

SR-BI inhibits ABCG1-stimulated net cholesterol efflux from cells to plasma HDL

Laurent Yvan-Charvet,^{1,*} Tamara A. Pagler,^{*} Nan Wang,^{*} Takafumi Senokuchi,^{*} May Brundert,[†] Hongna Li,^{*} Franz Rinninger,[†] and Alan R. Tall^{*}

Division of Molecular Medicine,^{*} Department of Medicine, Columbia University, New York, NY; University Hospital Hamburg Eppendorf,[†] Hamburg, Germany

Abstract This study compares the roles of ABCG1 and scavenger receptor class B type I (SR-BI) singly or together in promoting net cellular cholesterol efflux to plasma HDL containing active LCAT. In transfected cells, SR-BI promoted free cholesterol efflux to HDL, but this was offset by an increased uptake of HDL cholesteryl ester (CE) into cells, resulting in no net efflux. Coexpression of SR-BI with ABCG1 inhibited the ABCG1-mediated net cholesterol efflux to HDL, apparently by promoting the reuptake of CE from medium. However, ABCG1-mediated cholesterol efflux was not altered in cholesterol-loaded, SR-BI-deficient (SR-BI^{-/-}) macrophages. Briefly cultured macrophages collected from SR-BI^{-/-} mice loaded with acetylated LDL in the peritoneal cavity did exhibit reduced efflux to HDL. However, this was attributable to reduced expression of ABCG1 and ABCA1, likely reflecting increased macrophage cholesterol efflux to apolipoprotein E-enriched HDL during loading in SR-BI^{-/-} mice. **In conclusion, cellular SR-BI does not promote net cholesterol efflux from cells to plasma HDL containing active LCAT as a result of the reuptake of HDL-CE into cells. Previous findings of increased atherosclerosis in mice transplanted with SR-BI^{-/-} bone marrow probably cannot be explained by a defect in macrophage cholesterol efflux.**—Yvan-Charvet, L., T. A. Pagler, N. Wang, T. Senokuchi, M. Brundert, H. Li, F. Rinninger, and A. R. Tall. **SR-BI inhibits ABCG1-stimulated net cholesterol efflux from cells to plasma HDL.** *J. Lipid Res.* 2008. 49: 107–114.

Supplementary key words high density lipoprotein • ATP binding cassette transporter G1 • scavenger receptor class B type I • lecithin:cholesterol acyltransferase • liver X receptor • macrophage

HDL cholesterol levels are inversely correlated with the incidence of coronary artery disease (1). In part, the atheroprotective properties of HDL are thought to reflect the removal of excess cholesterol from macrophage foam cells by HDL or its apolipoproteins. ABCA1, the defective molecule in Tangier disease, promotes the efflux of cellular cholesterol to lipid-poor apolipoproteins and

initiates HDL formation by lipidating apolipoprotein A-I (apoA-I) in the liver and intestine (2, 3). However, ABCA1 interacts poorly with HDL₂ and HDL₃ particles, which constitute the bulk of the plasma HDL (4).

Two cell surface transporters have been described that can promote cholesterol efflux from cells to HDL. Scavenger receptor class B type I (SR-BI) facilitates the bidirectional flux of cholesterol between cells and HDL and can promote net cholesterol efflux to recombinant cholesterol-poor HDL particles (5, 6). More recently, ABCG1 was shown to mediate the net efflux of cellular free cholesterol (FC) to HDL (7, 8). In the presence of spherical plasma HDL particles, ABCG1 promoted net cholesterol efflux from macrophages and increased the accumulation of cholesteryl ester (CE) in medium as a result of LCAT-mediated cholesterol esterification (9). SR-BI binds HDL at the cell surface, and binding appears to have an essential role in the promotion of cholesterol exchange with HDL (10). In contrast, ABCG1 does not bind HDL directly but appears to act at the cell surface to increase the availability of cholesterol to a variety of different acceptors, including HDL and cyclodextrin (7).

Transplantation of bone marrow from SR-BI deficient (SR-BI^{-/-}) mice into LDL receptor^{-/-} or apoE^{-/-} mice has shown an increase in atherosclerosis, suggesting a protective function of macrophage SR-BI (11–13). Although an initial study reported no major effect of SR-BI deficiency on macrophage cholesterol efflux to HDL (11), subsequent work has shown a defect in cholesterol efflux to HDL after in vivo [³H]cholesterol loading of macrophages (13), suggesting that the increase in atherosclerosis could be related to defective macrophage cholesterol efflux. The present study was designed to compare the roles of SR-BI and ABCG1 in promoting mass cholesterol efflux from macrophages to HDL. In addition, we determined whether there might be a cooperative interaction between SR-BI and ABCG1 in promoting cholesterol efflux.

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¹To whom correspondence should be addressed.
e-mail: ly2159@columbia.edu

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METHODS

Animals

SR-BI^{-/-} mice were described previously (14). Animals, fed a regular chow diet, were housed at Columbia University Medical Center according to animal welfare guidelines.

Plasma HDL preparation

HDL (density = 1.063–1.21g/ml) was isolated by preparative ultracentrifugation from normolipidemic human plasma (HDL) or from wild-type (WT) and SR-BI^{-/-} murine plasma (WT HDL and SR-BI^{-/-} HDL, respectively) and stored in PBS containing 1 mM EDTA (15). SR-BI^{-/-} HDL particles exhibited an increased size with higher amounts of cholesterol compared with WT HDL (14, 15).

Cell transfection and culture

HEK293 cells at a density of 1×10^6 cells/well were transiently transfected with similar amounts of control empty vector (pcDNA 3.1⁺), murine SR-BI cDNA alone combined with empty vector (1:1) (5), murine ABCG1 cDNA alone combined with empty vector (1:1) (7), or both SR-BI and ABCG1 cDNA (1:1) using LipofectAMINE 2000 according to the manufacturer's instructions (Invitrogen). Then, cells were incubated for different times in DMEM containing 0.2% BSA with 100 μ g HDL protein/ml. Because cells do not directly promote the efflux of CE to HDL, we next confirmed that the increased CE accumulated in medium was dependent on the LCAT reaction. HDLs were pretreated with 2 mM LCAT inhibitor [diethyl *p*-nitrophenyl phosphate (E600); Sigma-Aldrich] for 0.5 h at room temperature, then HDL was added directly to the efflux medium as shown in Fig. 2C below (final concentration of E600 in the medium was 0.2 mM).

Mouse peritoneal macrophages

Peritoneal macrophages from SR-BI^{-/-} and WT mice were collected at 3 days after an intraperitoneal injection of thioglycollate and seeded on 24-well plates in DMEM supplemented with 10% FBS for 1 h before treatment. RNA interference to suppress ABCG1 expression in mouse macrophages was performed as described previously (16). Briefly, mouse peritoneal macrophages from WT and SR-BI^{-/-} mice were transfected with small interfering RNA (siRNA) and LipofectAMINE 2000 (Invitrogen) for 6 h, then medium was replaced overnight with DMEM containing 10% FBS. The next day, the cells were treated in DMEM plus 0.2% BSA with or without 50 μ g/ml acetylated low density lipoprotein (AcLDL) and 2 μ M of a liver X receptor (LXR) agonist (TO901317) for 16 h before cholesterol efflux studies to induce the expression of ABCG1 (17). In addition, peritoneal macrophages were loaded *in vivo* to study cholesterol efflux with minimal culture times, as described previously (13). Briefly, WT and SR-BI^{-/-} mice were injected intraperitoneally with AcLDL (10 mg/kg body weight; two times at an interval of 12 h) at 24 h before macrophages were harvested. After isolation, cells were seeded for 1 h in DMEM plus 10% FBS, nonadherent cells were washed off, and cholesterol efflux was then determined by the addition of 100 μ g protein/ml HDL in DMEM plus 0.2% BSA.

Cholesterol mass analysis

Cholesterol efflux was performed for 6 h in DMEM containing 0.2% BSA in the presence or absence of human HDL or murine (WT and SR-BI^{-/-}) HDL (100 μ g protein/ml). After incubation with HDL, the lipid fractions were extracted from the collected medium with hexane or from cells using hexane-isopropanol

(3:2, v/v) in the presence of stigmaterol (5 μ g/sample) added as the internal standard. After drying under N₂ gas, the mass of FC dissolved in hexane was subjected to gas-liquid chromatography. Total cholesterol (TC) was determined after saponification, and CE was calculated as the difference between TC and FC. The HDL-mediated net cholesterol efflux was calculated by subtraction of the cholesterol mass of the medium cultured with or without cells. The reduction in cholesterol mass in cells was determined by subtracting the cholesterol mass for cells incubated in medium with or without HDL.

RNA analysis

Total RNA was extracted from macrophages as described previously (18), and cDNA was synthesized from 1 μ g of total RNA with SuperScript reverse transcriptase (Life Technologies, Inc.). Real-time PCR was performed as described previously (19). β -Actin RNA expression was used to account for variability in the initial quantities of mRNA.

Western blot analysis

Macrophages were scraped in 10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 0.1% SDS, and 1% Triton X-100 in the presence of protease inhibitors (Roche Molecular Biochemicals). The protein content was determined by the Bio-Rad protein assay. Aliquots of 20 μ g of protein preparations were resolved by 10% SDS-PAGE and transferred onto nitrocellulose membranes. Primary antibodies for ABCG1 and SR-BI were purchased from Santa Cruz Biotechnology (Santa Cruz, CA) and Novus Biologicals (Littleton, CO). Specific protein signals were revealed using the ECL detection system (Amersham Biosciences).

RESULTS

ABCG1 but not SR-BI mediates net cholesterol efflux from HEK293 cells to plasma HDL

To compare the ability of SR-BI and ABCG1 to promote net cholesterol efflux to HDL, we transiently transfected HEK293 cells with the murine SR-BI and ABCG1 cDNAs. Overexpression of SR-BI resulted in up to a 4-fold increase of FC concentration in medium compared with empty vector-transfected cells after 6 h of incubation with HDL (Fig. 1A). However, there was also a decrease in the amount of HDL CE accumulating in medium after 6 h and thus no significant change in TC (Fig. 1B, C). Similar results were obtained when twice as much SR-BI cDNA was transfected into cells, indicating that the results were not likely a reflection of low levels of SR-BI expression (data not shown). Experiments in which either cells were labeled with [³H]cholesterol or HDL containing [³H]cholesterol was added in medium confirmed that SR-BI promoted a bidirectional flux of cholesterol under the conditions of our experiment (data not shown). In contrast to results with SR-BI, overexpression of ABCG1 led to a 2-fold increase in TC in medium, in large part attributable to a 2-fold increase in CE content (Fig. 1B, C). This was similar to previous findings, in which it was shown that the increase in HDL-CE induced by ABCG1 was dependent on LCAT activity (9). Thus, ABCG1 but not SR-BI mediated net cholesterol efflux to plasma HDL. One possible explanation for this difference might be that while

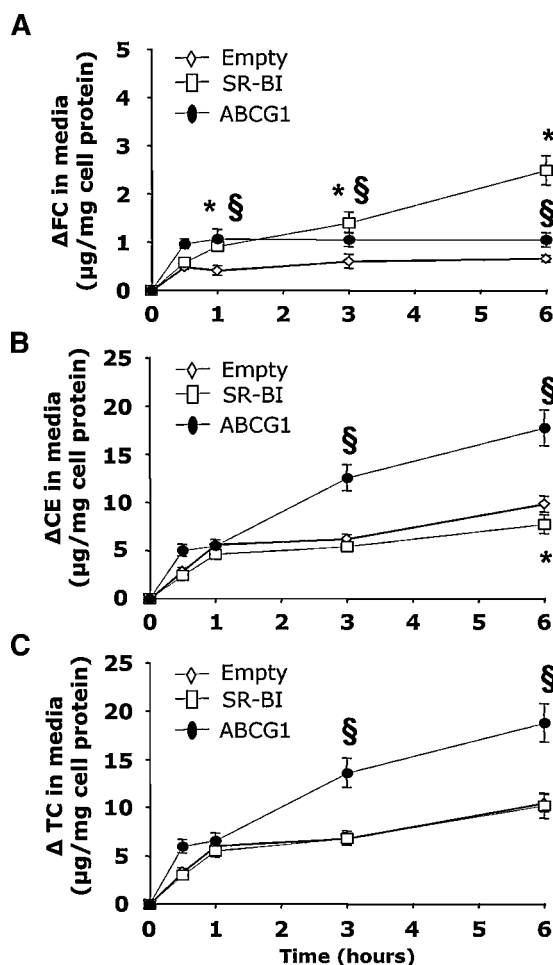


Fig. 1. Overexpression of ABCG1-mediated but not scavenger receptor class B type I (SR-BI)-mediated net cholesterol efflux to HDL. HEK293 cells were transiently transfected with plasmid constructs expressing ABCG1, SR-BI, or empty vector, and cholesterol efflux was initiated by the addition of HDL to medium [100 $\mu\text{g}/\text{ml}$ HDL protein containing $0.75 \pm 0.08 \mu\text{g}$ free cholesterol (FC)/ml medium and $6.04 \pm 0.65 \mu\text{g}$ cholesteryl ester (CE)/ml medium]. The increased FC (A), CE (B), and total cholesterol (TC; C) in medium were determined for the indicated periods of time. Values are means \pm SEM of two independent experiments. * $P < 0.05$, SR-BI overexpression versus empty vector; § $P < 0.05$, ABCG1 overexpression versus empty vector.

promoting FC efflux, SR-BI also enhanced the selective uptake of HDL-CE into cells.

SR-BI inhibits ABCG1-stimulated CE accumulation in medium in an LCAT-dependent manner

We next determined whether SR-BI might facilitate ABCG1-mediated net cholesterol efflux by tethering HDL at the cell surface. To evaluate this possibility, we coexpressed SR-BI and ABCG1 in HEK293 cells and determined net cholesterol efflux to HDL. Paradoxically, SR-BI coexpression with ABCG1 completely reversed the ABCG1-mediated increased CE accumulation in medium to the level of the vector control (Fig. 2B). This could not be attributed to a difference in ABCG1 expression levels, because it was similar in cells transfected with ABCG1

alone or ABCG1 plus SR-BI (Fig. 2A). Preincubation of HDL with an LCAT inhibitor reduced TC efflux only in empty vector and SR-BI-transfected cells (Fig. 2C). The reduction of HDL-CE accumulation in medium was incomplete ($\sim 50\%$), likely because of a partial reversal of LCAT inhibition when the inhibitor was diluted into medium for the efflux experiments, as described previously (9). By contrast, TC efflux was not significantly inhibited in ABCG1-transfected cells when LCAT activity was inhibited (impaired CE formation was compensated by an increase in FC accumulation), and inhibition of LCAT activity substantially reduced the ability of SR-BI to reverse the ABCG1-dependent increase in medium CE accumulation (Fig. 2C). The different effects of LCAT inhibition between SR-BI and ABCG1 are not totally understood. One interpretation of these results is that SR-BI reverses the LCAT-dependent accumulation of CE in medium in empty cells and in cells expressing ABCG1 by promoting the selective uptake of HDL-CE into cells. Thus, ABCG1 promotes cholesterol efflux and the subsequent esterification by LCAT in HDL, but in cells overexpressing both ABCG1 and SR-BI, there appears to be a futile cycle involving the reuptake of HDL-CE into cells by SR-BI.

Selective disruption of ABCG1 but not SR-BI in peritoneal macrophages causes decreased cholesterol efflux

We next determined the role of endogenous SR-BI in macrophages in promoting cholesterol efflux and possibly giving rise to a futile cycle of HDL-CE reuptake when endogenous ABCG1 is induced by LXR activation. Under basal conditions, there was no significant effect of SR-BI deficiency on cholesterol efflux to HDL in medium (Fig. 3A). WT macrophages loaded with AcLDL plus TO901317 to induce ABCG1 exhibited a large increase in FC (8-fold) and CE (2-fold) accumulation in medium after 6 h of incubation with HDL (Fig. 3A). Again, HDL-induced cholesterol efflux was not significantly affected by the deletion of SR-BI. In cholesterol-loaded, LXR-activated cells, partial knockdown of ABCG1 by siRNA ($\sim 70\%$) (16) resulted in a 30% reduction in TC efflux to HDL in medium (Fig. 3A), along with an equivalent reduction in the change of cellular cholesterol mass (data not shown), and this was not affected by SR-BI deficiency (Fig. 3A). These experiments indicate that endogenous SR-BI does not influence either basal or LXR-induced ABCG1-dependent cholesterol efflux to HDL in macrophages. The lack of evidence for an SR-BI-dependent futile cycle of HDL-CE reuptake in cells with induced expression of ABCG1 did not parallel that seen in transfected HEK293 cells, most likely because of much lower expression levels of SR-BI in cholesterol-loaded macrophages compared with transfected HEK293 cells (Fig. 4C).

Decreased cholesterol efflux from SR-BI^{-/-} macrophages after in vivo cholesterol loading

SR-BI^{-/-} macrophages showed decreased cholesterol efflux to HDL after in vivo cholesterol loading of macrophages by [³H]cholesterol, suggesting that SR-BI ex-

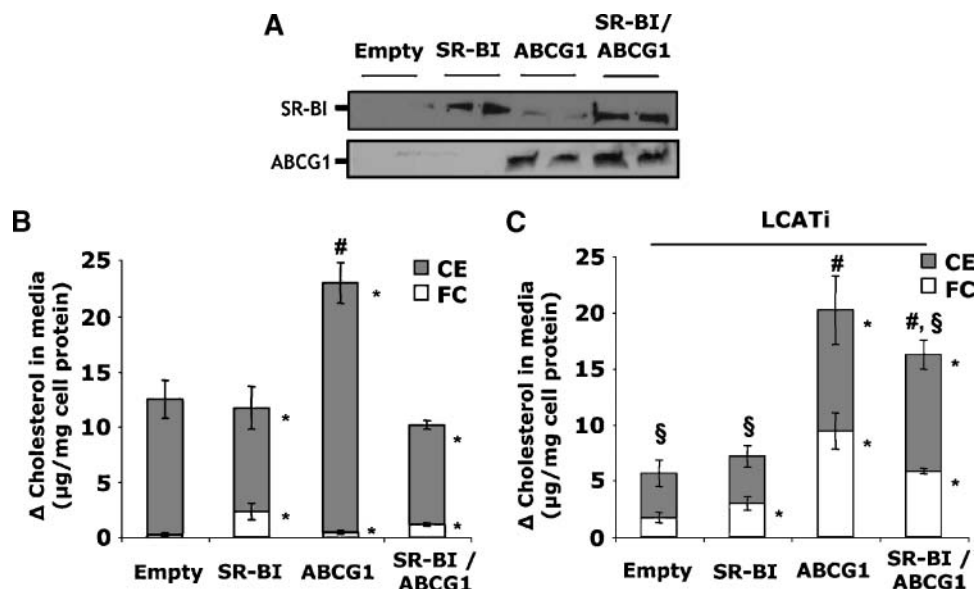


Fig. 2. Cholesterol efflux from HEK293 cells cotransfected with ABCG1 and SR-BI cDNAs. Cells were transiently transfected with plasmid constructs expressing SR-BI, ABCG1, or empty vector, and cholesterol efflux was initiated by the addition of HDL to medium (100 µg/ml HDL protein) for 6 h. **A:** Immunoblot of SR-BI and ABCG1 in HEK293 transfected cells. **B:** Increased cholesterol mass in medium. **C:** Effect of an LCAT inhibitor (E600; 2 mM) on net cholesterol efflux to HDL. The data represent means \pm SEM of two independent experiments performed in triplicate. * $P < 0.05$, significant difference in FC and CE versus empty vector; # $P < 0.05$, significant difference in TC versus empty vector; § $P < 0.05$, significant difference in TC versus LCAT-untreated cells.

pression might be reduced during prolonged ex vivo cell culture in control macrophages (13). Therefore, we wished to exclude the possibility that the lack of effect of SR-BI deficiency on cholesterol efflux might be the result of the reduced expression of this transporter during cell culture. Macrophages were loaded by intraperitoneal injection of AcLDL, and net cholesterol efflux to HDL was measured after cultures of WT or SR-BI^{-/-} macrophages for 1 h. In contrast to the experiment shown in Fig. 3A, macrophage SR-BI deficiency resulted in an ~40% reduction of TC efflux to HDL under basal or AcLDL-loaded conditions (Fig. 3B). Equivalent results were obtained when cellular cholesterol content was analyzed (Fig. 3C). One interpretation of these findings might be that SR-BI expression levels are decreased during ex vivo macrophage culture (13). However, we did not find any change in SR-BI mRNA and protein expression between freshly isolated or cultured macrophages (Fig. 4A, B). Thus, we next considered the possibility that the deficiency of SR-BI might lead to the reduced expression of other genes involved in cholesterol efflux. Indeed, we found reduced expression of ABCG1 mRNA and protein and reduced expression of ABCA1 and sterol-regulatory element binding protein 1c mRNA in SR-BI^{-/-} cells (Fig. 5A, B). These findings indicated that the reduced cholesterol efflux to HDL after in vivo AcLDL loading of SR-BI^{-/-} cells was likely the result of the reduced expression of ABCG1 and was not a direct result of SR-BI deficiency. Moreover, there appeared to be reduced expression of several LXR target genes in macrophages isolated from SR-BI^{-/-} mice.

HDL particles from SR-BI^{-/-} mice promote cholesterol efflux and reduce LXR-mediated gene expression in macrophages

We recently found that large, apoE-rich HDL particles accumulating in subjects with homozygous genetic deficiency of cholesteryl ester transfer protein or in subjects treated with the cholesteryl ester transfer protein inhibitor torcetrapib have a increased ability to promote cholesterol efflux from macrophages in an ABCG1-dependent manner (9, 20). Similarly, HDL from SR-BI^{-/-} mice is known to be a large apoE-rich particle enriched in cholesterol (14) and was recently reported to have greater ability to promote cholesterol efflux from macrophages than control HDL (15). This suggested the hypothesis that enhanced cholesterol efflux from macrophages as a result of increased levels of large, apoE-rich HDL might promote cholesterol efflux during in vivo AcLDL loading in the peritoneal cavity of SR-BI^{-/-} mice, leading to reduced expression of ABC transporters. To test this hypothesis, we first confirmed the enhanced cholesterol efflux capacity of HDL from SR-BI^{-/-} mice (used at the same protein concentration, 50 µg/ml) in both WT and SR-BI^{-/-} cells (Fig. 6A). Large apoE-rich HDL particles from SR-BI^{-/-} mice exhibited a significant 1.4-fold increase in FC and an ~1.3-fold increase in CE accumulation in medium compared with WT HDL. Similar results were obtained in WT and SR-BI^{-/-} macrophages, ruling out a role of macrophage SR-BI in these findings (Fig. 6A). Furthermore, compared with WT HDL, incubation with SR-BI^{-/-} HDL caused a more pronounced decrease in macrophage ABCA1 and ABCG1 mRNA expression (Fig. 6B, C). These

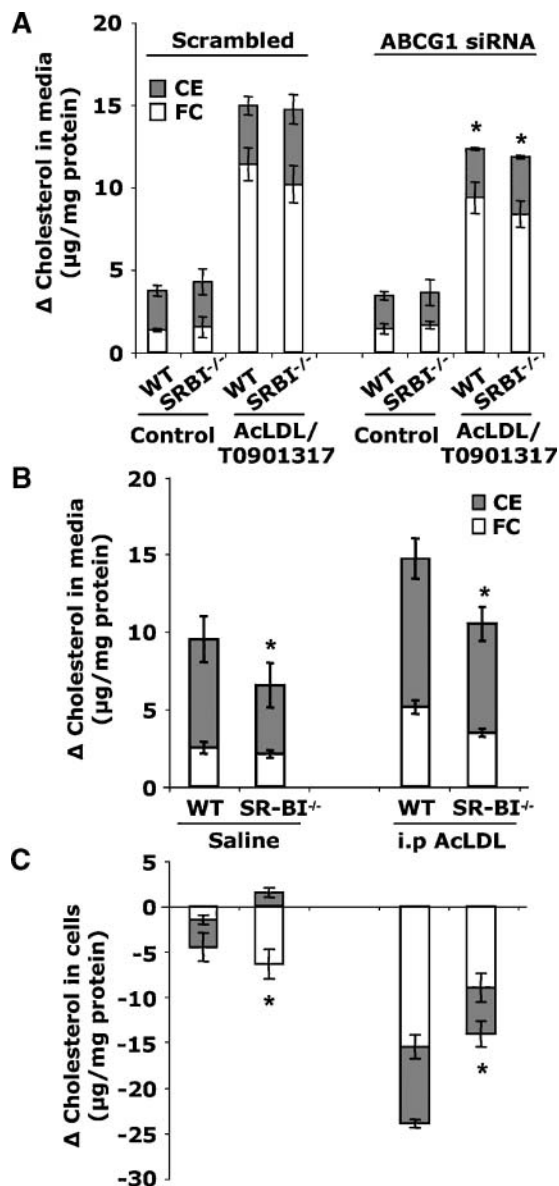


Fig. 3. Effect of induction or suppression of ABCG1 expression on HDL-mediated cholesterol efflux from SR-BI deficient (SR-BI^{-/-}) macrophages. **A:** Mouse peritoneal macrophages were transfected with small interfering RNA (siRNA) against ABCG1 or scrambled control siRNA before in vitro loading with acetylated low density lipoprotein (AcLDL; 50 µg/ml) plus TO901317 (2 µM) for 16 h in 0.2% BSA. Net cholesterol mass in medium was determined at 6 h after the addition of HDL (100 µg/ml HDL protein containing 0.75 ± 0.08 µg FC/ml medium and 6.04 ± 0.65 µg CE/ml medium). **B, C:** Mouse peritoneal macrophages were loaded in vivo by injection of AcLDL in the peritoneal cavity of wild-type (WT) and SR-BI^{-/-} mice. Then, macrophages were harvested and cholesterol mass determination was performed with minimal culture times (1 h). **B:** Net cholesterol mass in medium was determined at 6 h after the addition of HDL (100 µg/ml HDL protein). **C:** Cholesterol removal from cells by HDL was estimated by changed intracellular cholesterol mass in the cells. Values are means ± SEM of two independent experiments. * *P* < 0.05, significant difference versus cells transfected with scrambled control siRNA or versus WT macrophages.

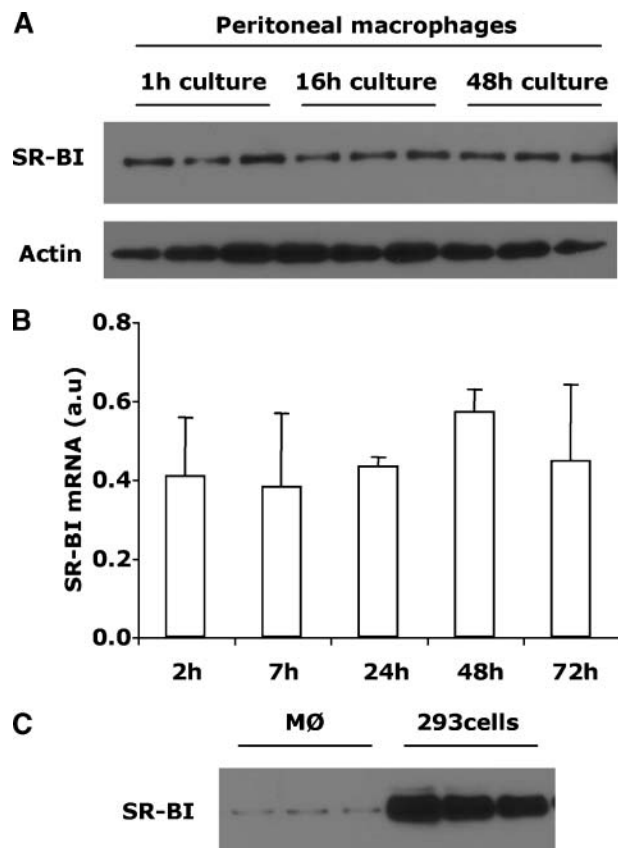


Fig. 4. Macrophage SR-BI mRNA and protein levels during cell culture and compared with HEK293 cells overexpressing SR-BI. **A, B:** Mouse peritoneal macrophages were cultured in DMEM plus 10% FBS, and cells were harvested at different times for mRNA and protein analysis. **A:** Representative immunoblot of SR-BI. **B:** SR-BI transcript levels were quantified and normalized to β-actin RNA amount. Values are means ± SEM of one experiment performed in triplicate. a.u., arbitrary units. **C:** Immunoblot of SR-BI in WT macrophages and in HEK293 cells overexpressing SR-BI.

experiments raise the possibility that the lower levels of expression of ABCA1 and ABCG1 (Fig. 5) were likely the result of the enhanced promotion of cholesterol efflux from macrophages by SR-BI^{-/-} HDL and were not a consequence of macrophage SR-BI deficiency.

DISCUSSION

Most contemporary reviews describe both ABCG1 and SR-BI as promoting net cholesterol efflux from foam cells to plasma HDL (21–24). Our study confirms and extends earlier findings suggesting that macrophage SR-BI does not play a significant role in promoting net cholesterol efflux to plasma HDL (11, 15). In contrast, ABCG1 promoted net cholesterol efflux from transfected cells and cholesterol-loaded macrophages to plasma HDL. A recent study of in vivo reverse cholesterol transport from macrophages into the plasma and stool has shown individual and additive roles of ABCG1 and ABCA1 in this process, but no role of SR-BI (25). A previous report of reduced

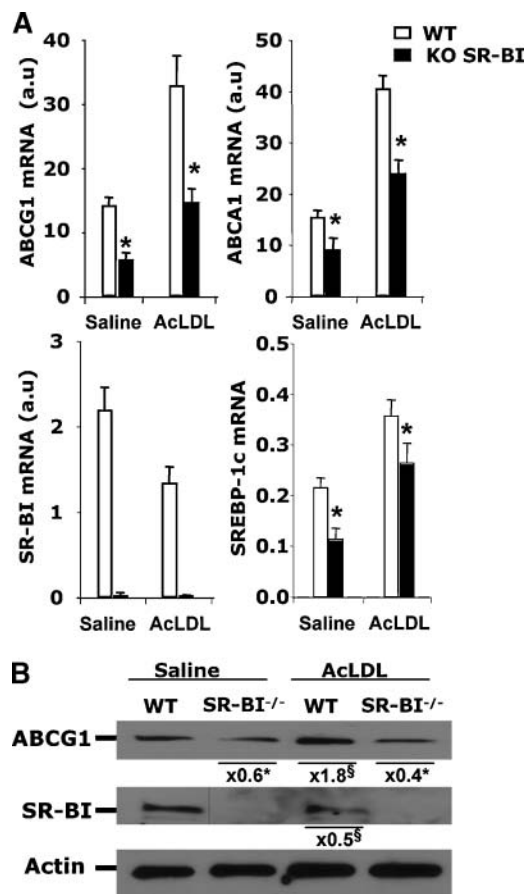


Fig. 5. Effect of in vivo cholesterol loading on liver X receptor (LXR) target gene expression in SR-BI^{-/-} macrophages. Mouse peritoneal macrophages were loaded in vivo by injection of AcLDL in the peritoneal cavity of WT and SR-BI^{-/-} mice. Then, macrophages were harvested, seeded for 1 h, and used for mRNA and protein determinations. **A:** ABCG1, ABCA1, SR-BI, and sterol-regulatory element binding protein (SREBP)-1c transcripts levels were quantified and normalized to β -actin RNA amount. Values are means \pm SEM of two independent experiments. a.u., arbitrary units. * $P < 0.05$, significant difference versus WT macrophages. **B:** Immunoblot of SR-BI and ABCG1 in WT and SR-BI^{-/-} macrophages under basal and stimulated conditions. The fold change values indicate change in knockout cells versus WT cells (* $P < 0.05$) or in loaded cells versus unloaded cells ([§] $P < 0.05$) from two independent experiments.

cholesterol efflux to HDL after in vivo loading of SR-BI^{-/-} macrophages was confirmed (13) but unexpectedly was shown to reflect the reduced expression of ABCG1 and ABCA1 in these macrophages, most likely as a result of the downregulation of LXR target genes secondary to enhanced ABCG1-dependent efflux of cholesterol by the large, apoE-rich HDL accumulating in SR-BI^{-/-} mice (14, 15).

The present study proposes some mechanistic insight into why SR-BI does not promote net cholesterol efflux to plasma HDL. Whereas a number of previous studies have shown that SR-BI promotes isotopic cholesterol efflux from cells to HDL or serum (5, 6, 10), the present study shows that the net mass cholesterol efflux to plasma HDL is not increased, most likely because although SR-BI

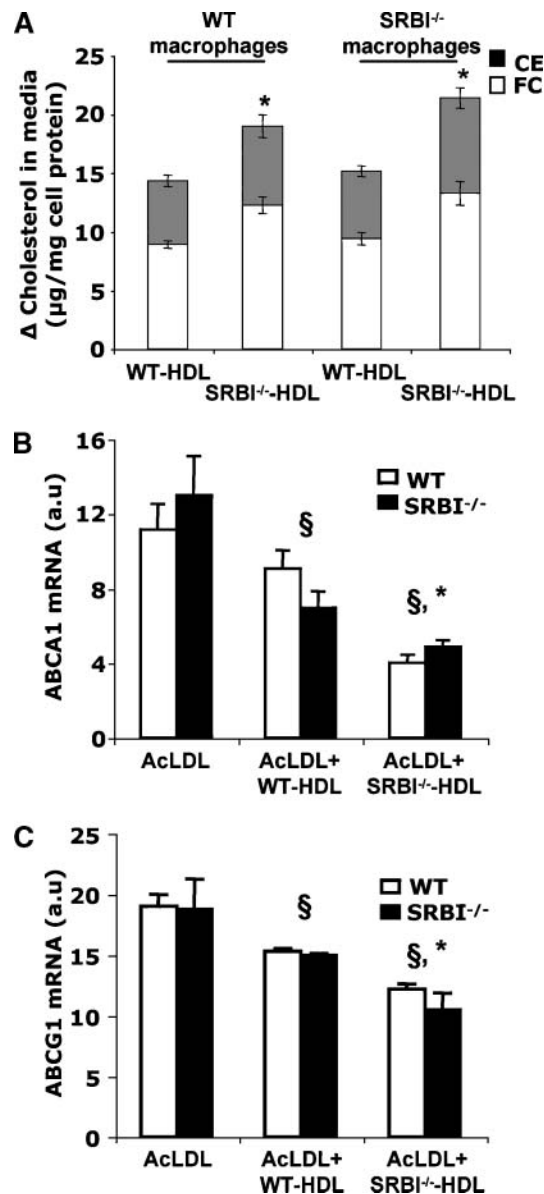


Fig. 6. Effect of SR-BI^{-/-} HDL on cholesterol efflux and LXR target gene expression. WT and SR-BI^{-/-} peritoneal macrophages were loaded with AcLDL (50 μ g/ml) for 16 h. **A:** Net cholesterol mass in medium was determined at 8 h after the addition of WT and SR-BI^{-/-} HDL (at the same protein concentration, 50 μ g/ml). **B, C:** ABCA1 and ABCG1 transcript levels were quantified and normalized to β -actin RNA amount. Values are means \pm SEM of one experiment performed in triplicate. a.u., arbitrary units. * $P < 0.05$, significant difference versus WT HDL; [§] $P < 0.05$, significant difference versus AcLDL condition.

promotes FC efflux, it also enhances the uptake of HDL-CE, so that there is no net increase in total HDL cholesterol in medium. Thus, even though the ongoing LCAT reaction in HDL would provide a gradient for SR-BI-facilitated FC efflux from cells, this would likely be counterbalanced by the uptake of HDL-CE by SR-BI-mediated selective uptake. Although not promoting net cholesterol efflux, these changes could perhaps lead to a change in cellular cholesterol homeostasis, because HDL-CE entering the cell would not initially play a regulatory role

corresponding to that resulting from the efflux of FC. In contrast to these observations in macrophages, it is likely that the ability of SR-BI to mediate FC exchange with cells (5) is important in the rapid clearance of macrophage-derived cholesterol in the liver and its excretion into bile (26).

We also considered the possibility that SR-BI might facilitate ABCG1-mediated cholesterol efflux by tethering HDL near the cell surface. This possibility was refuted in cells overexpressing both transporters; rather, a futile cycle appeared to operate whereby increased HDL-CE accumulating in medium as a result of the combined activities of ABCG1 and LCAT was returned to the cell by SR-BI-mediated selective uptake. A similar competing role has been observed between SR-BI and ABCA1 in mediating cholesterol efflux to HDL (27). Such futile cycles could have physiological relevance in cells or tissues expressing SR-BI and ABCG1 or ABCA1, such as macrophages and liver (4, 28, 29). However, we found that SR-BI had no effect on basal or LXR-induced ABCG1-dependent cholesterol efflux to HDL in macrophages, probably because macrophages express high levels of ABCG1 but low levels of SR-BI. Whereas cholesterol loading of macrophages induces ABCG1 as a result of LXR activation (17), SR-BI is not an LXR target and is repressed in cholesterol-loaded cells (30). This may prevent a futile competition between SR-BI and ABCG1. Moreover, preliminary results in liver homogenates suggest that ABCG1 is not expressed with SR-BI on the cell surface (data not shown), suggesting that they would not be in competition in the liver. Although SR-BI and ABCG1 are both highly expressed in the adrenal, SR-BI is localized to specialized microvillar channels, where it acts to promote the selective uptake of HDL lipids (31). Thus, it is possible that the futile cycle created by the joint overexpression of SR-BI and ABCG1 in HEK293 cells is avoided under physiological conditions.

Several different laboratories have reported increased atherosclerosis in atherosclerosis-prone mice transplanted with SR-BI^{-/-} bone marrow (11–13). The mechanisms responsible for this effect have not been completely clarified, but it has been presumed that this might be related to an in vivo defect in cholesterol efflux to HDL (13). Indeed, such a defect was shown in an ex vivo cholesterol efflux experiment using SR-BI^{-/-} macrophages that had been loaded with [³H]cholesterol in the peritoneal cavity. Our results confirm similar findings using in vivo loading with AcLDL but suggest that they were caused by reduced expression of the LXR target genes ABCA1 and ABCG1 in SR-BI^{-/-} macrophages, secondary to higher levels of efflux of cholesterol or related LXR-activating sterols induced in vivo by the large apoE-rich HDL of SR-BI deficiency. Because the HDL was similar in mice transplanted with WT or SR-BI^{-/-} bone marrow (11–13), we believe that reduced macrophage cholesterol efflux cannot account for the increase in atherosclerosis in the latter group. This suggests a novel antiatherogenic function of SR-BI in bone marrow-derived cells. For example, SR-BI on Sertoli cells has been shown to enhance the clearance of apoptotic spermatocytes by

recognition of phosphatidylserine (32), and if macrophage SR-BI had a similar function in clearing apoptotic cells, it could be antiatherogenic during early lesion development (13). ■

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