Selective uptake of HDL cholesteryl esters and cholesterol efflux from mouse peritoneal macrophages independent of SR-BI

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Abstract **Scavenger receptor class B type I (SR-BI) mediates the selective uptake of HDL cholesteryl esters (CEs) and facilitates the efflux of unesterified cholesterol. SR-BI expression in macrophages presumably plays a role in atherosclerosis. The role of SR-BI for selective CE uptake and cholesterol efflux in macrophages was explored. Macrophages and HDL originated from wild-type (WT) or SR-BI knockout (KO; homozygous) mice. For uptake, macrophages were incubated in medium containing ^{125}I-/^{3}H-labeled HDL. For lipid removal, [^{3}H]cholesterol efflux was analyzed using HDL as acceptor. Selective uptake of HDL CE ([^{3}H]cholesteryl oleyl ether − ^{125}I-tyramine cellobiose) was similar in WT and SR-BI KO macrophages. Radiolabeled SR-BI KO-HDL yielded a lower rate of selective uptake compared with WT-HDL in WT and SR-BI KO macrophages. Cholesterol efflux was similar in WT and SR-BI KO cells using HDL as acceptor. SR-BI KO-HDL more efficiently promoted cholesterol removal compared with WT-HDL from both types of macrophages. Macrophages selectively take up HDL CE independently of SR-BI. Additionally, in macrophages, there is substantial cholesterol efflux that is not mediated by SR-BI. Therefore, SR-BI-independent mechanisms mediate selective CE uptake and cholesterol removal. SR-BI KO-HDL is an inferior donor for selective CE uptake compared with WT-HDL, whereas SR-BI KO-HDL more efficiently promotes cholesterol efflux.**—Brundert, M., J. Heeren, M. Bahar-Bayansar, A. Ewert, K. J. Moore, and F. Rinninger. **Selective uptake of HDL cholesteryl esters and cholesterol efflux from mouse peritoneal macrophages independent of SR-BI.** ***J. Lipid Res.*** **2006.** 47: **2408–2421.**

Supplementary key words high density lipoprotein • scavenger receptor class B type I • reverse cholesterol transport

HDL levels in plasma are inversely related to the risk of atherosclerosis in humans (1). A physiological role of HDL in cholesterol homeostasis in vivo is established (2). HDL and its precursors presumably remove cholesterol from peripheral tissues. After esterification in plasma, HDL-associated cholesteryl esters (CEs) are delivered to other lipoprotein fractions (3) or tissues (4). One mechanism that mediates the direct delivery of HDL CE to organs is the selective lipid uptake pathway (4). In this process, HDL CEs are internalized by cells independently of the uptake of HDL holoparticles. Physiologically, this lipid uptake appears to be important for the delivery of cholesterol to steroidogenic tissues for hormone synthesis and to the liver for disposal via bile (4).

Scavenger receptor class B type I (SR-BI) is a cell surface receptor that mediates selective HDL CE uptake in cultured cells (5). In rodents, SR-BI is most abundantly expressed in liver and in steroidogenic organs (5), which are the tissues most actively engaged in selective HDL lipid uptake in vivo (4). The role of SR-BI in HDL metabolism was addressed in vivo (6–8). In mice with a targeted homozygous null mutation in the gene encoding SR-BI (SR-BI KO), plasma HDL cholesterol is increased and no selective HDL CE uptake by the liver is observed; in contrast, substantial hepatic selective CE uptake is detected in wild-type (WT) littermates (7, 8). Together, this evidence suggests that SR-BI is a receptor that mediates HDL selective CE uptake in vivo.

Distinct from lipid internalization, SR-BI has a function in cellular cholesterol removal as well. In Chinese hamster ovary cells, SR-BI may facilitate HDL-mediated cholesterol efflux (9). Cholesterol removal rates correlate closely with SR-BI expression levels in these cells. Therefore, a dual function of SR-BI in lipid homeostasis was suggested (i.e., it facilitates the efflux of unesterified cholesterol and mediates the uptake of HDL-associated CE) (2).

Macrophages are detected in atherosclerotic lesions of the vessel wall, and cholesterol accumulation leads to their conversion to foam cells (10). HDL selective CE uptake (11, 12) and more recently SR-BI expression (9, 13) by

Manuscript received 21 March 2006 and in revised form 2 August 2006.

Published, JLR Papers in Press, August 22, 2006.
DOI 10.1194/jlr.M600136-JLR200

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macrophages have been established. The dominant function of SR-BI in arterial wall macrophages (i.e., to mediate the uptake of HDL-associated CE or facilitate the efflux of unesterified cholesterol) is controversial at present. In bone marrow transplantation studies in mice, macrophage SR-BI expression protected the vessel wall from atherosclerosis (14–16). In contrast, an SR-BI deficiency was associated with an increased atherosclerotic lesion area in the murine aorta (17). These studies provide evidence for a role of macrophage SR-BI expression in the pathogenesis of atherosclerosis.

Besides the function of SR-BI in cellular lipid metabolism, the expression of this receptor has a substantial impact on the composition of HDL particles in plasma (6). HDL from SR-BI-deficient mice is enriched in unesterified cholesterol and contains more apolipoprotein E (apoE) compared with HDL from WT rodents (6, 7, 17–19). Additionally, SR-BI KO-HDL particles have on average a larger diameter compared with WT-HDL (6, 18).

In this study, the role of SR-BI in macrophage cholesterol homeostasis was explored. Macrophages and HDL originated from SR-BI KO (homozygous) and WT mice (6). Initially, the question was addressed whether cellular HDL selective CE uptake can be detected in the absence of SR-BI expression. Additionally, the contribution of SR-BI to cellular cholesterol efflux was investigated. These studies explore the issue of whether macrophages have a mechanism that mediates selective HDL CE uptake and efflux of cholesterol that is independent from SR-BI. Because the composition of WT-HDL and of SR-BI KO-HDL are substantially different, both HDL fractions were evaluated as donors for selective CE uptake and as acceptors for cellular cholesterol (6, 17). The results provide evidence for a mechanism distinct from SR-BI that mediates selective HDL lipid uptake by macrophages. Also, an SR-BI-independent mechanism promotes cholesterol efflux. A surprising result was that SR-BI KO-HDL yields substantial differences in CE uptake and cholesterol efflux in macrophages compared with WT-HDL.

MATERIALS AND METHODS

Mice

Mice with a targeted null mutation in the SR-BI gene (SR-BI KO; SR-BI$^{-/-}$) or the CD36 gene (CD36 KO; CD36$^{-/-}$) and their respective WT littermates were described previously (6, 7, 20). Only WT or homozygous mutant animals were used in this study. All protocols were approved by the local committee on laboratory animal care.

Lipoprotein preparation

HDL (d = 1.063–1.21 g/ml) was isolated from WT murine plasma (WT-HDL) and in parallel from SR-BI KO murine plasma (SR-BI KO-HDL) by sequential ultracentrifugation (7). HDL_3 (d = 1.125–1.21 g/ml), LDL (d = 1.019–1.05 g/ml), and acetylated LDL (acetyl-LDL) were prepared from human plasma (21, 22). HDL and HDL_3 were labeled with ^{125}I-tyramine cellobiose (^{125}I-TC) and [^{3}H]cholesteryl oleyl ether ([^{3}H]CEt; Amersham) (23).

Mouse peritoneal macrophages

Macrophages were collected from WT, SR-BI KO, or CD36 KO mice 3 days after an intraperitoneal injection of thioglycollate (3.85%, 1.0 ml) (24, 25). Cells were plated onto six-well dishes (Nunc) at 3×10^6 cells/well in DMEM (Life Technologies) containing FBS (10%; Life Technologies), penicillin (50 IU/ml), streptomycin (50 μg/ml), and glutamine (2 mM). Four hours after plating, nonadherent cells were removed by washing in PBS (1×). Macrophages were cultured (37°C, 24 h) in DMEM containing FBS, antibiotics, and glutamine (see above). Finally, this medium was aspirated, the cells were washed, and the medium was replaced by DMEM containing BSA (5 mg/ml), antibiotics, and glutamine (see above). Cells were cultured at 37°C in this medium for 24 h.

BHK cells

BHK cells were transfected with the plasmid pBK-CMV-hSR-BI containing the full-length human SR-BI cDNA or with the respective vector (pBK-CMV; Stratagene) (26).

Preincubation of the cells

Before initiating the assays, cells were preincubated (37°C, 30 min) in serum-free and lipoprotein-free medium (21). These preincubations were done to allow internalization or dissociation of membrane-associated serum or protein components originating from culture or from cell secretion. After aspiration of the medium, the cells were washed in PBS (2×). Thereafter, preincubation was performed (37°C, 30 min) in DMEM containing BSA (5 mg/ml) and antibiotics (see above).

Uptake of doubly radiolabeled HDL by macrophages and BHK cells

Cells were incubated at 37°C in DMEM containing BSA (5 mg/ml) and the respective ^{125}I-TC-/[^{3}H]CEt-labeled HDL; the lipoprotein concentrations and incubation times are indicated in the figure legends (21). After these incubations, the medium was aspirated and the cells were washed in PBS (4×). Then DMEM containing BSA (5 mg/ml) and unlabeled human HDL_3 (100 μg HDL_3/ml) was added for a "chase" incubation (37°C, 2.0 h) to remove reversibly cell-associated tracers (21). After this chase period, the medium was aspirated and the cells were washed again in PBS (1×). The cells were then released from the wells with trypsin/EDTA solution (0.5 g/l trypsin and 0.2 g/l EDTA; 1.0 ml/well). Trypsin activity was quenched by the addition of PBS supplemented with excess BSA (50 mg/ml, 4°C). The cell suspensions were transferred to tubes with a PBS (4°C) rinse of the wells. The cells then were pelleted by centrifugation (2,000 *g*, 4°C, 15 min) followed by aspiration of the supernatant. Thereafter, the pellet was resuspended in PBS (4°C, 5.0 ml) followed by centrifugation (2,000 *g*, 4°C, 15 min). The final cell pellet was dissolved in NaOH solution (0.1 N, 1.0 ml) and sonicated, and aliquots were used for protein determination (27), direct ^{125}I radioassay, and ^{3}H liquid scintillation counting after lipid extraction (21).

[^{3}H]cholesterol efflux from macrophages

Macrophages were incubated (37°C, 24 h) in DMEM supplemented with [^{3}H]cholesterol (0.5 μCi/ml; New England Nuclear), FBS, antibiotics, and glutamine (see above) (24). After labeling, the cells were equilibrated overnight in DMEM containing BSA (5 mg/ml) and antibiotics (see above). After labeling and equilibration, macrophages were washed with PBS (1×). This was followed by incubation (37°C) in DMEM containing BSA (5 mg/ml), murine HDL, human HDL_3, or purified delipidated human apoA-I (4). At the indicated time intervals, 55 μl of

medium was taken, and after precipitation at 6,000 *g* for 10 min to remove cell debris and cholesterol crystals, radioactivity in a 50 μl aliquot of supernatant was determined by scintillation counting. Finally, the cells were lysed in NaOH solution and the radioactivity of an aliquot was determined. Cholesterol efflux is expressed as percentage of the radioactivity released from cells into the medium relative to the total radioactivity in cells and medium (24).

^{125}I-TC-HDL binding to macrophages

Briefly, after chilling (4°C) and washing (PBS, 4°C), macrophages were incubated (4°C, 2.0 h) in DMEM (pH 7.4) containing BSA (5 mg/ml), HEPES (10 mM), and the indicated concentration of ^{125}I-TC-HDL (28). Thereafter, the cells were washed with PBS (4°C) and digested with NaOH solution (0.1 M). Aliquots of the suspension were assayed for ^{125}I radioactivity and for protein (27).

Immunoblot analysis

Postnuclear supernatants were prepared from cells (26). These samples or HDL were fractionated by SDS-PAGE (10%) under reducing conditions. Thereafter, the proteins were transferred to nitrocellulose membranes. Finally, the membranes were incubated in buffer containing an anti-mouse SR-BI antiserum (29), an anti-mouse CD36 antiserum (30), or specific antibodies directed against murine apoA-I (Biodesign), apoE (Acris), or β-actin (Biodesign). IgG binding was detected using a peroxidase-conjugated goat anti-rabbit IgG (Amersham). Antibody binding was visualized by ECL detection (Amersham) and autoradiography.

Miscellaneous

Briefly, in some cases, cholesterol, phospholipid, and triglyceride were measured with enzymatic assays (Roche) and protein was analyzed as described (27). In other cases, the cholesterol content of macrophage lipid extracts was determined using the Amplex Red Cholesterol Kit (Invitrogen) and protein was analyzed with the Micro BCA Kit (Pierce).

Total mRNA was extracted from cells using the NucleoSpin II Kit (Macherey-Nagel). To obtain cDNA, reverse transcription was performed using oligo(dT) primer, polymerase, and RNase inhibitor according to the manufacturer's recommendations. PCR was performed using the following primers (SR-BI forward, GGCGCATAAAGCCTCTGGCCAC; SR-BI reverse, CGGGTCTATGCGGACATTCTTGAGC; β2-microglobulin forward, GGCCTGTATGCTATCCAGAA; β2-microglobulin reverse, TGCAGGCGTATGTATCAGTC). Standard cycle conditions were

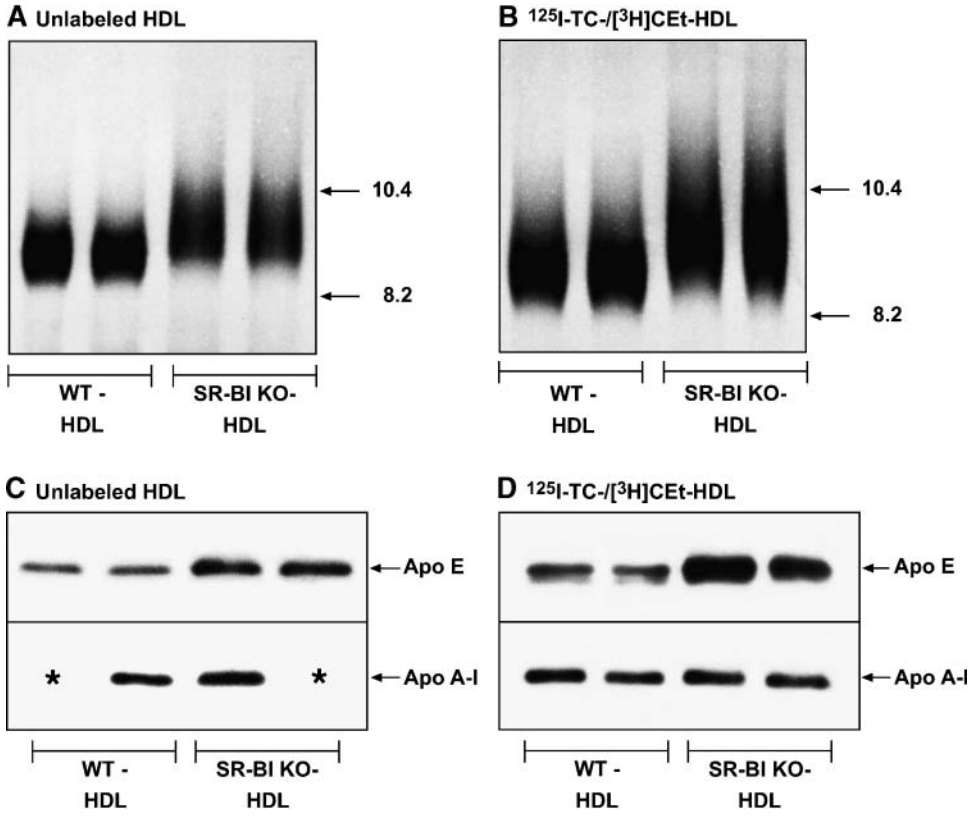

Fig. 1. HDL analysis by nondenaturing polyacrylamide gradient gel electrophoresis and immunoblots. A, B: Unlabeled (10 μg; A) and doubly radiolabeled (14 μg; B) wild-type (WT)-HDL and scavenger receptor class B type I (SR-BI) knockout (KO)-HDL were subjected to nondenaturing polyacrylamide gradient gel electrophoresis. The migration positions of standard proteins (nm) are shown (arrows). Two independent experiments yielded qualitatively identical results. C, D: Unlabeled (C) and doubly radiolabeled (B) WT-HDL and SR-BI KO-HDL were immunoblotted using apolipoprotein E (apoE)- and apoA-I-specific antibodies. In C, 5 μg of HDL protein (for apoE) and 0.2 μg HDL protein (for apoA-I) were loaded. In D, 1.7 μg of HDL protein (for apoE) and 0.2 μg of HDL protein (for apoA-I) were loaded. Three independent experiments yielded qualitatively identical results. Asterisks indicate that no sample was loaded. CEt, cholesteryl oleyl ether; TC, tyramine cellobiose.

95°C for 10 min, followed by 35 cycles of 95°C for 30 s, 66°C for 1 min (β2-microglobulin) or 70°C for 1 min (SR-BI), and 72°C for 1 min. PCR products (SR-BI, 315 bp; β2-microglobulin, 252 bp) were separated on agarose gels.

HDL was analyzed by nondenaturing polyacrylamide gradient gel (4–15%) electrophoresis, and HDL size was estimated by comparison with standard proteins (31).

Statistics and calculations

Values shown are means ± SEM. Student's *t*-test was used to calculate significance.

^{125}I-TC-/[^{3}H]CEt-labeled HDL uptake by cells is shown in terms of apparent HDL particle uptake, expressed as HDL protein (4, 21). This is done to compare the uptake of both tracers on a common basis. The uptake of HDL holoparticles is represented by equal uptake of both tracers. The difference in uptake between [^{3}H]CEt and ^{125}I-TC yields the apparent selective HDL CE uptake.

RESULTS

WT and SR-BI KO mice

Peritoneal macrophages and lipoproteins originated from SR-BI KO (homozygous) mice or from their WT littermates (6, 7). In WT mice, total plasma cholesterol was 98.5 ± 3.6 mg/dl, and in SR-BI KO animals, the respective value was 190.5 ± 7.7 mg/dl (n = 17 mice; $P < 0.0001$). This increase in total cholesterol in SR-BI KO mice is primarily attributable to an increase in HDL cholesterol (data not shown) (7).

Size and composition of unlabeled and doubly radiolabeled murine HDL

HDL was isolated in parallel from plasma from WT and SR-BI KO mice and thereafter radiolabeled in the protein moiety with ^{125}I-TC and in the lipid moiety with [^{3}H]CEt (23).

HDL particle size was estimated by nondenaturing polyacrylamide gel electrophoresis (**Fig. 1A, B**) (31). Migration of unlabeled and radiolabeled SR-BI KO-HDL was slower than that of WT-HDL, indicating a larger apparent particle size. This increase in the size of SR-BI KO-HDL is consistent with previous studies (6, 18).

The chemical analysis of WT-HDL and SR-BI KO-HDL revealed for unlabeled and radiolabeled SR-BI KO-HDL an enrichment in cholesterol compared with WT-HDL (**Table 1**) (7). This increase in total cholesterol is primarily attributable to an increment of unesterified cholesterol (7, 17–19). The protein composition of both HDL preparations was analyzed on immunoblots (Fig. 1C, D). SR-BI KO-HDL contained substantially more apoE compared with WT-HDL, and this observation is consistent with previous studies (6, 18). ApoA-I was detected in similar quantities in WT-HDL and SR-BI KO-HDL.

SR-BI mRNA and protein expression of macrophages

SR-BI mRNA levels and SR-BI protein expression of macrophages isolated from WT or SR-BI KO mice were examined (**Fig. 2**). SR-BI mRNA was detected in macrophages from WT mice using conventional RT-PCR, whereas no expression was measurable in cells from SR-BI KO animals (Fig. 2A). Similar expression levels were detected for the reference gene (i.e., β2-microglobulin) in both groups of cells. To explore SR-BI protein expression, postnuclear supernatants from WT and SR-BI KO macrophages were immunoblotted using an SR-BI-specific antiserum (Fig. 2B) (29). In WT cells, a signal corresponding to SR-BI was detected. In contrast, in proteins originating from mutant macrophages, the respective band was not visible. Transfected BHK cells with high SR-BI expression served as a positive control (Fig. 2B) (26). In summary, SR-BI is expressed in WT macrophages, based on mRNA and protein detection (32).

TABLE 1. Chemical composition of HDL preparations

HDL Preparations	WT-HDL	SR-BI KO-HDL
	% of total mass	
Unlabeled HDL		
Total cholesterol	11.7 ± 1.0[a]	17.9 ± 1.9[a]
Unesterified cholesterol	3.1 ± 0.2[b]	10.1 ± 1.1[b]
Esterified cholesterol	8.8 ± 0.8	7.9 ± 0.9
Phospholipid	20.4 ± 2.0	21.1 ± 2.4
Triglyceride	1.4 ± 0.4	1.1 ± 0.4
Protein	66.4 ± 2.8	59.9 ± 4.3
Unesterified-total cholesterol	0.26	0.56
^{125}I-TC-/[^{3}H]CEt-HDL		
Total cholesterol	13.4 ± 0.5[c]	18.3 ± 0.8[c]
Unesterified cholesterol	3.3 ± 0.1[d]	10.0 ± 0.6[d]
Esterified cholesterol	10.0 ± 0.5	8.3 ± 0.8
Phospholipid	24.4 ± 0.5	22.1 ± 1.0
Triglyceride	0.52 ± 0.07	0.41 ± 0.05
Protein	61.7 ± 1.0	59.2 ± 1.7
Unesterified-total cholesterol	0.24	0.54

Wild-type (WT)-HDL, scavenger receptor class B type I (SR-BI) knockout (KO)-HDL, ^{125}I-tyramine cellobiose (TC)-/[^{3}H]cholesteryl oleyl ether (CEt)-WT-HDL, and ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL were prepared as described in Materials and Methods. Thereafter, cholesterol, unesterified cholesterol, phospholipids, triglyceride, and protein were analyzed. Results are presented as fraction of total mass. Values are means ± SEM of four (unlabeled HDL) or nine (radiolabeled HDL) independent preparations; within each preparation, measurements were done in duplicate or triplicate.

[a] $P = 0.01$.
[b] $P = 0.0013$.
[c] $P = 0.0001$.
[d] $P < 0.0001$.

Uptake of murine HDL by macrophages

To investigate lipoprotein uptake, macrophages were incubated in medium containing ^{125}I-TC-/[^{3}H]CEt-labeled HDL (4, 21). Then, cellular internalization of HDL tracers was analyzed and expressed in terms of apparent HDL particle uptake. Selective CE uptake from HDL by cells is calculated as the difference in apparent HDL particle uptake between [^{3}H]CEt and ^{125}I-TC.

Dose-response curves for HDL uptake by macrophages are shown in **Fig. 3**. WT cells were incubated in medium containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL (Fig. 3A). Uptake of HDL-associated ^{125}I-TC and [^{3}H]CEt increased in a dose-dependent manner (data not shown). As expected, apparent HDL particle uptake attributable to [^{3}H]CEt was in excess of that attributable to ^{125}I-TC; the difference in

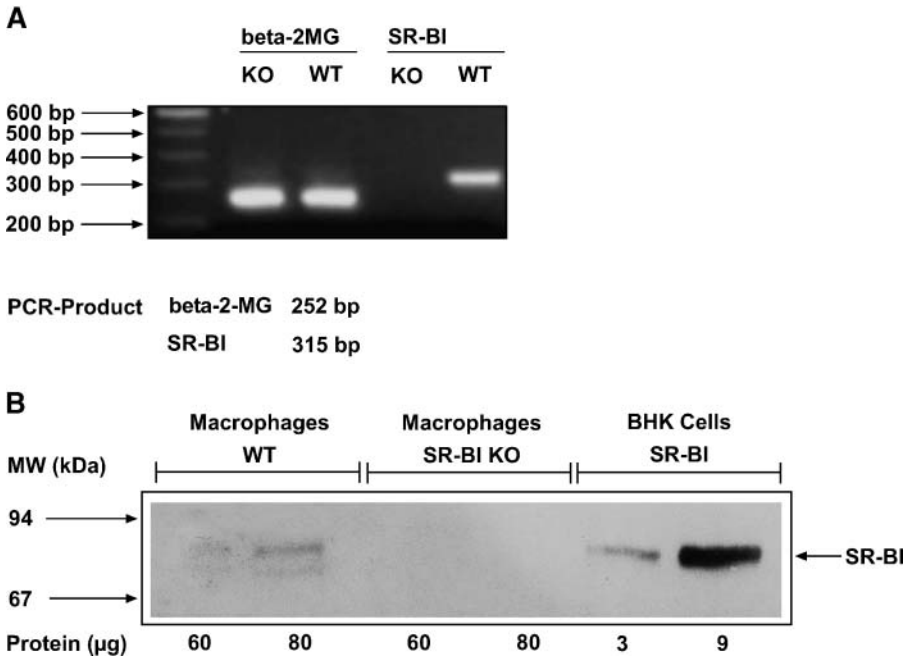

Fig. 2. SR-BI mRNA levels and immunoblot analysis for SR-BI in macrophages from WT or SR-BI KO mice and in BHK cells. Macrophages were prepared from WT or SR-BI KO mice, and BHK cells with SR-BI expression were generated as described in Materials and Methods. A: Expression of SR-BI and β2-microglobulin (beta-2-MG) mRNA was determined by RT-PCR. Shown are results from one representative experiment from a total of three. B: Postnuclear supernatants were prepared, and the indicated protein mass was fractionated by SDS-PAGE (10%). After transfer to nitrocellulose, the proteins were immunoblotted with an anti-SR-BI antiserum as described in Materials and Methods. Two similar blots yielded qualitatively identical results. MW, molecular mass.

uptake between both tracers yields the apparent selective CE uptake, and this is presented in Fig. 3A. This selective CE uptake increased in an HDL concentration-dependent manner. In this experiment (Fig. 3A), from total cellular [^{3}H]CEt uptake, only 3.9% to 6.0% were internalized via HDL holoparticle internalization as represented by ^{125}I-TC (4). In parallel, WT macrophages were incubated in the presence of ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL (Fig. 3A). Also in this case, a dose-dependent increase in selective CE uptake ([^{3}H]CEt − ^{125}I-TC) was detected, although the respective rate was significantly lower compared with radiolabeled WT-HDL in WT cells.

Additionally, macrophages from SR-BI KO mice were incubated in medium containing either ^{125}I-TC-/[^{3}H]CEt-WT-HDL or ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL (Fig. 3B). In the presence of ^{125}I-TC-/[^{3}H]CEt-WT-HDL, selective HDL CE uptake increased in a dose-dependent manner. Selective lipid uptake from ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL by SR-BI KO macrophages was lower compared with that from ^{125}I-TC-/[^{3}H]CEt-WT-HDL (Fig. 3B). Remarkably, all rates for HDL selective CE uptake were similar in WT and SR-BI KO macrophages (i.e., in cells with or without SR-BI expression) (Fig. 3).

Kinetics for macrophage HDL uptake are presented in **Fig. 4**. WT macrophages were incubated in medium containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL, and selective CE uptake ([^{3}H]CEt − ^{125}I-TC) increased in a time-dependent manner (Fig. 4A). In parallel, WT macrophages were incubated in the presence of ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL (Fig. 4A). Under these conditions as well, a time-dependent increase in HDL selective CE uptake ([^{3}H]CEt − ^{125}I-TC) was observed, although the respective rate was significantly lower compared with selective lipid uptake from ^{125}I-TC-/[^{3}H]CEt-WT-HDL by WT macrophages.

In parallel, macrophages from SR-BI KO mice were incubated in medium containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL or ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL (Fig. 4B). Also in these cases, a time-dependent increase in selective HDL CE uptake ([^{3}H]CEt − ^{125}I-TC) was detected. Radiolabeled SR-BI KO-HDL yielded a lower rate of selective CE uptake compared with labeled WT-HDL in SR-BI KO macrophages. Again, the macrophage SR-BI deficiency was associated with similar rates of selective HDL CE uptake compared with cells with SR-BI expression (Fig. 4A vs. B).

Regulation of HDL selective CE uptake

Macrophages were cultured under cholesterol-poor conditions in this study. Cholesterol deprivation upregulates HDL selective CE uptake by macrophages (12). The possibility was considered that the results shown in Figs. 3 and 4 were observed exclusively under these experimental conditions. To investigate regulation, WT or SR-BI KO macrophages were cultured in the absence or presence of acetyl-LDL to increase cell cholesterol (**Fig. 5**) (22). In WT cells incubated without acetyl-LDL, cell cholesterol was 42.7 μg/mg cell protein; in the presence of acetyl-LDL, the respective value increased to 70.3 μg/mg cell protein.

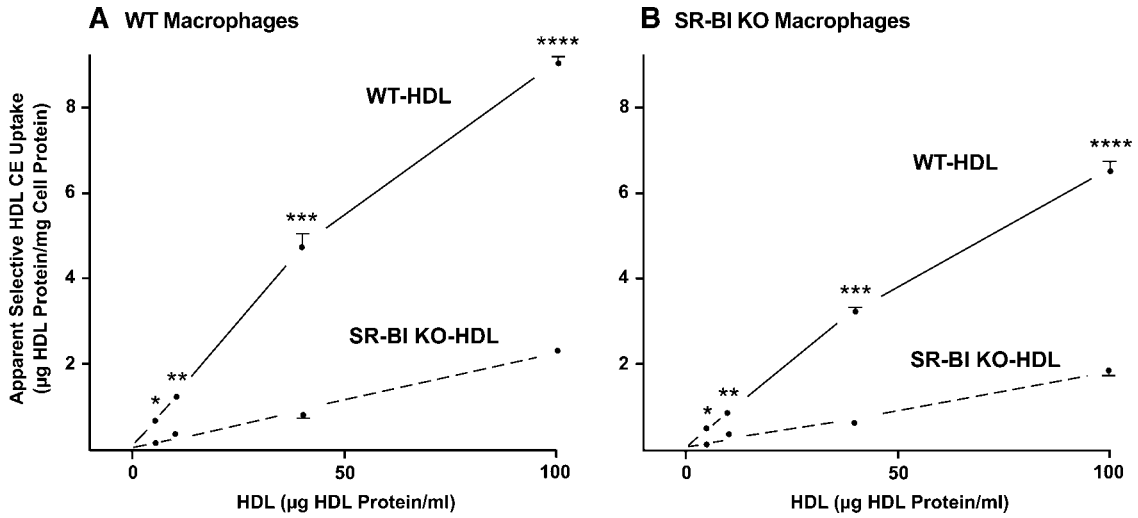

Fig. 3. Dose-response curves for selective cholesteryl ester (CE) uptake from ^{125}I-TC-/[^{3}H]CEt-WT-HDL and ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL by WT or SR-BI KO macrophages. Macrophages from WT (A) and SR-BI KO (B) mice were incubated (37°C, 4.0 h) in DMEM containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL or ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL; the respective HDL concentrations are given on the abscissae. Then, cellular tracer content and apparent HDL particle uptake (^{125}I-TC, [^{3}H]CEt) were analyzed, and apparent selective HDL CE uptake ([^{3}H]CEt − ^{125}I-TC) was calculated. Values are means ± SEM of three independent incubations. Where no error bars are shown, the SEMs were smaller than the respective symbols. For A, * $P = 0.0007$, ** $P = 0.0014$, *** $P = 0.0086$, **** $P = 0.0001$; for B, * $P = 0.0045$, ** $P = 0.0071$, *** $P = 0.0064$, **** $P = 0.0026$. Two independent similar experiments yielded qualitatively identical results.

After the loading period, macrophages were incubated in medium containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL or ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL (Fig. 5). In WT cells, acetyl-LDL loading decreased selective HDL CE uptake ([^{3}H]CEt − ^{125}I-TC), and this was true for ^{125}I-TC-/[^{3}H]CEt-WT-HDL and ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL (Fig. 5A). Analogously, in SR-BI KO macrophages, cholesterol loading decreased selective HDL CE uptake ([^{3}H]CEt − ^{125}I-TC) using both ^{125}I-TC-/[^{3}H]CEt-WT-HDL and ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL as lipoprotein preparations (Fig. 5B).

Selective HDL CE uptake by cells with high SR-BI expression

^{125}I-TC-/[^{3}H]CEt-WT-HDL and ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL yield substantial differences in rates of selective CE uptake in macrophages. However, in this model, SR-BI expression is lower compared with other cell types (Fig. 2B). Therefore, the issue was addressed whether the different rates of selective CE uptake from WT-HDL and SR-BI KO-HDL in macrophages were unique to this experimental model. Additionally, the question was raised

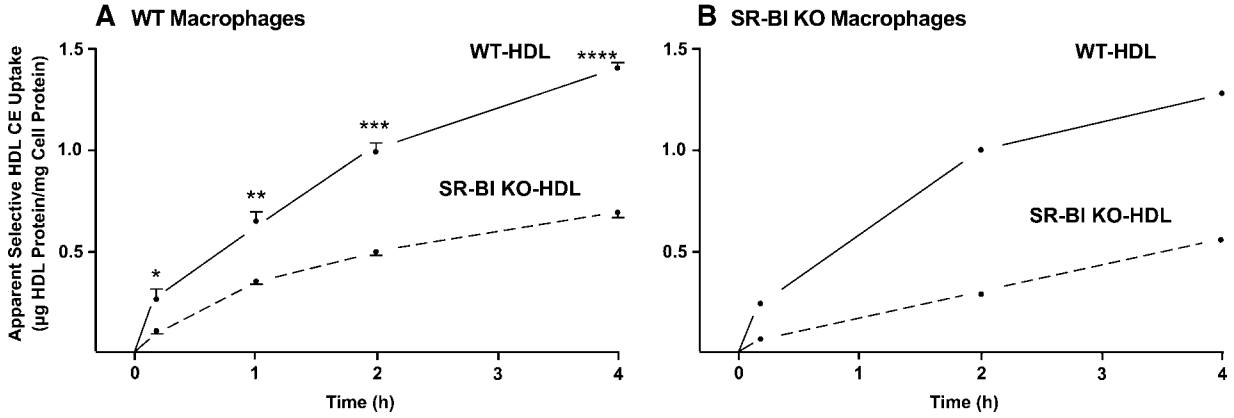

Fig. 4. Kinetics for selective CE uptake from ^{125}I-TC-/[^{3}H]CEt-WT-HDL and ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL by WT or SR-BI KO macrophages. Macrophages from WT (A) and SR-BI KO (B) mice were incubated (37°C, 10 min, 1.0 h, 2.0 h, or 4.0 h) in medium containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL (20 µg HDL protein/ml) or ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL (20 µg HDL protein/ml). Then, cellular tracer content and apparent HDL particle uptake (^{125}I-TC, [^{3}H]CEt) were analyzed, and apparent selective CE uptake ([^{3}H]CEt − ^{125}I-TC) was calculated as described in Materials and Methods. Values are means ± SEM of three (A) or two (B) incubations. In A, where no error bars are shown, the SEMs were smaller than the respective symbols. In B, variation from the mean was <8%. For A, * $P = 0.02$, ** $P = 0.004$, *** $P = 0.0003$, **** $P < 0.0001$. Two independent similar experiments yielded qualitatively identical results.

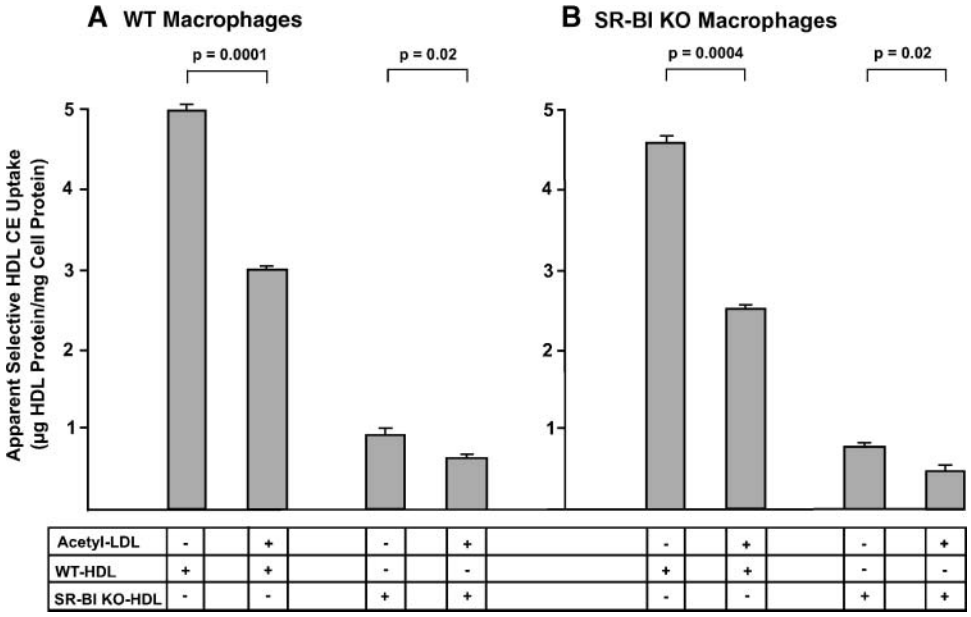

Fig. 5. Regulation of selective CE uptake from ^{125}I-TC-/[^{3}H]CEt-WT-HDL and ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL by macrophages prepared from WT or SR-BI KO mice. Macrophages from WT (A) and SR-BI KO (B) mice were incubated (37°C, 20 h) in medium in the absence or presence of acetylated LDL (acetyl-LDL; 50 μg protein/ml). Preincubation (37°C, 0.5 h) was followed by incubation (37°C, 4.0 h) in medium containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL (40 μg HDL protein/ml) or ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL (40 μg HDL protein/ml). Then, cellular tracer content and apparent HDL particle uptake (^{125}I-TC, [^{3}H]CEt) were analyzed. Shown is apparent selective HDL CE uptake (i.e., the difference between [^{3}H]CEt and ^{125}I-TC). Values are means ± SEM of three incubations. One independent similar experiment yielded qualitatively identical results.

whether radiolabeled WT-HDL and SR-BI KO-HDL interact similarly or differently with SR-BI. Stably transfected BHK cells with very high SR-BI expression and control BHK cells (vector) with no detectable SR-BI were incubated in parallel in medium containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL or ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL (**Fig. 6**) (26). In control BHK cells, ^{125}I-TC-/[^{3}H]CEt-WT-HDL yielded a higher rate of selective CE uptake ([^{3}H]CEt − ^{125}I-TC) compared with ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL. In BHK cells with high SR-BI expression, HDL selective CE uptake increased compared with that in control BHK cells (Fig. 6). However, also in these BHK cells, selective CE uptake from ^{125}I-TC-/[^{3}H]CEt-WT-HDL was in excess of uptake from ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL. Thus, irrespective of cellular SR-BI expression and in independent experimental models, SR-BI KO-HDL yields a lower rate of selective CE uptake.

Cholesterol efflux from macrophages

To explore the role of SR-BI for lipid efflux, cell cholesterol of WT and SR-BI KO macrophages was radiolabeled with [^{3}H]cholesterol (9, 24). Then, a [^{3}H]cholesterol efflux assay was initiated in the presence of WT-HDL or SR-BI KO-HDL as lipid acceptor.

In HDL dose-response curves for [^{3}H]cholesterol efflux from WT macrophages, WT-HDL promoted a dose-dependent increase in [^{3}H]cholesterol removal (**Fig. 7A**). Qualitatively identical, SR-BI KO-HDL facilitated a dose-dependent lipid efflux as well, although the respective rate was quantitatively significantly higher compared with WT-HDL in these WT cells. In macrophages from SR-BI KO mice, [^{3}H]cholesterol efflux was also explored (Fig. 7B). Lipid removal from these SR-BI-deficient macrophages was quantitatively similar compared with that in WT cells; again, SR-BI KO-HDL more efficiently stimulated [^{3}H]cholesterol efflux from these SR-BI-deficient macrophages.

Human HDL_3 as donor for selective CE uptake and as acceptor for cell cholesterol

Human HDL is frequently used to investigate cholesterol homeostasis of murine macrophages (11, 14–16, 25). Therefore, selective CE uptake from human ^{125}I-TC-/[^{3}H]CEt-HDL_3 by murine WT and by SR-BI KO macrophages was explored (**Fig. 8**). In WT macrophages, uptake of both ^{125}I-TC and [^{3}H]CEt increased in a dose-dependent manner, and the difference ([^{3}H]CEt − ^{125}I-TC) yielded a dose-dependent apparent selective CE uptake from HDL_3 (Fig. 8A). In SR-BI KO macrophages, ^{125}I-TC-/[^{3}H]CEt-HDL_3 yielded a dose-dependent increase in apparent selective CE uptake as well, and the respective rate was not significantly different from that of WT cells (Fig. 8B).

Using human HDL_3 and human apoA-I as sterol acceptors, [^{3}H]cholesterol efflux from murine WT and SR-BI KO macrophages was investigated. Dose-response curves for cellular [^{3}H]cholesterol efflux with HDL_3 as lipid acceptor are presented in **Fig. 9**. Increasing concentra-

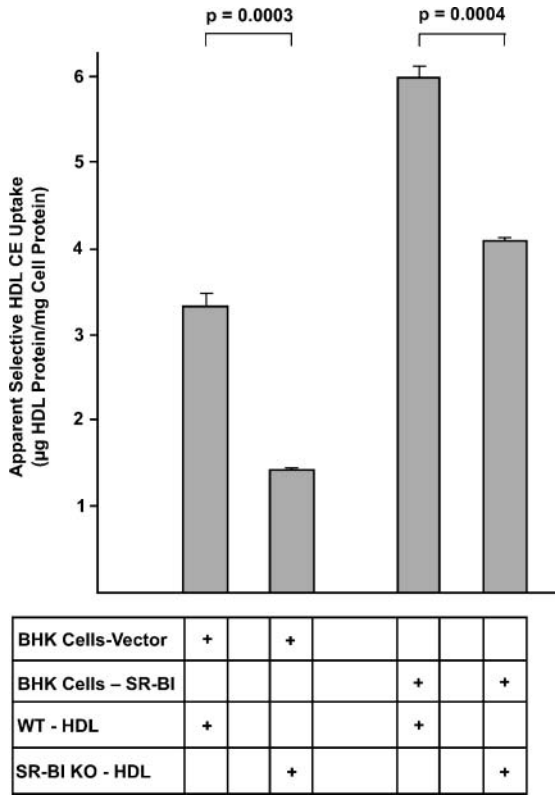

Fig. 6. Selective CE uptake from ^{125}I-TC-/[^{3}H]CEt-WT-HDL and ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL by BHK cells without or with SR-BI expression. Control BHK cells (vector) or BHK cells with SR-BI expression were incubated (37°C, 4.0 h) in medium containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL (40 µg HDL protein/ml) or ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL (40 µg HDL protein/ml). Then, cellular tracer content and apparent HDL particle uptake were analyzed. Apparent HDL selective CE uptake was calculated as the difference in apparent particle uptake between [^{3}H]CEt and ^{125}I-TC. Values are means ± SEM of three incubations. One independent identical and one independent similar experiment yielded qualitatively identical results.

tions of HDL_3 in the medium dose-dependently stimulated the cellular efflux of [^{3}H]cholesterol. The rates of [^{3}H]cholesterol flux were similar for macrophages isolated from WT and SR-BI KO mice (Fig. 9). In addition to HDL_3, lipid-free apoA-I (20 µg protein/ml) time-dependently (1.0–6.0 h) stimulated the efflux of [^{3}H]cholesterol in WT and SR-BI KO macrophages (data not shown). Again, no difference in [^{3}H]cholesterol efflux between WT and SR-BI KO macrophages was detected when apoA-I was the lipid acceptor.

Macrophage cholesterol mass flux

Concerning the interaction between macrophages and HDL, this study shows that macrophages internalize esterified cholesterol from HDL selectively. In parallel, HDL promotes the efflux of unesterified cellular cholesterol. These observations are based on experiments using radioisotopes; therefore, no conclusion concerning the net mass cholesterol flux between macrophages and HDL can be made. To address net mass cholesterol flux between HDL and cells under the experimental conditions of this study, WT and SR-BI KO macrophages were incubated (37°C) in medium containing unlabeled WT-HDL or SR-BI KO-HDL (**Table 2**). After 2 or 300 min of incubation, cells were harvested and cell cholesterol mass was analyzed. Between 2 and 300 min of incubation, a decrease in macrophage cholesterol mass was observed under all conditions. However, SR-BI KO-HDL more efficiently promoted a decline in cell cholesterol compared with WT-HDL in both WT and SR-BI KO macrophages (Table 2).

CD36 and macrophage HDL metabolism

The class B scavenger receptor CD36 is expressed in macrophages (33). This receptor protein binds HDL with high affinity but mediates selective HDL CE, presumably at a low rate compared with SR-BI (34, 35). To explore a possible role of CD36 in selective CE uptake and in cholesterol efflux, the expression of this protein in macrophages isolated from WT mice or from SR-BI KO animals was investigated on immunoblots (**Fig. 10**) (30). CD36 expression was detected in WT and SR-BI KO macrophages, and the respective signal was identical in both cell types.

The function of CD36 in selective HDL CE uptake by macrophages was addressed directly. Macrophages were isolated from WT mice and from CD36 KO (homozygous) animals (20). WT or CD36-deficient cells were incubated in medium containing ^{125}I-TC-/[^{3}H]CEt-HDL_3 (**Fig. 11**). Increasing concentrations of radiolabeled HDL_3 yielded a dose-dependent increase in cellular uptake of ^{125}I-TC and [^{3}H]CEt; as result, apparent selective HDL_3 CE uptake ([^{3}H]CEt − ^{125}I-TC) increased in an HDL_3 concentration-dependent manner. The rate of selective HDL_3 CE uptake was very similar in macrophages isolated from WT and CD36 KO mice (Fig. 11).

HDL binding to macrophages

HDL binding to the cell surface is established (28). WT-HDL and SR-BI KO-HDL had different effects on HDL uptake and on cholesterol efflux from macrophages. To address the issue of whether these differences are related to variations in HDL binding to the cell membrane, binding of ^{125}I-TC-HDL to macrophages was explored (**Fig. 12**). WT and SR-BI KO macrophages were incubated (4°C) in medium containing ^{125}I-TC-WT-HDL or ^{125}I-TC-SR-BI KO-HDL. ^{125}I-TC-WT-HDL and ^{125}I-TC-SR-BI KO-HDL bound in a dose-dependent manner to both types of macrophages. Binding of ^{125}I-TC-SR-BI KO-HDL quantitatively was higher compared with ^{125}I-TC-WT-HDL. No difference in ^{125}I-TC-HDL binding to macrophages with or without SR-BI expression was observed (Fig. 12).

To explore the binding of human ^{125}I-TC-HDL_3 to murine cells, macrophages isolated from WT or SR-BI KO mice were incubated (4°C, 2.0 h) in medium containing human ^{125}I-TC-HDL_3 (10, 20, 40, 100, and 200 µg HDL_3/ml) (data not shown). Binding of this tracer preparation to both types of cells was very similar. Thus, murine HDL and human HDL_3 yielded qualitatively identical results with respect to binding to cells.

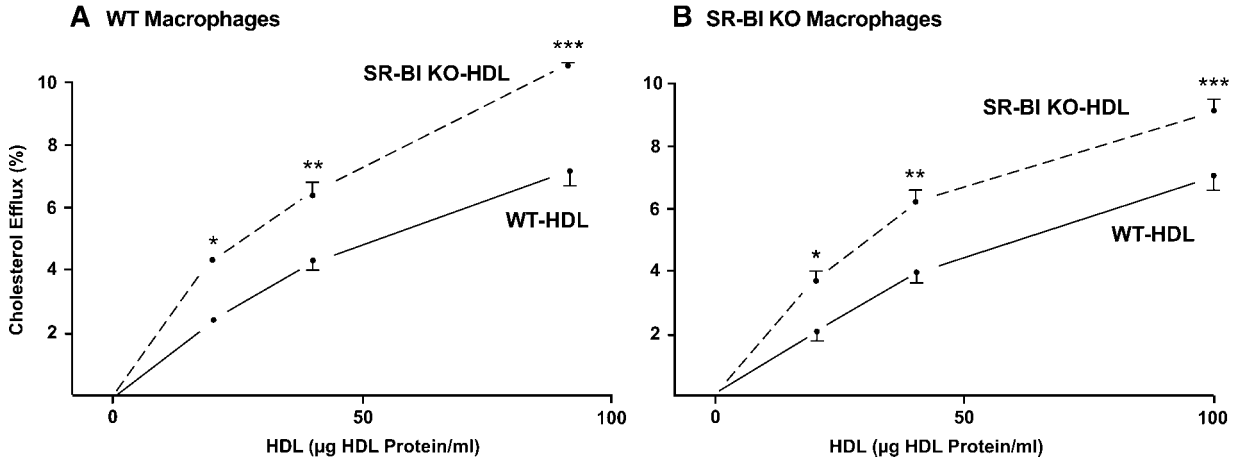

Fig. 7. [^{3}H]cholesterol efflux from WT and SR-BI KO macrophages, and dose-response curves for WT-HDL and SR-BI KO-HDL. The cell cholesterol of macrophages isolated from WT (A) and SR-BI KO (B) mice was labeled with [^{3}H]cholesterol. Then, macrophages were incubated (37°C, 5.0 h) in medium containing WT-HDL or SR-BI KO-HDL; the respective HDL concentrations are indicated on the abscissae. [^{3}H]cholesterol efflux was analyzed as described in Materials and Methods. Values are means ± SEM of three incubations. Where no error bars are shown, the SEMs were smaller than the respective symbols. For A, * $P = 0.0002$, ** $P = 0.008$, *** $P = 0.002$; for B, * $P = 0.003$, ** $P = 0.007$, *** $P = 0.03$. Two independent similar experiments yielded qualitatively identical results.

DISCUSSION

Murine peritoneal macrophages selectively took up HDL-associated CE in this investigation. This result is consistent with previous observations in this cell type (11, 12, 25). Macrophages express SR-BI, and this was confirmed here in terms of mRNA and protein (11, 13, 15, 32, 36). Macrophages with and without SR-BI expression selectively took up HDL CE in this study, and the respective rates for this lipid internalization were similar in the absence or presence of this HDL receptor. HDL selective CE uptake was regulated as a function of cell cholesterol in macrophages with and without SR-BI expression. These observations provide evidence that macrophages have a mechanism for selective CE uptake that is distinct from SR-BI.

These results regarding an SR-BI-independent mechanism for HDL selective CE uptake are consistent with pre-

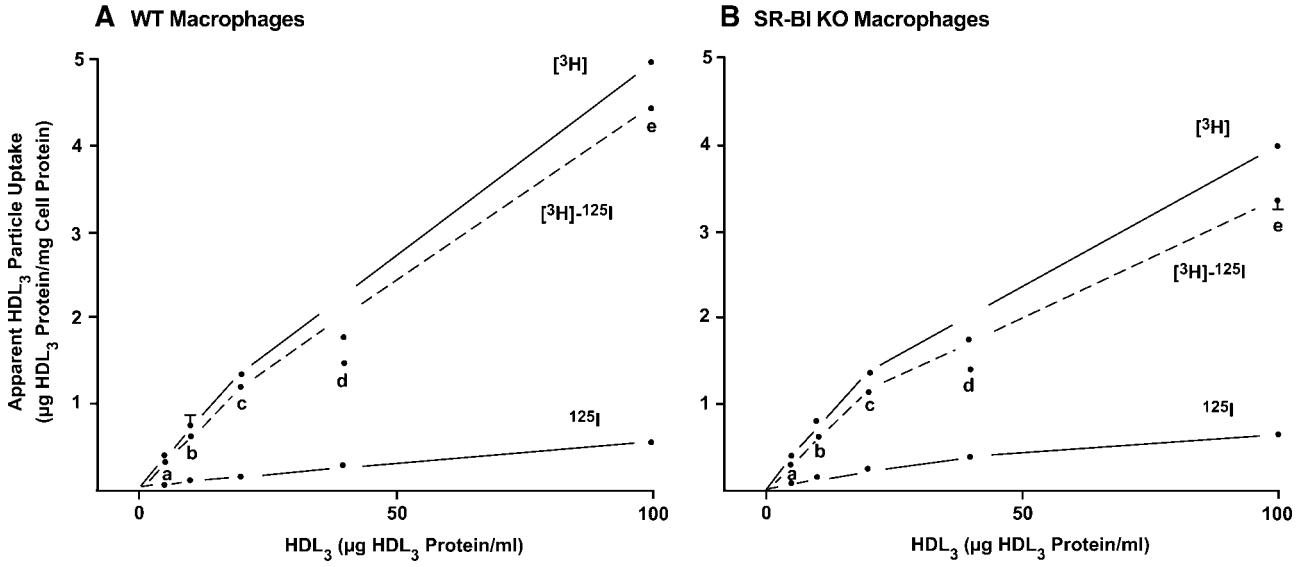

Fig. 8. Uptake of human ^{125}I-TC-/[^{3}H]CEt-HDL$_3$ by WT or SR-BI KO macrophages. Macrophages from WT (A) and SR-BI KO (B) mice were incubated (37°C, 4.0 h) in medium containing human ^{125}I-TC-/[^{3}H]CEt-HDL$_3$; the respective HDL$_3$ concentrations are given on the abscissae. Then, cellular tracer content and apparent HDL$_3$ particle uptake were analyzed. ^{125}I represents apparent HDL$_3$ particle uptake according to ^{125}I-TC-labeled protein; [^{3}H] demonstrates apparent particle uptake attributable to [^{3}H]CEt; and [^{3}H]−^{125}I shows the difference (i.e., apparent selective CE uptake). Values are means ± SEM of three independent incubations. Where no error bars are shown, the SEMs were smaller than the respective symbols. For selective uptake: [a] $P = 0.23$, [b] $P = 0.86$, [c] $P = 0.53$, [d] $P = 0.059$, [e] $P = 0.15$. One independent experiment yielded qualitatively identical results.

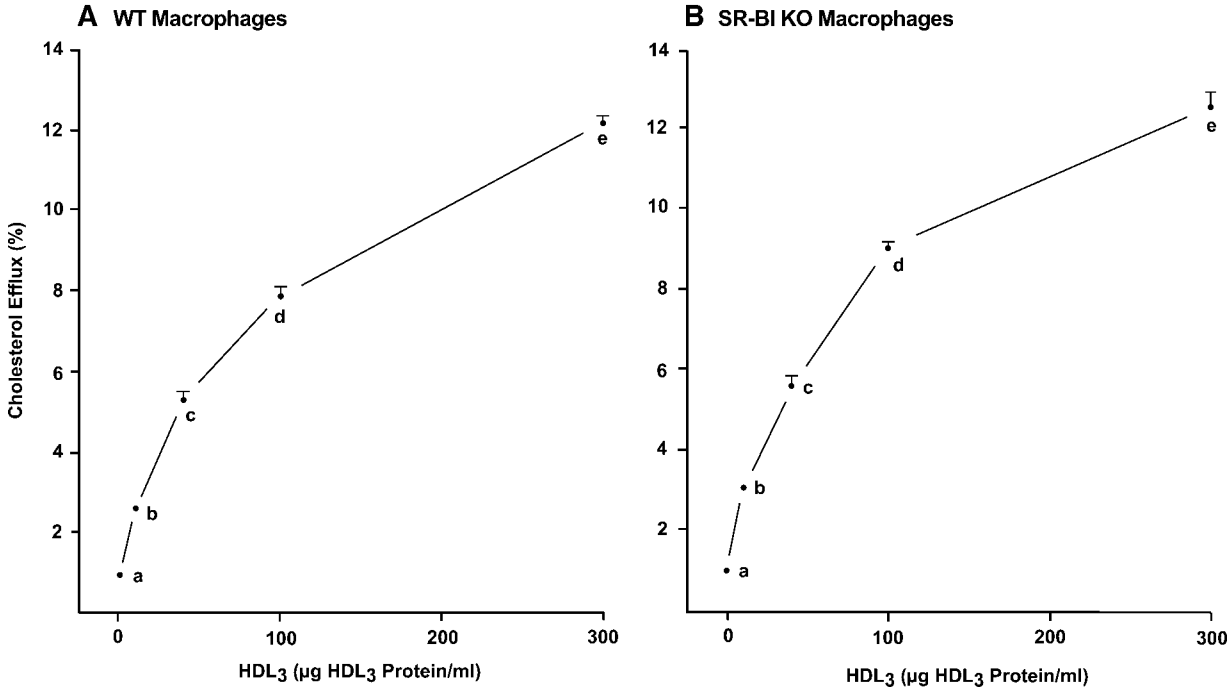

Fig. 9. [^{3}H]cholesterol efflux from WT and SR-BI KO macrophages, and dose-response curves for human HDL_3. The cell cholesterol of macrophages from WT (A) and SR-BI KO (B) mice was labeled with [^{3}H]cholesterol. Then, macrophages were incubated (37°C, 5.0 h) in medium containing human HDL_3; the respective HDL_3 concentrations are given on the abscissae. [^{3}H]cholesterol efflux was analyzed as described in Materials and Methods. Values are means ± SEM of three incubations. Where no error bars are shown, the SEMs were smaller than the respective symbols. [a] $P = 0.10$, [b] $P = 0.005$, [c] $P = 0.53$, [d] $P = 0.01$, [e] $P = 0.38$. Two independent similar experiments yielded qualitatively identical results.

vious conclusions. Lipoprotein lipase and hepatic lipase stimulated selective CE uptake in vitro to a similar extent independent of cellular SR-BI expression levels (26). More relevant, in vivo selective HDL CE uptake by adrenals was observed in mutant SR-BI KO mice (7). In summary, the results presented here as well as other in vitro and in vivo experiments provide evidence for a cellular mechanism for HDL selective CE uptake distinct from SR-BI.

HDL from WT mice is different from the respective preparation isolated from SR-BI KO animals (6, 7, 17–19). SR-BI KO-HDL, which contains large HDL particles, is enriched in free cholesterol and apoE, and this was confirmed here for this lipoprotein fraction whether it was radiolabeled or not. Because of these differences, both HDL preparations were used as ligands for the selective CE uptake pathway. SR-BI KO-HDL donated CE to cells at lower rates compared with WT-HDL. This result was true irrespective of the SR-BI expression level or the cell model. In cells without (i.e., SR-BI KO macrophages) and in cells with (i.e., transfected BHK cells) high SR-BI expression, SR-BI KO-HDL yielded a low rate of selective uptake. In contrast, WT-HDL donated CE at substantially higher rates to the respective model. Prominent differences between SR-BI KO-HDL and WT-HDL were an enrichment of the former fraction in unesterified cholesterol and apoE and the larger average size of SR-BI KO-HDL (6, 7, 17–19). These differences in physical properties and composition apparently have a profound effect on the rates of selective CE uptake by a given cell. This variation in the rate of selective CE uptake between WT-HDL and SR-BI KO-HDL was true whether selective CE uptake was mediated by SR-BI or by an SR-BI-independent mechanism. Therefore, these differences in selective CE uptake must be explained by features of the ligand particle and not by the cellular mechanism that mediates its internalization.

Which mechanism facilitates the SR-BI-independent selective HDL CE uptake at the molecular level? Besides SR-BI, another member of the class B scavenger receptor family (i.e., the multiligand receptor CD36) is expressed in macrophages (33). CD36 binds HDL but presumably

TABLE 2. Macrophage cholesterol mass after incubation with HDL

Macrophages	HDL	Cell Cholesterol: Incubation Time of 2 min	Cell Cholesterol: Incubation Time of 300 min
		μg/mg cell protein	
WT	WT-HDL	210	195
WT	SR-BI KO-HDL	178	152
SR-BI KO	WT-HDL	150	136
SR-BI KO	SR-BI KO-HDL	163	136

Macrophages from WT or SR-BI KO mice were incubated (37°C, 2 or 300 min) in medium containing unlabeled WT-HDL or SR-BI KO-HDL (40 μg HDL protein/ml). Thereafter, the cells were harvested and cellular cholesterol and protein were analyzed as described in Materials and Methods. Values are means of two independent incubations. Variation from the mean was <6%. Two independent similar experiments yielded qualitatively identical results.

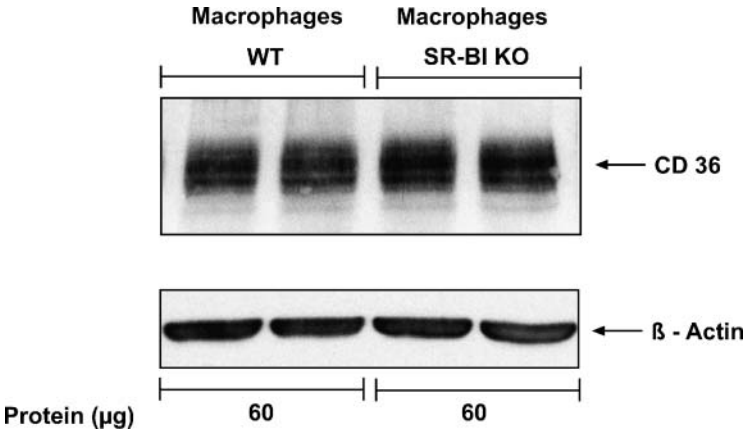

Fig. 10. Immunoblot analysis for CD36 in WT or SR-BI KO macrophages. Macrophages were isolated from WT or SR-BI KO mice, and postnuclear supernatants were prepared. Then, proteins were fractionated by SDS-PAGE (10%). After transfer to nitrocellulose, the proteins were immunoblotted with an anti-mouse CD36 antiserum or with a β-actin antibody, as described in Materials and Methods. Two similar blots yielded qualitatively identical results.

mediates HDL selective CE uptake at a low rate compared with SR-BI (34, 35). In this study, CD36 expression was identical in macrophages isolated from WT and SR-BI KO mice. Additionally, selective CE uptake from HDL was identical in macrophages prepared from WT and CD-deficient mice (CD36 KO). These observations argue against a role of CD36 in the SR-BI-independent selective HDL CE uptake. A novel efflux-recapture mechanism may mediate selective HDL CE uptake in adipocytes (37). Accordingly, CEs that are associated with the plasma membrane are captured by apoE. ApoE-associated CEs are finally internalized by cells in a mechanism that involves the low density lipoprotein receptor-related protein. Assuming that both macrophages and adipocytes express this pathway, then it is possible that this mechanism has a function in selective HDL CE uptake in SR-BI-deficient cells. Lipid-lipid interactions between the phospholipid monolayer of the lipoprotein surface and the cellular plasma membrane may play a role in the selective transfer of lipids to cells (38). The results of this study are compatible with this model. However, the SR-BI-independent HDL selective CE uptake observed here in macrophages may be mediated by several mechanisms simultaneously. Additional receptor proteins that facilitate selective lipid uptake may be defined in the future.

Removal of unesterified [^{3}H]cholesterol from macrophages was similar in this study in the absence or presence of SR-BI. This was true whether HDL or apoA-I was the cholesterol acceptor. This observation is consistent with recent studies (15, 39). Therefore, in macrophages, a mechanism distinct from SR-BI mediates cholesterol efflux. Quantitatively, the contribution of these pathways is substantial (39). Besides SR-BI, several alternative mechanisms for cellular cholesterol removal have been defined (2). One pathway is the efflux of unesterified cholesterol via aqueous diffusion, although quantitatively this route is inefficient

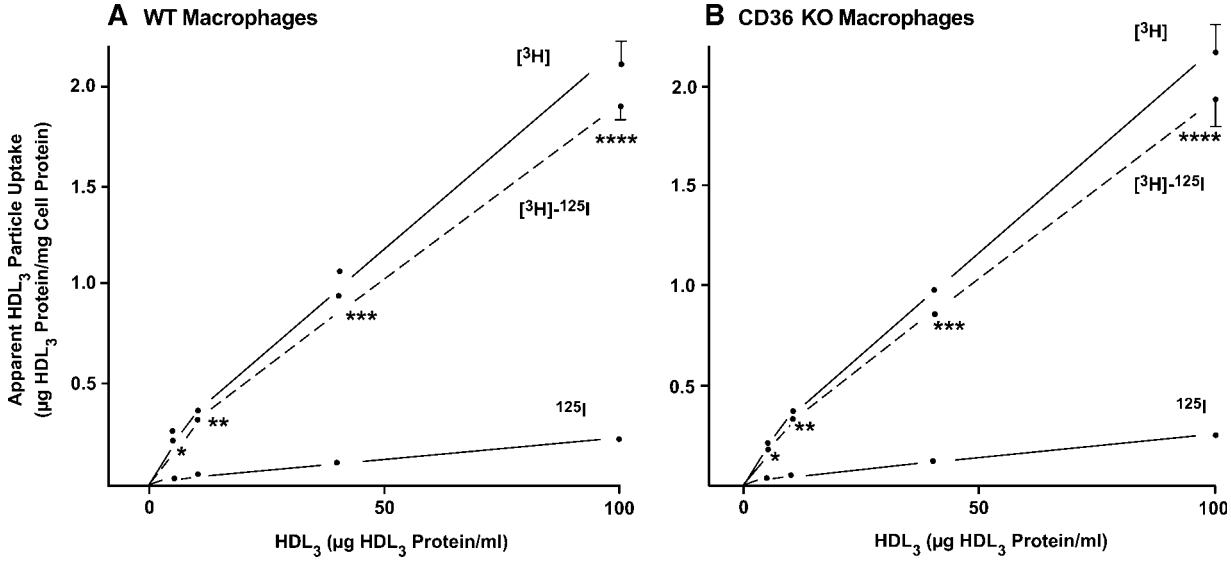

Fig. 11. Uptake of human ^{125}I-TC-/[^{3}H]CEt-HDL$_3$ by WT or CD36 KO macrophages. Macrophages from WT (A) and CD36 KO (B) mice were incubated (37°C, 4.0 h) in medium containing human ^{125}I-TC-/[^{3}H]CEt-HDL$_3$; the respective HDL$_3$ concentrations are given on the abscissae. Then, cellular tracer content and apparent HDL$_3$ particle uptake were analyzed. ^{125}I represents apparent HDL$_3$ particle uptake according to ^{125}I-TC-labeled protein; [^{3}H] demonstrates apparent particle uptake attributable to [^{3}H]CEt; and [^{3}H]−^{125}I shows the difference (i.e., apparent selective CE uptake). Values are means ± SEM of three independent incubations. Where no error bars are shown, the SEMs were smaller than the respective symbols. For selective uptake, * $P = 0.083$, ** $P = 0.43$, *** $P = 0.013$, **** $P = 0.85$.

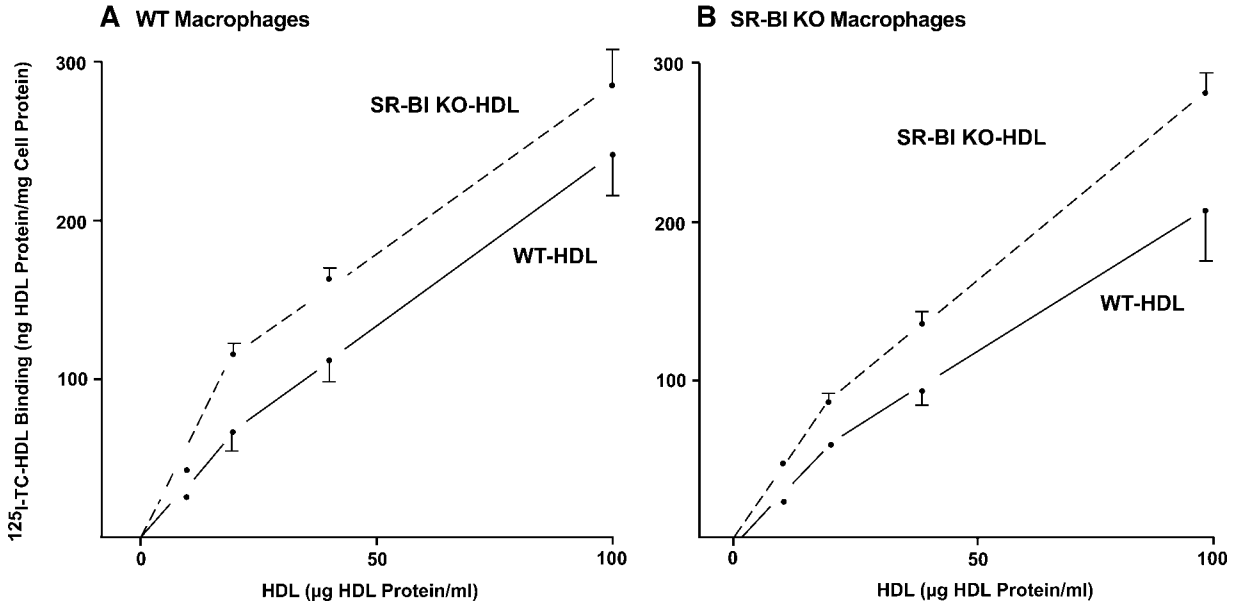

Fig. 12. Binding of ^{125}I-TC-WT-HDL and ^{125}I-TC-SR-BI KO-HDL to WT or SR-BI KO macrophages. Macrophages from WT (A) and SR-BI KO (B) mice were incubated (4°C, 2.0 h) in DMEM containing ^{125}I-TC-WT-HDL or ^{125}I-TC-SR-BI KO-HDL; the respective HDL concentrations are given on the abscissae. Then, cellular tracer association was analyzed. Values are means ± SEM of three independent incubations. Where no error bars are shown, the SEMs were smaller than the respective symbols. One independent experiment yielded qualitatively identical results.

(2). ABCA1 mediates both cholesterol and phospholipid efflux; however, lipid-poor apoA-I, and not HDL, appears to be the preferred cholesterol acceptor for this protein (40). Therefore, a direct role of ABCA1 in the HDL-mediated and SR-BI-independent cholesterol efflux from macrophages is unlikely. ABCG1 and ABCG4 are members of the ABC transporter family as well, and these molecules may mediate cholesterol efflux from macrophages (41). These transporters stimulate cholesterol efflux preferentially to HDL as acceptor, so it seems possible that these molecules may play a role in cholesterol efflux in SR-BI-deficient macrophages. Additionally, it cannot be excluded that several pathways distinct from SR-BI mediate cholesterol efflux from macrophages simultaneously. In summary, the data presented here suggest that SR-BI-independent mechanisms play a major role for cholesterol efflux in macrophages.

Acceptors for excess cell cholesterol are apoA-I, the dominant protein component of HDL, and HDL itself (2). In this study, murine WT-HDL, murine SR-BI KO-HDL, human HDL_3, and human apoA-I accelerated cholesterol removal from murine macrophages. However, SR-BI KO-HDL more efficiently promoted cholesterol efflux compared with WT-HDL. Thus, analogous to HDL uptake, quantitatively the effects of both murine HDL preparations on cholesterol efflux differ significantly. These observations imply that the physical properties (i.e., size) and/or composition of a given HDL particle determine its ability to accept excess cellular cholesterol. Relevant in this context are recent results obtained with HDL_2 originating from cholesteryl ester transfer protein (CETP)-deficient patients (42). These particles are larger and are enriched in cholesterol compared with HDL_2 from controls. HDL_2 from CETP-deficient patients stimulates cholesterol efflux from macrophages at a higher rate than control HDL_2. Both these data (42) and the results of this study point to the role of HDL particle size and composition in the ability of a given lipoprotein to facilitate cholesterol efflux from macrophages.

Initially, the interaction between macrophages and HDL (i.e., HDL selective CE uptake and cholesterol efflux) was analyzed with radioisotopes. However, these results do not yield conclusions with respect to the net effect of this interaction on cell cholesterol mass. The question was raised whether net cholesterol uptake or net cholesterol efflux from macrophages occurs under these conditions. Here, an incubation of macrophages in medium containing HDL yielded a net decrease in cell cholesterol mass. This reduction was more pronounced using SR-BI KO-HDL, and this result is consistent with [^{3}H]cholesterol efflux from macrophages.

To define in more detail a potential mechanism by which SR-BI KO-HDL more efficiently stimulates cholesterol removal from macrophages and induces a greater decrease in cell cholesterol mass compared with WT-HDL, binding (4°C) of ^{125}I-TC-HDL was explored. Binding of ^{125}I-TC-SR-BI KO-HDL to macrophages was higher compared with that of ^{125}I-TC-WT-HDL, and this was true for both WT and SR-BI KO macrophages. Quantitatively, ^{125}I-TC-WT-HDL and ^{125}I-TC-SR-BI KO-HDL binding to WT and SR-BI KO macrophages was similar. The increased binding of ^{125}I-TC-SR-BI KO-HDL to both types of macrophages is one potential explanation for why this lipoprotein fraction more efficiently promotes cellular cholesterol efflux.

In bone marrow transplantation experiments with mice, macrophage SR-BI expression protected against atherosclerosis (14–16). In contrast, in the vessel wall of SR-BI-deficient mice, atherogenesis was accelerated (17). Therefore, macrophage SR-BI expression has an essential role in atherosclerosis. This study is compatible with the hypothesis that the atheroprotective effect of SR-BI is not mediated by a direct role of this protein in HDL cholesterol metabolism of macrophages. SR-BI-independent mechanisms may compensate for the function of this receptor, at least in murine macrophages. However, SR-BI is a multiligand receptor for HDL, LDL, oxidized LDL, acetylated LDL, and small unilamellar vesicles (43). An SR-BI-mediated uptake of these molecules by macrophages could have a role in atherogenesis and may explain the protective effect of this receptor on atherosclerosis in rodents.

This investigation was supported by Research Grant Ri 436/8-1 from the Deutsche Forschungsgemeinschaft (Bonn, Germany). The assistance of Mrs. S. Ehret, Mrs. B. Keller, Mrs. B. Schulz, and Mrs. M. Thiel in these studies is gratefully acknowledged. CETP was a kind gift from Dr. Richard E. Morton and Mrs. Diane Greene.

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