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SR-BI, a Multifunctional Receptor in Cholesterol Homeostasis and Atherosclerosis

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Abstract

The HDL receptor scavenger receptor class B type I (SR-BI) plays crucial roles in cholesterol homeostasis, lipoprotein metabolism and atherosclerosis. Hepatic SR-BI mediates reverse cholesterol transport (RCT) by the uptake of HDL cholesterol for routing to the bile. Through the selective uptake of HDL lipids, hepatic SR-BI modulates HDL composition and preserves HDL atheroprotective functions of mediating cholesterol efflux and minimizing inflammation and oxidation. Macrophage and endothelial cell SR-BI inhibits the development of atherosclerosis by mediating cholesterol trafficking to minimize atherosclerotic lesion foam cell formation. SR-BI signaling also helps limit inflammation and cell death c 2017 Published by Elsevier Ltd. and mediates efferocytosis of apoptotic cells in atherosclerotic lesions thereby preventing vulnerable plaque formation. SR-BI is emerging as a multifunctional therapeutic target to reduce atherosclerosis development.

Keywords

SR-BI; atherosclerosis; inflammation; cell death; efferocytosis

Introduction

Increased apolipoprotein B containing lipoproteins (LDL and VLDL) and decreased and/or dysfunctional HDL are risk factors for atherosclerosis[1], the underlying cause of heart

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attack and stroke. Arterial wall retention of apoB containing lipoproteins promotes endothelial cell dysfunction, resulting in recruitment of blood monocytes into the intima[1]. Intima monocytes differentiate into macrophages, which internalize modified LDL cholesterol to become foam cells, the hallmark of the fatty streak phase of atherosclerosis[1]. HDL and its major apolipoprotein, apoAI, prevent foam cell formation by stimulating cholesterol removal from macrophages and by reducing oxidative modification of apoB containing lipoproteins[1]. The removal of cholesterol from cells by HDL or apoAI is the first step in the RCT process, wherein the cholesterol is delivered to the liver for excretion into the bile[1]. The HDL receptor, SR-BI (See Glossary) was first isolated and identified by Calvo et al.[2] as a sequence closely related to CD36 and LIMPII analogous-1, and the gene, now called *SCARB1*, was initially named CLA-1. Acton and colleagues[3] demonstrated that SR-BI binds HDL with high affinity and mediates the selective uptake of HDL cholesteryl ester (CE) into the liver. SR-BI also mediates the bidirectional flux of free cholesterol (FC) between cells and HDL[4, 5]. The influx of HDL CE and FC by hepatic SR-BI and subsequent routing to bile is a major route of delivery of peripheral cholesterol to the liver for excretion, in both mice and humans[1]. Hepatic SR-BI also mediates the selective uptake of HDL lipid hydroperoxides[6, 7]. By mediating uptake of HDL lipids, SR-BI preserves HDL function[7, 8].

SR-BI is also expressed in macrophages and endothelial cells where it functions to reduce atherosclerosis, in mice[9–12, 13Vaisman, 2015 #15]. SR-BI reduces foam cell formation by mediating cholesterol removal from macrophages[14, 15]. As atherosclerosis progresses from a fatty streak to advanced lesions, there are increased inflammatory events resulting from enhanced oxidative stress[1]. The enhanced inflammation increases cell death and efficient efferocytosis of dead cells by macrophages and is required to prevent vulnerable lesion formation, which can lead to plaque rupture and clinical cardiovascular events[1]. SR-BI mediates anti-inflammatory and prosurvival signaling in macrophages and endothelial cells[11, 16] and macrophage SR-BI mediates the efferocytosis of apoptotic cells[11]. Thus, evidence has mounted that SR-BI is a multifunctional receptor, and this review focuses on the mechanisms by which SR-BI protects against atherosclerosis development.

Effects of Hepatic SR-BI on Atherosclerosis and Plasma Lipoproteins

Evidence has mounted that SR-BI expression impacts the development of atherosclerosis, and modulation of plasma lipoproteins and mediation of reverse cholesterol transport (RCT) by hepatic SR-BI likely contribute to the effects on atherosclerosis. Compared to wild type (WT) controls, SR-BI knockout mice have increased plasma cholesterol consisting primarily of enlarged HDL (Figure 1, Key Figure) enriched with FC and apoE[17]. Importantly, consumption of a western diet for 20 weeks to induce atherosclerosis development results in significantly more atherosclerosis compared to WT mice[18]. Administration of a high fat diet containing cholesterol to SR-BI knockout mice results in marked accumulation of HDL particles in the VLDL size range that are FC and apoE enriched, raising the possibility that the decreased RCT and resulting dysfunctional HDL contributes to the atherosclerosis development[19]. Interestingly, deletion of SR-BI in apoE^{-/-} mice consuming a chow diet causes accelerated atherosclerosis resulting in coronary artery occlusion, myocardial infarction, and premature death at 5 to 8 weeks of age[20, 21]. These mice have severe

hypercholesterolemia ($\approx 1000\text{mg/dl}$) with markedly increased lipoprotein FC and HDL particles in the size range of LDL and VLDL[21, 22] and studies suggest that the accelerated atherosclerosis is in part due to their abnormal lipoproteins[15, 23]. Recently, Fuller and colleagues[24] showed that SR-BI/LDLR double knockout mice versus LDLR^{-/-} mice also have abnormal lipoprotein profiles leading to severe coronary atherosclerosis, myocardial infarction, and decreased survival rate (average lifespan = 9.4 weeks) when fed a modified Western diet containing high cholesterol (1.25%). The accelerated atherosclerosis that occurs in these SR-BI knockout mouse models is due in part to the loss of hepatic SR-BI, which mediates the last step in RCT, the removal of HDL CE and FC for routing to bile. This is substantiated by studies demonstrating that liver specific knockdown of SR-BI in mice increases the development of atherosclerosis,[19] and by other studies showing that overexpression of hepatic SR-BI in LDLR^{-/-} mice reduces atherosclerosis[25]. In humans, HDL CE is transferred by CETP to VLDL for conversion to LDL, which is internalized by hepatic LDL receptors. However, it has been estimated that at least 33% of HDL CE is directly taken up by hepatic SR-BI in humans[26]. Consistent with a role for SR-BI in hepatic uptake of HDL-C in humans (Figure 1), subjects carrying loss of CE transport function variants (i.e. P376L, P297S) in the *SCARB1* gene have elevated HDL cholesterol in the form of enlarged, apoE-enriched particles[14, 27]. Interestingly, heterozygous carriers of the P376L *SCARB1* gene variant have been reported to have an increased risk of coronary heart disease (odds ratio = 1.79, $p < 0.05$)[27]. Recent studies[28] revealed structurally by protein homology modeling how SR-BI transports HDL CE (Box 1). In addition to uptake of CE from HDL, hepatic SR-BI may also play a role in the clearance of VLDL. Studies showed that clearance of VLDL was reduced by 67% in SR-BI^{-/-} versus WT mice,[29] and that hepatic SR-BI binds VLDL. SR-BI internalizes VLDL via a mechanism involving lipoprotein lipase and heparin sulfate proteoglycans[30]. Consistent with SR-BI mediating VLDL clearance, deletion of SR-BI in apoE^{-/-} and hypomorphic apoE mice increases plasma levels of VLDL and IDL[22, 31]. The increase in remnant lipoproteins also likely contributes to the increased atherosclerosis in SR-BI deficient mice as IDL/VLDL can induce foam cell formation without need of oxidative modification[32]. Recent studies by Yang and colleagues demonstrated that SR-BI mediates the uptake of lipids from the proatherogenic particle Lp(a)[33]. In addition, the *in vivo* clearance of Lp(a) was decreased in SR-BI deficient mice and overexpression of hepatic SR-BI markedly increased the clearance of Lp(a)[33]. Importantly, studies showed that human carriers of 6 different *SCARB1* gene variants had increased plasma levels of Lp(a), and the variants had reduced ability to mediate the uptake of Lp(a) CE[34].

Hepatic SR-BI Uptake of HDL Lipids Preserves the Atheroprotective Functions of HDL

Evidence has mounted that HDL function is a better marker than HDL-C levels for risk of coronary heart disease[44–46]. Thus, it is likely that the dysfunctional nature of the HDL from SR-BI knockout mouse models and human carriers of the *SCARB1* gene P376L variant contribute to the enhanced atherosclerosis development. Studies have shown that HDL from either SR-BI knockout mice or human carriers of the *SCARB1* gene P376L variant does not have an impaired ability to mediate the efflux of radiolabeled FC from

cells[8, 27]. However, it should be kept in mind that the flux of cholesterol between cells and HDL is bidirectional and measurement of efflux may not reflect the ability of HDL to promote a net reduction in macrophage cholesterol stores[47]. Indeed, the cholesterol content was 2.7-fold higher in cholesterol-enriched macrophages incubated with HDL from SR-BI^{-/-} versus WT mice (Figure 1) consuming a chow diet[8]. Furthermore, HDL particles that are in the VLDL size range from SR-BI^{-/-} mice fed a high fat diet (1.25% cholesterol) were as efficient as acetylated LDL in enriching macrophages with cholesterol[19]. Taken together, the studies suggest that SR-BI deletion leads to increased influx of HDL cholesterol into macrophages, which could be due to increased influx of FC and/or selective uptake of CE. In addition, it is possible that the holoparticle uptake of SR-BI^{-/-} mouse HDL is increased due to apoE enrichment and/or oxidative modification[7, 48]. HDL also prevents thrombosis by preventing platelet activation and by minimizing platelet cholesterol content. Platelets from SR-BI^{-/-} mice and human carriers of the *SCARB1* gene P297S variant are heavily enriched with FC and are prothrombotic (Figure 1), compared to control platelets[49, 50]. This suggests that HDL in both SR-BI^{-/-} mice and human carriers of the *SCARB1* gene P297S variant is impaired in the ability to limit platelet cholesterol content and activation. Studies have shown that SR-BI knockout mice have increased oxidative stress as exemplified by increased plasma levels F2-isoprostanes and protein carbonyls suggesting that their HDL is impaired in preventing oxidation (Figure 1) [7]. Indeed, SR-BI deficient mice have decreased plasma PON1 activity, which is essential to HDL preventing LDL oxidation[7]. Interestingly, recent studies have shown that hepatocyte SR-BI is critical in determining the capacity of HDL to acquire newly secreted PON1 and thus, maintain its anti-oxidative capacity[51]. HDL also reduces the oxidation status of LDL by acting as a sink for lipid hydroperoxides transferred from LDL[52].

Studies in SR-BI^{-/-} mice have shown that hepatic SR-BI mediates the selective removal of lipid hydroperoxides from HDL,[6, 7] thereby preventing the accumulation of oxidized lipids in HDL and increasing HDL capacity to accept lipid hydroperoxies from LDL (Figure 1). The HDL enzyme, LCAT, maintains the gradient of FC flow from peripheral tissues to plasma in RCT by esterifying HDL FC[1]. The loss of hepatic SR-BI leads to compositional changes in HDL including increased sphingomyelin, which markedly reduces the ability of LCAT to bind HDL resulting in accumulation of toxic FC in HDL resulting in reduced cholesterol efflux capacity and RCT[22, 53, 54]. In addition, studies have shown that an atheroprotective function of HDL is to promote endothelial cell migration and limit apoptosis[1, 16]. Recent studies have shown that overexpression of hepatic ABCA1 decreases expression of SR-BI resulting in HDL that has an impaired ability to induce endothelial cell migration and reduce cell death (Figure 1)[55]. Importantly, forced expression of SR-BI in hepatic ABCA1 transgenic restored the ability of HDL to prevent apoptosis[55]. Taken together, hepatic SR-BI protects against atherosclerosis by modulating HDL composition and preserving its function (Figure 1).

SR-BI and Atherosclerotic Lesion Foam Cell Formation

Bone marrow transplantation studies have established that hematopoietic cell SR-BI functions in preventing atherosclerosis. Transplantation of either apoE^{-/-} or LDLR^{-/-} mice with hematopoietic cells deficient in SR-BI results in accelerated atherosclerosis

development, compared to mice receiving control bone marrow[9–12]. The cause of the increased atherosclerosis that occurs with hematopoietic cell SR-BI deficiency is localized to the arterial wall as there are no effects on plasma cholesterol and lipoprotein distribution. In addition, studies demonstrated that transplantation of mice that are null for SR-BI and express low levels of apoE (SR-BI^{-/-} apoE-hypomorphic) with SR-BI^{+/+} versus SR-BI^{-/-} bone marrow markedly reduces coronary atherosclerosis and myocardial infarction[13]. While SR-BI is expressed in a number of bone marrow cell types including B and T lymphocytes, studies suggest lymphocyte SR-BI does not impact the development of atherosclerosis. Earlier studies[56] showed that deletion of T and B lymphocytes in SR-BI^{-/-}apoE^{-/-} mice does not affect the extent of atherosclerosis and survival rate. Furthermore, treatment of SR-BI^{-/-} apoE-hypomorphic mice with the immunosuppressant, FTY720, which reduces the number of circulating lymphocytes does not reduce the development of atherosclerosis. In addition, there is no difference in the number of T and B cells in atherosclerotic lesions containing SR-BI^{+/+} versus SR-BI^{-/-} cells[11]. It is likely that macrophage SR-BI is critical to the atheroprotective effects of hematopoietic cell SR-BI as macrophages are the most abundant cell type in atherosclerotic lesions and macrophage SR-BI expression impacts cholesterol homeostasis, inflammation and efferocytosis.

One mechanism by which macrophage SR-BI could reduce atherosclerosis is by functioning in the removal of cholesterol from macrophages (Figure 2). Indeed, a number of studies have shown that SR-BI mediates cholesterol efflux from human macrophages. In addition, in cholesterol loaded human monocyte-derived macrophages ABCA1 and SR-BI were the main mechanisms of cholesterol efflux to HDL, and ABCG1 was not a critical cholesterol transporter[57]. Consistent with these observations, other studies showed that incubation of human monocyte-derived macrophages with testosterone, which upregulated SR-BI with no change in ABCA1 levels, greatly reduces the cholesterol stores in the presence of HDL[58]. Furthermore, the use of BLT-1 to block cholesterol transport via SR-BI significantly reduced cholesterol efflux from cholesterol-enriched THP-1 macrophages[59]. Importantly, cholesterol enriched macrophages from human carriers of the *SCARB1* gene P297S variant exhibited significantly reduced cholesterol efflux to HDL, compared to macrophages from control subjects[14]. The role of SR-BI in mediating cholesterol efflux from mouse macrophages is somewhat controversial. Studies have suggested that ABCA1 and ABCG1 are the main mechanisms of cholesterol efflux from mouse macrophages, with SR-BI only making a minor contribution[60–62]. However, other studies using mouse macrophages have shown that inhibition of SR-BI cholesterol transport significantly reduces cholesterol efflux to HDL[63]. In addition, overexpression of SR-BI and caveolin-1 in RAW macrophages increased the cholesterol efflux to HDL, whereas overexpression of ABCG1 had only modest effects[64]. These inconsistent findings are probably due to differences in cholesterol efflux conditions (efflux time, HDL dose, cholesterol-enrichment), SR-BI expression, and/or mouse macrophage type[47, 62, 65]. The flux of cholesterol between cellular SR-BI and HDL is bidirectional and is regulated by the cholesterol status and phospholipid species of both the cell and HDL[4, 5]. In particular, the use of cholesterol-normal cells does not test the role of SR-BI in cholesterol transport. Interestingly, examination of the cholesterol content of cholesterol-enriched mouse peritoneal macrophages after long term incubation

with HDL, so that CE hydrolysis becomes relevant, demonstrated a marked impairment in cholesterol mobilization from SR-BI^{-/-} versus control macrophages[15] (Figure 2).

A major pathway for clearance of cytoplasmic CE involves autophagosome trafficking of CE to lysosomes for hydrolysis to FC and efflux via ABCA1[66] (Figure 2). In addition, studies have shown that efficient autophagy in macrophages reduces atherosclerosis[67]. Interestingly, recent studies demonstrated that autophagy is impaired in SR-BI deficient mouse macrophages in the setting of infection, and the autophagy induction by SR-BI[68] involves the formation of subcellular membrane cholesterol domains (Box 1) raising the possibility that SR-BI regulates the mobilization of cytoplasmic cholesterol for availability to ABCA1 for efflux. Consistent with this possibility, the mobilization of CE to lipid-free apoAI is markedly impaired in SR-BI^{-/-} macrophages compared to control cells, even with similar ABCA1 levels (Figure 2) [15]. In addition, SR-BI localizes to macrophage lysosomes, and SR-BI deficient macrophages contain lysosomes that are FC enriched and neutral lipid engorged[15]. Furthermore, SR-BI trafficks to lysosomes with Rab7,[69] a protein that is critical in facilitating the fusion of autophagosomes with lysosomes[70]. A role for SR-BI in clearance of cytoplasmic CE is also substantiated by studies showing that the foam cells of atherosclerotic lesions containing SR-BI deficient versus control cells have 3% and 40% of their cytoplasm not occupied by lipid, respectively[15]. In summary, there is abundant evidence to support a role for macrophage SR-BI in maintaining cholesterol homeostasis (Figure 2).

Recent studies suggest that endothelial cell SR-BI functions in reducing atherosclerotic lesion foam cell formation. Specific overexpression of SR-BI in endothelial cells decreased atherosclerosis in both apoE^{-/-} and WT mice[71]. While the effects of endothelial cell overexpression of SR-BI on atherosclerosis are likely due in part to decreased plasma cholesterol and increased HDL-C, the arterial endothelial cell SR-BI may also contribute to the reduced atherosclerosis. These and other studies[71, 72] have shown that a major portion of the endothelial cell transcytosis of HDL from the apical to basolateral side is mediated by SR-BI, suggesting that SR-BI provides HDL to the subendothelium to promote cholesterol efflux from macrophages. Importantly, basolateral SR-BI mediated the uptake of HDL cholesterol for transport to the apical side, suggesting that endothelial cell SR-BI removes cholesterol released to HDL from intimal macrophages[71]. Furthermore, recent studies have shown that endothelial cell SR-BI mediates the uptake and transcytosis of HDL in lymphatic vessels to effectively remove cholesterol from peripheral tissue, thereby raising the possibility that lymphatic SR-BI also reduces foam cell formation in atherosclerotic lesions[73].

Role of SR-BI Signaling in Preventing Inflammation and Atherosclerosis

Inflammatory events are critical to the progression of fatty streaks to advanced atherosclerotic lesions, and studies suggest that SR-BI protects against atherosclerosis by regulating the inflammatory response. Compared to LDLR^{-/-} mice, SR-BI/LDLR double knockout mice fed an atherogenic diet have increased plasma levels of proinflammatory IL-6 and TNF- α [24] (Figure 3). In addition, apoE^{-/-} mice transplanted with SR-BI deficient versus apoE^{-/-} bone marrow have increased serum levels of proinflammatory cytokines

including IL-1 β , IL-6, and TNF- α [11]. Proinflammatory Ly6C^{hi} versus anti-inflammatory Ly6C^{lo} monocytes preferentially migrate into the subendothelial space and convert into proinflammatory M1 macrophages[1, 74, 75]. Interestingly, recent studies showed that SR-BI/LDLR double knockout mice have increased numbers of proinflammatory Ly6C^{hi} monocytes and decreased numbers of anti-inflammatory Ly6C^{lo} monocytes, compared to LDLR^{-/-} mice [24]. Consistent with SR-BI regulating macrophage conversion to inflammatory M1 versus anti-inflammatory M2 phenotype, exposure of SR-BI deficient versus WT cells to LPS results in increased expression of proinflammatory cytokines (TNF- α , IL-1 β , and IL-6) and decreased expression of anti-inflammatory, TGF- β [76]. These same studies showed that SR-BI regulates macrophage inflammation by reducing NF- κ B, P38, and JNK signaling (Figure 3)[76]. A number of SR-BI ligands have been shown to regulate the macrophage inflammatory response. Interaction of PON1 with SR-BI reduced the conversion of macrophages to the proinflammatory M1 phenotype[77]. In addition, HDL interaction with SR-BI reduces the inflammatory response to LPS in human macrophages by markedly reducing NF- κ B activation[78]. Furthermore, PI3K/Akt signaling in macrophages regulates NF- κ B activation (Figure 3)[76, 79], and recent studies have shown that the interaction of macrophage SR-BI with apoptotic cells activates PI3K/Akt signaling (Figure 3) and induces expression of anti-inflammatory cytokines(IL-10, TGF- β)[11]. In addition, HDL activates PI3K/Akt signaling in macrophages, which is mediated by SR-BI and involves interaction with its adaptor protein, PDZK1(Box 1), and activation of S1P receptor 1(S1PR1) signaling[40]. Thus, a likely mechanism by which SR-BI regulates inflammation is by activating Akt phosphorylation leading to reduced NF- κ B activation (Figure 3)[40].

Limiting endothelial cell inflammation is critical to reducing monocyte adhesion and recruitment into intima thereby preventing lesion progression. SR-BI interaction with HDL prevents endothelial cell inflammation, by controlling eNOS activation and expression of the antioxidant enzyme, 3-beta-hydroxysteroid-delta 24-reductase (DHCR24)[80, 81]. SR-BI mediated production of nitric oxide (NO) and DHCR24 leads to less TNF- α stimulated NF- κ B activation, resulting in reduced endothelial cell expression of inflammatory monocyte adherence proteins and chemokines (VCAM-1, ICAM-1, MCP-1), thereby reducing monocyte recruitment into the intima[80, 82]. In activating eNOS, HDL binding to SR-BI activates Src, which leads to Akt activation, and Akt then phosphorylates eNOS[83]. SR-BI signaling of eNOS activation requires the C-terminal transmembrane domain, which binds plasma membrane cholesterol (Box 1), and the C-terminal cytoplasmic PDZ-interacting domain that binds to the SR-BI adaptor protein PDZK1[39, 42]. HDL sphingosine 1-phosphate (S1P) also activates eNOS in endothelial cells, and a recent study demonstrated that S1P promotes interaction of SR-BI with S1PR1 to activate S1PR1[84]. This study raises the interesting possibility that SR-BI controls cellular inflammation by controlling signaling via other receptors as part of a protein complex.

Effects of SR-BI Signaling on Apoptosis Susceptibility and Efferocytosis in Atherosclerosis

Studies have shown that SR-BI induction of eNOS activity and Akt phosphorylation promote endothelial cell survival and proliferation, thereby maintaining endothelial cell

barrier integrity[16, 85, 86]. In addition, HDL PON1 interaction with SR-BI decreased apoptotic and necrotic cell death in cultures of proinflammatory M1 macrophages (Figure 3) [77]. SR-BI activation of Akt likely plays a role in preventing macrophage apoptosis, as Akt promotes survival by inducing the phosphorylation of Bad, which prevents Bad from inhibiting the anti-apoptotic factor BCL-XL (Figure 3) [87]. Moreover, SR-BI interaction with HDL inhibits oxidized LDL induced endoplasmic reticulum stress, as evidenced by decreased phosphorylation of eukaryotic translation initiation factor 2 α and reduced levels of CHOP and Bip[88]. Consistent with a role for SR-BI in preventing cell death in atherosclerotic lesions, transplantation of either LDLR^{-/-} or apoE^{-/-} mice with SR-BI deficient bone marrow results in markedly increased numbers of lesion TUNEL positive cells, compared to lesions in mice receiving control bone marrow[11]. The increased number of dead cells in lesions containing SR-BI deficient cells was due in part to increased susceptibility to apoptosis as SR-BI deficient versus control lesions contained more macrophages that stained positive for active caspase 3 (Figure 3)[11].

Recent studies have shown that macrophage SR-BI mediates the efferocytosis of apoptotic cells[11]. Macrophage SR-BI recognizes apoptotic cell phosphatidylserine as a ligand, and is localized to phagosomes (Figure 3)[11]. Using both in vivo and in vitro assays, it was shown that SR-BI^{-/-} macrophages are impaired in mediating the efferocytosis of apoptotic cells compared to WT controls[11]. Macrophage SR-BI interacts with and activates Src leading to activation of PI3K and Rac1 to induce the phagocytosis of apoptotic cells. The efferocytosis of apoptotic cells by SR-BI also activates Akt, which promotes phagocyte survival and reduces inflammation [11, 89]. Interestingly, in vitro pharmacological activation of Rac1 corrected the defective efferocytosis in SR-BI-deficient macrophages, raising the possibility that impaired SR-BI signaling affects other efferocytosis pathways (Figure 3). Consistent with this possibility, macrophage SR-BI deficient cells have increased levels of proinflammatory HMGB1 that interacts with Src, preventing association with plasma membrane receptors, which results in decreased Rac1 activation[90]. Importantly, hematopoietic SR-BI deficiency led to a marked accumulation of atherosclerotic lesion apoptotic cells that were not associated with macrophages, demonstrating that macrophage SR-BI is critical for the efferocytosis of apoptotic cells in atherosclerotic lesions[11]. Impaired engulfment of apoptotic cells in atherosclerotic lesions leads to secondary necrosis and unresolved inflammation (Figure 3), and this increased cell death causes thinning of the fibrous cap, which is characteristic of the vulnerable plaque that leads to plaque rupture and thrombosis, and causes the development of clinical ischemic cardiovascular events[1]. Consistent with SR-BI-mediated efferocytosis preventing plaque instability, atherosclerotic lesions of mice reconstituted with SR-BI deficient vs. WT hematopoietic cells had increased necrotic area and thinning of the fibrous cap[11].

Concluding Remarks

Studies have established the atheroprotective role of the HDL receptor SR-BI, in mouse models. As the main pathway of hepatic removal of HDL cholesterol in mice, SR-BI is critical for the delivery of cholesterol to bile and in preserving HDL functions. Hepatic SR-BI is also crucial in the clearance of remnant lipoproteins and atherogenic Lp(a), in mice. In macrophages and endothelial cells, SR-BI protects against atherosclerosis by minimizing

foam cell formation and by regulating signaling pathways involved in efferocytosis, cell survival, and inflammation. Recent genomic analysis studies suggest that SR-BI is atheroprotective in humans, as carriers of loss of function *SCARB1* variants are at increased risk of CVD[27]. The accumulation of enlarged HDL in plasma in carriers of *SCARB1* variants substantiates that hepatic SR-BI mediates uptake of HDL cholesterol in humans[14, 27]. Thus far, studies have not adequately addressed whether the HDL from humans carriers of loss of function *SCARB1* variants is dysfunctional. However, the observation that their platelets have increased cholesterol content and are prothrombotic[14] suggests that their HDL has less cholesterol efflux capacity. As in mice, recent studies have demonstrated that human carriers of loss of function *SCARB1* variants have increased plasma Lp(a) suggesting that hepatic SR-BI also mediates clearance of proatherogenic Lp(a) in humans[34]. However, future studies need to address whether hepatic SR-BI also contributes to the clearance of atherogenic remnant lipoproteins in humans. Macrophages from human carriers of loss of function *SCARB1* variants are defective in cholesterol efflux[14] suggesting that like in mice, macrophage SR-BI protects against atherosclerosis at the level of the arterial wall. SR-BI deficient macrophages have impaired autophagy in response to stress[68], and defective autophagy accelerates atherosclerosis. Future studies need to establish the mechanisms by which SR-BI impacts autophagy in both human and mouse macrophages, and whether its role is relevant to CE mobilization, inflammation, cell death, and development of atherosclerosis. SR-BI decreases inflammatory Ly6C^{hi} monocytes in mice[24]. Studies need to examine if this is also the case in humans as enhanced monocytes is proatherogenic, the mechanisms by which SR-BI controls monocytes also need to be defined. Taken together SR-BI is a multifunctional receptor against atherosclerosis making it a viable therapeutic target. Identification of the detailed mechanism by which SR-BI functions including its adaptor proteins, signaling molecules, and its transcriptional regulators will be important to provide the basis for new therapeutic approaches for atherosclerosis.

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Glossary

ATP-binding cassette transporter A1 (ABCA1) and G1 (ABCG1)

ABCA1/ABCG1 are transmembrane proteins that utilize ATP binding and hydrolysis to transport various substrates across cellular membranes. ABCA1 and ABCG1 mediate cholesterol efflux to apoA1 and HDL, respectively

Autophagy

is a regulated cellular self-degrading process to effectively clear damaged organelles and proteins in response to stress. Damaged constituents are isolated from the rest of the cell within double-membrane vesicles (i.e autophagosomes). Autophagosomes fuse with lysosomes for degradation of the constituents. Autophagy also operates in the clearance of cytoplasmic cholesteryl ester (CE), where the CE is packaged in autophagosomes and

trafficked to lysosomes for hydrolysis to free cholesterol and subsequent release from the cell by cholesterol transporters

Cytokines

Small proteins that are produced and secreted by different cell types to modulate the immune response to inflammation, infection, and trauma. Proinflammatory cytokines include tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and IL-6. Anti-inflammatory and cell survival cytokines include IL-10 and transforming growth factor- β (TGF- β)

Efferocytosis

An immune process in which apoptotic cells and their debris are engulfed by professional phagocytes (i.e. macrophages) for efficient clearance and resolution of inflammation

Lecithin-cholesterol acyltransferase (LCAT)

is an enzyme that binds to lipoproteins, mainly HDL, and esterifies free cholesterol to cholesteryl ester

Lipoprotein (a) (Lp(a))

is a proatherogenic lipoprotein that consists of apolipoprotein a bound to LDL via a disulfide bridge with apolipoprotein B100. Lp(a) is the main carrier of oxidized phospholipids in plasma

Nuclear factor kappa B (NF- κ B)

is a protein complex that is activated in response to stress, oxidized LDL, and lipopolysaccharide to enhance the transcription of inflammatory cytokines. Ligands that bind SR-BI, including HDL, PON1, apoptotic cells, and sphingosine 1-phosphate, reduce the inflammatory response by activating Akt and reducing activation of NF- κ B

Paraoxonase/arylesterase 1 (PON1)

is an enzyme that hydrolyzes many molecules including lactones and prevents the oxidation of LDL

Phosphoinositide 3-kinase (PI3K)

it phosphorylates phosphoinositides at the 3 position of the inositol ring leading to membrane recruitment and activation of protein kinase B (Akt)

Scavenger Receptor class B type I (SR-BI)

is a multiple ligand receptor and is critical in facilitating hepatic selective uptake of cholesteryl esters from HDL without whole HDL particle endocytosis

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Box 1**SR-BI Structure and Function**

Understanding the structure of SR-BI is critical to developing approaches to prevent atherosclerosis. SR-BI is a hairpin-looped structure with two short transmembrane domains, two cytoplasmic tails, and a large extracellular loop. Studies using SR-BI/CD36 chimeric receptors demonstrated that the extracellular domain binds to multiple ligands and is critical to CE selective uptake and the bi-directional flux of free cholesterol[35]. Binding of HDL to the extracellular loop is not essential for cholesterol efflux, but subdomains of the N-terminal half of the extracellular domain are required for cholesterol efflux and organization of plasma membrane cholesterol domains[36]. Binding of HDL to the extracellular domain is required for CE transport, and subdomains of C-terminal half of the extracellular domain operate in selective uptake of CE[36]. Studies [28] that determined the high-resolution crystal structure of the CD36 family protein, lysosomal integral membrane protein type-2, revealed structurally by protein homology modeling how SR-BI transports HDL CE. A large cavity traverses the entire length of the SR-BI molecule, and mutagenesis of SR-BI showed that the cavity is a lipophilic tunnel through which CE is delivered from the bound lipoprotein to the outer leaflet of the plasma membrane[28]. Using fluorescence resonance energy transfer, Sahoo and colleagues determined that SR-BI forms oligomers at the C-terminal region of the extracellular domain[37]. In addition, a glycine dimerization motif in the N-terminal transmembrane domain is critical to SR-BI oligomerization, and CE transport is proportional to the degree oligomerization[38].

The C-terminal cytoplasmic and transmembrane domains are critical to SR-BI signaling. The C-terminal cytoplasmic domain contains a PDZ-binding domain that interacts with the adaptor protein, PDZK1. In endothelial cells, activation of eNOS involves interaction of SR-BI with Src, and then activation of Src leads to activation of PI3K, Akt, and eNOS[39]. PDZK1 interaction with SR-BI is not required for Src interaction with SR-BI, but is essential to Src activation[39]. In macrophages, PDZK1 is also required for HDL/SR-BI induced activation of Akt[40]. In hepatocytes, PDZK1 interaction maintains steady state levels of SR-BI by preventing its degradation[41]. SR-BI acts as a membrane cholesterol sensor and the C-terminal transmembrane domain binds to cholesterol[42]. Using a SR-BI C-terminal trans-membrane Q445A point mutant that has reduced interaction with cholesterol, it has been demonstrated that SR-BI interaction with membrane cholesterol is not critical for interaction with Src or PDZK1, but is required for activation of Src[43]. Future studies are needed to determine the mechanism of how the interaction of SR-BI with membrane cholesterol activates Src.

Trends

- SR-BI mainly functions as a receptor for HDL. Loss of SR-BI mediated uptake of HDL lipids results in impaired ability of HDL to promote cholesterol efflux and prevent inflammation.
- Deficiency of SR-BI impairs autophagy in macrophages in response to infection, which likely affects cell death, inflammation, and cholesterol mobilization.
- Macrophage SR-BI acts as an apoptotic cell receptor, and enhances efferocytosis via Src/PI3K/Rac1 signaling, thereby limiting atherosclerotic lesion necrotic core size, inflammatory response, and vulnerable plaque formation in mice.
- Macrophages isolated from humans carrying a loss of function variant of *SCARB1* (P297S) exhibit impaired cholesterol efflux to HDL.
- Human genetic studies confirm that SR-BI is atheroprotective, as carriers of the loss of function P376L *SCARB1* variant are at increased risk of CVD.

Outstanding Questions Box

- Does SR-BI function in the clearance of remnant lipoproteins in humans?
- What are the consequences of loss of function *SCARB1* mutations on HDL function in humans?
- How does SR-BI regulate autophagy in macrophages?
- Does macrophage SR-BI function in autophagy in the setting of atherosclerosis?
- What proteins interact with SR-BI to mediate signaling pathways involved in inflammation, cell death, and cholesterol mobilization?
- How does SR-BI regulate monocytoysis in mice, and does increased monocytoysis occur in humans carrying loss of function *SCARB1* mutations?

Figure 1. Hepatic SR-BI: Selective Uptake of HDL Lipids Regulates HDL Function

Hepatic SR-BI mediates the selective uptake of HDL lipids including cholesteryl ester (CE) and lipid hydroperoxides (LOOH), thereby modulating HDL composition and function. Loss of hepatic SR-BI function results in the accumulation of enlarged, dysfunctional HDL in plasma. The cholesterol-enriched HDL has reduced capacity to promote the net efflux of cholesterol from macrophages. The HDL has increased LOOH content and decreased PON1 activity thereby reducing its anti-oxidative function. The enlarged HDL particle is impaired in mediating pro-survival and anti-inflammatory signaling. The reduced ability of enlarged HDL to remove cholesterol from cells results in platelets that are cholesterol engorged and prothrombotic.

Figure 2. SR-BI Functions in Preventing Macrophage Foam Cell Formation by Mobilizing Cholesterol

Macrophages have three major proteins that mediate the efflux of free cholesterol (FC) including SR-BI, ABCA1, and ABCG1. SR-BI and ABCG1 efflux cholesterol to HDL, whereas ABCA1 transports phospholipid and cholesterol to plasma-derived lipid-poor apoA1 or to apoE that is secreted by macrophages. In human macrophages, ABCA1 and SR-BI are the main cholesterol efflux pathways. Cytoplasmic cholesteryl ester (CE) is cleared by two pathways: 1) CE can be hydrolyzed by neutral CE hydrolase (NCEH) and the FC trafficked to the plasma membrane for efflux; 2) Cytoplasmic CE is also packaged into autophagosomes that fuse with lysosomes, where the CE is hydrolyzed by lysosomal acid lipase and the FC trafficked for release from the cell.

Figure 3. SR-BI Regulates Macrophage Inflammation, Apoptosis, and Efferocytosis

In response to stressors such as oxidized LDL and inflammatory cytokines, NF- κ B is activated thereby enhancing the transcription of proinflammatory cytokines (i.e. IL-1 β , TNF- α , and IL-6). Ligands that bind SR-BI including HDL, PON1, apoptotic cells, and sphingosine 1-phosphate reduce the inflammatory response by activating Akt and reducing activation of NF- κ B leading to increased secretion of anti-inflammatory IL-10 and TGF- β . When SR-BI signaling and activation of Akt is compromised, the enhanced production of inflammatory cytokines and reactive oxygen species (ROS) promotes apoptosis. Macrophage SR-BI mediates the efferocytosis of apoptotic cells via Src/PI3K/Akt/Rac1 signaling, leading to phagocyte survival and an anti-inflammatory response. Impaired efferocytosis via SR-BI promotes secondary necrosis, phagocyte death, and unresolved inflammation promoting formation of the atherosclerotic vulnerable plaque. MΦ, macrophage; PS, phosphatidylserine; AC, apoptotic cells.