Regulation of scavenger receptor class B type I in hamster liver and Hep3B cells by endotoxin and cytokines

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Abstract **Multiple changes in HDL metabolism occur during infection and inflammation that could potentially impair the antiatherogenic functions of HDL. Scavenger receptor class B type I (SR-BI) promotes cholesterol efflux from peripheral cells and mediates selective uptake of cholesteryl ester into hepatocytes, thereby playing a pivotal role in reverse cholesterol transport. We studied the effect of endotoxin (lipopolysaccharide, LPS) and cytokines [tumor necrosis factor (TNF) and interleukin 1 (IL-1)] on hepatic SR-BI mRNA and protein levels in Syrian hamsters. LPS significantly decreased SR-BI mRNA levels in hamster liver. This effect was rapid and sustained, and was associated with a decrease in hepatic SR-BI protein levels. High cholesterol diet did not change hepatic SR-BI mRNA levels, and LPS was able to decrease SR-BI mRNA levels during high cholesterol feeding. TNF and IL-1 decreased SR-BI mRNA levels in the liver, and the effects of TNF and IL-1 were additive. TNF and IL-1 also decreased SR-BI levels in Hep3B hepatoma cells. More importantly, TNF and IL-1 decreased the uptake of HDL cholesteryl ester into Hep3B cells. In addition, we studied the effect of LPS on SR-BI mRNA in RAW 264.7 cells, a macrophage cell line. LPS rapidly decreased SR-BI mRNA levels in RAW 264.7 cells, but the effect was not sustained and did not lead to a reduction in SR-BI protein levels. Our results suggest that the decrease in hepatic SR-BI levels due to LPS and cytokines during infection and inflammation may decrease selective uptake of cholesteryl ester into the liver and result in impaired reverse cholesterol transport.**—Khovidhunkit, W., A. H. Moser, J. K. Shigenaga, C. Grunfeld, and K. R. Feingold. **Regulation of scavenger receptor class B type I in hamster liver and Hep3B cells by endotoxin and cytokines.** ***J. Lipid Res.*** **2001.** 42: **1636–1644.**

Supplementary key words acute-phase response • HDL metabolism • lipopolysaccharide • RAW 264.7 macrophages • reverse cholesterol transport

Lipoproteins are a heterogeneous group of lipid-transporting particles whose levels are associated with atherosclerosis. Whereas plasma levels of LDL cholesterol directly correlate with the risk of coronary artery disease, the relationship between plasma levels of HDL cholesterol and risk is inverse, indicating that HDL is antiatherogenic. There are several mechanisms by which HDL may protect against atherogenesis, and one of the most extensively studied and accepted hypotheses suggests that HDL plays a role in removing excess cholesterol from peripheral cells and returning it to the liver for excretion (1). This process, known as reverse cholesterol transport (RCT), consists of multiple steps and involves a number of enzymes, transfer proteins, and cell surface receptors (2, 3).

RCT begins when HDL removes cholesterol from cells in an unesterified form. Once transferred onto HDL, unesterified cholesterol is converted to cholesteryl ester by the enzyme LCAT. Cholesteryl ester in HDL particles may be returned to the liver by several mechanisms. First, cholesteryl ester in HDL may be exchanged for triglyceride in triglyceride-rich lipoproteins by CETP. Cholesteryl ester in triglyceride-rich lipoproteins can then be taken up by the hepatocyte via the LDL receptor and/or LDL receptor-related protein (LRP). Second, cholesteryl ester in HDL may be delivered directly into hepatocytes by endocytosis of HDL particles through the LDL receptor, LRP, or yet to be elucidated HDL receptors. Last, cholesteryl ester in HDL may be preferentially taken up by hepatocytes without degradation of apolipoproteins, leaving the particle intact. This latter process is known as selective uptake of cholesteryl ester.

Selective uptake of cholesteryl ester from HDL has been documented in several in vitro and in vivo studies (4–6), and is now known to be mediated by scavenger receptor class B type I (SR-BI) (7). SR-BI is a cell surface receptor initially shown to bind modified LDL (8). It was later demonstrated that SR-BI could bind HDL and mediate the selective uptake of cholesteryl ester from HDL into hepatocytes (7). Moreover, SR-BI may play an additional

Abbreviations: APR, acute-phase response; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-1, interleukin 1; LPDS, lipoprotein-deficient serum; LPS, lipopolysaccharide; LRP, LDL receptor-related protein; RCT, reverse cholesterol transport; SR-BI, scavenger receptor class B type I; TNF, tumor necrosis factor.

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role in cholesterol removal from peripheral cells (9, 10). Animal studies have shown that manipulating SR-BI levels affects HDL metabolism and the development of atherosclerosis (11–18), making SR-BI one of the key proteins in these processes. Identification of factors that regulate SR-BI in vivo should provide insights into the mechanisms by which HDL metabolism and RCT are affected in physiological and pathological conditions.

Evidence has suggested a possible relationship between chronic infection/inflammation and atherosclerosis (19). During infection and inflammation, a variety of changes in HDL metabolism occur (20). HDL cholesterol levels decrease during infection and inflammation. In addition, we and others have shown that endotoxin and cytokines decrease plasma levels and activities of several key proteins involved in HDL metabolism and RCT, including LCAT, CETP, hepatic lipase, and phospholipid transfer protein (21–26). In contrast, circulating levels of secretory phospholipase A_2, serum amyloid A, and apolipoprotein J increase (27–29). All of these changes can have profound effects on HDL metabolism and potentially affect RCT. Although SR-BI is one of the key receptors in RCT, how SR-BI is regulated in vivo by endotoxin and cytokines is unknown. In this study, we examined whether endotoxin and cytokines, major inducers of inflammation, affect SR-BI levels in hamster liver, in Hep3B human hepatoma cells, and in RAW 264.7 cells, a murine macrophage cell line.

MATERIALS AND METHODS

Materials

Endotoxin [lipopolysaccharide (LPS) from *Escherichia coli* serotype 055:B5] was purchased from Difco Laboratories (Detroit, MI). Recombinant human tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) were purchased from R&D Systems (Minneapolis, MN). Rabbit polyclonal antibody against the last 14 amino acid of the carboxy terminus of the mouse SR-BI (7) was kindly provided by H. H. Hobbs (Dallas, TX). In some experiments, anti-SR-BI antibody was purchased from Novus Biologicals (Littleton, CO). Supplies for immunoblot analysis and [^{3}H]cholesteryl oleyl ether (58.0 Ci/mmol) were purchased from Amersham-Pharmacia (Piscataway, NJ).

Animal experiments

We used hamsters as an animal model because HDL metabolism in hamsters resembles that of humans (30). In addition, changes in lipid and lipoprotein metabolism during infection/inflammation in hamsters have already been characterized (31). Male Syrian hamsters (approximately 140–180 g) were obtained from Charles River Laboratories (Wilmington, MA) and provided with rodent chow and water ad libitum. In cholesterol feeding experiments, animals were fed a 3% cholesterol diet for 4 days. Animals were injected intraperitoneally with the indicated doses of endotoxin, TNF, IL-1, or TNF and IL-1. Control animals were injected with normal saline. At the dose of 100 μg of LPS per 100 g body weight, animals become toxic, serum amyloid A is induced, and circulating cytokine levels are expected to be in the septic range (32). However, this dose is far below the lethal dose (LD_{50}~ 5 mg/100 g body weight) required to cause death in rodents in our laboratory. Because LPS and cytokines can cause anorexia, food was withdrawn after the injection. At the indicated time points, the animals were killed with halothane, plasma was collected, 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 (San Francisco, CA), and were performed in accordance with their guidelines.

Cell culture experiments

Hep3B human hepatoma cells and RAW 264.7 murine macrophages were obtained from the American Type Culture Collection (Manassas, VA) and maintained in MEM supplemented with 10% fetal bovine serum under 5% CO_2. For the mRNA and protein experiments, cells were plated until confluent. LPS or cytokine was added to MEM containing BSA in the absence of serum, and at the 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 (33). Poly(A)$^+$ RNA was quantified by measuring absorption at 260 nm and 10 μg of RNA was loaded on 1% agarose-formaldehyde gels and electrophoresed. The uniformity of sample applications was checked by ultraviolet visualization of the acridine orange-stained gels before transfer to Nytran membranes. We and others have found that LPS, TNF, and IL-1 increase actin mRNA levels in the liver by 2- to 5-fold in rodents (31, 34). LPS also produced a 2-fold increase in hepatic mRNA levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and a 2.6-fold increase in cyclophilin mRNA (35); therefore, the mRNA levels of actin, GAPDH, and cyclophilin, which are widely used for normalizing data, cannot be used to study LPS or cytokine-induced regulation of proteins in the liver. However, the differing direction of the changes in mRNA levels (increased for some proteins, decreased for some proteins, and no change for other proteins), the magnitude of alterations (up to 90% decrease), and the relatively small standard error of the mean make it unlikely that the changes observed were due to unequal loading of mRNA. RNA blots were hybridized with a ^{32}P-labeled SR-BI cDNA probe generated by RT-PCR from mouse liver (oligonucleotide primers: upper, 5′-ATGCAGGTC CATGAAGCTGAC-3′ and lower, 5′CTATAGCTTGGCTTCTTGC AGC-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 with a Bio-Rad (Hercules, CA) imaging densitometer.

Isolation of membranes and Immunoblot analysis

Frozen hamster tissues were homogenized in homogenization buffer containing 20 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 0.2 M sucrose, and 5% (v/v) protease inhibitor cocktail, and centrifuged at 8,000 *g* for 15 min. For cultured cells, after extensive washing, scraped cells were repeatedly frozen and thawed in homogenization buffer, using liquid N_2, and centrifuged to remove cell debris. The supernatant was collected and centrifuged at 100,000 *g* for 60 min. The membrane pellets were resuspended in 0.1 M phosphate buffer, pH 7.5, and protein concentration was measured. Membrane proteins were resolved on a 7.5% polyacrylamide gel and transferred to Hybond-P polyvinylidene difluoride membrane. For immunodetection, the blots were blocked in phosphate-buffered saline-0.1% Tween containing 5% nonfat dry milk before incubation with an anti-SR-BI antibody. Donkey anti-rabbit antibody conjugated with horseradish peroxidase was used as a secondary antibody. The blots were visualized with an enhanced chemiluminescence (ECL) Plus detection system and subjected to autoradiography. Quantification of the signals was performed by densitometry.

Isolation and labeling of HDL_3

HDL_3 (d = 1.125–1.21 g/ml) and lipoprotein-deficient serum (LPDS, d > 1.25 g/ml) were isolated from fasting sera of normal human subjects by ultracentrifugation and extensively dialyzed as previously described (36). HDL_3 was radiolabeled with [^{3}H]cholesteryl oleyl ether, a nondegradable, intracellularly trapped marker, using a glass fiber exchange method. HDL labeled with a cholesteryl ether analog has been shown to have similar metabolic and biological functions as native HDL (4). Briefly, 100 μCi of [^{3}H]cholesteryl oleyl ether was applied onto the glass microfiber filter in a glass scintillation vial and dried with N_2. One milliliter of HDL_3 (~5 mg/ml) and 5 ml of LPDS (as a source of CETP) were added and the vial was rotated gently for 24 h at 37°C. HDL_3 was recovered after ultracentrifugation and dialysis. The cholesterol/protein ratio of HDL_3 was ~0.27, and the ^{3}H activity of radiolabeled HDL_3 was ~29,000 cpm/μg cholesterol.

Cholesteryl ester uptake experiments

Uptake of radiolabeled HDL_3 was performed as previously described (37). Hep3B human hepatoma cells were plated overnight in serum-containing medium. On the following day, cells were incubated in MEM containing 10% LPDS with or without cytokines or LPS for 48 h. Cells were then preincubated in MEM containing 0.1% BSA for 30 min before incubation with radiolabeled HDL_3 (20 μg of protein per ml) for 4 h. EDTA (1 mM) was added to the medium to inhibit the activity of classic lipoprotein receptors. Unlabeled HDL_3 (60–100 μg/ml) was added for chase incubation for 2 h to remove reversibly cell-associated tracers. Cells were extensively washed with phosphate-buffered saline and dissolved in 0.1 N NaOH for 30 min followed by sonication. Aliquots were used to determine ^{3}H radioactivity and DNA content. The uptake of cholesteryl ester was expressed as nanograms of cholesteryl ester per microgram of DNA.

Biochemical determinations

Total and free cholesterol concentrations were measured by enzymatic assays (Wako, Osaka, Japan). Protein concentrations were determined by a modified Lowry protein method (Pierce, Rockford, IL). DNA content of cells was determined by a fluorometric method (38).

Statistics

Data are presented as means ± SEM. Comparisons between groups were performed by a Student's *t*-test. *P* values less than 0.05 were considered significant.

RESULTS

Effect of LPS on lipids and lipoprotein levels

LPS administration to hamsters (100 μg/100 g body weight) resulted in a significant decrease in plasma HDL cholesterol levels at 16 h (58 ± 3 mg/dl in control group, n = 6, and 44 ± 3 mg/dl in LPS-treated group, n = 7, 25% decrease, $P < 0.005$). In our previous study using 50 μg of LPS per 100 g body weight (21), we showed that HDL cholesterol levels decreased by 41%, 35%, 13%, and 17% at 4, 8, 16, and 24 h, respectively, after LPS injection. Similarly, both total and LDL cholesterol levels in this study also increased significantly after LPS injection as previously observed (21) (total cholesterol = 94 ± 8 mg/dl in control group and 146 ± 13 mg/dl in LPS-treated group, $P < 0.01$; LDL cholesterol = 13 ± 5 mg/dl in control group and 77 ± 12 mg/dl in LPS-treated group, $P < 0.001$). Plasma triglyceride levels increased after LPS injection but did not reach significance (109 ± 10 mg/dl in control group and 128 ± 21 mg/dl in LPS-treated group, $P = 0.45$).

Effect of LPS on hepatic SR-BI mRNA and protein levels in hamsters

A single dose of 100 μg of LPS per 100 g body weight rapidly decreased hepatic SR-BI mRNA levels in hamsters as shown in **Fig. 1A** and **B**. LPS produced a maximum decrease in SR-BI mRNA levels at 8–16 h (75% decrease), and the levels continued to be significantly suppressed 24 h after LPS administration.

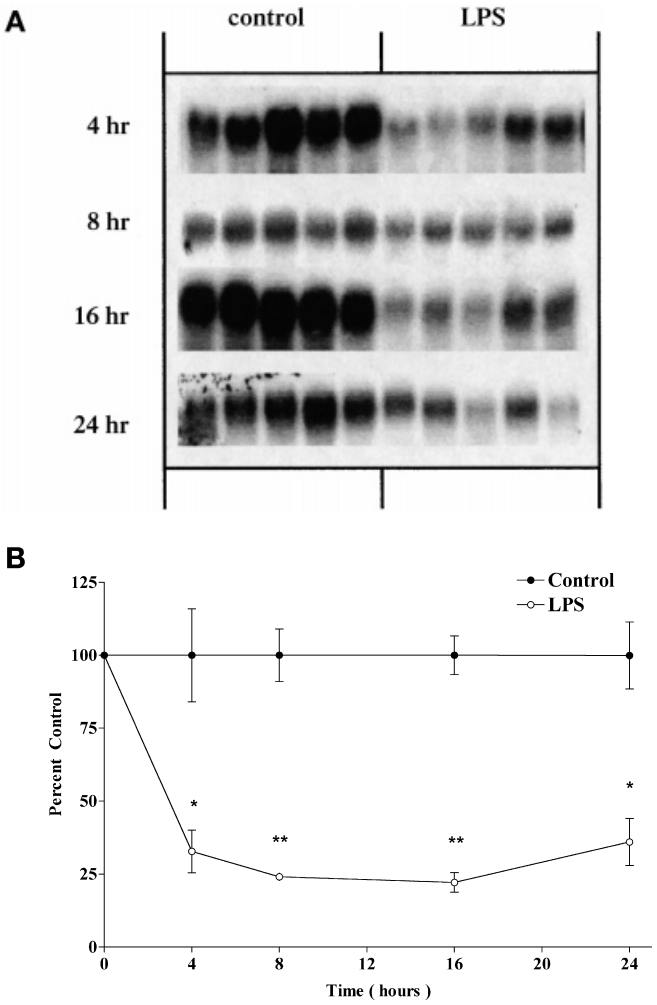


Fig. 1. Time course of the effect of LPS on SR-BI mRNA in hamster liver. Syrian hamsters were injected with LPS (100 μg/100 g body weight). At the indicated time points, livers were harvested and RNA was isolated. A: Poly(A)$^+$ RNA (10 μg in each lane) was hybridized with ^{32}P-labeled cDNA probes and bands were analyzed by agarose gel electrophoresis followed by autoradiography as described in Materials and Methods. B: The SR-BI bands were quantitated by densitometry. Data are presented as percent change versus control (mean ± SEM). n = 5 in each group at each time point. * $P < 0.005$; ** $P < 0.001$.

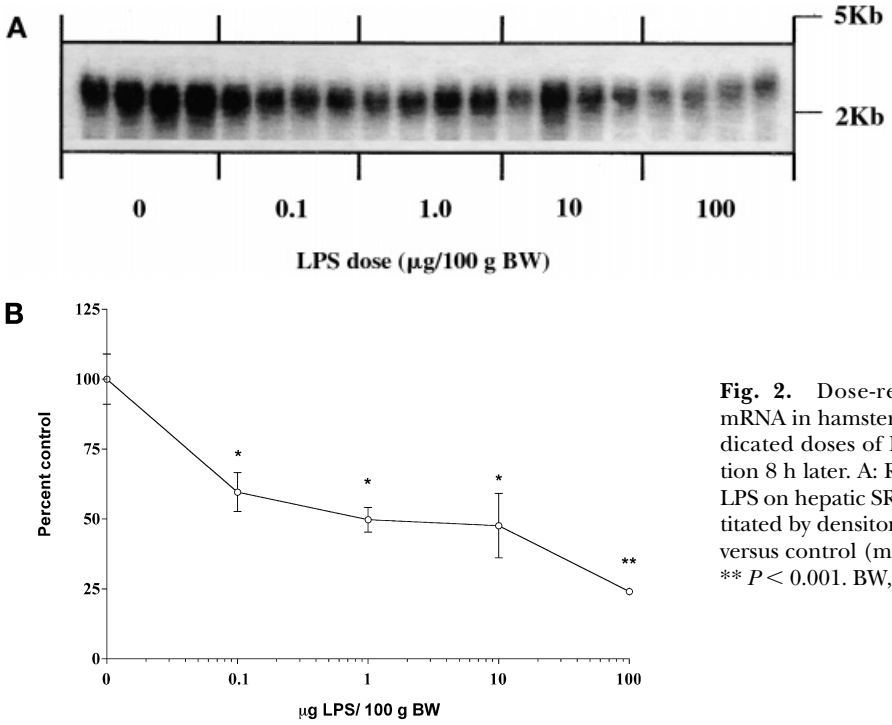


Fig. 2. Dose-response curve of the effect of LPS on SR-BI mRNA in hamster liver. Syrian hamsters were injected with the indicated doses of LPS and livers were harvested for mRNA isolation 8 h later. A: RNA blot showing the effect of different doses of LPS on hepatic SR-BI mRNA levels. B: The SR-BI bands were quantitated by densitometry and data are presented as percent change versus control (mean ± SEM). n = 4 in each group. * $P < 0.025$; ** $P < 0.001$. BW, Body weight.

The dose-response curve of LPS effects on hepatic SR-BI mRNA levels was examined at the 8-h time point. LPS administration resulted in a dose-dependent decrease in hepatic SR-BI mRNA levels (**Fig. 2A** and **B**). The levels were significantly decreased by low doses of LPS, which did not cause the animals to appear ill. A half-maximal decrease in hepatic SR-BI mRNA levels of hamsters was produced by approximately 0.1 μg of LPS per 100 g body weight.

Because LPS induced a reduction in SR-BI mRNA in hamster liver, we next investigated whether SR-BI protein levels in the liver also are decreased. We determined the protein levels of hepatic SR-BI at the time of the maximal decrease of mRNA levels. Sixteen hours after LPS injection (100 μg/100 g body weight), SR-BI protein levels in the liver were significantly decreased (80% decrease) compared with controls (**Fig. 3A** and **B**). In contrast, SR-BI protein levels in the adrenal and the testis, two tissues that highly express SR-BI, increased in response to LPS but the increases did not achieve significance (data not shown).

Effect of diet and LPS on hepatic SR-BI mRNA levels

A high cholesterol diet has been shown to decrease hepatic SR-BI mRNA levels in rats (39); therefore, we next investigated whether a high cholesterol diet had a similar effect on hepatic SR-BI mRNA levels in hamsters. After 4 days of a 3% cholesterol diet, the hepatic levels of SR-BI mRNA were not significantly different from those of hamsters fed a regular chow diet (**Fig. 4A** and **B**). As described above, LPS significantly decreased hepatic SR-BI mRNA levels in hamsters fed regular chow. In addition, LPS also significantly decreased hepatic SR-BI mRNA levels in animals fed a high cholesterol diet (Fig. 4A and B).

Effect of TNF and IL-1 on hepatic SR-BI mRNA levels

Cytokines mediate many of the effects of LPS, and therefore we next examined the effect of cytokines on

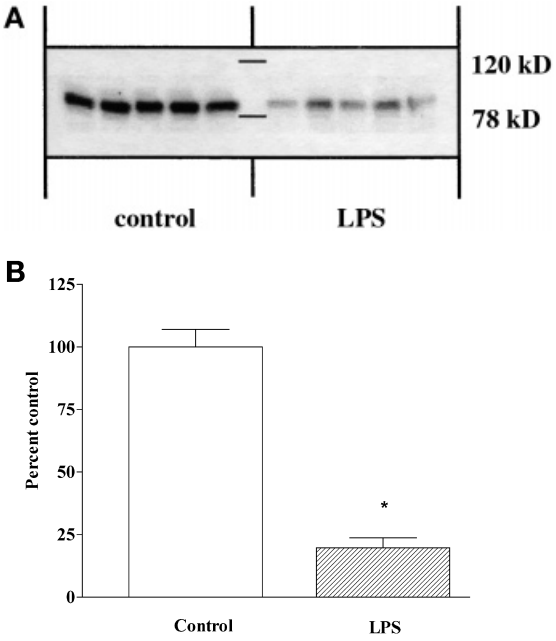
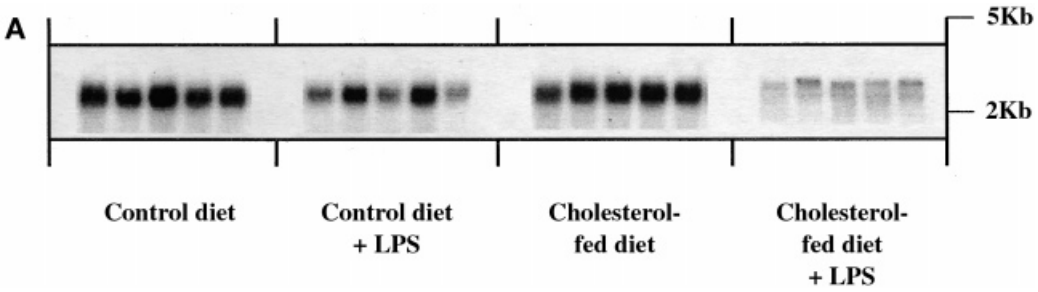


Fig. 3. The effect of LPS on SR-BI protein in hamster liver. Syrian hamsters were injected with normal saline or LPS (100 μg/100 g body weight) and 16 h later, livers were harvested for preparation of liver membranes. A: Fifty micrograms of liver membrane was loaded in each lane and immunoblot analysis was performed as described in Materials and Methods. B: The SR-BI bands were quantitated by densitometry and data are presented as percent change versus control (mean ± SEM). n = 5 in each group. * $P < 0.001$.



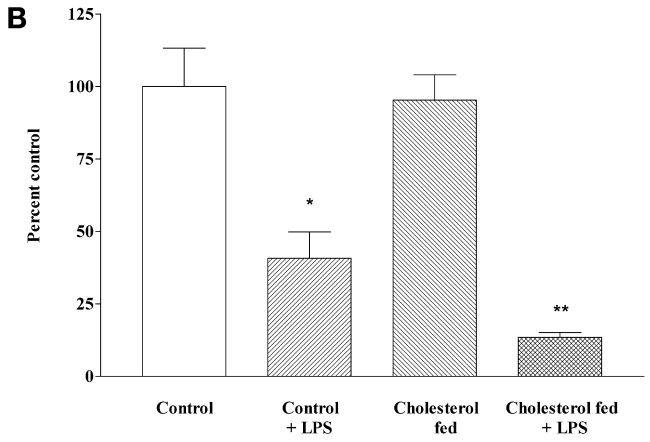
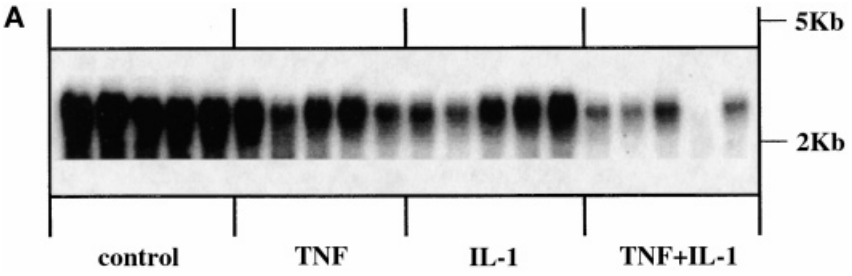


Fig. 4. The effect of diet and LPS on SR-BI mRNA in hamster liver. Syrian hamsters were fed regular chow or a 3% cholesterol diet for 4 days, followed by injection with normal saline or LPS (100 μg/100 g body weight). The liver was harvested 16 h later for mRNA isolation. A: RNA blots showing the effect of a high cholesterol diet and LPS on hepatic SR-BI mRNA levels. B: The SR-BI bands were quantitated by densitometry and data are presented as percent change versus control (mean ± SEM). n = 5 in each group. The difference between the control group and the cholesterol-fed group is not statistically significant ($P = 0.8$). * $P < 0.01$; ** $P < 0.001$.

hepatic SR-BI mRNA levels. The effects of TNF (25 μg/100 g body weight), IL-1 (1 μg/100 g body weight), and a combination of TNF and IL-1 (25 μg TNF and 1 μg IL-1/100 g body weight) on hepatic SR-BI mRNA levels are shown in **Fig. 5A** and **B**. TNF produced a 50% decrease in SR-BI mRNA levels; IL-1 induced a 60% reduction in hepatic SR-BI mRNA levels. In addition, the combination of TNF and IL-1 administration resulted in a further decrease in SR-BI mRNA levels (90%), suggesting an additive effect.

Effect of TNF and IL-1 on SR-BI levels and cholesteryl ester uptake in Hep3B cells

In the liver, SR-BI is expressed in both hepatocytes and Kupffer cells (39). To determine whether the decrease in



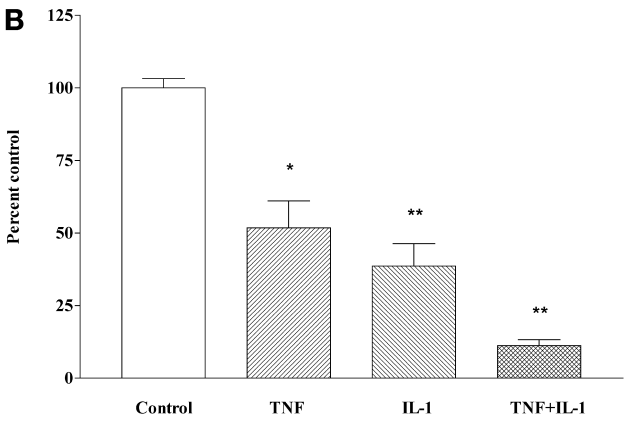


Fig. 5. The effect of cytokines on SR-BI mRNA in hamster liver. Syrian hamsters were injected with normal saline, TNF-α (25 μg/100 g body weight), IL-1β (1 μg/100 g body weight), or a combination of TNF-α and IL-1β (25 μg of TNF-α and 1 μg of IL-1β/100 g body weight) and livers were harvested 8 h later for mRNA isolation. A: RNA blot showing the effect of TNF and/or IL-1 on hepatic SR-BI mRNA levels. B: The SR-BI bands were quantitated by densitometry and data are presented as percent change versus control (mean ± SEM). n = 5 in each group except in the TNF + IL-1 group (n = 4). * $P = 0.001$; ** $P < 0.001$.

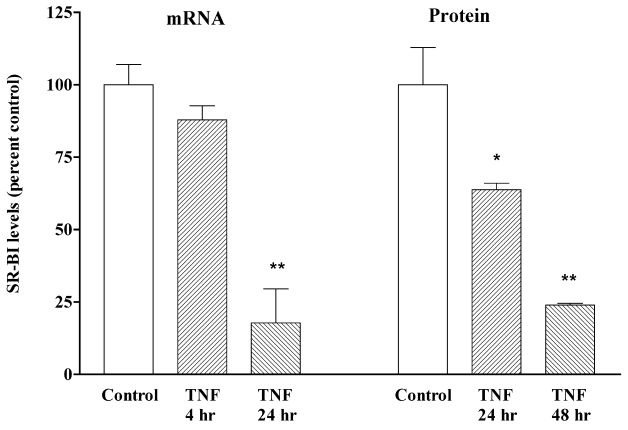


Fig. 6. The effect of TNF on SR-BI mRNA and protein levels in Hep3B cells. Hep3B cells were grown until confluent. TNF (10 ng/ml) was incubated with cells in MEM with 0.1% BSA in the absence of serum, and at the indicated time points cells were harvested. A: The effect of TNF on SR-BI mRNA levels at 4 and 24 h. n = 4 in each group at each time point. B: The effect of TNF on SR-BI protein levels at 24 and 48 h. n = 3 in each group at each time point. * $P < 0.05$; ** $P < 0.01$.

SR-BI levels in the liver was due to a decrease in SR-BI on the hepatocyte, we studied the effect of cytokines in Hep3B human hepatoma cells. In agreement with our in vivo data, treatment of Hep3B cells with TNF (10 ng/ml) resulted in a significant decrease in SR-BI mRNA and proteins levels. There was a 12% decrease in SR-BI mRNA levels at 4 h and an 82% decrease at 24 h (**Fig. 6**). For SR-BI protein levels, there was a 36% decrease at 24 h and a 76% decrease at 48 h (Fig. 6). A similar degree of reduction of SR-BI levels between hamster liver and Hep3B cells suggests that the decrease in vitro reflects the in vivo changes.

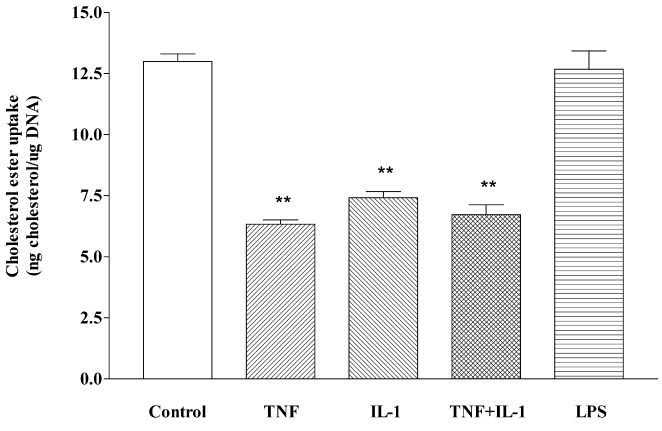


Fig. 7. The effect of cytokines on cholesteryl ester uptake from HDL. HDL_3 was isolated and radiolabeled as described in Materials and Methods. Hep3B cells were incubated with TNF-α (10 ng/ml), IL-1β (10 ng/ml), TNF-α and IL-1β (10 ng/ml each), LPS (1 μg/ml), or no cytokine/LPS for 48 h before incubation with radiolabeled HDL_3 (20 μg/ml) for 4 h. Unlabeled HDL_3 (60 μg/ml) was added for 2 h and cells were lysed in 0.1 N NaOH and sonicated. Aliquots were used to determined radioactivity and DNA content. ** $P < 0.001$ compared with control.

SR-BI on the hepatocyte promotes cholesteryl ester uptake from HDL. To determine the significance of the decrease in SR-BI levels in the liver and in Hep3B cells, we performed cholesteryl ester uptake experiments using HDL_3 that had been labeled with a cholesteryl ether analog. Preincubation of TNF (10 ng/ml), IL-1 (10 ng/ml), or a combination of TNF and IL-1 (10 ng/ml each) with Hep3B cells resulted in a decrease in uptake of HDL_3 cholesteryl ether analog (**Fig. 7**), indicating that the cytokine-induced decrease in SR-BI resulted in a decrease in cholesteryl ester uptake by cells. Preincubation of LPS (10–1,000 ng/ml) with Hep3B cells, in contrast, did not decrease the uptake of cholesteryl ester from HDL_3 (Fig. 7), confirming previous studies that immortalized cell lines, such as Hep3B and HepG2 cells, typically respond to cytokines, but not LPS (40–42).

Effect of LPS on SR-BI mRNA levels in RAW 264.7 cells

Studies have implicated the SR-BI in promoting cholesterol movement out of cells (9, 10). This process of cholesterol efflux is the initial step of RCT and helps prevent cholesterol accumulation in peripheral cells, such as macrophages (43). We therefore studied the effect of LPS in RAW 264.7 cells, a murine macrophage cell line. As shown in **Fig. 8**, LPS rapidly decreased SR-BI mRNA levels (40% decrease by 2 h) and the maximum effect was reached by 4 h (90% decrease). In contrast to the LPS-induced decrease in SR-BI levels in the liver, the decrease in SR-BI mRNA levels in RAW 264.7 cells was not sustained. In addition, only high doses of LPS (100 and 1,000 ng/ml), not lower doses (1 and 10 ng/ml), significantly decreased SR-BI mRNA levels (data not shown). Moreover, we did not detect a decrease in SR-BI protein levels after cells were incubated with LPS (up to 1,000 ng/ml) at 4, 8, or 24 h (data not shown). Overall, the results suggest that, in con-

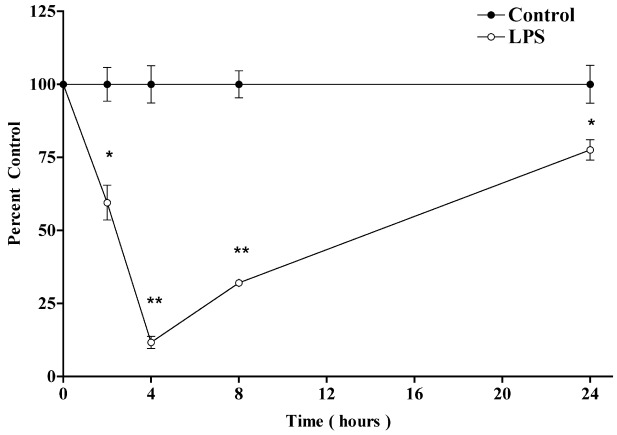


Fig. 8. The effect of LPS on SR-BI mRNA in RAW 264.7 cells. RAW 264.7 cells were grown until confluent. LPS (100 ng/ml) was incubated with cells in MEM with 2% bovine serum albumin in the absence of serum, and at the indicated time points cells were harvested for RNA isolation. n = 4 in each group at each time point. * $P < 0.025$; ** $P < 0.001$.

trast to the effect on the liver, the reduction of SR-BI levels in RAW 264.7 cells in response to LPS was transient and did not lead to a reduction in SR-BI protein levels.

DISCUSSION

SR-BI is a scavenger receptor initially shown to bind native and modified LDL (8). Later, SR-BI was shown to bind HDL and mediate selective uptake of HDL cholesteryl ester (7). SR-BI is abundantly expressed in the liver, adrenal glands, and gonads, tissues in which selective uptake of HDL cholesteryl ester has been previously demonstrated (6). In steroidogenic tissues, such as the adrenals and gonads, SR-BI plays a role in delivering cholesteryl ester for steroid hormone synthesis. In the liver, SR-BI is involved in selective uptake of cholesteryl ester, one of the key steps in RCT. Moreover, SR-BI may play an additional role in RCT by mediating the removal of cholesterol from peripheral tissues.

The role of SR-BI in HDL metabolism and RCT is supported by a number of in vivo studies (11–15). Overexpression of SR-BI in the liver resulted in a profound reduction of plasma levels of HDL cholesterol in mice (11–13). Concomitantly, there is an increase in selective uptake of HDL cholesteryl ester into the liver, resulting in an increase in biliary cholesterol secretion (11). In addition, mice in which SR-BI had been deleted or dramatically reduced showed increased plasma HDL cholesterol levels (14, 15). Furthermore, the reduction of hepatic SR-BI expression was associated with decreased selective uptake of HDL cholesteryl ester (15). Collectively, these studies support an important role for the SR-BI in RCT by mediating selective uptake of HDL cholesteryl ester at the hepatic level.

Previously, it has been shown that there are multiple changes of proteins involved in HDL metabolism and RCT during the acute-phase response (APR) (21–29). Specifically, a decrease of two proteins, LCAT and CETP, could potentially lead to a decrease in RCT. Because SR-BI is one of the key proteins in RCT, it was logical to determine whether SR-BI expression was also decreased during the APR. We hypothesized that SR-BI levels might be decreased during the APR, and, therefore, studied the hepatic expression of SR-BI using LPS and cytokines, known inducers of the APR.

In this study, we demonstrated that LPS reduced the hepatic mRNA levels of SR-BI in hamsters, and the reduction was rapid and sustained. The decrease in hepatic mRNA levels of SR-BI was also associated with a decrease in SR-BI protein levels. In addition, treatment of hamsters with TNF or IL-1 produced a reduction in hepatic mRNA levels of SR-BI. When TNF and IL-1 were given simultaneously, the reduction of hepatic levels of SR-BI was additive and the degree of the reduction was similar to that observed with LPS treatment.

Diet has been shown to regulate hepatic SR-BI levels in some studies (39, 44). In rats, feeding a diet enriched with cholesterol, olive oil, and cholic acid for 2 weeks led to a decrease in SR-BI protein levels in hepatocytes (39). In hamsters, a diet enriched with polyunsaturated fatty acids resulted in an increase in hepatic SR-BI protein levels (44). In another study in which mice were fed a high cholesterol or high fat diet for 4 weeks, however, there was no significant change in hepatic SR-BI levels (45). Our study, in which hamsters were fed a high cholesterol diet for 4 days, did not show changes in hepatic SR-BI mRNA levels. The difference between our results and those of others could represent species differences, differences in diet composition, or possibly a shorter period of cholesterol feeding. It is of note, however, that our protocol of cholesterol feeding has been shown previously to regulate a number of genes involved in cholesterol metabolism in hamster liver (46, 47). Nevertheless, LPS was able to decrease hepatic SR-BI mRNA levels in animals fed a high cholesterol diet, similar to that observed with a regular diet.

In the liver, both hepatocytes and Kupffer cells express SR-BI (39). SR-BI on the hepatocyte is responsible for the selective uptake of HDL cholesteryl ester; however, the role of SR-BI on Kupffer cells in cholesteryl ester uptake and RCT is unknown. In this study, we have shown that the decrease in SR-BI levels in hamster liver could be accounted for, at least in part, by a decrease in the hepatocyte. More importantly, the reduction in SR-BI levels in the hepatocyte was associated with the decrease in the uptake of cholesteryl ester from HDL.

The reduction of SR-BI due to LPS and cytokines may have several consequences. During the APR, the reduction in hepatic expression of SR-BI may decrease selective uptake of cholesteryl ester into hepatocytes (as demonstrated by our in vitro studies of hepatocytes), limiting cholesterol removal by the liver, and thus making cholesterol available to peripheral cells for host defense. For example, cholesterol has been shown to regulate lymphocyte activation and proliferation (48, 49). In addition, the APR is associated with cellular damage, and the processes of repair and regeneration of peripheral cells require cholesterol for new membrane synthesis. Decreasing RCT could facilitate the cellular responses required for surviving acute injuries, infection, or inflammation.

Although the acute decrease in hepatic SR-BI levels during the APR may be beneficial to the host, a prolonged reduction of SR-BI in the liver may result in undesirable consequences. SR-BI-deficient mice are susceptible to atherosclerosis, which may be related to decreased RCT (16, 17). In agreement with the concept that SR-BI protects against atherosclerosis, hepatic overexpression of SR-BI significantly reduced atherosclerosis in cholesterol-fed LDL receptor-deficient mice (18). It is possible that the acute reduction of SR-BI in the liver during the APR may decrease RCT and redirect cholesterol to peripheral cells for host defense, but a prolonged reduction of hepatic SR-BI, which might occur in chronic infection or inflammation, may impair RCT, which could result in an increased risk for atherosclerosis.

SR-BI has been demonstrated to promote cholesterol efflux from peripheral cells (9, 10). Overexpression of SR-BI in CHO cells increased cholesterol efflux, and the efflux

rates of different types of cells correlated with the levels of SR-BI expression. However, the significance of SR-BI in mediating cholesterol efflux in vivo is presently unknown. In this study, we demonstrated that LPS decreased mRNA levels of SR-BI in RAW 264.7 cells. However, the reduction was rather short-lived, and high doses of LPS were required to produce a reduction (Fig. 8), which is in contrast to the effect of LPS on hepatic SR-BI mRNA, which was prolonged and sensitive to low doses of LPS (Figs. 1 and 2). In addition, it did not decrease the protein levels. A study of human monocyte-derived macrophages has shown that LPS decreased the protein levels of Cla-1, a human homolog of SR-BI (50). Nevertheless, incubation of LPS with macrophages did not result in a change in cholesterol efflux compared with control cells (51). Failure to detect the effect of LPS on cholesterol efflux may, in fact, be due to a transient effect of LPS on SR-BI levels.

In summary, we have demonstrated that LPS and cytokines decreased SR-BI levels in the liver and in hepatocyte and macrophage cultures. These changes, which may be beneficial initially for the host, may impair selective uptake of HDL cholesteryl ester and thereby decrease RCT, increasing the risk for atherosclerosis.

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