Downregulation of liver X receptor-α in mouse kidney and HK-2 proximal tubular cells by LPS and cytokines

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Abstract The acute-phase response (APR) suppresses type II nuclear hormone receptors and alters the expression of their target genes involved in lipid metabolism in the liver and heart. Therefore, we examined the expression of liver X receptor/retinoid X receptor (LXR/RXR) and their target genes in kidney from mice treated with lipopolysaccharide (LPS) and in human proximal tubular HK-2 cells treated with interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). We found that LXRα and RXRα expression was suppressed by LPS in kidney and by IL-1β or TNF-α in HK-2 cells. The decrease in LXRα/RXRα expression was associated with a decrease in the expression of several LXRα target genes [apolipoprotein E (apoE), ABCA1, ABCG1, and sterol-regulatory element binding protein-1c (SREBP-1c)] and a decrease in ligand-induced apoE expression. Moreover, IL-1β and TNF-α significantly reduced liver X receptor response element (LXRE)-driven transcription as measured by LXRE-linked luciferase activity. However, overexpression of LXRα/RXRα only partially restored the cytokine-mediated reduction in LXRE-linked luciferase activity. Additionally, expression of the LXR coactivators peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) and steroid receptor coactivator-2 (SRC-2) was decreased by IL-1β or TNF-α. We conclude that the APR suppresses the expression of both nuclear receptors LXRα/RXRα and several LXRα coactivators in kidney, which could be a mechanism for coordinately regulating the expression of multiple LXR target genes that play important roles in lipid metabolism in kidney during the APR.—Wang, Y., A. H. Moser, J. K. Shigenaga, C. Grunfeld, and K. R. Feingold. **Downregulation of liver X receptor-α in mouse kidney and HK-2 proximal tubular cells by LPS and cytokines. *J. Lipid Res.* 2005.** 46: **2377–2387.**

Supplementary key words lipopolysaccharide • acute-phase response • lipid metabolism

Infection and inflammation induce the acute-phase response (APR), which leads to dramatic alterations in lipid and lipoprotein metabolism (1, 2). Hypertriglyceridemia (3, 4), decreased high density lipoprotein cholesterol levels (5, 6), enhanced lipolysis, and decreased fatty acid oxidation (7, 8) are some of the changes that are often observed during the APR. These alterations in lipoprotein metabolism are believed to confer immediate protection to the host from further detrimental damage during the acute stage of infection (1, 2). In many instances, these metabolic alterations are attributable to increased or decreased transcription of genes that control lipid metabolism, and they are often induced by proinflammatory cytokines such as tumor necrosis factor (TNF) or interleukin-1 (IL-1) (1, 2). Although the underlying mechanisms remain to be identified, we and others have shown that cytokine-induced suppression of many lipid genes during the APR is achieved through the downregulation of type II nuclear hormone receptors (9–13).

Several nuclear hormone receptors, including peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR), and farnesoid X receptor (FXR), belong to the type II receptor superfamily (14). These receptors bind to DNA as obligate heterodimers with the retinoid X receptor (RXR) (15). Unlike the steroid receptors that shuttle between the cytoplasm and the nucleus, most RXR heterodimers are constitutively in the nucleus. In the absence of ligand, the heterodimers are believed to be bound to DNA and complexed with corepressor proteins. Ligand binding induces a structural change that dissociates the corepressor proteins, recruits the coactivator proteins, and promotes the transcription of target genes (16).

Among these nuclear hormone receptors, LXR plays an important role in regulating the transcription of genes responsible for cholesterol metabolism and homeostasis, in-

Manuscript received 5 April 2005 and in revised form 21 July 2005 and in re-revised form 9 August 2005.

Published, JLR Papers in Press, August 16, 2005.
DOI 10.1194/jlr.M500134-JLR200

Abbreviations: apoE, apolipoprotein E; APR, acute-phase response; FXR, farnesoid X receptor; IL, interleukin; LPS, lipopolysaccharide; LXR, liver X receptor; LXRE, liver X receptor response element; PGC-1, peroxisome proliferator-activated receptor γ coactivator 1; PPAR, peroxisome proliferator-activated receptor; PTC, proximal tubular cell; RXR, retinoid X receptor; SRC, steroid receptor coactivator; SREBP-1c, sterol-regulatory element binding protein-1c; TNF, tumor necrosis factor.

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cluding apolipoprotein E (apoE), ABCA1, ABCG1, ABCG5, ABCG8, cholesterol 7α-hydroxylase, and scavenger receptor class B type I (17–25). In addition, LXR has been implicated in the regulation of the lipogenic pathway, because sterol-regulatory element binding protein-1c (SREBP-1c) is a direct target of LXR (26–28).

LXR exists in two forms, LXRα and LXRβ. LXRα is highly expressed in several metabolically active tissues, such as liver, intestine, adipose tissue, and macrophages, whereas LXRβ is ubiquitously expressed in most tissues (17, 18). The kidney plays a central role in the excretion of waste material from the body while simultaneously maintaining plasma metabolite homeostasis. Within the renal cortex, the proximal tubular cells (PTCs) are known to have highly active lipid and glucose metabolism, because these cells need energy to reabsorb salts, water, and metabolic substances through active transport processes (29–31). Therefore, it is likely that LXR may be involved in the regulation of lipid and glucose metabolism within these cells. Indeed, both LXR isoforms are expressed in the kidney, including PTCs (32, 33). Using genome-wide expression profiling analysis of tissues obtained from mice fed an LXR agonist, Steffensen et al. (34) recently identified a large number of putative LXR target genes in kidney. Although most of these genes were involved in lipid, cholesterol, and carbohydrate metabolism, LXR appeared to be involved in a variety of previously unreported biological functions. This implies that LXR may play a broader regulatory role than hitherto recognized in this metabolically active organ.

Previously, we have shown that hepatic mRNA levels of LXR and RXR together with their target genes were rapidly decreased by lipopolysaccharide (LPS) and proinflammatory cytokines in rodents during the APR (9). Given the potential regulatory roles of LXR in kidney, we hypothesized that LXR/RXR expression in kidney, particularly in metabolically active PTCs, is also suppressed during the APR. This may in turn affect the LXR target genes involved in lipid metabolism. To test this hypothesis, we examined the expression of LXR and RXR together with their cognate target genes in kidney from mice treated with LPS. We also examined the expression of these genes in a human PTC line, HK-2 cells, treated with proinflammatory cytokines.

MATERIALS AND METHODS

Materials

LPS (*Escherichia coli* 55:B5) was obtained from Difco and freshly diluted to the desired concentration in pyrogen-free 0.9% saline. Cell growth medium, which is made of keratinocyte serum-free medium (basal medium) supplemented with 5 ng/ml recombinant epidermal growth factor and 0.05 mg/ml bovine pituitary extract, was purchased from Invitrogen. Cytokines (human TNF-α, human IL-1β, and human IL-6) were from R&D Systems and were freshly diluted to the desired concentrations in basal medium containing 0.1% BSA. Tri-Reagent, 22*R*-hydroxysterol, and fatty acid-free BSA were from Sigma. Effectene transfection reagent was purchased from Qiagen. [α-^{32}P]dCTP (3,000 Ci/mmol) was purchased from Perkin-Elmer Life Sciences. Oligo(dT)-cellulose type 77F was from Amersham Biosciences.

Animals

Eight week old female C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were maintained in a barrier room with a normal 12 h light cycle and were provided with mouse chow and water ad libitum. For a typical experiment, mice were anesthetized with halothane and were injected intraperitoneally with 100 μg of LPS in saline or with saline alone. The dose of LPS used in this study had significant effects on triglyceride and cholesterol metabolism but was nonlethal, because the half-maximally lethal dose for LPS in rodents is ~50 mg/kg body weight (35). Food was withdrawn immediately after the injection of LPS, because LPS induces anorexia in rodents (36). At the indicated time points, mice were euthanized with an overdose of halothane and kidneys were excised and stored at −80°C. All experiments were performed according to protocols approved by the Animal Studies Subcommittee of the San Francisco Veterans Affairs Medical Center.

Cell culture experiments

HK-2, a human PTC line, was obtained from the American Type Culture Collection (Rockville, MD). The cells were originally derived from a primary human PTC culture transformed with human papilloma virus 16 E6/E7 genes (37). The cells retain a phenotype indicative of well-differentiated PTC and have been reported to reproduce experimental results obtained with freshly isolated PTCs (37). The cells were maintained in growth medium in a 75 cm^2 flask. Fresh medium was added to cells every 3 days until confluence was achieved. All experiments were done using cells at passage 10 or below. For experiments, cells were seeded in 100 mm Falcon dishes at a density of 2×10^6 cells/dish. After 48 h of incubation, the cells were washed once with phosphate-buffered saline, and fresh growth medium was added in the presence of appropriate cytokine. Cells were harvested at the indicated time points.

Isolation of RNA and Northern blot analysis

Total RNA from mouse was isolated from 100 mg of snap-frozen kidney tissue using Tri-Reagent. Poly(A)$^+$ RNA was purified using oligo(dT) cellulose and quantified by measuring absorption at 260 nm. Ten micrograms of poly(A)$^+$ RNA was denatured and electrophoresed on a 1% agarose/formaldehyde gel. Total RNA from HK-2 cells was isolated from cells in a 100 mm dish using the Tri-Reagent method and was resuspended in diethyl pyrocarbonate-treated water. Thirty micrograms of total RNA was denatured and electrophoresed on a 1% agarose/formaldehyde gel. The uniformity of sample loading was checked by ultraviolet visualization of the ethidium bromide-stained gel before electrotransfer to Nytran membranes (Schleicher and Schuell, Keene, NH). Prehybridization, hybridization, and washing procedures were performed as described previously (12). Membranes were probed with [α-^{32}P]dCTP-labeled cDNAs using the Rediprime™ II Random Priming Labeling System (Amersham). mRNA levels were detected by autoradiography or by Personal FX phosphoimager (Bio-Rad) and quantified using Quantity One software (Bio-Rad). RXRα cDNA was a gift from Dr. Daniel D. Bikle (University of California, San Francisco). RXRβ, RXRγ, LXRα, and LXRβ cDNAs were kindly provided by Dr. David J. Mangelsdorf (University of Texas Southwestern Medical Center, Dallas, TX). ApoE cDNA was kindly provided by Dr. Robert Raffai (University of California, San Francisco). Sterol 27-hydroxylase cDNA was kindly provided by Dr. David Russell (University of Texas Southwestern Medical Center). Primer sequences used for the generation of cDNA probes for Northern blot analysis are listed in **Table 1**.

TABLE 1. Primer sequences used for the generation of cDNA probes for Northern blotting

Gene	Accession Number	Forward Primers (5′–3′)	Reverse Primers (5′–3′)
ABCA1	X75926	TCTCTGCTATCTCCAACCTCATC	ACGTCTTCACCAGGTAATCTGAA
ABCG1	NM_009593	GAAGACCTGCACTGCGACATC	GCTGCGAGGTGATGTAGTCATT
Thyroid receptor-associated protein 220	AF283812	GCAAGGTGTCTCAGAACCC	CCAGCAGCATCTGCAATAAG
SRC-1	U40396	CCTCAGATGCAGCAGAATGTC	GTGGTTATTCAGTCAGTAGCTG
SRC-2	XM_011633	GCAACCACTCCTCAGGGTAG	CTGCTGGACTCCTGGCTCAG
SRC-3	NM_008679	GCGCCAGAGATATGAAACAATGC	GGCTGCCCATCATCTGTACATTC
CREB binding protein	NM_004380	CTGCCTCCCAAGCACTGAATC	CAAACAGGACAGTCATGTCGTG
PGC-1α	AF049330	GACCACAAACGATGACCCTCC	GCCTCCAAAGTCTCTCTCAGG

PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; SRC, steroid receptor coactivator.

Quantitative real-time PCR

First-strand cDNA was synthesized from 1 μg of total RNA (from mouse or HK-2 cells) with random hexamer primers using the Advantage® RT-for-PCR kit (BD Biosciences Clontech, San Diego, CA). The real-time PCR contained, in a final volume of 20 μl, 20 ng of reverse-transcribed total RNA, 300 nM forward and reverse primers, and 10 μl of 2× SYBR Green PCR Master Mix (Stratagene, La Jolla, CA). PCR was carried out on 96-well plates using the Mx3000P™ real-time PCR System (Stratagene). The relative amounts of all mRNAs were calculated using the comparative threshold cycle (C_T) method. 36B4 mRNA was used as the invariant control for all experiments. All primer sequences used for real-time quantitative PCR are listed in **Table 2**.

Transfection studies and luciferase assay

HK-2 cells were plated on six-well plates at a density of 5×10^5 cells/well in the growth medium. After 24 h, cells were incubated in the basal medium for 1 h and were then transfected with DNA-Effectene complex containing 10 μg/ml Effectene and 2 μg/ml liver X receptor response element (LXRE)-luciferase vector (pTK-LXRE3-LUC), with or without 0.1 μg/ml expression vectors for LXRα and RXRα (pCMX-hLXRα and pCMX-hRXRα, respectively; kindly provided by Dr. David J. Mangelsdorf, University of Texas Southwestern Medical Center). Expression of pCMX-luciferase was not affected by either IL-1β or TNF-α. Cells were incubated with the DNA-Effectene complex in the basal medium at 37°C for 6 h. After the DNA-Effectene complex was removed, the cells were incubated with fresh growth medium for 18 h. Transfected cells were then treated with appropriate cytokine. After 24 h of incubation, transfected cells were harvested with lysis buffer (Promega, Madison, WI), and aliquots of the lysates were assayed for luciferase using a Wallac VICTOR^{2}™ 1420 Multilabel Counter (Perkin-Elmer Life Sciences).

Statistical analysis

Data are expressed as means ± SEM of experiments with four to five animals or three to four plates for each group or time point. The difference between two experimental groups was analyzed using the Student's *t*-test. Differences among multiple groups were analyzed using one-way ANOVA with the Dunnett's post hoc test. $P < 0.05$ was considered significant.

RESULTS

LPS decreases LXRα mRNA level in mouse kidney

We first determined the effect of LPS administration in mice on the mRNA levels of LXRs and RXRs in kidney at various times. As shown in **Fig. 1A**, 4 h after LPS administration (100 μg/mouse), LXRα mRNA level was decreased by >60%. The levels of RXRα and RXRβ were also decreased by 50% and 67%, respectively. However, LXRβ mRNA level was not significantly altered. RXRγ was not detectable in kidney in either control or LPS-treated mice.

By 16 h after LPS treatment, the downregulation of LXRα expression was still observed (Fig. 1B). However, the suppressive effect of LPS treatment on RXRα and RXRβ expression was no longer apparent by 16 h. Thus, administration of LPS in mice resulted in a rapid decrease in LXRα expression in kidney that was sustained, whereas suppression of RXRα and RXRβ was of relatively short duration.

To determine whether the diminished expression of LXRα in kidney is associated with the reduced transcription of LXRα-regulated genes, we next examined the effect of LPS on the mRNA levels of genes that are involved in cholesterol metabolism. As shown in **Fig. 2A**, LPS treatment decreased the expression of apoE, ABCA1, and ABCG1 by 40, 75, and 90%, respectively, in mouse kidney compared with controls. Furthermore, LPS treatment decreased the LXRα target gene SREBP-1c, a transcription factor that

TABLE 2. Primer sequences used for real-time quantitative PCR

Gene	Accession Number	Forward Primers (5′–3′)	Reverse Primers (5′–3′)
mSREBP-1a	NM_011480	GGCCGAGATGTGCGAAC	GTTGATGAGCTGGAGCATGT
mSREBP-1c	NM_011480	AGCTGTCGGGGTAGCGTCTG	GAGAGTTGGCACCTGGGCTG
mSREBP-2	AF374267	GCGTTCTGGAGACCATGGA	ACAAAGTTGCTCTGAAAACAAATCA
hSREBP-1c	BC063281	CGGAGCCATGGATTGCAC	CTTCAAGAGAGGAGCTCAATGTGG
hPGC-1β	NM_133263	CCAAGACCAGCAGCTCCTA	CCACTGTCAAGGTCTGCTCA
h/m36B4	M17885	GCGACCTGGAAGTCCAACTAC	ATCTGCTGCATCTGCTTGG

h, human; m, mouse; SREBP, sterol-regulatory element binding protein.

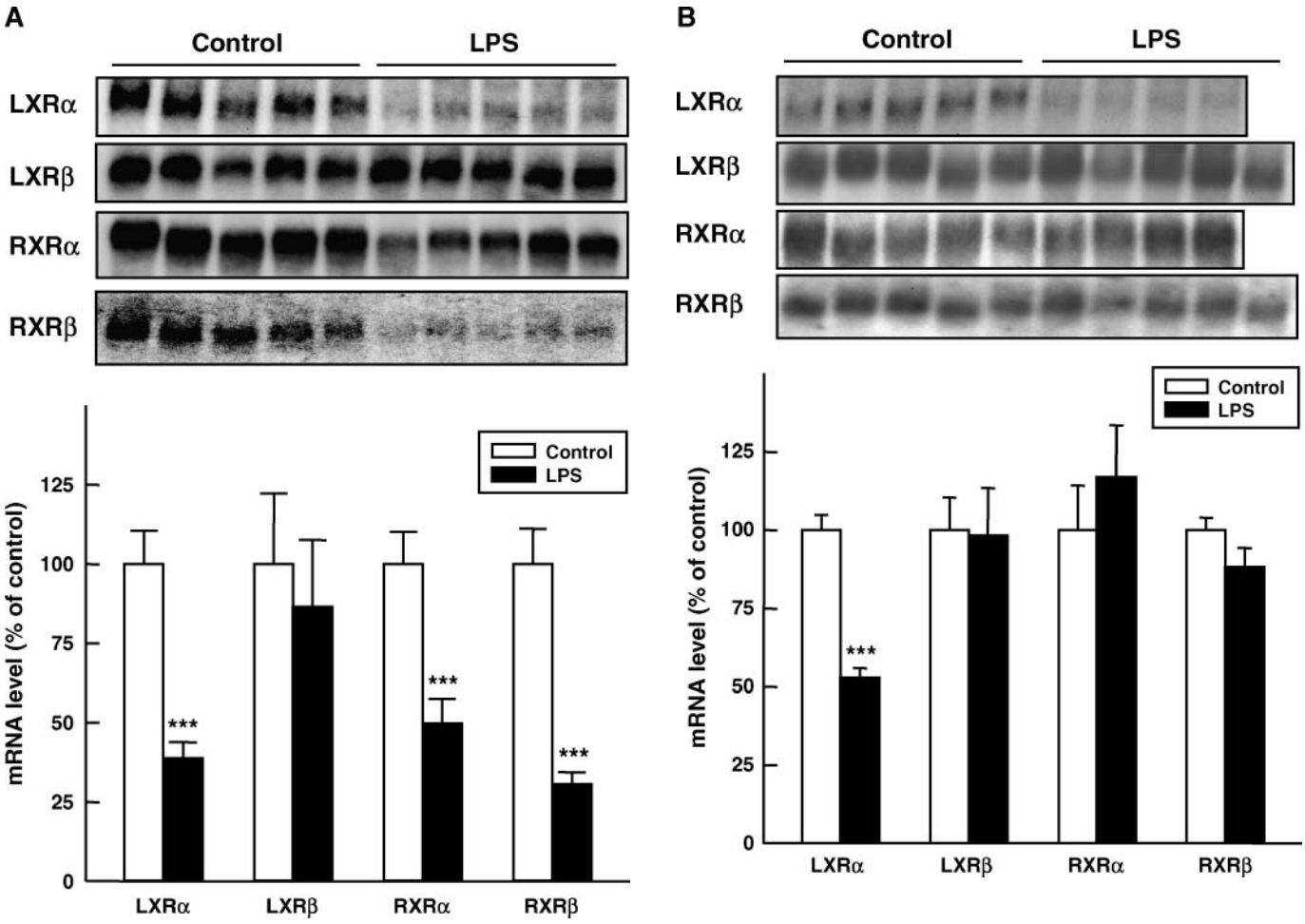

Fig. 1. Effect of lipopolysaccharide (LPS) on nuclear hormone receptors in mouse kidney. Mice were injected intraperitoneally with either saline or LPS (100 μg/mouse). The animals were euthanized at 4 h (A) or 16 h (B) after LPS administration. Poly(A)$^+$ RNA was prepared from the kidneys, and Northern blot analysis was carried out as described in Materials and Methods. The calculated expression levels in the bottom panels are based on absolute RNA loaded per lane. LXRα, liver X receptor α; LXRβ, liver X receptor β; RXRα, retinoid X receptor α; RXRβ, retinoid X receptor β. Data (means ± SEM, n = 4–5) are expressed as percentages of control values. *** $P < 0.001$ versus control.

enhances the expression of genes required for fatty acid synthesis, by 78% (Fig. 2B).

Interestingly, the mRNA level of sterol 27-hydroxylase, an enzyme responsible for the synthesis of a putative LXR ligand, 27-hydroxycholesterol, was decreased by 43% (Fig. 2A). In contrast, the levels of two other SREBP-related transcription factors, SREBP-1a and SREBP-2, were not significantly altered (data not shown). Thus, a number of LXRα-regulated genes, as well as a gene that could produce an LXR ligand, were suppressed during the LPS-induced APR in mouse kidney.

IL-1β and TNF-α decrease LXRα mRNA in HK-2 human PTCs

Because the kidney is a heterologous organ with distinct functions along each nephron segment, and the PTCs are among the most metabolically active cells in the kidney, we further examined the effect of proinflammatory cytokines on the expression of LXRs/RXRs and their target genes in PTCs. Human HK-2 cells were treated with cytokines at 10 ng/ml for 24 h, and total RNA was harvested for Northern blot analysis. As shown in **Fig. 3**, IL-1β and TNF-α decreased LXRα mRNA to 25% and 38% of control values, respectively. RXRα mRNA was also reduced significantly in the presence of IL-1β or TNF-α. However, these cytokines did not alter the expression of LXRβ and RXRβ in HK-2 cells. RXRγ was not detected in these cells. Moreover, LXR expression was not affected by treatment of cells with IL-6 (data not shown).

IL-1β and TNF-α are mainly secreted by various immune cells, including macrophages, in response to LPS during the APR. Although unlikely, we also determined whether LPS has a direct suppressive effect on LXRα expression in the PTCs by incubating HK-2 cells in the presence of LPS for 24 h. As expected, LXRα expression was not changed even at the highest concentration of LPS (100 ng/ml) (data not shown). Thus, IL-1β and TNF-α, but not IL-6 or LPS, decreased LXRα and RXRα expression in HK-2 cells.

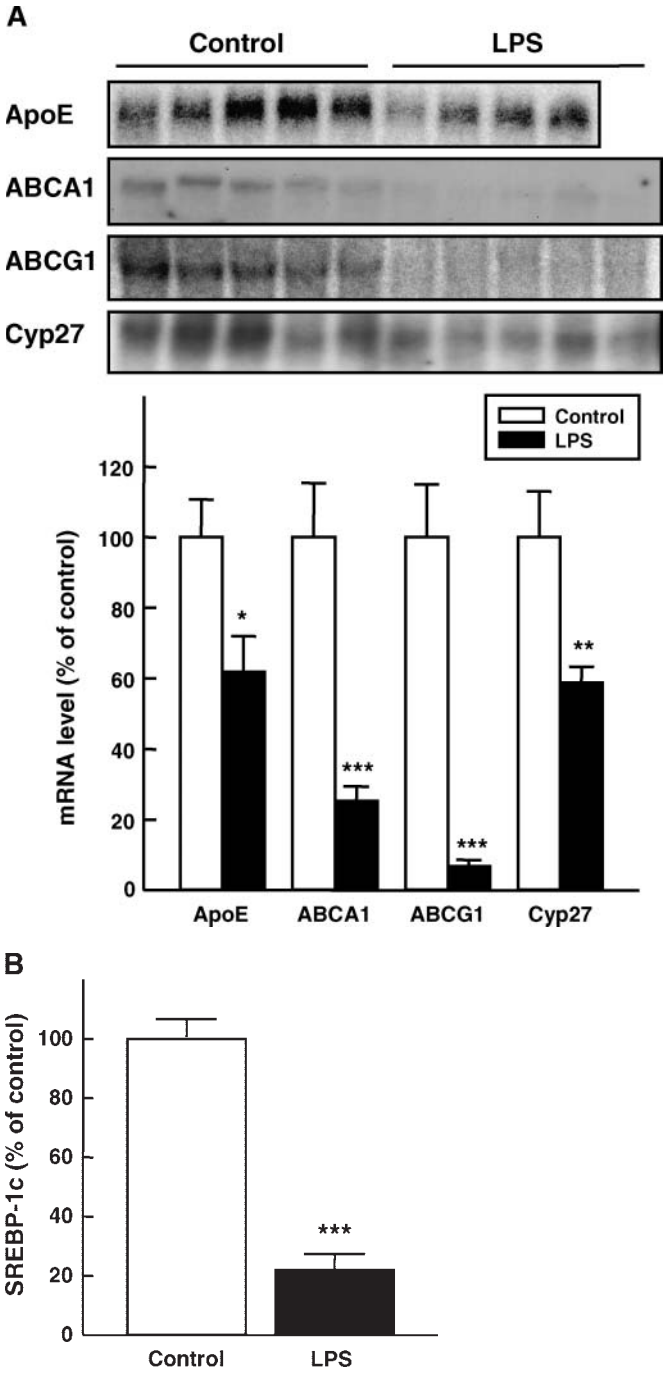

Fig. 2. Effect of LPS on genes involved in cholesterol and fatty acid metabolism in mouse kidney. Mice were injected intraperitoneally with either saline or LPS (100 μg/mouse). The animals were euthanized at 16 h after LPS administration. Poly(A)$^+$ RNA was prepared from the kidneys. Quantitation of genes involved in cholesterol metabolism was carried out by Northern blot analysis (A), and quantitation of sterol-regulatory element binding protein-1c (SREBP-1c) mRNA was carried out by QPCR (B), as described in Materials and Methods. ApoE, apolipoprotein E; Cyp27, sterol 27-hydroxylase. Data (means ± SEM, n = 4–5) are expressed as percentages of control values. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ versus control.

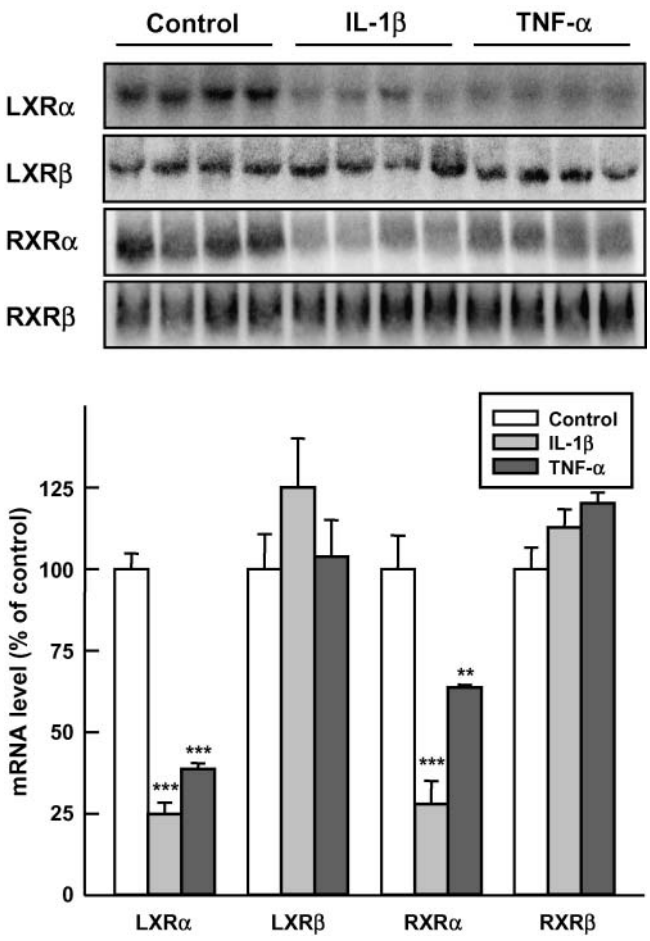

Fig. 3. Effect of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) on nuclear hormone receptors in HK-2 proximal tubular cells. HK-2 cells were plated on 100 mm Petri dishes in growth medium (2×10^6 cells/dish). After 48 h of incubation, cells were washed once with phosphate-buffered saline, and medium was replaced with fresh growth medium plus the appropriate cytokine at 10 ng/ml. After 24 h of incubation, total RNA was isolated from each dish, and Northern blot analysis was performed as described in Materials and Methods. Data (means ± SEM, n = 3) are expressed as percentages of control values. *** $P < 0.001$, ** $P < 0.01$ versus control.

We also examined the dose-response curves and time course of the effects of IL-1β and TNF-α on LXRα expression in HK-2 cells. As shown in **Fig. 4**, at the 24 h time point, IL-1β induced a dose-dependent decrease in LXRα mRNA level, with a half-maximal decrease in LXRα at 10 pg/ml. TNF-α treatment also decreased LXRα mRNA level at very low concentrations. Thus, the decrease in LXRα expression induced by IL-1β or TNF-α is very sensitive. The concentrations of the cytokines (up to 10 ng/ml) used in the culture experiments represent those in the serum after LPS administration and are similar to the levels observed with sepsis.

Furthermore, IL-1β and TNF-α were able to decrease LXRα expression as early as 4 h after treatment (**Fig. 5**). These data indicate that the suppression of LXRα expression by IL-1β and TNF-α in HK-2 cells occurred rapidly and was sustained, similar to what is seen in vivo.

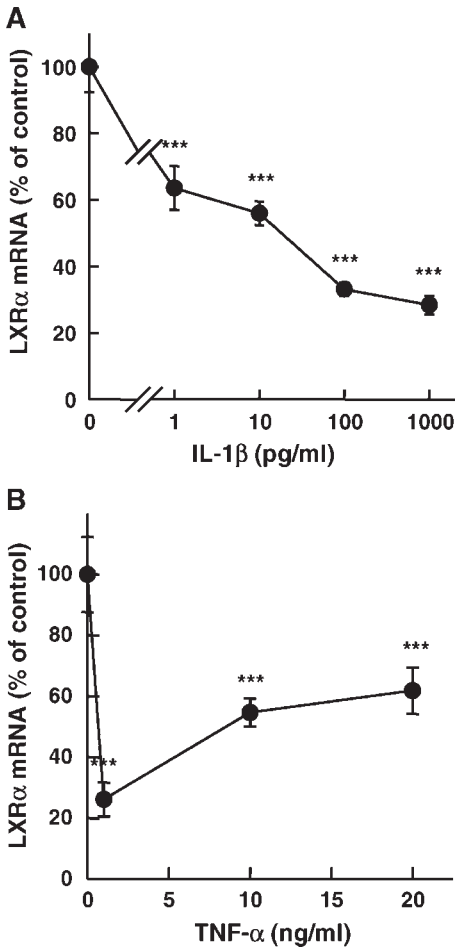

Fig. 4. Concentration curve of the effect of IL-1β and TNF-α on LXRα expression in HK-2 cells. HK-2 cells were cultured for 24 h with various concentrations of IL-1β (A) or TNF-α (B) as indicated. Total RNA was isolated, and Northern blot analysis was performed as described in Materials and Methods. Data (means ± SEM, n = 3) are expressed as percentages of control values. *** $P < 0.001$ versus control.

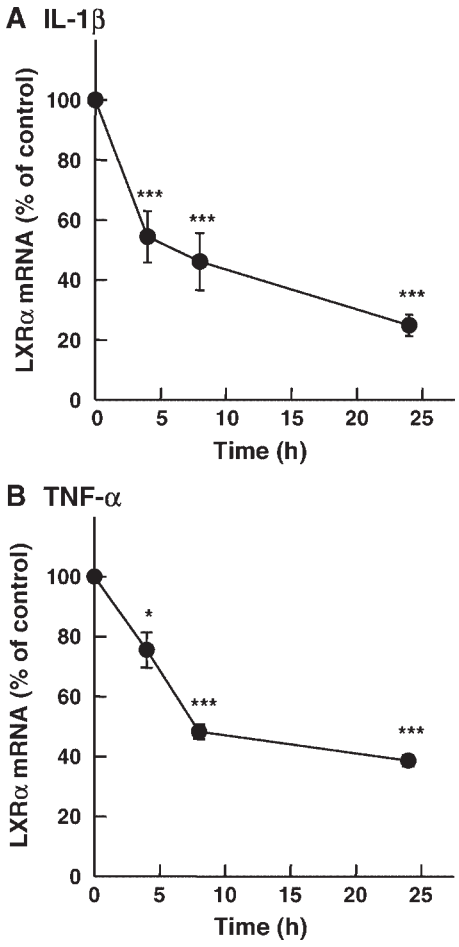

Fig. 5. Time course response curve of the effect of IL-1β and TNF-α on LXRα expression in HK-2 cells. HK-2 cells were cultured with 10 ng/ml IL-1β (A) or TNF-α (B) at various time points as indicated. Total RNA was isolated, and Northern blot analysis was performed as described in Materials and Methods. Data (means ± SEM, n = 3) are expressed as percentages of control values. *** $P < 0.001$, * $P < 0.05$ versus control.

IL-1β and TNF-α decrease LXR transcription and the expression of the LXRα-regulated genes SREBP-1c and apoE in HK-2 cells

To determine the effects of IL-1β and TNF-α on the expression of genes regulated by LXRα, we performed transfection studies in HK-2 cells using an LXRE construct linked to luciferase. As shown in **Fig. 6**, treatment of transfected cells with IL-1β caused a 70% reduction in the activity of luciferase linked to LXRE compared with controls. TNF-α also reduced LXR-driven luciferase activity by 33%. Therefore, IL-1β and TNF-α suppressed the expression of LXRE-regulated transcription in HK-2 cells.

We next examined whether the expression of genes that are known to be regulated by LXRα was also affected by IL-1β and TNF-α in HK-2 cells. As shown in **Fig. 7A**, treatment of cells with IL-1β and TNF-α for 24 h decreased SREBP-1c mRNA levels by 58% and 56%, respectively. Likewise, IL-1β and TNF-α decreased apoE mRNA levels by 69% and 32%, respectively (Fig. 7B). Moreover, when HK-2 cells were treated with a ligand of LXRα, 22*R*-hydroxysterol, apoE expression was increased by 70%, yet IL-1β was still able to significantly inhibit 22*R*-hydroxysterol-induced expression of apoE (Fig. 7C). These results suggest that treatment of HK-2 cells with IL-1β and TNF-α suppressed both the basal and ligand-induced expression of LXRα-regulated target genes in HK-2 cells.

Overexpression of LXRα/RXRα in HK-2 cells partially restores the cytokine-mediated reduction in LXRE-linked luciferase activity

The active transcription of LXRE-driven genes depends on the coordinated action of both LXR/RXR and coactivator proteins. To determine whether the loss of LXRα/RXRα is the sole factor responsible for the cytokine-mediated reduction in LXRE-driven gene transcription, we cotransfected LXRα/RXRα expression plasmids together with the LXRE-linked luciferase construct in HK-2 cells, followed by cytokine treatment. As shown in **Fig. 8**, in LXRE-only transfected cells (basal group), IL-1β markedly suppressed LXRE-linked luciferase activity to 31% of con-

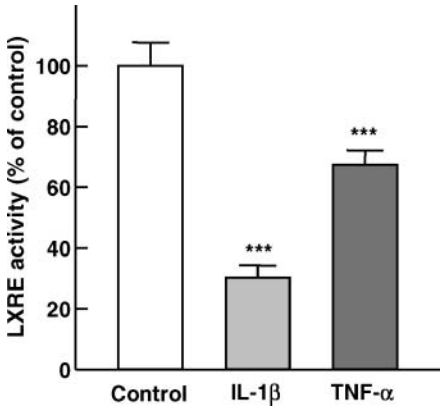

Fig. 6. Effect of IL-1β and TNF-α on liver X receptor response element (LXRE)-linked luciferase activity in transfected HK-2 cells. HK-2 cells were plated on six-well plates (5×10^5 cells/well) in culture medium. After 24 h of incubation, cells were transfected with a LXRE-linked luciferase vector as described in Materials and Methods. The next day, cells were incubated in fresh growth medium in the presence of 10 ng/ml IL-1β or TNF-α for 24 h. At the end of the incubation, cells were harvested in a lysis buffer, and luciferase activity was determined. Data (means ± SEM, n = 3) are expressed as percentages of control values. *** $P < 0.001$ versus control.

trol, whereas TNF-α decreased luciferase activity to 70% of control. Cotransfection of LXRα/RXRα (0.1 μg/ml) in HK-2 cells (LXRα + RXRα group) resulted in a 6-fold increase in the LXRE-linked luciferase activity compared with the basal control. However, overexpression of both LXRα and RXRα partially reversed the inhibition of LXRE luciferase activity by either IL-1β or TNF-α; with IL-1β, ~50% of the cytokine-mediated reduction in LXRE-luciferase activity was prevented by overexpression of LXRα/RXRα, whereas with TNF-α, ~66% was prevented. These data suggest that cytokine-induced reduction in LXRα/RXRα expression in HK-2 cells plays a role in the cytokine-induced inhibition of LXRE-driven gene expression, but that other factors also contribute to the changes.

Coactivators, interacting with transcription factors, are required for active gene transcription. Therefore, we determined the effect of IL-1β and TNF-α on coactivator mRNA levels. As shown in **Fig. 9**, although mRNA levels of several coactivators [CREB binding protein, peroxisome proliferator-activated receptor γ coactivator 1β (PGC-1β), steroid receptor coactivator-1 (SRC-1), and thyroid receptor-associated protein 220] were not altered by cytokine treatment, both PGC-1α and SRC-2 mRNA levels were decreased by 40–60% by either IL-1β or TNF-α treatment. Thus, a decrease in coactivators, coupled with the decrease in LXRα/RXRα, together could be responsible for the reduction in LXRE-driven gene transcription during the APR.

DISCUSSION

During infection and inflammation, a wide range of metabolic changes occur as part of the APR (38, 39). These

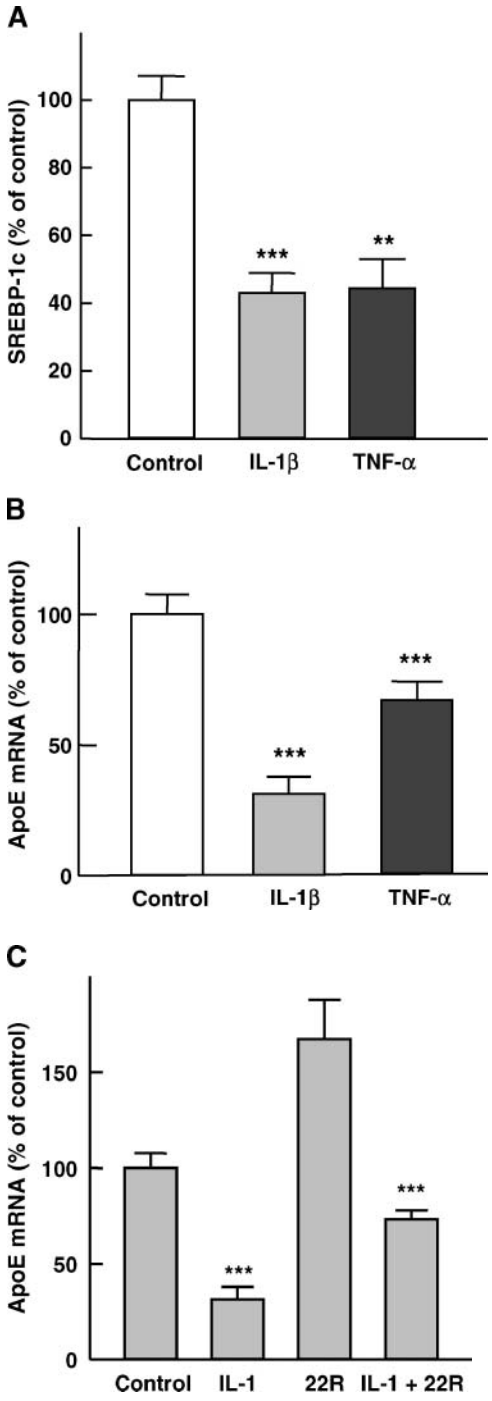

Fig. 7. Effect of IL-1β and TNF-α on SREBP-1c and apoE in HK-2 cells. HK-2 cells were plated on 100 mm Petri dishes in growth medium (2×10^6 cells/dish). After 48 h of incubation, cells were washed once with phosphate-buffered saline, and medium was replaced with fresh growth medium plus the appropriate cytokine at 10 ng/ml. After 24 h of incubation, total RNA was isolated from each dish. Quantitation of SREBP-1c mRNA (A) was carried out by quantitative real-time PCR, and quantitation of apoE mRNA (B) was carried out by Northern blot analysis, as described in Materials and Methods. C: Cells were pretreated with 10 μM 22*R*-hydroxysterol (22R) for 16 h, followed with 24 h of incubation with fresh growth medium with or without 10 pg/ml IL-1β and 10 μM 22*R*-hydroxysterol. Total RNA was isolated, and Northern analysis of apoE was performed. Data (means ± SEM, n = 3) are expressed as percentages of control values. *** $P < 0.001$, ** $P < 0.05$ versus control.

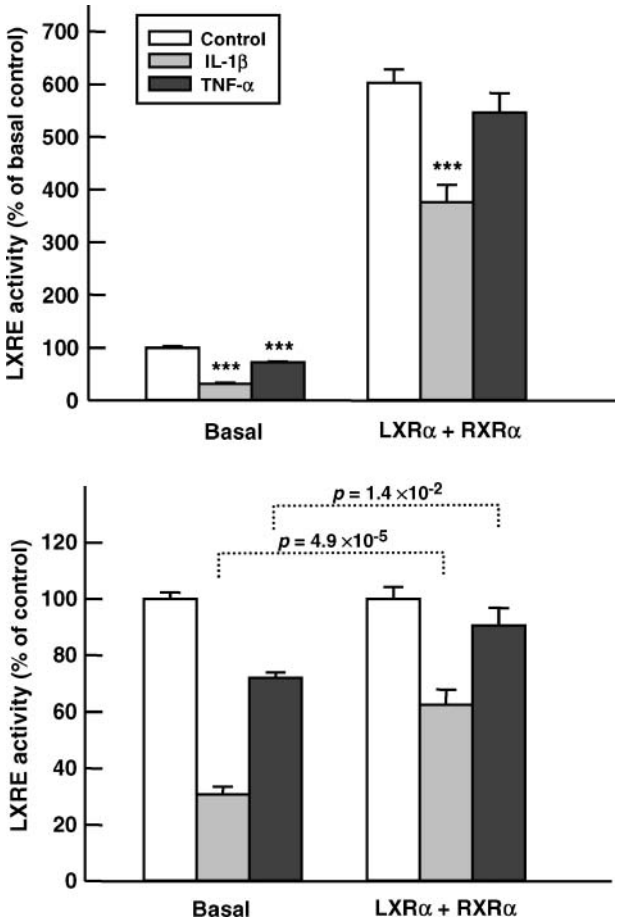

Fig. 8. Partial recovery of cytokine-induced inhibition of LXRE-linked luciferase activity in HK-2 cells cotransfected with LXRα and RXRα. HK-2 cells were transfected with 2 μg/ml LXRE-linked luciferase plasmid with (LXRα + RXRα group) or without (basal group) 0.1 μg/ml expression vectors for LXRα and RXRα, as indicated. Top: Data (means ± SEM, n = 3) for the LXRE activity expressed as percentages of basal control values. *** $P < 0.001$ versus control. Bottom: LXRE activity shown as a percentage of control values in each group. *P* values show the comparison between groups in the presence of cytokines.

changes are accompanied by alterations in the plasma levels of acute-phase proteins: positive acute-phase proteins, such as C-reactive protein and serum amyloid A, show increases in plasma levels; negative acute-phase proteins, such as albumin and transferrin, show decreases in plasma levels (38, 39). The APR is mediated by proinflammatory cytokines such as TNF, IL-1, and IL-6 (40). These cytokines play important roles in the transcriptional regulation of the positive and negative acute-phase proteins (40).

Although extensive studies have centered on the transcriptional regulation of positive acute-phase proteins (for reviews, see 41, 42), much less is known about the regulation of negative acute-phase proteins. Recently, we and others have shown that several type II nuclear hormone receptors are downregulated by proinflammatory cytokines during the APR, suggesting that the decreased nuclear hormone receptors may contribute to the changes in the transcription of many negative acute-phase proteins involved in lipid metabolism (1, 2). For example, we found that the expression of PPARs and RXRs is suppressed in liver and heart during the APR (9, 13). A decrease in PPARα level was associated with reduced expression of fatty acid transport protein and carnitine palmitoyltransferase I, which are responsible for transporting fatty acids into cytosol and further into mitochondria, respectively. As a result, limited amounts of fatty acids within mitochondria are available for β-oxidation, and ATP generation is compromised. Likewise, a decrease in hepatic LXR expression may be associated with a reduced plasma level of cholesteryl ester transfer protein during the APR (43). Thus, cytokine-induced suppression of nuclear hormone receptors may provide an explanation for some of the alterations in lipid metabolism during the APR.

In this study, we show that LPS suppressed LXRα/RXRα expression in mouse kidney. The decrease in LXRα mRNA level in kidney occurred rapidly (within 4 h) and was sustained during the late stage of the APR (up to 16 h). Consistent with the reduction in LXRα expression, we also found that levels of cognate LXRα target genes, such as apoE, ABCA1, ABCG1, and SREBP-1c, were decreased during the APR. Moreover, the expression of RXRα, the obligate LXRα binding partner, was also decreased during the early stage of the APR. Thus, these results, together with our previous finding that FXR expression in mouse kidney is reduced during the APR (12), lend further credence to our notion that downregulation of type II nuclear hormone receptors may be one important mechanism to regulate lipid metabolism in mouse kidney during the APR.

Because the kidney is a heterologous organ with distinct functions along each nephron segment, we further examined the effect of proinflammatory cytokines on the expression of LXRs/RXRs and their target genes in cultured HK-2 PTCs. In this study, we found that suppression of LXRα in HK-2 cells was mediated by the proinflammatory cytokines IL-1β and TNF-α in a dose- and time-dependent manner. The decrease in LXRα mRNA levels by IL-1β and TNF-α was accompanied by a marked reduction in LXRE-driven transcription, as measured by LXRE-linked luciferase activity and cognate LXRα target gene expression (SREBP-1c and apoE) in HK-2 cells. Interestingly, a recent study by Ruan et al. (44) also demonstrated that IL-1β inhibits LXR expression in human kidney mesangial cells. Therefore, IL-1 and TNF appear to be the major mediators for the negative regulation of nuclear hormone receptors in the kidney. It is noteworthy that in the present study, we examined a limited number of cognate LXRα target genes, whose biological functions have been elucidated extensively in liver and macrophages. In a recent study using genome-wide expression profiling analysis of tissues obtained from mice fed an LXR agonist, Steffensen et al. (34) identified a large number of putative LXR target genes in kidney. Thus, one may speculate that downregulation of LXR in mouse kidney may play a broader regulatory role in many genes yet to be recognized during the APR.

LXRα-mediated gene transcription requires the con-

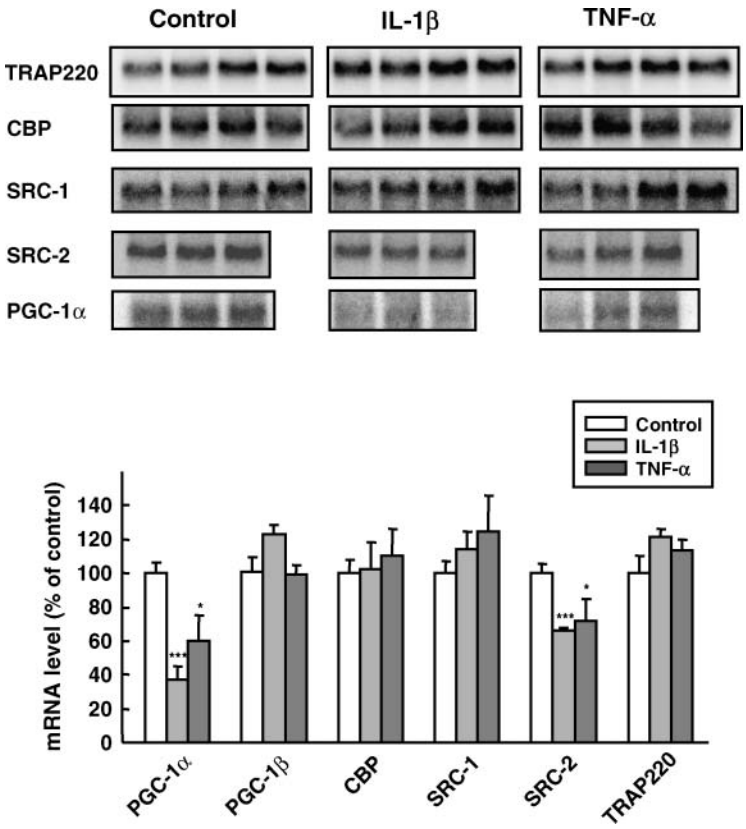

Fig. 9. Effect of IL-1β and TNF-α on LXRα coactivators in HK-2 cells. HK-2 cells were plated on 100 mm Petri dishes in growth medium (2×10^6 cells/dish). After 48 h of incubation, cells were washed once with phosphate-buffered saline, and medium was replaced with fresh growth medium plus the appropriate cytokine at 10 ng/ml. After 24 h of incubation, total RNA was isolated from each dish. Quantitation of mRNA for peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), CREB binding protein (CBP), steroid receptor coactivator-1 (SRC-1), SRC-2, and thyroid receptor-associated protein 220 (TRAP220) was carried out by Northern blot analysis, and quantitation of PGC-1β mRNA was carried out by quantitative real-time PCR, as described in Materials and Methods. SRC-3 mRNA was not detected in HK-2 cells. Data (means ± SEM, n = 3–4) are expressed as percentages of control values. *** $P < 0.001$, * $P < 0.05$ versus control.

certed action of transcription factors and coactivator proteins. Downregulation of the transcription factors LXRα/RXRα during the APR provides a plausible explanation for the reduction in LXRα-mediated gene transcription. However, our cotransfection study showed that even when LXRα/RXRα were constitutively expressed in HK-2 cells, cytokine-induced reduction in LXRα-mediated gene transcription (measured by LXRE-linked luciferase activity) was only partially recovered. Therefore, other factors, in addition to LXRα/RXRα, may be required for the complete recovery of LXRE-linked luciferase activity in the presence of cytokines. Consistent with this, we found that the expression of PGC-1α and SRC-2, two potential coactivators required for active LXRα-mediated gene transcription, were also suppressed by IL-1β and TNF-α treatment in HK-2 cells. Thus, LXRα-mediated gene transcription during the APR appears to be regulated at least at the levels of both transcription factors and coactivators, which leads to a coordinated biological reprogramming in response to infection and injury. On the one hand, downregulation of type II nuclear hormone receptors during the APR, such as LXRα and PPARα, provides a sensitive and specific pathway to regulate the expression of their target lipid genes and thus effectively alter lipid metabolism. On the other hand, selective downregulation of coactivators during the APR, such as PGC-1 and SRC, allows the functional integration of multiple nuclear hormone receptors, which share common coactivators for active transcription. This may lead to simultaneous alteration in several interlocking pathways in a tissue- and time-specific manner. Indeed, we observed that the expression of PGC-1α, a coactivator for both PPARα and LXRα, was also suppressed in both mouse liver and kidney during the APR (unpublished data). Apparently, gene regulation during the APR is a multifactorial process, and further detailed study is required to define the regulatory roles of coactivators in this complex process.

A decrease in the expression of LXRα target genes may provide an explanation for some of the changes in lipid metabolism during the APR. Although much of the research to date into the role of LXRα in the kidney has been centered on the mesangium and glomerulus, it is increasingly recognized that pathology within the tubulointerstitium is ultimately more predictive of the renal outcome (45). Indeed, in the diverse forms of acute renal injury, including the sepsis syndrome, both free cholesterol and cholesteryl esters accumulate within the proximal tubular epithelium (46–50). It has been suggested that PTC cholesterol accumulation is an integral component of the kidney's response to tissue injury, so that PTCs increase resistance to superimposed nephrotoxic attack and thus acquire cytoresistance (48, 49, 51). Although the underlying mechanisms remain to be defined, cholesterol accumulation within PTCs during sepsis syndrome may partly be attributable to a decrease in cellular cholesterol efflux (46). Because LXRα regulates the expression of genes involved in cholesterol efflux, it is possible that a decrease in LXRα/RXRα activity would result in a decrease in cholesterol efflux from the PTCs. Consistent with this notion, our current study showed that a decrease in LXRα expression in the kidney was associated with a decrease in the expression of apoE, ABCA1, and ABCG1,

which may contribute to a decrease in cholesterol efflux in PTCs in the sepsis syndrome. Further functional studies will be necessary to address the potential roles of LXR/RXR and their putative target genes in lipid metabolism in PTCs during the APR.

In summary, our study showed that expression of LXRα/RXRα was suppressed by LPS in mouse kidney and by IL-1β or TNF-α in HK-2 cells. This was associated with reduced expression of LXRα target genes involved in cholesterol metabolism and lipogenic activity. Furthermore, this study suggests that downregulation of type II nuclear hormone receptors, together with their coactivators, may contribute to the negative APR in multiple metabolically active organs, including liver and kidney. Because acute renal failure occurs in >50% of patients with sepsis syndrome, unraveling the mechanisms responsible for the changes in lipid metabolism within the kidney tubulointerstitium may offer better understanding of renal pathophysiology during the APR. Ultimately, the use of LXR-selective agonists and antagonists may provide new approaches to modulate renal function during sepsis.

This work was supported by grants from the Research Service of the Department of Veterans Affairs and by National Institutes of Health Grant AR-050629. The authors thank Dr. Biao Lu for a critical reading of the manuscript.

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