

Endotoxin down-regulates ABCG5 and ABCG8 in mouse liver and ABCA1 and ABCG1 in J774 murine macrophages: differential role of LXR

Weerapan Khovidhunkit,^{1,*} Arthur H. Moser,^{*} Judy K. Shigenaga,^{*,†} Carl Grunfeld,^{*,†} and Kenneth R. Feingold^{2,*}

Metabolism Section,^{*} Department of Veterans Affairs Medical Center, San Francisco, CA 94121; and Department of Medicine,[†] University of California, San Francisco, CA 94143

Abstract Several of the ATP binding cassette (ABC) transporters have recently been shown to play important roles in reverse cholesterol transport (RCT) and prevention of atherosclerosis. In the liver, ABCG5 and ABCG8 have been proposed to efflux sterols into the bile for excretion. ABCG5 and ABCG8 also limit absorption of dietary cholesterol and plant sterols in the intestine. In macrophages, ABCA1 and ABCG1 mediate cholesterol removal from these cells to HDL. Many of these ABC transporters are regulated by the liver X receptor (LXR). We have previously shown that endotoxin (lipopolysaccharide) down-regulates LXR in rodent liver. In the present study, we examined the *in vivo* and *in vitro* regulation of these ABC transporters by endotoxin. We found that endotoxin significantly decreased mRNA levels of ABCG5 and ABCG8 in the liver, but not in the small intestine. When endotoxin or cytokines (tumor necrosis factor and interleukin-1) were incubated with J774 murine macrophages, the mRNA levels of ABCA1 were decreased. This effect was rapid and sustained, and was associated with a reduction in ABCA1 protein levels. Endotoxin and cytokines also decreased ABCG1 mRNA levels in J774 cells. Although LXR is a positive regulator of ABCA1 and ABCG1, we did not observe a reduction in protein levels of LXR or in binding of nuclear proteins to an LXR response element in J774 cells. The decrease in ABCG5 and ABCG8 levels in the liver as well as a reduction in ABCA1 and ABCG1 in macrophages during the host response to infection and inflammation coupled with other previously described changes in the RCT pathway may aggravate atherosclerosis.—Khovidhunkit, W., A. H. Moser, J. K. Shigenaga, C. Grunfeld, and K. R. Feingold. **Endotoxin down-regulates ABCG5 and ABCG8 in mouse liver and ABCA1 and ABCG1 in J774 murine macrophages: differential role of LXR.** *J. Lipid Res.* 2003. 44: 1728–1736.

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In epidemiological studies, plasma levels of HDL cholesterol are inversely correlated with the risk of coronary artery disease; therefore, HDL is postulated to protect against atherosclerosis (1). Reverse cholesterol transport (RCT) is an HDL-mediated pathway by which cholesterol is removed from peripheral cells and transported to the liver for excretion and/or catabolism (2, 3). Cholesterol efflux, the first step of RCT, begins when HDL or its major apolipoprotein, apoA-I, accepts cholesterol from cells. Cholesterol on HDL is returned to the liver by several routes. Once delivered to the liver, most cholesterol is metabolized by a series of hepatic enzymes into bile acids. The remaining cholesterol is excreted directly into the bile, along with bile acids.

Accumulating evidence has suggested a possible relationship between atherosclerosis and chronic infections and inflammatory diseases (4). How infections and inflammatory states, especially those outside the arterial wall, can promote atherosclerosis is not clear. However, it is known that the acute-phase response (APR) is induced during infection and inflammation. During the APR, multiple alterations in lipid and lipoprotein metabolism occur (5). Plasma triglyceride levels increase, and there is an increase in small, dense LDL (6). These particular changes have been recognized as risk factors for atherosclerosis.

Considerable evidence also suggests that, during the APR, RCT is impaired. First, HDL cholesterol levels decrease in most species (7). Second, we and others have shown that

Abbreviations: ABC, ATP binding cassette; APR, acute-phase response; cpt-cAMP, 8-(4-chlorophenylthio) adenosine 3', 5'-cyclic monophosphate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-1, interleukin-1; LPS, lipopolysaccharide; LXR, liver X receptor; RCT, reverse cholesterol transport; RXR, retinoid X receptor; TNF, tumor necrosis factor.

¹ Present address for W. Khovidhunkit: Endocrinology and Metabolism Unit, Department of Medicine, King Chulalongkorn Memorial Hospital, Chulalongkorn University, Bangkok, Thailand.

² To whom correspondence should be addressed.
e-mail: kfeingld@itsa.ucsf.edu

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levels of several proteins involved in RCT pathway decrease, including lecithin:cholesterol acyltransferase, cholesterol ester transfer protein, phospholipid transfer protein, hepatic lipase, and scavenger receptor class B type I (8–14). Third, there is an impairment of both cholesterol efflux from cells and cholesterol ester uptake into the liver (14–17). Fourth, a series of enzymes that catabolize cholesterol into bile acids, and several proteins in the bile acid transport pathway, are down-regulated (18–21). Collectively, several steps in RCT are impaired during the APR that may contribute to the increased risk of atherosclerosis.

Recently, several key proteins in the RCT pathway have been discovered, a number of which are members of the ATP-binding cassette (ABC) transporter superfamily (22). ABCA1 and ABCG1 are ABC transporters that are involved in the movement of cholesterol from cells to HDL and its apolipoproteins (23–28). ABCG5 and ABCG8 regulate sterol absorption from the small intestine and its excretion from the liver into the bile (29–32).

Identification of factors that regulate ABC transporters should provide insights into the mechanisms by which RCT is affected in physiological and pathological states. Previously, our laboratory has shown that in rodents, hepatic mRNA levels of liver X receptor (LXR) and retinoid X receptor (RXR) were rapidly decreased in response to endotoxin (33). LXR is a nuclear hormone transcription factor that heterodimerizes with RXR and with activation increases the expression of ABCA1, ABCG1, ABCG5, and ABCG8 (25, 29, 34–38). We therefore hypothesized that endotoxin treatment would decrease these ABC transporters in the liver. In the present study, we report that endotoxin decreases mRNA levels of ABCG5 and ABCG8 in mouse liver. In J774 murine macrophages, endotoxin and cytokines decrease levels of ABCA1 and ABCG1. However, there was no reduction in levels of LXR or in binding of nuclear proteins to an LXR response element in macrophages. Thus, while LXR may mediate the decrease in these proteins in the liver, these data demonstrate that, in macrophages, the effects of endotoxin on the transporters may not be mediated through LXR.

MATERIALS AND METHODS

Materials

Endotoxin [lipopolysaccharide (LPS) from *Escherichia coli* serotype 055:B5] was purchased from Difco Laboratories (Detroit, MI). Recombinant tumor necrosis factor α (TNF α) and interleukin-1 β (IL-1 β) were purchased from R and D Systems (Minneapolis, MN). Polyclonal antibody against ABCA1 was purchased from Novus Biologicals (Littleton, CO). Antibodies against LXR α and LXR β were purchased from Affinity Bioreagents (Golden, CO). Antibodies against RXR α , RXR β , and RXR γ were purchased from Santa Cruz Biotechnologies (Santa Cruz, CA). 8-(4-Chlorophenylthio) adenosine 3', 5'-cyclic monophosphate (cpt-cAMP), 22(*R*)-hydroxycholesterol, and all other chemicals were obtained from Sigma (St. Louis, MO). Supplies for immunoblot analysis were purchased from Amersham Biosciences (Piscataway, NJ).

Animal experiments

C57BL/6 mice (~6–8 weeks of age) were obtained from Jackson Laboratory (Bar Harbor, ME) and provided with rodent chow and water ad libitum. Animals were injected intraperitoneally with indicated doses of endotoxin (0.1–100 μ g/mouse), whereas control animals were injected with normal saline. The highest dose of endotoxin used in our study (100 μ g/animal) was able to induce the APR but is far below the lethal dose (LD₅₀ ~5 mg/100 g body weight) required to cause death in rodents in our laboratory. Because endotoxin can cause anorexia, food was withdrawn from endotoxin-injected mice and control mice after the injection. At indicated time points, the animals were euthanized using halothane, and the tissue was excised and stored at -80°C . The animal procedures were approved by the Animal Studies Subcommittee of the San Francisco Veterans Affairs Medical Center, and were performed in accordance with the guidelines.

Cell culture experiments

J774 murine macrophages were obtained from the American Type Culture Collection and maintained in minimum essential media supplemented with 10% fetal bovine serum under 5% CO₂. In some experiments, cells were incubated with 0.3 mM cpt-cAMP or 10 μ M 22(*R*)-hydroxycholesterol before being treated with endotoxin or cytokines. Endotoxin or cytokines was incubated with cells in media containing human serum albumin in the absence of serum, and at indicated time points, cells were washed and harvested.

Isolation of RNA and RNA blot analysis

Isolation of RNA and RNA blot analyses were performed as previously described (14). Total or poly(A)⁺ RNA was quantified by measuring absorption at 260 nm, and equal amounts of RNA were loaded on 1% agarose-formaldehyde gels and electrophoresed. The uniformity of sample applications was checked by UV visualization of the acridine orange-stained gels before transfer to Nytran membranes. Because endotoxin increased hepatic mRNA levels of actin, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and cyclophilin in rodent liver (39–41), the mRNA levels of actin, GAPDH, and cyclophilin, which are widely used for normalizing data, cannot be used to study endotoxin regulation of proteins in the mouse liver. However, the differing directions of changes in mRNA levels (increased for some proteins, and decreased for some proteins), the magnitude of alterations (8-fold increase or up to 75% decrease), and the relatively small standard error of the mean (SEM) make it unlikely that the changes observed were due to unequal loading of mRNA. RNA blots were hybridized with ³²P-labeled cDNA probes generated by RT-PCR from the mouse liver (ABCG5, ABCG8, and ABCA1) or J774 murine macrophages (ABCG1) using the following primers: ABCG5 upper primer, 5'-TGC CCT TTC TGA GTC CAG AG-3' and lower primer, 5'-GTG CTC TTT CAA TGT TCT CCA G-3'; ABCG8 upper primer, 5'-ATG AGC TGG AAG ACG GGC TG-3' and lower primer, 5'-GCC AGT GAG AGC AAG GCT GA-3'; ABCA1 upper primer, 5'-TCT CTG CTA TCT CCA ACC TCA TC-3' and lower primer, 5'-ACG TCT TCA CCA GGT AAT CTG AA-3'; ABCG1 upper primer, 5'-GAA GAC CTG CAC TGC GAC ATC-3' and lower primer, 5'-GTT GCA TTG CGT TGC GTT AGT C-3'. After washing, the blots were exposed to X-ray films for various durations to ensure that measurements were done on the linear portion of the curve, and the bands were quantified using the Bio-Rad imaging densitometer.

Immunoblot analysis

J774 cells were scraped into cold PBS and centrifuged to get cell pellets. Lysis buffer containing 10 mM HEPES (pH 7.9), 0.5% Nonidet P40, 1.5 mM MgCl₂, 0.2 M sucrose, 10 mM KCl,

0.5 mM dithiothreitol, and 1% (v/v) protease inhibitor cocktail (Sigma) was incubated with cells for 5 min at 4°C. The supernatant was collected after centrifugation and the protein concentrations were measured. For immunoblot analysis of LXR and RXR, nuclear proteins were isolated as previously described (33). Proteins were resolved on polyacrylamide gels and transferred to Hybond-P PVDF membrane as previously described (14). For immunodetection, the blots were blocked in PBS/0.1% Tween containing 5% nonfat dry milk before incubation with a primary antibody and a secondary antibody conjugated with horseradish peroxidase. The blots were visualized using the ECL Plus chemiluminescence detection system and subjected to autoradiography. Quantification of the signals was performed by densitometry.

Electrophoretic mobility shift assay

Nuclear extracts were prepared as described above, and an electrophoretic mobility shift assay was performed as previously described (33). The oligonucleotides corresponding to the LXR response element of ABCA1 5'GCG CAG AGG TTA CTA TCG GTC AAA3' (36) and the mutated oligonucleotides 5' GCG CAG TAG TTA CTA TCA CAC AAA3' were used.

Statistics

Data are presented as mean \pm SEM. Comparisons between groups were performed using a Student's *t*-test. *P* values less than 0.05 were considered significant.

RESULTS

Effect of endotoxin on mRNA levels of ABCG5 and ABCG8 in mouse liver and small intestine

Our laboratory has previously shown that endotoxin decreased hepatic mRNA and protein levels of LXR α in hamsters (33). We have obtained similar results in mouse liver (data not shown). Because ABCG5 and ABCG8 are regulated by LXR α , we examined whether these two transporters were affected by endotoxin.

A single dose of 100 μ g of endotoxin rapidly decreased hepatic mRNA levels of ABCG5 and ABCG8 in mice as shown in **Fig. 1A** and **B**. The mRNA levels started to decrease at 4–8 h and continued to be significantly suppressed for 24 h after endotoxin administration. Although the chronological pattern and the magnitude of the reduction of hepatic mRNA levels of both ABCG5 and ABCG8 were relatively similar, slight differences in the degree of the inhibition suggest that the mechanisms might not be completely identical.

The dose response curve of the effects of endotoxin on hepatic mRNA levels of ABCG5 and ABCG8 was examined at the 16 h time point. Administration of endotoxin resulted in a dose-dependent decrease in hepatic mRNA levels of both ABCG5 and ABCG8 (**Fig. 2A, B**). The levels were significantly decreased by low doses of endotoxin. A half-maximal decrease in hepatic levels of ABCG5 and ABCG8 was produced by \sim 0.1 μ g and 2.0 μ g of endotoxin, respectively.

In addition to the liver, ABCG5 and ABCG8 are also expressed in the small intestine. Therefore, we examined whether endotoxin injection decreased intestinal mRNA levels of these two transporters. In contrast to those observed in the liver, we found that there were no changes in

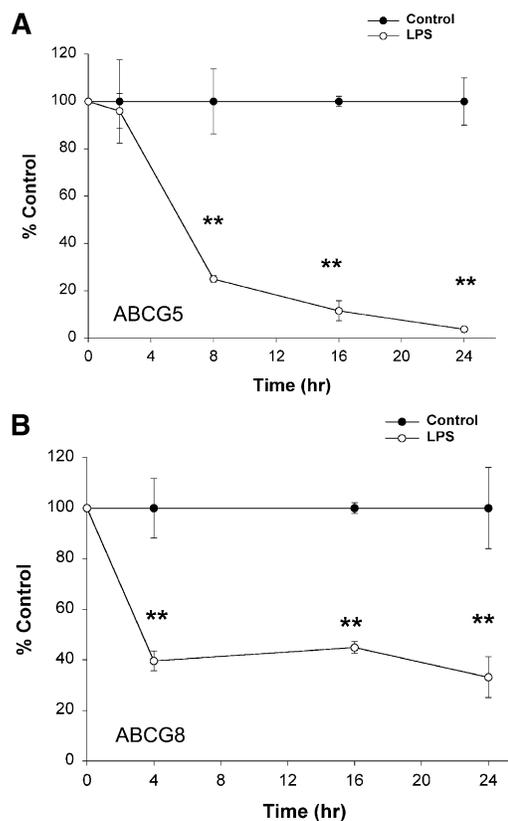


Fig. 1. Time course of the effect of endotoxin [lipopolysaccharide (LPS)] on ATP binding cassette (ABC)G5 mRNA (A) and ABCG8 mRNA (B) in mouse liver. Mice were injected with endotoxin (100 μ g). At indicated time points, livers were harvested and RNA was isolated. Poly(A)⁺ RNA was hybridized with a ³²P-labeled cDNA probe, and bands were analyzed by agarose gel electrophoresis followed by autoradiography as described in Materials and Methods. Data are presented as percent change versus control (mean \pm SEM). *n* = 4–5 in each group at each time point. ** *P* < 0.01.

the mRNA levels of both ABCG5 and ABCG8 in the small intestine (ABCG5 expression in the control group was 100 \pm 2% and in the endotoxin group was 79 \pm 12%, *P* = nonsignificant; and ABCG8 expression in the control group was 100 \pm 6% and in the endotoxin group was 115 \pm 13%, *P* = nonsignificant). ABCA1 mRNA levels in the small intestine were also not decreased in response to endotoxin (100 \pm 15% in the control group and 166 \pm 25% in the endotoxin group, *P* = nonsignificant).

To further determine whether the different results were due to differential regulation of LXR/RXR between the small intestine and the liver, we examined the mRNA levels of LXR and RXR in the small intestine in response to endotoxin. We found no significant changes in LXR β or RXR α mRNA in the small intestine, and did not detect LXR α , RXR β , or RXR γ (data not shown). Additionally, PPAR α and PPAR γ were not detected, and PPAR β / σ was abundantly expressed, but was not changed by endotoxin treatment. These results suggest that the difference in the responsiveness of ABCG5 and ABCG8 between the liver and the small intestine may be due to differential regulation of LXR and RXR by endotoxin in different tissues.

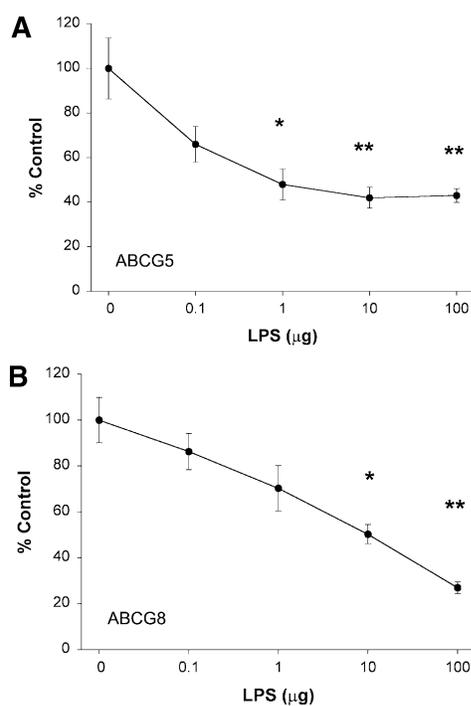


Fig. 2. Dose response curve of the effect of endotoxin (LPS) on ABCG5 mRNA (A) and ABCG8 mRNA (B) in mouse liver. Mice were injected with endotoxin (100 µg). At 16 h, livers were harvested and RNA was isolated. Poly(A)⁺ RNA was hybridized with a ³²P-labeled cDNA probe, and bands were analyzed by agarose gel electrophoresis followed by autoradiography as described in Materials and Methods. Data are presented as percent change versus control (mean ± SEM). n = 4–5 in each group at each time point. * *P* < 0.05, ** *P* < 0.01.

Effect of endotoxin on mRNA levels of ABCA1 in J774 murine macrophages

In macrophages, ABCA1 plays a critical role in apolipoprotein-mediated cholesterol efflux (23, 24). We found that incubation of endotoxin with J774 murine macrophages resulted in a decrease in ABCA1 mRNA levels (Fig. 3). The maximal decrease in mRNA levels of ABCA1 was observed 8 h after endotoxin and was relatively sustained until 24 h (Fig. 3A). There was also a concentration-dependent decrease in mRNA levels of ABCA1 (Fig. 3B). It is of note that the half-maximal decrease in the mRNA levels of ABCA1 was produced by the concentrations of endotoxin between 1–10 ng/ml, which corresponds to the concentrations of endotoxin found in the circulation during sepsis (42).

Effect of endotoxin on mRNA levels of ABCG1 in J774 cells

Besides ABCA1, ABCG1 is another ABC transporter that is expressed in macrophages and is involved in cholesterol efflux (25). We examined whether endotoxin had similar effects on ABCG1. As shown in Fig. 4A and B, endotoxin decreased mRNA levels of ABCG1 in J774 cells in a time-dependent and concentration-dependent manner. The degree of reduction of mRNA levels of ABCG1, however, was relatively small compared with that observed with ABCA1 (Fig. 3A, B).

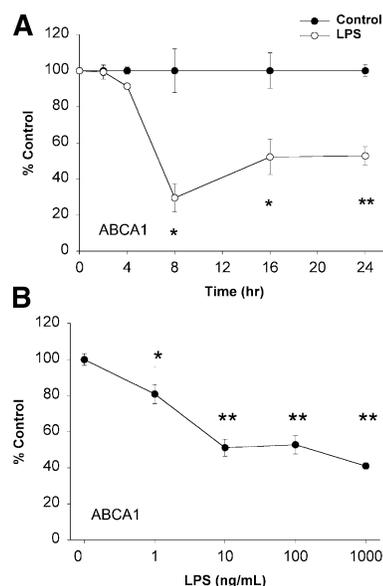


Fig. 3. Time course (A) and dose response curve (B) of the effect of endotoxin (LPS) on ABCA1 mRNA in J774 macrophages. Cells were incubated with indicated concentrations of endotoxin, and at indicated time points, cells were harvested and RNA was isolated. Total RNA was hybridized with a ³²P-labeled cDNA probe, and bands were analyzed by agarose gel electrophoresis followed by autoradiography as described in Materials and Methods. Data are presented as percent change versus control (mean ± SEM). n = 3 in each group at each time point. * *P* < 0.05, ** *P* < 0.01.

Effect of TNF and IL-1 on mRNA levels of ABCA1 and ABCG1 in J774 cells

Because cytokines mediate many of the effects of endotoxin, we next examined the effect of cytokines on mRNA levels of ABCA1. Both TNF and IL-1 (100 ng/ml) produced a ~50% decrease in ABCA1 mRNA levels (Fig. 5A). Similarly, TNF and IL-1 were able to decrease mRNA levels of ABCG1 (Fig. 5B).

Effect of cAMP, oxysterols, and endotoxin on mRNA levels of ABCA1 and ABCG1

Both cAMP and oxysterols are known inducers of ABCA1; however, the mechanism of induction is different. Oxysterols also induce ABCG1. To determine whether endotoxin can decrease mRNA levels of ABCA1 and ABCG1 in the presence of an inducer, we incubated J774 cells with cpt-cAMP, a cAMP analog, or 22(*R*)-hydroxycholesterol, an oxysterol, before treating cells with endotoxin. We found that cpt-cAMP and 22(*R*)-hydroxycholesterol both induced ABCA1 mRNA levels, but the magnitude was different. cpt-cAMP increased ABCA1 mRNA levels by 15- to 20-fold, whereas 22(*R*)-hydroxycholesterol increased ABCA1 mRNA levels by ~40% (Fig. 6A). In our studies, the mRNA levels of ABCG1 were not significantly changed with either cpt-cAMP or 22(*R*)-hydroxycholesterol (Fig. 6B).

Treatment with endotoxin did not decrease ABCA1 mRNA levels when ABCA1 was induced by cpt-cAMP, but endotoxin was able to cause an ~50% reduction in the mRNA levels of ABCA1 when ABCA1 was induced by 22(*R*)-hydroxycholesterol (Fig. 6A). Endotoxin also de-

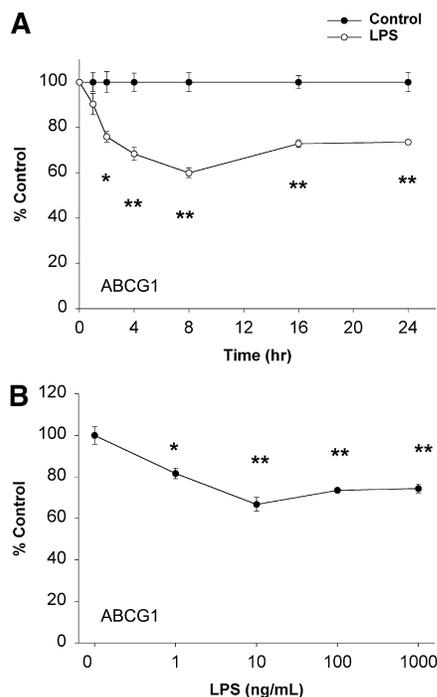


Fig. 4. Time course (A) and dose response curve (B) of the effect of endotoxin (LPS) on ABCG1 mRNA in J774 macrophages. Cells were incubated with indicated concentrations of endotoxin and, at indicated time points, cells were harvested and RNA was isolated. Total RNA was hybridized with a ^{32}P -labeled cDNA probe, and bands were analyzed by agarose gel electrophoresis followed by autoradiography as described in Materials and Methods. Data are presented as percent change versus control (mean \pm SEM). $n = 3$ in each group at each time point. * $P < 0.05$, ** $P < 0.01$.

creased mRNA levels of ABCG1 when 22(*R*)-hydroxycholesterol was present, but not cpt-cAMP (Fig. 6B).

Effect of endotoxin, TNF, and IL-1 on ABCA1 protein levels in J774 murine macrophages

It has been reported that in different tissues, the mRNA levels of ABCA1 do not always correlate with the protein levels (43). Because of the reduction in mRNA levels of ABCA1 in macrophages, we investigated whether protein levels of ABCA1 were also decreased. As shown in Fig. 7, endotoxin, TNF, and IL-1 all produced a significant decrease in the protein levels of ABCA1. Due to lack of reactive antibodies against ABCG1, the protein levels in J774 cells were not investigated.

Effect of endotoxin on protein levels of LXR and RXR in J774 cells

Our laboratory has previously shown that, in the liver, endotoxin treatment is associated with a decrease in mRNA levels, proteins levels, and binding activities of LXR and RXR (33). Since ABCA1 and ABCG1 are activated by LXR, and endotoxin was able to block the induction of ABCA1 and ABCG1 by 22*R*-hydroxycholesterol, an LXR ligand, we next examined whether a decrease in ABCA1 and ABCG1 in macrophages was due to a decrease in LXR. We, however, found no changes in the protein levels of either

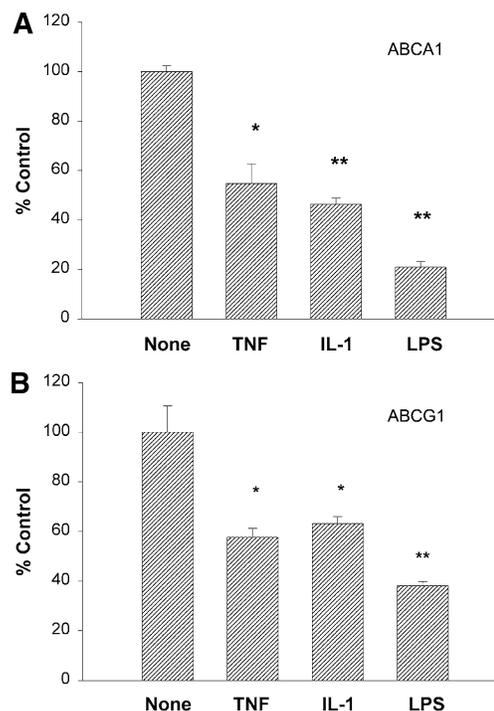


Fig. 5. The effect of tumor necrosis factor (TNF), interleukin-1 (IL-1), and endotoxin (LPS) on ABCA1 mRNA (A) and ABCG1 mRNA (B) levels in J774 macrophages. TNF (100 ng/ml), IL-1 (100 ng/ml), or endotoxin (100 ng/ml) was incubated with cells in minimal essential media with 2.5% human serum albumin in the absence of serum, and 24 h later, cells were harvested for RNA isolation as described in Materials and Methods. $n = 3$ in each group. * $P < 0.05$, ** $P < 0.01$.

LXR α or LXR β in J774 cells when cells were treated with endotoxin (Fig. 8A).

LXR is a nuclear hormone receptor that heterodimerizes with RXR. We next determined whether endotoxin affected the protein levels of RXR in J774 cells. We detected all three isoforms of RXR in these cells, RXR α , RXR β , and RXR γ . Endotoxin decreased the protein levels of RXR α , whereas it increased the protein levels of RXR β and RXR γ (Fig. 8A). We performed an electrophoretic mobility shift assay using the oligonucleotides that correspond to the LXR response element found in the ABCA1 promoter region to determine the binding of LXR/RXR heterodimers. However, we found that there was no difference between nuclei preparations from control cells and cells treated with endotoxin in the ability to bind the oligonucleotides (Fig. 8B). Altogether, these data did not support the hypothesis that the decrease in ABCA1 levels in J774 macrophages was primarily mediated through a reduction in either LXR or RXR.

DISCUSSION

A number of proteins in the ABC transporter superfamily have recently been discovered to play a role in cholesterol efflux and RCT (22). ABCA1 is a transporter on the plasma membrane that translocates cholesterol and phos-

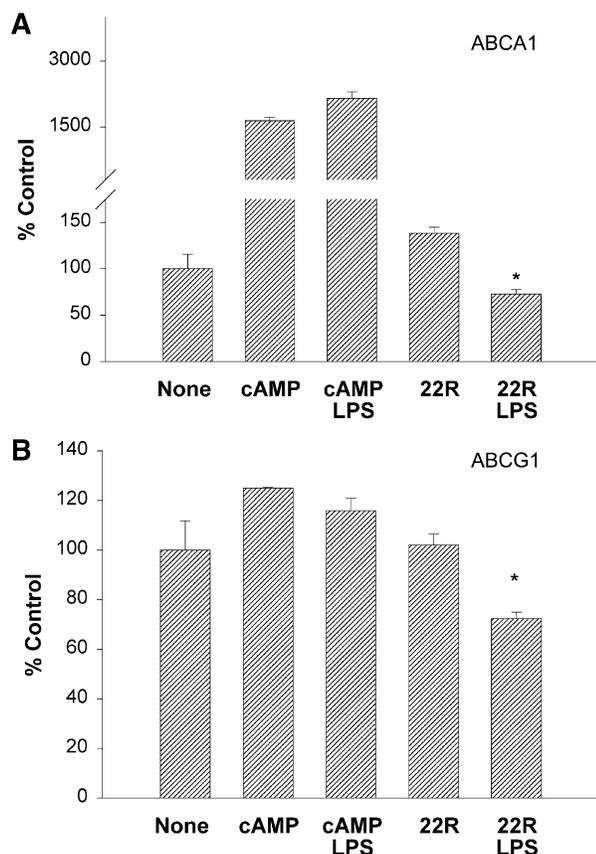


Fig. 6. The effect of 8-(4-chlorophenylthio)adenosine 3', 5'-cyclic monophosphate (cpt-cAMP) and 22(*R*)-hydroxycholesterol in the presence or absence of endotoxin (LPS) on ABCA1 mRNA (A) and ABCG1 mRNA (B) levels in J774 macrophages. J774 cells were pretreated with 0.3 mM cpt-cAMP or 10 μ M 22(*R*)-hydroxycholesterol before treatment with endotoxin (100 ng/ml) for 24 h. Cells were harvested for RNA isolation as described in Materials and Methods. $n = 3$ in each group. cAMP denotes cpt-cAMP and 22*R* denotes 22(*R*)-hydroxycholesterol. * $P < 0.05$ compared with 22(*R*)-hydroxycholesterol alone.

pholipid out of the cells. Mutations of ABCA1 result in defects in apolipoprotein-mediated cholesterol efflux as found in Tangier disease and familial HDL deficiency (23, 44–47). These patients have low levels of HDL, and in some cases, premature atherosclerosis has been reported (48). ABCG1 is another ABC transporter that is involved in cholesterol efflux (25). In contrast to ABCA1, ABCG1 is located primarily inside the cells. ABCG5 and ABCG8 are two ABC half-transporters that form a heterodimer in the small intestine and in the liver (29, 49). In the small intestine, the ABCG5-ABCG8 heterodimers efflux plant sterols as well as dietary cholesterol out of the intestinal cells, whereas in hepatocytes, they efflux those sterols into the bile (31, 32, 50). Mutations in ABCG5 or ABCG8 are the cause of sitosterolemia, a disorder characterized by xanthomatosis and premature atherosclerosis due to uncontrolled absorption of sterols and failure to excrete them into the bile. In addition to defects in plant sterol metabolism, patients with sitosterolemia also have abnormal cholesterol metabolism. An increase in cholesterol absorption as

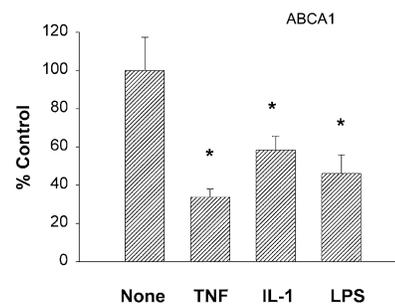


Fig. 7. The effect of endotoxin (LPS) on ABCA1 protein levels in J774 macrophages. Endotoxin (100 ng/ml) was incubated with cells in minimal essential media with 2.5% human serum albumin in the absence of serum, and 24 h later, cells were harvested for protein isolation as described in Materials and Methods. $n = 3$ in each group. * $P < 0.05$.

well as a defect in cholesterol excretion into the bile has been noted in these patients (51, 52).

Our laboratory has previously reported that endotoxin decreased LXR and RXR in rodent liver. Because ABCG5 and ABCG8 are positively regulated by LXR (29, 38), we investigated the regulation of ABCG5 and ABCG8 by endotoxin in the liver. As expected, we found that both ABCG5 and ABCG8 were coordinately down-regulated. Our data suggest that cholesterol excretion into the bile could be impaired during the APR. Previously, we and others have shown that during infection, there is a reduction in sev-

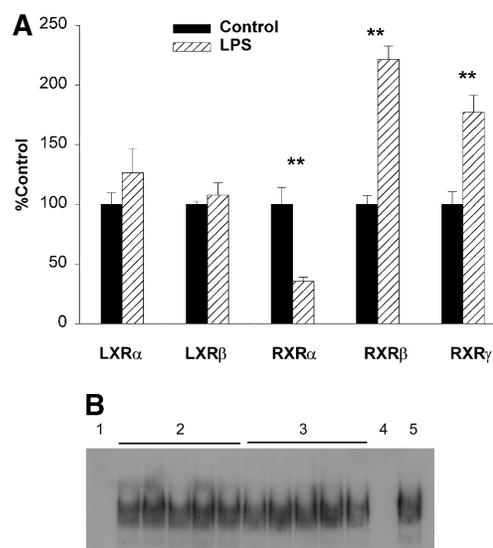


Fig. 8. The effect of endotoxin (LPS) on protein levels of liver X receptor (LXR) α , LXR β , retinoid X receptor (RXR) α , RXR β , and RXR γ (A) and binding activities of LXR/RXR (B) in J774 macrophages. J774 cells were incubated with endotoxin (100 ng/ml) for 24 h, and nuclei fractions of the cells were prepared for immunoblot analysis and/or electrophoretic mobility shift assay as described in Materials and Methods. $n = 3$ –5 in each group at each time point. ** $P < 0.01$. B: 1, no nuclear extract; 2, five different samples of nuclear extract from control cells; 3, five different samples of nuclear extract from endotoxin-treated cells; 4, unlabeled specific oligonucleotides were included at 100-fold excess for competition; 5, unlabeled nonspecific (mutated) oligonucleotides were included at 100-fold excess for competition.

eral key enzymes involved in bile acid synthesis, including CYP7A, CYP7B, and CYP27 (18, 19). In addition, there is a decrease in bile salt export pump and multidrug resistance-related protein 2, two main proteins involved in canalicular bile salt secretion, resulting in decreased bile salt excretion and cholestasis (53–55). Therefore, it appears that the majority of cholesterol excretion through bile acids is impaired during infection. Taken together, during infection not only is cholesterol excretion decreased, but there is also coordinate reduction in nearly every step of cholesterol catabolism and excretion into the bile. This reduction is likely the mechanism of cholestasis commonly observed during sepsis (20, 54). The reduction in cholesterol excretion could help retain cholesterol in the body for use during infection.

In macrophages, ABCA1 and ABCG1 are involved in cholesterol efflux. Previous studies from our laboratory and others have shown that during the APR, changes in HDL particles resulted in impairment of cholesterol efflux from cells to HDL (15, 16). Treatment of mouse peritoneal macrophages with interferon- γ caused a decrease in ABCA1 mRNA levels and cholesterol efflux (56). A recent study in RAW cells also reported that endotoxin treatment down-regulated ABCA1 mRNA levels (57); however, data on protein levels of ABCA1 were not provided. A study comparing mRNA and protein levels of ABCA1 in different tissues has shown that in some tissues, the abundance of mRNA levels and protein levels are not well correlated (43). For example, in spleen and thymus, the mRNA levels of ABCA1 were low, but the levels of protein found were higher than expected (43). Therefore, we examined both mRNA levels and protein levels of ABCA1 in response to endotoxin treatment in our studies using J774 cells. We found that endotoxin decreased both ABCA1 mRNA and protein levels. We also provide new information that TNF and IL-1 treatment, like endotoxin, could decrease ABCA1 mRNA and protein levels. Collectively, our data on ABCA1 as well as ABCG1 provide the mechanism for previous findings that cholesterol efflux was impaired during the APR (16, 57).

In macrophages, both ABCA1 and ABCG1 are up-regulated by LXR, but we found that the effect could not be explained by a decrease in LXR. Data from immunoblot analysis showed that both isoforms of LXR, LXR α and LXR β , did not decrease with endotoxin. When RXR, a partner of LXR heterodimerization, was examined in J774 cells, changes in the levels of the three isoforms of RXR were different. Endotoxin decreased RXR α but increased RXR β and RXR γ isoforms. Because the relative abundance of different LXR and RXR isoforms vary among different cell types, an electrophoretic mobility shift assay was performed to detect the binding of LXR/RXR heterodimers to the LXR response element of ABCA1. Again, we did not find differences between nuclei preparations from control cells or cells treated with endotoxin. Therefore, our data did not support the hypothesis that a decrease in ABCA1 levels in J774 cells was mediated through a reduction in either LXR or RXR. A recent study using an inhibitor of NF- κ B reported that NF- κ B might be involved in the down-regulation of ABCA1 by endotoxin (57).

Our current study demonstrates that endotoxin, TNF, and IL-1 decreased ABCA1 mRNA and protein levels in J774 macrophages. A study in RAW264.7 cells using endotoxin also reported similar changes in mRNA levels (57). Another study using mouse peritoneal macrophages reported that interferon- γ decreased ABCA1 (56). However, in human THP-1 cells, ABCA1 was found to be up-regulated by endotoxin (58). It is not clear whether the discrepancy is due to different cell types, species specificity, or other unknown factors. It is of note that the up-regulation of ABCA1 by endotoxin in THP-1 cells was also reported to be mediated by an LXR-independent mechanism (58).

In summary, our study showed that endotoxin down-regulated ABCG5 and ABCG8 in the liver. This effect likely impairs cholesterol excretion from hepatocytes into the bile. Endotoxin also decreased ABCA1 and ABCG1 in macrophages, thus providing the mechanism for previous findings that cholesterol efflux from macrophages was impaired during the APR. While a decrease in LXR may mediate the decrease in levels of these proteins in the liver, the down-regulation of ABCA1 by endotoxin in macrophages is not likely to be mediated by LXR. The present study adds to the accumulating evidence that multiple steps in RCT are impaired during the APR. Although decreased RCT may redirect cholesterol to peripheral cells (e.g., leukocytes) to acutely promote host defense, prolongation of impaired RCT may contribute to an increased risk of atherosclerosis observed in chronic infections and inflammatory states. **FIG**

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