Kupffer cells modulate splenic interleukin-10 production in endotoxin-induced liver injury after partial hepatectomy

Kiyotaka Kurachi¹, Shohachi Suzuki¹,*, Takanori Sakaguchi¹, Yoshihiro Yokoi¹, Hiroyuki Konno¹, Satoshi Baba², Satoshi Nakamura¹

¹Second Department of Surgery, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu 431-3192, Japan
²Second Department of Pathology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu 431-3192, Japan

1. Background/Aims: This study was conducted to investigate the implication of Kupffer cells and the spleen in interleukin (IL)-10 production in endotoxin-induced liver injury after hepatectomy.

Methods: Rats were divided into five groups: the S group, sham-operation; the SG group, sham-operation followed by intravenous gadolinium chloride (GdCl₃; 7 mg/kg) administration to inhibit Kupffer cell function; the H group, two-thirds hepatectomy; the HG group, hepatectomy and subsequent GdCl₃ administration; the HGS group, hepatectomy and splenectomy with GdCl₃ administration. Lipopolysaccharide (1.5 mg/kg) was intravenously administered for each group 48 h after surgery.

Results: GdCl₃ treatment significantly suppressed the elevation of plasma tumor necrosis factor (TNF)-α levels by lipopolysaccharide administration with completely inhibited induction of hepatic TNF-α and IL-10 mRNAs. In the HG group, marked increase in plasma IL-10 levels associated with enhanced splenic IL-10 mRNA was observed 1 h after lipopolysaccharide administration when compared to those in the H and HGS groups. Plasma TNF-α/IL-10 ratio 1 h after lipopolysaccharide administration was higher in the order of H, HGS and HG groups. Hepatic parenchymal damage and the 24-h mortality were lowest in group HG, followed by groups HGS and H.

Conclusions: Kupffer cells after hepatectomy may aggravate endotoxin-induced liver injury via down-regulation of IL-10 production in the spleen.

© 2002 European Association for the Study of the Liver. Published by Elsevier Science B.V. All rights reserved.

Keywords: Kupffer cell; Spleen; Gadolinium chloride; Tumor necrosis factor-α; Interleukin-10; Endotoxin; Endotoxemia; Hepatectomy; Cytokine; Lipopolysaccharide

1. Introduction

Extended hepatectomy is performed for radical resection of hepatobiliary tract malignancies [1]. Recent advances in operative procedures and perioperative management have led to reduced mortality after major hepatectomy [2], but liver failure associated with post-operative infections still remains a major contributor to mortality [1,2]. Endotoxin derived from gram-negative bacteria has been reported to be a major causal factor in endotoxemia and sepsis in experimental or clinical investigations with massive hepatectomy [3–5]. Endotoxin has harmful effects on various organs through the induction of inflammatory mediators, including several cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 [6–8]. To maintain homeostasis, IL-10 acts as an anti-inflammatory cytokine and down-regulates inflammatory products such as pro-inflammatory cytokines, reactive oxygen species, and nitric oxide derivatives [9–11]. There are some reports on Kupffer cells, resident macrophages in the liver, as the main source of circulating IL-10 in response to endotoxin [12–14]. In addition, splenocytes are also able to release IL-10 after stimulation with endotoxin [15]. Recent studies indicated that the balance of pro- and anti-inflammatory cytokines was closely related to the severity and outcome of sepsis [16,17]. However, proof of pro- and anti-inflammatory cytokines in endotoxin-induced liver injury after partial hepatectomy,
and the role of Kupffer cells and the spleen under those conditions, has not sufficiently been demonstrated.

This study was conducted to determine the role of Kupffer cells and the spleen in pro- and anti-inflammatory cytokine responses in endotoxin-induced liver injury after partial hepatectomy. To address this issue, we determined the profile of plasma and tissue levels of TNF-α and IL-10 expression after a sublethal dose of endotoxin administration after partial hepatectomy combined with Kupffer cell blockade or splenectomy.

2. Materials and methods

Male Wistar rats (Japan SLC, Hamamatsu, Japan), weighing 180–200 g were fasted for 12 h before the experiments and given free access to water. The experimental protocols followed the Institution’s and the National Research Council’s criteria for the care and use of animals in research. Animals were subjected to either laparotomy alone as a sham-operation, or two-thirds hepatectomy performed according to the method of Higgins and Anderson [18] under light ether anesthesia. Their abdominal incisions were closed with 3-0 silk. Rats were divided into five groups: the S group, sham-operation alone; the SG group, sham-operation followed by intravenous treatment with gadolinium chloride (GdCl₃ 7 mg/kg) administration immediately and 24 h after surgery to inhibit Kupffer cell function; the H group, two-thirds hepatectomy alone; the HG group, hepatectomy with subsequent GdCl₃ administration; the HGS group, hepatectomy and simultaneous splenectomy with subsequent GdCl₃ administration. As endotoxin, lipopolysaccharide (LPS) (Escherichia coli O26: B6; Difco, Detroit, MI) was injected via the penile vein 48 h after surgery. The dose of LPS (1.5 mg/kg) was a sublethal dose for normal animals without any mortality [12,19]. Blood and tissue samples were obtained before and 1 and 4 h after LPS administration. Blood samples were centrifuged at 3000 rpm for 15 min and plasma samples were stored at -84°C until assays. Data at each time point were obtained from five animals and a total of 15 animals were included in each group. Another series of animals (n = 15 rats in each group) was used to assess the mortality rate for 24 h after LPS administration.

2.1. Measurement of plasma ALT activities

Plasma alanine aminotransferase (ALT) levels were measured to assess hepatic parenchymal damage 1 and 4 h after LPS administration using a Hitachi 736 autoanalyzer (Hitachi Ltd., Tokyo, Japan).

2.2. Measurement of plasma TNF-α and IL-10 levels

Determinations of plasma TNF-α and IL-10 levels were performed using a TNF-α test kit (Genzyme Co., Cambridge, MA) and a rat IL-10 test kit (BioSource International, Camarillo, CA) based on an enzyme-linked immunosorbent assay.

All samples were tested in duplicate. The plate was read on a microplate reader (EL 340, Bio-tek Instruments, Inc., Winooski, VT).

2.3. Extraction of hepatic and splenic tissue mRNA and Northern blot analysis

Extraction of total RNA of the liver and the spleen was performed using TRIzol reagent (Life Technologies, Grand Island, NY) according to the manufacturer’s instructions. For Northern blotting, 30 μg of total RNA was separated on 1% agarose gels containing formaldehyde. Subsequently, gels were capillary-transferred to a nylon membrane Hybond-N (Amer-sham Life Sciences, Arlington Heights, IL) and fixed using ultraviolet light. The membranes were hybridized for 12 h with a 32P-labeled TNF-α probe at 42°C in a buffer containing 50% formamide, 1 × Denhardt’s solution, 5 × standard saline citrate, 0.5% sodium dodecyl sulfate and 100 μg/ml salmon sperm DNA. After hybridization, the membrane was stringently washed, with the final washing being performed at 65°C for 10 min with 0.1 × standard saline citrate and 0.1% sodium dodecyl sulfate. The membranes were then subjected to autoradiography. The same membranes were sequentially rehybridized with IL-10 and β-actin probes. The signal intensity was quantified using an image scanner (GT-9000, EPSON, Tokyo, Japan) and image analysis software (NIH Image ver. 1.5, Bethesda, MD). The densitometric values of TNF-α and IL-10 mRNAs were normalized by calculating the ratio of the signal intensities to that of β-actin mRNA. The probes for TNF-α and β-actin were previously described [12]. The rat IL-10 probe (485 bp) was prepared by the reverse transcription polymerase chain reaction (RT-PCR) method as reported by Goggins et al. [20]. The sequence of the RT-PCR-amplified fragment was confirmed after subcloning into a pCR2.1 vector (Invitrogen, Carlsbad, CA).

2.4. Histological assessment

The resected specimens were fixed in 10% formalin and embedded in paraffin. Four-μm sections were made and stained with hematoxylin-eosin for histological examination.

2.5. Statistical analysis

All results are expressed as the mean ± SD. Statistical analyzes were performed using a statistical software package (Statview 4.5; Abacus Concepts, Berkeley, CA). The Cox-Mantel test was used to assess differences in survival rates. For the other parameters, differences among groups were assessed using one-way analysis of variance with Fisher’s post hoc test. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Mortality rate

No death occurred for animals in the sham-operation groups with LPS administration. Animals in the H group poorly tolerated LPS, as shown by 67% mortality rate in the 24 h after LPS administration (P < 0.01, S and SG groups versus the H group). The mortality rates after LPS administration in the HG and HGS groups were reduced up to 20 and 33%, respectively, and the former showed a significant difference when compared with that in the H group (P < 0.01) (Table 1).

3.2. Plasma ALT levels

There were no significant differences in the plasma ALT levels of sham-operation groups before and after LPS administration. Among hepatectomy groups, the H group showed significantly higher plasma ALT levels 4 h after LPS injection than those in the HG and HGS groups at the corresponding time (P < 0.01) (Fig. 1A).

3.3. Plasma TNF-α levels

Plasma TNF-α levels just before LPS administration were less than 10 pg/ml in all groups. Plasma TNF-α levels 1 h after LPS administration were significantly elevated with higher values in all groups when compared with those
before LPS. In sham-operation groups, intravenous GdCl₃ administration significantly inhibited the elevation of plasma TNF-α levels 1 h after LPS administration (P < 0.01). Among the groups with hepatectomy, plasma TNF-α levels 1 h after LPS administration in the H group were highest (8039 ± 278 pg/ml) with a significant difference compared with those in the HG and HGS groups (P < 0.01). The HGS group showed the lowest plasma IL-10 levels 1 h after LPS administration among the hepatectomy groups.

3.4. Plasma IL-10 levels

Plasma IL-10 levels before LPS administration were below 30 pg/ml in the sham-operation group, and were around 100 pg/ml before LPS administration in hepatectomy groups (Fig. 1C). In sham operation groups, GdCl₃ treatment significantly suppressed the elevation of plasma IL-10 levels 1 h after LPS administration (P < 0.01). In groups with hepatectomy, plasma IL-10 levels increased more than 1500 and 600 pg/ml 1 and 4 h after LPS administration, respectively, with a significant difference compared with those before LPS administration (P < 0.01). Plasma IL-10 levels 1 h after LPS administration in the HG group were significantly higher than those in the H and HGS groups (P < 0.01). The HGS group showed the lowest plasma IL-10 levels 1 h after LPS administration among the hepatectomy groups.

TNF-α to IL-10 ratio in plasma. As shown in Fig. 1D, the ratio of plasma TNF-α levels to plasma IL-10 levels 1 h after LPS administration in the H group (3.62 ± 1.50) was significantly higher than those in the HGS (2.25 ± 0.15) and HG (1.24 ± 0.15) groups (P < 0.01). There was a significant difference between the HGS and HG groups (P < 0.01). The order of the ratio was consistent with the order of the mortality and severity of liver damage by plasma ALT levels.

3.5. Expression of TNF-α and IL-10 mRNAs in the liver

Hepatic TNF-α and IL-10 mRNAs before LPS administration were undetectable in all groups (Fig. 2A). LPS stimulation up-regulated hepatic TNF-α and IL-10 mRNAs around 100 pg/ml before LPS administration in hepatectomy groups (Fig. 1C). In sham operation groups, GdCl₃ treatment significantly suppressed the elevation of plasma IL-10 levels 1 h after LPS administration (P < 0.01). In groups with hepatectomy, plasma IL-10 levels increased more than 1500 and 600 pg/ml 1 and 4 h after LPS administration, respectively, with a significant difference compared with those before LPS administration (P < 0.01). Plasma IL-10 levels 1 h after LPS administration in the HG group were significantly higher than those in the H and HGS groups (P < 0.01). The HGS group showed the lowest plasma IL-10 levels 1 h after LPS administration among the hepatectomy groups.

TNF-α to IL-10 ratio in plasma. As shown in Fig. 1D, the ratio of plasma TNF-α levels to plasma IL-10 levels 1 h after LPS administration in the H group (3.62 ± 1.50) was significantly higher than those in the HGS (2.25 ± 0.15) and HG (1.24 ± 0.15) groups (P < 0.01). There was a significant difference between the HGS and HG groups (P < 0.01). The order of the ratio was consistent with the order of the mortality and severity of liver damage by plasma ALT levels.

3.5. Expression of TNF-α and IL-10 mRNAs in the liver

Hepatic TNF-α and IL-10 mRNAs before LPS administration were undetectable in all groups (Fig. 2A). LPS stimulation up-regulated hepatic TNF-α and IL-10 mRNAs.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Operation and treatment</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>Sham ope</td>
<td>0/15 (0)</td>
</tr>
<tr>
<td>SG</td>
<td>Sham ope + GdCl₃</td>
<td>0/15 (0)</td>
</tr>
<tr>
<td>H</td>
<td>Hepatectomy</td>
<td>10/15 (67)* **</td>
</tr>
<tr>
<td>HG</td>
<td>Hepatectomy + GdCl₃</td>
<td>3/15 (20)</td>
</tr>
<tr>
<td>HGS</td>
<td>Hepatectomy + GdCl₃ + splenectomy</td>
<td>5/15 (33)</td>
</tr>
</tbody>
</table>

* P < 0.01 versus S and SG groups; and ** P < 0.01 versus HG group.
mRNAs within 1 h in the S and H groups. Increased hepatic TNF-α mRNA levels in the H group were maintained for 4 h after LPS administration, whereas a decline in hepatic TNF-α mRNA levels in the S group was observed 4 h after LPS administration. Treatment with GdCl₃ completely inhibited the induction of TNF-α and IL-10 mRNAs 1 and 4 h after LPS administration in the SG, HG and HGS groups. IL-10 mRNA levels 4 h after LPS administration were slightly decreased in the H and S groups. Splenectomy did not affect the inhibitory effect of GdCl₃ on the TNF-α and IL-10 mRNA expression (Figs. 2B,C).

3.6. Expression of TNF-α and IL-10 mRNAs in the spleen

The HG group showed a tiny accumulation of TNF-α mRNA in the spleen even before LPS administration (Fig. 3A). Up-regulated TNF-α mRNA 1 h after LPS injection was maintained for up to 4 h, irrespective of GdCl₃ treatment in all groups (Fig. 3B). In the S, SG and H groups, IL-10 mRNA expression showed a similar profile to TNF-α mRNA after LPS administration. Splenic IL-10 mRNA levels in the HG group were more up-regulated 1 h after LPS administration when compared with those in the H group ($P < 0.01$) (Fig. 3C).

3.7. Histological findings

There was no focal necrosis or damages to liver tissues before LPS administration in all groups and sham-operation groups after LPS administration. Four hours after LPS administration, diffuse congestion and focal necrosis with neutrophil infiltration were seen in the midzonal to periportal areas in the H group (Fig. 4A). Damage to a lesser extent was observed in liver tissues in the HG and HGS groups (Figs. 4B,C). Depletion of the marginal zones of lymphoid follicles, congestion and marked neutrophil accumulation was observed in the spleens in the H group (Fig. 5A). Regardless of LPS administration, the histological damage was mild in the HG group (Fig. 5B).

4. Discussion

One of the most serious morbidity events after hepatectomy is liver failure resulting from infection [1,2]. Endotoxin from gram-negative bacteria acts as a major causative agent in the pathogenesis of sepsis, producing various inflamma-
inflammatory cytokine production [9–12], and may potentially protect against endotoxin-induced liver injury [22–25]. However, little information is available on the role of Kupffer cells and the spleen in the regulation of pro- and anti-inflammatory cytokine production in endotoxin-induced liver injury after partial hepatectomy.

We demonstrated that activated Kupffer cells and the spleen were the main sources of TNF-α production in the present study. Since treatment with GdCl3 completely abolished the induction of TNF-α mRNA in the liver and inhibited the elevation of plasma TNF-α levels after LPS administration, modulation of TNF-α production in the liver, especially Kupffer cells, leads to changes in circulating TNF-α levels. The spleen is also considered to be one of the main sites producing TNF-α in response to LPS stimulation, because splenic TNF-α mRNA expression was enhanced after LPS administration, while elevation of plasma TNF-α levels was suppressed by splenectomy. Interestingly, the peak plasma TNF-α levels after LPS administration observed in the HGS group were similar to those in the S group. Nevertheless, animals in the HGS group showed liver damage after LPS administration, whereas those in sham-operation groups did not. Therefore, TNF-α seems not to be the sole causative factor in the development of endotoxin-induced liver injury after hepatectomy.

Recent studies have revealed the important role of IL-10 as an anti-inflammatory cytokine, it exerts a protective effect in endotoxemia and sepsis [26]. IL-10 inhibits the activation of nuclear factor kappa B (NF-κB), a crucial transcription factor for pro-inflammatory molecules. Inhibition of NF-κB activation results in the down-regulation of pro-inflammatory cytokines (TNF-α, IL-1, IL-6) [27,28], chemokines (IL-8, monocyte chemoattractant protein-1), and adhesion molecules like intercellular adhesion molecule (ICAM)-1 [16,29]. Other inflammatory mediators, such as reactive oxygen species and nitrogen derivatives, are also suppressed by IL-10. Moreover, D’Amico and associates [30] demonstrated that IL-10 generates functional decoy receptors for chemokines on monocyte lineages, and scavenges chemokines by trapping them without signal transduction. These functions of IL-10 may be useful to shut down the vicious inflammatory circle. IL-10 is mainly released from Kupffer cells [12–14], splenocytes [15], monocytes/macrophages [31] and T cells [32]. In our study, Kupffer cell elimination by GdCl3 treatment completely inhibited IL-10 mRNA induction in the liver after LPS administration. Activated Kupffer cells are mainly implicated in hepatic IL-10 production. Moreover, we confirmed in this study that circulating IL-10 is derived from the spleen, as well as the liver, because IL-10 mRNA was induced in the spleen in response to LPS administration (Fig. 3C). Surprisingly, the HG group showed enhanced splenic IL-10 mRNA induction by LPS administration, resulting in the marked increase of plasma IL-10 levels when compared with the H group. Kupffer cells during liver regeneration may have the potential to regulate splenic mediators such as TNF-α, IL-1β, IL-6 and IL-8, reactive oxygen species, and nitric oxide derivatives [3,6,9–12]. Complicated interaction among these mediators leads to the development of organ failure and the mortality associated with sepsis. Previous studies demonstrated that activated Kupffer cells and the spleen were closely implicated in endotoxin-induced liver injury after partial hepatectomy through the augmentation of TNF-α production [3,12,16,21]. Because the large Kupffer cells are selectively eliminated by GdCl3 treatment, these type of cell may be important target for prevention of endotoxin-induced liver injury [19]. In the cytokine family, IL-10 is known as an anti-inflammatory cytokine that efficiently blocks pro-

Fig. 4. Histological findings of the liver. Diffuse congestion and focal necrosis with neutrophil infiltrations were seen in the midzonal to periporal areas (arrows) in H group 4 h after LPS administration (A). Damage to a lesser extent was observed in liver tissues of the HG (B) and HGS groups (C). (Hematoxylin-eosin; original magnification 150×).
IL-10 production in response to LPS administration. Ayala et al. also indicated that Kupffer cells interfere with splenic lymphocyte function during sepsis [33]. Conceivably, constituent cells in the spleen may compensate for the function of Kupffer cells after GdCl₃ treatment, and may be associated with IL-10 production.

TNF-α has been recognized as an important component of the early signaling pathways that facilitate hepatocyte proliferation after partial hepatectomy [6]. Rai and associates [6] reported that GdCl₃ pretreatment causes sustained overexpression of hepatic TNF-α mRNA and transient overexpression of circulating TNF-α protein within 24 h after partial hepatectomy. In negative regulators of TNF-α, regenerative induction of IL-10 was abolished but transforming growth factor (TGF)-β₁ induction was unaltered after partial hepatectomy in animals with GdCl₃ pretreatment. These three cytokines can be produced by Kupffer cells in normal liver [3,6,21,34], but the other liver cell populations may be predominately responsible for TNF-α and TGF-β₁ production after partial hepatectomy in GdCl₃–treated animals [6]. The profiles of TNF-α and IL-10 production after hepatectomy were different between their and our experiments, although we did not determine hepatic expression of TGF-β₁. The main reason on this discrepancy is that GdCl₃ treatment was performed after partial hepatectomy and implication of cytokines in our study was determined before and 1 and 4 h after LPS administration at 48 h after surgery.

In contrast with the beneficial effects of IL-10, some studies assumed a link between circulating IL-10 levels and high mortality in septic conditions [22,35]. In our study, peak values of plasma IL-10 levels after LPS stimulation in hepatectomy groups were three times those in sham-operation groups. Although peak plasma IL-10 levels were highest in the HG group, mortality and liver damage in this group was lowest among the hepatectomy groups. Therefore, it is difficult to predict the severity of disease from the circulating IL-10 level alone. Walley and associates [17] demonstrated that the balance of pro- and anti-inflammatory cytokines was closely related to the severity and outcome of sepsis. In this study, elevation of IL-10 and reduction of TNF-α by treatment with GdCl₃ prevented liver injury and improved survival after LPS administration. The mortality in endotoxin-induced injury after two-thirds hepatectomy was lowest in group HG, followed by groups HGS and H. The degree of hepatic parenchyma damage due to LPS administration in each group was the same as the order in mortality. The ratio of plasma TNF-α levels to plasma IL-10 levels in the H group was significantly lower than those in the HG and HGS groups (P < 0.05) (Fig. 1D). Thus, the balance between pro- and anti-inflammatory cytokines seems to be more reliable for the prediction of disease severity and mortality in the pathophysiological conditions associated with endotoxia or sepsis.

In conclusion, our results indicated that activated Kupffer cells after partial hepatectomy may aggravate endotoxin-induced liver injury via down-regulation of IL-10 production. The balance of pro- and anti-inflammatory cytokines generated by Kupffer cells and the spleen may be important in the modulation of endotoxin-induced liver injury after partial hepatectomy.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research (No. 12671218) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References


