# Reduced Atherosclerotic Lesions in Mice Deficient for Total or Macrophage-Specific Expression of Scavenger Receptor-A

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*Abstract*—The absence of the scavenger receptor A (SR-A)-I/II has produced variable effects on atherosclerosis in different murine models. Therefore, we examined whether SR-AI/II deficiency affected atherogenesis in C57BL/6 mice, an inbred strain known to be susceptible to diet-induced atherosclerotic lesion formation, and whether the deletion of macrophage SR-AI/II expression would modulate lesion growth in C57BL/6 mice and LDL receptor (LDLR)$^{-/-}$ mice. SR-AI/II–deficient (SR-AI/II$^{-/-}$) female and male mice on the C57BL/6 background were challenged with a butterfat diet for 30 weeks. No differences were detected in plasma lipids between SR-AI/II$^{-/-}$ and SR-AI/II$^{+/+}$ mice, whereas both female and male SR-AI/II$^{-/-}$ mice had a tremendous reduction (81% to 86%) in lesion area of the proximal aorta compared with SR-AI/II$^{+/+}$ mice. Next, to analyze the effect of macrophage-specific SR-AI/II deficiency in atherogenesis, female C57BL/6 mice were lethally irradiated, transplanted with SR-AI/II$^{-/-}$ or SR-AI/II$^{+/+}$ fetal liver cells, and challenged with the butterfat diet for 16 weeks. In a separate experiment, male LDLR$^{-/-}$ mice were reconstituted with SR-AI/II$^{-/-}$ or SR-AI/II$^{+/+}$ fetal liver cells and challenged with a Western diet for 10 weeks. No significant differences in plasma lipids and lipoprotein profiles were noted between the control and experimental groups in either experiment. SR-AI/II$^{-/-}$→C57BL/6 mice, however, had a 60% reduction in lesion area of the proximal aorta compared with SR-AI/II$^{+/+}$→C57BL/6 mice. A similar level of reduction (60%) in lesion area was noted in the proximal aorta and the entire aorta en face of SR-AI/II$^{-/-}$→LDLR$^{-/-}$ mice compared with SR-AI/II$^{+/+}$→LDLR$^{-/-}$ mice. These results demonstrate in vivo that SR-AI/II expression has no impact on plasma lipid levels and that macrophage SR-AI/II contributes significantly to atherosclerotic lesion formation. (*Arterioscler Thromb Vasc Biol*. 2000;20:2593-2599.)

**Key Words:** atherosclerosis ■ macrophages ■ scavenger receptor type A ■ foam cells formation ■ fetal liver cell transplantation

Scavenger receptors are a family of integral membrane glycoproteins that mediate binding and uptake of native and modified lipoproteins by the macrophage.[1,2] The first scavenger receptor cloned was the bovine scavenger receptor,[3] or scavenger receptor type A (SR-AI/II). The SR-AI/II are trimeric integral membrane glycoproteins[4,5] that are generated through alternative splicing of a single gene.[6] The uptake of modified-LDL cholesterol by SR-AI/II has been proposed to play a key role in the pathogenesis of atherosclerosis by promoting lipid accumulation and foam cell formation by the macrophage.[7] The SR-AI/II is expressed predominantly by macrophages[8,9] and is widely expressed by macrophages and foam cells of atherosclerotic lesions.[10–12] However, smooth muscle cells and endothelial cells may also express the SR-AI/II,[13,14] particularly in the presence of oxidative stress and certain growth factors in vitro[15] and in vivo.[16] The relative contributions of SR-AI/II expression by macrophages, endothelial cells, and smooth muscle cells to atherosclerotic lesion formation in vivo remain uncertain.

The development of mice with targeted disruption of the SR-AI/II gene provided an important model in which to examine the role of the SR-AI/II in atherosclerosis in vivo,[17] yet the results of these studies have been conflicting.[17–19] The absence of SR-AI/II in apoE-deficient (apoE$^{-/-}$) mice induced a 58% reduction in lesion size compared with control apoE$^{-/-}$ mice.[17] In contrast, mice deficient for both the SR-AI/II (SR-AI/II$^{-/-}$) and the LDL receptor (LDLR$^{-/-}$) had 28% and 23% reductions in aortic lesion area after 4 and 12 weeks, respectively, of a high-fat diet containing 1.25% cholesterol and 0.5% cholic acid compared with LDLR$^{-/-}$ mice wild-type for the SR-AI/II.[18] Although both of these studies support a proatherogenic role for the SR-AI/II, it is unclear why elimination of the SR-AI/II had a more pronounced effect on atherosclerosis in the apoE$^{-/-}$ mice than in the LDLR$^{-/-}$ mice.[20] Furthermore, apoE Leiden transgenic mice null for SR-AI/II showed a nonsignificant trend for an increase in lesion area with the development of more complex

lesions,[19] leading these authors to suggest that apoE modulates the effect of SR-AI/II deficiency on atherosclerosis.[19,21] Although it is possible that the role of the scavenger receptor in atherosclerosis is more relevant in the setting of apoE deficiency than in the presence of apoE or in LDLR deficiency, other factors, such as genetic background, may explain these results.[22]

In the present study, we investigated whether SR-AI/II deficiency affects atherogenesis in C57BL/6 mice, a strain of mice that is known to be susceptible to diet-induced atherosclerotic lesion formation, and whether the deletion of macrophage SR-AI/II expression modulates lesion growth in C57BL/6 mice and LDLR$^{-/-}$ mice. In recent years, we and others have demonstrated the usefulness of murine bone marrow transplantation to examine the role of macrophage gene expression in atherosclerotic lesion formation.[23–27] We recently extended this approach by using fetal liver cell (FLC) transplantation to reconstitute C57BL/6 mice with lipoprotein lipase–null macrophages.[28] After lethal irradiation, FLC transplantation results in reconstitution of the entire hematopoietic system.[29] Because SR-AI/II is expressed by macrophages, but not by other cells of the hematopoietic system, FLC transplantation will result in a de facto macrophage-specific knockout of SR-AI/II. Here, we used a similar approach to study the impact of macrophage SR-AI/II on atherosclerosis and lipoprotein metabolism.

In the present study, male and female SR-AI/II$^{-/-}$ mice on the C57BL/6 background fed an atherogenic diet for 30 weeks showed dramatic protection from atherosclerosis. In addition, the transplantation of SR-AI/II$^{-/-}$ FLCs into lethally irradiated C57BL/6 mice or LDLR$^{-/-}$ mice resulted in significant reductions in atherosclerotic lesion size, independent of changes in plasma lipids. These results demonstrate that macrophage SR-AI/II expression promotes foam cell formation and atherosclerosis in vivo and highlight the importance of genetic background in murine studies of atherosclerosis and lipoprotein metabolism.

## Methods

### Animal Procedures

Mice with targeted disruption of the SR-AI/II gene[17,30] were used after reaching at least the sixth backcross into C57BL/6 background. Recipient C57BL/6 and LDLR$^{-/-}$ mice were originally purchased from Jackson Laboratories Inc, and LDLR$^{-/-}$ mice were used at the 10th backcross or higher into C57BL/6 background. All mice were maintained in microisolator cages and fed a rodent chow diet that contained 4.5% fat (PMI No. 5010) and autoclaved acidified (pH 2.8) water. Atherogenic diets were (1) butterfat diet, containing 19.5% butterfat, 1.25% cholesterol, and 0.5% cholic acid (ICN) and (2) Western-type diet, containing 21% fat and 0.15% cholesterol (Teklad). Animal care and experimental procedures were performed according to the regulations of the Vanderbilt University Animal Care Committee.

### FLC Collection

FLCs were collected as described previously.[28] Briefly, female and male SR-AI/II$^{+/-}$ mice were mated, and on day 14 of gestation, the pregnant mice were killed and the livers were dissected from the embryos. A single-cell suspension was prepared in RPMI-1640 medium (GIBCO BRL) containing 2% FCS by passing liver tissue through syringes fitted with G21 and G25 needles, sequentially. The FLCs were cryopreserved in RPMI-1640 containing 10% DMSO and 25% FCS. To identify SR-AI/II genotype and the sex of the fetuses, the DNA from tail tissues was amplified by PCR with primer sets specific for SR-AI/II or *Zfy* gene of the Y chromosome as described previously.[28]

### FLC Transplantation

FLCs were thawed rapidly at 37°C, washed in RPMI-1640 containing 2% FBS, and counted. Eight-week-old female C57BL/6 and male LDLR$^{-/-}$ mice were lethally irradiated (9 Gy) from a cesium γ source, and 4 hours later, $5 \times 10^6$ cells in 0.3 mL RPMI-1640 medium was injected into the tail vein. Recipient mice were fed the rodent chow diet for 8 weeks during reconstitution of hematopoietic cells and then challenged with the atherogenic diets.

### Serum Lipid and Lipoprotein Analysis

Mice were fasted for 4 hours, and blood samples were collected via retro-orbital venous plexus puncture with the animals under metofane anesthesia. The serum concentrations of total cholesterol and triglycerides were determined with Sigma Chemical Co kits 352 and 339 adapted for microtiter plate assay. HDL cholesterol concentration was measured on an automated ACE analyzer with the Direct HDL Test (10981; Schiapparelli Biosystems, Inc). To analyze the serum lipoprotein profile, serum was subjected to fast-performance liquid chromatography (FPLC) analysis using a Superose 6 column from Pharmacia on an HPLC system model 600 (Waters) as previously described.[28]

### Immunocytochemistry

To detect macrophages and the SR-AI/II protein, 5-μm serial cryosections of the proximal aorta were fixed in cold acetone, immersed in PBS (pH 7.2), and incubated overnight at 4°C with either a monoclonal rat antibody to mouse SR-AI/II, 2F8 (a gift of Dr Siamon Gordon, University of Oxford, Oxford, UK)[31] or a rat antibody to mouse macrophages, MOMA-2 (Accurate Chemical & Scientific Corp).[32] The sections were treated with goat biotinylated antibodies to rat IgG (PharMingen) and incubated with avidin-biotin complex labeled with alkaline phosphatase (Vector Laboratory). Enzyme was visualized with Fast Red TR/Naphthol AS-NX substrate (Sigma Chemical Co). Nonimmune rat serum in the place of primary antibody was used as a negative control. Photomicroscopy was performed on a Zeiss Axiophot with Plan-FLUAR objectives

### Quantification of Arterial Lesions

Mice were killed while under anesthesia, and 30 mL saline was flushed through the left ventricle. The entire aorta was dissected for en face preparation as previously described.[33] The heart with the proximal aorta was embedded in OCT and snap-frozen in liquid N$_2$. Cryosections of the proximal aorta that were 10 μm thick were prepared starting at the aortic sinus and continuing 300 μm distally, according to the method of Paigen et al,[34] adapted for computer analysis.[23] Sections were stained with oil red O and counterstained with hematoxylin. The images of the aorta were captured and analyzed with an imaging system (KS 300 Release 2.0; Kontron Electronik GmbH).

### Statistical Analysis

Mean serum cholesterol levels that represented an average serum cholesterol level for individual mice fed the study diets were used for analysis of the correlation between serum cholesterol levels and the extent of atherosclerosis (SigmaStat 2.0; Jandel Scientific Inc). The statistical significance of differences in mean aortic lesion areas between the groups was determined with the Student's *t* test.

## Results

To analyze the role of SR-AI/II in atherosclerotic lesion formation, SR-AI/II$^{-/-}$ mice were backcrossed into the C57BL/6 background. Next, 10-week-old littermate mice were separated according to the PCR-based genotype (Figure I; please see http://atvb.ahajournals.org) into the experimental groups of SR-AI/II$^{-/-}$ females (n=13) and males (n=19) and control groups of SR-AI/II$^{+/+}$ females (n=9) and males (n=9), and these mice were challenged with the butterfat diet
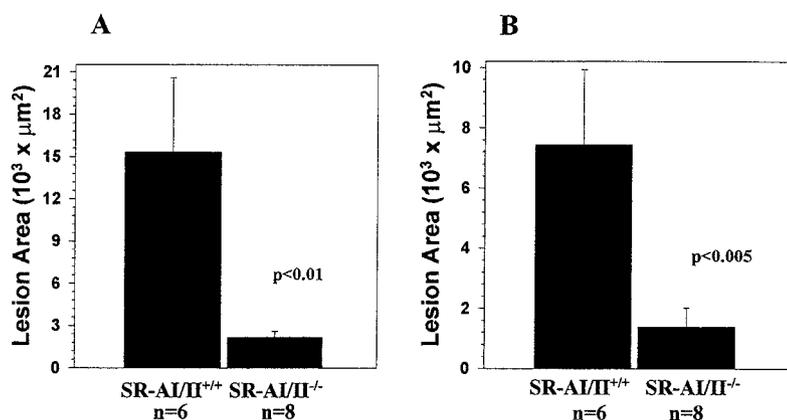
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## B

**Figure 1.** Quantification of atherosclerotic lesion area in the proximal aorta of SR-AI/II$^{+/+}$ and SR-AI/II$^{-/-}$ C57BL/6 mice. The extent of atherosclerotic lesions was quantified after 30 weeks of the butterfat diet using oil red O–stained cross sections of the proximal aorta of females (A) and males (B) with image analysis. Fifteen alternate 10-mm sections from the proximal aorta were analyzed for each mouse (n represents number of mice per group). Data are represented as the average (mean±SEM) lesion area per group measured in 15 sections per mouse.

for 30 weeks. Body weight measurements of the mice did not show significant differences between the groups at baseline or in the course of the diet (data not shown). In both females and males, total plasma cholesterol and triglyceride levels increased on the butterfat diet with no sustained differences between the groups at the major time points (Tables I and II; please see http://atvb.ahajournals.org). After 30 weeks on the butterfat diet, mean±SEM serum cholesterol levels in female experimental and control mice were 148±17 and 145±9 mg/dL, respectively, and in the male experimental and control mice, the levels were 167±19 and 156±11 mg/dL, respectively.

The extent of atherosclerosis in the proximal aorta was examined after 30 weeks on the diet. All of the mice developed moderate fatty streak lesions localized exclusively in the proximal aorta. These lesions contained predominantly macrophage-derived foam cells, as determined by immunocytochemistry with the monoclonal antibody to mouse macrophages, MOMA-2 (data not shown). Quantitative analysis of the extent of atherosclerosis in the proximal aorta revealed that the mean±SEM atherosclerotic lesion area in SR-AI/II$^{-/-}$ females was reduced by 86% compared with wild-type females (2153±427 versus 15295±4248 $\mu$m$^2$/section, $P<0.006$; Figure 1A). A similar level of lesion reduction (81.5%) was found in male SR-AI/II$^{-/-}$ mice compared with wild-type males (1377±292 versus 7424±2508 $\mu$m$^2$/section, $P<0.005$; Figure 1B). Thus, in the setting of prolonged exposure to an atherogenic diet, C57BL/6 mice null for SR-AI/II expression are dramatically protected from the development of macrophage-derived foam cells and atherosclerosis.

To examine the contribution of macrophage SR-AI/II expression in atherogenesis, mice chimeric for macrophage SR-AI/II expression were created through FLC transplantation.[28] Two separate experiments were performed with 2 different murine models: C57BL/6 and LDLR$^{-/-}$ mice. First, to examine the impact of macrophage SR-AI/II on lesion formation under conditions inducing a moderate increase in the plasma cholesterol level, female 8-week-old C57BL/6 mice were lethally irradiated (9 Gy) and transplanted with female SR-AI/II$^{-/-}$ (experimental group, n=15) or with SR-AI/II$^{+/+}$ (control group, n=18) FLCs. After 8 weeks on a normal chow diet, the mice were challenged with the butterfat diet for 16 weeks. In a separate experiment, to study the contribution of macrophage SR-AI/II in the setting of severe hypercholesterolemia characterized by elevated levels of

LDL cholesterol, 8-week-old male LDLR$^{-/-}$ mice were transplanted with male SR-AI/II$^{-/-}$ (experimental group, n=13) or SR-AI/II$^{+/+}$ (control group, n=15) FLCs. After 8 weeks of a normal chow diet, the LDLR$^{-/-}$ recipient mice were challenged with the Western diet for 10 weeks.

Body weights and serum lipid levels were determined at regular intervals during the course of the experiments. While on the normal chow diet, the C57BL/6 and LDLR$^{-/-}$ recipient mice had a slight increase in body weight without differences between the groups (data not shown). Interestingly, both C57BL/6 and LDLR$^{-/-}$ mice reconstituted with SR-AI/II$^{-/-}$ macrophages gained significantly more body weight after 12 and 8 weeks on the atherogenic diets, respectively, than control SR-AI/II$^{+/+}\rightarrow$C57BL/6 and SR-AI/II$^{+/+}\rightarrow$LDLR$^{-/-}$ mice (Figure 2). Eight weeks after transplantation, no differences in serum cholesterol and triglyceride levels were detected between the experimental and control groups in either experiment with the normal chow diets (Tables III and IV; please see http://atvb.ahajournals.org). As expected, the atherogenic diets induced moderate hypercholesterolemia in the C57BL/6 mice and a more severe hypercholesterolemia in the LDLR$^{-/-}$ mice, but no sustained differences in serum cholesterol and triglyceride levels were noted between the control and experimental groups in either experiment (Tables III and IV; please see http://atvb.ahajournals.org). After 16 weeks on the butterfat diet, mean±SEM serum cholesterol levels of C57BL/6 experimental and control mice were 154±8 and 162±10 mg/dL, respectively, and in the LDLR
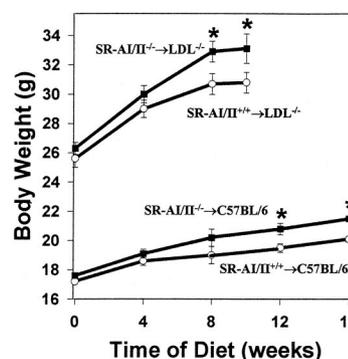


**Figure 2.** Body weight of C57BL/6 and LDLR$^{-/-}$ mice transplanted with SR-AI/II$^{+/+}$ and SR-AI/II$^{-/-}$ FLCs at different time points of the diet. Mice were fasted for 4 hours, and the body weight was measured. *$P<0.05$ vs SR-AI/II$^{+/+}\rightarrow$C57BL/6 and SR-AI/II$^{+/+}\rightarrow$LDLR$^{-/-}$ mice, respectively.
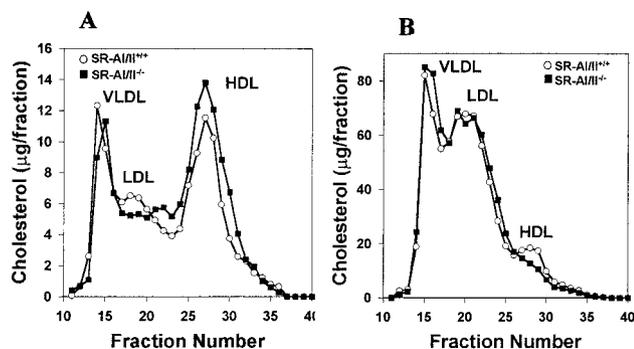
**Figure 3.** Lipoprotein distribution in C57BL/6 (A) and LDLR$^{-/-}$ (B) mice transplanted with SR-AI/II$^{+/+}$ and SR-AI/II$^{-/-}$ FLCs after 16 and 10 weeks of the atherogenic diet, respectively. Mice were fasted for 4 hours. The lipoprotein distribution was determined by FPLC followed by cholesterol analysis of each fraction. Data are represented as an average (n=3) of the percent of total cholesterol per fraction. Fractions 14 to 17 contain VLDL; fractions 18 to 24 contain IDL/LDL; and fractions 25 to 30 contain HDL. Fractions 31 to 40 include non–lipoprotein-associated proteins.



**Figure 4.** Atherosclerotic lesions in the proximal aorta of C57BL/6 mice transplanted with SR-AI/II$^{+/+}$ or SR-AI/II$^{-/-}$ FLCs. The extent of atherosclerotic lesions was quantified after 16 weeks of the butterfat diet with cross sections of the proximal aorta stained with oil red O. Data are presented as the average of mean lesion area per group measured in 15 sections per mouse.

experimental and control mice, levels were 765±24 and 753±25 mg/dL, respectively.

Examination of the distribution of cholesterol among the serum lipoprotein fractions by size-exclusion chromatography in recipient C57BL/6 mice after 16 weeks of the butterfat diet revealed an accumulation of cholesterol in the VLDL/IDL range and a relative decrease in the HDL cholesterol compared with chow-fed C57BL/6 mice (data not shown), with no differences between the experimental and control groups (Figure 3A). The HDL cholesterol levels were similar in SR-AI/II$^{+/+}$→C57BL/6 and SR-AI/II$^{-/-}$→C57BL/6 mice (56±4 and 50±2 mg/dL, respectively; $P$=0.25). After 10 weeks of the Western diet, the cholesterol distribution among the serum lipoprotein fractions in recipient LDLR$^{-/-}$ mice had changed dramatically with a massive accumulation of cholesterol in the VLDL/IDL/LDL range and a decrease in the HDL fraction compared with chow-fed LDLR mice (data not shown) (Figure 3B). However, no significant differences in lipoprotein profiles were detected between the groups. The HDL cholesterol levels in SR-AI/II$^{-/-}$→LDLR$^{-/-}$ mice (102±5 mg/dL) did not differ compared with SR-AI/II$^{+/+}$→LDLR$^{-/-}$ mice (104±6 mg/dL; $P$=0.73). Therefore, the lack of macrophage SR-AI/II expression had no impact on serum lipid levels or lipoprotein profiles under these dietary conditions.

After 16 weeks on the butterfat diet, the extent of atherosclerosis in the proximal aortas of the transplanted C57BL/6 mice was determined. Examination of oil red O–stained cross sections of the proximal aorta revealed fatty streaks lesions, which consisted almost exclusively of macrophage-derived foam cells, as determined through immunocytochemical staining with MOMA-2 (data not shown). Quantitative analysis of the extent of atherosclerotic lesions in the proximal aorta revealed that mean±SEM lesion area in SR-AI/II$^{-/-}$→C57BL/6 mice was reduced by 60% compared with SR-AI/II$^{+/+}$→C57BL/6 mice (13436±1894 and 33464±5465 $\mu$m$^2$/section, respectively; $P$<0.0006; Figure 4). Thus, C57BL/6 mice reconstituted with SR-AI/II$^{-/-}$ macrophages are protected from atherosclerosis compared with SR-AI/II$^{+/+}$→C57BL/6 mice.
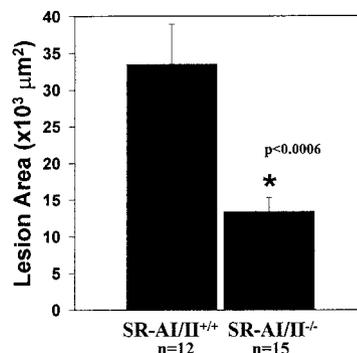
Analysis of cross sections from the proximal aorta of LDLR$^{-/-}$ mice after 10 weeks of the Western diet demonstrated large atherosclerotic lesions consisting predominantly of macrophage-derived foam cells. Immunocytochemical analysis of serial sections of the proximal aorta for staining with monoclonal antibodies specific for mouse macrophages, MOMA-2, (Figures 5A and 5B) or the SR-AI/II protein (2F8) revealed that macrophage-derived foam cells in SR-AI/II$^{+/+}$→LDLR$^{-/-}$ mice colocalized with SR-AI/II protein (Figure 5C), whereas macrophages from SR-AI/II$^{-/-}$→LDLR$^{-/-}$ mice did not react with the 2F8 antibody (Figure 5D). Quantitative analysis of the extent of atherosclerosis in sections from the proximal aorta demonstrated a 60% reduction in lesion area of SR-AI/II$^{-/-}$→LDLR$^{-/-}$ mice compared with SR-AI/II$^{+/+}$→LDLR$^{-/-}$ mice (62129±8047 and 156000±19659 $\mu$m$^2$/section ±SEM, respectively; $P$<0.0003; Figure
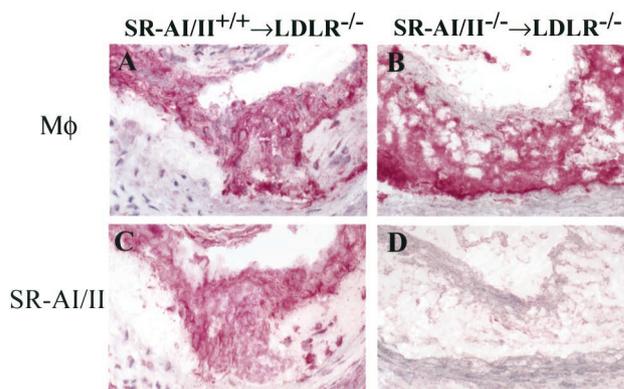


**Figure 5.** Detection of macrophages and SR-AI/II protein in the proximal aorta of SR-AI/II$^{+/+}$→LDLR$^{-/-}$ (A and C) or SR-AI/II$^{-/-}$→LDLR$^{-/-}$ (B and D) mice after 8 weeks of the Western diet. Cross sections are stained with rat monoclonal antibody to macrophages, MOMA-2 (A and B), or with rat monoclonal antibody to SR-AI/II, clone 2F8 (C and D), followed by biotinylated goat anti-rat IgG and then with avidin-biotin complex labeled with alkaline phosphatase. The enzymatic activity was visualized with Fast Red TR/Naphthol AS-NX substrate. As a negative control, the primary antibody was omitted during the incubation of some sections and resulted in no staining (data not shown). Note SR-AI/II expression in control mice is colocalized with macrophages, whereas macrophages in SR-AI/II$^{-/-}$→C57BL/6 mice do not stain for SR-AI/II (40×).
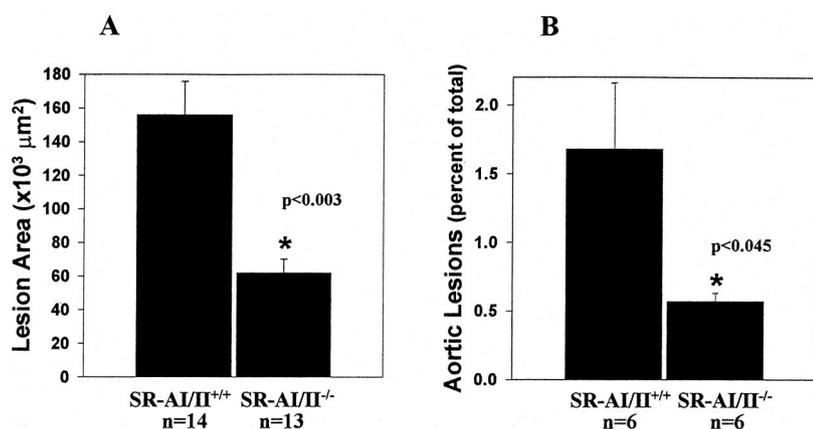
## A



## B



**Figure 6.** Atherosclerotic lesion area in the proximal aorta (A) and entire aorta en face (B) of LDLR$^{-/-}$ mice transplanted with SR-AI/II$^{+/+}$ and SR-AI/II$^{-/-}$ FLCs. The extent of atherosclerotic lesions was quantified after 10 weeks of the Western diet with cross sections of the proximal aorta stained with oil red O (A) and aorta en face stained with Sudan IV (B).

6A). Analysis of the extent of atherosclerosis in the aortas en face yielded similar results with a 65% reduction in the lesion area of SR-AI/II$^{-/-}$→LDLR$^{-/-}$ mice compared with SR-AI/II$^{+/+}$→LDLR$^{-/-}$ mice (0.58±0.06% and 1.68±0.48%, respectively; $P<0.045$; Figure 6B). Hence, LDLR$^{-/-}$ mice reconstituted with SR-AI/II$^{-/-}$ macrophages were protected from atherosclerosis under dietary conditions that induced severe hypercholesterolemia and accelerated lesion formation.

## Discussion

The present study provides strong evidence that macrophage SR-AI/II expression promotes atherogenesis and foam cell formation in vivo. It has been previously reported that SR-AI/II deficiency reduces the extent of atherosclerosis by 58% in apoE$^{-/-}$ mice[17] and by 22% to 28% in LDLR$^{-/-}$ mice.[18] In contrast, apoE Leiden transgenic mice null for SR-AI/II showed a nonsignificant trend for and increase in lesion area with more complex lesions,[19] leading these authors to suggest that apoE modulates the effect of SR-AI/II deficiency on atherosclerosis.[19,21] Alternatively, other factors, such as a mixed genetic background, may explain these conflicting results, because the mating of SR-AI/II$^{-/-}$ mice (129/ICR) with LDLR$^{-/-}$ mice (129/C57BL/6) or apoE Leiden mice (C57BL/6) produced hybrids, which may differ in the segregation of atherosclerosis susceptibility loci.[34]

The SR-AI/II$^{-/-}$ mice were crossed onto the C57BL/6 background, a strain that is susceptible to diet-induced atherosclerosis.[34] To analyze the impact of SR-AI/II expression on atherosclerosis in vivo, female and male SR-AI/II$^{+/+}$ and SR-AI/II$^{-/-}$ mice on C57BL/6 background were challenged with the butterfat diet for 30 weeks. SR-AI/II$^{-/-}$ mice were protected from atherosclerosis with an ≈80% reduction in lesion area of the proximal aorta compared with SR-AI/II$^{+/+}$ mice (Figure 1), in the absence of significant differences in plasma lipids between the groups. This remarkable effect of SR-AI/II on lesions was detected in both female and male mice, even though, as expected, females had bigger (2-fold) lesions.[35] Recent studies in apoE$^{-/-}$ mice support the notion that lipid oxidation increases with age and duration of hypercholesterolemia.[36] The SR-AI/II may assume a greater role in atherogenesis with prolonged exposure to a high-fat high-cholesterol diet. These results demonstrate that SR-AI/II has a crucial role in atherosclerotic lesion formation in C57BL/6 mice.

Macrophages are the major, but not the only, cell type expressing the SR-AI/II. Therefore, to dissect the impact of macrophage SR-AI/II on atherosclerotic lesion formation, mice chimeric for macrophage SR-AI/II were generated through transplantation with SR-AI/II$^{-/-}$ FLCs. Female C57BL/6 mice were lethally irradiated, reconstituted with SR-AI/II$^{-/-}$ or SR-AI/II$^{+/+}$ FLCs, and challenged with the butterfat diet for 16 weeks. The dietary intervention induced a moderate increase in serum lipids and prominent fatty streak lesions located exclusively in the proximal aorta. The C57BL/6 mice reconstituted with SR-AI/II$^{-/-}$ macrophages had a 60% reduction in lesion area compared with SR-AI/II$^{+/+}$→C57BL/6 mice (Figure 4). Consequently, the resistance to atherosclerosis in SR-AI/II$^{-/-}$ mice may be accounted for mainly by macrophage SR-AI/II expression. Finally, to analyze the contribution of macrophage SR-AI/II in lesion formation under conditions of severe hypercholesterolemia and elevated levels of LDL cholesterol, LDLR$^{-/-}$ mice were reconstituted with SR-AI/II$^{-/-}$ macrophages and fed with the Western diet for 10 weeks. The diet induced severe hypercholesterolemia and fatty streak lesions in the proximal aorta and distributed throughout the aorta. Similar to C56BL/6 mice, LDLR$^{-/-}$ mice reconstituted with SR-AI/II$^{-/-}$ macrophages had a 60% reduction in lesion area compared with SR-AI/II$^{+/+}$→LDLR$^{-/-}$ mice as assessed with 2 independent techniques: measurement of lesion size in cross sections of the proximal aorta and in the entire aorta en face (Figure 6). The 60% reduction in the extent of atherosclerosis seen in the SR-AI/II$^{-/-}$→LDLR$^{-/-}$ mice is greater than the 22% to 28% reduction in lesion area previously described in SR-AI/II$^{-/-}$ LDLR$^{-/-}$ mice[18] but similar to the 58% reduction seen in SR-AI/II$^{-/-}$ apoE$^{-/-}$ mice.[17] Our findings suggest that the mixed genetic background may have contributed to the smaller reduction in lesion area previously reported in LDLR$^{-/-}$ mice[18] and to the lack of an effect of the SR-AI/II on atherosclerosis seen in apoE Leiden transgenic mice.[19] Furthermore, these results indicate that the presence or absence of apoE does not modulate the effect of macrophage SR-AI/II expression on atherosclerosis, as previously suggested,[19,21] because the reductions in atherosclerosis seen for these SR-AI/II$^{-/-}$ mice of the wild type for apoE are virtually identical to the results for apoE$^{-/-}$ mice.[17] These data emphasize the importance of the stage of atherosclerosis and genetic background of mice in an experimental design to elucidate the role of SR-AI/II expression in lesion formation.

Targeted disruption of the class B scavenger receptor CD36 has recently been reported to protect against atherosclerotic lesion development in apoE$^{-/-}$ mice.[37] CD36 differs structurally from SR-AI/II and is more widely expressed. CD36 has a broad ligand specificity, binding to oxidized LDL, fatty acids, anionic phospholipids, and the proteins collagen and thrombospondin.[38] Like the SR-AI/II, CD36 mediates binding and uptake of modified lipoproteins by the macrophage and has been shown to mediate foam cell formation in vitro and in vivo.[21] The SR-AI/II recognizes only extensively modified LDL, and in vitro studies have demonstrated that the ability of SR-AI/II$^{-/-}$ macrophages to mediate the uptake of acetylated LDL was reduced by 80%, whereas the uptake of copper-oxidized LDL (Cu-OxLDL) was reduced by only 30%.[39] In contrast, CD36$^{-/-}$apoE$^{-/-}$ macrophages showed a 60% reduction in the uptake of Cu-OxLDL and a 52% reduction in uptake of acetylated LDL.[37] Furthermore, recent in vitro studies indicate that CD36 is the major receptor for the uptake of myeloperoxidase-modified LDL.[37,40] Despite these apparent differences in specificity for uptake of modified lipoproteins, the extents of reduction in atherosclerosis in apoE$^{-/-}$ mice null for SR-AI/II or CD36 were similar.[17,37] Thus, both the SR-AI/II and CD36 play important roles in atherosclerosis and foam cell formation in vivo, and there appears to be little redundancy in the scavenger receptor system, because the absence of either SR-AI/II or CD36 is not compensated for by the presence of the other.

No sustained significant differences in serum lipid levels were detected in SR-AI/II$^{-/-}$ mice or C57BL6 and LDLR$^{-/-}$ mice reconstituted with SR-AI/II$^{-/-}$ macrophages, compared with the SR-AI/II$^{+/+}$ control mice. In contrast, SR-AI/II$^{-/-}$/apoE$^{-/-}$ mice had a 46% higher plasma cholesterol level compared with SR-AI/II$^{+/+}$/apoE$^{-/-}$ mice.[17] Perhaps the increase in the plasma cholesterol levels in SR-AI/II$^{-/-}$/apoE$^{-/-}$ mice may point to a function of the SR-AI/II that requires apoE. Alternatively, this may be due to differences related to the mixed genetic background of the apoE$^{-/-}$ mice.[17] Consistent with our results, the absence of the SR-AI/II did not change plasma lipids in mice with a mixed genetic background (129/ICR) fed a normal chow or high-fat diet.[17] Our results support the hypothesis that SR-AI/II expression does not significantly affect serum cholesterol and triglyceride levels.

In response to a high-fat diet, both C57BL/6 and LDLR$^{-/-}$ mice reconstituted with SR-AI/II$^{-/-}$ macrophages had small but significant increases in body weight compared with SR-AI/II$^{+/+}$→C57BL/6 and SR-AI/II$^{+/+}$→LDLR$^{-/-}$ mice. Although this finding was unexpected, it is interesting because modified LDL acts as a ligand for the nuclear hormone receptor peroxisome proliferator-activated receptor-γ,[41,42] which promotes adipogenesis.[43] The absence of SR-AI/II expression in macrophages may in effect redirect modified LDL to other cell acceptors located in adipose tissue cells. This may explain our findings that C57BL/6 and LDLR$^{-/-}$ mice reconstituted with SR-AI/II$^{-/-}$ macrophages had a gain in body weight compared with SR-AI/II$^{+/+}$→C57BL/6 and SR-AI/II$^{+/+}$→LDLR$^{-/-}$ mice.

In conclusion, our results demonstrate that both male and female C57BL/6 mice null for SR-AI/II are protected from atherosclerosis. When fed the butterfat diet, they had an 80% reduction in aortic lesion area compared with wild-type mice. This resistance to atherosclerosis may be in large part accounted for by macrophage expression of SR-AI/II, because both C57BL/6 and LDLR$^{-/-}$ mice reconstituted with SR-AI/II$^{-/-}$ macrophages had 60% reductions in aortic lesion size, respectively. The present results demonstrate in vivo that macrophage SR-AI/II expression promotes foam cell formation and atherosclerosis.

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# Arteriosclerosis, Thrombosis, and Vascular Biology

**Reduced Atherosclerotic Lesions in Mice Deficient for Total or Macrophage-Specific Expression of Scavenger Receptor-A**

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**Fig. I.** *Identification of SR-AI/II Genotype in mice by PCR.* DNA samples were extracted from mouse tail and amplified by PCR using a primer set specific for mouse SR-AI/II gene (primer S21, CAA GTG ATA CAT CTC AAG GTC and primer S27, CTG TAG ATT CAC GGA CTC TG) and Neo insert (primer Neo707, GAG GAG TAG AAG GTG GCG CGA A). Amplification was performed in a RapidCycler (Idaho Technology, Idaho Falls, OH) with the following parameters: 40 sec at $94^{\circ}$C for the first cycle, and then 30 cycles of 15 sec at $94^{\circ}$C, 35 sec at $55^{\circ}$C, and 55 sec at $72^{\circ}$C. The first and the last lanes are molecular weight markers (100 bp ladder, Promega Corp), lanes 2-5 are control samples, SR-AI/II$^{+/+}$, SR-AI/II$^{+/-}$, SR-AI/II$^{-/-}$ and no template, respectively. The lanes 6-13 are DNA samples of the pups from the same litter. Wild type mice showed a 325 bp band, SR-AI/II$^{-/-}$ mice had a 440 bp band, heterozygous mice had both bands.

**Table 1. Total Serum Cholesterol and Triglyceride Levels in Female SR-AI/II$^{+/+}$ and SR-AI/II$^{-/-}$ Mice in the Course of Butterfat Diet**

| Group of Animals | Serum Lipid | Baseline | 4 weeks of diet | 6 weeks of diet | 12 weeks of diet | 15 weeks of diet | 27 weeks of diet | 30 weeks of diet |
|---|---|---|---|---|---|---|---|---|
| **SR-AI/II$^{+/+}$** N = 13 | Chol. | 88 ± 4 | 148 ± 17 | 153 ± 30 | 141 ± 14 | 166 ± 15 | 169 ± 16 | 148 ± 17 |
| | Trigl. | 39 ± 3 | 69 ± 8 | 30 ± 4 | 35 ± 4 | 34 ± 3 | 99 ± 5 | 67 ± 4 |
| **SR-AI/II$^{-/-}$** N = 9 | Chol. | 87 ± 3 | 145 ± 3 | 154 ± 4 | 137 ± 8 | 162 ± 6 | 148 ± 5 | 145 ± 9 |
| | Trigl. | 42 ± 4 | 62 ± 3 | 30 ± 2 | 31 ± 3 | 32 ± 2 | 93 ± 4 | 62 ± 2 |

Values are in mg/dl (Mean ± SEM). The number of animals in each group is indicated by *n*. The differences are statistically not significant between the groups at any time point.

**Table 2. Total Serum Cholesterol and Triglyceride Levels in Male SR-AI/II$^{+/+}$ and SR-AI/II$^{-/-}$ Mice in the Course of Butterfat Diet**

| Group of Animals | Serum Lipid | Baseline | 4 weeks of diet | 6 weeks of diet | 12 weeks of diet | 15 weeks of diet | 27 weeks of diet | 30 weeks of diet |
|---|---|---|---|---|---|---|---|---|
| SR-AI/II$^{+/+}$ n = 9 | Chol. | 95 ± 2 | 154 ± 3 | 163 ± 5 | 129 ± 14 | 164 ± 13 | 154 ± 12 | 167 ± 19 |
| | Trigl. | 50 ± 2 | 73 ± 5 | 28 ± 2 | 20 ± 3 | 54 ± 2 | 74 ± 2 | 73 ± 5 |
| SR-AI/II$^{-/-}$ n = 19 | Chol. | 103 ± 4 | 166 ± 4 | 187 ± 5* | 165 ± 6 | 179 ± 5 | 154 ± 6 | 156 ± 11 |
| | Trigl. | 59 ± 5 | 69 ± 2 | 24 ± 2 | 26 ± 2 | 44 ± 6 | 85 ± 2 | 78 ± 5 |

Values are in mg/dl (mean ± SD). The number of animals in each group is indicated by *n*. *The difference is statistically significant (p < 0.05) compare to the control group, SR-AI/II$^{+/+}$ mice, at that time point.

**Table 3. Total Serum Cholesterol and Triglyceride Levels in C57BL/6 Mice after Transplantation with SR-AI/II$^{+/+}$ or SR-AI/II$^{-/-}$ Fetal Liver Cells**

| Group of animals | Serum lipid | Baseline | Baseline Chow Diet | 4 weeks Butterfat Diet | 8 weeks Butterfat Diet | 12 weeks Butterfat Diet | 16 weeks Butterfat Diet |
|---|---|---|---|---|---|---|---|
| SR-AI/II$^{+/+}$→C57BL/6 n = 15 | Chol. | 99 ± 2 | 87 ± 4 | 129 ± 7 | 169 ± 7 | 163 ± 7 | 162 ± 10 |
| | Trigl. | 57 ± 5 | 51 ± 3 | 48 ± 4 | 81 ± 4 | 59 ± 5 | 59 ± 5 |
| SR-AI/II$^{-/-}$→C57BL/6 n = 18 | Chol. | 98 ± 2 | 92 ± 2 | 115 ± 4 | 155 ± 5 | 156 ± 7 | 154 ± 8 |
| | Trigl. | 55 ± 3 | 55 ± 2 | 68 ± 5* | 80 ± 5 | 47 ± 4 | 47 ± 4 |

Values are in mg/dl (Mean ± SEM). The number of animals in each group is indicated by *n*. *The differences are statistically significant (p<0.05) compared to SR-AI/II$^{+/+}$→C57BL/6 mice at that time point.

**Table 4. Total Serum Cholesterol and Triglyceride Levels in Male LDLR$^{-/-}$ Mice after Transplantation with SR-AI/II$^{+/+}$ or SR-AI/II$^{-/-}$ Fetal Liver Cells**

| Group of animals | Serum lipid | Baseline | Baseline Chow diet | 4 weeks Western Diet | 8 weeks Western Diet | 10 weeks Western Diet |
|---|---|---|---|---|---|---|
| SRAI/II$^{+/+}$→LDLR$^{-/-}$ | Cholesterol | 283 ± 7 | 240 ± 8 | 724 ± 33 | 929 ± 36 | 753 ± 25 |
| n = 17 | Triglycerides | 104 ± 4 | 139 ± 8 | 172 ± 13 | 290 ± 40 | 285 ± 42 |
| SRAI/II$^{-/-}$→LDLR$^{-/-}$ | Cholesterol | 277 ± 10 | 230 ± 9 | 645 ± 26 | 888 ± 35 | 765 ± 24 |
| n = 13 | Triglycerides | 113 ± 5 | 124 ± 5 | 169 ± 9 | 328 ± 27 | 324 ± 45 |

Values are in mg/dl (Mean ± SEM). The number of animals in each group is indicated by *n*. Differences between groups are not statistically significant at any time point.