Influence of the HDL Receptor SR-BI on Lipoprotein Metabolism and Atherosclerosis

Bernardo L. Trigatti, Monty Krieger, Attilio Rigotti

Abstract—The scavenger receptor class B type I (SR-BI) was the first molecularly well-defined cell-surface HDL receptor to be described. SR-BI mediates selective HDL cholesterol uptake by formation of a productive lipoprotein/receptor complex, which requires specific structural domains and conformation states of apolipoprotein A-I present in HDL particles. SR-BI is abundantly expressed in several tissues, including the liver, where its expression is regulated by various mechanisms, including the transcriptional activity of nuclear receptors. The importance of SR-BI in overall HDL cholesterol metabolism and its antiatherogenic activity in vivo has been definitively established by SR-BI gene manipulation in mice. Remarkably, SR-BI/apolipoprotein E double-knockout mice develop complex coronary artery disease, myocardial infarction, and heart failure. Additional studies should help to define the importance of SR-BI in human health and disease. (Arterioscler Thromb Vasc Biol. 2003;23:1732-1738.)

Key Words: scavenger receptor class B type I ■ selective uptake ■ regulation ■ knockout mouse ■ coronary heart disease

Scavenger receptors are cell-surface transmembrane proteins that can bind modified lipoproteins, such as acetylated LDL and oxidized LDL. These receptors were initially reported in cultured macrophages in which they mediate cholesterol uptake from modified lipoproteins, leading to the formation of lipid-loaded macrophages that resemble the characteristic foam cells present in atherosclerotic lesions.1,2 Because LDL modified by oxidation may play an important role in foam cell formation during atherogenesis,3 macrophage scavenger receptors have been considered to be potentially key contributors to the pathogenesis of atherosclerotic cardiovascular disease.

The wide and complex ligand binding activities of scavenger receptors suggested the existence of multiple classes of these cell-surface proteins. In fact, over the past decade, several classes of cDNAs encoding different types of scavenger receptors, which are expressed in various cell types including macrophages, have been cloned.2,4 Both in vitro and in vivo experiments have provided strong support for the role of these cell-surface receptors in a variety of physiolog-
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transport, HDL metabolism, and atherosclerotic cardiovascular disease (see also reference 6 and references cited therein).

SR-BI and Cellular Cholesterol Transport

SR-BI was discovered during the study of a novel scavenger receptor activity in Chinese hamster ovary cells (reference 6 and references cited therein). The SR-BI cDNA encodes a 509 amino acid protein, which contains 1 putative membrane-spanning hydrophobic domain near each end of the protein and a large extracellular region with a cysteine-rich carboxy-terminal half and multiple potential N-glycosylation sites. SR-BI is indeed a highly N-glycosylated 82-kDa protein that is also modified by fatty acylation.6,7

SR-BI binds to a variety of ligands, including modified (acetylated or oxidized) lipoproteins, maleylated bovine serum albumin, advanced glycation end-product modified proteins, anionic phospholipids, and apoptotic cells.6,8 SR-BI also binds native lipoproteins such as HDL, LDL, and VLDL.6 Most studies of SR-BI have focused on its activity as a cell-surface HDL receptor.5,6 HDLs comprise a variety of particles with different sizes, lipid and protein compositions, and densities.9 The most abundant protein component of HDL is apolipoprotein A-I (apoA-I). The larger, cholesteryl ester–rich, lower density, spherical α-HDL particles bind more tightly to SR-BI than higher density HDLs, lipid-poor pre-β-HDL,10,11 or lipid-free apoA-I, whose binding is difficult to assess because of high nonspecific binding to cultured cells.10,12,13 The binding of reconstituted discoidal apoA-I complexes by SR-BI exhibits a very high affinity (higher than that of α-HDL) and requires either the amino- or the carboxy-terminal amphipathic helices of apoA-I.10 Assays using apoA-I fragments and a model peptide have indicated that amphipathic helices are critical for the binding of apolipoproteins to SR-BI.12 Taken together, these studies may explain the ability of SR-BI to bind a wide variety of lipoprotein classes and suggest that the conformation/organization of apoA-I in HDL particles is critical for the formation of a productive HDL/SR-BI interaction.10,11,13,15 The relative contents of apoA-I and apoA-II in spherical HDL particles may also affect their interactions with SR-BI.16,17

In addition to binding lipoproteins, SR-BI can mediate selective lipoprotein cholesterol uptake.8 Selective lipid uptake was initially discovered during analysis of HDL metabolism in vivo in rodents. Selective uptake involves cholesterol delivery from plasma HDL to tissues (especially the liver and steroidogenic tissues) without HDL particle degradation.18,19 Studies using purified SR-BI reconstituted into artificial liposomes have established that SR-BI by itself can mediate HDL binding and selective lipid uptake without the requirement of other proteins or specialized cellular structures.20 Additionally, SR-BI–mediated uptake of lipids other than cholesterol, including phospholipids, triglycerides, and α-tocopherol, has been described.8 Additional analysis of SR-BI–mediated selective uptake has indicated that SR-BI facilitates lipid uptake by a two-step mechanism involving an initial productive lipoprotein binding step followed by an efficient lipid transfer step.12,14,21,22 Additional studies will be required to determine if, in some tissues, SR-BI–mediated retroendocytosis of HDL particles during selective lipoprotein cholesterol ester uptake also plays a role in this process.23 The detailed molecular interactions between lipoproteins and SR-BI that result in productive binding and lipid transport remain to be established. The recent discovery of chemical inhibitors of the transfer of lipids mediated by SR-BI should provide new mechanistic insights into this lipid uptake pathway.22

In some types of cultured cells, SR-BI seems to be concentrated in plasma membrane caveolae.6 It has been reported that caveolae are the sites where SR-BI–mediated selective cholesterol uptake occurs before irreversible internalization of cholesterol into intracellular compartments.24 The formation of a caveolin-containing multiprotein complex may participate in the transport of cholesteryl esters taken up via SR-BI from the cell surface into intracellular membranes.25 However, recent studies in some cell types have suggested that expression of one major form of caveolin, caveolin-1, which is a principle protein component of many caveolae, does not affect26 or decreases27,28 SR-BI–mediated selective uptake of HDL lipids and that localization of SR-BI in caveolae-like domains may not be required for SR-BI–mediated selective cholesteryl ester uptake.20,29 SR-BI expression induces the formation of specialized structures called microvillar channels in cultured cells and in steroidogenic tissues in vivo.30,31 These structures seem to be the sites of SR-BI–mediated selective uptake in these cells. The roles of diverse specialized membrane domains in SR-BI–mediated lipid transport between HDL and cells remain to be defined.

In addition to mediating selective lipid uptake from lipoproteins to cells, SR-BI can mediate the bidirectional movement of unesterified cholesterol between lipoproteins and cells, the extent of which seems to depend on the cholesterol concentration gradient between HDL particles and the plasma membrane.32,33 Indeed, the expression levels of SR-BI correlate with the rate of free cholesterol efflux to HDL in a wide variety of cell lines.32 However, the relevance of SR-BI–mediated cholesterol efflux for HDL metabolism in vivo remains to be determined.

Regulation of Hepatic SR-BI Expression

Studies in rodents suggest that SR-BI expression in the liver is especially important for HDL metabolism and overall cholesterol homeostasis in vivo. Under basal conditions, most hepatic SR-BI expression is detected in parenchymal cells.6,34–36 Although SR-BI can be found in the canalicular domains of hepatocytes in mice overexpressing hepatic SR-BI27 and cultured hepatocyte couplets23 as well as in isolated plasma membrane fractions enriched in the canalicular surface,38,39 there have been conflicting reports about the polarized expression of SR-BI (exclusively sinusoidal or sinusoidal and canalicular) in hepatocytes of wild-type animals.35,36,39
Hepatic SR-BI expression can be regulated by a variety of dietary, hormonal, metabolic, and pharmacological manipulations. In the hamster, dietary plant-derived polyunsaturated fatty acids have been shown to stimulate hepatic SR-BI expression and HDL cholesteryl ester uptake, whereas dietary myristic acid decreases liver SR-BI levels in association with increased plasma HDL levels. SR-BI expression is inversely regulated by cellular α-tocopherol concentrations in the HepG2 human hepatic cell line and by dietary vitamin E supply in the mouse liver. Whereas streptozotocin-induced diabetes in hypercholesterolemic rats increases SR-BI protein levels, which correlates with reduced plasma HDL, insulin-treated diabetic hamsters exhibit lower levels of SR-BI compared with similar hamsters injected with saline only. In rats, estrogen administration at pharmacologic levels suppresses hepatic SR-BI expression by an indirect effect that is abolished by hypophysectomy and dependent on the estrogen-induced increase in LDL receptor activity. Additional studies have also reported decreased hepatic SR-BI protein expression after cholesterol feeding in the rat. However, studies with hamsters and mice did not find that hepatic SR-BI levels or HDL cholesteryl ester transport were regulated by changes in dietary cholesterol.

In the liver, a soluble multiple PDZ-domain–containing protein, PDZK1 (also called CLAMP), binds to the cytoplasmic C-terminus of SR-BI and seems to play an important role in controlling the intracellular transport, polarized expression, stability, and activity of SR-BI. Coexpression of PDZK1/CLAMP with SR-BI in transfected cells affects both the stability of SR-BI as well as the efficiency of conversion of HDL cholesteryl esters taken up via SR-BI to intracellular unesterified cholesterol. Mutations in the C-terminus of SR-BI that abolish binding to PDZK1/CLAMP prevent cell-surface expression of the receptor in the liver. Indeed, the plasma cholesterol levels of a PDZK1/CLAMP KO mouse are increased ~1.8-fold, similar to the increase in plasma cholesterol in SR-BI KO mice. Furthermore, a small PDZK1–associated protein, SPAP/DD96/MAP17, can regulate PDZK1 levels, and hepatic DD96 overexpression can alter plasma HDL cholesterol levels.

Recently, in vivo and in vitro studies have indicated the importance of nuclear receptors in the regulation of hepatic SR-BI expression. For instance, the administration of cholic acid, an activator of the farnesoid X receptor (FXR), in mice significantly increased hepatic SR-BI messenger RNA and protein levels. The FXR-dependent regulation of SR-BI in the murine liver was additionally demonstrated by the lowering of SR-BI expression in FXR knockout (KO) mice as well as the lack of cholic acid–induced upregulation of SR-BI in FXR-deficient animals. In addition, liver X receptors, the liver receptor homolog 1, the peroxisome proliferator–activated receptor γ, and the hepatocyte nuclear factor 4-α have all been reported to stimulate hepatic SR-BI gene expression.

Because of the importance of hepatic SR-BI expression for HDL homeostasis and the multiple regulatory effects of the peroxisome proliferator–activated receptor α (PPAR-α) on lipid metabolism, the activation of this additional nuclear receptor by its ligands (eg, fibrates) might have important consequences for SR-BI expression levels in the liver. Indeed, fibrate treatment suppresses SR-BI protein, but not mRNA, expression in the murine liver, and the reduced SR-BI activity is apparently responsible for altered HDL metabolism in drug-treated animals (enlarged HDL particle size). The posttranscriptional regulatory effect of fibrates on hepatic SR-BI protein levels was not observed in PPAR-α-deficient mice, demonstrating that this regulation requires normal PPAR-α expression. The normalization of hepatic SR-BI expression in fibrate-treated mice by adenoviral SR-BI gene transfer abolished the fibrate-mediated HDL particle size enlargement. Taken together, these findings show a functionally relevant effect of fibrate-dependent hepatic SR-BI regulation on HDL metabolism in mice. Thus, the potential significance of fibrate-induced PPAR-α activation on human SR-BI and HDL metabolism in fibrate-treated patients requires additional investigation.

**SR-BI and HDL Metabolism**

A series of in vitro and in vivo studies of the activity, expression, and regulation of SR-BI provided strong indirect evidence of its relevance for HDL metabolism. Studies in intact animals involving SR-BI gene manipulation using adenovirus-mediated gene transfer, transgenesis, and gene targeting in mice definitively established the importance of SR-BI as a functional HDL receptor in vivo. Hepatic overexpression of SR-BI is associated with decreased plasma

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**Image Description:**

Occlusive coronary artery atherosclerosis in SR-BI/apoE dKO mice. While young apoE KO mice have normal coronary arteries (top), SR-BI/apoE KO mice (bottom) develop extensive coronary artery stenosis with lipid, extracellular matrix, and fibrin deposition. Scale bars = 40 μm. From reference 74.
TABLE 1. Features of SR-BI/apoE Double-Knockout Mice: A Novel Model of Coronary Heart Disease

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
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<tbody>
<tr>
<td>Lipid-rich, fibrin-rich, occlusive, coronary arterial lesions</td>
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<tr>
<td>Multiple myocardial infarctions</td>
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<tr>
<td>Cardiac dysfunction</td>
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<td>Enlarged hearts</td>
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<td>Reduced contractility and relaxation</td>
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<td>Reduced ejection fraction</td>
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<tr>
<td>Ischemic and atrioventricular electrocardiographic abnormalities</td>
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<tr>
<td>Premature death</td>
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levels of HDL cholesterol, increased HDL cholesteryl ester clearance, and increased biliary cholesterol content and transport of cholesterol from the liver into the bile. Furthermore, SR-BI KO mice and mice with attenuated hepatic expression of SR-BI exhibit elevated plasma HDL cholesterol concentrations, reduced selective HDL cholesterol clearance, decreased adrenal cholesterol content, and decreased bile cholesterol concentration and secretion. The ratio of unesterified to esterified cholesterol in the plasma of SR-BI KO mice is significantly higher than that of SR-BI-positive controls. Normal unesterified to esterified cholesterol ratios are restored when the SR-BI KO mouse is treated with the antioxidant, lipid-lowering drug probucol; however, the mechanism by which this occurs has not been determined. SR-BI also plays a critical role in controlling plasma HDL and tissue α-tocopherol levels, which may have implications for the abnormal phenotypes associated with SR-BI deficiency in gene-targeted mice.

SR-BI and Atherosclerotic Cardiovascular Disease
Experiments using transgenic and KO mice have established that SR-BI expression protects against atherosclerosis. Transgene or adenosine-mediated hepatic overexpression of SR-BI markedly reduces atherosclerosis in various murine models of the disease. In addition, complete disruption of the SR-BI gene in both the chow-fed apoE-deficient (SR-BI/apoE double-KO [dKO] mice) and Western diet-fed LDL receptor-deficient (SR-BI/LDLR dKO) models substantially accelerates the onset of atherosclerosis as does attenuated SR-BI expression in LDLR-deficient mice. Bone marrow transplantation experiments have established that expression of SR-BI in bone marrow–derived cells is responsible, in part, for the atheroprotective effects of SR-BI. Furthermore, SR-BI KO mice develop atherosclerosis when fed a Western-type high-fat/high-cholesterol diet. Taken together, these studies clearly demonstrated the antiatherogenic activity of SR-BI in the mouse.

Remarkably, SR-BI/apoE dKO mice fed a normal chow diet develop at a very early age complex occlusive coronary artery lesions containing cholesterol clefts and fibrin deposits (Figure, Table 1). These mice exhibit multiple patchy myocardial infarctions with variable sizes and associated substantial myocardial fibrosis and die at a very young age (~6 weeks). Their demise is associated with substantial cardiac dysfunction. They exhibit severe hemodynamic and electrocardiographic abnormalities. Anesthesia induces substantial conductance abnormalities (eg, bradycardias and atrioventricular blocks), reminiscent of anesthetica effects in humans with heart disease. These severe cardiac phenotypes are not observed in apoE KO mice heterozygous for the SR-BI/null mutation (B. Trigatti, A. Braun, and M. Krieger, unpublished observations, 2000) and have not been reported in the case of attenuated SR-BI expression (~50% of wild-type hepatic SR-BI expression) in doubly homozygous SR-BIatt/apoE KO mice. Thus, the SR-BI/apoE dKO mouse might be a particularly useful animal model to investigate the mechanisms underlying the development of complex coronary artery disease, myocardial infarction, and heart failure. It may also be useful for preclinical testing of potential genetic or pharmacological therapies for coronary heart disease. Indeed, treatment with the drug probucol can prevent all of the early-onset atherosclerosis and cardiac abnormalities in the SR-BI/apoE dKO mouse.

SR-BI may protect against atherosclerosis by any one or a combination of the following mechanisms (Table 2): (1) it can mediate the hepatic uptake and biliary secretion of HDL cholesterol and therefore may suppress atherosclerosis by stimulating overall reverse cholesterol transport; (2) its expression in macrophage foam cells in atherosclerotic plaques may affect the flux of cholesterol between cells and HDL; (3) it may prevent accumulation of atherogenic lipoproteins in plasma; (4) it may contribute to α-tocopherol–mediated atheroprotection; (5) it may be involved in nitric oxide–mediated atheroprotection by mediating HDL-dependent endothelial NO synthase activation in vascular endothelial cells; and (6) its expression may influence arterial oxygen supply by controlling red blood cell maturation and preventing anemia. A striking phenotype of SR-BI KO mice is anemia and the appearance of inclusions in circulating red blood cells, which is exacerbated (100% reticulocytosis) under hypercholesterolemic conditions (eg, feeding SR-BI single KO mice a high-cholesterol diet or in SR-BI/apoE dKO mice). These inclusions have the characteristics of phagolysosomes, and their accumulation suggests a cholesterol-dependent block in their expulsion. These abnormalities are consistent with impaired red blood cell maturation and can be reversed by removal of cholesterol from the cells in vitro or in vivo and prevented by probucol treatment of the SR-BI/apoE dKO mice.

Conclusion
The discovery and additional characterization of the HDL receptor SR-BI have provided new insights into cholesterol...
and lipoprotein metabolism. In addition, the discovery that abnormal lipoprotein metabolism in SR-BI KO mice is the apparent cause of their female infertility has raised the possibility that dyslipidemia may underlie some forms of human female infertility.64–68 Analysis of SR-BI function in vivo has not only established the importance of SR-BI in HDL metabolism, it has led to a novel small animal model of human coronary heart disease and heart failure, the SR-BI/apoE dKO mouse. A series of studies examining the association of polymorphisms in the SR-BI gene and alterations in lipoprotein metabolism and related physiological parameters has suggested that SR-BI also may play an important role in humans91–97; however, additional studies will be required to establish with precision the significance of SR-BI in human physiology and disease.

Acknowledgments
This study was supported by the NIH Heart, Lung, and Blood Institute, the Canadian Institute for Health Research, Heart and Stroke Foundation of Ontario and Natural Science and Engineering Research Council from Canada, and Fondo Nacional de Desarrollo CientificoyTecnológico from Chile. The authors acknowledge the contributions of many colleagues and collaborators who have been involved in their studies on SR-BI.

References


46. Souidi M, Combettes-Souverain M, Milliat F, Eckhardt ER, Audas O, Dubras S, Parquet M, Ferezou J, Lutton C. Hamsters predisposed to sucrose-induced cholesterol gallstones (LPN strain) are more resistant to excess dietary cholesterol than hamsters that are not sensitive to cholelithiasis induction. *J Nutr.* 2001;131:1803–1811.


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Arterioscler Thromb Vasc Biol. 2003;23:1732-1738; originally published online August 14, 2003;
doi: 10.1161/01.ATV.0000091363.28501.84
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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