Fukushima J. Med. Sci.,
Vol. 49, No. 1, 2003
[Review Article]

ADHESION MOLECULES AND CXC CHEMOKINES IN ENDOTOXIN-INDUCED LIVER INJURY

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(Received March 31, 2003, accepted April 10, 2003)

Abstract: Interactions between leukocytes and sinusoidal endothelial cells are known to be involved in the pathogenesis of acute liver injury. Various adhesion molecules and chemokines play key roles in these cell-to-cell interactions, and the expression of these adhesion molecules and the production of chemokines are regulated by inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interferon-γ (IFN-γ). We have shown that the expression of intercellular adhesion molecule-1 (ICAM-1) on cultured rat sinusoidal endothelial cells stimulated with TNF-α increases in a dose-dependent manner. The number of neutrophils that adhered to sinusoidal endothelial cells pretreated with TNF-α also increased in a dose-dependent manner and significantly decreased upon incubation with an anti-ICAM-1 antibody. In endotoxin-induced rat liver injury, the number of neutrophils infiltrating the sinusoids increased after serum TNF-α, macrophage inflammatory protein-2 (MIP-2) and cytokine-induced neutrophil chemoattractant (CINC) reached their peak levels. In addition, the level of ICAM-1 expression on sinusoidal endothelial cells greatly increased from 8 h after exposure to endotoxin, and these cells were adhered to neutrophils that expressed both LFA-1 and Mac-1. Moreover, lipo-prostaglandin E1 (PGE1) reduced the extent of liver injury, and also reduced the number of neutrophils that infiltrated the liver, was reduced the production of MIP-2 and CINC, but not that of TNF-α, in rats injected with endotoxin.

Keywords: ICAM-1, MIP-2, CINC, endotoxin, lipo-PGE1

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INTRODUCTION

Cellular adhesion and recognition mechanisms are among the most basic requirements for the evolution of multicellular organisms. Such cell-to-cell or cell-to-matrix interactions involve an antigen-independent receptor-ligand interaction mediated by adhesion molecules. Adhesion molecules so far indentified have been classified into six major families according to common structural features, i.e., the integrin, immunoglobulin, selectin, sialomucin, cadherin, and link protein familes [1, 2, 3]. In addition, the expression of several adhesion molecules is known to be regulated by various cytokines such as TNF-α, interleukin-1 (IL-1) and interferon-γ (IFN-γ) [4, 5]. In the liver, inflammatory cells pass through the sinusoidal endothelial cells and accumulate at inflammatory sites. Consequently, leukocyte-sinusoidal endothelial cell interaction may play an important role in the control of immune responses [6]. In liver injury, the expression of adhesion molecules on sinusoidal endothelial cells is up-regulated and the production of inflammatory cytokines increases [7, 8]. On the other hand, chemokines are a family of low-molecular-weight proteins that are not only responsible for the recruitment of inflammatory cells from the vasuculature but also involved in the activation of adhesion molecules (9). The chemokines are separated into four distinct structural families by their relative positioning of cysteines at the amino terminus, i.e., CC chemokines, ELR (Glu-Leu-Arg)-CXC chemokines, non-ELR-CXC chemokines and CX3C cemokines (Table 1). Macrophage inflammatory protein-2 (MIP-2) and cytokine-induced neutrophil chemoattractant (CINC), both of which belong to the family of CXC chemokines, have been shown to be the major neutrophil chemotactic factors that cause activation of neutrophils in liver injury (10-11). These chemokines have also been shown to be induced by inflammatory mediators such as endotoxin, TNF-α and IL-1 (12, 13). In this review, we describe the relationships between changes in adhesion molecules on sinusoidal endothelial cells and level of cytokines and chemokines *in vitro* and *in vivo*.

Expression of adhesion molecules on sinusoidal endothelial cells

Immunohistochemical analysis using biopsy specimens obtained from patients with liver disease and healthy controls has revealed that various adhesion molecules are expressed on sinusoidal endothelial cells [13-15]. As shown in Table 2, ICAM-1 and ICAM-2 (belonging to the immunoglobulin superfamily), leukocyte function-associated-3 (LFA-3), very late antigen-5 (VLA-5) (belonging to the integrin family) and vascular adhesion protein 1 (VAP-1) (belonging to the link protein family) are expressed on sinusoidal endothelial cells in normal liver. On the other hand, in patients with acute or chronic liver disease, the expression of these adhesion molecules is upregulated and new adhesion molecules such as E-selectin or P-selectin are expressed. In particular, ICAM-1 and vascular cell adhesion molecule-

1 (VCAM-1) expression on sinusoidal endothelial cells is markedly enhanced in inflammed liver tissue. The expression of other adhesion molecules, such as cadherin or neural cell adhesion molecule (NCAM), which are known to be expressd on vascular endothelial cells, on sinusoidal endothelial cells has not been confirmed.

The ligands of these adhesion molecules exist primarily on the surfaces of leukocytes [4], suggesting that interactions between sinusoidal endothelial cells and leukocytes via adhesion molecules play an important role in the pathogenesis of liver injury. Adhesion pathways between leukocytes and sinusoidal endothelial cells, such as the E-selectin ligand-1 (ESL-1)-E-selectin pathway, P-selectin glycoprotein ligand-1 (PSGL-1)-P-selectin pathway, LFA-1/Mac-1-ICAM-1 pathway and VLA-4-VCAM-1 pathway, are involved in leukocyte infiltration and hepatocyte injury. Recently, Fas-mediated apoptosis and perforin-mediated injury have been reported to be associated with the pathogenesis of hepatocyte injury in chronic hepatitis due to hepatitis B or hepatitis C virus [16-18]. Cell-to-cell adhesionis needed in these systems, and these adhesion pathways play a crucial role in Fas-mediated apoptosis and perforin-mediated injury.

Cytokines and adhesion molecules

The expression of adhesion molecules on sinusoidal endothelial cells is mediated by several substances such as thrombin, lipopolysaccharide (LPS) and histamine, and various cytokines. E-selectin, ICAM-1 and VCAM-1 are up-regulated by inflammatory cytokines such as TNF-α, IL-1 and IFN-γ [19]. These cytokines are induced by adjacent Kupffer cells, lymphocytes, sinusoidal endothelial cells, activated platelets that have adhered to the damaged sinusoidal endothelial cells, and splenocytes, and they probably promote the adhesion of leukocytes to sinusoidal endothelial cells [7, 20-23]. P-selectin is expressed on the surfaces of Weibel-Palade bodies in vascular endothelial cells and is rapidly induced by histamine and thrombin [24].

In our study in which we examined the expression of ICAM-1 in cultured rat sinusoidal endothelial cells using the immunogold technique and a cytofluorometer, we found that ICAM-1 expression increased 8 h after stimulation by either TNF-α or IL-1α in a dose-dependent manner [25]. Kinetics analysis has revealed that the expression level of ICAM-1 on sinusoidal endothelial cells treated with these cytokines gradually increased from the beginning of stimulation to 24 h after the start of stimulation [25].

In addition, neutrophils adhered to cultured rat hepatic sinusoidal endothelial cells treated with these cytokines *in vitro*, and the number of neutrophils adhering to sinusoidal endothelial cells pretreated with TNF-α for 8 h increased in a dose-dependent manner. Moreover, the number of neutrophils that adhered to the cells significantly decreased upon incubation with an anti-ICAM-1 antibody (Fig. 1).

These findings are similar to those for other vascular endothelial cells and, therefore, like vascular endothelial cells, hepatic sinusoidal endothelial cells may be

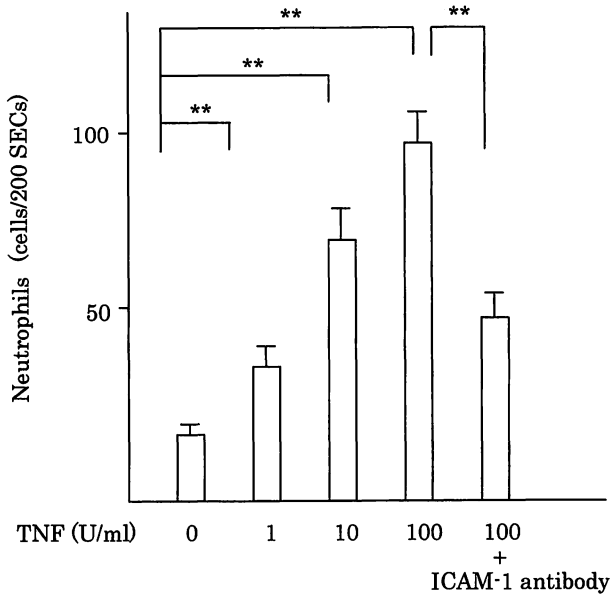

Fig. 1. Number of neutrophils that had adhered to cultured rat sinusoidal endothelial cells treated with tumor necrosis factor-α (*TNF-α*) for 8 h. Following TNF-α treatment, neutrophils were co-cultured with sinusoidal endothelial cells for 20 min. After washing three times, adhered neutrophils were stained with chloroacetate esterase, and the number of neutrophils that had adhered to 200 sinusoidal endothelial cells (*SECs*) was counted. $^{**}p<0.01$.

closely involved in the inflammation associated with liver disease via surface expression of various adhesion molecules.

We have also examined the relationships of expression of adhesion molecules on sinusoidal endothelial cells with infiltration of the liver by neutrophils and with serum levels of TNF-α, MIP-2, and CINC in rats with LPS-induced acute liver injury [26]. In that study, serum TNF-α peaked at 1 h and MIP-2 and CINC peaked at 8 h after exposure to LPS (2 mg/kg in a 0.2-ml solution administered intravenously). MIP-2 and CINC, which appeared to be induced in response to the increase in TNF-α, not only act as chemoattractants for neutrophils but also induce the translocation of Mac-1 from the intercellular storage pool onto the surfaces of neutrophils [27]. In our previous study [26] and as shown in Fig. 2a, b, the number of neutrophils infiltrating the sinusoids increased after serum TNF-α, MIP-2, and CINC had peaked, indicating that these cytokines may participate in the activation of neutrophils and up-regulation of adhesion molecules. Immunohistochemical studies have revealed that the expression level of ICAM-1 on sinusoidal endothelial cells and hepatocytes strongly increased from 8 h after exposure to LPS and that both LFA-1 and Mac-1 were expressed on neutrophils that have adhered to sinusoidal endothelial cells (Fig. 3). These findings suggest that interactions between neutrophils-and sinusoidal endothelial cells and between neutrophils and -

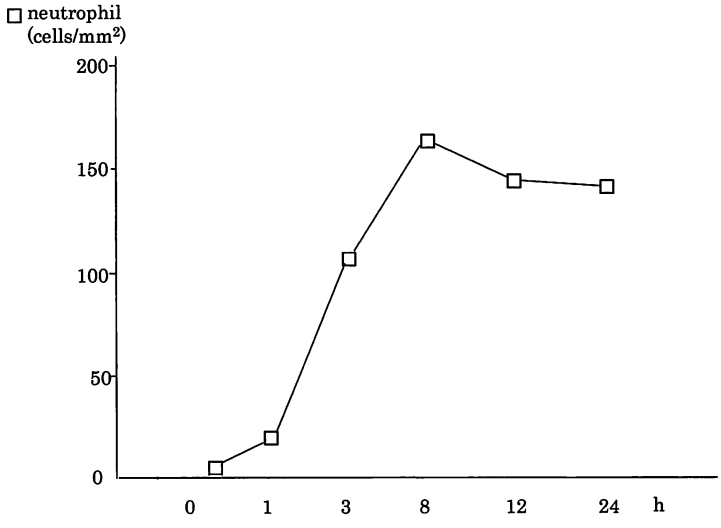

Fig. 2a

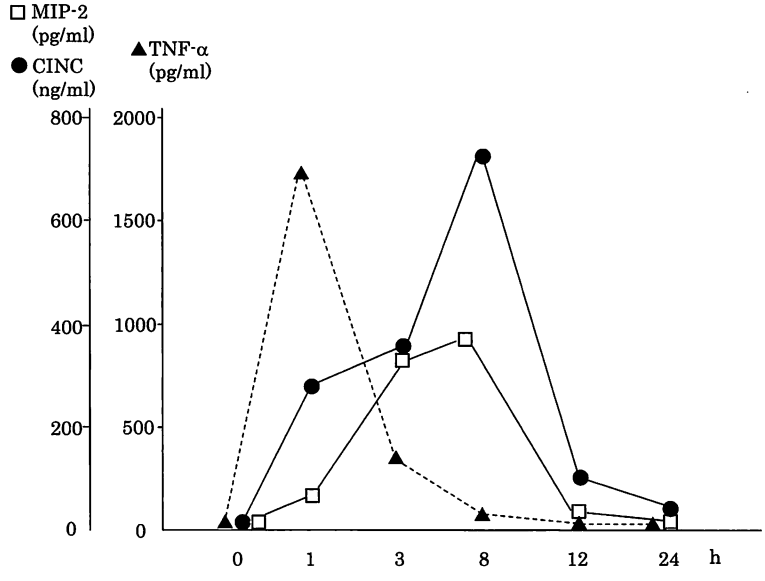

Fig. 2b

Fig. 2. a. Time course of changes in the number of infiltrating neutrophils in the liver after LPS injection (2 mg/kg). The numbers of neutrophils stained by chloroacetate-esterase were determined by microscopic examination and are expressed as means cells in five fields at a magnification of ×200.
b. Time courses of changes in serum TNF-α, MIP-2 and CINC levels in rats after LPS injection (2 mg/kg).

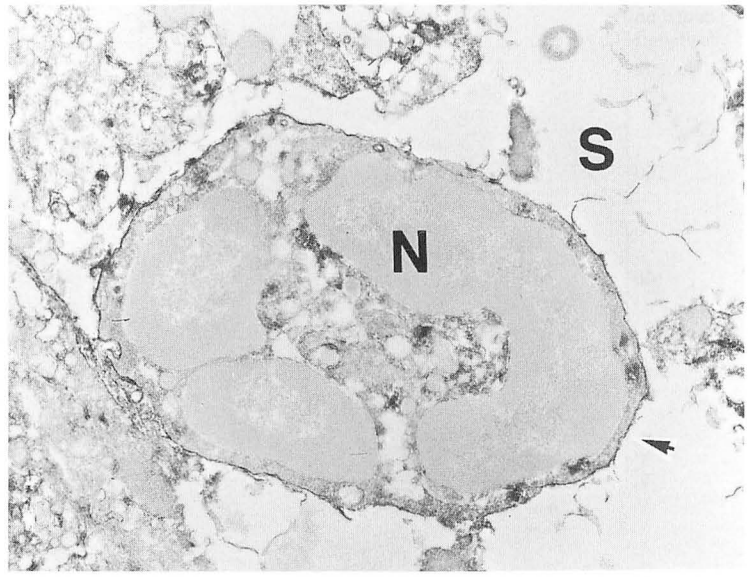

Fig. 3. Electron micrograph showing immunoreactive products for Mac-1 antibody on the surfaces of neutrophils (arrowheads) that had adhered to a degenerated sinusoidal endothelial cell after lipopolysaccharide treatment. *S*, sinusoid; *N*, neutrophil. ×14,000

hepatocytes occur via adhesion molecules and are related to inflammatory cytokines and chemokines such as TNF-α, MIP-2, and CINC, which play important roles in the pathogenesis of acute liver injury.

Effect of lipo-PGE1 on endotoxin-induced rat liver injury

Prostaglandin E1 (PGE1) is a substance known to protect hepatocytes through vasodilation, inhibition of platelet aggregation and regeneration of the liver (28-31). PGE1 has been used in studies to stimulate liver function and regenerate liver cells in patients with either fulminant hepatic failure or patients who have undergone liver transplantation (32, 33). PGE1 incorporated into lipid microspheres (lipo-PGE1) has been widely used to treat various vascular diseases because of its superior stability compared to that of free PGE1 and because of the fact that it causes less irritation to the upper respiratory tract and accumulates to a greater degree in the inflamed tissue (34, 35). PGE1 inhibits adherence and transendothelial migration of neutrophils *in vitro* (36). However, there have been few studies on the influence of lipo-PGE1 on the release of chemokines (36, 37). We therefore examined the effects of lipo-PGE1 on the release of chemokine release in endotoxin-induced rat liver injury.

The kinetics of serum ALT levels in rats injected with 2 mg/kg of LPS was assessed at 1, 3, 8, 12 and 24 h after injection. The results are shown in Fig. 4a. Serum ALT levels in rats injected with LPS and lipo-PGE1 (2 μg/kg) were significantly lower at 8, 12 and 24 h after injection than those in rats injected with LPS alone. Serum ALT levels in rats measured at 8 h after injection with LPS and

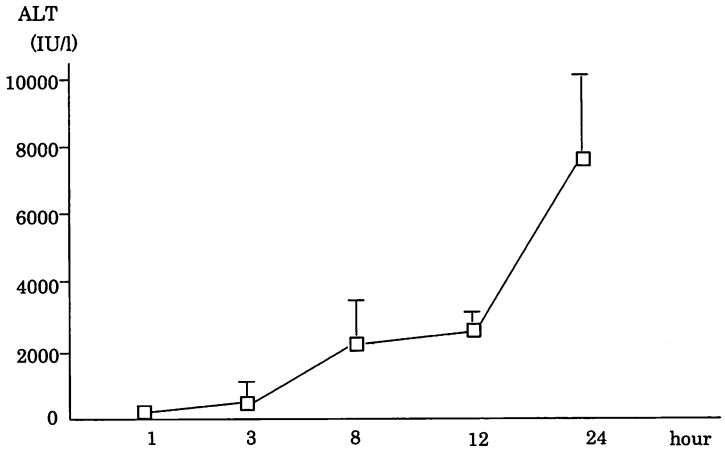

Fig. 4a

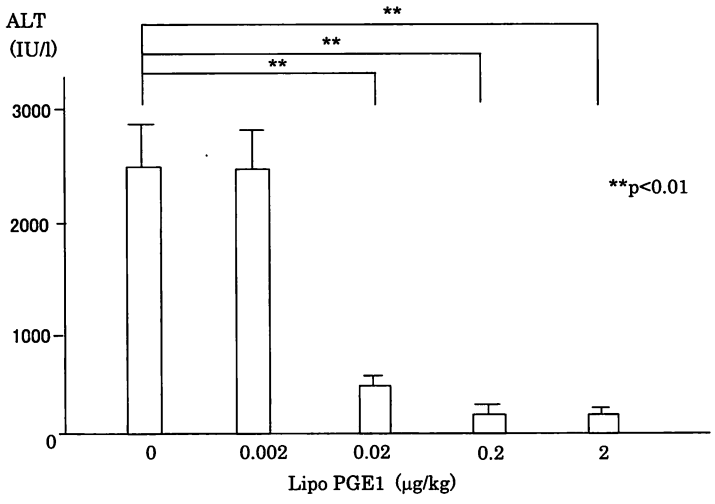

Fig. 4b

Fig. 4. a. Serum ALT levels in rats at 24 h after injection of LPS (2 mg/kg).
b. Serum ALT levels in rats at 8 h after injection of LPS and various concentrations of lipo-PGE1. ALT levels were significantly lower in rats injected with 0.02, 0.2 and 2 μg/kg of lipo PGE1 than in rats injected with LPS alone.

various concentrations of lipo-PGE1 ranging from 0.002 to 2 μg/kg significantly decreased in a dose-dependent manner (Fig. 4b).

Microscopic observation showed an increased infiltration of neutrophils in the sinusoids and a focalization of necrotic areas primarily in zone 2 of the hepatic lobules of rats injected with LPS alone and a similar pattern in rats injected with LPS and lipo PGE1. However, the number of neutrophils that had infiltrated the liver at 8 h after injection with LPS and various concentrations of lipo-PGE1

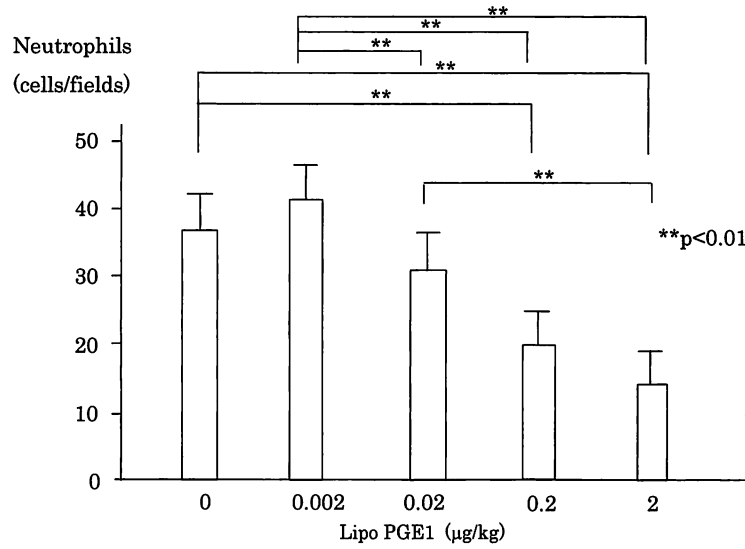

Fig. 5. Number of infiltrating neutrophils in the liver at 8 h after injection of LPS and various concentrations of lipo-PGE1. The number of infiltrating neutrophils in liver sections of rats injected with LPS and lipo-PGE1 decreased in a dose-dependent manner from 0.02 to 2 μg/kg of lipo-PGE1.

ranging from 0.002 to 2 g/kg significantly decreased in a dose-dependent manner compared with those observed in the liver of rats injected with LPS alone (Fig. 5). In addition, less extensive necrosis was observed in liver sections of rats injected with lipo-PGE1 than that in rats injected with LPS alone.

TNF-α levels had increased at 1 h after injection of LPS. However, no significant differences were observed at any time between the mean maximum levels in rats injected with LPS alone and in those injected with LPS and lipo-PGE1 (2 μg/kg) (Fig. 6a). The MIP-2 levels had increased at 1 h (75.9±42.2 IU/l), 3 h (342.9±35.9 pg/ml) and 8 h (358.3±23.4 pg/ml) after injection of LPS alone. In contrast, MIP-2 levels had decreased significantly at 3 h (141.4±95.5 pg/ml) and 8 h (44.9±44.7 pg/ml) in rats injected with LPS and lipo-PGE1 (2 μg/kg) (Fig. 6b). CINC levels in rats injected with LPS alone increased at 1 h (391.7±110.0 ng/ml), 3 h (327.7±28.5 ng/ml), and 8 h (723.3±29.0 ng/ml) after injection. In contrast, the levels had decreased significantly at 8 h (482.7±156.0 ng/ml) in rats injected with LPS and lipo-

Fig. 6. Time courses of changes in serum TNF-α, MIP-2 and CINC levels in rats injected with LPS alone and with LPS and lipo-PGE1 (2 μg/kg) measured at 1, 3, 8 and 12 h after injection.
a. TNF-α levels had increased at 1 h after the injection, but a significant difference was not observed at any time between TNF-α levels in rats injected with LPS alone and in rats injected with LPS and lipo-PGE1.
b. MIP-2 levels had significantly decreased at 3 and 8 h after injection of LPS and lipo-PGE1 compared with the levels after injection of LPS alone.
c. CINC levels had significantly decreased at 8 h after injection of LPS and lipo-PGE1 compared with the levels after injection of LPS alone.

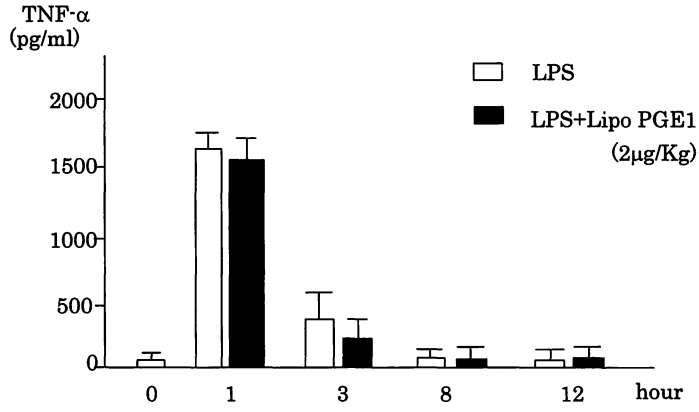

Fig. 6a

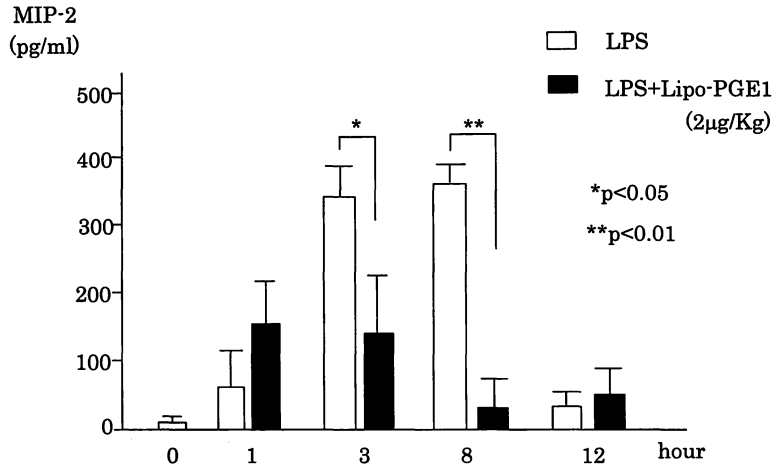

Fig. 6b

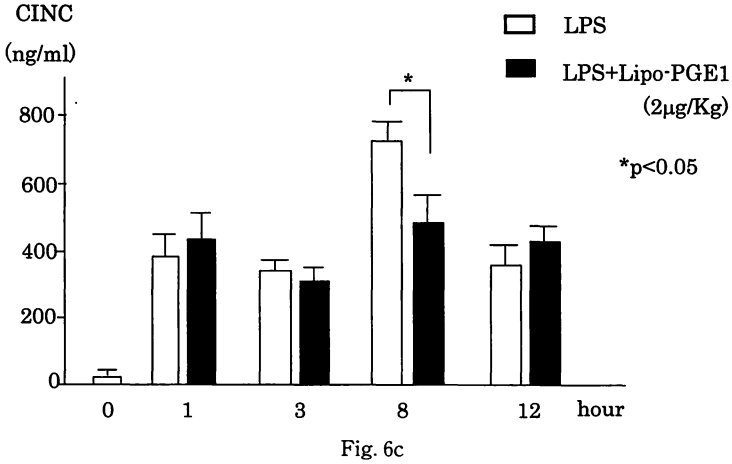

Fig. 6c

PGE1 (Fig. 6c).

Previous studies have suggested the infiltration of the injured liver by neutrophils is induced by endotoxins. Studies using rats have shown that neutrophil infiltration in the liver is regulated by chemotactic substances such as MIP-2 and CINC (38, 39). PGE1 has been reported to have hepatoprotective effects through regulation of the production of inflammatory cytokines (40). The present study clearly showed that lipo-PGE1 at physiological concentrations significantly reduces serum levels of MIP-2 and CINC, but not TNF-α, and ultimately results in a reduction in the extent of necrosis in the injured rat liver induced by LPS.

A summary of our observations is shown in Fig. 7. Initially, inflammatory cytokines such as TNF-α, IL-1 and IFN-γ were induced by adjacent Kupffer cells, lymphocytes, sinusoidal endothelial cells, and platelets activated by the stimulation of LPS. The cytokines stimulated not only the expression of adhesion molecules on neutrophils, sinusoidal endothelial cells, and hepatocytes but also the production of chemokines such as MIP-2 and CINC by hepatocytes and sinusoidal endothelial cells. The activated neutrophils adhered to the sinusoidal endothelial cells and passed through them. The neutrophils that accumulated in areas of hepatocyte necrosis were regulated by local production of chemokines from hepatocytes. In the present study, lipo-PGE1 in the present study seemed to have a direct effect on hepatocytes via a reduction in the production of MIP-2 and CINC, given that no reduction in TNF-α level in sera was observed in rats with LPS-induced liver injury that had been treated with lipo-PGE1. Therefore, the observed reduction in the extent of necrosis in the liver of rats injected with LPS and treated with PGE1 might

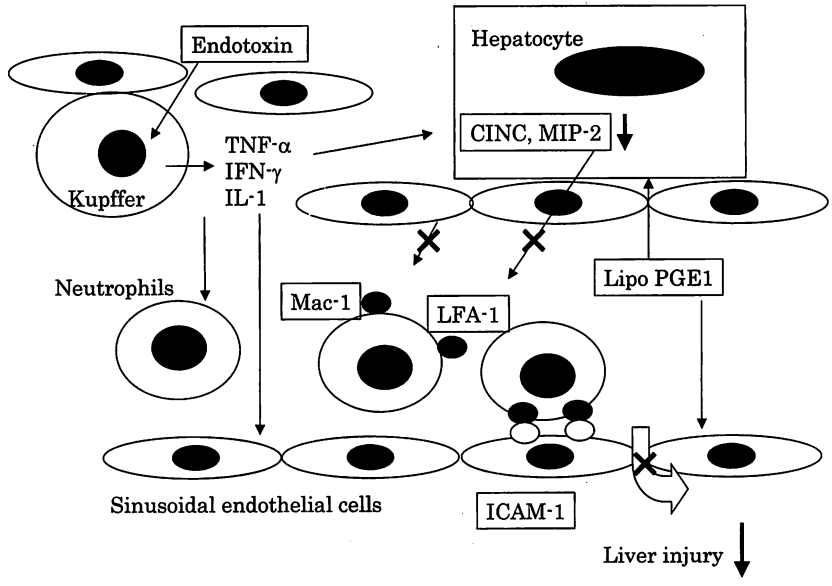

Fig. 7. Schema of adhesion molecules and chemokines in liver injury after injection of LPS and lipo-PGE1.

have been induced by prevention of the adhesion of neutrophils to endothelial cells or hepatocytes.

In conclusion, adhesion molecules and chemokines play key roles in many liver diseases, and lipo-PGE1 may have a benefical effect on acute liver injury. Further study is needed to elucidate the effects of lipo PGE1 on human liver diseases.

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