p21-activated Kinase-1 (PAK1) Inhibition of the Human Scavenger Receptor Class B, Type I Promoter in Macrophages Is Independent of PAK1 Kinase Activity, but Requires the GTPase-binding Domain*

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Scavenger receptor class B, type I (SR-BI), is a high density lipoprotein receptor that mediates the flux of cholesterol between high density lipoprotein and cells. Recent evidence suggests that SR-BI plays a role in atherosclerosis and that inflammatory mediators down-regulate SR-BI in the macrophage. The purpose of this study was to evaluate the ability of lipopolysaccharide (LPS) to down-regulate the activity of the human SR-BI promoter in the macrophage and to delineate the mechanisms involved. Experiments with cultured cells and in vitro derived macrophages showed that LPS has a powerful suppressive effect on SR-BI expression both in vitro and in vivo. Transient transfection studies demonstrated that LPS represses SR-BI promoter activity in the macrophage cell line RAW 264.7. Cotransfection with either a constitutively active p21-activated kinase-1 (PAK1) construct (T423E) or a kinase-deficient PAK1 construct (K299R) resulted in repression of the SR-BI promoter, similar to LPS. These results demonstrate that PAK1-mediated down-regulation of the SR-BI promoter is independent of PAK1 kinase activity and suggest that PAK1 mediates the LPS-induced decrease in promoter activity. Cotransfection with constitutively active Cdc42 or Rac expression constructs also resulted in down-regulation of the promoter; whereas the dominant-negative Cdc42 and Rac constructs elevated basal promoter activity and blunted the LPS response. Cotransfection of PAK1 constructs containing mutations in both the kinase domain and the Cdc42/Rac-binding domain attenuated the PAK1-mediated down-regulation of the promoter, suggesting that Rac and Cdc42 are required for PAK1-mediated decreases in SR-BI promoter activity. Deletion analysis and gel shift data suggest that LPS inhibits binding of a novel transcription factor to a myeloid zing finger protein-1-like element (−476 to −456) in the human SR-BI promoter. These results demonstrate that the PAK1 pathway down-regulates the SR-BI promoter and suggest that activation of this pathway may play an important role in cholesterol trafficking in the vessel wall.

The abbreviations used are: SR-BI, scavenger receptor class B, type I (SR-BI), 1 is a high density lipoprotein receptor that mediates both the influx (1–3) and efflux (4, 5) of cholesterol between high density lipoprotein and cells. Prominent expression of SR-BI has been observed in the liver and steroidogenic tissues (1, 2, 6, 7) and more recently in the macrophage and atherosclerotic vessel wall (2, 4, 8, 9). Although the contribution of SR-BI in the vessel wall to atherosclerosis is not known, hepatic overexpression of SR-BI reduces atherosclerosis in low density lipoprotein receptor-deficient mice fed a high fat diet. Because this effect is associated with reduced plasma high density lipoprotein, it has been suggested that SR-BI enhances hepatic uptake of high density lipoprotein cholesterol and thereby suppresses atherogenesis (10, 11). Consistent with this, crossing of SR-BI knockout mice with apoE knockout mice accelerates the onset of atherosclerosis (12), again suggesting an atheroprotective role for SR-BI.

A variety of stimuli have been demonstrated to regulate SR-BI expression. The hormones estrogen and adrenocorticotropic hormone have been observed to alter SR-BI expression (7, 13–15). In addition, modified low density lipoprotein has been shown to increase SR-BI in human monocyte-derived macrophages (8), whereas high cholesterol diet lowers SR-BI expression in rat liver parenchymal cells (15). Pro-inflammatory mediators such as lipopolysaccharide (LPS) have also been shown to down-regulate the mRNA and protein levels of SR-BI in the monocyte and macrophage (16). Despite a number of studies demonstrating regulation of SR-BI, relatively little is known about the basic mechanisms involved. Recent promoter studies have shown that members of the Sp1 transcription factor family are essential for transcription of the rat SR-BI gene in mouse Leydig tumor cells (17). It has also been shown that the sterol response element-binding protein activates transcription of the rat SR-BI promoter in variety of cell lines (18) and that steroidogenic factor-1 binds to and activates the human SR-BI promoter in mouse adrenocortical cells (6). More recently, it was shown that the ligand-activated peroxisome proliferator-activated receptor increases SR-BI expression in monocytes and macrophages (9).

The observation that pro-inflammatory stimuli such as LPS repress SR-BI expression has led to the hypothesis that inflammatory mediators contribute to atherogenesis by compromising cholesterol efflux from vascular macrophages. LPS is known to signal through Toll-like receptor-4 (19) and to trigger the activation of nuclear factor-κB (NF-κB) plus several MAPKs, the ERK, p38, and JNK proteins (20–24). In addition, LPS has

1, LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; PAK1, p21-activated kinase-1; DMEM, Dulbecco’s modified Eagle’s medium; NIK, NF-κB-inducing kinase; RSV, Rous sarcoma virus; AP-1, activator protein-1; MZF-1, myeloid zing finger protein-1; HSF, heat shock factor.

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PAK1 Inhibits SR-BI Promoter Activity

been shown to activate p21-activated kinase-1 (PAK1) in the macrophage, and PAK1 activation triggers nuclear accumulation of NF-κB (25).

PAK1, a member of the PAK family of serine/threonine kinases (26), is activated by the Rho family GTP-binding proteins Rac1 and Cdc42 (27, 28). Activation of these GTPases is induced by guanine nucleotide exchange factors, which catalyze the exchange of GDP for GTP (29). In addition to activation, guanine nucleotide exchange factors may regulate downstream signaling events of Rac1 and Cdc42 (30). These downstream signaling events include activation of many of the same pathways induced by LPS such as the JNK and p38 kinases (31–33). Although Rac1 and Cdc42 have multiple effectors, the Rac1- and Cdc42-mediated increase in p38 activity is dependent on PAK1 (32). Thus, there is accumulating evidence in the literature that the PAK1 pathway plays an important role in LPS-mediated signal transduction.

Many studies have suggested a role for PAK1 in cytoskeletal reorganization and cell motility (34–37), and several physiological signals such as thrombin, insulin, and epidermal growth factor have been reported to activate PAKs (28, 38, 39). However, the role of PAK1 in transcriptional regulation is not well understood. In this work, we studied the role of the Cdc42/Rac/PAK1 pathway in the LPS-induced regulation of the SR-BI promoter in macrophage RAW 264.7 cells.

MATERIALS AND METHODS

In Vivo Studies—Male C57BL/6 mice were injected intraperitoneally with 10 mg/kg LPS, and 48 h later, macrophages were harvested from the peritoneal cavity of LPS-treated animals. The cells were washed several times with cold RPMI 1640 medium (serum-free), and cell lysates were processed for Western blotting.

Cell Culture Studies—RAW 264.7 cells were grown to 80% confluency in 100-mm dishes in Dulbecco’s modified Eagle’s medium (DMEM, Life Technologies, Inc.) containing 10% fetal bovine serum. Growth medium was removed and replaced with DMEM containing 10% lipoprotein-deficient serum, and the cells were incubated for an additional 24 h. LPS (1 μg/ml) was added directly to fresh medium (DMEM containing 10% lipoprotein-deficient serum), and the cells were incubated for an additional 1, 3, 6, or 8 h and used for total RNA preparation or for an adhesion assay (36, 6, 12, 24, and 36 h) and used for protein extract preparation.

mRNA Preparation and Quantification—Total RNA was prepared using the QIAGEN RNaseasy mini kit, and DNase was treated according to the manufacturer’s protocol. mRNA expression was analyzed using real-time quantitative PCR on an Applied Biosystems Prism 7700 sequence detection system. Primer/probe sequences used for quantification were previously described (36, 38, and 35) and were provided by Dr. Jonathan Chernoff (Fox Chase Cancer Center, Philadelphia, PA). The pPB-Neo-NIK construct (provided by Jeffrey Marine, Pfizer) was generated by inserting 3 kilobase pairs of NIK coding sequence between the BamHI and XhoI sites of the pPB-Neo retroviral vector. The pCS, pRSV-fgal, and NF-κB response element constructs were kindly provided by Dr. Joseph Menetski (Pfizer).

Cell Transfections—The murine macrophage RAW 264.7 cell line was obtained from American Type Culture Collection (Manassas, VA) and maintained in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 300 μg/ml 1-glutamine. Cells were seeded overnight at 200,000 cells/well in 24-well tissue culture plates (Costar Corp.). The medium was then replaced with 0.5 ml/well phenol red-free DMEM supplemented with 5% charcoal/dextran-treated bovine serum (Hyclone Laboratories). Cells were transfected in triplicate for 6 h with 100 μl of Opti-MEM mixtures (Life Technologies, Inc.) containing a total of 0.5 μg of experimental plasmid, 0.05 μg of pRSV-fgal normalization plasmid, and 1 μl of LipofectAMINE 2000 (Life Technologies, Inc.). One ml of phenol red-free DMEM supplemented with 5% charcoal/dextran-treated bovine serum was added to each well after 6 h of transfection, and the cells were then treated with LPS (1 μg/ml; Escherichia coli serotype 0111:B4, Sigma) or saline as indicated for 16 h. In cotransfection experiments, controls were cotransfected with empty pG3-L3-Basic vector to keep total DNA concentrations constant (empty pG3-L3-Basic vector luciferase values were subtracted from total levels). Luciferase and ß-galactosidase samples were prepared with the luciferase assay system (Promega) and the Galacto-Star kit (Tropix Inc.), respectively. Luciferase and ß-galactosidase activities were then measured in a microplate luminesimeter (EG&G Berthold).

Nuclear Extracts—RAW 264.7 cells were treated with 1 μl/ml LPS or saline vehicle for 16 h. Cells were then rinsed with cold phosphate-buffered saline and lysed for 5 min in hypotonic buffer containing 0.2% Nonidet P-40, 20 mM HEPES, 20 mM NaF, 1 mM Na3VO4, 1 μM phosphatase and protease inhibitors (Roche Molecular Biochemicals), and 0.05% Triton X-100. Whole cell lysates were centrifuged at 10,000 × g for 10 min to remove cell debris. Protein concentration was determined in the supernatants using the Bradford assay (49). Lysates were solubilized in Laemmli sample buffer, and 40 μg of protein was loaded onto each lane of an 8–18% Tris/glycine gel (Novex) and subjected to electrophoresis. After electrophoresis, separated proteins were transferred to nitrocellulose membranes and immunoblotted with a 1:1500 dilution of a rabbit anti-SR-BI polyclonal antibody (catalog number 4034995, PV Biobiosystems), a 1:1000 dilution of a mouse anti-glyceraldehyde-3-phosphate dehydrogenase monoclonal antibody (clone 6C6, Advanced Immunotechnology) to verify equal amounts of protein loaded. After extensive washing with phosphate-buffered saline and 0.2% Tween 20, the blots were incubated with horseradish peroxidase-conjugated secondary antibodies (1:2000 dilution), and detection of immune complexes was carried out with the ECL Western blot detection system (Amersham Pharmacia Biotech).

Electrophoretic Mobility Shift Assays—Four double-stranded oligonucleotide probes spanning the SR-BI promoter from −564 to −413 were synthesized and labeled with 74 nucleotides using [γ-32P]ATP (3000 Ci/mmol). Labeled DNA probes were associated with 10 μg of nuclear extract protein for 30 min at room temperature. Samples were then separated on a 0.5% Triton borate/EDTA and 5% acrylamide gel for 1 h at 300V. Gels were dried and developed with Hyperfilm MP (Amersham Pharmacia Biotech). Further analysis of the LPS-responsive region was conducted as described above with three shorter overlapping double-stranded oligonucleotide probes (A: −497 to
**RESULTS**

**LPS Inhibits SR-BI Expression in Vitro and in Vivo**—LPS down-regulates SR-BI expression in human monocytes and macrophages in vitro (16). These data were confirmed using the macrophage-like RAW 264.7 cells. LPS (1 μg/ml) treatment of these cells resulted in a time-dependent decrease in SR-BI mRNA levels, with peak reductions at 6 h post-treatment (Fig. 1A). Western blot analysis of RAW 264.7 cells treated with LPS also indicated a dramatic reduction in SR-BI protein levels (Fig. 1B). *In vivo* confirmation of these results was obtained using peritoneal macrophages from LPS-treated C57BL/6 mice. Intraperitoneal injection of these animals with LPS (10 mg/kg) resulted in nearly complete elimination of SR-BI protein in peritoneal macrophages as determined by Western blot analysis, whereas it had very minimal effects on the levels of the β-actin control (Fig. 1C). Based on these findings, we conclude that LPS has a powerful negative effect on SR-BI expression in macrophage cells both *in vitro* and *in vivo*.

**LPS Inhibits SR-BI Promoter Activity**—LPS has been demonstrated to down-regulate SR-BI mRNA levels in human monocytes and macrophages (16); however, the effects of LPS on the transcriptional activity of the SR-BI promoter have not been studied. To evaluate the effect of LPS on SR-BI promoter activity, RAW 264.7 cells were transfected with the SR-BI −896/−157 promoter construct and treated for 16 h with LPS (1 μg/ml) or saline as a control. As shown in Fig. 2, the combined results from six independent experiments each performed in triplicate demonstrate that LPS treatment resulted in a significant (*p < 0.001*) reduction in SR-BI −896/−157 promoter activity. To delineate the LPS-responsive region, shortened promoter constructs were generated and transfected into RAW 264.7 cells. As shown in Fig. 3, LPS treatment resulted in a significant decrease in the activity of the −896/−157 and −564/−157 promoter constructs; however, LPS did not alter the activity of the shorter promoter constructs. These results indicate that the LPS-responsive region lies between −564 and −413 of the promoter.

**The Serine/Threonine Kinase PAK1 Inhibits SR-BI Promoter Activity**—Given that LPS has been demonstrated to activate PAK1 in RAW 264.7 cells (25) and that PAKs can activate important transcriptional regulatory cascades such as NF-κB, p38 MAPK, and JNK (25, 32, 33), the ability of PAK1 to down-regulate the SR-BI promoter was tested. As shown in Figs. 4 and 6, cotransfection of RAW 264.7 cells with the constitutively active PAK1 construct (T423E) and the SR-BI −896/−157 promoter construct resulted in a significant reduction in
promoter activity. Cotransfection of the kinase-deficient PAK1 construct (K299R) also significantly decreased the activity of the promoter (Figs. 4 and 6). These results demonstrate that PAK1-mediated down-regulation of the SR-BI promoter is independent of PAK1 kinase activity. No additional decrease in promoter activity was observed in PAK1-transfected cells when treated with LPS (data not shown), suggesting that PAK1 mediates the LPS-induced decrease in promoter activity.

Rac and Cdc42 Inhibit the SR-BI Promoter—To provide further evidence that the Cdc42/Rac/PAK1 pathway is involved in regulation of the SR-BI promoter, we tested the ability of known upstream activators of PAK1 to mimic the PAK1 response. It is well established that the small G-proteins Rac and Cdc42 activate PAK1 (27). To evaluate the role of these G-proteins in the regulation of the SR-BI promoter, RAW 264.7 cells were cotransfected with constitutively active or dominant-negative G-protein expression constructs and the SR-BI −896/−157 promoter construct. As demonstrated in Fig. 5, expression of the constitutively active forms of Cdc42 (Q61L) and Rac (V12) significantly inhibited promoter activity. As in the PAK1 transfectants, no additional decrease in promoter activity was observed when constitutively active Cdc42- or Rac-transfected cells were treated with LPS or when cells were transfected with both active G-protein and PAK1 constructs (data not shown). These findings suggest that Cdc42 and Rac mediate the LPS-induced down-regulation of the SR-BI promoter. Furthermore, as shown in Fig. 5, expression of the dominant-negative Cdc42 T17N mutant resulted in a 2-fold increase (p < 0.005) in the activity of the promoter, whereas cotransfection of the dominant-negative RacN17 mutant resulted in a smaller yet significant increase (p < 0.05) in the activity of the promoter. These dominant-negative G-protein constructs exert their negative effect by trapping the guanine nucleotide exchange factors, which are necessary for the dissociation of GDP and subsequent GTP binding that activate these G-proteins (29). These results suggest that endogenous active Cdc42 and Rac inhibit the SR-BI promoter under basal conditions. In addition, when cells that were cotransfected with the dominant-negative Cdc42 or Rac constructs were treated with LPS, the response to LPS was blunted. Although LPS treatment resulted in a reduction in promoter activity compared with the dominant-negative constructs alone, this decrease was not significantly different from that in the control cells transfected with the SR-BI promoter alone (Fig. 5).

Rac and Cdc42 Are Required for PAK1-mediated Down-regulation of SR-BI Promoter Activity—To evaluate the role of these G-proteins in PAK1-mediated down-regulation of the promoter, double mutant PAK1 constructs containing mutations in both the kinase domain (K299R) and the G-protein-binding domain (H83L and H86L) were cotransfected with the promoter construct. As shown in Fig. 6, PAK1-mediated decreases in SR-BI promoter activity were attenuated under both the kinase-deficient and constitutively active PAK1 conditions when the G-protein-binding domain was also mutated. These results demonstrate that PAK1-mediated decreases in SR-BI promoter activity are dependent on a functional G-protein-binding domain and suggest that the Cdc42- and Rac-induced decreases in promoter activity are due at least in part to interactions with PAK1.

NF-κB Is Not Involved in the LPS-mediated Down-regulation
of the SR-BI Promoter—It is well established that LPS activates NF-κB in the monocyte/macrophage (40, 41). More recently, PAK1 activity has been demonstrated to be required for activation of NF-κB in RAW 264.7 cells (25). To evaluate the role of NF-κB in the LPS-induced down-regulation of the SR-BI promoter, RAW 264.7 cells were cotransfected with a p65 expression construct. As shown in Fig. 7B, expression of p65 resulted in an 18-fold increase in an NF-κB response element-luciferase construct. However, cotransfection of the p65 expression construct had minimal effects on the activity of SR-BI promoter constructs (Fig. 7A). To further confirm that activation of the NF-κB pathway did not alter SR-BI promoter activity, an upstream activator of NF-κB, NIK, was coexpressed with the SR-BI promoter. As shown in Fig. 7D, overexpression of NIK resulted in a 5-fold increase in the activity of the NF-κB response element-luciferase construct. However, as in the p65 experiments, NIK had no effect on SR-BI promoter activity (Fig. 7C). These results suggest that activation of the NF-κB pathway does not alter SR-BI transcriptional activity.

LPS Inhibits Transcription Factor Binding to an MZF-1-like Element in the SR-BI Promoter—To further delineate the LPS-responsive region of the SR-BI promoter (−564 to −413) (Fig. 2), four double-stranded oligonucleotide probes spanning the LPS-responsive region were synthesized and labeled for gel shift analysis. As shown in Fig. 8A, DNA-protein complexes were observed with each of the four probes (GS1–4). However, GS-3 (−497 to −456) revealed a prominent DNA-protein complex under control conditions, which was inhibited in the LPS-treated cells. GS-3 was then further divided into three probes (A, B, and C) and evaluated by gel shift analysis. As shown in Fig. 8B, the protein-binding region was confined to probe B, spanning −476 to −456. As observed in GS-3 binding, treatment of cells with LPS inhibited protein binding to probe B. This binding was shown to be specific, as competition experiments with increasing concentrations of unlabeled probe B blocked formation of the complex, whereas increasing concentrations of unlabeled probes A and C had no effect on the DNA-protein complex (Fig. 8C). Transcription factor data base analysis of probe B revealed two putative cis-elements: a heat shock factor (HSF) element and an MZF-1 element (Fig. 9A). To determine whether these putative sites were mediating protein binding to probe B, point mutations at consensus sites were made and evaluated by gel shift analysis. As depicted in Fig. 9A, a point mutation of the HSF element (M1) did not alter protein binding, whereas a point mutation of the MZF-1 element (M2) blocked protein binding. These results are supported by analysis of probe C, which contained the HSF element, but not the MZF-1 element, and did not demonstrate DNA-protein interaction. However, two different consensus sequences for MZF-1 as identified by Morris et al. (42) failed to compete for binding with probe B (Fig. 9B), suggesting that MZF-1 is not a component of the DNA-protein complex. In addition, AP-1 and Sp1 consensus probes also failed to block DNA-protein interaction with probe B (Fig. 9C). To confirm that the MZF-1-like element was involved in the LPS response, the M2 point mutation was introduced in the SR-BI −896/−157 promoter construct. Transient transfection experiments with the M2 construct demonstrated a 30% decrease in the LPS-mediated repression of the promoter. Together, these results suggest that LPS inhibits the binding of a novel transcription factor that binds to an MZF-1-like element and activates transcription of the human SR-BI promoter.

DISCUSSION

Previous studies have documented SR-BI expression in human monocytes and macrophages (2, 8, 9) and in the murine macrophage cell line RAW 264.7 (4). It has also been shown that LPS down-regulates SR-BI mRNA and protein levels in monocytes and macrophages (16). The results in this study confirm these in vitro findings and demonstrate for the first time that LPS down-regulates SR-BI protein levels in macrophages in vivo. The finding in this study that LPS decreases SR-BI promoter activity in RAW 264.7 cells provided a system to begin elucidating the signaling pathways involved in LPS regulation of the SR-BI promoter in macrophage cells.

Although LPS signaling is complex and not completely un-
PAK1 Inhibits SR-BI Promoter Activity

Fig. 8. Gel shift analysis of the LPS-responsive region. A, nuclear extracts from RAW 264.7 cells treated for 16 h with LPS or saline as a control were incubated with four double-stranded oligonucleotide probes spanning the LPS-responsive region (GS1-4) as described under "Materials and Methods." GS-3 (−497 to −456) demonstrated a strong difference in DNA-protein complex formation, with decreased complex observed in the LPS-treated cells (arrow). B, GS-3 was further divided into three probes (A, B, and C) and evaluated by gel shift analysis. The protein-binding region was confined to probe B, spanning −476 to −456; and LPS inhibited protein binding to probe B. C, competition experiments with increasing concentrations of unlabeled probe B blocked formation of the complex, whereas increasing concentrations of unlabeled probes A and C had no effect on the DNA-protein complex. "B", labeled probe alone.
Fig. 9. Novel transcription factor-binding site in the LPS-responsive region. A, a putative HSF element and an MZF-1 element were identified in the LPS-responsive region (G5-3 probe B). Point mutations at consensus sites in these elements were made and evaluated by gel shift analysis using control nuclear extracts. Mutation of the HSF element (M1) did not alter protein binding, whereas mutation of the MZF-1 element (M2) blocked protein binding. B, a 100-fold molar excess of unlabeled AP-1 and Sp1 consensus probes also failed to block DNA-protein interaction with probe B, C, control.

responsive region of the human SR-BI promoter to −476 to −456. Importantly, the steroidogenic factor-1 site previously demonstrated to regulate human SR-BI promoter activity in adrenocortical cells (6) is located at positions −218 to −212 and does not appear to be a major mediator of basal transcriptional activity in RAW 264.7 cells, as significant decreases in basal activity were observed in the construct containing the steroidogenic factor-1 site (−255 to −157). Together, these observations indicate that transcriptional regulation of SR-BI is cell type-dependent.

Transcription factor data base analysis of the putative LPS-responsive region indicated that an MZF-1-like element was involved; and indeed, a point mutation in this element at position −464 mimicked the LPS-induced decrease in protein binding to the region. However, MZF-1 consensus probes were unable to compete for binding, suggesting that the MZF-1 protein was not a component of the DNA-protein complex inhibited by LPS. Given that this LPS-responsive region is G-rich, characteristic of Sp1-binding sites, and that Sp1 has been implicated in transcription of the rat SR-BI promoter (17), we tested the possibility that the LPS-responsive region was binding the Sp1 protein. However, gel shift competition assays with an Sp1 consensus probe indicated that Sp1 was not a member of the protein complex disrupted by LPS. In addition, experiments ruled out roles for AP-1 and NF-κB in the LPS-mediated reduction in promoter activity. Together, these results suggest that a novel transcription factor binds to −476 to −456 of the human SR-BI promoter and drives basal transcription in the macrophage. In addition, these results suggest that LPS disrupts binding of this factor, thereby inhibiting SR-BI transcription. Indeed, a point mutation in the MZF-1-like element, although not abolishing the LPS response, did reduce the LPS response, suggesting that the LPS effect is due in part to the MZF-1-like element. Further experiments are needed to determine the nature of the factor binding to the MZF-1-like element and to decipher additional LPS-responsive regions in the SR-BI promoter.

Although the extracellular milieu in an atherosclerotic plaque is complex, thus exposing foam cells to a multitude of stimuli, recent studies in platelets provide further evidence that PAK1 may be an important physiologic regulator of SR-BI in the atherosclerotic vessel. The coagulation protease, thrombin, has been shown to activate PAK1 in platelets (38). In addition, ligation of the thrombin protease-activated receptor (PAR-1) is known to activate Cdc42 and Rac in platelets (47). Importantly, the thrombin receptor has been detected in macrophages (48), and thrombin is likely present in the atherosclerotic vessel wall. These observations raise the possibility that thrombin-induced activation of PAK1 may be an important physiologic mechanism of SR-BI regulation in the foam cell and warrant further exploration into the role of PAK1 activity in cholesterol homeostasis in the atherosclerotic vessel wall.

In summary, we have documented that LPS and the PAK1 pathway down-regulate human SR-BI promoter activity in the macrophage. These results support the hypothesis that inflammatory mediators exacerbate foam cell formation via down-regulation of the SR-BI receptor in the macrophage.

Note Added in Proof—While this manuscript was under review, Khovidhuyk et al. (2001) J. Lipid Res. 42, 1636–1644 reported that LPS treatment in hamsters dramatically represses SR-BI mRNA and protein levels in liver, consistent with our results in macrophages.
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