Cellular Physiology of Cholesterol Efflux in Vascular Endothelial Cells

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Background—Of the cells that compose the atherosclerotic plaque, vascular endothelial cells are the most resistant to cholesterol accumulation. Cholesterol efflux pathways may play an important role in endothelial cholesterol homeostasis.

Methods and Results—We examined the global genetic response of endothelial cells to cholesterol and in particular the contribution of the cholesterol efflux proteins ATP-binding cassette transporter A1 (ABCA1), ATP-binding cassette transporter G1 (ABCG1), and scavenger receptor B-I (SR-BI) to endothelial cell cholesterol efflux. The ABCG1 gene is induced in endothelial cells by cholesterol, whereas ABCA1 is not. Using specific chemical inhibitors of ABC transporters and SR-BI, we have shown that neither ABC transporters nor SR-BI is required for apolipoprotein A-1–mediated endothelial cholesterol efflux.

Conclusions—Endothelial cells may use nontraditional pathways for cholesterol efflux. (Circulation. 2004;110:●●●-●●●.)

Key Words: endothelium ■ cholesterol ■ genes

Endothelial dysfunction is a key factor in the pathogenesis of atherosclerosis and is characterized by an increased expression of leukocyte adhesion molecules, increased permeability of the endothelium to LDLs, and decreased bioavailability of nitric oxide. Endothelial cells do not, however, undergo the foam cell phenotype change seen in macrophages and smooth muscle cells in the atherosclerotic plaque, a change embodied by the unrestricted accumulation of cellular cholesterol. This is surprising because endothelial cells express receptors for oxidized lipoproteins (eg, CD36, LOX-1), receptors that are not subject to negative feedback regulation by cholesterol and are thought to underlie foam cell formation.

Numerous processes, including de novo synthesis of cholesterol, lipoprotein uptake, cholesterol esterification, and reverse cholesterol transport, control cellular cholesterol metabolism. Genes that govern reverse cholesterol transport may be particularly important in determining the fate of cells within the atherosclerotic plaque. Mutations in the gene encoding the ATP-binding cassette transporter A1 (ABCA1) cause cellular phospholipid and cholesterol efflux defects that result in a severe loss of plasma HDL. Low plasma HDL cholesterol is a risk factor for the development of atherosclerosis, but loss of ABCA1 activity specifically in macrophages may predispose them to foam cell formation and contribute directly to the development of atherosclerotic lesions. Because HDL is thought to have important effects on endothelial health, it is appealing to hypothesize that ABCA1 is active in endothelium. However, the evidence supporting a role for ABCA1 in endothelial cells is equivocal.

The ATP-binding cassette transporter G1 (ABCG1) is a poorly characterized half-transporter relative of ABCA1, originally described in macrophages. It is thought to participate in reverse cholesterol transport by promoting HDL subfraction 3 (HDL3)–mediated cellular cholesterol efflux. Like the full transporter ABCA1, transcriptional regulation of ABCG1 is controlled by the liver X receptor/retinoid X receptor (LXR/RXR) pathway of nuclear hormone receptors. This pathway is induced by specific hydroxylated derivatives of cholesterol, eg, 27-hydroxycholesterol, which can serve as a proxy indicator for the presence of cholesterol in cells. Overexpression of ABCG1 in mouse liver results in decreased plasma levels of cholesteryl esters and HDL cholesterol, although the mechanism for this effect is not known. A recent report shows that ABCG1 expression in the liver is highest in Kupffer cells (tissue macrophages) and endothelial cells and lowest in parenchymal cells.

The scavenger receptor B-I (SR-BI, also known as CLA-1 in humans) is an HDL receptor that can mediate selective uptake of HDL cholesteryl esters by cells but can also promote cellular free cholesterol efflux to HDL and the reorganization of a cholesterol oxidase–sensitive pool of cellular cholesterol. In endothelial cells, SR-BI is required...
for endothelial nitric oxide synthase (eNOS) activation by HDL. SR-BI has been localized within caveolae, cholesterol-rich plasma membrane microdomains that are abundant in endothelial cells.

In the present study we examined the expression of cholesterol-regulated genes in endothelial cells in general using functional genomics and the genes ABCA1, ABCG1, and SR-BI in particular. We also investigated the contribution of ABCA1, ABCG1, and SR-BI to endothelial cholesterol efflux.

**Methods**

**Cell Culture**

Pooled human umbilical vein endothelial cells (HUVECs) and human aortic endothelial cells (HAECs) were from Cambrex and were used before the fourth passage. Normal human skin fibroblasts were obtained by biopsy from a healthy human volunteer, and Tangier fibroblasts were from a patient with compound ABCA1 mutations (ABCA1 C1477R amino acid substitution and an ABCA1 IVS25+1 [G→C] mRNA splice site mutation). Fibroblasts were used before the 13th passage. COS cells were from Invitrogen. Cell medium for HUVECs and HAECs was EGM2 (Cambrex), and cell medium for human skin fibroblasts and COS cells was Dulbecco’s modified Eagle’s medium (Invitrogen), supplemented with 100 U/mL penicillin and 100 U/mL streptomycin (Invitrogen), nonessential amino acids (Invitrogen), and 10% FBS (Biomedia). Cell medium for HepG2 cells was the same as for fibroblasts, except that Dulbecco’s modified Eagle’s medium-F12 was used (Invitrogen). Bovine serum albumin (BSA), cholesterol, 22(R)-hydroxycholesterol, and 8-bromo-cAMP were from Sigma. For the SR-BI dose-response experiment, a variety of concentrations of cholesterol (0 to 50 μg/mL and 22(R)-hydroxycholesterol (0 to 5 μg/mL) were used. No significant cell death was observed through a light microscope at the reported doses of reagents.

**DNA Microarray**

We seeded 250 000 HUVECs in 100-mm cell culture dishes. At confluence, the medium was changed to serum-free medium containing 2 mg/mL BSA and either 20 μg/mL cholesterol in an ethanol carrier or the ethanol carrier alone and incubated for 24 hours. Total RNA was isolated with the use of the RNeasy Mini Kit (Qiagen). RNA was tested for quality on an agarose gel. We used 1 μg of total RNA on a HU95A DNA microarray (Affymetrix) containing probe sets for 12 000 gene sequences; results were analyzed with GeneSpring software (Silicon Genetics). Criteria for differential regulation by cholesterol treatment were set as ≥2-fold upregulation or downregulation at a probability value of <0.05 with n=3.

**Northern Blot**

We seeded 250 000 HUVECs, HAECs, and human skin fibroblasts on 100-mm cell culture plates. On reaching confluence, growth medium was changed to serum-free medium with 2 mg/mL BSA and either 20 μg/mL cholesterol in an ethanol carrier, 0.5 mM 8-bromo-cAMP, or ethanol carrier alone and incubated for 24 hours. Total RNA was isolated with the use of the RNeasy Mini Kit (Qiagen). cDNA was used as a control vector. COS cells were seeded at 100 000 cells per well in 12-well plates. At half-confluence, cellular cholesterol pools were radiolabeled for 24 hours. The kinetic study of cholesterol efflux was performed over 24 hours except in the case of the kinetic study. Efflux substrate was serum-free medium containing 1 mg/mL BSA, serum-free medium containing 1 mg/mL BSA and apolipoprotein A-I (apo-A) at 5 μg/mL (Biodesign), or serum-free medium containing 1 mg/mL BSA and 50 μg/mL HDL (Biodesign). The ABC inhibitor glyburide was purchased from Calbiochem, and a stock solution of 25 mg/mL was prepared in dimethyl sulfoxide (DMSO). A novel inhibitor of SR-BI function, BLT-1, was purchased as compound 5234221 from Chembridge, and a 100-mM stock solution was prepared in DMSO. Glyburide was added to the efflux medium for the final 24 hours of the experiment. BLT-1 was added to the efflux medium, and efflux was for 6 hours to limit cell toxicity. BLT-1 medium contained 5 mg/mL BSA and 0.5% DMSO to ensure solubility of the compound. Radioactivity was counted in both the efflux medium and the cells. Efflux was expressed as the radioactivity counted in the medium divided by the sum total of the radioactivity in the medium and the cells (n=3). In some cases, HUVEC cellular lipids from 12-well plates were isolated with 2:3 hexane:isopropanol and dried, and total cholesterol was measured with the Infinity total cholesterol kit (ThermoDNA) according to the manufacturer’s instructions.

**Transfections**

The ABCG1 expression vector was from the Invitrogen Genestorm library and contained a full-length ABCG1 cDNA tagged with a V5 epitope and a 6×His in a pCDNA 3.1 plasmid. The ABCA1 expression vector contained a full-length ABCA1 cDNA cloned in a pcDNA 3.0 plasmid. Empty pcDNA 3.0 plasmid containing no cDNA was used as a control vector. COS cells were seeded at 100 000 cells per well in 12-well plates. At half-confluence, cellular cholesterol pools were radiolabeled with 1 μCi/mL [3H]cholesterol. At confluence, medium was changed to OptiMEM (Invitrogen), and cells were transfected overnight with Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s instructions. At this point, cells were either incubated for 24 hours with 50 μg/mL HDL, to measure efflux or their cell lysate was isolated for use in an immunoblot.

**Immunoblotting**

Cell lysates were harvested in lysis buffer (20 mmol/L Tris, 0.5 mmol/L EDTA, 0.5 mmol/L EGTA, 0.5% Triton X-100, Complete protease inhibitors [Roche]), homogenized, and centrifuged for 10 minutes at 3500 rpm in a tabletop centrifuge at 4°C to pellet nuclei. The protein concentration of postnuclear supernatants was measured with the BCA protein assay (Pierce). We loaded 45 μg of cell lysate onto an 8% polyacrylamide gel. After electrophoresis, proteins were transferred to an Immobilon P membrane (Millipore). Primary anti-SR-BI antibody (Novus Biologicals catalog No. NB400-104B1) was used at a concentration of 1:750, anti-V5 antibody (Invitrogen) was used at 1:2500, anti-ABCA1 antibody was used at 1:500 (Novus Biologicals), anti-ABCG1 antibody was used at 1:100 (Santa Cruz Biotechnologies), and anti-HSP70 antibody was used at 1:1000 (Transduction Laboratories). Anti-mouse and anti-rabbit secondary antibodies coupled to horseradish peroxidase (Am-
ersham) were used at a concentration of 1:5000, and the donkey anti-goat secondary antibody (Santa Cruz Biotechnologies) was used at a concentration of 1:10 000. Signal was revealed with the use of an enhanced chemiluminescence kit (Pierce) and captured with X-Omat Blue XB-1 film (Kodak). ImageQuant software was used to quantify protein signals. For the dose-response experiments, semi-quantitative exposures were selected.

Results

Functional Genomics

We used a DNA microarray to search for changes in the expression of large numbers of HUVEC genes induced by cholesterol. Gene expression in HUVECs treated with 20 μg/mL cholesterol for 24 hours was compared with basal gene expression with the use of Affymetrix HU95A DNA microarrays. The Table shows those HUVEC mRNA transcripts found to be significantly upregulated or downregulated by cholesterol treatment. A large group of downregulated transcripts is required for de novo cholesterol biosynthesis. ABCG1, which has been proposed to enhance HDL-mediated cellular cholesterol and phospholipid efflux, was upregulated by cholesterol treatment. Other transcripts affected by cholesterol treatment are implicated in proliferation, inflammation, cytoskeletal function, and protein chaperone function. A number of HUVEC expressed sequence tags or otherwise uncharacterized transcripts were also affected by cholesterol treatment. The increase in cellular cholesterol did not increase the mRNA levels of the cell adhesion molecules intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and e-selectin (data not shown).

Expression and Regulation of the ABC Transporters ABCA1 and ABCG1

Because our microarray study showed ABCG1 to be upregulated by cholesterol and because ABCG1 is proposed to enhance cholesterol efflux, we further studied the regulation of this gene and its close relative ABCA1 in HAECs and HUVECs. We treated endothelial cells, normal fibroblasts, and Tangier fibroblasts with cholesterol or the LXR agonist...
22(R)-hydroxycholesterol. Total RNA from cells was analyzed by Northern blot with the use of specific radiolabeled ABCA1 and ABCG1 DNA probes (Figure 1A). In HUVECs and HAECs, cholesterol and 22(R)-hydroxycholesterol were potent inducers of ABCG1 expression, whereas ABCA1 expression was low or absent throughout. This pattern was reversed in fibroblasts, in which ABCA1 could be highly induced by cholesterol and 22(R)-hydroxycholesterol but ABCG1 levels remained low. Similar results were obtained at the protein expression level (Figure 1B). 8-Bromo-cAMP, which increases ABCA1 transcription in mouse macrophages, had no effect on endothelial or fibroblast ABC transporter expression (data not shown).

**ABCA1, ABCG1, and Endothelial Cholesterol Efflux**

To examine possible pathways utilized by endothelial cells for reverse cholesterol transport, we measured efflux to a variety of potential substrates. Efflux to BSA alone, apoA-I, and HDL₃ was measured in cholesterol-treated HUVECs, normal fibroblasts, and Tangier fibroblasts deficient in ABCA1 (Figure 2). ApoA-I stimulated cholesterol efflux in control fibroblasts but not in HUVECs or Tangier fibroblasts. This is consistent with the low ABCA1 expression seen in HUVECs and the absent ABCA1 activity in Tangier fibroblasts. HDL₃ stimulated cholesterol efflux in all cell types but was especially effective in promoting cholesterol efflux in ABCA1-expressing normal fibroblasts. In a dose-response experiment, we compared cholesterol efflux in cholesterol-treated normal fibroblasts with efflux in cholesterol-treated Tangier fibroblasts using 10, 25, 50, and 100 μg/mL HDL₃; efflux in normal fibroblasts was 2.45-, 2.69-, 2.15-, and 1.85-fold higher than in Tangier fibroblasts at these concentrations (data not shown). HAEC efflux was also tested and showed a pattern similar to that of HUVECs among efflux substrates (data not shown). Because HDL can mediate a bidirectional cholesterol flux and our standard efflux assay only measures the efflux component of total flux, we measured total cholesterol mass in cholesterol-treated HUVECs incubated with BSA alone or with HDL₃. Total cholesterol mass was significantly less in cholesterol-treated HUVECs...
incubated with HDL3 than in cells incubated with BSA alone, demonstrating that the net cholesterol flux between HUVECs and HDL3 is efflux (data not shown).

We took advantage of the fact that ABCG1 transcription is induced by 22(R)-hydroxycholesterol to determine whether ABCG1 expression could promote cholesterol efflux to HDL3. We treated HUVECs with increasing doses of the LXR agonist 22(R)-hydroxycholesterol, from 0 to 2.5 μg/mL, and measured cholesterol efflux to HDL3. No significant change in efflux was associated with 22(R)-hydroxycholesterol treatment (data not shown). A kinetic study of cholesterol efflux to HDL3 showed that endothelial cells had the same rate of efflux to HDL3 regardless of sterol treatment (Figure 3A). In contrast, normal fibroblasts had increased efflux when treated with various sterols (Figure 3B). We also treated HUVECs, normal fibroblasts, and Tangier fibroblasts with the ABC inhibitor glyburide and measured cholesterol efflux to HDL3. Glyburide can inhibit a broad range of ABC transporters, including ABCA1 and the CFTR protein. At high doses, glyburide inhibited cholesterol efflux in normal fibroblasts but not in Tangier fibroblasts, indicating that glyburide was indeed inhibiting ABCA1 activity (Figure 4). HUVEC efflux was not affected by glyburide treatment, suggesting that cholesterol efflux in endothelial cells was not dependent on ABC-type transporters that are sensitive to glyburide.

ABCA1, ABCG1, and COS Cell Cholesterol Efflux

To extend our observation that ABCG1 expression in HUVEC was not associated with cholesterol efflux, we transfected COS cells with ABCG1 and ABCA1 expression vectors and measured their efflux to apoA-I and HDL3 (Figure 5A and 5B). Cells transfected with ABCA1 showed increased expression of ABCA1 in immunoblots with the use of anti-ABCA1 antibody; this increased expression was associated with large increases in cholesterol efflux to apoA-I and HDL3 compared with cells transfected with empty vector. Immunoblots of lysates from cells transfected with tagged ABCG1 with an anti-V5 epitope antibody showed expression of a protein with a molecular weight of ~65 kDa. This is smaller than the predicted molecular weight of ABCG1 but is consistent with data from another group that used a recombinantly tagged ABCG1 construct. ABCG1-transfected COS cells had no increase in cholesterol efflux to HDL3 compared with cells transfected with empty vector.
We considered the possibility that an alternate efflux protein mediates endothelial cholesterol efflux to HDL₃. SR-BI is an HDL receptor that has been described in endothelial cells and is known to promote cellular cholesterol efflux. We compared the expression of SR-BI in HepG2 cells, normal fibroblasts, and HUVECs. HepG2 lysates show very high levels of SR-BI protein expression (Figure 6). SR-BI expression in HUVECs was present, although lower than in HepG2 cells. SR-BI expression in fibroblasts was undetectable. In HUVECs, cholesterol and 22(R)-hydroxycholesterol treatment decreased SR-BI expression. A dose-response experiment observing SR-BI expression in HUVECs treated with increasing concentrations of cholesterol and 22(R)-hydroxycholesterol was performed. In both cholesterol- and 22(R)-hydroxycholesterol–treated HUVECs, increasing sterol doses led to a decrease in SR-BI protein expression, to a maximum of ~65% of basal expression (Figure 7). To determine whether the SR-BI expressed in HUVECs increases HDL₃-mediated cholesterol efflux, we treated cells with the specific SR-BI inhibitor BLT-1 and measured cholesterol efflux to HDL₃. Nieland et al. characterized BLT-1, showing it to be a highly effective blocker of selective cholesteryl ester uptake from HDL by SR-BI, as well as the SR-BI component of HDL₃-mediated cellular cholesterol efflux. High doses of BLT-1 significantly attenuated cholesterol efflux to HDL₃ in control HepG2 cells but not HUVECs (Figure 8). These data suggest that cholesterol efflux to HDL₃ in HUVEC is independent of SR-BI.

**Discussion**

Cholesterol homeostasis in endothelial cells remains poorly understood. In contrast to other cells that form the atherosclerotic plaque (smooth muscle cells and macrophages), vascular endothelial cells do not accumulate cholesterol. We addressed this question by investigating the global genetic response of endothelial cells to cholesterol and by examining in particular the role of 3 prominent proteins thought to promote cellular cholesterol efflux: ABCA1, ABCG1, and SR-BI.

The DNA microarray study we performed on HUVEC RNA indicates that the endothelial cholesterol biosynthetic apparatus is profoundly affected by cholesterol treatment. Although many cholesterol biosynthesis genes are known to be regulated by sterol-responsive cis-acting DNA elements, the scale of the effect of cholesterol in HUVECs is notable, going beyond the rate-limiting step catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase (Table). The expression of galectin-3, a macrophage recruitment protein, was also induced in HUVECs by cholesterol treatment. Endothelial activation and leukocyte recruitment into the arterial intima is of prime importance in the pathogenesis of atherosclerosis. Hypercholesterolemia is a major risk factor for the development of atherosclerosis, and therefore the induction of HUVEC galectin-3 by cholesterol may have in vivo significance. However, we believe that if cholesterol were truly a strong proinflammatory stimulus for endothelial cells, an increase in the expression of classic markers of endothelial activation, such as vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and e-selectin, would also have been seen. Probe sets for these genes were present on the DNA microarray used in our experiments, but no significant changes in gene expression were detected. Other lipoprotein constituents...
blots to study the expression of ABCG1 and the related transporter ABCA1 in endothelial cells and fibroblasts. ABCG1 mRNA and protein expression in HUVECs and HAECs was inducible by cholesterol and the LXR ligand 22(R)-hydroxycholesterol, but ABCA1 expression was low or absent (Figure 1). In contrast, ABCG1 expression in fibroblasts was low, whereas ABCA1 could be strongly upregulated by cholesterol and 22(R)-hydroxycholesterol. Both the ABCA1 and ABCG1 genes are known to contain LXR/RXR-binding DNA consensus sequences that confer responsiveness to specific oxysterols. Because endothelial ABCG1 was inducible by cholesterol and 22(R)-hydroxycholesterol alike, it is likely that endothelial cells possess the hydroxylating enzymes that convert cholesterol to oxysterol LXR ligands. Other studies on the expression of 27-hydroxylase in endothelium support this conclusion as well.21,22 It is unclear why ABCA1 and ABCG1 are differentially regulated in endothelial cells and fibroblasts, but this may be related to tissue-specific expression of transcription factors that regulate these genes.

A functional assay for cholesterol efflux demonstrated no ABCA1 activity in HUVEC, consistent with its low expression, because HUVECs, like Tangier fibroblasts, were unable to efflux cholesterol to apoA-I (Figure 2). HUVECs and Tangier fibroblasts were able to efflux cholesterol to HDL, which is consistent with the 2-step model of cholesterol efflux proposed by Fielding et al.6 In this model, as lipid-poor apoA-I gains phospholipids from ABCA1, it gains the ability to promote cholesterol efflux through an ABCA1-independent pathway. Fibroblasts expressing normal ABCA1 still had higher efflux to HDL, than either Tangier fibroblasts or endothelial cells (Figure 2). It has been shown that ABCA1 has a higher affinity for lipid-poor apoA-I than HDL.23,24 Our data suggest that ABCA1 is able to enhance HDL-mediated cholesterol efflux over a range of HDL concentrations. Although this does not show a direct physical interaction between HDL and ABCA1, our data demonstrate that regardless of the specific mechanism, ABCA1 and HDL, can cooperate at some level to promote cholesterol efflux.

Using sterol induction and a chemical inhibitor of ABC transporters, we have shown that ABCG1 expression is not associated with cholesterol efflux to HDL, in endothelial cells (Figures 3 and 4). Furthermore, exogenous expression of ABCG1 in COS cells also failed to increase cholesterol efflux to HDL, (Figure 5). However, it is possible that coexpression of an as yet unknown heterodimeric partner of ABCG1 may be required for full functionality of the protein. This is in striking contrast to the work of Klucken et al.,7 which demonstrated an association between ABCG1 expression and cholesterol efflux to HDL, in macrophages. In vivo the functional interaction between HDL and ABCG1 could be indirect; by promoting cholesterol and phospholipid excretion into the bile, ABCG1 may promote liver uptake of HDL and lowering of plasma HDL-cholesterol.10

We also investigated the role of SR-BI in endothelial cholesterol efflux. SR-BI is being scrutinized increasingly for its antiatherogenic properties, which appear to stem from its key position in the reverse cholesterol pathway and its ability to provoke nitric oxide synthesis in endothelial cells.13 SR-BI is an

besides cholesterol, such as lysophosphatidylcholine,20 remain good candidates as mediators of endothelial activation.

Our DNA microarray also showed that cholesterol upregulated the expression of ABCG1 mRNA in HUVECs. ABCG1 has been implicated previously in HDL-mediated cholesterol and phospholipid efflux in macrophages.7 We used Northern

Figure 7. SR-BI expression decreases in HUVEC after cholesterol or 22(R)-hydroxycholesterol treatment. Cells were treated with varying doses of cholesterol (0 to 50 μg/mL) (A) or 22(R)-hydroxycholesterol (22OH) (0 to 5 μg/mL) (B) for 24 hours. Cell lysates were collected, and 45 μg of protein was loaded on an 8% polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane and immunoblotted to detect SR-BI expression. The membrane was stripped and immunoblotted to detect HSP70 expression as a control for loading. Semiquantitative exposures were selected, and densitometry was used to quantify bands. Data are expressed as a ratio of SR-BI signal to HSP70 signal, with the dose of 0 corresponding to 100% of initial SR-BI expression and the remaining doses scaled accordingly. Points represent mean ± SD; n = 3.

Figure 8. The SR-BI inhibitor BLT-1 attenuates cholesterol efflux to HDL, in HepG2 cells but not in HUVECs. Cellular cholesterol pools were radiolabeled, and the cells were loaded with 20 μg/mL cholesterol for 24 hours. Efflux was over 6 hours with 50 μg/mL HDL, and varying doses of BLT-1 from 0 to 0.5 mmol/L. Efflux was calculated as in Figure 2. Points represent mean ± SD; n = 3.
HDL receptor that mediates selective cholesteryl ester uptake from HDL but can also promote cellular free cholesterol efflux to HDL.\(^2\)\(^1\)\(^2\) Expression of SR-BI was high in liver-derived HepG2 cells, lower in HUVECs, and absent altogether in fibroblasts (Figure 6). SR-BI expression was decreased in a dose-dependent manner in HUVECs treated with cholesterol or 22(R)-hydroxycholesterol (Figure 7). SR-BI mediates the selective uptake of cholesteryl esters, and therefore downregulation of the SR-BI gene may protect HUVECs from excessive sterol uptake and accumulation. Keratinocytes behave in a fashion similar to endothelial cells, downregulating SR-BI in response to cholesterol and oxysterols.\(^2\)\(^5\) A specific chemical inhibitor of SR-BI, BLT-1, inhibited cholesterol efflux to HDL in HepG2 cells but not HUVECs (Figure 8). This is likely due to the higher expression of SR-BI in HepG2 cells, which itself implies that efflux may be more dependent on SR-BI in that cell type (Figure 6). Another study of SR-BI in porcine brain capillary endothelial cells showed a correlation between SR-BI expression and efflux to HDL, but this required high expression of SR-BI using cells transfected with SR-BI–bearing adenoviruses.\(^2\)\(^6\) Although SR-BI expression may not be high enough in endothelial cells to make SR-BI a major pathway for cholesterol efflux to HDL, this does not exclude SR-BI from playing a major role in HDL-endothelium eNOS signaling. Our finding that cholesterol and oxysterols can decrease expression of SR-BI may provide an additional mechanistic link between hypercholesterolemia and decreased endothelial function.

Our present study shows that endothelial cells have a strong ability to efflux cellular cholesterol to HDL\(_1\) that may be independent of ABCA1, ABCG1, and SR-BI expression or activity. Although some groups have already shown no ABCA1 activity in endothelial cell lines,\(^6\) the finding that modifying the expression or activity of ABCG1 and SR-BI has little influence on endothelial efflux is novel. There may be other mechanisms and genes that have been overlooked or have gone undiscovered that mediate endothelial cholesterol efflux. Efflux to HDL may occur simply through passive diffusion or microsomalization. In endothelial cells, oxysterol formation may itself be a means for removing excess cholesterol independent of HDL. We saw evidence of endothelial conversion of cholesterol to oxysterols. It has been suggested elsewhere that oxysterol formation may be the predominant mechanism for removal of excess brain sterols because the blood-brain barrier prevents normal reverse cholesterol transport from taking place.\(^2\)\(^6\) Further investigation into the mechanisms of endothelial-HDL interactions will be required to shed light on HDL\(_1\)-mediated cholesterol efflux in endothelium.

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**References**


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