Gene Transfer and Hepatic Overexpression of the HDL Receptor SR-BI Reduces Atherosclerosis in the Cholesterol-Fed LDL Receptor–Deficient Mouse

Karen F. Kozarsky, Mary H. Donahee, Jane M. Glick, Monty Krieger, Daniel J. Rader

Abstract—HDL cholesterol levels in humans are inversely correlated with the risk of atherosclerosis. The class B scavenger receptor type I (SR-BI) is the first molecularly well-defined HDL receptor, and hepatic overexpression of SR-BI in normal mice has been shown to result in decreased plasma HDL cholesterol levels. To determine whether SR-BI overexpression is proatherogenic or is protective against atherosclerosis, LDL receptor–deficient mice were placed on a high-fat/high-cholesterol diet for 2 or 12 weeks to induce atherosclerotic lesions of different stages and then were injected with a recombinant adenovirus encoding murine SR-BI. Transient hepatic overexpression of SR-BI in mice with both early and advanced lesions significantly decreased atherosclerosis. SR-BI expression was associated with markedly decreased HDL cholesterol and either unchanged or only modestly reduced non-HDL cholesterol levels; in all experiments, the mean HDL cholesterol levels were significantly correlated with atherosclerotic lesion size. These data suggest that interventions that promote HDL cholesterol transport and lower plasma HDL cholesterol levels can suppress atherosclerosis, even when initiated after significant lesion development. Thus, stimulation of hepatic SR-BI activity may provide a novel target for therapeutic intervention in atherosclerotic cardiovascular disease. (Arterioscler Thromb Vasc Biol. 2000;20:721-727.)

Key Words: adenovirus ■ HDL ■ receptors, lipoprotein ■ recombinant protein

Epidemiological data indicate a strong inverse association between HDL cholesterol levels and atherosclerotic cardiovascular disease in humans.1 The atheroprotective effect of HDL has been postulated to be due, in part, to a process called "reverse cholesterol transport,"2,3 in which excess cholesterol is removed from arterial cells and returned to the liver for excretion into the bile. In reverse cholesterol transport, HDL is proposed to remove excess unesterified cholesterol from cells in the arterial intima, after which this cholesterol is esterified by lecithin:cholesteryl acyltransferase in the plasma and then transported directly or indirectly to the liver for excretion into the bile. Indirect transport to the liver involves transfer of some of the cholesteryl ester of HDL to other lipoproteins by the cholesteryl ester transfer protein (CETP), with subsequent receptor-mediated endocytosis of these lipoproteins by the liver. Some species (eg, mice) do not make CETP, and this mechanism of indirect transport is not available. Direct transport to the liver involves the binding of HDL to the surfaces of hepatocytes and efficient transfer into the cells of the cholesteryl ester but not other HDL components (eg, its protein and phospholipid outer shell). This process, which is distinct from receptor-mediated endocytosis, is called "selective cholesterol uptake"4 and is also used for the delivery of HDL cholesteryl ester to steroidogenic tissue.

The HDL receptor scavenger receptor class B type I (SR-BI) plays an important role in mediating selective uptake of HDL cholesterol by hepatocytes and steroidogenic cells in vitro and in vivo.5–11 For example, mice with homozygous null mutations in the SR-BI gene exhibit a 2-fold increase in plasma cholesterol. As expected for animals lacking the major receptor for selective uptake of HDL cholesterol, most of the increase was due to accumulation in HDL particles of much greater than normal size, whereas there was virtually no increase in plasma apoA-I concentration.7 Furthermore, mice bearing an insertion in the promoter region of the SR-BI gene that, in the homozygous state, results in ∼50% of normal hepatic SR-BI expression exhibit increased plasma HDL cholesterol and reduced hepatic selective uptake of HDL cholesterol.8 In addition, hepatic overexpression of SR-BI in mice injected with a recombinant adenovirus encoding SR-BI results in markedly reduced levels of HDL cholesterol and increased biliary cholesterol content.10 Similar results have been obtained in transgenic mice overexpressing SR-BI in the liver.12,13 Therefore, hepatic SR-BI may mediate one of the
critical steps in reverse cholesterol transport, the direct delivery of HDL cholesteryl ester to the liver.

Because the concentration of murine plasma HDL cholesterol is inversely proportional to the level of hepatic SR-BI expression, the potential influence of SR-BI on atherogenesis has been unclear. Increased hepatic SR-BI activity may be expected to increase reverse cholesterol transport and protect against atherogenesis, yet the simultaneously decreased steady-state concentration of plasma HDL cholesterol might be proatherogenic. In addition, SR-BI can bind apoB-containing lipoproteins, in some circumstances can influence their plasma concentrations in vivo, and thus might influence atherosclerosis by affecting the levels of non-HDL cholesterol. To address these issues, we examined the effects of adenovirus-mediated gene transfer and transient hepatic overexpression of SR-BI on atherosclerosis in a murine model of atherosclerosis, the LDL receptor (LDLR)–deficient mouse fed a high-fat/high-cholesterol diet.

Methods

Mice and Diet

Female LDLR knockout (LDLR-deficient) mice crossed 10 times onto a C57BL/6 background were obtained from the Jackson Laboratory, Bar Harbor, Me. At either 2 weeks or 12 weeks before adenovirus infusion, mice were placed and maintained on a western-type diet containing 21% butterfat, 1% safflower oil, and 0.15% cholesterol obtained from Dyets, Inc. All procedures followed were in accordance with institutional guidelines.

Recombinant Adenoviruses

Ad.E1\(\Delta\) and Ad.mSR-BI have been described previously. Briefly, Ad.mSR-BI contains an expression cassette encoding a cytomegalovirus enhancer-promoter, murine SR-BI cDNA, and an SV40 polyadenylation site inserted into the E1 region of adenovirus; Ad.E1\(\Delta\) has the E1-region deletion with no inserted cassette. Both of the recombinant, replication-defective adenoviruses contain partial E3 deletions. Mice were injected via the tail vein with 1 mL of purified recombinant adenovirus in a volume of 0.1 mL. At the indicated times, blood was collected from the retro-orbital venous plexus into heparinized capillary tubes. Mice were euthanized and aortas harvested 4 weeks after adenovirus injection.

Plasma Cholesterol Assays/FPLC and Immunohistochemical Analysis of Hepatic mSR-BI Expression

The cholesterol, HDL cholesterol, apoA-I, and apoB concentrations in whole plasma were analyzed with a Cobas/Fara autoanalyzer as previously described. HDL cholesterol was measured with an assay kit (EZ HDL) from Sigma Diagnostics, Inc. HDL cholesterol levels in hyperlipidemic mouse plasma as assayed with this reagent were previously found to compare favorably with HDL cholesterol levels as determined by heparin-manganese precipitation and by analysis of mixtures of purified (human) lipoproteins. Non-HDL cholesterol was determined by subtracting HDL cholesterol from total cholesterol. The apoA-I and apoB assays (Sigma Diagnostics, Inc) used human standards. Fast protein liquid chromatography (FPLC) analysis was performed on individual plasma samples as previously described. In the 6-week feeding protocol, at 4 weeks after virus injection, mice were euthanized and livers were stained with an anti–SR-BI antibody to ensure that transgene expression was obtained in the SR-BI virus–infused mice. All of the mice injected with Ad.mSR-BI showed staining above the background (ie, endogenous SR-BI levels) at day 28 except for 1 mouse, which had no detectable increase in SR-BI expression and no changes in plasma HDL cholesterol levels. This mouse was excluded from the analysis. Livers from mice injected with Ad.E1\(\Delta\) were also stained with a polyclonal anti-adenovirus antibody to confirm adenovirus infection.

Quantification of Atherosclerotic Lesions

Mice were anesthetized and then gently perfused with PBS via a needle placed in the left ventricle, after which the aorta was removed and fixed in 10% buffered formalin/PBS for 3 days. Adventitial and adipose tissues were removed by careful dissection, and the outer curvature of the arch was cut longitudinally. For the 6-week feeding protocol, aortas were stained with oil red O solution (1.8% oil red O, wt/vol, in 60% isopropanol, filtered twice with a 0.2-\(\mu\)m filter) for 15 minutes and destained with 60% isopropanol for 5 minutes to eliminate background staining. The aortas were mounted in Aquamount on a glass slide. For the 16-week feeding protocol, the fixed aortas were briefly rinsed in 70% ethanol, stained for 6 minutes with Sudan IV (0.5% Sudan IV/35% ethanol/50% acetone), and destained for 5 minutes in 80% ethanol. Because the lesions were large in volume, these aortas did not have coverslips applied but instead were pinned onto a black wax surface. Images were captured, and the red-stained lesion area was quantified with Image Pro Plus image analysis software (Media Cybernetics). Each sample was quantified independently by 2 individuals, and the results were averaged. A comparison of lesion sizes between the control group and SR-BI–treated mice was performed with the nonparametric Mann-Whitney test and Prism software (6-week experiment) and a parametric test (16-week experiment; normal distribution).

Results

Effects of SR-BI Overexpression on Early Atherosclerotic Lesions

Two protocols were used to evaluate the effects of SR-BI overexpression on atherosclerosis in the fat-fed LDLR-deficient mouse. First, in 2 separate experiments, female LDLR-deficient mice were placed on the western-type diet for 2 weeks, injected with recombinant adenovirus, and maintained for another 4 weeks on the diet before the harvesting of their aortas for quantification of atherosclerosis (6-week feeding protocol). The recombinant, replication-defective adenoviruses used were a control virus containing no transgene expression cassette (Ad.E1\(\Delta\)) and a virus encoding the murine SR-BI cDNA under the control of the cytomegalovirus enhancer/promoter (Ad.mSR-BI). Figure 1 shows the cholesterol/lipoprotein profiles for mice sampled immediately before virus injection (day 0) and 7 days after injection. One group of mice received the control

Figure 1. FPLC analysis of plasma lipoproteins from western diet–fed LDLR-deficient mice. Plasma samples were drawn either before (○) or 7 days after (●) injection with either a control adenovirus (Ad.E1\(\Delta\), A) or the mSR-BI–expressing adenovirus (Ad.mSR-BI, B). The samples were subjected to FPLC separation on Superose 6 columns, and each fraction was assayed for its cholesterol content as described in Methods. The first peak, fractions 4 to 8, represents VLDL; the second peak, fractions 9 to 29, IDL/LDL; and the third peak, fractions 30 to 40, HDL.
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TABLE 1. Plasma Lipid and Protein Concentrations in LDLR-Deficient Mice as a Function of Time After Injection of Recombinant Adenovirus

<table>
<thead>
<tr>
<th>Virus</th>
<th>Day</th>
<th>HDL cholesterol</th>
<th>Non-HDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
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</tr>
<tr>
<td></td>
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<td>21</td>
<td>28</td>
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<tr>
<td>Ad.E1Δ</td>
<td>0</td>
<td>116.0±18.6</td>
<td>105.0±18.4</td>
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<tr>
<td></td>
<td>7</td>
<td>119.7±31.1</td>
<td>47.5±21.4†</td>
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<tr>
<td></td>
<td>14</td>
<td>99.7±26.3</td>
<td>72.5±24.0*</td>
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<tr>
<td></td>
<td>21</td>
<td>114.7±40.4</td>
<td>89.5±27.7</td>
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<td></td>
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<td>89.7±38.4</td>
<td>81.0±46.9</td>
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<tr>
<td>Mean</td>
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<td>72.6±25.5‡</td>
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<tr>
<td>Total cholesterol</td>
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<td>1502±209</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1152±237</td>
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<td>Non-HDL cholesterol</td>
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<td>Mean</td>
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<tr>
<td>ApoA-I</td>
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<td>7</td>
<td>44.8±7.7</td>
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<td></td>
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<tr>
<td>Mean</td>
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<tr>
<td>Triglyceride</td>
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</tr>
<tr>
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<td>28</td>
<td>363±117</td>
<td>386±345</td>
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<tr>
<td>Mean</td>
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<tr>
<td>ApoB</td>
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<td>378±63</td>
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<td>297±85</td>
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</tr>
<tr>
<td>Mean</td>
<td>336±85</td>
<td>316±111</td>
<td></td>
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</tbody>
</table>

Mean indicates mean postinjection levels (average of days 7, 14, 21, and 28). Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol. The values are expressed as mg/dL; the data are shown as average±SD; n=15 for Ad.E1Δ, n=11 for Ad.mSR-BI.

*P<0.05; †P<0.0005 for Ad.mSR-BI-injected mice vs control (Ad.E1Δ) mice; determined by t test.

‡P<0.01 for Ad.mSR-BI-injected mice vs control (Ad.E1Δ) mice.

virus (A) and the other Ad.mSR-BI (B). The plasma of un.injected LDLR-deficient mice on a western-type diet contains substantial amounts of IDL/LDL cholesterol, with lower but readily detectable amounts of VLDL and HDL cholesterol. Injection of the control virus, Ad.E1Δ, resulted in no alterations in HDL cholesterol (Figure 1A, Table 1) or in levels of apoA-I, the major protein component of HDL (Table 1). However, injection with Ad.mSR-BI substantially decreased HDL cholesterol on postinjection days 7 (Figure 1B; Table 1) and 14 (Table 1). These results are similar to the results previously observed in wild-type mice,10 in which Ad.mSR-BI injection caused a dramatic decrease in plasma HDL cholesterol, and also demonstrate that this decrease can occur even in the presence of substantially elevated circulating levels of LDL. ApoA-I, the major protein component of HDL, was more modestly reduced in the Ad.mSR-BI-injected mice (Table 1). Although it appears that there was a substantially smaller SR-BI overexpression–mediated reduction of apoA-I levels in western diet–fed LDLR-deficient mice than in chow-fed, wild-type mice,10 these observations were made in separate experiments using different viral stocks and could be due to a variety of experimental variations (eg, precise levels of SR-BI expression) and perhaps not to the differences in genotypes and diets. The mean HDL cholesterol levels after adenovirus injection were significantly lower in the SR-BI–overexpressing mice than in control mice (Table 1, P<0.01).

Compared with changes in HDL, the amounts of non-HDL lipoproteins, which contained most of the plasma cholesterol (>90%; Figure 1 and Table 1), changed much less dramatically in the SR-BI–overexpressing mice. Triglyceride levels increased in the SR-BI–overexpressing mice on day 7 (Table 1), simultaneously with the biggest decrease in HDL cholesterol and an increase in VLDL cholesterol (Figure 1). Plasma apoB levels (Table 1), which provide a marker for non-HDL lipoproteins, were slightly decreased after injection of Ad.mSR-BI, but only on day 14 was this decrease significantly lower than that for controls (P<0.05). The effects of SR-BI overexpression on non-HDL cholesterol were also small. Seven days after injection, a modest reduction in the relative amounts of cholesterol within the largest lipoproteins was determined by FPLC (VLDL and IDL/LDL, Figure 1A and 1B) and non-HDL cholesterol determined enzymatically (Table 1), but this occurred in both control and SR-BI–overexpressing groups of mice, and the IDL/LDL size particles were somewhat smaller in both groups (shift to the right in Figure 1), presumably as a result of general effects of adenovirus injection. Although there was a trend toward lower levels of non-HDL cholesterol in the SR-BI–overexpressing mice (Figure 1B and Table 1), neither the non-HDL cholesterol levels at the individual time points nor the mean value for all time points after injection (P>0.3) were significantly different from those of controls. Taken together, these data show that transient overexpression of SR-BI resulted in a significant, although transient, decrease in HDL cholesterol and a much smaller change in non-HDL cholesterol.

To determine whether hepatic overexpression of mSR-BI affected the course of atherogenesis in the 6-week feeding protocol, we euthanized the animals (15 controls, 11 Ad.mSR-BI–injected) 4 weeks after injection and quantitatively assessed the extent of atherosclerosis using an en face protocol, we euthanized the animals (15 controls, 11 Ad.mSR-BI–injected) 4 weeks after injection and quantitatively assessed the extent of atherosclerosis using an en face
mice (mean values of 297 366 and 86 637 animals exhibited significantly less lesion area than control mice receiving a modified feeding protocol. These mice were euthanized, and the aortic arches were analyzed for the presence of oil red O–stained (6-week) or Sudan IV–stained (16-week) lesions as described in Methods. The data are expressed as average values, with error bars designating the SEM. A, Ad.E1Δ, n = 15; Ad.mSR-BI, n = 9 for each group; *P = 0.034.

**Figure 2.** Atherosclerotic lesion size in aortas from LDLR-deficient mice maintained on the western-type diet for 6 (A) or 16 (B) weeks. LDLR-deficient mice were maintained on the 6-week (A) or 16-week (B) western diet feeding protocol (2 or 12 weeks before and then 4 weeks after adenovirus injection), then euthanized, and the aortic arches were analyzed for the presence of oil red O–stained (6-week) or Sudan IV–stained (16-week) lesions as described in Methods. The data are expressed as average values, with error bars designating the SEM. A, Ad.E1Δ, n = 15; Ad.mSR-BI, n = 9 for each group; *P = 0.034.

**Figure 3.** Correlation of mean HDL cholesterol levels with atherosclerotic lesion size. For both the 6-week feeding protocol (A) and the 16-week feeding protocol (B), the lesion size for each animal is shown as a function of mean HDL cholesterol level (see Tables 1 and 2). The lines represent linear regression analyses, and the r² values for the analyses are shown.

Effects of SR-BI Overexpression on More Advanced Atherosclerotic Lesions

To verify and extend these findings, we performed similar experiments with 2 additional groups of LDLR-deficient mice receiving a modified feeding protocol. These mice were placed on the western-type diet for 12 weeks before virus injection to determine whether hepatic SR-BI overexpression would have similar effects in animals with more advanced aortic arch lesions. The mice were euthanized 4 weeks after virus injection, a total of 16 weeks on diet. Table 2 shows the effects of SR-BI overexpression on plasma total cholesterol, non-HDL cholesterol, and HDL cholesterol levels. For HDL cholesterol, the effects of SR-BI overexpression were significant for 2 of the 3 postinjection times (days 4 and 28) as well as for the overall mean, whereas only the postinjection day 28 values for total and non-HDL cholesterol were significantly different in the SR-BI–overexpressing mice (Table 2). After the mice were killed, the perfused and fixed aortas were examined visually. Lesions in the mice were sufficiently large that the extent of atherosclerosis in the unstained, intact aortas could be assessed with a dissecting microscope (Figure 4).

Blind, semiquantitative estimates of unstained lesion sizes from the aortic arch through the carotid branch points by 3 observers showed a significant reduction in the SR-BI–overexpressing animals (P = 0.017, data not shown). The lesion areas in these samples were also quantified after Sudan IV staining (see Methods) and are shown in Figure 2B. As was observed with lesions in the 6-week feeding protocol, the SR-BI–overexpressing mice exhibited significantly smaller lesions than control mice (P = 0.034). The animal-by-animal correlations of atherosclerosis with mean HDL and non-HDL cholesterol levels (averaged over all postinjection measurements, both control and SR-BI–overexpressing animals) were striking. Both the mean HDL cholesterol levels (Figure 3B) and the mean non-HDL cholesterol levels were strongly correlated with atherosclerotic lesion size (P = 0.0008 and 0.0007 and r² = 0.5150 and 0.5207, respectively) when both groups of mice were pooled. In addition, the mean apoA-I levels after injection in the SR-BI–injected mice were
significantly lower than in those injected with control virus, 41 ± 5 mg/dL compared with 60 ± 6 mg/dL (P = 0.02). ApoA-I levels and lesion size were also directly correlated (P = 0.003; r² = 0.4419).

Discussion

We used adenovirus-mediated gene overexpression in the livers of LDLR-deficient mice fed a western diet to assess the influence of the HDL receptor SR-BI on lipoprotein metabolism and atherosclerosis. Consistent with our previous studies in wild-type mice fed a chow diet, we found that overexpression of SR-BI markedly reduced HDL cholesterol levels (eg, 55% reduction 7 days after injection), indicating that transient SR-BI overexpression influences HDL metabolism even in the setting of substantially elevated non-HDL cholesterol. These results provide further support for the proposal that hepatic SR-BI is an HDL receptor that plays a role in determining circulating levels of HDL.5–10,13,15

SR-BI has previously been shown to bind apoB-containing lipoproteins in vitro; however, the relatively low levels of apoB-containing lipoproteins in chow-diet–fed wild-type mice made it difficult to determine the effects of SR-BI overexpression in vivo in those animals. LDLR-deficient mice fed a high-fat/high-cholesterol diet have high levels of apoB-containing plasma lipoproteins. We found in these animals that compared with its effects on HDL cholesterol, transient hepatic overexpression of SR-BI modestly reduced the levels of apoB-containing lipoprotein cholesterol (non-HDL cholesterol) and apoB itself only at some time points after Ad.mSR-BI injection and did not have significant effects on the mean levels. Decreased non-HDL cholesterol levels due to SR-BI overexpression have been reported for SR-BI transgenic mice.12,13,15 Additional work will be necessary to determine whether the decreases in the apoB-containing lipoproteins were due to direct interactions of these lipoproteins with SR-BI or were indirect consequences of SR-BI overexpression (eg, secondary consequences of changes in plasma HDL). Our results indicate that transient overexpression of SR-BI via gene transfer has a much less pronounced effect on apoB-containing lipoprotein cholesterol levels in LDLR-deficient mice than does transgenic overexpression,12,13 suggesting that the effects of hepatic SR-BI overexpression on the metabolism of apoB-containing lipoproteins may be more complex than currently appreciated.

Strikingly, we found that transient hepatic overexpression of SR-BI significantly reduced atherosclerosis in western diet–fed LDLR-deficient mice at 2 different times of lesion development. Although additional studies will be necessary to define the molecular basis for the protective effects of SR-BI, several potentially interrelated mechanisms may account for this result. First, hepatic overexpression of SR-BI is expected to substantially increase the flux of HDL cholesterol through the reverse cholesterol transport pathway from the arterial wall to the liver and then out of the body via the bile.10,12,15,19 As a consequence, the rate of plaque deposition would be expected to decrease, and plaque regression might even occur. Second, a consequence of hepatic SR-BI overexpression may be the generation of altered HDLs with increased antiatherogenic properties (eg, enhanced cellular cholesterol efflux25 and/or greater access to the arterial intima26). Third, hepatic overexpression of SR-BI might alter the amounts or structures of non-HDL lipoproteins (eg, apoB-containing lipoproteins) and thus diminish their atherogenicity. Our analysis of the correlations between lipoprotein component levels and lesion sizes, especially HDL cholesterol levels (see Results), suggests that in this system, changes in HDL cholesterol levels, presumably due to increased flux through the reverse cholesterol transport pathway, are likely to have had a major impact on atherosclerosis. Although there was a trend toward reduced levels of non-HDL cholesterol in the SR-BI–overexpressing animals compared with controls, substantial animal-to-animal variation resulted in no statisti-
cally significant SR-BI overexpression–dependent differences in 1 of the 2 experimental protocols (6-week protocol).

Nevertheless, it is possible that changes in non-HDL lipoproteins influenced atherosclerosis in this study. In the 16-week protocol, there were strong correlations between lesion size of each animal and both mean HDL cholesterol and mean non-HDL cholesterol levels of each animal. Arai et al. reported studies of the effects on atherosclerosis of hepatic SR-BI overexpression in SR-BI transgenic mice bred into a heterozygous LDLR-deficient background. When these mice were fed a 1.25% cholesterol/choleic acid (Paigen) diet, the SR-BI transgenic mice developed less atherosclerosis than controls and had lower levels of HDL and non-HDL cholesterol. There are, however, a number of differences between the results of Arai et al and those reported here. In contrast to their results with the Paigen diet, when they fed mice a western-type diet similar to the one used here, they did not observe significant differences in non-HDL cholesterol levels or atherosclerotic lesion sizes in SR-BI–overexpressing and control animals. Furthermore, they found a strong correlation of atherosclerosis with non-HDL cholesterol but little correlation with HDL cholesterol, whereas we found a strong HDL cholesterol/lesion size correlation. Finally, it is possible that some of the differences in these studies arise because of different effects of chronic versus transient expression, different levels of hepatic SR-BI overexpression, or differences in the timing of SR-BI overexpression relative to the atherosclerotic disease process.

The results reported here represent another example of the positive association of HDL cholesterol levels with atherosclerosis. Previous reports from other laboratories have shown that transgenic overexpression of CETP reduces both HDL cholesterol levels and atherosclerosis in mice and that a deficiency of CETP is associated with increases in both HDL cholesterol levels and risk of coronary heart disease in humans. Furthermore, transgenic overexpression of lecithin:cholesterol acyltransferase increases both HDL cholesterol and atherosclerosis in mice. When considered with the antiatherogenic effects of apoA-I overexpression in mice but only modest proatherogenic effects of reduced apoA-I expression and the well-known inverse correlation of plasma HDL cholesterol levels and atherosclerosis in humans, these data highlight the complex interplay of lipoproteins, receptors, and plasma-modifying enzymes with atherosclerosis. The influence of decreasing or increasing HDL cholesterol levels on the risk for atherosclerosis clearly depends on the mechanisms by which the HDL cholesterol levels are modulated and thus may dramatically affect potential antiatherosclerosis therapies focused on HDL cholesterol levels.

In summary, adenovirus-mediated overexpression of murine SR-BI in LDLR-deficient mice fed a western-type diet significantly reduced atherosclerosis despite markedly reducing HDL cholesterol levels and only modestly lowering non-HDL cholesterol levels. This suggests that hepatic SR-BI expression can influence the rate of selective HDL cholesterol uptake in the liver and raises the possibility that hepatic SR-BI expression can also influence the overall rate of reverse cholesterol transport from arterial wall to liver. If so, these data would suggest that manipulation of the rate of reverse cholesterol transport by altering expression of an HDL receptor in vivo can have a positive impact on atherogenesis. Consistent with the results reported here are recent studies that have shown that elimination of SR-BI activity in apoE-deficient mice raises HDL cholesterol levels and dramatically accelerates atherogenesis. Furthermore, the data reported here demonstrate that intervention via hepatic SR-BI overexpression after the establishment of either early or more advanced atherosclerotic lesions is effective at reducing the progression of murine atherosclerosis. Because the tissue distribution and regulation of expression of SR-BI in humans or cultured human cells resembles that in mice, these findings raise the possibility that intervention targeted toward increasing hepatic SR-BI expression might provide a novel approach to the prevention and treatment of atherosclerotic cardiovascular disease.

Acknowledgments
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Deficient Mouse

Atherosclerosis in the Cholesterol-Fed LDL Receptor–Deficient Mouse

Karen F. Kozarsky, Mary H. Donahee, Jane M. Glick, Monty Krieger and Daniel J. Rader

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