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Bacterial Lipopolysaccharide Induces Uncoupling Protein-2 Expression in Hepatocytes by a Tumor Necrosis Factor-α-Dependent Mechanism

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The liver is a target for bacterial lipopolysaccharide (LPS) and participates in the metabolic response to endotoxemia. Recently published evidence indicates that LPS increases the expression of mitochondrial uncoupling protein-2 (UCP-2) mRNAs in several tissues, including the liver. Because hepatocytes in the healthy liver do not express UCP-2, LPS was thought to induce UCP-2 in liver macrophages, which express UCP-2 constitutively. However, the present studies of cultured peritoneal macrophages indicate that LPS reduces steady state levels of UCP-2 mRNAs in these cells. In contrast, UCP-2 mRNAs are induced in hepatocytes isolated from LPS treated rats and transfection of these hepatocytes with UCP-2 promoter-reporter constructs demonstrates substantial increases in UCP-2 promoter activity. LPS induction of hepatocyte UCP-2 expression is virtually abolished by prior treatment of rats with neutralizing antibodies to tumor necrosis factor α (TNF). Futhermore, TNFα treatment induces UCP-2 mRNA accumulation in primary cultures of hepatocytes from healthy rats. Thus, hepatocytes are likely to be important contributors to endotoxin-related increases in liver UCP-2 via a mechanism that involves the LPS-inducible cytokine, TNFα.

Exposure to sub-lethal doses of gut-derived bacterial products, such as lipopolysaccharide (LPS), provokes a systemic response that includes fever and increased oxygen consumption (1). The molecular mechanisms responsible for such LPS-related "hypermetabolism" remain poorly understood, although LPS-inducible pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF, cachectin) and interleukin 1-beta (IL-1β, endogenous pyrogen) are clearly involved (2, 3). Recently, several groups have identified novel mitochondrial uncoupling proteins (UCPs), which can promote increased oxygen consumption and heat production (i.e., hypermetabolism) by uncoupling mitochondrial electron transport from ATP synthesis (4-6). To date, genes encoding three distinct UCP's have been cloned (4, 5, 7). UCP-1 and UCP-3 are expressed predominately in adipose tissue and muscle. UCP-2 is expressed in these tissues but is also more ubquitous, with low levels of expression in several other tissues, including heart, liver, lung, pancreas, kidney, and brain (5). UCP-2 is also expressed constitutively in macrophages (4) and resident macrophages appear to account for most, if not all, of the UCP-2 mRNAs that are found in some tissues, such as the liver (8). Uncoupling proteins are believed to regulate energy homeostasis and thermogenesis in muscle and adipose tissues (7, 9, 10). However, at present, relatively little is known about the role(s) of uncoupling proteins in the physiology and pathophysiology of other organs, including the liver.

The liver is a sensitive target organ for many LPS-inducible cytokines, including TNF and TNF-regulated cytokines (11). After exposure to LPS, hepatic oxygen consumption increases (12), the expression of many hepatocyte genes is modified to affect changes in glucose, lipid and amino acid metabolism, and the synthesis of various acute-phase proteins is initiated (13-15). The present work tests the hypothesis that hepatocytes respond to LPS by up-regulating UCP-2 which, in turn, may contribute to the evolution of this "hypermetabolic" state.

MATERIALS AND METHODS

Materials. All chemicals were purchased from Sigma Co. (St. Louis, MO) with the following exceptions. Collagenase was obtained from Worthington (Freehold, NJ). Trizol reagent, tissue culture medium (Dulbecco's Modified Eagle's medium (DMEM) and Chee's medium), bovine collagen (Vitrogen 100), and plastic tissue culture dishes (Falcon) were purchased from GIBCO-BRL (Gaithersburg, MD). Chloroform and isopropyl alcohol from Baker (Phillispsburg,

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NJ). Agarose was obtained from Life Technologies (Gaithersburg, MD) and nylon membranes from Dupont (Boston, MA). Nylon mesh screens (Nitex 153) were purchased from Tetko Inc. (NY). Maxiprep columns were purchased from Qiagen (Valencia, CA). The PGL3-BA luciferase expression vector was obtained from Promega (WI). Murine UCP-2 cDNA was obtained from an adipocyte cDNA library and characterized in M.D. Lane's laboratory (Dept. of Biological Chemistry, John Hopkins University, Baltimore, MD). Neutralizing goat anti-mouse polyclonal anti-TNF antibody (Ab TNF) was provided by Dr. Greg Bagby (Department of Physiology, Louisiana State University, New Orleans, LA). This antibody has been well characterized (16) and we previously used it to neutralize TNF and inhibit liver regeneration after PH (17-20).

Animal studies. Adult (6-8 week old) male Sprague-Dawley rats (mean weight, 250 g) were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, MA), and adult (8 week old) male ob/ob mice and their lean (?/ob) littermates were obtained from Jackson Laboratories (Bar Harbor, Maine). Rats and mice were housed in temperature and light controlled chambers on a 12 h:12 h light-dark cycle, and provided with rodent chow and water *ad libitum*. To evaluate UCP-2 expression in liver cells, rats were divided in three groups: Group 1 was injected intraperitoneally (i.p.) with 0.5 ml of sterile, pyrogen-free saline, Group 2 received an i.p. injection of lipopolysacaride (LPS) from E. coli serotype 0111:B4 (0.5 mg LPS/kg body weight), and Group 3 received the same dose of LPS 2 hours after being pre-treated with anti-TNF antibodies (60 mg/kg body weight, i.p.). Some rats were sacrificed at different time points after treatment to harvest the entire liver. Others underwent *in situ* perfusion of the liver 24 h after treatment with LPS or vehicle so that hepatocytes could be isolated. In other studies, ob/ob mice and their lean litter mates were injected i.p. with either vehicle (phosphate buffered saline), or LPS (10 μg/mouse).

Peritoneal macrophages were also harvested from rats. For these studies, animals were injected i.p. with 3% thioglycollate. Four days later, they were sacrificed by CO_2 insuffication and the peritoneal cavity was lavaged immediately with sterile, pyrogen-free saline to obtain the thioglycollate-elicited peritoneal macrophages as described (21). All animal experiments fulfilled Johns Hopkins University and National Research Council criteria for humane care.

Hepatocyte isolation and culture. Hepatocytes were obtained from rat liver by *in situ* liver perfusion with collagenase. Briefly, the rats were anesthetized with Ketamyne (60 mg/kg) and Xylazine (2mg/kg) i.p., and the abdomen was opened with a midline incision under sterile conditions. After cannulation of the portal vein, the liver was perfused with Hank's EGTA solution, followed by 0.03% collagenase, and then removed from the carcass. Cells were suspended in Hanks solution, filtered and centrifuged. After washing the hepatocyte pellet, cells were resuspended in Hanks solution. An aliquot was removed to confirm the purity of the isolation and to assess viability by trypan blue staining (22). Such inspection typically indicated that preparations contained >97% viable cells, of which >99% were hepatocytes.

Cells were plated in bovine collagen (Vitrogen 100)-coated, plastic dishes (Falcon) at a density of 10×10^6 cells/10 cm dish, in 10 ml of Chee's medium. Cultures were kept in humidified incubators at 37^0C in 5% CO_2/95% O_2 and allowed to attach to the plates for 4 hours. Thereafter, cells were either harvested immediately (to assess the effects of *in vivo* LPS ± Ab TNF), plated for various times (0.5, 3, 6 or 24 h) with recombinant murine TNFα (600 ng/plate), or used for transfection experiments. At the time of harvest, cells were placed in RNA lysis buffer (Tryzol), flash frozen in liquid nitrogen, and kept at -80^0C until use.

Macrophage culture experiments. Peritoneal macrophages were plated on plastic dishes (Falcon) in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 10 mM HEPES (10^7 cells/dish). The cells were allowed to attach to the plates for 3-4 hours and then the medium was replaced. Cells were cultured over night at 37^0C in 5% CO_2/95% O_2 as described (21). The next morning, cultures were treated either with LPS (1 μg/ml) or an equal volume of pyrogen-free, normal saline. After 90 minutes, cells were harvested into RNA lysis buffer, flash frozen, and saved at -80^0C until RNA isolation.

RNA extraction and Northern blot analysis. Total RNA was isolated from whole liver or from cell cultures as described by Chomszenski and Sacchi (23). In each cell culture experiment, total RNA was isolated from cells pooled from 2 different plates/time point. In all studies, RNA was quantified first by UV absorption at 260 nm and then RNA quality and concentration were confirmed by electrophoresis on denaturating agarose mini-gels followed by staining with 0.04% methylene blue staining.

To evaluate treatment-related differences in UCP-2 expression, RNA (20 μg/lane) was fractionated by agarose gel electrophoresis, transferred by capillary blotting to nylon membranes, and hybridized with ^{32}P-labeled UCP-2 cDNA at 42^0C for 18 hours. After washing at a moderate stringency, blots were evaluated by phosphoimager and then exposed to x-ray film with intensifying screens. To confirm lane-lane equivalency of RNA, each membrane was stripped and re-examined for the expression of 18 S RNA.

All studies in intact rats and all cell culture experiment were repeated at least twice. RNA from each preparation was evaluated by Northern blot analysis. Phosphoimage results of each blot were normalized to the expression of 18 S RNA on the same membrane, and the normalized UCP-2 signal intensity was expressed as a percentage of the saline-treated control on the same blot. Results of different blots were averaged to calculate the mean (and standard error) induction of UCP-2 mRNA.

Hepatocyte transfection experiments. A DNA fragment containing 3.3 kb of 5′ flanking sequence of the UCP-2 gene (−3263 to +39) was subcloned into the *Sac*I and *Hin*dIII site of PGL3-BA luciferase expression vector, giving rise to pUcp2Luc-3.3. The pUcp2Luc-3.3 and CMV-β-gal constructs were purified for transfection by using Qiagen maxiprep column. The transfection procedure of hepatocytes was described by D. S. Pasco et al. (24). Hepatocytes (1×10^6 cells/dish) were plated in 60-mm plastic culture dishes with 5 ml of medium. The culture medium was DMEM/F12 (1:1) containing gentamycin sulfate (25 μg/ml) and amphotericin B (0.7 μg/ml), supplemented with insulin (5 μg/ml), dexamethasone (0.1 μM) and 4% fetal bovine serum. The medium was replaced every 24 hr and cultures were maintained at 37^0C in a humidified atmosphere containing 5% CO2 and 95% air. At 24 hr of culture, 3 μg of UCP-2 promoter constructs were cotransfected by calcium phosphate precipitation along with 3 μg of carrier DNA and 3 μg CMV-β-gal constructs into hepatocytes (25). After 8 hr, the plates were washed twice with warm medium. Thirty six hours later the cells were harvested and assayed for luciferase and β-galactosidase activity.

RESULTS

Effect of LPS on the Expression of UCP-2 by Hepatocytes

Northern blot analysis was used to evaluate the effect of LPS on liver UCP-2 expression in adult male and female rats. As shown in Fig 1a, little UCP-2 mRNA was detected in healthy animals of either gender before LPS treatment. However, in both sexes some induction of UCP-2 was apparent as early as 1 h after LPS injection and steady state UCP-2 mRNA levels had increased by at least 30 fold by 24 h after LPS injection (Fig. 1b). Female rats tended to exhibit a greater induction of UCP-2 than male rats. Because of possible gender-related differences in UCP-2 expres-

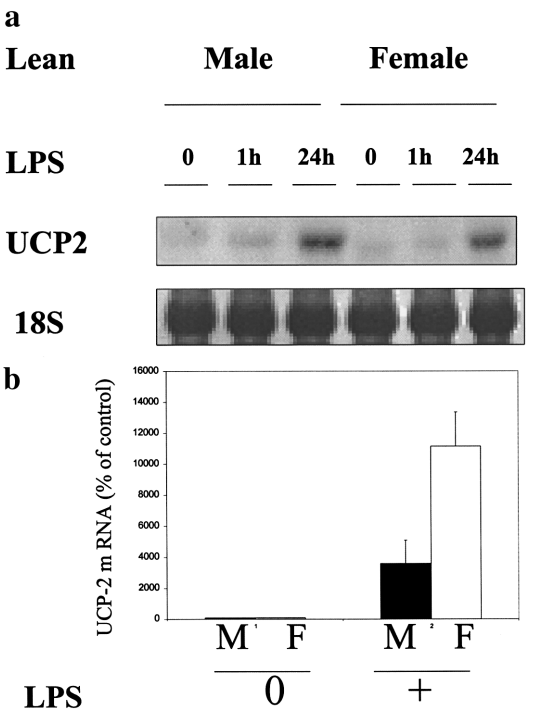

FIG. 1. Changes in hepatic expression of UCP-2 mRNA after LPS injection. Male and female rats were injected with sterile, pyrogen-free saline or LPS (0.5 mg/kg body weight), and total liver RNA was isolated before or 1 and 24 h after treatment. RNA (20 μg/lane) from each rat was separated by electrophoresis on agarose gels under denaturating conditions, transferred to membranes, and hybridized with ^{32}P-labeled cDNA for UCP-2. After stringent washings, blots were exposed to x-ray film with intensifying screens. (a) A representative Northern blot is shown. Bottom panel shows 18S RNA expression on this blot. (b) Graphic representation of phosphoimager analysis results from 3 experiments. On each blot, UCP-2 expression was normalized to 18S expression and expressed as the per centage of UCP-2 expression in the same gender at time 0 on the same blot. The mean ± SE of results from the different blots is shown ($p < 0.01$ for 24 h post-LPS vs time zero).

sion, all subsequent studies were performed in male rats.

Others have reported that macrophages, but not hepatocytes, express UCP-2 in the healthy liver (8) and, thus, UCP-2 is barely detected by Northern blot analysis of total liver RNA before treatment with LPS. However, the abundance of UCP-2 mRNAs in the liver after LPS treatment suggested to us that the gene may have been up-regulated in hepatocytes, because these cells account for more than 85% of the liver mass. To evaluate this possibility, collagenase perfusion of the liver was done to isolate hepatocytes from LPS-treated rats and saline-treated controls. As others have reported (8), we found little, if any, UCP-2 mRNA in hepatocytes harvested from control rats. However, hepatocytes isolated 24 h after *in vivo* LPS treatment expressed high levels of UCP-2 mRNA (Fig. 2, top panel). The mean induction of UCP-2 m RNA levels in hepatocytes isolated from LPS treated rats in 3 separate experiments was 8.6 ± 1.5 fold higher than that of controls ($p < 0.01$ for LPS-treated vs control).

Although the primary hepatocyte suspensions and cultures were not overtly contaminated with liver nonparenchymal cells, it is conceivable that a few resident macrophages may have been present. Therefore, subsequent studies were done to determine if LPS might also induce UCP-2 expression in macrophages. As shown in the middle panel of Fig. 2, unlike hepatocytes, thioglycollate-elicited peritoneal macrophages express UCP-2 message constitutively. Also, in contrast to hepatocytes which up-regulate UCP-2 mRNAs when challenged with LPS, macrophages down-regulate UCP-2 expression by more than 50% within 90 minutes of exposure to LPS. Taken together, the results shown in Fig. 2 support the concept that UCP-2 is an inducible gene in hepatocytes and indicate that it is up-regulated in these cells following exposure to LPS.

To determine if LPS may regulate expression mediated by the UCP-2 promoter, hepatocytes from control and LPS pretreated rats were transfected with luciferase reporter constructs driven by 3.3 kb of 5′ flanking sequence of the UCP-2 gene. As shown in Fig. 3, little reporter gene activity was demonstrated following transfection of control hepatocytes with this construct. However, very different results were noted when the 3.3 kb promoter-reporter construct was transfected into hepatocytes from LPS-treated rats. In the latter, reporter gene expression increased more than 10 fold, indicating that an LPS-responsive nucleotide sequence is located within -3.3 kb of the transcription start site in the UCP-2 gene and suggesting that LPS induces

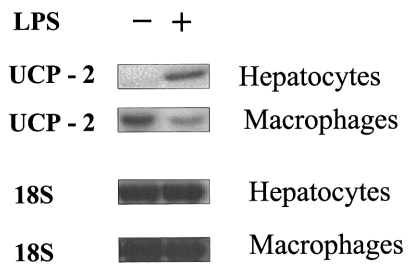

FIG. 2. Cell-specific changes in UCP-2 expression after LPS exposure. Rats were injected with LPS (0.5 mg/kg, i.p.) or a similar volume of saline. 24 h later, primary hepatocytes were isolated by *in situ* liver perfusion with collagenase. Hepatocytes were plated on collagen-coated plastic dishes in serum-free medium for 4 hours and then harvested for RNA isolation. Different rats were injected with thioglycollate to elicit peritoneal macrophages. Five days later, the rats were sacrificed and the macrophages harvested, plated on plastic dishes in serum-supplemented medium for 16 h and then treated with either vehicle or LPS (1 μg/ml) for 90 minutes. Cells were then harvested for isolation of total cellular RNA. Northern blot analysis (20 μg/lane) was used to evaluate UCP-2 expression in hepatocytes (top panel) and macrophages (second panel) before (−) and after (+) exposure of the animal (for hepatocytes) or cells (for macrophages) to LPS. Results from representative blots are shown. The bottom two panels demonstrate the 18 S RNA expression on the same two blots.

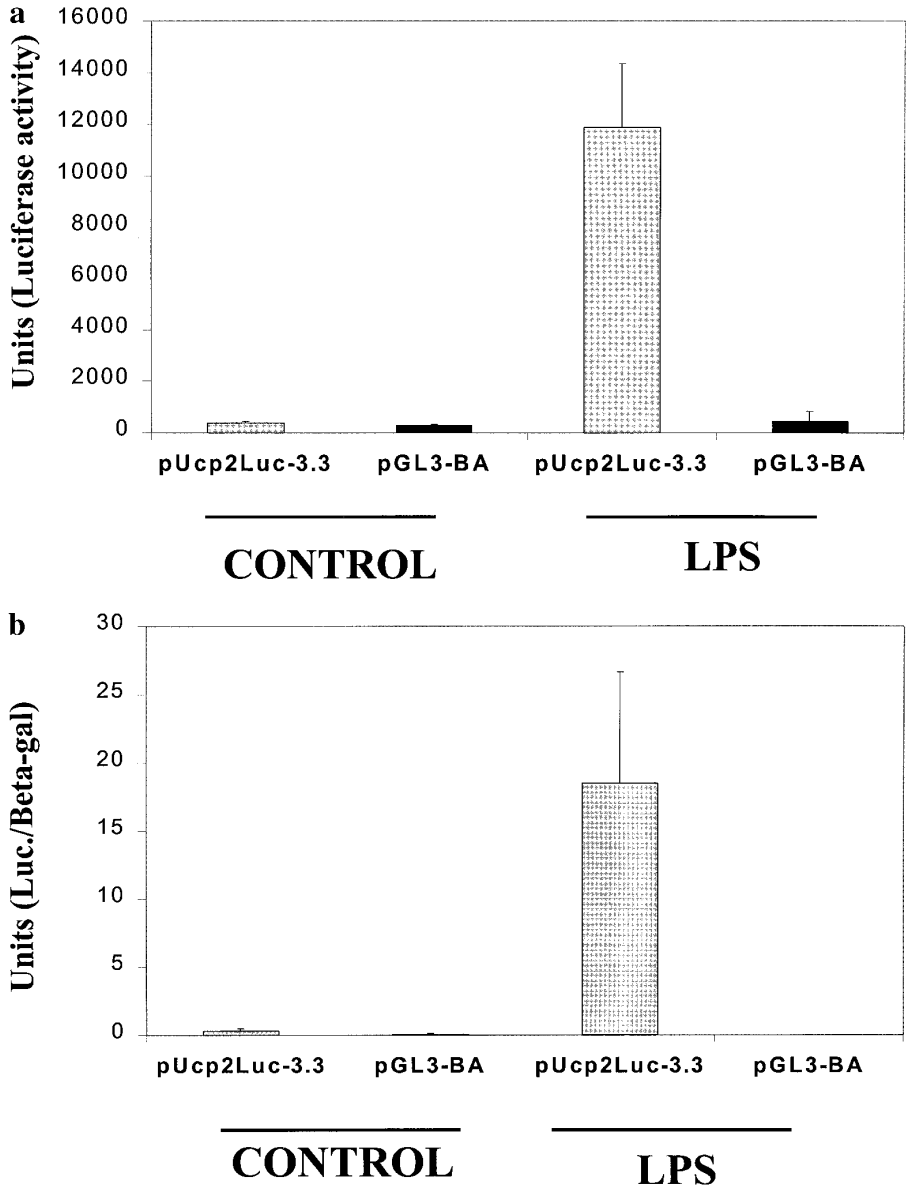

FIG. 3. UCP-2 promoter activity in rat primary hepatocytes. The 3.3kb DNA fragment containing UCP-2 promoter was subcloned into a luciferase expression vector (pGL3-BA) as described under Materials and Methods. Hepatocytes isolated from either normal or LPS-treated rats were transiently transfected with pUcp2Luc-3.3 and pGL3-BA. 3 μg of pUcp2Luc-3.3 or pGL3-BA was cotransfected by calcium phosphate precipitation along with 3 μg of CMV-β-gal and 3 μg of carrier DNA. Cell extracts were prepared and assayed for luciferase and β-galactosidase assay. (a) Relative luciferase activity of pUcp2Luc-3.3 was calculated by normalization to equal cell number. (b) Relative luciferase activity of pUcp2Luc-3.3 was normalized to β-galactosidase activity. Each data point is the mean ± SEM from three replicates.

UCP-2 expression by promoting increased transcription of the gene.

Role of TNF in LPS-Induction of UCP-2 mRNA Expression

Although hepatocytes may have receptors for LPS or LPS-binding protein (26), many LPS-associated responses are regulated by LPS-induced cytokines, such as tumor necrosis factor α (TNFα) or TNF-related cytokines. Indeed, TNFα has been implicated as a proximal mediator of many hepatic responses to LPS (11), and, thus, may regulate the induction of UCP-2 after LPS exposure. To evaluate this possibility, rats were pretreated with neutralizing goat antibodies to murine TNF or control, nonimmune goat anti-mouse IgG 2 hours before receiving vehicle or LPS.

As before, LPS increased expression of UCP-2 mRNA in hepatocytes from control rats (Fig. 4, lane 1). Notably, pre-treatment with neutralizing antibodies to TNF significantly inhibited this LPS-related induction of

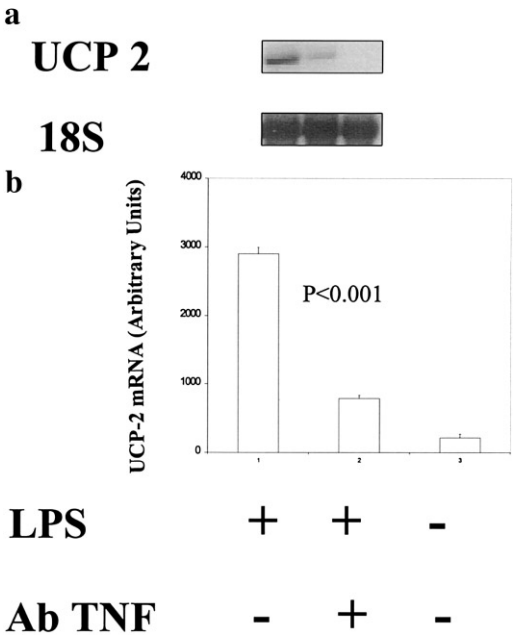

FIG. 4. Effect of TNF neutralization on hepatic UCP-2 induction by LPS. Using the techniques described earlier, RNA was isolated from hepatocytes that had been harvested from saline-treated control rats, rats that had been pre-treated with neutralizing anti-TNF antibodies 2 h before receiving LPS or LPS-treated rats that had been pre-treated with nonspecific goat anti-rodent IgG. This experiment was repeated twice. (a) A representative Northern blot demonstrating the effects of LPS and anti-TNF antibody treatments is shown. The bottom panel shows 18S RNA expression on this blot. (b) Graphic representation of the data from both experiments. In each study, UCP-2 expression was evaluated by phosphoimager analysis. Results were normalized to the 18 S RNA expression in the same study and are expressed as the mean ± SE arbitrary units.

UCP-2 (Fig. 4, lane 2). Phosphoimage analysis of blots from two separate experiments demonstrated a 70-80% reduction in UCP-2 expression after anti-TNF antibody treatment. Thus, the LPS-related induction of UCP-2 appears to involve the proinflammatory cytokine, TNFα.

To evaluate the role of TNFα in the induction of UCP-2 more directly, primary hepatocytes were harvested from normal rats and incubated with recombinant murine TNFα for varying periods of time (i.e., 0, 0.5, 3, 6, or 24 h). At the end of each incubation period, RNA was isolated and UCP-2 mRNA expression was evaluated by Northern blot analysis. Little UCP-2 message was detected in either experimental group until 24 h. However, at this time point, steady state levels of UCP-2 mRNA in TNF-treated cultures were 2-3 fold greater than in control cultures (Fig. 5). Thus, taken together, results of *in vivo* neutralization studies with anti-TNF antibodies (Fig. 4) and results from *in vitro* experiments with recombinant TNFα (Fig. 5) both suggest that TNF plays an important role in the up-regulation of UCP-2 message expression in hepatocytes.

UCP-2 Induction and LPS-Related Liver Injury

Mitochondrial uncoupling proteins are believed to compromise the efficiency of ATP synthesis by uncoupling mitochondrial oxidative phosphorylation (7, 9, 10). Since ATP depletion promotes cellular necrosis (27), LPS-related induction of UCP-2 may be involved in the pathogenesis of endotoxin liver injury. If UCP-2 contributes to LPS-induced liver injury, then UCP-2 may be relatively over-expressed in situations that enhance the hepatotoxic effects of endotoxin. We previously reported that genetically obese mice (ob/ob) and rats (fa/fa) were particularly vulnerable to endotoxin-related liver injury (28). To determine if this increased sensitivity to LPS-induced liver injury correlated with increased expression of UCP-2, liver RNA from ob/ob mice and their lean (?/ob) littermates were evaluated by Northern blot analysis. As shown in Fig. 6, steady state mRNA levels of UCP-2 were greater in ob/ob mice than controls before LPS treatment. LPS injection (10 μg/mouse) resulted in UCP-2 induction in both lean and obese mice. However, ob/ob mice expressed substantially more UCP-2 mRNA than controls even 24 h after LPS exposure. Similar results were obtained when UCP-2 expression was compared in fa/fa rats and their lean littermates (data not shown). Thus, these experiments showed a positive correlation between

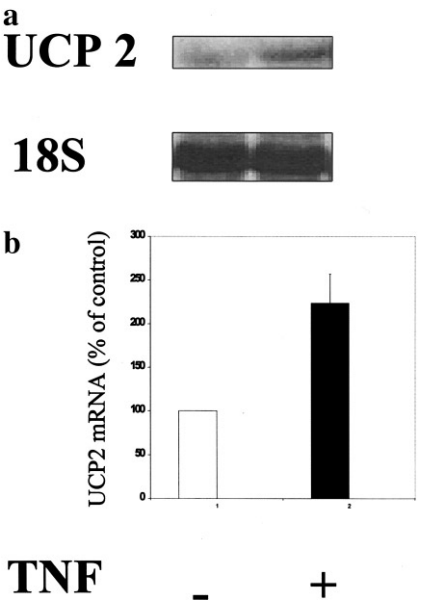

FIG. 5. Effect of TNFα on UCP-2 expression in primary cultures of rat hepatocytes. Hepatocytes were isolated from normal rats and cultured for varying times (0, 0.5, 3, 6, or 24 hours) in serum free medium with murine recombinant TNFα (600 ng/ml) or sterile saline. Total RNA was isolated and evaluated by Northern blot analysis. (a) A representative blot illustrating UCP-2 mRNA in control cultures and in TNF-treated cultures at the end of 24 h treatment is shown. The bottom panel demonstrates 18 S RNA expression on the same blot. (b) Graphic representation of the phosphoimager analysis of data from duplicate experiments.

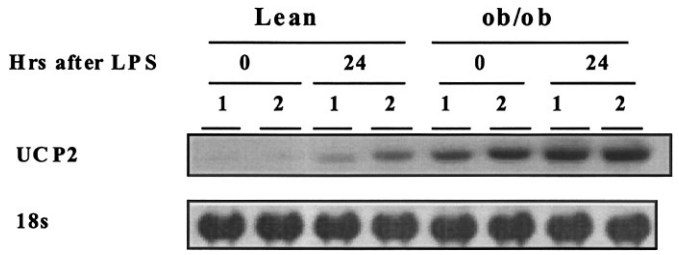

FIG. 6. Changes in hepatic expression of UCP-2 after LPS injection in lean and ob/ob mice. Northern blot analysis of UCP-2 mRNA levels before (0) and 24 h after the mice were treated with lipopolisaccharide (10 μg LPS i.p.). Total liver RNA from 2 mice/group were analyzed at each time point (20 μg/lane). Top panel, uncoupling protein-2 (UCP-2). Bottom panel, 18 S RNA on the same blot.

UCP-2 expression and sensitivity to LPS-related liver injury.

DISCUSSION

The liver is a target for bacterial endotoxins and plays a central role in the metabolic response to sepsis (11,13-15). The present results confirm a recent report that mitochondrial uncoupling proteins are induced in the liver following exposure to endotoxin (29) and provide novel evidence that LPS differentially regulates UCP-2 gene expression, inducing UCP-2 mRNAs in hepatocytes but repressing UCP-2 message expression in macrophages. Because hepatocytes do not express UCP-2 mRNA constitutively (8), the finding that UCP-2 is an LPS-inducible gene in hepatocytes was unanticipated. Furthermore, these data refute an earlier hypothesis which suggested that LPS increased liver UCP-2 expression primarily by inducing UCP-2 in hepatic macrophages (29).

In addition, the present work identifies a specific LPS-inducible cytokine, i.e., TNFα, that is involved in the induction of UCP-2 mRNA during endotoxemia by showing that neutralizing antibodies to TNFα inhibit LPS-related induction of the gene *in vivo* and that recombinant TNFα increases hepatocyte UCP-2 mRNA levels *in vitro.* Transfection experiments with UCP-2 promoter constructs indicate that UCP-2 promoter activity is increased after exposure to LPS, suggesting a potential molecular mechanism by which TNFα may induce UCP-2 expression. Computer assisted analysis of nucleotide sequences in the proximal 3.3 kb of the UCP-2 promoter identified potential consensus motifs for C/EBP and AP-1, transcription factors that are activated by TNF and TNF-regulated cytokines, such as IL-6 (11). Thus it is plausible that LPS may promote the transcriptional activation of UCP-2 via a TNF-dependent mechanism.

Others have suggested that LPS-related induction of UCP-2 is likely to contribute to thermogenesis during sepsis (29). However, since our results demonstrate that UCP-2 message is up-regulated in hepatocytes after LPS, it is conceivable that this gene may also mediate other responses to endotoxin. For example, since mitochondrial uncoupling proteins are known to inhibit oxidative phosphorylation (30), increased UCP-2 activity could result in decreased efficiency of hepatocyte ATP synthesis. This, in turn, may promote hepatocyte ATP depletion, leading to liver necrosis in situations where the demand for ATP exceeds ATP supply. Hence, UCP-2 induction may play a role in the pathogenesis of endotoxin liver injury. In support of this concept, we noted a positive correlation between liver UCP-2 expression and vulnerability to LPS-related liver injury. Genetically obese rodents, which exhibit liver injury when challenged with low doses of LPS (28), express high levels of hepatic UCP-2 mRNA. While these findings do not establish a definitive cause-effect relationship between UCP-2 and hepatocyte necrosis, they justify further study of this possibility.

Alternatively, hepatocyte UCP-2 induction could represent an adaptive response to the increased oxidant stress that follows endotoxin exposure (14). Negre-Salvayre et al. have shown that UCP-2 can modulate hydrogen peroxide generation and, in their studies, increased UCP-2 activity resulted in decreased hydrogen peroxide production (31). Thus, the up-regulation of UCP-2 in hepatocytes may be a consequence, rather than a cause, of LPS-related liver injury. If chronic oxidant stress contributes to UCP-2 induction in hepatocytes, this may explain why basal expression of UCP-2 is increased in obese rodents with fatty livers because these animals have higher than normal blood levels of TNF even before they are challenged with exogenous LPS (28). Ironically, if the primary "purpose" of UCP-2 induction is an anti-oxidant defense mechanism, this seemingly beneficial response could increase hepatocyte vulnerability to necrosis by compromising the efficiency of ATP synthesis. Thus, the ultimate physiological implication of UCP-2 induction may be dictated by the environment in which this response occurs.

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