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ORIGINAL ARTICLE

Protective effect of recombinant human IL-1Ra on CCl_4-induced acute liver injury in mice

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Abstract

AIM: To evaluate the effects of positive regulation of recombinant human interleukin 1 receptor antagonist (rhIL-1Ra) on hepatic tissue recovery in acute liver injury in mice induced by carbon tetrachloride (CCl_4).

METHODS: Acute liver damage was induced by injecting 8-wk-old mice with CCl_4 1 mL/kg (1:3 dilution in corn oil) intraperitoneally (ip). Survival after liver failure was assessed by injecting 8-wk-old mice with a lethal dose of CCl_4 2.6 mL/kg (1:1 dilution in corn oil) ip. Mice were subcutaneously injected with 1 mg/kg recombinant human IL-1Ra twice a day after CCl_4 treatment for 5 d. Serum alanine amino transferase (ALT) and aspartate aminotransferase (AST) levels were determined with a commercial assay kit. Serum IL-1β, IL-1Ra levels were measured by enzyme-linked immunosorbent assay kit. Quantitative real-time polymerase chain reaction was used to determine liver IL-1β, IL-1Ra and IL-6 expression during CCl_4-induced acute liver injury. Liver sections were stained with hematoxylin-eosin. A histology-injury grading system was used to evaluate the degree of necrosis after acute liver injury. Proliferating cell nuclear antigen (PCNA) staining was used to evaluate the role of rhIL-1Ra in promoting hepatocyte proliferation.

RESULTS: Quantitative analysis showed a higher level of IL-6 mRNA expression and reduced serum AST and ALT levels in the livers of the rhIL-1Ra-treated group at the early phase of CCl_4-induced acute liver injury. Histological examination indicated a decrease in centrilobular necrotic areas in mice treated with rhIL-1Ra, and a novel role of rhIL-1Ra in promoting hepatocyte proliferation was also supported by an increase of PCNA staining. All these results, accompanied by a strong survival benefit in rhIL-1Ra-treated *vs* PBS-treated groups, demonstrated that rhIL-1Ra administration ameliorated the histological damage and accelerated the regeneration and recovery process of the liver.

CONCLUSION: rhIL-1Ra could be further developed as a novel therapeutic agent for the treatment of acute liver injury because of its ability to reduce hepatocellular damage and facilitate liver regeneration.

Key words: Recombinant human interleukin 1 receptor antagonist; Carbon tetrachloride; Liver injury; Hepatocyte proliferation

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INTRODUCTION

The interleukin (IL)-1 family includes the structurally related proteins IL-1α, IL-1β, and interleukin 1 receptor antagonist (IL-1Ra) that bind to the same cell surface receptor. However, IL-1Ra functions as a competitive inhibitor of IL-1α and IL-1β[1]. IL-1α and IL-1β play a key role in inflammation[2]; in response to stimuli such as inflammatory agents, infections, or microbial endotoxins, a dramatic increase of IL-1 mediated by macrophages and various other cell types is seen[3,4]. IL-1Ra is a naturally occurring anti-inflammatory protein; it competitively blocks the binding of IL-1α and IL-1β to type I IL-1 receptor, but exerts no agonist activity[5,6]. IL-1Ra has been shown to inhibit the effects of IL-1 both *in vitro* and *in vivo*, and as an acute-phase protein, it can reduce the severity of several animal models of inflammatory disease[7]. Serum levels of IL-1Ra can rise dramatically during different inflammatory and noninflammatory conditions[8]. Human IL-1Ra is synthesized as a 177 amino acid precursor that contains a 25 amino acid signal sequence plus a 152 amino acid mature region[9,10].

Acute administration of carbon tetrachloride (CCl_4) is used to establish an experimental model of severe hepatocellular damage involving generation of oxidative stress and recruitment of inflammatory cells[11-13], which is reported to induce liver architectural and functional damage[14-16]. Liver regeneration involves a complex regulated response to CCl_4-induced acute liver injury[17,18].

In this study, we aimed to examine the effects of IL-1Ra as an acute phase protein in reducing hepatic injury and accelerating hepatocyte proliferation following CCl_4 administration. Although the anti-inflammatory effect of IL-1Ra has been described, the contribution of this cytokine to protect from the liver injury remains unclear. The present findings indicate that IL-1Ra is a critical factor that shows a potent antihepatotoxic activity in recovery of hepatocellular necrosis and in acceleration of liver regeneration during injury. IL-1Ra could provide a novel therapeutic approach by stimulating liver regeneration.

MATERIALS AND METHODS

Animals

Procedures were performed in male C57BL/6 mice (purchased from SLC Shanghai, China) 8 wk after birth, maintained in a conventional clean facility and in accordance with the National Animal Care and Use Committee.

Cytokine and reagents

Recombinant human IL-1Ra (rhIL-1Ra) was obtained from Dr Wei Han's Laboratory at the School of Pharmacy, Shanghai Jiao Tong University. Endotoxin level of the rhIL-1Ra was under 0.1 EU/μg. CCl_4 was purchased from Sigma, USA.

Acute liver injury and lethal dose performance

Acute liver injury was induced by injecting 8-wk-old mice with CCl_4 1 mL/kg (1:3 dilution in corn oil) intraperitoneally (ip). A lethal dose was administered by injecting 8-wk-old mice with CCl_4 2.6 mL/kg (1:1 dilution in corn oil) ip.

rhIL-1Ra and PBS injection

Mice were subcutaneously injected with 1 mg/kg rhIL-1Ra (diluted to 0.5 mg/mL with PBS) twice a day after CCl_4 administration for 5 d because human and murine IL-1Ra show an overall homology of 77% with no apparent species specificity[19]. The first rhIL-1Ra injection was performed at 1 h after CCl_4 treatment. The control group of mice was subcutaneously injected with the same volume of PBS.

Serum aspartate aminotransferase and alanine amino transferase

Serum aspartate aminotransferase (AST) and alanine amino transferase (ALT) levels were determined with a commercial assay kit (Nanjing Jiancheng Biological Technology, Inc., China). Enzyme activities were expressed as an international unit per liter (IU/L).

Enzyme-linked immunosorbent assay

Serum IL-1β and IL-1Ra level were measured by enzyme-linked immunosorbent assay (ELISA) kit (R&D system, Minneapolis, MN, USA) according to the manufacturer's instructions.

Histology-injury grading

Formalin-fixed, paraffin-embedded liver sections were stained with hematoxylin-eosin for the histological investigations. To evaluate the degree of necrosis after acute liver injury we created an injury grading score (Grade I - IV) based on severity of necrotic lesions in the liver parenchyma (Table 1).

Proliferating cell nuclear antigen staining

For proliferating cell nuclear antigen (PCNA) immunohistochemical staining, de-paraffinized sections of liver blocks were used. Liver tissues were fixed for 24 h in neutral buffered formalin, processed routinely and embedded in wax. Immunohistochemical staining was performed as previously described[16]. The sectioned liver tissues were stained using a mouse monoclonal antibody against PCNA and the SABC Staining Kit (Wuhan Boster Biological Technology, Wuhan, China) according to manufac-

Table 1 Injury grade

No. of mice	Day + 2[1]	Day + 3[1]	Day + 5[1]	Day + 7[1]
Group A (rhIL-1Ra)				
1	Ⅲ	Ⅱ	Ⅰ	0
2	Ⅲ-Ⅳ	Ⅰ	Ⅰ	0
3	Ⅲ	Ⅰ	0	0
4	Ⅲ	Ⅱ	Ⅰ	0
5	Ⅲ-Ⅳ	Ⅰ	0	Ⅰ
6	Ⅳ	Ⅰ	0	0
Group B (PBS)				
1	Ⅲ-Ⅳ	Ⅱ-Ⅲ	Ⅰ-Ⅱ	0
2	Ⅳ	Ⅲ	Ⅱ	Ⅰ
3	Ⅲ-Ⅳ	Ⅲ	Ⅱ	0
4	Ⅳ	Ⅱ-Ⅲ	Ⅱ	0
5	Ⅳ	Ⅲ	Ⅱ	Ⅰ
6	Ⅲ-Ⅳ	Ⅲ	Ⅰ	Ⅰ

[1]Day of sacrifice after CCl_4 1 mL/kg (1:3 dilution in corn oil) ip treatment. Injury grading with respect to severity of necrosis in liver parenchyma: Grade 0: Normal histology; Grade Ⅰ: Presence of degenerated hepatocytes with only rare foci of necrosis; Grade Ⅱ: Mild centrilobular necrosis around the central vein, occupying only a part of Rappaport's zone Ⅲ; Grade Ⅲ: Established necrosis limited to zone Ⅲ; Grade Ⅳ: Extensive, confluent centrilobular necrosis involving Rappaport's zone Ⅲ and Ⅱ. rhIL-1Ra: Recombinant human interleukin 1 receptor antagonist.

turer's protocol, then subjected to photomicroscopic observation (NIS-Elements Basic Research, Nikon Eclipse 50i, Kanagawa, Japan).

Quantitative real-time polymerase chain reaction

Total RNA was obtained from the liver of mice and was prepared using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA). The quantification and qualification of RNA were determined by UV absorbance and electrophoresis in 1.2% agarose. RNA quality was satisfied when the 28s rRNA banding was twice the intensity of the 18s rRNA without significant smearing of the rRNA bands. Quantitative real-time polymerase chain reaction (RT-PCR) reactions were performed with the MJ chromo 4 RT-PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA). Specific primers were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA, USA) and their sequences are listed as follows: IL-1β (sense) 5'TGAGCACCTTCTTTTCCTTC3', IL-1β (anti-sense) 5'GTTCATCTCGGAGCCTGTAG3'; IL-1Ra (sense) 5'AGACCTTGTGTCCTGTTTAGC3', IL-1Ra (anti-sense) 5'GGTCAATAGGCACCATGTCT3'; IL-6 (sense) 5'CCACTCCCAACAGACCTGTCTATAC3', IL-6 (anti-sense) 5'CACAACTCTTTTCTCATTTCCACGA3'; β-actin (sense) 5'AGCCTTCCTTCTTGGGTATG3', β-actin (anti-sense) 5'GTGTTGGCATAGAGGTCTTTAC3'. For the RT-PCR reaction, the following procedure was followed. Total RNA (5 μg) was used as a template for synthesizing the first-strand of cDNA with M-MuLV reverse transcriptase (MBI Fermentas, Vilnius, Lithuania) in a 20 mL reaction volume. PCR reactions were carried out by adding 100 × diluted cDNAs, 100 nmol/L of each primer, and SYB Premix Ex *Taq* (TaKaRa, Dalian, China) in 20 μL reactions. PCR conditions were optimized using Opticon monitor 3 software (Bio-Rad Laboratories) and involved the following steps: 95℃ for 5 min, 1 cycle; 95℃ for 5 s and 60℃ for 30 s, 40 cycles. Final data were analyzed with Opticon monitor 3 software (Bio-Rad Laboratories), presented as ratios to β-actin for each time point.

Statistical analysis

Results are expressed as mean ± SD. Statistically significant differences over time in the same treatment group, or among different treatment groups at a single time point, were determined by Student's *t* test. $P < 0.05$ was considered to be statistically significant. Results from survival experiments were analyzed using the log-rank test and expressed as Kaplan-Meier survival curves.

RESULTS

IL-1β, IL-1Ra and IL-6 expression during CCl_4-induced acute liver injury

Expression of IL-1β mRNA decreased in the first 12 h, and reached its lowest point at day 1.5 (Figure 1A). In contrast, expression of IL-1Ra mRNA was rapidly induced and reached a peak within 12 h following 1 mL/kg CCl_4 administration (Figure 1B). Serum level of IL-1β did not increase so rapidly (Figure 1C). We found that serum IL-1Ra enhanced markedly after CCl_4 administration (Figure 1D), induced by generation of oxidative stress and recruitment of inflammatory cells. We confirmed that an adequate ratio of serum IL-1Ra to IL-1 was crucial to the recovery of liver injury (Figure 1E), and we found that the ratio reached a peak at day 1.5 after CCl_4 administration. Furthermore, the expression of IL-6 mRNA was also stimulated by excessive treatment with rhIL-1Ra (Figure 1F).

rhIL-1Ra protects mice from acute hepatocellular damage

CCl_4-induced acute liver injury that results in a quantifiable liver damage recovers naturally within 7 d, as mice sacrificed 7 d after CCl_4 injection appear with normal liver histology. To confirm the role of rhIL-1Ra in protecting from hepatic damage, we investigated the effect of rhIL-1Ra on CCl_4-induced acute liver injury. Mice were subcutaneous injected with rhIL-1Ra and PBS after CCl_4 administration. Animals were sacrificed 1, 3, 5 and 7 d after CCl_4 administration for AST and ALT determination. The serum level of ALT or AST rapidly elevated to reach a peak at day 1 then decreased thereafter in PBS-treated control mice, while rhIL-1Ra treatment significantly inhibited the elevation of ALT and AST from day 1 to day 5 (Figure 2A and B). The reduction of serum AST and ALT indicated that rhIL-1Ra has a direct protective effect on hepatocytes. To evaluate the effect of rhIL-1Ra on hepatocellular necrosis and inflammation, histological changes in the liver after CCl_4 administration with or without rhIL-1Ra treatment were examined by histology-injury grading (Table 1). Liver sections from PBS-treated animals showed hepatocellular necrosis and inflammation at day 3 after CCl_4 administration; in contrast, liver sections from the rhIL-1Ra-treated group demonstrated only mild hepatocellular necrosis and in-

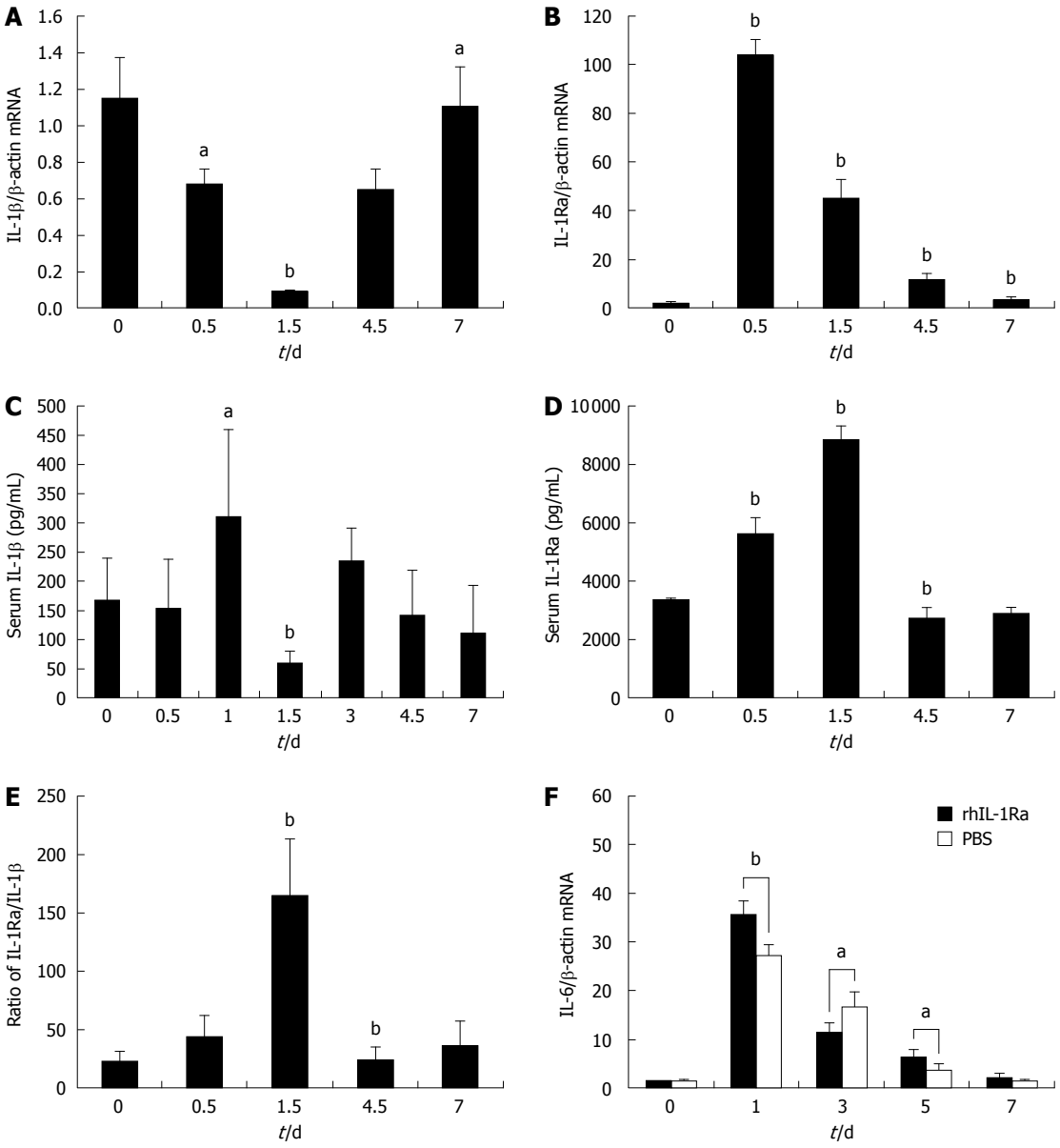


Figure 1 Interleukin (IL)-1β, interleukin 1 receptor antagonist (IL-1Ra) and IL-6 expression after carbon tetrachloride (CCl_4) (1 mL/kg) administration. A: Quantification of IL-1β mRNA levels; B: IL-1Ra mRNA levels; C: Serum IL-1β; D: Serum IL-1Ra; E: Ratio of serum level of IL-1Ra to IL-1β; F: Quantification of IL-6 mRNA levels of the livers treated with recombinant human IL-1Ra (rhIL-1Ra) or PBS. $^aP < 0.05$, $^bP < 0.01$.

flammation was dramatically decreased. We found the necrotic areas were significantly diminished around the central vein (Figure 2D and E) and centrilobular regions (Figure 2C) in rhIL-1Ra-treated mice at day 3. However, rhIL-1Ra did not cause any liver injury to healthy mice (Figure 2F). These findings indicate that rhIL-1Ra has a potent anti-hepatotoxic activity in reducing hepatocellular necrosis around the central vein.

rhIL-1Ra promotes hepatocyte proliferation from an early phase

We also investigated the proliferation of hepatocytes by immunostaining of PCNA in sections of liver tissue at days 2 and 3. Our PCNA staining confirmed that the number of positive cells increased sharply at day 2 (Figure 3C). Great numbers of hepatocytes (Figure 3E) could be detected in the liver sections of rhIL-1Ra-treated mice at day 3, which demonstrated that rhIL-1Ra significantly increased the number of $PCNA^+$ cells. In contrast, PBS-treated mice showed a much fewer number of $PCNA^+$ cells (Figure 3D and F). In our study, we also confirmed rhIL-1Ra was unable to induce hepatocyte proliferation (Figure 3A and B) in normal mice. Numbers of $PCNA^+$ cells (Figure 3G) in at least 12 mm^2 tissue sections were counted for each mouse, which showed that mice receiving rhIL-1Ra after CCl_4 injection gained the potent advantage of accelerating hepatocyte proliferation from an early phase.

rhIL-1Ra increases probability of survival after a lethal dose performance

In dose-response experiments, we found that 2.6 mL/kg

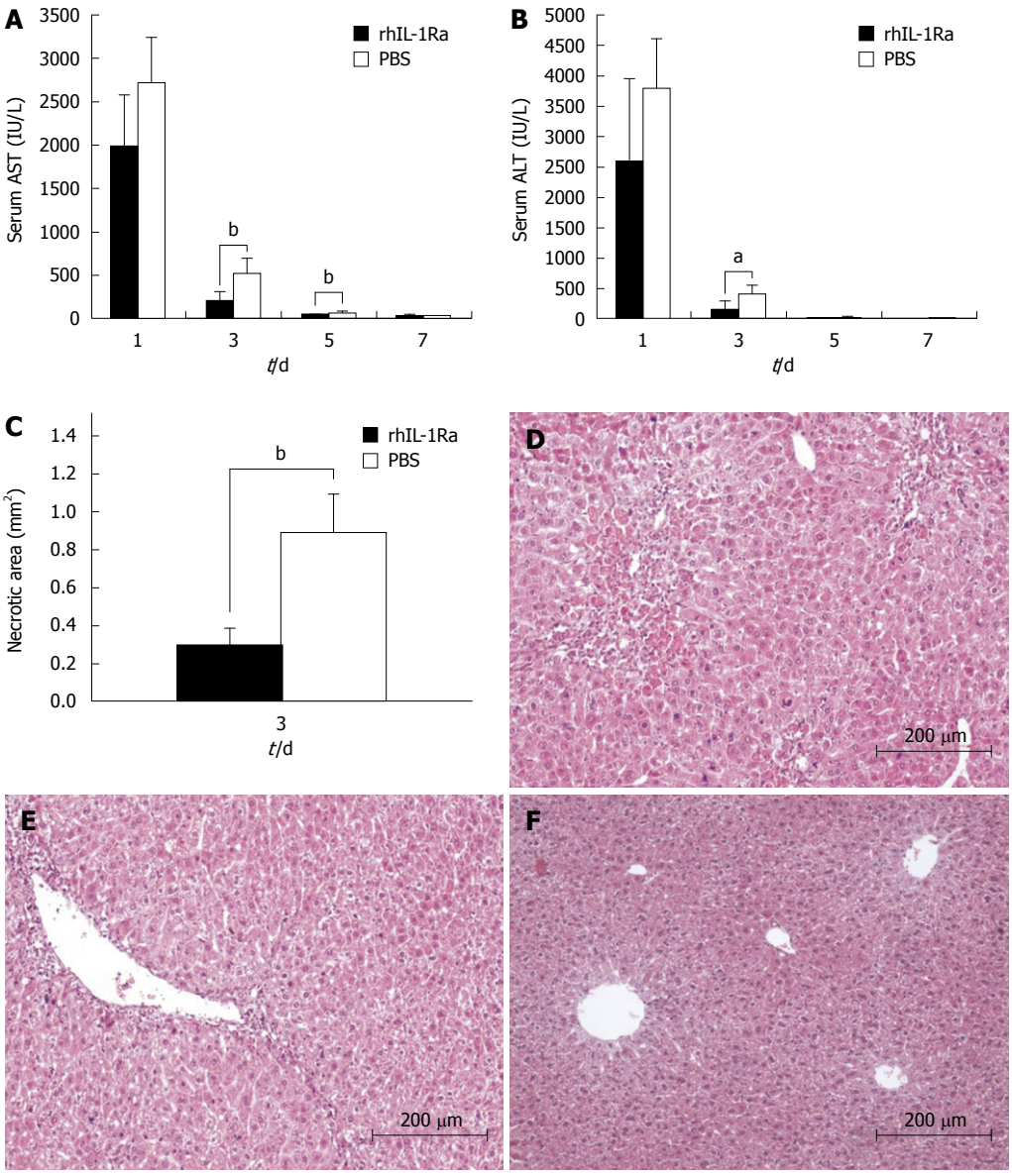
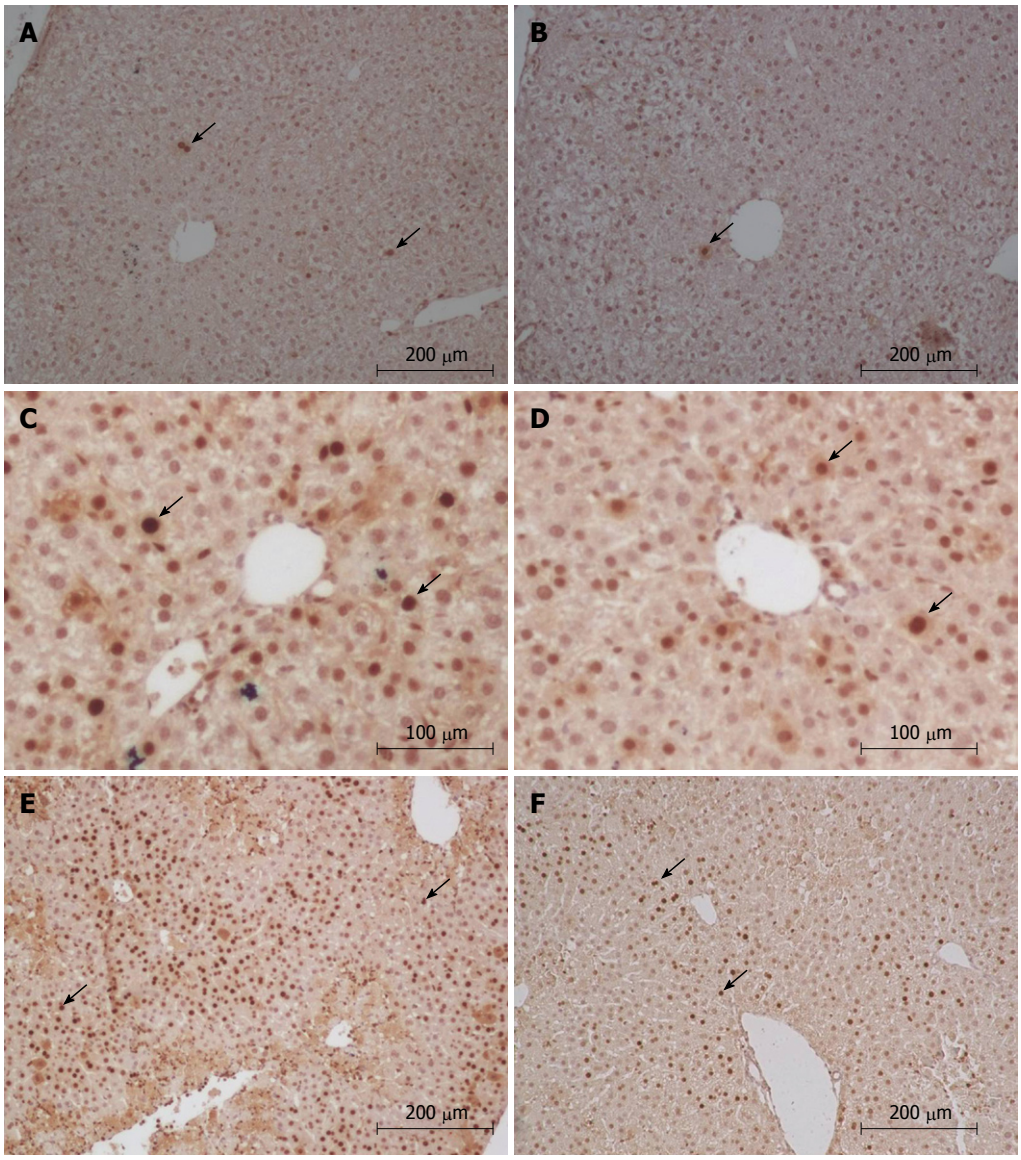


Figure 2 Acute liver injury (1 mL/kg CCl_4 administration) ± rhIL-1Ra. A: Serum aspartate aminotransferase (AST); B: Serum alanine amino transferase (ALT), with rhIL-1Ra or PBS; C: Necrotic areas. Representative findings from at least 12 mm^2 tissue sections were counted for each mouse; D-F: Hematoxylin and eosin (HE) stained liver sections; D: Group received PBS at day 3 after CCl_4 administration, shows necrosis with clusters of inflammatory cells around central vein (original magnification, × 100); E: Group received rhIL-1Ra at day 3 after CCl_4 administration, demonstrates mostly histological recovery with only inconspicuous necrosis still remaining around central vein and very few inflammatory cells are present (original magnification, × 100); F: Normal liver histology with rhIL-1Ra treatment, which shows no difference from normal liver tissue histology. $^aP < 0.05$, $^bP < 0.01$.

CCl_4 is a median lethal dose (mortality 50%, data not shown) within 24 h. rhIL-1Ra treatment in the CCl_4-induced acute liver failure model offers a survival benefit in treated mice, increasing the probability of survival significantly from 10.0% to 55.0% at day 3 after CCl_4 injection ($P = 0.006$, Figure 4).

DISCUSSION

The model of acute intoxication with CCl_4 has been used for decades to investigate the response of acute and chronic liver injury, because the elementary lesions caused by this hepatotoxin replicate those seen in most cases of human liver diseases. Pro-inflammatory cytokines such as IL-6 and IL-1β are believed to play a key role in the pathogenesis of CCl_4-induced liver injury[8,18,20,21], which make it a good model for us to study signal transduction and cell cycle events in a synchronized manner *in vivo*. The CCl_4-induced acute liver injury model is generated with 1 mL/kg dose for a typical hepatic injury, which would function as a strong regenerative stimulus. Regarding liver damage, IL-1Ra plays a critical role in the prevention of fatty liver and hypercholesterolemia under inflammatory conditions[22-24]. In this study, we investigated the severity of CCl_4-induced acute liver injury in mice after rhIL-1Ra treatment; the results demonstrate that rhIL-1Ra hypodermal injection affords protection from liver injury.



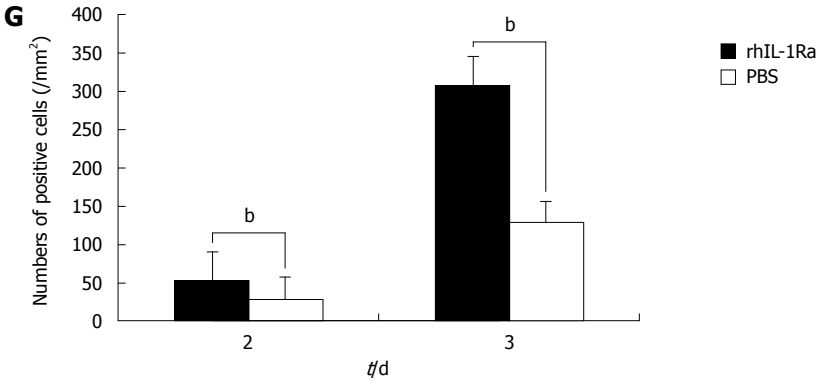


Figure 3 Immunostaining of proliferating cell nuclear antigen (PCNA) shows rhIL-1Ra promotes hepatocyte proliferation. A: Normal liver (original magnification, × 100); B: Normal liver in rhIL-1Ra treated mice (original magnification, × 100); C: Group received rhIL-1Ra at day 2 after CCl_4 administration, numerous $PCNA^+$ hepatocytes in centrilobular areas and scattered $PCNA^+$ hepatocytes at the edge of hepatocellular necrosis (original magnification, × 200); D: Group received PBS at day 2 after CCl_4 administration, few $PCNA^+$ hepatocytes in centrilobular areas at day 2 (original magnification, × 200); E: Group received rhIL-1Ra at day 3 after CCl_4 administration, numerous positive cells in centrilobular areas around central vein (original magnification, × 100); F: Group received PBS at day 3 after CCl_4 administration shows fewer numbers of positive cells (original magnification, × 100); G: Numbers of $PCNA^+$ cells after CCl_4 administration with rhIL-1Ra or PBS, at least 12 mm^2 tissue sections were counted for each mouse. Arrows point to PCNA+ hepatocytes. $^bP < 0.01$.

The balance between IL-1β and IL-1Ra has been extensively studied in a variety of experimental animal models of disease; either local overproduction of IL-1β or underproduction of IL-1Ra predisposes to the devel-

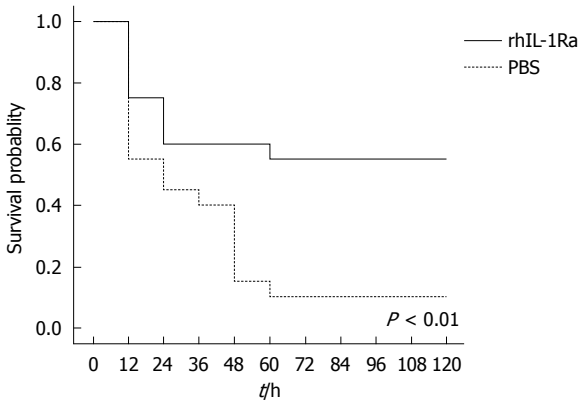


Figure 4 rhIL-IRa treatment increased probability of survival after a lethal dose administration of CCl_4 (2.6 mL/kg). Mice were administered with either PBS as a control (n = 20) or rhIL-1Ra (n = 20) twice a day for 5 d. Survivals were scored twice a day for 5 d, and the results were analyzed using the log-rank test and expressed as the Kaplan-Meier survival curves. P = 0.006.

opment of disease and the therapeutic administration of IL-1Ra is efficacious in preventing tissue damage[25]. Serum IL-1β and IL-1Ra are mediators of inflammation. Down-regulation in the release of the pro-inflammatory cytokine IL-1β and the up-regulation of its antagonist (IL-1Ra) may be a part of the inflammatory response to infection[8]. It is necessary to functionally inhibit the biologic effects of IL-1β on hepatocytes. We confirmed that liver IL-1β mRNA originally decreased in the first 12 h after CCl_4 treatment, and reached its lowest level at day 1.5. On the other hand, liver IL-1Ra mRNA increased to its highest level at day 1.5. These results show that the liver has a strong power to trigger compensatory growth mechanisms, which suppress the liver IL-1β expression. Several observations have emphasized that an adequate ratio of IL-1Ra to IL-1β is protective in inflammatory or immune liver disease[26-28]. Both serum IL-β and serum IL-1Ra were elevated after CCl_4 treatment, but serum IL-1Ra increased 100-fold greater than IL-1β at day 1.5. Serum IL-1Ra to IL-1β ratio was much higher in contrast to normal mice, which suggests a beneficial effect of IL-1Ra on CCl_4-induced acute liver injury. By analyzing the ELISA data of IL-1Ra and IL-1β, we found that serum IL-1β increased after CCl_4 treatment, which simultaneously stimulated the production of IL-1Ra as a compensatory reflection. The effects of IL-1Ra on blocking receptor binding of IL-1β during the acute-phase response may serve to suppress the inflammatory consequences of early IL-1β release after CCl_4-induced acute liver injury; with subsequent recovery, serum IL-1Ra decreased.

The level of serum aminotransferases is a very important marker to judge the severity of acute hepatic injury. After CCl_4 treatment the level of serum AST and ALT was significantly elevated, and attenuated by rhIL-1Ra in our experiment. The histological studies also showed that rhIL-1Ra inhibited inflammation and necrosis, which are the most common characteristics of CCl_4-induced liver damage. These findings suggest that rhIL-1Ra protects hepatocytes from the oxidative damage caused by CCl_4, which is likely due to inhibition of the pro-inflammatory mediators.

Serum IL-1Ra can rise dramatically during different inflammatory and non-inflammatory conditions such as sepsis[29] and chronic rheumatic diseases[30-32]. In addition, it plays a crucial role in regulating IL-1 signaling in various inflammatory states. IL-1Ra deficiency has been associated with major metabolic dysfunctions[33,34]. Serum levels of IL-1Ra were found to correlate with serum IL-6 concentrations[35], and administration of either IL-1 or IL-6 to patients increased the circulating levels of IL-1Ra[36,37]. Liver is a recognized target organ for pro-inflammatory cytokines such as TNFα, IL-1 and IL-6[38,39]. Regarding liver regeneration, IL-6 is thought to result in enhanced transcription, triggering hepatocytes to leave their quiescent state (G0) and enter a prereplicative phase (G1). Expression of IL-6 appears to be essential for the priming of hepatocytes[40,41]. Previous studies showed that IL-1Ra production was enhanced by IL-1β, and increasing IL-1β and IL-6 exhibits a strong stimulatory effect on the acceleration of IL-1Ra expression[8]. IL-6 is a marked signal to trigger liver regeneration, as demonstrated in a previous study[42], and in this current study, we found that the production of IL-6 could also be enhanced by excessive rhIL-1Ra treatment. *In vitro,* IL-1β inhibits hepatocyte proliferation[43]. Isoda *et al*[33] found that mRNA levels of IL-1β were significantly elevated in livers of IL-1Ra-/- mice, and liver growth is also inhibited by hepatocyte proliferation inhibitor and IL-1β[44,45]. It is of particular interest that excessive rhIL-1Ra inhibits the activity of IL-1β, which rapidly and significantly increased the number of $PCNA^+$ hepatocytes.

IL-1Ra is well tolerated clinically and has a short half-life, making it an ideal protective agent for acute hepatocellular damage and for accelerating liver regeneration. Healthy humans are the most sensitive indicators of IL-1 agonist activity: 1 ng/kg of intravenous IL-1β produces symptoms[46]. In contrast, the intravenous infusion of 10 mg/kg of IL-IRa in healthy humans, a 10 million-fold molar excess, gives no effect[47]. Histopathological studies showed that rhIL-1Ra-treated healthy mice show no changes in contrast with normal liver, and excessive rhIL-1Ra treatment could not induce hepatocellular proliferation in healthy mice. We used very classical methods to judge whether rhIL-1Ra has an effect on repairing the damage of mice CCl_4-induced acute liver injury and accelerating liver regeneration. The work indicates that rhIL-1Ra administration accelerates recovery from acute CCl_4-induced liver injury. To confirm that rhIL-1Ra dramatically prevented CCl_4-induced liver injury, a lethal dose of CCl_4 was used, and rhIL-1Ra treatment resulted in survival benefits in mice with acute hepatic failure. This work indicates that rhIL-1Ra accelerates recovery from acute CCl_4-induced liver injury and offers a strong survival advantage in injured mice.

Although additional studies are necessary to confirm this effect in humans, our findings provide a rationale

to develop new pharmacological strategies in the clinical management of patients with acute liver injury. This approach might also provide a novel therapeutic tool for regenerative liver cell therapy.

COMMENTS

Background

Interleukin 1 receptor antagonist (IL-1Ra) is a member of the interleukin 1 cytokine family and is a major anti-inflammatory cytokine. It acts as a natural inhibitor of the pro-inflammatory cytokines IL-1α and IL-1β through its action of blocking the binding of interleukin-1 to cell-surface receptors, and modulates a variety of IL-1-related immune and inflammatory responses. Although the anti-inflammatory effect of IL-1Ra has been described, the contribution of this cytokine in protecting from liver injury remains unclear.

Research frontiers

IL-1Ra is well tolerated clinically and has a short half-life, making it an ideal protective agent for acute hepatocellular damage and for accelerating liver regeneration. The authors used very classical methods to judge whether recombinant human IL-1Ra has effects on repairing the damage of carbon tetrachloride (CCl_4)-induced acute liver injury in mice and on accelerating liver regeneration.

Innovations and breakthroughs

The authors aimed to examine the effects of IL-1Ra as an acute phase protein in accelerating hepatocyte proliferation following CCl_4-induced liver injury. The results indicate that IL-1Ra is a critical factor that shows a potent antihepatotoxic activity in recovery of hepatocellular necrosis and accelerates liver regeneration during injury. IL-1Ra could provide a novel therapeutic approach by stimulating liver regeneration.

Applications

Although additional studies are necessary to confirm this effect in humans, the authors' findings provide a rationale to develop new pharmacological strategies in the clinical management of patients with acute liver injury. This approach might also provide a novel therapeutic tool for regenerative liver cell therapy.

Peer review

It is interesting and well-written. I suggest the authors perform electron microscopic examination in order to investigate the ultrastructural features of the hepatocytes of normal compared to recombinant human IL-1Ra-treated mice, and CCL_4-compared to recombinant human interleukin 1 receptor antagonist-treated mice.

REFERENCES

1 **Arend WP**. Interleukin 1 receptor antagonist. A new member of the interleukin 1 family. *J Clin Invest* 1991; **88**: 1445-1451
2 **Dinarello CA**. Biologic basis for interleukin-1 in disease. *Blood* 1996; **87**: 2095-2147
3 **Dinarello CA**. Interleukin-1 and interleukin-1 antagonism. *Blood* 1991; **77**: 1627-1652
4 **Dinarello CA**. The interleukin-1 family: 10 years of discovery. *FASEB J* 1994; **8**: 1314-1325
5 **Dripps DJ**, Brandhuber BJ, Thompson RC, Eisenberg SP. Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. *J Biol Chem* 1991; **266**: 10331-10336
6 **Granowitz EV**, Clark BD, Mancilla J, Dinarello CA. Interleukin-1 receptor antagonist competitively inhibits the binding of interleukin-1 to the type II interleukin-1 receptor. *J Biol Chem* 1991; **266**: 14147-14150
7 **Cominelli F**, Nast CC, Clark BD, Schindler R, Lierena R, Eysselein VE, Thompson RC, Dinarello CA. Interleukin 1 (IL-1) gene expression, synthesis, and effect of specific IL-1 receptor blockade in rabbit immune complex colitis. *J Clin Invest* 1990; **86**: 972-980
8 **Gabay C**, Smith MF, Eidlen D, Arend WP. Interleukin 1 receptor antagonist (IL-1Ra) is an acute-phase protein. *J Clin Invest* 1997; **99**: 2930-2940
9 **Arend WP**, Malyak M, Guthridge CJ, Gabay C. Interleukin-1 receptor antagonist: role in biology. *Annu Rev Immunol* 1998; **16**: 27-55
10 **Gabay C**, Porter B, Fantuzzi G, Arend WP. Mouse IL-1 receptor antagonist isoforms: complementary DNA cloning and protein expression of intracellular isoform and tissue distribution of secreted and intracellular IL-1 receptor antagonist in vivo. *J Immunol* 1997; **159**: 5905-5913
11 **Slater TF**. Free-radical mechanisms in tissue injury. *Biochem J* 1984; **222**: 1-15
12 **Poli G**. Liver damage due to free radicals. *Br Med Bull* 1993; **49**: 604-620
13 **Johnson SJ**, Hines JE, Burt AD. Macrophage and perisinusoidal cell kinetics in acute liver injury. *J Pathol* 1992; **166**: 351-358
14 **Sasaki S**, Yoneyama H, Suzuki K, Suriki H, Aiba T, Watanabe S, Kawauchi Y, Kawachi H, Shimizu F, Matsushima K, Asakura H, Narumi S. Blockade of CXCL10 protects mice from acute colitis and enhances crypt cell survival. *Eur J Immunol* 2002; **32**: 3197-3205
15 **Morimoto J**, Yoneyama H, Shimada A, Shigihara T, Yamada S, Oikawa Y, Matsushima K, Saruta T, Narumi S. CXC chemokine ligand 10 neutralization suppresses the occurrence of diabetes in nonobese diabetic mice through enhanced beta cell proliferation without affecting insulitis. *J Immunol* 2004; **173**: 7017-7024
16 **Kalinichenko VV**, Bhattacharyya D, Zhou Y, Gusarova GA, Kim W, Shin B, Costa RH. Foxf1 +/- mice exhibit defective stellate cell activation and abnormal liver regeneration following CCl4 injury. *Hepatology* 2003; **37**: 107-117
17 **Steinman L**, Martin R, Bernard C, Conlon P, Oksenberg JR. Multiple sclerosis: deeper understanding of its pathogenesis reveals new targets for therapy. *Annu Rev Neurosci* 2002; **25**: 491-505
18 **Morio LA**, Chiu H, Sprowles KA, Zhou P, Heck DE, Gordon MK, Laskin DL. Distinct roles of tumor necrosis factor-alpha and nitric oxide in acute liver injury induced by carbon tetrachloride in mice. *Toxicol Appl Pharmacol* 2001; **172**: 44-51
19 **Dana MR**, Yamada J, Streilein JW. Topical interleukin 1 receptor antagonist promotes corneal transplant survival. *Transplantation* 1997; **63**: 1501-1507
20 **DeCicco LA**, Rikans LE, Tutor CG, Hornbrook KR. Serum and liver concentrations of tumor necrosis factor alpha and interleukin-1beta following administration of carbon tetrachloride to male rats. *Toxicol Lett* 1998; **98**: 115-121
21 **Howren MB**, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med* 2009; **71**: 171-186
22 **Hardardóttir I**, Grünfeld C, Feingold KR. Effects of endotoxin and cytokines on lipid metabolism. *Curr Opin Lipidol* 1994; **5**: 207-215
23 **Khovidhunkit W**, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. *J Lipid Res* 2004; **45**: 1169-1196
24 **Feige U**, Hu YL, Gasser J, Campagnuolo G, Munyakazi L, Bolon B. Anti-interleukin-1 and anti-tumor necrosis factor-alpha synergistically inhibit adjuvant arthritis in Lewis rats. *Cell Mol Life Sci* 2000; **57**: 1457-1470
25 **Arend WP**. The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev* 2002; **13**: 323-340
26 **Sekiyama KD**, Yoshiba M, Thomson AW. Circulating pro-inflammatory cytokines (IL-1 beta, TNF-alpha, and IL-6) and IL-1 receptor antagonist (IL-1Ra) in fulminant hepatic failure and acute hepatitis. *Clin Exp Immunol* 1994; **98**: 71-77
27 **Gramantieri L**, Casali A, Trerè D, Gaiani S, Piscaglia F, Chieco P, Cola B, Bolondi L. Imbalance of IL-1 beta and IL-1 receptor antagonist mRNA in liver tissue from hepatitis C virus (HCV)-related chronic hepatitis. *Clin Exp Immunol*

1999; **115**: 515-520
28 **Conti F**, Breton S, Batteux F, Furlan V, Houssin D, Weill B, Calmus Y. Defective interleukin-1 receptor antagonist production is associated with resistance of acute liver graft rejection to steroid therapy. *Am J Pathol* 2000; **157**: 1685-1692
29 **Granowitz EV**, Santos AA, Poutsiaka DD, Cannon JG, Wilmore DW, Wolff SM, Dinarello CA. Production of interleukin-1-receptor antagonist during experimental endotoxaemia. *Lancet* 1991; **338**: 1423-1424
30 **Prieur AM**, Kaufmann MT, Griscelli C, Dayer JM. Specific interleukin-1 inhibitor in serum and urine of children with systemic juvenile chronic arthritis. *Lancet* 1987; **2**: 1240-1242
31 **Gabay C**, Gay-Croisier F, Roux-Lombard P, Meyer O, Maineti C, Guerne PA, Vischer T, Dayer JM. Elevated serum levels of interleukin-1 receptor antagonist in polymyositis/dermatomyositis. A biologic marker of disease activity with a possible role in the lack of acute-phase protein response. *Arthritis Rheum* 1994; **37**: 1744-1751
32 **Suzuki H**, Takemura H, Kashiwagi H. Interleukin-1 receptor antagonist in patients with active systemic lupus erythematosus. Enhanced production by monocytes and correlation with disease activity. *Arthritis Rheum* 1995; **38**: 1055-1059
33 **Isoda K**, Sawada S, Ayaori M, Matsuki T, Horai R, Kagata Y, Miyazaki K, Kusuhara M, Okazaki M, Matsubara O, Iwakura Y, Ohsuzu F. Deficiency of interleukin-1 receptor antagonist deteriorates fatty liver and cholesterol metabolism in hypercholesterolemic mice. *J Biol Chem* 2005; **280**: 7002-7009
34 **Matsuki T**, Horai R, Sudo K, Iwakura Y. IL-1 plays an important role in lipid metabolism by regulating insulin levels under physiological conditions. *J Exp Med* 2003; **198**: 877-888
35 **De Benedetti F**, Pignatti P, Massa M, Sartirana P, Ravelli A, Martini A. Circulating levels of interleukin 1 beta and of interleukin 1 receptor antagonist in systemic juvenile chronic arthritis. *Clin Exp Rheumatol* 1995; **13**: 779-784
36 **Bargetzi MJ**, Lantz M, Smith CG, Torti FM, Olsson I, Eisenberg SP, Starnes HF Jr. Interleukin-1 beta induces interleukin-1 receptor antagonist and tumor necrosis factor binding protein in humans. *Cancer Res* 1993; **53**: 4010-4013
37 **Tilg H**, Trehu E, Atkins MB, Dinarello CA, Mier JW. Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. *Blood* 1994; **83**: 113-118
38 **Michalopoulos GK**, DeFrances MC. Liver regeneration. *Science* 1997; **276**: 60-66
39 **Fausto N**, Campbell JS, Riehle KJ. Liver regeneration. *Hepatology* 2006; **43**: S45-S53
40 **Diehl AM**, Rai RM. Liver regeneration 3: Regulation of signal transduction during liver regeneration. *FASEB J* 1996; **10**: 215-227
41 **Streetz KL**, Luedde T, Manns MP, Trautwein C. Interleukin 6 and liver regeneration. *Gut* 2000; **47**: 309-312
42 **Gabay C**, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; **340**: 448-454
43 **Boulton R**, Woodman A, Calnan D, Selden C, Tam F, Hodgson H. Nonparenchymal cells from regenerating rat liver generate interleukin-1alpha and -1beta: a mechanism of negative regulation of hepatocyte proliferation. *Hepatology* 1997; **26**: 49-58
44 **LaBrecque D**. Liver regeneration: a picture emerges from the puzzle. *Am J Gastroenterol* 1994; **89**: S86-S96
45 **Friedman JM**, Chung EY, Darnell JE Jr. Gene expression during liver regeneration. *J Mol Biol* 1984; **179**: 37-53
46 **Tewari A**, Buhles WC Jr, Starnes HF Jr. Preliminary report: effects of interleukin-1 on platelet counts. *Lancet* 1990; **336**: 712-714
47 **Granowitz EV**, Porat R, Mier JW, Pribble JP, Stiles DM, Bloedow DC, Catalano MA, Wolff SM, Dinarello CA. Pharmacokinetics, safety and immunomodulatory effects of human recombinant interleukin-1 receptor antagonist in healthy humans. *Cytokine* 1992; **4**: 353-360

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