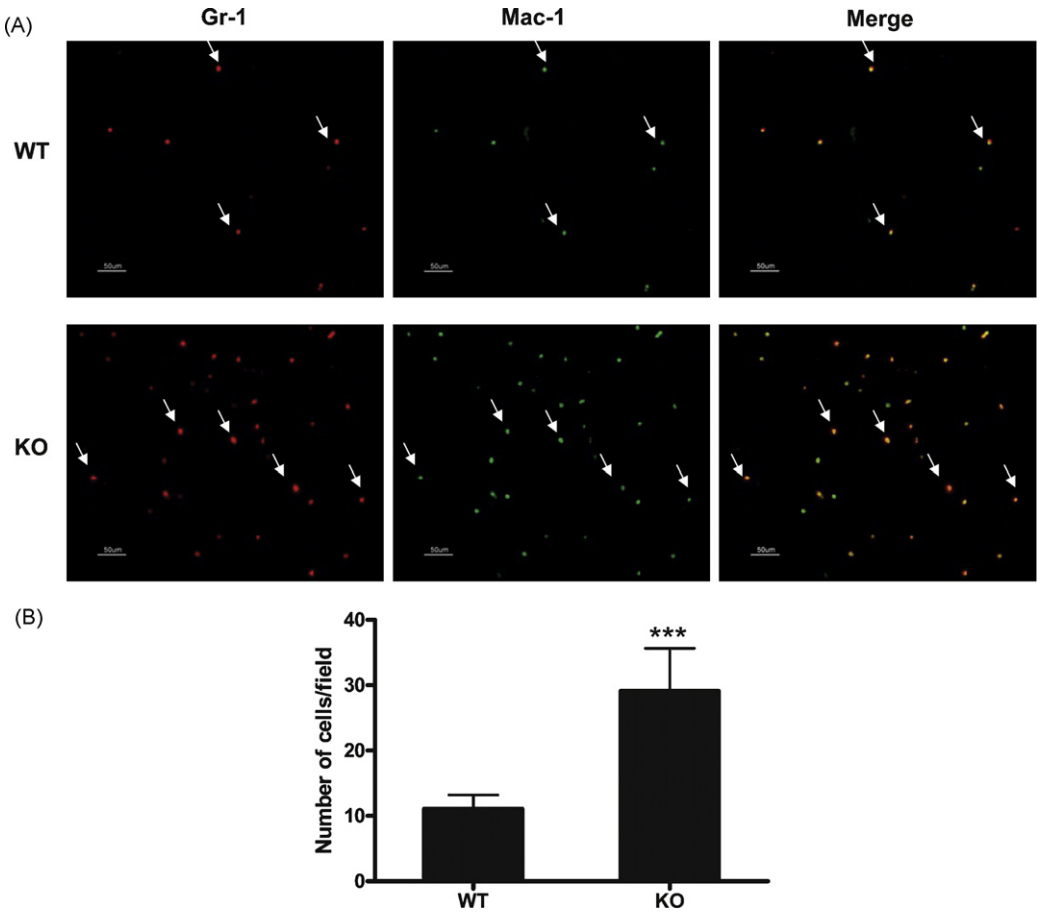

Fig. 2 – Neutrophils show increased infiltration after halothane treatment. Livers were obtained 12 h after halothane treatment of WT and IL-10 KO mice. (A) Immunofluorescence staining for the Gr-1 (red), Mac-1 (green) and merge of the two immunofluorescence for cryostat sections. Arrows indicate typical Gr-1+ Mac-1+ neutrophils. (200×, final magnification). (B) Mean number of Gr-1+ Mac-1+ neutrophils in sections. Results are representative of five experiments. *** $P < 0.001$.

STAT3 (Tyr705), STAT1 and phospho-STAT1 (Thr701) were visualized using antibodies from BD Bioscience.

2.9. Statistical analysis

All results are expressed as mean ± S.D. Statistical comparisons between two groups were made using Student's *t*-test after analysis of variance. The level of significance was set to $\alpha = 0.05$. All tests were double-sided.

3. Results

3.1. Enhanced liver injury in IL-10 KO mice after halothane administration

HILI was studied in C57BL/6 mice and IL-10 KO mice. 30 mmol/kg halothane resulted in a slight elevation of serum ALT activity in WT C57BL/6 mice as reported previously [3]. However, the serum ALT levels of IL-10 KO mice were significantly higher 24 h after halothane administration, although no significant difference was observed 12 h after halothane administration (Fig. 1A). In line with ALT results, TUNEL assay of liver sections demonstrated more abundant apoptotic nuclei in IL-10 KO mice than in WT controls (Fig. 1B).

3.2. Increased neutrophil infiltration in IL-10 KO mice after halothane administration

Since the predominant role of neutrophils in HILI has been emphasized [3], we then stained liver sections for neutrophil infiltration 12 and 24 h after halothane treatment. Twelve hours after halothane treatment, cryostat sections of liver from WT and IL-10 KO mice were stained for observation of the neutrophil markers Mac-1 and Gr-1. Fig. 2A shows Mac-1+Gr-1+ cells in the sections. When compared with controls, a significant increase (approximately three fold) in mean neutrophil number in IL-10 KO mice was observed (Fig. 2B). Similar results were observed 24 h after halothane administration (data not shown). These observations indicated that increased liver neutrophil infiltration in IL-10 KO mice may account for the increased liver injury.

3.3. Elevation of neutrophil chemokine IL-8 in IL-10 KO mice after halothane administration

We next examined which factors may contribute to neutrophil infiltration in halothane treated IL-10 KO mice. Hepatic mRNA expression of neutrophil migration and activation related factors including TNF-α, IL-8, IL-6 and iNOS were determined by quantitative real-time PCR 12 h after halothane treatment. Among these factors, the expression of IL-8, a well recognized neutrophil chemokine, was strongly elevated in IL-10 KO mice (Fig. 3A) while the levels of other factors were not significantly changed (data not shown). We also detected the expression of IL-8 receptors in both WT and IL-10 KO mice. We found increased IL-8Rb expression in IL-10 KO mice while there was no significant difference in IL-8Ra expression between the groups (Fig. 3B and C). These results suggested that increased neutrophil infiltration in IL-10 KO mice after halothane treatment may be mediated by enhanced expression of neutrophil chemoattractant factor IL-8 and its receptor IL-8Rb.

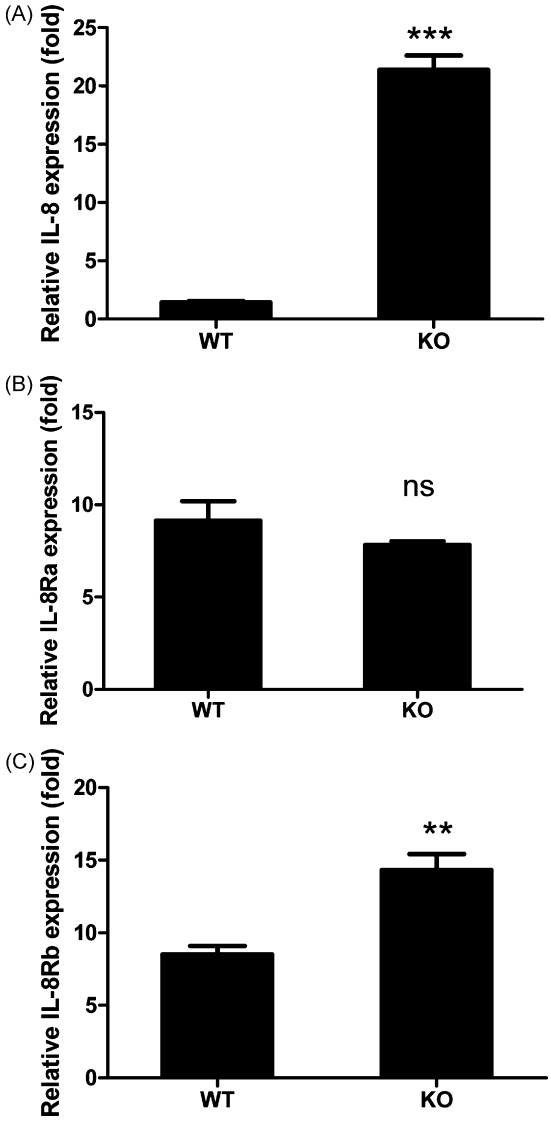

Fig. 3 – Expression of hepatic IL-8 and IL-8 receptors. WT and KO mice were treated with halothane as stated in Fig. 1. After 12 h, mice were sacrificed and livers were removed for RNA extraction. mRNA levels of (A) IL-8 (B), IL-8Ra and (C) IL-8Rb were determined using real-time PCR. Results are shown as mean ± S.D. of five mice in each group. Results are representative of four experiments. *** $P < 0.001$, ** $P < 0.01$.

3.4. P38 MAPK and NF-κB are involved in high IL-8 expression in halothane treated IL-10 KO mice

Previous studies suggested that IL-8 expression depends upon several signaling pathways, including some involving P38 MAPK and NF-κB. We first measured DNA binding activity of NF-κB using EMSA. A significant increase in DNA binding activity of NF-κB was observed in halothane treated IL-10 KO mice as compared with that in WT mice (Fig. 4A and C). Next,

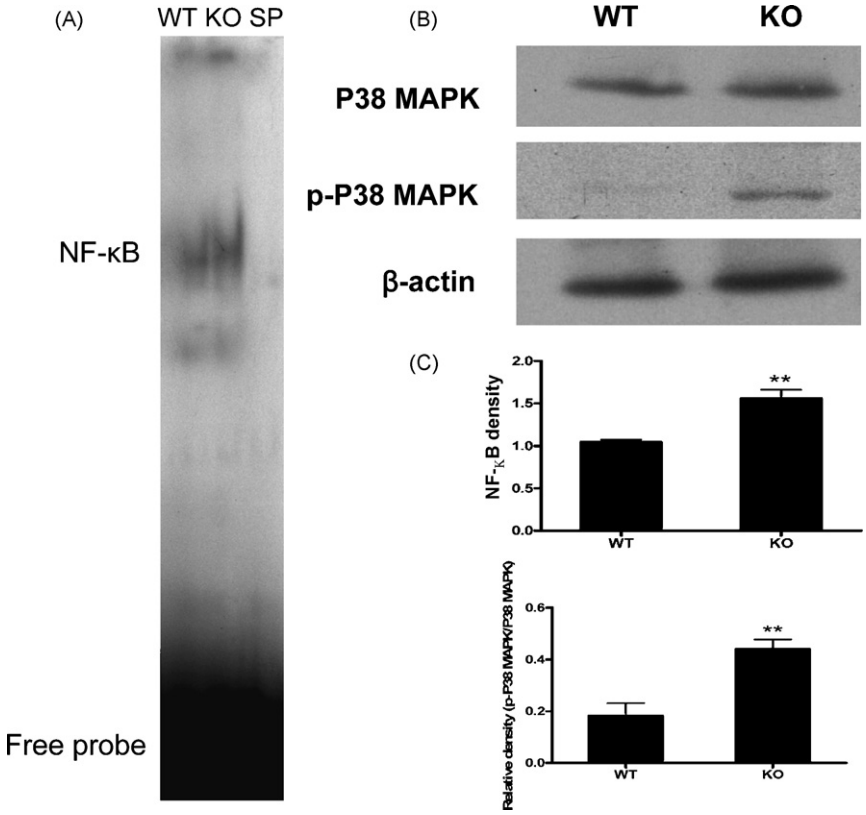

Fig. 4 – NF-κB and P38 MAPK signaling pathway measurement. WT and KO mice were treated with halothane as stated in Fig. 1. After 12 h, mice were sacrificed. (A) Liver nuclear extracts were subjected to NF-κB-specific EMSA. Specificity of the NF-κB and DNA binding reaction was confirmed by incubation of liver nuclear extracts with unlabeled ('cold') oligonucleotides for NF-κB labeled with SP. (B) Liver cell lysates were immunoblotted with anti-P38 MAPK and anti-p-P38 MAPK antibodies. (C) The band intensities or ratio of band intensities were quantified and calculated using Biorad-Image for Windows Program (Biorad GS-800 Calibrated Densitometer). Results are representative of five experiments. ** $P < 0.01$.

total P38 and phosphorylated P38 (p-P38) MAPK levels were measured using western blot analysis. p-P38 MAPK levels were significantly higher in halothane treated IL-10 KO mice than in WT mice (Fig. 4B and C). These observations showed that lack of IL-10 increased IL-8 expression in a P38 MAPK- and NF-κB-dependent manner in HILI.

3.5. *IL-10 deficiency increases STAT1 and STAT3 activation in HILI*

Activation of STATs plays many critical roles in the liver. Among various STATs, the roles of STAT1 and STAT3 in liver injury inflammation and damage have been well documented [10]. The influence of IL-10 deficiency on STAT1 and STAT3 after halothane treatment was then investigated. We found that the activation of STAT1 and STAT3 was increased in halothane treated IL-10 KO mice as compared with that in WT mice (Fig. 5A and B).

3.6. *Exogenous IL-10 protects susceptible Balb/c mice from HILI*

To confirm the protective role of IL-10 in the development of HILI, we treated halothane susceptible Balb/c mice with recombinant IL-10 (rIL-10), and measured ALT levels 24 h after halothane administration. The ALT levels of control Balb/c mice reached as high as 600U/L, similar to values published in a previous report [3]. In contrast, rIL-10 treatment significantly suppressed the elevation of ALT induced by halothane (Fig. 6). Therefore, IL-10 protected susceptible Balb/c mice from HILI.

4. Discussion

The major finding of the present study is that the absence of IL-10 enhances HILI. Increased IL-8 expression and neutrophil infiltration may account for the enhanced liver injury. Activation of NF-κB and P38 MAPK may be responsible for the enhanced IL-8 production in halothane treated IL-10 deficient mice. In addition, exogenous rIL-10 protects a susceptible mouse strain from HILI.

The liver is an organ with predominant innate immunity, playing an important role, not only in host defenses against invading microorganisms and tumor transformation, but also in liver injury and repair [11]. The involvement of cytokines in DILI has been well documented. Among these, IL-10 shows comprehensive hepatoprotective effects. IL-10 protects mice

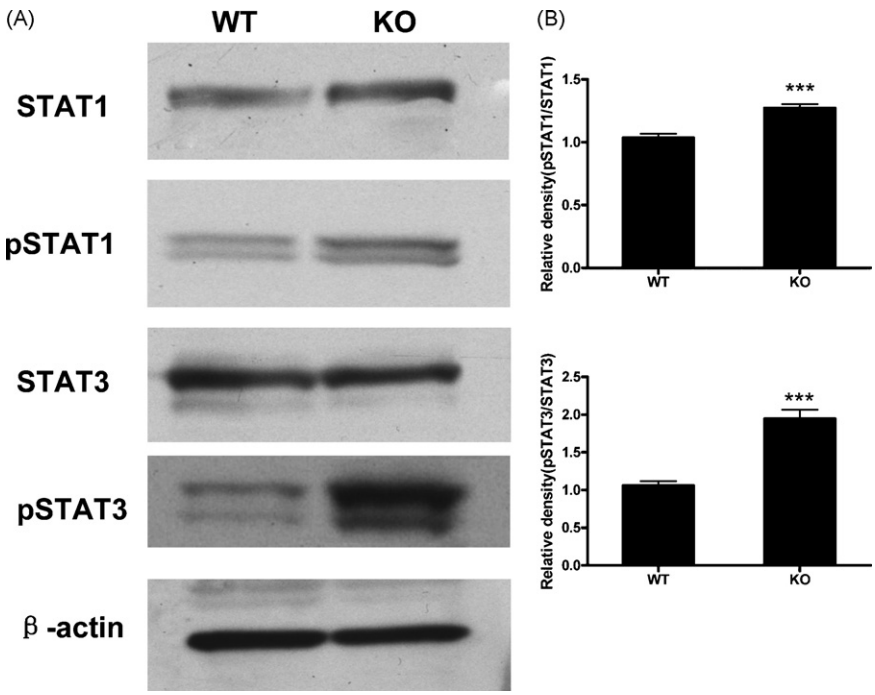

Fig. 5 – Activation of STAT1 and STAT3. WT and KO mice were treated with halothane as stated in Fig. 1. After 12 h, mice were sacrificed. (A) Liver cell lysates were immunoblotted with anti-STAT1, anti-p-STAT1, anti-STAT3 and anti-p-STAT3 antibodies. (B) The ratios of band intensities were quantified and calculated using Biorad-Image for Windows Program (Biorad GS-800 Calibrated Densitometer). Results are representative of five experiments. *** $P < 0.001$.

from a variety of liver injuries induced by Concanavalin A (ConA), APAP, LPS and CCl4 [7,12–14]. In the present study, a similar phenomenon was observed in HILI when lack of IL-10 enhanced the severity of HILI, while IL-10 treatment significantly reduced the elevation of ALT after halothane treatment.

The infiltration of neutrophils was implied in many inflammatory conditions involving liver inflammation and damage. In CCl4 induced liver injury, inflammatory infiltrates consisting mostly of neutrophils were observed in the centrilobular area [13]. The role of neutrophils in the pathogenesis of hepatic ischemia/reperfusion injury was confirmed by pretreatment with a functional inactivation monoclonal antibody against neutrophils, which significantly protected the liver from reperfusion injury and allowed a complete recovery of the hepatic ATP content [15,16]. Similar results were observed in LPS induced liver injury. Neutrophil infiltration was an early occurrence in the liver after exposure to hepatotoxic doses of LPS, while neutrophil depletion protected against liver injury from LPS [17]. In HILI, the amount of infiltrating neutrophils was correlated with the severity of liver damage in different mouse strains. Neutrophil depletion by anti-neutrophil serum reduced the severity of HILI in a susceptible strain [3]. Similarly, we found the severity of liver injury was correlated with the number of infiltrating neutrophils in the liver, thus confirming the pathogenic role played by neutrophils in HILI.

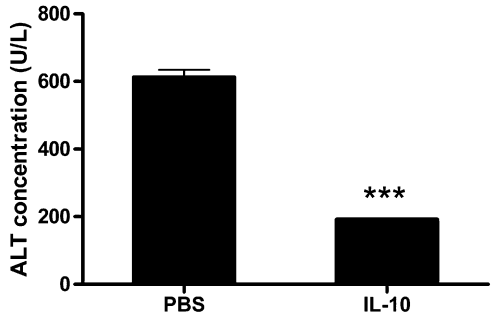

Fig. 6 – IL-10 protects susceptible Balb/c mice from HILI. PBS or rIL-10 treated Balb/c mice (8∼10 weeks old, male) were injected i.p. with 30 mmol/kg body weight of halothane. Serum ALT levels were determined 24 h later. Results are shown as mean ± S.D. of five mice per group, and individual samples were assayed in triplicate. Results are representative of three experiments. *** $P < 0.001$.

IL-10 is a potent inhibitor of IL-8 synthesis and appears to play an auto-regulatory role [18–20]. Our observation of increased IL-8 expression in IL-10-/- mice with halothane challenge supports the notion of an inhibitory effect of IL-10 on IL-8 production. IL-8 is the first CXC chemokine that was discovered and characterized [21], and was observed to be a potent chemoattractant for neutrophils both in vitro and in vivo [22,23]. IL-8 is produced by numerous cell types including monocytes, neutrophils, basophils, eosinophils, etc [24]. IL-8 exerts its effects upon neutrophils via two different cell surface receptors initially named IL-8 receptors type A and B (IL-8Ra and IL-8Rb) [25,26]. In terms of chemotaxis, there appears to be a division of labor amongst the receptors. IL-8Rb responds to picomolar concentrations of IL-8 and is thought to initiate the migration of neutrophils distant from the site of inflammation, whilst IL-8Ra mediates a signal in response to

higher concentrations of IL-8 such as those encountered at the inflammatory site [27]. Our observation of the upregulation of IL-8Rb in the liver suggests a predominant role for IL-8Rb in halothane induced neutrophil infiltration and liver injury.

Several signaling pathways regulate IL-8 production. The promoter element of IL-8 contains an NF-κB element that is required for activation in all cell types studied [28]. Chromatin immunoprecipitation shows that binding of NF-κB to the endogenous IL-8 promoter and subsequent recruitment of RNA polymerase II occur quickly, within one-half hour of stimulation [29]. In addition, P38 MAPK significantly contributes to IL-8 expression as proven by observations made in small molecule inhibitor treatment assays and in P38-deficient mice [29,30]. The P38 MAPK pathway regulates a specific, post-transcriptional step in IL-8 gene expression [29]. Previous studies have indicated that IL-10 suppresses NF-κB activation in hepatic ischemia/reperfusion injury [14]. The inhibitory effects upon P38 MAPK activation by IL-10 were also reported [31]. In the present study, the absence of IL-10 increased NF-κB and P38 MAPK activity after halothane administration, indicating that the increased IL-8 production may be mediated by enhanced NF-κB and P38 MAPK activation.

The activation of STATs has been observed in various liver disease models. In particular, STAT1 and STAT3 are considered most important in liver injury and repair [10]. STAT1 is activated in ConA-induced hepatitis (CIH) and LPS/D-galactosamine-induced liver damage. Disruption of the STAT1 gene abolishes the diseases, suggesting that STAT1 plays an essential role in hepatic inflammation and injury [32–34]. STAT3 has been shown to play key roles in acute phase response, protecting against liver injury, and promoting liver regeneration [32,35]. Previous studies have suggested that IL-10 inhibits the activation of STAT1 in the liver, by inducing SOCS2 and SOCS3 [36]. Here we found the activation of STAT1 and STAT3 in halothane treated IL-10 KO mice, suggesting the important roles they play in the disease. However, further studies should be carried out to clarify the detailed functions of STAT1 and STAT3 in the pathogenesis of HILI.

In summary, we report increased susceptibility to HILI in IL-10 KO mice. In addition, we find upregulation of neutrophil chemokine IL-8 and IL-8Rb after halothane treatment in IL-10 KO mice. Activation of NF-κB and P38 MAPK pathways might account for the IL-8 upregulation in these mice. Furthermore, exogenous IL-10 administration reduces the severity of HILI in susceptible Balb/c mice, further confirming the protective role of IL-10 in HILI.

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REFERENCES

[1] Ibanez L, Perez E, Vidal X, Laporte JR. Prospective surveillance of acute serious liver disease unrelated to infectious, obstructive, or metabolic diseases: epidemiological and clinical features, and exposure to drugs. J Hepatol 2002;37:592–600.
[2] Temple RJ, Himmel MH. Safety of newly approved drugs: implications for prescribing. Jama 2002;287:2273–5.
[3] You Q, Cheng L, Reilly TP, Wegmann D, Ju C. Role of neutrophils in a mouse model of halothane-induced liver injury. Hepatology 2006;44:1421–31.
[4] Wright R, Eade OE, Chisholm M, Hawksley M, Lloyd B, Moles TM, et al. Controlled prospective study of the effect on liver function of multiple exposures to halothane. Lancet 1975;1:817–20.
[5] Farrell GC, Frost L, Tapner M, Field J, Weltman M, Mahoney J. Halothane-induced liver injury in guinea-pigs: importance of cytochrome P450 enzyme activity and hepatic blood flow. J Gastroenterol Hepatol 1996;11:594–601.
[6] Lind RC, Gandolfi AJ, Hall PD. Age and gender influence halothane-associated hepatotoxicity in strain 13 guinea pigs. Anesthesiology 1989;71:878–84.
[7] Bourdi M, Masubuchi Y, Reilly TP, Amouzadeh HR, Martin JL, George JW, et al. Protection against acetaminophen-induced liver injury and lethality by interleukin 10: role of inducible nitric oxide synthase. Hepatology 2002;35:289–98.
[8] Bourdi M, Eiras DP, Holt MP, Webster MR, Reilly TP, Welch KD, et al. Role of IL-6 in an IL-10 and IL-4 double knockout mouse model uniquely susceptible to acetaminophen-induced liver injury. Chem Res Toxicol 2007;20:208–16.
[9] Nagaki M, Naiki T, Brenner DA, Osawa Y, Imose M, Hayashi H, et al. Tumor necrosis factor alpha prevents tumor necrosis factor receptor-mediated mouse hepatocyte apoptosis, but not fas-mediated apoptosis: role of nuclear factor-kappaB. Hepatology 2000;32:1272–9.
[10] Gao B. Cytokines, STATs and liver disease. Cell Mol Immunol 2005;2:92–100.
[11] Gao B, Jeong WI, Tian Z. Liver: An organ with predominant innate immunity. Hepatology 2008;47:729–36.
[12] Santucci L, Fiorucci S, Chiorean M, Brunori PM, Di Matteo FM, Sidoni A, et al. Interleukin 10 reduces lethality and hepatic injury induced by lipopolysaccharide in galactosamine-sensitized mice. Gastroenterology 1996;111:736–44.
[13] Louis H, Van Laethem JL, Wu W, Quertinmont E, Degraef C, Van den Berg K, et al. Interleukin-10 controls neutrophilic infiltration, hepatocyte proliferation, and liver fibrosis induced by carbon tetrachloride in mice. Hepatology 1998;28:1607–15.
[14] Yoshidome H, Kato A, Edwards MJ, Lentsch AB. Interleukin-10 suppresses hepatic ischemia/reperfusion injury in mice: implications of a central role for nuclear factor kappaB. Hepatology 1999;30:203–8.
[15] Jaeschke H, Farhood A, Smith CW. Neutrophils contribute to ischemia/reperfusion injury in rat liver in vivo. Faseb J 1990;4:3355–9.
[16] Jaeschke H, Farhood A, Bautista AP, Spolarics Z, Spitzer JJ, Smith CW. Functional inactivation of neutrophils with a Mac-1 (CD11b/CD18) monoclonal antibody protects against ischemia-reperfusion injury in rat liver. Hepatology 1993;17:915–23.
[17] Hewett JA, Schultze AE, VanCise S, Roth RA. Neutrophil depletion protects against liver injury from bacterial endotoxin. Lab Invest 1992;66:347–61.
[18] Chen CC, Manning AM. TGF-beta 1, IL-10 and IL-4 differentially modulate the cytokine-induced expression of IL-6 and IL-8 in human endothelial cells. Cytokine 1996;8:58–65.
[19] de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991;174:1209–20.

[20] Ehrlich LC, Hu S, Peterson PK, Chao CC. IL-10 down-regulates human microglial IL-8 by inhibition of NF-kappaB activation. Neuroreport 1998;9:1723–6.
[21] Yoshimura T, Matsushima K, Tanaka S, Robinson EA, Appella E, Oppenheim JJ, et al. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. Proc Natl Acad Sci USA 1987;84:9233–7.
[22] Beaubien BC, Collins PD, Jose PJ, Totty NF, Hsuan J, Waterfield MD, et al. A novel neutrophil chemoattractant generated during an inflammatory reaction in the rabbit peritoneal cavity in vivo. Purification, partial amino acid sequence and structural relationship to interleukin 8. Biochem J 1990;271:797–801.
[23] Jose PJ, Collins PD, Perkins JA, Beaubien BC, Totty NF, Waterfield MD, et al. Identification of a second neutrophil-chemoattractant cytokine generated during an inflammatory reaction in the rabbit peritoneal cavity in vivo. Purification, partial amino acid sequence and structural relationship to melanoma-growth-stimulatory activity. Biochem J 1991;278(Pt 2):493–7.
[24] Pease JE, Sabroe I. The role of interleukin-8 and its receptors in inflammatory lung disease: implications for therapy. Am J Respir Med 2002;1:19–25.
[25] Murphy PM, Tiffany HL. Cloning of complementary DNA encoding a functional human interleukin-8 receptor. Science 1991;253:1280–3.
[26] Holmes WE, Lee J, Kuang WJ, Rice GC, Wood WI. Structure and functional expression of a human interleukin-8 receptor. Science 1991;253:1278–80.
[27] Chuntharapai A, Kim KJ. Regulation of the expression of IL-8 receptor A/B by IL-8: possible functions of each receptor. J Immunol 1995;155:2587–94.
[28] Roebuck KA. Regulation of interleukin-8 gene expression. J Interferon Cytokine Res 1999;19:429–38.
[29] Hoffmann E, Dittrich-Breiholz O, Holtmann H, Kracht M. Multiple control of interleukin-8 gene expression. J Leukoc Biol 2002;72:847–55.
[30] Allen M, Svensson L, Roach M, Hambor J, McNeish J, Gabel CA. Deficiency of the stress kinase p38alpha results in embryonic lethality: characterization of the kinase dependence of stress responses of enzyme-deficient embryonic stem cells. J Exp Med 2000;191:859–70.
[31] Kontoyiannis D, Kotlyarov A, Carballo E, Alexopoulou L, Blackshear PJ, Gaestel M, et al. Interleukin-10 targets p38 MAPK to modulate ARE-dependent TNF mRNA translation and limit intestinal pathology. Embo J 2001;20:3760–70.
[32] Hong F, Jaruga B, Kim WH, Radaeva S, El-Assal ON, Tian Z, et al. Opposing roles of STAT1 and STAT3 in T cell-mediated hepatitis: regulation by SOCS. J Clin Invest 2002;110:1503–13.
[33] Siebler J, Wirtz S, Klein S, Protschka M, Blessing M, Galle PR, et al. A key pathogenic role for the STAT1/T-bet signaling pathway in T-cell-mediated liver inflammation. Hepatology 2003;38:1573–80.
[34] Kim WH, Hong F, Radaeva S, Jaruga B, Fan S, Gao B. STAT1 plays an essential role in LPS/D-galactosamine-induced liver apoptosis and injury. Am J Physiol Gastrointest Liver Physiol 2003;285:G761–8.
[35] Cressman DE, Diamond RH, Taub R. Rapid activation of the Stat3 transcription complex in liver regeneration. Hepatology 1995;21:1443–9.
[36] Shen X, Hong F, Nguyen VA, Gao B. IL-10 attenuates IFN-alpha-activated STAT1 in the liver: involvement of SOCS2 and SOCS3. FEBS Lett 2000;480:132–6.