Repression of Farnesoid X Receptor during the Acute Phase Response

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The acute phase response is associated with changes in the hepatic expression of genes involved in lipid metabolism. Nuclear hormone receptors that heterodimerize with retinoid X receptor (RXR), such as thyroid receptors, peroxisome proliferator-activated receptors, and liver X receptors, modulate lipid metabolism. We recently demonstrated that these nuclear hormone receptors are repressed during the acute phase response induced by lipopolysaccharide (LPS), consistent with the known decreases in genes that they regulate. In the present study, we show that LPS significantly decreases farnesoid X receptor (FXR) mRNA in mouse liver as early as 8 h after LPS administration, and this decrease was dose-dependent with the half-maximal effect observed at 0.5 μg/100 g of body weight. Gel-shift experiments demonstrated that DNA binding activity to an FXR response element (IRE) is significantly reduced by LPS treatment. Supershift experiments demonstrated that the shifted protein-DNA complex contains FXR and RXR. Furthermore, the expression of FXR target genes, SHP and apoCII, were significantly reduced by LPS (70 and 60%, respectively). Also, LPS decreases hepatic LRH expression in mouse, which may explain the reduced expression of CYP7A1 in the face of SHP repression. In Hep3B human hepatoma cells, both tumor necrosis factor (TNF) and interleukin-1 (IL-1) significantly decreased FXR mRNA, whereas IL-6 did not have any effect. TNF and IL-1 also decreased the DNA binding activity to an IRE response element and the expression of SHP and apoCII. Importantly, TNF and IL-1 almost completely blocked the expression of luciferase activity linked to a FXR response element promoter construct transfected into Hep3B cells. Together with our earlier studies on the repression of RXRs, peroxisome proliferator-activated receptors, LXR, thyroid receptors, constitutive androstane receptor, and pregnane X receptor, these results suggest that decreases in nuclear hormone receptors are major contributors to the decreased gene expression that occurs in the negative acute phase response.

The acute phase response (APR) is induced during infection, inflammation, and injury and is associated with a wide range of metabolic changes (1). Among these, changes in lipid metabolism have received much attention due to the link between infection/inflammatory diseases and atherosclerosis (2–6). The characteristic changes in lipid metabolism during the APR include hypertriglycerideremia (7), decreases in serum high density lipoprotein cholesterol levels (8, 9), increased hepatic cholesterol synthesis, inhibition of bile acid synthesis (10), increased hepatic fatty acid synthesis, and decreased hepatic fatty acid oxidation and ketogenesis (11, 12). These changes are mediated by alterations in gene expression caused by pro-inflammatory cytokines including TNFα, IL-1β, and IL-6 (10). However, the underlying mechanism by which these cytokines regulate gene transcription is not well understood, especially for the negative acute phase proteins.

Nuclear hormone receptors are ligand-activated transcription factors that are involved in various biological processes including development and physiological homeostasis (13). Small lipophilic molecules such as steroids, thyroid hormones, vitamin D, and retinoids bind to and activate these receptors to exert their physiological effects by regulating the transcription of specific genes (13–15). These receptors share common structural features, including central, highly conserved DNA binding domains and carboxyl-terminal ligand binding domains (13–15). They can be divided into four major subgroups based on their dimerization and DNA binding properties. Type II receptors are characterized by their DNA binding as a heterodimer with the 9-cis-retinoic acid receptor (RXR) (13, 16). This group includes the peroxisome proliferator-activated receptor (PPAR), retinoic acid receptor, vitamin D receptor, liver X receptor (LXR), and thyroid hormone receptors (TRs) (13, 17).

The farnesoid X receptor (FXR) was once an orphan receptor, and recently, it was found that bile acids are the ligands for FXR (18–20). FXR forms an obligate heterodimer with RXR and, thus, belongs to the Type II nuclear receptor subgroup. FXR has been shown to bind to FXR response elements (FXRE) composed of two inverted hexanucleotide repeats (AGGTCA spaced by one nucleotide (IR-1) (21). Chenodeoxycholic acid (CDCA), the most potent ligand for FXR, induces the ideal bile acid-binding protein (I-BABP) (18, 22), bile salt export pump (23), phospholipid transfer protein (PLTP) (21, 24), apolipoprotein CII (apoCII) (25), and SHP (26).

Activation of FXR down-regulates the expression of CYP7A1 via the action of SHP protein (26). FXR-induced SHP binds to and inactivates the liver receptor homolog 1 (LRH), a transcrip-
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FXR expression is decreased during the acute phase response.

Expression of two proteins that are regulated by FXR, bile salt export pump (BSEP) (31) and phospholipid transfer protein (PLTP) (32), are decreased during the APR. Therefore, we hypothesized that the bile acid receptor FXR is also suppressed during APR, affecting lipid/cholesterol metabolism. In the present study, we demonstrate that LPS and pro-inflammatory cytokines TNFα and IL-1β decrease the expression of FXR, its DNA binding activity to FXR response element (IR1), and transcription by FXR in studies of mice in vivo and the Hep3B human hepatoma cells in vitro, which is accompanied by decreased expression of FXR-regulated genes, SHP, and apocII.

The data suggest that altered FXR activity may contribute to the changes in lipid and cholesterol metabolism that occur during the APR.

EXPERIMENTAL PROCEDURES

Materials—LPS (Escherichia coli 55:B5) was obtained from Difco and freshly diluted to the desired concentration in pyrogen-free 90% saline. Minimum essential medium (MEM) was purchased from Fisher. Cytokines (human TNFα, human IL-1β, and human IL-6) were from R&D Systems and were freshly diluted to desired concentrations in serum-free MEM media containing 0.1% bovine serum albumin (fatty acid-free). Tri-Reagent and fatty acid-free bovine serum albumin were from Tri-Reagent (Sigma). DNA-protein complexes were separated by electrophoresis on a 4% polyacrylamide gel in 0.5 M Tris-buffered EDTA at 4°C. 32P-labeled oligonucleotide probes were end-labeled with T4-polyadenylate kinase (Amersham Biosciences). DNA-protein complexes were separated by electrophoresis (constant voltage of 200 V) on a 5% nondenaturing polyacrylamide gel in 0.5× Tris-buffed EDTA at 4°C. The gel was dried, exposed to x-ray film, and quantified by densitometry. Glyceraldehyde-3-phosphate dehydrogenase was used as a control probe. LRH-1 cDNA was kindly provided by Dr. Kristina Schoonjans (Institut de Genetique et de Moleculaire et Cellulaire, Universite Louis Pasteur, Paris, France). Mouse and human FXR, SHP, and apocII probes were prepared by PCR using the following primers: FXR-5′-GCT CAC TGT CGN CAA GTG ACC-3′ (upper), 5′-CCA NGA CAT CAG CAT CTC AGC-3′ (lower); SHP-5′-AGG GTG CTG CCC ATG CCA G-3′ (upper), 5′-GAG CTC CAG AAA AGC ATG TC-3′ (lower); apocII 5′-GCC AAG GAG GTT GCC AAA G-3′ (upper), 5′-GCT GTG ATG CGA GCA A-3′ (lower).

Preparation of Nuclear Extracts—Nuclear extracts were prepared according to Neish et al. (33). Briefly, cells were disrupted in a sucrose-buffered HEPES buffer containing 0.5% Nonidet P-40 as a detergent, protease inhibitors, and dithiothreitol. After disruption by 5 min of incubation on ice and centrifugation, nuclear proteins were separated in a NaCl-HEPES buffer and re-suspended in a glyceral-containing buffer. All the procedures were carried out on ice. Protein quantification was determined by the Bradford assay (Bio-Rad), and yields were similar in control and cytokine-treated groups.

Electromobility Shift Assay—10 µg of crude nuclear extract were incubated on ice for 30 min with 6× 10^4 cpm of 32P-labeled oligonucleotides in 15 µl of binding buffer consisting of 20% glycerol, 25 mM Tris-HCl, pH 7.5, 40 mM KCl, 0.5 mM MgCl2, 0.1 mM EDTA, 1 mM dithiothreitol, 2 µg of poly(dI-dC), and 1 µg of salmon sperm DNA. Double-stranded oligonucleotide probes were end-labeled with T4-polyadenylate kinase (Amersham Biosciences) in the presence of 50 µCi of [γ-32P]dATP and purified on a Sephadex G-25 column (Amersham Biosciences). DNA-protein complexes were separated by electrophoresis (constant voltage of 200 V) on a 5% nondenaturing polyacrylamide gel in 0.5× Tris-buffed EDTA at 4°C. The gel was dried, exposed to x-ray film, and quantified by densitometry. In the competition assay, a 100-fold molar excess of the specific or mutated unlabeled oligonucleotide was preincubated on ice for 1 h with 10 µg of mRNA extract from control cells. The following oligonucleotides were used: IR1, 5′-GATCGCCAGGGTGAATAACCTCGGGG-3′; mut-IR1, 5′-GATCGCCAGGGTGAATAACCTCGGGA-3′. Transfection Studies—Hep3B cells were grown overnight in 35-mm plates and washed twice with serum-free medium. DNA-Lipofectin complex containing 1.5 µg/mL FXRE-luciferase vector (a gift from Dr. Menge), University of Texas Southwestern Medical Center, Dallas, TX), 0.5 µg/mL of pRSV-β-galactosidase vector (a gift from Dr. Allan Pollack, Nephrology section at Veterans Affairs Medical Center, San Francisco, CA), and 5 µg/mL Lipofectin (Invitrogen) were allowed to form at room temperature for 15 min. The cells were overlaid with the DNA-Lipofectin complex and incubated at 37°C for 4–6 h. After washing the cells with serum-free medium, fresh growth medium containing 10% fetal bovine serum was added.

Cell Culture—Hep3B cells were maintained in MEM medium supplemented with 10% fetal bovine serum in 75-cm² flasks. Cells were seeded in 100-mm dishes at a concentration of 2×10^5 cells/dish. After an overnight incubation, cells were washed twice with phosphate-buffered saline (Ca²⁺- and Mg²⁺-free) and trypsinized before seeding. For typical experiments, cells were seeded in 100-mm dishes at a concentration of 2×10^5 cells/dish. After an overnight incubation, cells were washed twice with phosphate-buffered saline, and medium was replaced with fresh MEM (without serum) plus 0.1% bovine albumin and the appropriate cytokine concentration. For transfection assays, 1.5×10^5 cells were used/well in 6-well plates. RNA Isolation and Northern Blot Analysis—Total RNA from mouse kidney tissue was isolated from 300–400 mg of snap-frozen whole liver and ~100 mg of kidney tissue using Tri-Reagent (Sigma). Poly(A)+ RNA was subsequently purified using oligo(D) cellulose. RNA was quantified by measuring absorption at 260 nm. 10 µg of poly(A)+ RNA were denatured and electrophoresed on a 1% agarose, formaldehyde gel. Total RNA from Hep3B was isolated from a 100-mm dish by the Tri-Reagent method and resuspended in diethyl pyrocarbonate-treated water. 30 µg of total RNA was denatured and electrophoresed as described above. The uniformity of sample loading was checked by UV visualization of the ethidium bromide-stained gel before electrophoresis to Nytro membrane (Schleicher & Schuell). Prehybridization, hybridization, and washing procedures were performed as described previously (34). Membranes were probed with [α-32P]dCTP-labeled cDNAs using the random priming technique (Amersham Biosciences). mRNA levels were detected by exposure of the membrane to x-ray film and quantified by densitometry. Glyceraldehyde-3-phosphate dehydrogenase was used as a control probe. LRH-1 cDNA was kindly provided by Dr. Kristina Schoonjans (Institut de Genetique et de Moleculaire et Cellulaire, Universite Louis Pasteur, Paris, France). Mouse and human FXR, SHP, and apocII probes were prepared by PCR using the following primers: FXR-5′-GCT CAC TGT CGN CAA GTG ACC-3′ (upper), 5′-CCA NGA CAT CAG CAT CTC AGC-3′ (lower); SHP-5′-AGG GTG CTG CCC ATG CCA G-3′ (upper), 5′-GAG CTC CAG AAA AGC ATG TC-3′ (lower); apocII 5′-GCC AAG GAG GTT GCC AAA G-3′ (upper), 5′-GCT GTG ATG CGA GCA A-3′ (lower).

RESULTS

LPS Decreases FXR mRNA Level—We initially determined the effect of LPS administration on the FXR mRNA levels in...
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Because hepatic expression of FXR is down-regulated by LPS administration in mouse liver, next we examined if FXR expression is altered in the intestine and the kidney, which also express FXR. As in the liver, LPS administration for 16 h caused a significant decrease (−55%) in the level of FXR mRNA in the kidney (Fig. 1C). However, FXR mRNA was not reduced in the intestine (−130%), suggesting that LPS causes tissue-specific responses in terms of FXR expression. These results indicate that LPS-induced APR reduces expression of FXR in mouse liver and kidney but not in the intestine.

LPS Administration Decreases the DNA Binding Activity of FXR—Nuclear hormone receptors exert their effect on transcriptional regulation by binding to their cognate response element in the promoter region of target genes. To determine whether the reduction of FXR mRNA caused by LPS administration affects the DNA binding activity of FXR, we isolated nuclei from mouse liver and performed the electrophoretic gel mobility shift assay using a 32P-labeled DNA oligonucleotide containing FXR response element IR-1. As shown in Fig. 2A, two major FXR-IR1 complexes were observed in the control samples. LPS administration significantly decreased the binding of proteins in the nuclear extract from mouse liver to IR1 when compared with the control. LPS decreased FXR-IR1 binding by −75 and −50% for the upper and the lower band, respectively (Fig. 2B). Competition with 100-fold molar excess of specific oligonucleotide (WT), but not with mutated oligonucleotide (Mut), abolished the complex formation of radiolabeled IR1 with FXR (Fig. 2A), demonstrating the specificity of the two complexes. Furthermore, we demonstrated that the complexes contain FXR and RXR. They were supershifted with anti-FXR (SS1, second lane) and anti-RXR (SS2, third lane) antibody (Fig. 3). The migration of FXR-DNA complex was not affected by nonspecific IgG (fifth lane).

LPS Administration Reduces mRNA Levels of SHP and ApoCII—To determine whether the decreased binding of FXR to DNA in hepatic nuclear extracts from LPS-treated mouse is associated with reduced transcription of FXR-regulated genes, we investigated the effect of LPS administration on the mRNA levels of SHP and apoCII, two of genes that are known to be regulated by FXR. SHP is a key mediator of the FXR effect on the transcriptional regulation of CYP7A1 gene, and SHP is induced by FXR activation. As shown in Fig. 4, LPS administration decreases the expression of SHP by −70% and apoCII by 60% in mouse liver when compared with control. These data demonstrate that FXR-regulated genes are also repressed during LPS-induced APR in the mouse.

LRH Expression Decreases during APR—It is well established that FXR down-regulates CYP7A1 expression as a feedback mechanism to maintain homeostasis of bile acid metabolism (26, 27). This is achieved by FXR-induced stimulation of SHP expression (26). SHP binds to and inactivates LRH, which is required for the transcriptional activation of CYP7A1 (26). Therefore, based on the above results showing a decrease in FXR and SHP, it is reasonable to expect an increase in the level of CYP7A1 during the APR. However, our previous studies demonstrated that the expression of Cyp7A1 is down-regulated during the APR induced by LPS administration in vivo (38).
Thus, we determined the effect of LPS on the expression of LRH in mouse liver. As shown in Fig. 5, LPS administration for 16 h caused an $65\%$ reduction in the LRH mRNA levels in mouse liver. This result suggests that the LPS-induced decrease in LRH may play a major role in determining the transcriptional regulation of CYP7A1.

TNF and IL-1, but Not IL-6, Decrease FXR mRNA in Hep3B Human Hepatoma Cells—It is well known that the physiological effect of LPS is mediated by pro-inflammatory cytokines such as TNF, IL-1, and IL-6. To determine whether these cytokines also decrease FXR mRNA in vitro, as LPS does in mouse liver, human hepatoma cell line Hep3B cells were treated with cytokines at 10 ng/ml for 24 h, and RNA was isolated for Northern analysis. As shown in Fig. 6, TNF treatment decreased FXR mRNA to less than 20% that of the control level. Also, IL-1 decreased FXR mRNA by 60%. However, IL-6 did not affect FXR expression in Hep3B cells (data not shown). These results suggest that the effect of LPS on FXR expression is mediated by TNF and IL-1.

TNF Reduces the DNA Binding Activity of FXR in Hep3B Cells—Because the inflammatory cytokine TNF causes a reduction in the FXR mRNA, we next determined if TNF also causes a similar change in the DNA binding activity of nuclear extracts from human hepatoma cells to an IR-1 FXR response element. For this experiment, Hep3B cells were treated with TNF at 10 ng/ml. After 24 h the nuclei were isolated, and an electromobility shift assay was conducted as described under “Experimental Procedures.” As shown in Fig. 7A, TNF treatment at 10 ng/ml in Hep3B cells reduced the binding of FXR to IR1, confirming the in vivo result. The reduction of DNA binding activity was $75$ and $55\%$ for the upper and the lower band, respectively (Fig. 7B). Competition with 100-fold molar excess of IR1 oligonucleotide (WT), but not of mutated oligonucleotide (Mut), abolished the shift of DNA band (Fig. 7A), indicating the complex specificity. Furthermore, the FXR-DNA complex showed supershifting after incubation of control nuclear extract with anti-FXR antibodies (Fig. 8). These results indicate that the decrease in FXR expression is associated with a decline in the DNA binding activity of FXR during the acute phase caused by the proinflammatory cytokine TNF in Hep3B cells.

TNF and IL-1 Decrease the Expression of FXR-regulated Genes SHP and ApoCII—We next examined if the expression levels of two genes that are known to be regulated by FXR are also affected by TNF and IL-1 in Hep3B human hepatoma cells. Treatment of Hep3B cells with TNF and IL-1 at 10 ng/ml for 24 h decreased SHP mRNA level by $60$ and $50\%$, respec-
Hepatic total RNA was prepared 16 h after saline or LPS administration (100 μg of LPS/mouse) from mouse liver. Northern blot analysis was performed as described under “Experimental Procedures” using SHP and apoCII cDNAs. Data (means ± S.E., n = 4–5) are expressed as a percentage of controls. *, p < 0.05 versus control.

Fig. 4. Effect of LPS on the expression of FXR target genes SHP and apoCII. Hepatic total RNA was prepared 16 h after saline or LPS administration (100 μg of LPS/mouse) from mouse liver. Northern blot analysis was performed as described under “Experimental Procedures” using SHP and apoCII cDNAs. Data (means ± S.E., n = 4–5) are expressed as a percentage of controls. *, p < 0.05 versus control.

Fig. 5. LPS administration decreases the expression of LRH in mouse liver. Hepatic total RNA was prepared 16 h after saline or LPS administration (100 μg of LPS/mouse) from mouse liver. Northern blot analysis was performed as described under “Experimental Procedures.” Data (means ± S.E., n = 4–5) are expressed as a percentage of controls. *, p < 0.05 versus control.

Inhibition of FXRE-Luciferase Activity by TNF and IL-1—To further elucidate the effect of cytokine treatment on the expression of genes regulated by FXR, we next carried out the transfection studies using a FXRE construct linked to luciferase. The FXRE-Luc construct contains five copies of FXR response element with basal promoter activity. Luciferase activity was not observed in the absence of FXR stimulation. Therefore, basal luciferase activity was stimulated with a natural ligand of FXR, chenodeoxycholic acid. As shown in Fig. 10, both TNF and IL-1 almost completely abolished the activity of luciferase linked to FXRE when compared with control. This result clearly demonstrates that TNF and IL-1 treatment of Hep3B cells inhibits the expression of FXRE-regulated genes.

DISCUSSION

Infection, inflammation, and trauma induce a wide range of metabolic changes in the liver as part of APR and result in the altered concentration of plasma proteins that are collectively called acute phase proteins (1, 37). During the APR, the levels of proteins such as C-reactive protein and serum amyloid A increase (positive acute phase proteins), whereas the levels of proteins such as albumin and transferrin decrease (negative acute phase proteins) (1, 37, 38). It is believed that positive acute phase proteins play a role in protecting the host by neutralizing foreign agents to minimize tissue damage (39). Inflammatory cytokines such as TNF, IL-1, and IL-6 mediate the APR and the transcriptional regulation of positive and negative acute phase proteins. Activation of the TNF/IL-1 receptors stimulates membrane sphingomyelinase to convert sphingomyelin to ceramide, which leads to the activation and translocation of transcription factors activator protein-1 and TR, constitutive androstane receptor, and pregnane X receptor including HNF-4, RXRs, retinoic acid receptors, LXRs, PPARs, and suggested that they might be involved in the repression of several negative acute phase proteins. For example, a decrease in PPARs may contribute to the reduced expression of acyl-CoA synthetase (29, 34) and carnitine palmitoyltransferase I (45) during the APR. Similarly, a decrease in LXR, LRH, and SHP may contribute to the decrease in cholesteryl ester transfer protein (46). Because many of the proteins regulated by nuclear hormone receptors are involved in the metabolism of triglycer-
ide, cholesterol, and bile acids, it is likely that repression of key nuclear receptors during the APR may be one of the mechanisms accounting for the changes in lipid metabolism.

In the present study, we show that the induction of the APR by LPS administration decreases the level of FXR mRNA in mouse liver. The decrease of FXR mRNA was significant as early as 8 h after LPS administration. Comparing this to our previous study, which showed that RXR protein levels were significantly decreased as early as 2 h (29), the decrease of FXR mRNA is preceded by the repression of RXR. We also investigated the level of FXR mRNA in the kidney and the intestine, two other organs involved in bile acid metabolism. Interestingly, FXR mRNA expression was also down-regulated in the kidney but not in the intestine, indicating that the response of FXR mRNA to LPS is tissue-specific. In the liver, FXR mRNA is decreased by low doses of LPS (the half-maximal dose for FXR mRNA was ~0.5 μg/100g of body weight), indicating that the reduction in FXR mRNA is a highly sensitive response to LPS. Furthermore, in vitro experiments with Hep3B cells show that TNF and IL-1, but not IL6, decreased the level of FXR mRNA, suggesting that the decrease of FXR mRNA by LPS in mouse liver is mediated by the action of the inflammatory cytokines TNF and IL-1.

The decrease in FXR mRNA levels in the liver during the APR may influence the transcription of its target genes. Our study shows that FXR binding to FXR response element IRI is decreased after LPS and cytokine treatment in the nuclear extract from mouse liver and Hep3B cells, respectively. In the electrophoresis mobility shift assay, we were able to observe two bands that were shifted from the unbound free probe, and both bands were supershifted by the FXR antibody. The presence of two bands could result from the presence of other proteins such as RXR, coactivators, and/or corepressors. Also, the supershift experiment confirms that RXR is a partner of FXR.

The decrease of FXR binding to the response element could result in the reduced transcription of target genes such as I-BABP (intestinal bile acid-binding protein) (22), BSEP (23), PLTP (21, 24), and apoCII (25). For example, the hepatic expression of BSEP, one of the FXR target genes, has been reported to be down-regulated by LPS, IL-1, and TNF administration (31). Also, Jiang and Bruce (32) report that LPS administration decreased plasma activity of PLTP and its mRNA level in the liver by ~66%. Transfection experiment in the present study clearly demonstrates that TNF and IL-1 almost completely block FXRE-linked luciferase activity, consistent with the decreased expression of FXR target genes by TNF and IL-1. These results agree with our finding that apoCII mRNA level is decreased by ~60%, which is comparable with the changes in PLTP and BSEP.

A decrease in the expression of FXR target genes may provide an explanation for some of the changes in the lipid metabolism during the APR. For example, APR-induced down-regulation of PLTP may disturb the transfer of phospholipids and cholesterol from TG-rich lipoproteins to high density lipoprotein and contribute to the increase of low density lipoproteins. In the present study, we show that expression of apoCII is decreased during the APR. ApoCII is a surface component of

![FIG. 7. Effect of LPS treatment on binding of Hep3B cell nuclear extracts to FXR response element IRI.](image)

![FIG. 8. Protein binding to IRI contains FXR.](image)
chylomicrons, very low density lipoproteins, and high density lipoproteins and plays an important role in plasma lipid metabolism as an activator of lipoprotein lipase (47). Indeed, apoCII deficiency has been linked to the increased plasma levels of triglycerides (48). Therefore, lower apoCII expression during by the APR may contribute to the increased levels of plasma triglycerides that occur during the APR.

Altered expression of FXR target genes during the APR could also affect bile acid synthesis and metabolism. When the high levels of bile acids accumulate in the body, FXR is activated by its natural ligands and negatively regulates the transcription of cholesterol 7α-hydroxylase (CYP7A1) as a compensatory mechanism (27, 28). This negative regulation by FXR is mediated by the induction of SHP, an orphan nuclear receptor that inhibits LRH activation of CYP7A1 expression (26). Our present study shows that FXR repression resulted in the reduced level of SHP during the APR, which could theoretically lead to increased levels of CYP7A1 during the APR. However, our previous work demonstrated that CYP7A1 is down-regulated during the LPS-induced APR in Syrian hamsters (36). There are a number of possible explanations for the decrease in CYP7A1. First, we showed that the level of LRH (mouse homologue of CYP7A1 promoter-binding factor), a nuclear hormone receptor that is required for the transcription of CYP7A1, is also decreased during the APR; a decrease in LRH would decrease the transcription level of CYP7A1 despite a reduction in SHP. Little is known about the transcriptional regulation of LRH, and more studies need to be conducted to further understand the implications of altered expression of LRH during the APR. A second mechanism is that CYP7A1 is regulated by other nuclear hormone receptors including HNF-4 (49, 50), chicken ovalbumin upstream promoter-transcription factor II (50, 51), RXR (51), and LXRs (52, 53). Transcription of CYP7A1 is induced by transfection with HNF-4 alone in HepG2 cells and chicken ovalbumin upstream promoter-transcription factor II cotransfection with HNF-4 had a synergistic effect (50). RXRs bind to a DR1 motif in the promoter region and transactivate gene transcription (51). In rats and mice, LXR is activated in response to oxysterols and induces CYP7A1 transcription to facilitate the excretion of excess cholesterol from the body in the form of bile acids (52, 53). We and others demonstrate that RXR, LXR, and HNF-4 are decreased during the APR (29, 45), conferring limitations on the amount of the transcriptional activators for CYP7A1. A recent report on repression of CYP7A1 by TNF via the HNF-4 site in the bile acid response element also supports the idea of HNF-4 being another key player in the transcriptional regulation of CYP7A1 (54). Finally, repression of CYP7A1 expression by high levels of bile acids, but not by FXR ligands, in SHP-null mice suggests that alternative mechanisms exist for the feedback regulation of CYP7A1 besides FXR/SHP pathway (55). Taken together with other studies (56, 57) that suggest redundant regulatory system for bile acid biosynthesis, it is, thus, not surprising that decreased levels of SHP would not necessarily result in the increased expression of CYP7A1 during the APR in the face of independent decreases in LRH, HNF-4, LXRs, and RXRs.

The effects of LPS are mediated by its stimulation of various immune cells to synthesize and secrete cytokines including TNF, IL-1, and IL-6 (1). TNF, IL-1, and IL-6 have all been shown to play important roles in regulating the increased expression of positive APR proteins. However, in our studies in...
which TNF and IL-1 inhibit the expression of nuclear hormone receptors, IL-6 does not have any effect (58). Similarly in the present study, TNF and IL-1 caused a decrease in the mRNA levels of FXR and SHP, whereas IL-6 did not have any significant effect. The results suggest that TNF and IL-1 are major mediators for the negative regulation of nuclear hormone receptors and that IL-6 is not involved in this process.

In summary, the present study demonstrates that LPS causes a marked decrease in FXR mRNA and activity in mouse liver. This was associated with reduced expression of FXR target genes SHP and apoCII. Furthermore, cytokines TNF and IL-1 caused a similar decrease in FXR expression, DNA binding activity, transcription, and target genes in Hep3B cells. Coupled with previous studies on the repression of RXRs, retinoic acid receptors, PPARs, LXRs, TR, constitutive androstane receptor, and pregnane X receptor, the results with FXR suggest that decreases in type II nuclear hormone receptors are major contributors to the negative APR. Considering the crucial physiological role of FXR in the feedback regulation on bile acid synthetic pathway and in the metabolism of cholesterol and lipoproteins, our study helps us to understand the underlying mechanisms of the changes in lipid metabolism during the APR.

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