Biochemical and Biophysical Research Communications **255,** 143–149 (1999)
Article ID bbrc.1999.0160, available online at http://www.idealibrary.com on IDEAL®

Kupffer Cell-Mediated Down Regulation of Rat Hepatic CMOAT/MRP2 Gene Expression

Jun-ichi Nakamura,* Toshirou Nishida,* Ken'ichiro Hayashi,† Norifumi Kawada,‡ Shigeyuki Ueshima,* Yuichi Sugiyama,§ Toshinori Ito,* Kenji Sobue,† and Hikaru Matsuda*

**First Department of Surgery, Osaka University Medical School, and †Department of Neurochemistry and Neuropharmacology, Biomedical Research Center, Osaka University Medical School, Yamadaoka 2-2, Suita, Osaka 565-0871, Japan; ‡The Third Department of Internal Medicine, Osaka City University Medical School, Asahimachi 1-5-7, Abeno, Osaka 565-0871, Japan; and §Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

Received December 10, 1998

Lipopolysaccharides (LPS) induces intrahepatic cholestasis and canalicular multispecific organic anion transporter (CMOAT/MRP2) plays a central role in hepatic bilirubin transport. This study examined the role of Kupffer cell in LPS-induced cholestasis. Rats were injected intravenously with LPS. Kupffer cells were inactivated with gadolinium chloride (Gd). CMOAT/MRP2 mRNA expression was time- and dose-dependently decreased by LPS injection with a decrease in bile flow and an increase in serum bilirubin level. Gd pretreatment inhibited decrease in CMOAT/MRP2 mRNA and bile flow, and increase in serum bilirubin. Kupffer cell-conditioned medium decreased CMOAT/MRP2 expression. Addition of anti-IL-1 or anti-TNFα antibody restored CMOAT/MRP2 expression, whereas IL-1 and TNFα decreased the expression. MAP kinases were activated by addition of the conditioned medium, and addition of PD98059 or SB203580 restored CMOAT/MRP2 expression. These results suggest that LPS activates Kupffer cells to secrete IL-1 and TNFα, which in turn activate MAP kinases and decrease CMOAT/MRP2 expression.

The liver plays a major role in the elimination of bilirubin, bile acids and various xenobiotics. Sepsis and septic conditions are frequently accompanied by cholestasis. Because extrahepatic infections, bacteremia and systemic inflammatory response syndrome are associated with cholestasis, it is postulated that these conditions are mediated in part by inflammatory cytokines (1,2). The histologic picture of the liver is consistent with intrahepatic cholestasis and minor abnormalities in hepatic morphology are frequent, and, thus, perturbations in hepatobiliary transport are considered to be closely linked to these conditions (3). Endotoxin, which is lipopolysaccharides (LPS) in the wall of gram-negative bacteria, induces similar conditions (4). LPS are well-known stimulants of cytokine production, and some cytokines such as TNFα decrease bile flow, increase serum bilirubin, and cause cholestasis (4–7). Kupffer cells activated by LPS secrete many cytokines such as TNFα and interleukin-1 (IL-1) (8). *In vivo* application of LPS decreases bile acid transport (2) and organic anion transport (7). Recently, LPS has been shown to decrease expression of some transport proteins for bile acids and bilirubin (1,9).

Hepatocytes take up many endogenous and exogenous substances via carrier-mediated systems and secrete them into bile canaliculi. The unidirectional ATP-driven systems for bile acids and organic anions are major determinants of bile flow and may represent the rate-limiting step in overall hepatobiliary transport from blood into bile (10). The liver canalicular membrane contains a unique set of ATP-dependent export proteins (11). Disturbances in the canalicular ATP-dependent organic anion transport (CMOAT/MRP2) result in cholestasis and hyperbilirubinemia (10–13). LPS and TNFα are suggested to decrease hepatocyte expression of CMOAT/MRP2 mRNA and protein (9). However, the precise mechanisms of LPS-induced cholestasis are not fully understood.

The purpose of this study was to clarify the role of Kupffer cells and their secretagogues in hepatic CMOAT/MRP2 expression under septic conditions.

MATERIALS AND METHODS

Chemicals

Lipopolysaccharides (LPS: Escherichia coli; Serotype 0127: B8) and gadolinium (III) chloride hexahydrate (Gd) were purchased from Sigma Chemical (St. Louis, MO). Anti-bilirubin-UDP-glucuronosyl-

0006-291X/99 $30.00

transferase antibody is gifted by Dr. Jayanta Roy-Chowdhury (Marion Bessin Liver Research Center, Albert Einstein College of Medicine, NY). PD98059, a specific inhibitor of extracellular signal-regulated kinase (ERK), and SB203580, a specific inhibitor of p38 mitogen-activated protein kinase (p38 MAPK), were obtained from from Calbiochem (Cambridge, MA). All chemicals used were of analytical grade.

Animals

Male Sprague-Dawley rats weighing 250g were used. The rats provided with water and standard laboratory chow ad libitum. The rats were anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneally), then intravenously injected with various doses of LPS dissolved in sterile saline (0~1 mg/kg body weight of LPS and 1 ml/kg body weight). Animals injected with an equal volume of sterile saline were used for the control. At 0, 2, 4, 6, 12, 18, 24, 48, 72, and 168 hours after LPS injection, the common bile duct was cannulated with a PE-10 polyethylene tube (inner diameter: 0.28 mm; outer diameter: 0.61 mm; Becton Dickinson Co., Franklin Lakes, NJ) to measure the bile flow rate. Bile was collected in tubes for 10 min each and then weighed. For biochemical analyses, small pieces were taken from the livers at indicated times and stored at −80°C until use. Before sampling liver tissues, blood samples were obtained from the inferior vena cava with heparinized syringes at indicated times. The plasma levels of total bilirubin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined with an auto-analyzer (Hitachi 7150, Hitachi, Tokyo, Japan). To examine the involvement of Kupffer cells, Gd (7 mg/kg body weight) dissolved in sterile saline, or an equal volume of sterile saline (1 ml/kg body weight) as a control was injected intravenously for 2 days before LPS injection (14).

Preparation of Hepatocytes and Kupffer Cells

Hepatocytes and Kupffer cells were separately prepared from rat livers. Hepatocytes were isolated from fed rats by perfusing the liver with 0.06% collagenase solution from the portal vein (15). The hepatocytes were suspended in Williams' E medium (GIBCO BRL, Rockville, MD) supplemented with 10% fetal calf serum (GIBCO BRL) at a cell density of 1×10^6 cells/ml, and cultured at 37°C and in 5% CO_2 and 95% O_2. The viability of the hepatocytes examined by trypan blue dye exclusion test were more than 90%. Kupffer cells were prepared from rat livers by essentially the same method as that of Kawada *et al.* (16). Kupffer cells were suspended in Williams' E containing 10% fetal calf serum at a cell density of 3×10^6 cells/ml, and cultured at 37°C and in 5% CO_2 and 95% O_2. The purity and viability of Kupffer cells were more than 95% and 95%, respectively.

Preparation of Conditioned Medium

After Kupffer cells were incubated in Williams' E medium containing 10% fetal calf serum for 48 hours, they were well washed with phosphate-buffered saline (PBS) and incubated in medium containing 300 ng/ml LPS dissolved in saline or equal amount of saline for 1, 2, 4, 6, and 8 hours. The Kupffer cell-conditioned medium incubated with 300 ng/ml LPS for the indicated time was added to hepatocytes, which were, then, incubated at 37°C. After 12-hour incubation, total RNA was extracted from the hepatocytes. In neutralization experiments, after addition of 10 mg/ml (final concentration) goat immunoglobulin, 10 mg/ml anti-rat TNFα antibody, 30 μl of solubilized anti-mouse IL-1α antibody, or both antibodies, the Kupffer cell-conditioned medium was preincubated at 37°C for 1 hour and, then, added to hepatocytes. After 12-hour incubation, total RNA was extracted from the hepatocytes. In MAPK blocking experiments, hepatocytes were incubated in each Kupffer cell-conditioned medium containing 0.2% DMSO as a control, or a final concentration of 20 nM PD98059, or 10 nM SB203580. After 12-hour incubation at 37°C, total RNA was extracted from the hepatocytes.

Northern Blotting

Total RNAs were extracted from liver or cultured hepatocytes under indicated conditions using ISOGEN RNA extraction kit (Nippon Gene, Tokyo, Japan). A CMOAT/MRP2 cDNA (GenBank D86086) fragment (expanding from 4035 to 4407) was used as a probe to monitor the expression of the mRNA. Probes were ^{32}P-labeled on the antisense strands and used for hybridization as described previously (17). To quantify the applied RNAs, ribosomal RNAs were stained with 0.02% methylene blue and the mRNA content was also quantitated by densitometry.

Kinase Assay

The cultured cells were well washed with ice-cold PBS and were lysed in the lysis buffer (20 mM Tris-HCl, pH 7.5, 137 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1% Triton X-100, 50 mM NaF, 1 mM Na_3VO_4, 50 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin and 1 μg/ml pepstatin). The amounts of ERK, c-Jun N-terminal kinase (JNK), and p38 MAPK in the cell lysates were estimated by Western blotting with specific antibodies against the respective protein kinases, and their activities were measured as described previously (17).

Statistics

The results are expressed as the mean ± standard error (SEM; n = 3) unless otherwise indicated. Statistical analysis was performed using the Kruskal-Wallis test and one-way as well as repeated-measure analysis of variance (ANOVA) with the Scheffe' post-hoc test. *P* values less than 0.05 were considered significant.

RESULTS

In vivo Effects of LPS on CMOAT/MRP2 Expression

One-shot intravenous injection of LPS (from 0.01 to 1 mg/Kg body weight) did not cause systemic hypotension and lethality in rats. Light microscopy revealed mild infiltration of mononuclear cells in sinusoids and cytoplasmic vacuolization in some perivenous hepatocytes in the livers treated with LPS without any evidence of a massive necrotic and apoptotic area.

Expression of CMOAT/MRP2 mRNA was time-dependently and dose-dependently decreased by LPS injection (Fig. 1A and 1B). The nadir of CMOAT/MRP2 mRNA levels appeared to be around 12 hours after LPS injection. No decrease in hepatic CMOAT/MRP2 mRNA levels was detected after saline injection. Similar but less marked results were obtained for expression of Na^+-taurocholate cotransporting (NTCP) mRNA and organic anion transporting protein (OATP) mRNA (data not shown). The hepatic protein levels of bilirubin-UDP-glucuronosyltransferase, enzyme locating in ER, were unchanged throughout the experiments (data not shown). Injection of 0.01-1.0 mg/kg LPS led to an insignificant increase in the serum levels of AST and ALT (data not shown). However, LPS injection significantly increased the plasma level of bilirubin from 0.071 ± 0.008 pg/ml (n = 3) before its

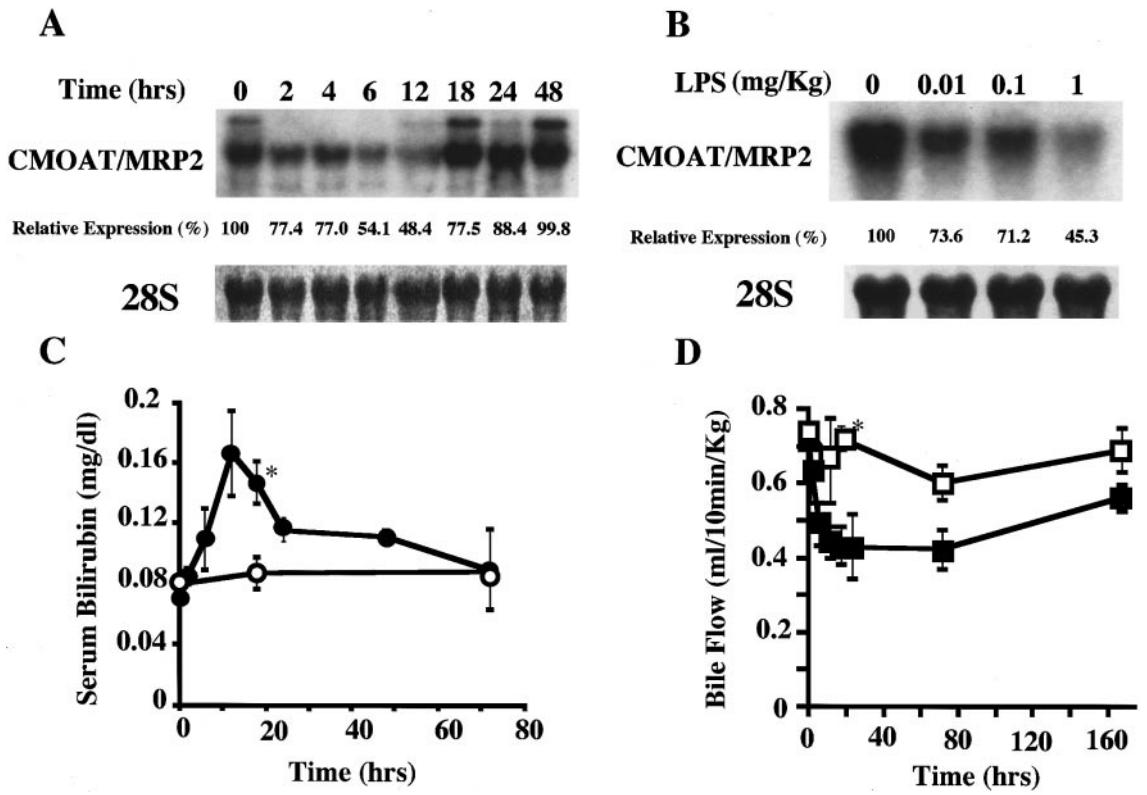

FIG. 1. Changes in serum bilirubin level, bile flow rate and hepatic CMOAT/MRP2 mRNA expression. (**A**) Time-course of northern blotting. Rats were intravenously injected with 1 mg/kg body LPS and hepatic CMOAT/MRP2 mRNA was measured. Typical northern blotting and mean values of data obtained by the densitometory were shown (n = 3). (**B**) Dose dependency. Various amounts of LPS were intravenously injected into rats. After 12 hours, hepatic CMOAT/MRP2 mRNA expression was measured. **C** and **D:** Rats were intravenously infused with 1 mg/kg body LPS (●, ■) or saline as a control (○, □). (**C**) Serum levels of bilirubin. Significant differences between the two groups (*) are indicated by repeated-measure ANOVA with the Scheffe's test ($P = 0.0480$). (**D**) Bile flow rates. Significant differences between the two groups (*) are indicated ($P = 0.0020$).

injection to 0.166 ± 0.027 pg/ml (n = 3) after 24 hrs, then, the plasma bilirubin level gradually decreased (Fig. 1C). The bile flow rate rapidly decreased after LPS injection, reached its nadir at 18 hrs after LPS injection, then gradually recovered (Fig. 1D). Neither increase in the plasma level of bilirubin nor decrease in bile flow rate was observed for saline-injected rats. The changes in the plasma bilirubin level and bile flow appeared to reflect those in hepatic CMOAT/MRP2 mRNA levels.

Gd Pretreatment Blocks Decrease in CMOAT/MRP2 Expression

The involvement of Kupffer cells in the LPS-induced decrease in the hepatic level of CMOAT/MRP2 mRNA was examined using Gd. Inactivation of Kupffer cells by Gd was confirmed by the fact that Gd pretreatment completely inhibited LPS-induced inducible nitric oxide synthetase (iNOS) immunoreactivity in Kupffer cells (data not shown). Gd pretreatment attenuated the increase in the plasma level of bilirubin and the decrease in bile flow rate after LPS injection (Fig. 2 A and 2B). In the liver, Gd pretreatment suppressed the decrease in hepatic CMOAT/MRP2 mRNA levels induced by LPS (Fig. 2C). Gd treatment itself had no effect on the plasma level of bilirubin, bile flow rate and hepatic CMOAT/MRP2 mRNA expression. These results suggest that Kupffer cell activation by LPS is involved in the LPS-induced decrease in hepatic CMOAT/MRP2 mRNA levels.

Kupffer Cell-Conditioned Medium Decreases Hepatic CMOAT/MRP2 mRNA Levels

Next, we examined which substances secreted from activated Kupffer cells were involved in the suppression of hepatic CMOAT/MRP2 mRNA expression using the Kupffer cell-conditioned medium. The medium, when incubated with LPS but not when incubated with saline, caused a decrease in the hepatic levels of CMOAT/MRP2 mRNA expression, although LPS itself had no effect on hepatic CMOAT/MRP2 mRNA expression (Fig. 3A and 3B). It is noteworthy that the Kupffer

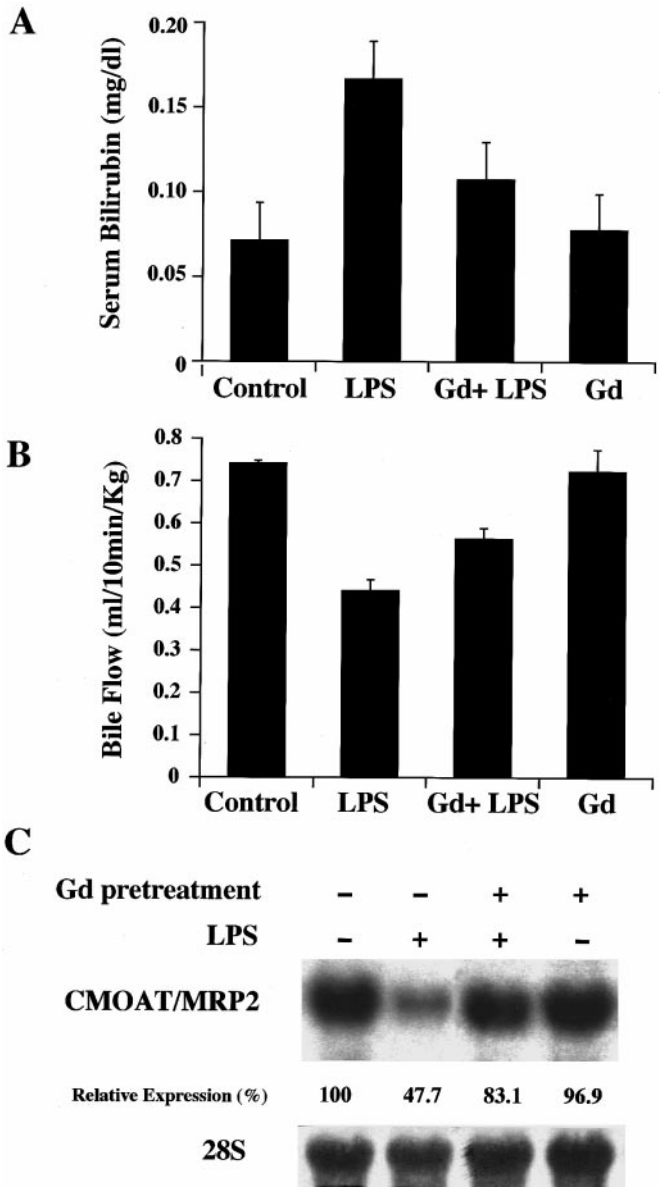

FIG. 2. Effects of Gd pretreatment. Gd (7 mg/kg body weight) was injected intravenously for two days before LPS injection. (**A**) After 12 hours, serum bilirubin levels were measured. Significant difference is found between the Sham and LPS groups ($P = 0.0281$), borderline significance between the LPS and Gd+LPS groups ($P = 0.0703$), and no significant difference between Sham and Gd+LPS groups ($P = 0.5217$). (**B**) Bile flow rates. Significant difference is found between the Sham and LPS groups ($P = 0.0017$) and between the LPS and Gd+LPS groups ($P = 0.0282$), and no significant difference between Sham and Gd+LPS groups ($P = 0.0688$). (**C**) Northern Blotting. Typical northern blotting and mean values of data obtained by the densitometory were shown (n = 3). Control; saline injection. LPS; LPS 1 mg/kg injection. Gd+LPS; Gd pretreatment and LPS 1 mg/kg injection. Gd; Gd pretreatment and saline injection.

cell-conditioned medium incubated with LPS for 1-2 hours already had substances suppressing hepatic CMOAT/MRP2 mRNA expression.

Cytokines Decreases Hepatic CMOAT/MRP2 mRNA Levels

Activated Kupffer cells secrete many substances after LPS stimulation, and TNFα and IL-1 are major secretagogues during 1 or 2 hours incubation with LPS (Kawada unpublished data and Ref. 8 & 17). Addition of anti-IL-1α antibody attenuated LPS-induced suppression of hepatocyte CMOAT/MRP2 mRNA expression (Fig. 4A). Addition of anti-TNFα antibody had a little effect on LPS-induced suppression of CMOAT/MRP2 mRNA expression. Restoration of hepatocyte CMOAT/MRP2 mRNA expression was more predominantly by addition of both antibodies. On the other hand, addition of TNFα, IL-1, or both to isolated hepatocytes suppressed hepatocyte CMOAT/MRP2 mRNA expression (Fig. 4B). These results suggested that IL-1, and partly TNFα, were responsible for LPS-induced and Kupffer cell-mediated hepatocyte CMOAT/MRP2 mRNA suppression.

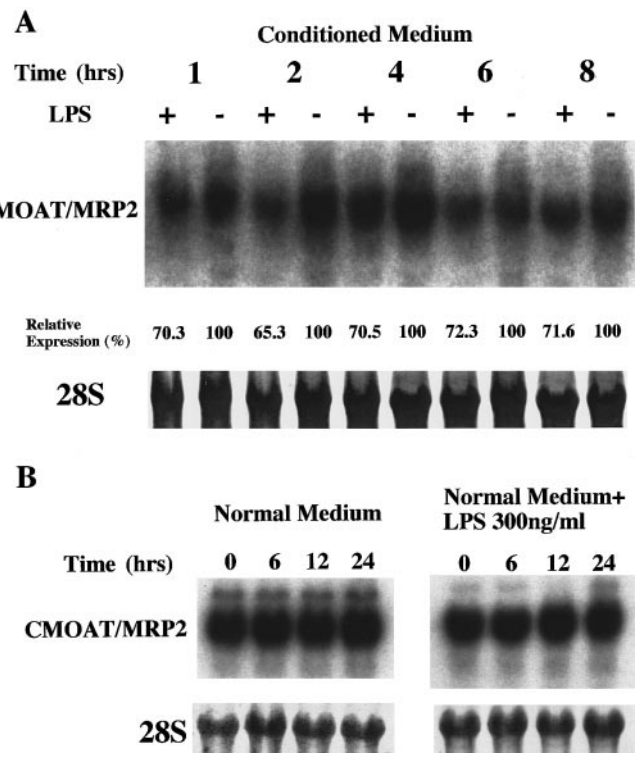

FIG. 3. Decrease of hepatocyte CMOAT/MRP2 expression by the Kupffer cell-conditioned medium. (**A**) Kupffer cells were incubated in Williams' E containing 300 ng/ml LPS (+) or an equal amount of saline (−) without fetal calf serum for the indicated times. Each culture medium was collected and added to the prepared hepatocytes. Typical northern blotting was shown. In data obtained by the densitometory, relative values of CMOAT/MRP2 mRNA expression in the presence of LPS to those in its absence were shown (n = 3). (**B**) Hepatocytes were incubated in Williams' E medium without 10% fetal calf serum containing 300 ng/ml LPS (right panel) or an equal amount of sterilized saline (left panel) for 12 hours at 37°C, and total RNA was extracted.

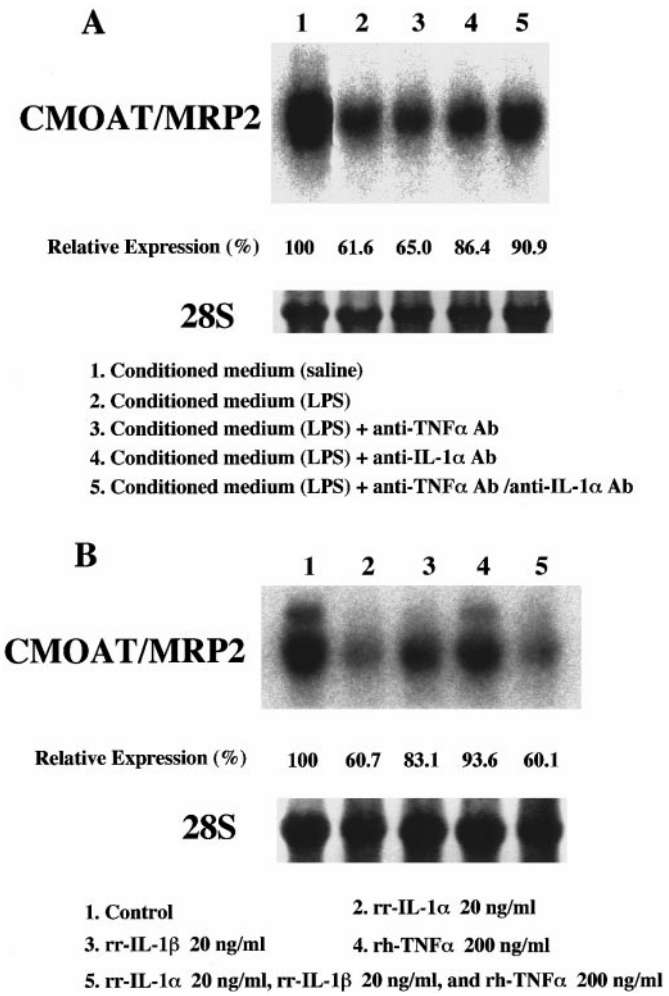

FIG. 4. Effects of IL-1 and TNFα on hepatocyte CMOAT/MRP2 expression. (**A**) Neutralization experiments. After addition of goat immunoglobulin (Lane 2), anti-rat TNFα antibody (Lane 3), anti-mouse IL-1α antibody (Lane 4), or both antibodies (Lane 5), the Kupffer cell-conditioned medium was preincubated at 37°C for 1 hour, then added to hepatocytes. (**B**) Stimulation experiments. Hepatocytes were incubated in Williams' E medium containing no additives (Lane 1), rr-ILα 20 ng/ml (Lane 2), rr-ILβ 20 ng/ml (Lane 3), rh-TNFα 200 ng/ml (Lane 4), or all (Lane 5). Typical northern blotting and mean values of data obtained by the densitometory were shown (n = 3).

Activated MAP Kinases Involved in CMOAT/MRP2 Downregulation

Because TNFα and IL-1 have various intracellular signaling pathways including the MAP kinase pathways (19–21) and because the MAP kinases have been reported to be one of the intracellular targets of LPS (22), we examined their involvement using kinase assay. Immunoblotting confirmed that the presence of 44 and 42 kDa ERK, JNK and p38 MAPK and showed that 44 kDa ERK was the most abundant in the hepatocytes (data not shown). The Kupffer cell-conditioned medium incubated with LPS greatly activated the MAP kinases, while serum exchange slightly stimulated three of them (Fig. 5A). Among three kinases, ERK activation appeared to be most activated. Activation of JNK in the presence of the conditioned medium was less significant than that in the presence of anisomicin. The involvement of these MAP kinases in LPS-induced suppression of CMOAT/MRP2 mRNA expression was examined using their specific inhibitors. Additions of PD98059, an inhibitor of ERK, and SB203580, an inhibitor of p38 MAPK, partly restored hepatocyte CMOAT/MRP2 mRNA expression (Fig. 5B). These results suggested that activation of at least two MAP kinases, ERK and p38 MAPK, are involved in LPS-induced and Kupffer cell-mediated suppression of hepatocyte CMOAT/MRP2 mRNA expression.

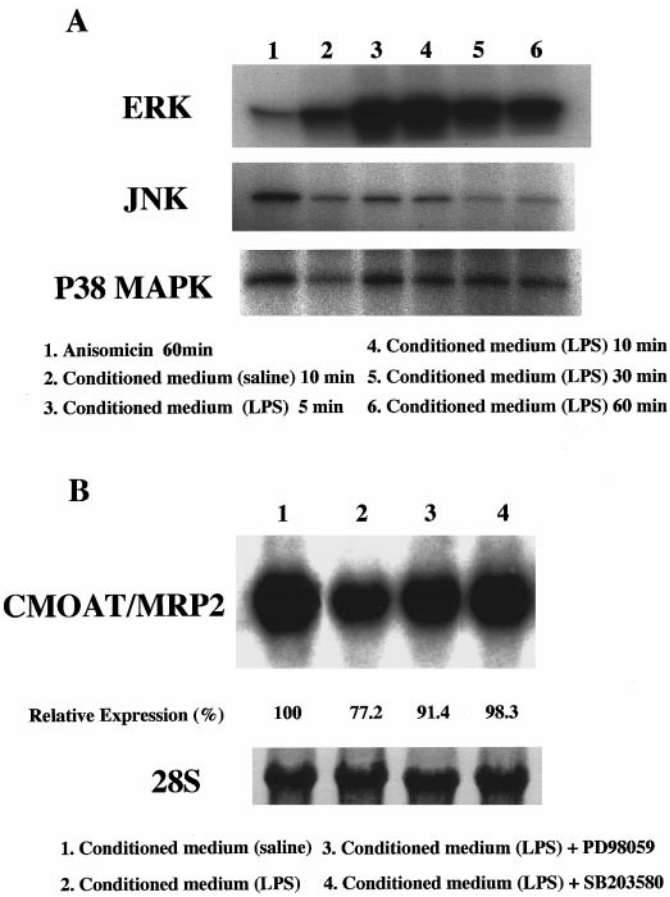

FIG. 5. Activation of MAP kinases. (**A**) Kinase Assay. The activities of ERK, JNK, and p38 MAPK in hepatocytes were measured after addition of 10 ng/ml anisomicin (Lane 1) and the Kupffer cell-conditioned medium treated with saline (Lane 2) or with 300 ng/ml LPS (Lanes 3–6) at the indicated times. (**B**) Northern blotting. Hepatocytes were incubated in the Kupffer cell-conditioned medium treated with saline (Lane 1) or with 300 ng/ml LPS containing 0.2% DMSO (Lane 2), 20 nM PD98059 (Lane 3), or 10 nM SB203580 (Lane 4). Typical northern blotting and mean values of data obtained by the densitometory were shown (n = 3).

DISCUSSION

A bolus injection of a large quantity of LPS may induce systemic hypotension and shock, which may result in hypoxic hepatic injuries, however, even without systemic hypotension and shock, cholestatic liver injury may occur. In the present investigation, the dose of LPS was set at levels which do not cause hypotension. The concentrations of LPS used are postulated to be within the range of portal endotoxin concentration during septic conditions. At these doses and concentrations, the increase in serum bilirubin and decrease in the bile flow were confirmed without any increase in the serum levels of AST and ALT. These conditions may correspond to cholestatic liver dysfunction in the clinical settings.

Previous studies indicated that Kupffer cells play an important role in hepatic injury caused by several different mechanisms including LPS-induced morphological changes in the liver (23,24). As the addition of LPS to isolated hepatocytes has no effect, liver injury under septic conditions is not thought to be directly induced by LPS. Gd pretreatment restored hepatic CMOAT/MRP2 mRNA expression, and inhibited cholestasis. The Kupffer cell-conditioned medium treated with LPS contained substances that cause suppression of hepatocyte CMOAT/MRP2 mRNA expression. These results have suggested that substances secreted from activated Kupffer cells may decrease hepatic CMOAT/MRP2 mRNA expression and bile flow and increase serum bilirubin.

Kupffer cells are resident macrophages in the liver, which can produce a large variety of cytokines including IL-1, IL-6, IL-8, IL-10, IL-12, TNFα, IFNα, IFNγ, G-CSF, and GM-CSF (18). Among these cytokines, IL-1 and TNFα are secreted at the early phase after expose to stimuli such as LPS (25). TNFα or IL-1 decreases the expression of CMOAT/MRP2 mRNA, and anti-TNFα and/or anti-IL-1α antibodies restored it. The effects of IL-1 and anti-IL-1α antibody appeared to be predominant over those of TNFα and anti-TNFα antibody, respectively (Fig. 4), suggesting that IL-1 is a stronger suppressant of hepatic CMOAT/MRP2 mRNA expression than TNFα. Thus, IL-1 secreted from Kupffer cells may be responsible for LPS-induced suppression of hepatic CMOAT/MRP2 mRNA expression.

Understanding the mechanisms of LPS-induced suppression of hepatic genes is important for elucidating the mechanisms of liver dysfunction and functional changes of the liver under septic conditions. LPS may directly and indirectly activate intracellular signaling pathways including the death signaling and MAP kinase pathways (22,26,27). We showed that Kupffer cells-conditioned medium activates the MAP kinases in the hepatocytes, especially ERK and p38 MAPK (Fig. 5). Although expression of many genes is regulated by the MAP kinases including the ERK kinase-NF kB pathway, most reported genes are upregulated by these kinases and only a few reports have described the mechanisms of downregulation (28–30). IL-6 downregulates the hepatic albumin gene via C/EBP-LAP, transcriptional activators (31,32). Recently, the downregulation of NTCP gene expression by LPS is shown to be mediated via tandem reductions in the nuclear binding activity of hepatocyte nuclear factor (HNF) 1 and Footprint B binding protein (FpB BP) (33). The 5′-flanking region of rat CMOAT/MRP2 was recently cloned and sequenced with putative binding sites of consensus sequences for Y-Box, GC-Box, AP1, CBF, CDP2, C/EBPα, EFIA, HNF-1, c-Myb, PEA3 and Sp1 (34). However, the mechanism of transcriptional downregulation of hepatic CMOAT/MRP2 under septic conditions remains unknown. The regulation of hepatic gene expression and its clinical relevance are of interest and are under investigation.

ACKNOWLEDGMENTS

The authors thank Dr. Jayanta Roy-Chowdhury (Marion Bessin Liver Research Center, Albert Einstein College of Medicine, NY) for his kind gift of anti bilirubin-UDP-glucuronosyltransferase antibody. The authors also thank Dr. Yasuo Uchiyama (Department of Cell Biology & Anatomy I, Osaka University Medical School) for his helpful discussions. This work was supported in part by Grants for Scientific Research No. 08671366 and 09671305 from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1. Green, R. M., Beier, D., and Gollan, J. L. (1996) *Gastroenterology* **111,** 193–198.
2. Moseley, R. H., Wang, W., Takeda, H., Lown, K., Shick, L., Ananthanarayanan, M., and Suchy, F. J. (1996) *Am. J. Physiol.* **271,** G137–G146.
3. Zimmerman, H. J., Fang, M., Utili, R., Seef, L. B., and Hoofnagle, J. Jaundice due to bacterial infection. (1979) *Gastroenterology* **77,** 362–374.
4. Moseley, R. H. Sepsis-associated cholestasis. (1997) *Gastroenterology* **112,** 302–306.
5. Whiting, J. F., Green, R. M., Rosenbluth, A. B., and Gollan, J. L. (1995) *Hepatology* **22,** 1273–1278.
6. Roelofsen H., van der Veere, C. N., Ottenhoff, R., Schoemaker, B., Jansen, P. L. M., and Oude Elferink, R. P. J. (1994) *Gastroenterology* **107,** 1075–1084.
7. Roelofsen, H., Schoemaker, B., Bakker, C., Ottenhoff, R., Jansen, P. L. M., and Oude Elferink, R. P. J. (1995) *Am. J. Physiol.* **269,** G427–G434.
8. Decker, K. (1990) *Eur. J. Biochem.* **192,** 245–261.
9. Trauner, M., Arrese, M., Soroka, C. J., Ananthanarayanan, M., Koeppel, T. A., Schlosser, S. F., Suchy, F. J., Keppler, D., and Boyer, J. L. (1997) *Gastroenterology* **113,** 255–264.
10. Muller, M., and Jansen, P. L. M. (1997) *Am. J. Physiol.* **272,** G1285–G1303.
11. Keppler, D., and Arias, I. M. (1997) *FASEB J.* **11,** 15–18.
12. Plaulusma, C. C., Bosma, P. J., Zaman, G. J. R., Bakker, C. T. M., Otter, M., Scheffer, G. L., Scheper, R. J., Borst, P., and Oude Elferink, R. P. J. (1996) *Science* **271,** 1126–1128.
13. Paulusma, C. C., Kool, M., Bosma, P. J., Scheffer, G. L., Borg,

F. T., Scheper, R. J., Tytgat, G. N. J., Borst, P., Baas, F., and Oude Elferink, R. P. J. (1997) *Hepatology* **25,** 1539–1542.

14. Lazar, G. (1973) *J. Reticuloendothelial Society* **13,** 231–237.
15. Rizzardini, M., Zappone, M., Villa, P., Gnocchi, P., Sironi, M., Diomede, L., Meazza, C., Monshouwer, M., and Cantoni, L. (1998) *Hepatology* **27,** 703–710.
16. Kawada, N., Mizoguchi, Y., Kobayashi, K., Monna, T., Morisawa, S., Ueda, N., Omoto, Y., Takahashi, Y., and Yamamoto, S. (1992) *Gastroenterology* **103,** 1026–1033.
17. Hayashi, K., Saga, H., Chimori, Y., Kimura, K., Yamanaka, Y., and Sobue, K. (1998) *J. Biol. Chem.* **273,** 28860–28867.
18. Cavaillon, J. M. (1994) *Biomed. Pharmacother.* **48,** 445–453.
19. Liu, Z., Hsu, H., Goeddel, D. V., and Karin, M. (1996) *Cell.* **87,** 565–576.
20. Malinin, N. L., Boldin, M. P., Kovalenko, A. V., and Wallach, D. (1997) *Nature* **385,** 540–544.
21. Lo, Y. Y. C., Luo, L., McCulloch, C. A. G., and Cruz, T. F. (1998) *J. Biol. Chem.* **273,** 7059–7065.
22. Han, J., Lee, J. D., Bibbs, L., and Ulevitch, R. J. (1994) *Science* **265,** 808–811.
23. Sarphie, T. G., D'Souza, N. B., and Deaciuc, I. V. (1996) *Hepatology* **23,** 788–796.
24. Adachi, Y., Bradford, B. U., Gao, W., Bojes, H. K., and Thurman, R. G. (1994) *Hepatology* **20,** 453–460.
25. Chensue, S. W., Terebuh, P. D., Remick, D. G., Scales, W. E., and Kunkel, S. (1991) *Am. J. Pathol.* **138,** 395–402.
26. Bohlinger, I., Leist, M., Gantner, F., Angermuller, S., Tiegs, G., and Wendel, A. (1996) *Am. J. Pathol.* **149,** 1381–1393.
27. Nagata, S. (1997) *Cell* **88,** 355–365.
28. Read, M. A., Whitley, M. Z., Gupta, S., Pierce, J. W., Best, J., Davis, R. J., and Collins, T. (1997) *J. Biol. Chem.* **272,** 2753–2761.
29. Kumar, A., Middleton, A., Chambers, T. C., and Metha, K. D. (1998) *J. Biol. Chem.* **273,** 15742–15748.
30. Guan, Z., Baier, L. D., and Morrison, A. R. (1997) *J. Biol. Chem.* **272,** 8083–8089.
31. Descombes, P., and Schibler, U. (1991) *Cell.* **67,** 569–579.
32. Trautwein, C., Rakemann, T., Pietrangelo, A., Plumpe, J., Montosi, G., and Manns, M. P. (1996) *J. Biol. Chem.* **271,** 22262–22270.
33. Tauner, M., Arrese, M., Lee, H., Boyer, J. L., and Karpen, S. J. (1998) *J. Clin. Invest.* **101,** 2092–2100.
34. Kauffmann, H. M., and Schrenk, D. (1998) *Biochem. Biophys. Res. Commun.* **245,** 325–331.