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Dual Role for Scavenger Receptor Class B, Type I on Bone Marrow-Derived Cells in Atherosclerotic Lesion Development

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The function of scavenger receptor class B, type I (SR-BI) in the liver as a high-density lipoprotein receptor that promotes the selective uptake of cholesteryl esters is well defined. Its role in macrophages, however, is primarily unknown, because it functions in the uptake of (modified) lipoproteins as well as the secretion of cholesterol to high-density lipoproteins. In this study, the biological role of SR-BI on bone marrow-derived cells, including macrophages, in lipid metabolism and atherosclerosis was assessed by selective disruption of SR-BI in bone marrow in two established models of atherosclerosis: low-density lipoprotein (LDL) receptor-deficient mice that develop extensive atherosclerosis on a Western-type diet and wild-type mice that develop fatty streak lesions when fed a high-cholesterol diet containing 0.5% cholate. The presence of SR-BI in bone marrow-derived cells in LDLr−/− mice decreased lesion development after 9 and 12 weeks of Western-type diet feeding, indicating that macrophage SR-BI protects against lesion development. At 6 weeks, no significant effect of SR-BI in bone marrow-derived cells on lesion development was observed. Interestingly, after only 4 weeks of Western-type diet feeding of transplanted LDLr−/− mice and in wild-type mice on a high-cholesterol/cholate diet, the presence of SR-BI in bone marrow-derived cells increased the development of small fatty streak lesions. It thus appears that, depending on the stage of atherosclerotic lesion development, SR-BI in bone marrow-derived cells is either pro-atherogenic or anti-atherogenic, indicating a unique dual role in the pathogenesis of atherosclerosis. ***(Am J Pathol 2004, 165:785–794)***

Macrophage-derived foam cells play an important role in all stages of atherosclerotic lesion development.[1] Macrophage-derived foam cells are the predominant constituent of the fatty streak, the early atherosclerotic lesion. In advanced atherosclerotic lesions, foam cells are detected as clusters of cells surrounding a core of lipid and necrotic material, where they modulate the stability of the atherosclerotic lesion. Macrophage-derived foam cells develop as a result of excessive accumulation of lipoprotein-derived cholesterol.[2] Because macrophages are incapable of limiting their uptake of modified lipoproteins via scavenger receptors, they heavily depend on cholesterol efflux mechanisms for maintaining cholesterol homeostasis within the cell.[3–6] First, as proposed by Phillips and colleagues,[7] driven by a concentration gradient, cholesterol can efflux from cells by passive diffusion to extracellular acceptor particles, such as high-density lipoprotein (HDL). In addition, cholesterol is effluxed to extracellular lipid-free apolipoprotein (apo) acceptors, such as apoAI, facilitated by the ATP-binding cassette transporter 1 (ABCA1).[8,9] Furthermore, it has been demonstrated that the rate of cholesterol efflux from various cell types correlates with the expression of scavenger receptor class B, type I (SR-BI).[10–13] SR-BI is an 82-kd glycosylated plasma membrane protein, capable of binding a wide array of native and modified lipoproteins.[14–16] SR-BI is abundantly expressed in liver and steroidogenic tissues, where it mediates the selective uptake of cholesteryl esters from HDL.[14,17,18] Several lines of evidence, however, suggest that it can also stimulate the bi-directional flux of free cholesterol between cells and extracellular lipoprotein particles.

SR-BI is expressed in lipid-laden macrophages in human and murine atherosclerotic lesions,[10,19,20] indicating that SR-BI might play an important role locally in the arterial wall, in addition to its systemic role in determining serum HDL cholesterol levels by mediating hepatic uptake and biliary secretion of HDL cholesterol. Locally in the arterial wall, SR-BI expression by macrophages may protect against atherosclerosis by stimulating cholesterol efflux and preventing foam cell formation. On the other hand, its function in the uptake of both modified and native lipoproteins might enhance foam cell formation rendering macrophage SR-BI a proatherogenic factor.[15,21]

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Macrophages, present in atherosclerotic lesions, primarily depend on infiltration from bone marrow-derived monocytes into the arterial wall. Therefore, in the present study, we investigated the effects of selective disruption of SR-BI in bone marrow-derived cells and thus macrophages on atherosclerotic lesion development in two established models of atherosclerosis: low-density lipoprotein (LDL) receptor-deficient mice that develop extensive atherosclerosis on a Western-type diet and wild-type (WT) mice that develop fatty streak lesions when fed a high-cholesterol diet containing 0.5% cholate. SR-BI on bone marrow-derived cells protected against the development of atherosclerosis in LDL receptor deficient ($LDLr^{-/-}$) mice fed a Western-type diet for 9 and 12 weeks. While this article was under preparation, two articles appeared indicating that SR-BI in bone marrow-derived cells lowered atherosclerotic lesion development in LDLr−/−[22] and apoE−/−[23] mice. In addition, we now demonstrate that SR-BI in bone marrow-derived cells facilitates the development of small fatty streak lesions in LDLr−/− mice fed a Western-type diet for only 4 weeks and in WT mice fed a high-cholesterol/cholate diet. These data support a unique dual role for SR-BI on bone marrow-derived cells in atherosclerotic lesion development.

Materials and Methods

Mice

Class B, type I scavenger receptor (SR-BI)-deficient mice were kindly provided by Dr. M. Krieger (Massachusetts Institute of Technology, Cambridge, MA).[24] Mice are on a mixed C57BL6/129 background. Heterozygous SR-BI-deficient mice were cross-bred to generate homozygous SR-BI−/− knockout progeny and SR-BI+/+ WT littermates. The presence of the targeted and WT SR-BI alleles was assessed by polymerase chain reaction (PCR) amplification of DNA extracted from tail biopsies (primers 5′-GAT-GGG-ACA-TGG-GAC-ACG-AAG-CCA-TTC-T-3′ and 5′-TCT-GTC-TCC-GTC-TCC-TTC-AGG-TCC-TGA-3′). Homozygous LDL receptor knockout[25] (LDLr−/−) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) as mating pairs and bred at the Gorlaeus Laboratory, Leiden, The Netherlands. Mice were housed in sterilized filter-top cages and given unlimited access to food and water. Mice were maintained on sterilized regular chow, containing 4.3% (w/w) fat with no added cholesterol (RM3; Special Diet Services, Witham, UK), or were fed a semisynthetic Western-type diet containing 15% (w/w) fat and 0.25% (w/w) cholesterol (Diet W; Hope Farms, Woerden, The Netherlands) or a semisynthetic high-cholesterol/cholate diet containing 15% (w/w) fat, 1% (w/w) cholesterol, and 0.5% cholate (Diet N, Hope Farms).[26] Drinking water was supplied with antibiotics (83 mg/L ciprofloxacin and 67 mg/L polymyxin B sulfate) and 6.5 g/L sucrose. Animal experiments were performed at the Gorlaeus Laboratories of the Leiden/Amsterdam Center for Drug Research in accordance with the National Laws. All experimental protocols were approved by the Ethics Committee for Animal Experiments of Leiden University.

Bone Marrow Transplantation

To induce bone marrow aplasia, female LDLr−/− and WT mice were exposed to a single dose of 9 Gy (0.19 Gy/min, 200 kV, 4 mA) total body irradiation, using an Andrex Smart 225 Röntgen source (YXLON Int., Copenhagen, Denmark) with a 6-mm aluminum filter, 1 day before transplantation. Bone marrow was isolated by flushing the femurs and tibias from female SR-BI−/− mice or female WT littermates with phosphate-buffered saline (PBS). Single-cell suspensions were prepared by passing the cells through a 30-μm nylon gauze. Irradiated recipients received 0.5×10^7 bone marrow cells by intravenous injection into the tail vein.

Assessment of Chimerism

The hematological chimerism of the transplanted mice was determined in genomic DNA from bone marrow by PCR. Two oligonucleotides were used for PCR amplification to detect both the WT and the null mutant SR-BI gene simultaneously, as described above.

Lipid Analyses

After an overnight fasting-period, ~100 μl of blood was drawn from each individual mouse by tail bleeding. The concentrations of free cholesterol and cholesteryl esters in serum were determined using enzymatic colorimetric assays (Roche Diagnostics, Mannheim, Germany). The distribution of lipids over the different lipoproteins in serum was determined by fractionation of 30 μl of serum of each mouse using a Superose 6 column (3.2 × 30 mm, Smart-System; Pharmacia, Uppsala, Sweden). Total cholesterol content of the effluent was determined using enzymatic colorimetric assay (Roche Diagnostics). Erythrocyte cholesterol content was analyzed using enzymatic colorimetric assays after lipid extraction. Briefly, 30 μl of washed and packed erythrocytes were extracted by subsequent addition of 100 μl of methanol and 100 μl of chloroform. The extract supernatants were evaporated under nitrogen, redissolved in 30 μl of ethanol, and used for analysis.

Histological Analysis of the Aortic Root

To analyze the development of atherosclerosis at the aortic root, transplanted LDLr−/− mice were sacrificed after 4, 6, 9, and 12 weeks of feeding the Western-type diet, respectively. Transplanted WT mice were fed a high-cholesterol/cholate diet for 10 or 12 weeks before sacrifice. The arterial tree was perfused *in situ* with PBS (100 mmHg) for 20 minutes via a cannula in the left ventricular apex. The heart plus aortic root and descending aorta were excised and stored in 3.7% neutral-buffered formalin (Formal-fixx; Shandon Scientific Ltd., UK). The athero-

sclerotic lesion areas in oil red O-stained cryostat sections of the aortic root were quantified using the Leica image analysis system, consisting of a Leica DMRE microscope coupled to a video camera and Leica Qwin Imaging software (Leica Ltd., Cambridge, UK). Mean lesion area (in μm^2) was calculated from 10 oil red O-stained sections, starting at the appearance of the tricuspid valves. For morphological analysis, sections were stained with Masson's Trichrome Accustain according to manufacturer's instructions (Sigma).

Macrophage Cholesterol Efflux Studies

Thioglycollate-elicited macrophages were isolated from SR-BI+/+ and SR-BI−/− mice 5 days after injection of 1 ml of Brewer's thioglycollate medium (Difco, Detroit, MI). After washing, cells were seeded on 24-well plates at a density of 0.5×10^6 cells in 500 μl of Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (w/v) bovine calf serum, 2 mmol/L L-glutamine, 100 μg/ml streptomycin, and 100 IU/ml penicillin. After 1 hour, nonadherent cells were removed by washing. After 2 days in culture, the cells were washed and incubated with 0.5 μCi/ml ^{3}H-cholesterol (Amersham Biosciences, Buckinghamshire, UK) in DMEM/0.2% bovine serum albumin for 24 hours at 37°C to load the cells with cholesterol. In addition, an *in vivo* method was developed to load peritoneal macrophages with ^{3}H-cholesterol, allowing the study of cholesterol efflux with minimal culture times. Briefly, SR-BI+/+ and SR-BI−/− mice, fed regular chow diet, were injected intraperitoneally with 1 ml of 3% Brewer's thioglycollate medium (Difco). After 5 days, the elicited SR-BI+/+ and SR-BI−/− peritoneal macrophages were labeled *in vivo* by intraperitoneal injection of ^{3}H-cholesterol. The injection sample was prepared by dissolving 6.25 μCi of ^{3}H-cholesterol in 6.25 μl of ethanol and subsequent addition of 500 μl PBS at 37°C. At 3.5 hours after injection, peritoneal macrophages were harvested and seeded on 24-well plates at a density of 0.5×10^6 cells in 500 μl of DMEM/0.2% bovine serum albumin. After 1 hour, nonadherent cells were removed by washing.

Cholesterol efflux from macrophages, loaded with cholesterol *in vitro* or *in vivo*, was subsequently studied by incubation of the cells with DMEM/0.2% free fatty acid (FFA) free bovine serum albumin alone, or supplemented with either 10 μg/ml of apolipoprotein AI (Calbiochem) or 50 μg/ml of human HDL (isolated according to Redgrave and colleagues[27]). After a 24-hour efflux period, radioactivity in the cells and medium was determined by liquid scintillation counting. Cholesterol efflux is defined as $(dpm_{medium}/dpm_{cell} + dpm_{medium}) \times 100\%$.

Macrophage Association Studies

β-Very low-density lipoprotein (VLDL) was isolated from rats that were fed a diet containing 2% cholesterol, 5% olive oil, and 0.5% cholate (Hope Farms) for 2 weeks. After overnight fasting, blood was collected from the abdominal aorta and β-VLDL was isolated using a discontinuous potassium bromide gradient, as described by Redgrave and colleagues.[27] The fraction of $d < 1.006$ = g/ml was isolated and dialyzed against bovine serum albumin/1 mmol/L ethylenediaminetetraacetic acid. The β-VLDL consisted for 14.6 ± 2.1% of triacylglycerols, 15.8 ± 1.1% phospholipids, 49.4 ± 3.1% cholesteryl esters, 9.9 ± 1.0% free cholesterol, and 10.3 ± 0.7% protein and displayed β-mobility on agarose gels. The isolated β-VLDL was labeled with ^{125}I at pH = 10.0 according to McFarlane,[28] modified as described earlier.[29] Human HDL was isolated by differential ultracentrifugation as described by Redgrave and colleagues[27] and subsequently labeled with ^{3}H-cholesterol oleate (Amersham) by exchange from donor particles as reported previously.[30] Thioglycollate-elicited macrophages were isolated 5 days after injection of 1 ml of Brewer's thioglycollate medium. After washing, cells were either directly used for association studies or seeded on 24-well plates as described above and used for association studies after 3 days in culture. Association of β-VLDL or HDL to the SR-BI+/+ and SR-BI−/− macrophages was determined by incubation with the indicated amounts of ^{125}I-β-VLDL or ^{3}H-CE HDL in DMEM/2% bovine serum albumin at 37°C. After 3 hours of incubation, the cells were washed and lysed in 0.1 mol/L NaOH, and cell protein content was determined according to Lowry and colleagues[31] Finally, the cell-associated radioactivity was determined.

Statistical Analyses

Statistical analyses were performed using the unpaired Student's *t*-test (Instat GraphPad software, San Diego, CA).

Results

Selective Disruption of SR-BI on Bone Marrow-Derived Cells in WT and LDL Receptor-Deficient Mice

To assess the role of bone marrow-derived SR-BI in atherosclerotic lesion development, we used the technique of bone marrow transplantation to selectively disrupt SR-BI in hematopoietic cells. Bone marrow from previously generated SR-BI-deficient mice was transplanted into either WT or LDLr−/− mice, which represent established models for the development of atherosclerosis. Successful reconstitution of recipients with hematopoietic donor cells was established by PCR-assisted amplification using primers specific for the WT and the null mutant SR-BI gene (Figure 1). Genomic DNA isolated from the recipient mice, transplanted with bone marrow from SR-BI knockout mice contained a prominent band indicative of the disrupted allele, whereas only a faint WT band was visible. The control transplanted mice only displayed the WT band, indicating that the bone marrow transfer was successful.

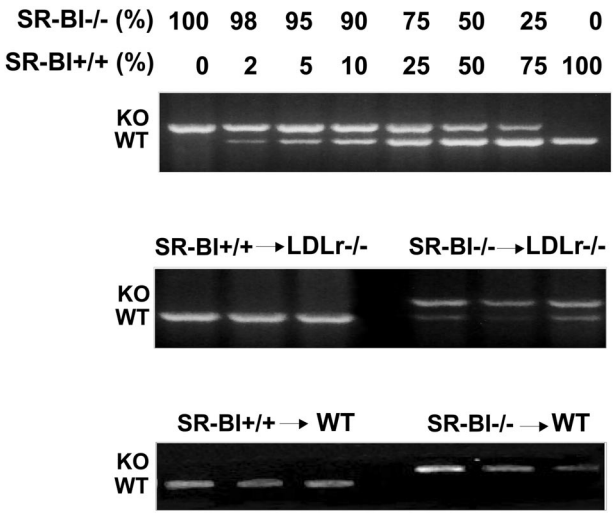

Figure 1. Verification of success of bone marrow transplantation. Verification of successful reconstitution with donor hematopoietic cells by PCR amplification of the WT and the null mutant SR-BI gene using genomic DNA isolated from bone marrow.

Effect of Disruption of SR-BI on Bone Marrow-Derived Cells in LDLr−/− Mice

During the course of the experiment, the effects of disruption of SR-BI in bone marrow-derived cells in LDLr−/− mice on serum lipid levels were carefully monitored. On regular chow diet, the majority of the cholesterol in LDLr−/− mice is transported by LDL and HDL (Figure 2). Disruption of SR-BI in bone marrow-derived cells did not significantly affect serum cholesterol levels (Table 1) nor the distribution of cholesterol over the lipoproteins in the circulation. To induce atherosclerotic lesion development, the transplanted mice were fed a Western-type diet, containing 0.25% cholesterol and 15% fat, starting at 8 weeks after transplantation. On challenging the mice with the Western-type diet, serum cholesterol levels increased approximately sixfold in both groups of mice, primarily because of an increase in VLDL and LDL cholesterol (Table 1, Figure 2). Under these conditions, also no effect of SR-BI disruption in bone marrow-derived cells on serum cholesterol levels was observed. Further-

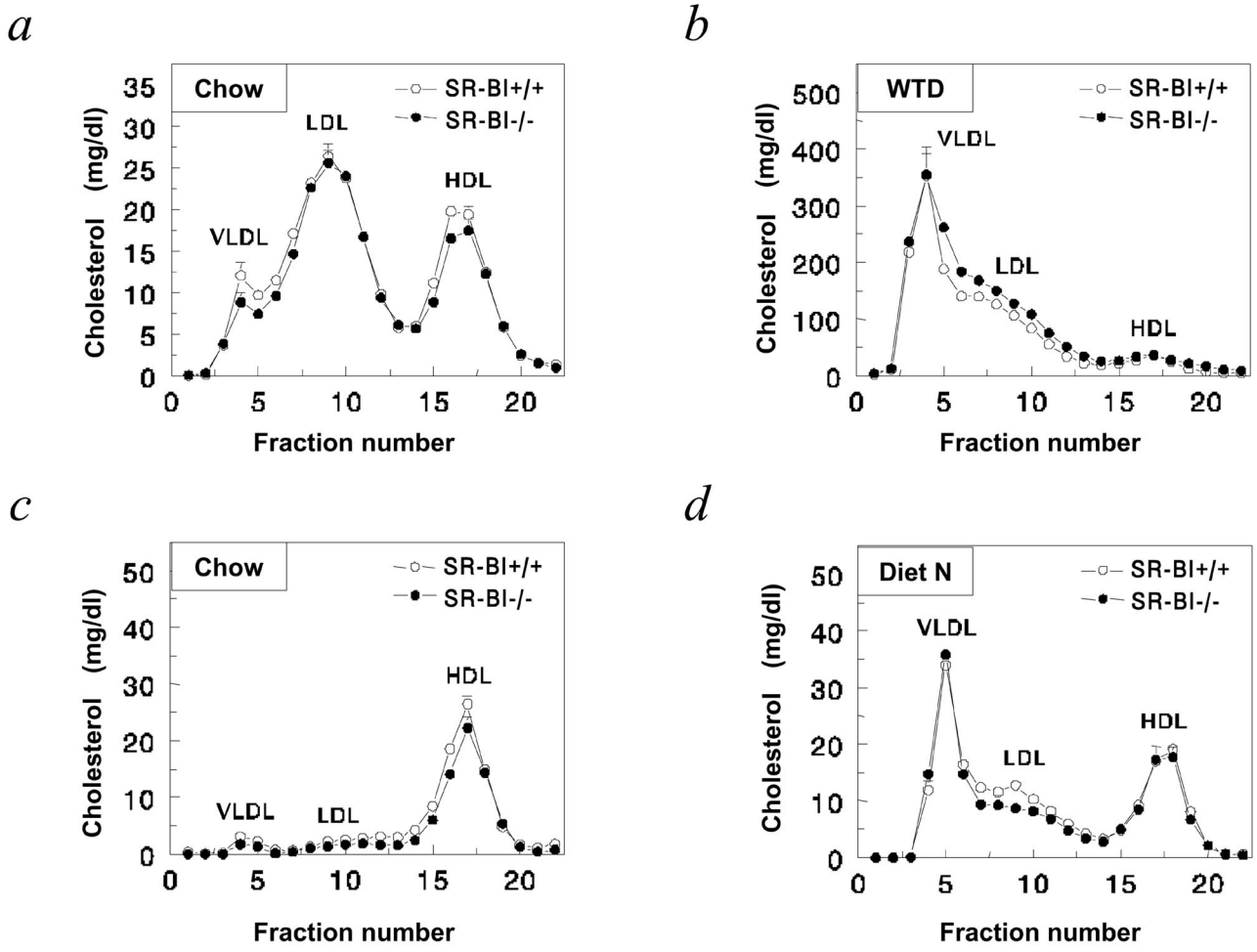

Figure 2. Effect of disruption of SR-BI in bone marrow-derived cells on serum cholesterol distribution in WT and LDL receptor-deficient (LDLr−/−) mice. Blood samples were drawn after an overnight fast at 8 weeks after transplant while feeding regular chow diet (chow) and at 16 weeks after bone marrow transplantation after 8 weeks of feeding a high-cholesterol/cholate (diet N) or Western-type diet (WTD). Sera from individual mice were loaded onto a Superose 6 column and fractions were collected. Fractions 3 to 7 represent VLDL; fraction 8 to 14, LDL; and fractions 15 to 19, HDL, respectively. The distribution of cholesterol over the different lipoproteins in LDLr−/− (**a** and **b**) or WT recipients (**c** and **d**) transplanted with SR-BI+/+ (**open circles**) or SR-BI−/− (**filled circles**) bone marrow is shown. Values represent the mean ± SEM of at least eight mice. No statistically significant differences were observed.

Table 1. Effect of Disruption of SR-BI in Bone Marrow-Derived Cells on Serum Lipid Levels in LDLr−/− and Wild-Type Mice

Mice	Time (weeks)	Diet	Free cholesterol (mg/dl)	Cholesteryl esters (mg/dl)	HDL cholesterol (mg/dl)
SR-BI+/+ → LDLr−/−	Baseline	Chow	102 ± 6	347 ± 34	ND
	8	Chow	113 ± 5	407 ± 24	205 ± 10
	16	WTD	468 ± 27	2659 ± 143	392 ± 45
SR-BI−/− → LDLr−/−	Baseline	Chow	95 ± 5	388 ± 26	ND
	8	Chow	105 ± 4	404 ± 19	184 ± 14
	16	WTD	541 ± 69	2690 ± 263	468 ± 51
SR-BI+/+ → WT	Baseline	Chow	24 ± 2	116 ± 12	ND
	8	Chow	33 ± 2	118 ± 8	78 ± 4
	16	Diet N	61 ± 5	295 ± 11	62 ± 7
SR-BI−/− → WT	Baseline	Chow	24 ± 2	103 ± 5	ND
	8	Chow	27 ± 2	109 ± 7	64 ± 6
	16	Diet N	46 ± 3	292 ± 14	58 ± 5

Serum lipids were measured in LDLr−/− and SR-BI+/+ wild-type (WT) mice before transplantation (baseline) and at 8 and 16 weeks after transplantation with SR-BI+/+ or SR-BI−/− bone marrow. At 8 weeks after transplantation, the regular chow diet was switched to a Western-type diet (WTD) or a high-cholesterol/cholate diet (diet N). Data represent mean ± SEM of at least eight mice. No significant differences between SR-BI+/+ → LDLr−/− *versus* SR-BI+/+ → LDLr−/− were noticed, while also the SR-BI+/+ → WT *versus* SR-BI+/+ → WT were not significantly different.

more, the cholesterol content of erythrocytes was comparable in both groups (0.59 ± 0.05 μg/μl in SR-BI+/+ → LDLr−/− mice *versus* 0.63 ± 0.06 μg/μl in SR-BI−/− → LDLr−/− chimeras).

Atherosclerotic lesion development was analyzed in the aortic root of SR-BI+/+ → LDLr−/− mice and in SR-BI−/− → LDLr−/− chimeras after 4, 6, 9, and 12 weeks of Western-type diet feeding (Figure 3). After 4 and 6 weeks of diet feeding initial lesions are observed with isolated macrophage foam cells. After 9 weeks, lesions have progressed to intermediate lesions with multiple foam cell layers, small extracellular lipid pools, and a thin fibrous cap. After 12 weeks, advanced lesions are observed with a (extracellular) lipid core and fibrotic layer and frequently observed calcification, cholesterol clefts, and necrosis. Disruption of SR-BI in bone marrow-derived cells induced lesion development after 9 and 12 weeks on the Western-type diet. At 9 weeks, the mean atherosclerotic lesion area was increased twofold as a result of SR-BI deficiency in bone marrow-derived cells [588 ± 81 × 10^3 μm^2 in SR-BI−/− → LDLr−/− mice (n = 10) *versus* 288 ± 49 × 10^3 μm^2 in SR-BI+/+ → LDLr−/− mice (n = 18), P = 0.0034]. At 12 weeks, the mean atherosclerotic lesion area was increased 1.5-fold as a result of SR-BI deficiency in bone marrow-derived cells from 480 ± 57 × 10^3 μm^2 in SR-BI+/+ → LDLr−/− mice (n = 12) to 736 ± 48 × 10^3 μm^2 in SR-BI−/− → LDLr−/− mice (n = 23), P = 0.0055. Morphological analysis of sections stained with Masson's Trichrome revealed that lesions of mice reconstituted with SR-BI−/− bone marrow appear more advanced with larger necrotic cores (Figure 3c). At 6 weeks, representing a time point with initial lesions, the effect of absence of SR-BI in bone marrow-derived cells on lesion development was only marginal and failed to reach statistical significance [211 ± 32 × 10^3 μm^2 in SR-BI−/− → LDLr−/− mice (n = 6) *versus* 151 ± 34 × 10^3 μm^2 in SR-BI+/+ → LDLr−/− mice (n = 11), P = 0.2618]. Interestingly, after only 4 weeks of Western-type diet feeding disruption of SR-BI in bone marrow-derived cells resulted in a twofold reduction in mean atherosclerotic lesion area [49 ± 9 × 10^3 μm^2 in SR-BI−/− → LDLr−/− mice (n = 5) *versus* 99 ± 14 × 10^3 μm^2 in SR-BI+/+ → LDLr−/− mice (n = 5), P = 0.0125]. Because SR-BI facilitated atherosclerosis in LDLr−/− mice after only 4 weeks of Western-type diet feeding, in contrast to the protective role at 9 and 12 weeks, this prompted us to study the effect in a second model of atherosclerosis with small fatty streak lesions: WT mice on a high-cholesterol/cholate diet.

Effect of Disruption of SR-BI in Bone Marrow-Derived Cells in WT Mice

On regular chow diet, the majority of the cholesterol in WT mice is transported by HDL (Figure 2). Disruption of SR-BI in bone marrow-derived cells did not significantly affect serum cholesterol levels (Table 1) or the distribution of cholesterol over the lipoproteins in the circulation (Figure 2). To induce atherosclerotic lesion development, the transplanted mice were fed a high-cholesterol diet, containing 1% cholesterol, 15% fat, and 0.5% cholate, starting at 8 weeks after transplantation. On challenging the mice with the high-cholesterol/cholate diet, serum cholesterol levels increased approximately threefold in both groups of mice, primarily because of an increase in VLDL and LDL cholesterol (Table 1, Figure 2).

Atherosclerotic lesion development was analyzed in the aortic root of SR-BI+/+ → WT mice and in SR-BI−/− → WT chimeras after 10 weeks of high-cholesterol/cholate diet feeding (Figure 4). As observed in the LDLr−/− mice fed the Western-type diet for only 4 weeks, disruption of SR-BI in bone marrow-derived cells inhibited atherosclerotic lesion development in WT mice [8.3 ± 1.8 × 10^3 μm^2 in SR-BI+/+ → WT mice (n = 9) *versus* 3.0 ± 0.6 × 10^3 μm^2 in SR-BI−/− → WT mice (n = 7), P = 0.0256]. In a separate independent transplantation experiment also a threefold decrease in lesion size was observed in the absence of bone marrow-derived SR-BI after 12 weeks of the high-cholesterol/cholate diet feeding [56 ± 14 × 10^3 μm^2 in SR-BI+/+ → WT mice (n = 6) *versus* 18 ± 4 × 10^3 μm^2 in SR-BI−/− → WT mice (n = 6), P = 0.0237]. Thus, apparently also under these

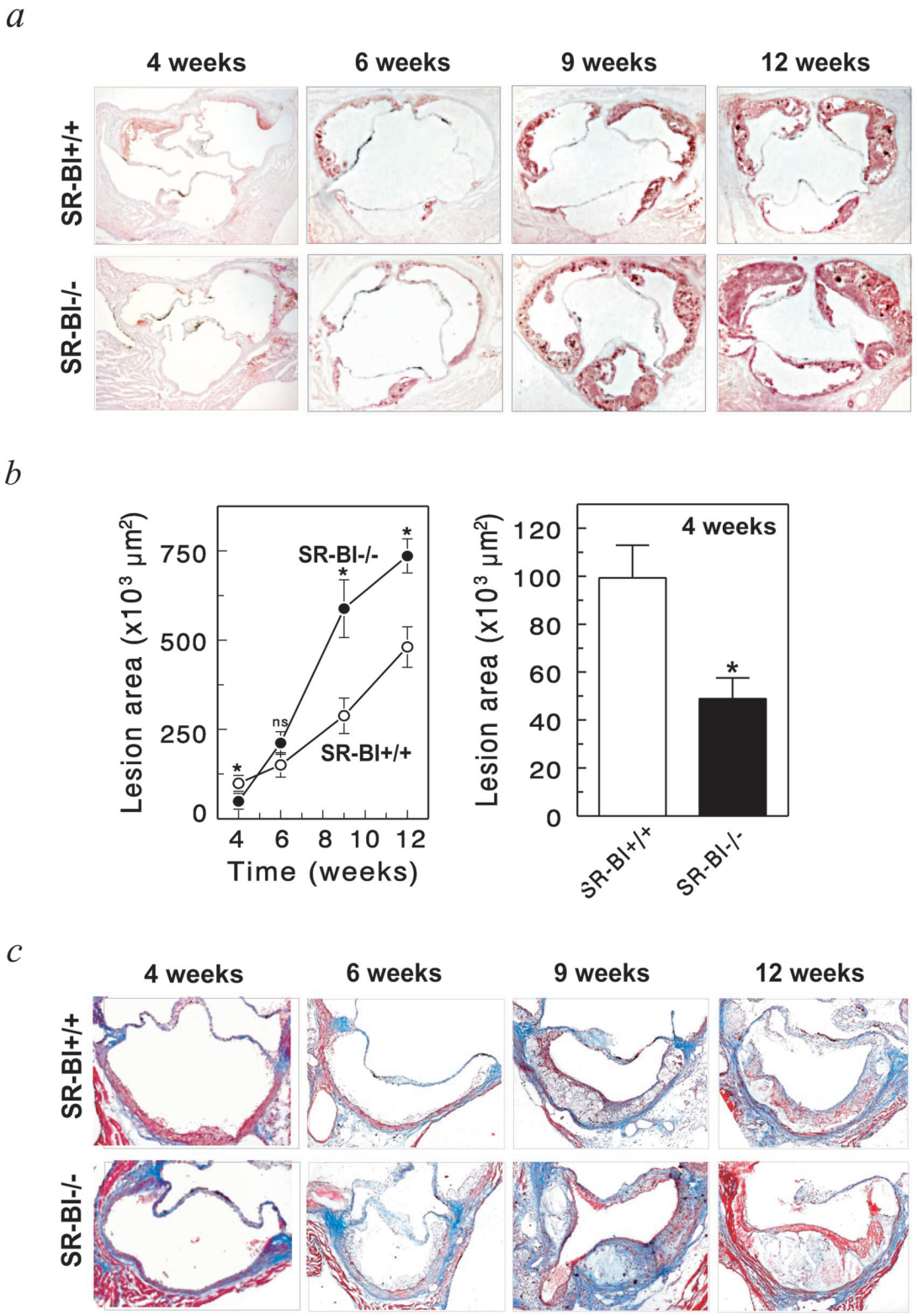

Figure 3. Disruption of SR-BI in bone marrow-derived cells induces atherosclerotic lesion development in LDLr−/− mice. Formation of atherosclerotic lesions was determined at the aortic root of SR-BI+/+ → LDLr−/−, and SR-BI−/− → LDLr−/− chimeras that were fed a Western-type diet (0.5% cholesterol, 15% fat) for 4, 6, 9, and 12 weeks, respectively. **a** and **b:** The mean lesion area was calculated from oil red O-stained cross-sections of the aortic root at the level of the tricuspid valves. Values represent the mean ± SEM of at least six mice. *, Statistically significant difference, $P < 0.01$, as compared to SR-BI+/+ → LDLr−/− mice. **c:** Morphological staining of atherosclerotic lesions in the aortic root with Masson's Trichrome Accustain, which stains cytoplasm and muscle fibers red and collagen blue. Original magnifications, ×50.

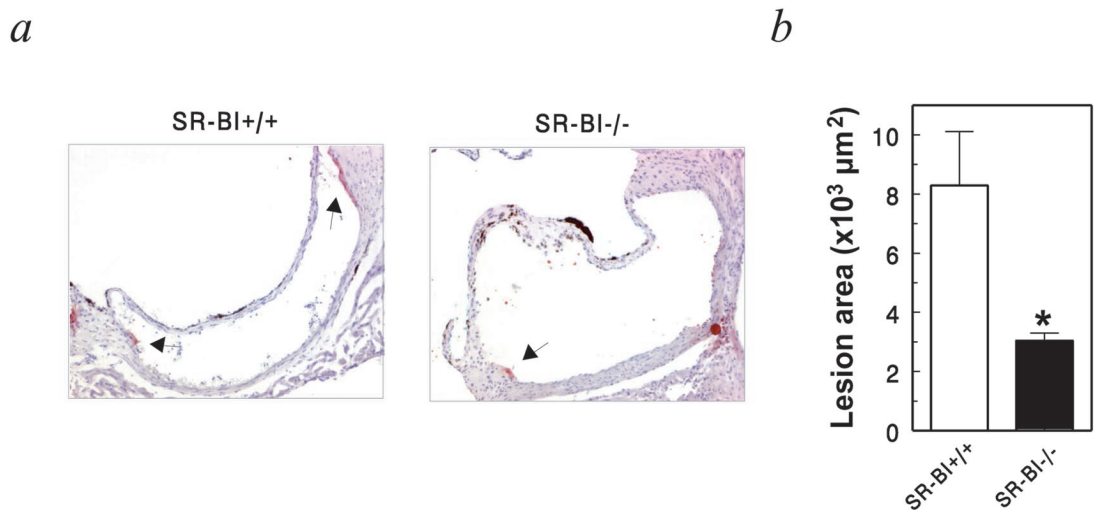

Figure 4. Disruption of SR-BI in bone marrow-derived cells inhibits atherosclerotic lesion development in WT mice. **a:** Formation of atherosclerotic lesions was determined at the aortic root of SR-BI+/+ → WT and SR-BI−/− → WT chimeras that were fed a high-cholesterol/cholate diet (1% cholesterol, 15% fat, 0.5% cholate) for 10 weeks. **b:** The mean lesion area was calculated from oil red O-stained cross-sections of the aortic root at the level of the tricuspid valves. Values represent the mean ± SEM of at least seven mice. *, Statistically significant difference of $P < 0.01$, as compared to SR-BI+/+ → WT. Original magnifications, ×100.

conditions the presence of SR-BI on bone marrow-derived cells facilitated lesion formation.

Dual Role of Macrophage SR-BI in Cellular Cholesterol Homeostasis

SR-BI is a multifunctional receptor capable of binding a wide array of native and modified lipoproteins. To investigate the relative importance of macrophage SR-BI in the uptake of atherogenic lipoproteins and efflux of cholesterol, we analyzed the effect of macrophage SR-BI deficiency on the association of β-VLDL and HDL, as well as the efflux of cholesterol to apoAI and HDL (Figure 5). Interestingly, deficiency for SR-BI resulted in a reduced association of the atherogenic lipoprotein β-VLDL to macrophages (Figure 5a). Surprisingly, no effect of SR-BI deficiency was observed on cholesterol efflux or association of HDL-CE using macrophages that had been cultured for 3 days (data not shown). However, using freshly isolated macrophages, we did observe a 28% reduction of HDL-CE association in the absence of macrophage SR-BI (Figure 5b). Therefore, to analyze the effect of macrophage SR-BI deficiency on cholesterol efflux under conditions with minimized cultures times, thioglycollate-elicited macrophages were loaded *in vivo* with ^{3}H-cholesterol by intraperitoneal injection of ^{3}H-cholesterol. After isolation of the macrophages, cholesterol efflux was subsequently studied *in vitro*. Interestingly, under these conditions macrophage SR-BI deficiency resulted in 20% reduction of cholesterol efflux to HDL, whereas efflux to apoAI was not affected (Figure 5c). Thus, depending on the culture conditions and the extracellular lipoproteins present, SR-BI might either mediate the uptake of cholesterol-rich lipoproteins or induce cholesterol efflux to

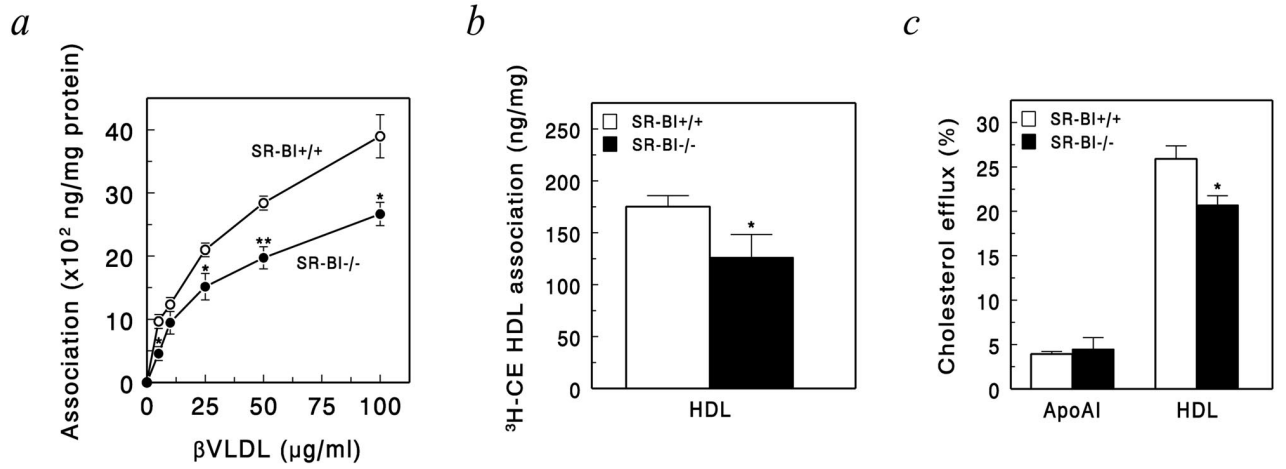

Figure 5. Dual role for macrophage SR-BI in cellular cholesterol homeostasis. **a:** Association of ^{125}I-βVLDL to SR-BI+/+ and SR-BI−/− peritoneal macrophages after 3 hours of incubation at 37°C at the indicated concentrations after 3 days in culture ($n = 4$). **b:** Association of 50 µg/ml of ^{3}H-cholesteryl ester-labeled HDL to freshly isolated peritoneal macrophages after 3 hours of incubation at 37°C ($n = 3$). **c:** ApoAI (10 µg/ml) and HDL (50 µg/ml) induced cellular cholesterol efflux from *in vivo* ^{3}H-cholesterol-labeled peritoneal macrophages isolated from SR-BI+/+ or SR-BI−/− mice, analyzed for 24 hours ($n = 3$). Values are means ± SEM of three or four individual mice. Statistically significant difference of *, $P < 0.05$ and **, $P < 0.01$ as compared to SR-BI+/+ macrophages.

HDL, indicating a dual role for macrophage SR-BI in cellular cholesterol homeostasis.

Discussion

Several lines of evidence indicate an anti-atherogenic role for scavenger receptor BI (SR-BI) in atherogenesis. Huszar and colleagues[32] showed that LDL receptor-deficient mice with an attenuated expression of SR-BI are more susceptible to atherosclerotic lesion development. Furthermore, disruption of SR-BI in WT[33] as well as in LDLr−/− mice[22] results in a highly increased susceptibility to atherosclerotic lesion development. When crossbred onto the apolipoprotein E knockout (apoE−/−) background, SR-BI deficiency leads to severe cardiac dysfunction and premature death.[34,35] Hepatic overexpression of SR-BI, on the other hand, protects against the development of atherosclerosis.[36–38]

This anti-atherogenic function of SR-BI can primarily be attributed to its role in the uptake of HDL cholesteryl esters by the liver. SR-BI, however, is also expressed in lipid-laden macrophages in atherosclerotic lesions,[10,19,20] indicating that SR-BI might play an additional important role locally in the arterial wall. In this study we show that SR-BI in bone marrow-derived cells, including macrophages, directly affects atherosclerotic lesion development. Interestingly, SR-BI in bone marrow-derived cells lowered atherosclerotic lesion development in LDLr−/− mice after 9 and 12 weeks of Western-type diet feeding. At 6 weeks, no significant effect was observed, while after only 4 weeks of Western-type diet-feeding SR-BI in bone marrow-derived cells facilitated atherogenesis. This was confirmed in WT mice that only develop small fatty streak lesions on a high-cholesterol/cholate diet. While this article was under preparation, two articles appeared demonstrating that SR-BI in bone marrow-derived cells lowered atherosclerotic lesion development in LDLr−/−[22] and apoE−/−[23] mice. Our data in LDLr−/− mice at 9 and 12 weeks of Western-type diet feeding are in agreement with this interpretation. However, we currently show that SR-BI in bone marrow-derived cells has a dual role in atherosclerotic lesion development and that, depending on the stage of atherosclerotic lesion development, SR-BI in bone marrow-derived cells is either anti-atherogenic or pro-atherogenic.

SR-BI is a multifunctional receptor capable of binding a wide array of native and modified lipoproteins. Its primary function is promoting the selective uptake of cholesteryl esters from HDL.[39] In addition to its role as a HDL receptor, SR-BI functions as a binding-site for atherogenic lipoproteins, including native LDL and modified LDL.[14–16] Adenoviral overexpression of SR-BI in liver reduces serum VLDL and LDL cholesterol levels in C57BL/6 mice[36,40] and reverses fibrate-induced hypercholesterolemia in apoE-deficient mice.[41] Furthermore, SR-BI transgenics display reduced VLDL and LDL levels,[37,42] whereas disruption of SR-BI in apoE−/− mice results in an increase in circulating VLDL and LDL levels.[34] Thus, SR-BI may also play a role in the metabolism of apoB-containing lipoproteins *in vivo*. In this study we show that the association of β-VLDL, a highly atherogenic lipoprotein, to macrophages in the presence of SR-BI is increased. Previously, we have shown that in the absence of the LDL receptor, a class B scavenger receptor, most likely SR-BI, is the primary receptor for the association of β-VLDL to macrophages and contributes to selective uptake of cholesteryl esters from β-VLDL.[43] This function as a receptor for the uptake of cholesteryl esters from atherogenic apoB-containing lipoproteins is expected to induce foam cell formation and thus facilitate atherosclerotic lesion development. Indeed, the presence of macrophage SR-BI does facilitate atherosclerotic lesion development in LDLr−/− mice fed a Western-type diet for only 4 weeks and in WT mice on a high-cholesterol/cholate diet.

In LDLr−/− mice fed Western-type diet for 9 and 12 weeks, however, a protective role in atherosclerotic lesion development was observed. De la Llera-Moya and colleagues[10] were the first to demonstrate that overexpression of SR-BI in cultured cells increases the rate of cholesterol efflux from cells to HDL particles. Furthermore, the rate of cholesterol efflux directly correlates with the level of SR-BI expression on a variety of cultured cells. In agreement, we found that cholesterol efflux from *in vivo* cholesterol-loaded SR-BI-deficient macrophages to HDL is impaired, whereas no effect on efflux to apoAI could be demonstrated. This is consistent with the generally accepted model that lipid-free apoAI is the ligand for cholesterol efflux via ABCA1, whereas SR-BI effluxes cholesterol to fully lipidated HDL.[44,45] The function of SR-BI as a mediator of cholesterol efflux could explain the observed increase in atherosclerosis in the absence of macrophage SR-BI as observed in LDLr−/− mice. Several lines of evidence support that SR-BI can mediate the bidirectional movement of free cholesterol (FC) between cells and lipoproteins down a FC concentration gradient.[10–12,46] Depending on the direction of the FC concentration gradient, either net efflux or net influx of cholesterol will occur. This leads to a unique function of macrophage SR-BI, that it can facilitate initial lesion formation and inhibit more advanced lesion formation when foam cells are heavily loaded with free cholesterol.

In addition to SR-BI, macrophages express ABCA1 and apoE that play an important role in sterol efflux pathways.[3–6] Previously, we have shown that macrophage ABCA1 deficiency[47] as well as apoE deficiency[48] induce atherosclerotic lesion development. Chen and colleagues[49] showed that SR-BI inhibits ABCA1-mediated cholesterol efflux by facilitating the reuptake of cholesterol (but not phospholipid) effluxed to apoAI. Furthermore, enhanced SR-BI expression in macrophages inhibits apoE-mediated cholesterol efflux by accelerating the degradation of newly synthesized apoE.[50] Depending on the stage of atherosclerotic lesion development, these pathways might potentially interact differently, thereby modulating the direction of net sterol flux in macrophages.

The pathogenesis of atherosclerosis involves a complicated sequence of events in which various cell types, including endothelial cells, smooth muscle cells, and

macrophages interact.[1] In addition to its function in bidirectional cholesterol transport, SR-BI has also been implicated in the delivery of anti-oxidants as α-tocopherol,[51,52] endothelium- and nitric-oxide-dependent arterial relaxation,[53,54] and the removal of apoptotic cells.[55] Progression of atherosclerotic lesions is characterized by an ongoing chronic inflammatory reaction and extensive cellular necrosis and apoptosis. It is therefore possible that impaired phagocytic activity of lesion macrophages because of the absence of SR-BI may have led to impaired clearance of apoptotic material, thereby inducing more excessive inflammatory responses and more rapid progression of the atherosclerotic lesion in absence of bone marrow-derived SR-BI.

Finally, other bone marrow-derived cells expressing SR-BI, in addition to macrophages, might have contributed to the effects at different stages of atherosclerotic lesion development. SR-BI deficiency is associated with impaired erythrocyte maturation as a result of increased cellular cholesterol levels.[56] Selective disruption of SR-BI in bone marrow-derived cells, however, did not affect the erythrocyte cholesterol content. This is in agreement with recent data from Covey and colleagues.[22] Recently, it was also shown that human platelets express CLA-1, the human homologue of SR-BI and that the levels of CLA-1 expression correlated inversely with platelet aggregation.[57] No evidence of thrombosis was found in atherosclerotic lesions of mice reconstituted with SR-BI-deficient bone marrow. It is thus unlikely that altered erythrocyte or platelet function might have contributed to atherosclerotic lesion development in mice reconstituted with SR-BI-deficient bone marrow.

In conclusion, we have demonstrated that SR-BI in bone marrow-derived cells, including macrophages, has a dual role in atherosclerotic lesion development. This will probably also be relevant for the pathogenesis of atherosclerosis in humans. CLA-1, the human homologue of murine SR-BI is strongly induced on differentiation from monocytes into macrophages.[19,20] Furthermore, in human carotid atherosclerotic lesions, CLA-1-positive staining is observed in the subendothelial region and the lipid core, co-localizing with specific macrophage markers.[19,20] Thus, high levels of CLA-1/SR-BI are present in both human and murine atherosclerotic lesions, suggesting also a possible dual role for this scavenger receptor in atherosclerotic lesion development in humans.

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