Both in vitro and in vivo studies of scavenger receptor class B type I (SR-BI) have implicated it as a likely participant in the metabolism of HDL cholesterol. To investigate the effect of SR-BI on atherogenesis, we examined two lines of SR-BI transgenic mice with high (10-fold increases) and low (2-fold increases) SR-BI expression in an inbred mouse background hemizygous for a human apolipoprotein (apo) B transgene. Unlike non-HDL cholesterol levels that minimally differed in the various groups of animals, HDL cholesterol levels were inversely related to SR-BI expression. Mice with the low expression SR-BI transgene had a 50% reduction in HDL cholesterol, whereas the high expression SR-BI transgene was associated with 2-fold decreases in HDL cholesterol as well as dramatic alterations in HDL composition and size including the near absence of α-migrating particles as determined by two-dimensional electrophoresis. The low expression SR-BI/apo B transgenics had more than a 2-fold decrease in the development of diet-induced fatty streak lesions compared with the apo B transgenics (4448 ± 1908 μm²/aorta to 10133 ± 4035 μm²/aorta; p < 0.001), whereas the high expression SR-BI/apo B transgenics had an atherogenic response similar to that of the apo B transgenics (14692 ± 7238 μm²/aorta) but 3-fold greater than the low SR-BI/apo B mice (p < 0.001). The prominent anti-atherogenic effect of moderate SR-BI expression provides in vivo support for the hypothesis that HDL functions to inhibit atherogenesis through its interactions with SR-BI in facilitating reverse cholesterol transport. The failure of the high SR-BI/apo B transgenics to have similar or even greater reductions in atherogenesis suggests that the changes resulting from extremely high SR-BI expression including dramatic changes in lipoproteins may have both pro- and anti-atherogenic consequences, illustrating the complexity of the relationship between SR-BI and atherogenesis.

Among the oldest and most consistently replicated finding concerning the relationship between lipoproteins and risk for atherosclerotic heart disease is the inverse association of HDL cholesterol and coronary artery disease (1, 2). Numerous studies have attempted to explain the properties of HDL that result in this relationship. A major postulated anti-atherogenic property of HDL involves the HDL particle participating in the metabolism of cholesterol through its transport of this lipid from the periphery to the liver, a process termed reverse cholesterol transport (3). In addition, it has been proposed that the particle itself participates in inhibiting pro-atherogenic processes in the plasma and/or at the vessel wall via a variety of mechanisms including the inhibition of lipid peroxidation, cytokine-induced expression of adhesion molecules, and procoagulation processes (4–7).

A significant advance in the understanding of how HDL may participate in cholesterol transport has been the identification and functional analyses of the scavenger receptor class B type I (SR-BI). This is the first HDL receptor to be well defined at the molecular level, and in vivo and in vitro studies have supported its participation in the selective uptake of HDL cholesterol (8–16). Many of the insights concerning the properties of this receptor in HDL metabolism have come from studies where SR-BI expression in mice has been altered by either somatic cell gene transfer or germ line manipulations. Transgene-mediated overexpression of SR-BI in mice resulted in dramatic reductions in plasma HDL (15, 16), whereas adenovirus-mediated overexpression of SR-BI was associated with both a decrease in plasma HDL as well as a substantial increase in biliary cholesterol (9). These SR-BI overexpression results are consistent with studies of mice with decreases in SR-BI expression caused by gene targeting (12, 14) where a marked increase in plasma HDL was noted in animals both heterozygous and homozygous for the targeted allele. Taken together, the in vivo and in vitro studies of SR-BI have consistently supported the suggested role of this molecule in reverse cholesterol transport.

Although the mouse is relatively resistant to diet-induced atherogenesis, a variety of transgenic and gene knockout mice have been created over the last several years with increased atherogenic susceptibility. Mice overexpressing human apo B transgenes with prominent diet-induced hypercholesterolemia have been a useful background to assess the impact on atherogenesis of other putative pro-or anti-atherogenic genetic manipulations (17, 18). The prominent diet-induced hypercholesterolemia and ensuing heightened atherosclerosis susceptibility of the apo B transgenics is the consequence of...

The abbreviations used are: HDL, high density lipoprotein; SR-BI, scavenger receptor class B type I; LDL, low density lipoprotein; LDLr, LDL receptor; apo, apolipoprotein; FPLC, fast protein liquid chromatography; HDLc, HDL cholesterol.

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creased synthesis and decreased clearance of human apo B containing lipoproteins. FVB mice, the background strain of mice in this study, do not develop atherosclerosis even when fed the atherogenic diet, whereas C57/BL6 mice do develop lesions on this diet. This difference allows us to study the well investigated mechanism of apo B-containing lipoprotein atherogenesis in the absence of an unknown mechanism that may be involved in the atherogenesis of C57/BL6 mice.

In the present study investigating the effect of SR-BI overexpression on diet-induced atherosclerosis, aortic fatty streak lesions were quantitatively assessed in animals hemizygous for a human apo B transgene compared with animals that, in addition to the human apo B transgene, contain SR-BI transgenes expressing at two different levels. These studies revealed that moderate increases in SR-BI expression, accompanied by moderate decreases in HDL concentration in the SR-BI/apo B transgenics, were associated with a significant reduction in atherogenesis. In contrast, significant increases in SR-BI expression accompanied by dramatic changes in HDL concentration, size, and composition were associated with an atherogenic susceptibility similar to animals exclusively expressing the human apo B transgene.

MATERIALS AND METHODS

Transgenic Mice—The two lines of SR-BI transgenic mice used in this study were created in an FVB background and expressed the transgene specifically in the liver at levels approximately 2-fold (low SR-BI) and 10-fold (high SR-BI) higher than those of control FVB mice (16). The transgene in these mice was a construct where the human apo A-I promoter had been fused to an SR-BI genomic fragment at its initiation codon. The human apo B transgenic line (apo B) created and maintained in an inbred FVB background has previously been described (18). Hemizygous high or low SR-BI transgenic mice were bred with hemizygous apo B mice to produce mice that either were nontransgenic, hemizygous for one transgene (high or low SR-BI or apo B), or doubly hemizygous for both transgenics (high SR-BI/apo B and low SR-BI/apo B). All mice used in this study were female.

Total RNA Isolation and RNase Protection Assays—Total RNA (10 μg) isolated from livers using RNA Stat 60 (Teltest Inc., Friendswood, TX) were subjected to RNase protection assays. The expression of the endogenous SR-BI, the SR-BI transgene, and the LDL receptor (LDLR) gene was determined as described previously (16).

Diet and Apolipoprotein and Lipoprotein Analyses—Mice were fed Purina mouse chow (number 5001) until 6 weeks of age and then fed an atherogenic diet consisting of 1.25% cholesterol, 0.5% cholic acid, and 15% fat for 18 weeks (19). Blood samples from the tail vein after an overnight fast were collected at 6 weeks of age and 4 weeks after initiation of the atherogenic diet.

Plasma levels of lipids, lipoproteins, and apolipoproteins were determined as described previously (16). Briefly, total cholesterol was determined by an enzymatic colorimetric assay using a kit (number 112756B Roche Molecular Biochemicals). Free cholesterol was determined using a commercially available kit (Wako, Osaka, Japan). Triglyceride and phospholipid concentrations were measured with Triglyceride/GB kit number 450032 (Roche Molecular Biochemicals) and Phospholipid B reagent (Wako), respectively. HDL cholesterol was measured after selective precipitation of non-HDL lipoproteins by dextran sulfate and magnesium chloride. Plasma levels of mouse apo A-I, apo B, and human apo B were determined by enzyme-linked immunosorbent assays as described previously (16, 20).

Two-dimensional nondenaturing electrophoresis and antibody blotting was carried out as described previously (21). Briefly, the first and second gels were used for the separation of lipoproteins. The proteins were separated on 5% polyacrylamide gradient gel (13%–16%) using a Tris-glycine buffer. After transfer to nitrocellulose membranes (Micron Separation Incorporated, Westboro, MA), the samples were treated with biotinylated rabbit polyclonal antibody to murine apo AI (Biodesign, Kennebunk, ME) in 10 mM phosphate buffer, pH 7.0 containing 2% milk. APO AI-containing HDL species were visualized with [125I]-labeled streptavidin (Amersham Pharmacia Biotech), and the nitrocellulose membranes were exposed to Fuji XLS film at −70 °C. Gradient gel electrophoresis and particle size distribution was determined by computer-aided scanning densitometry as described (16).

Pooled plasma samples of animals of each genotype were combined and fractionated using two tandem Superose 6 columns (Amersham Pharmacia Biotech) as described previously (22). Fractions containing either non-HDL or HDL were pooled and concentrated with Centricon concentrators (Amicon Inc., Beverly, MA) for further lipid composition and apolipoprotein analyses.

Atherosclerosis Lesion Area—After 18 weeks of feeding the atherogenic diet, female mice of the various genotypes were sacrificed, and their hearts were collected. Aortic sectioning, lipid staining, and lesion scoring were performed as described previously with some modification (18). Briefly, the heart and attached aorta were first perfused with phosphate-buffered saline and then perfused with diluted tissue embedding medium (Tissue-Tek O.C.T. (Miles Inc., Elkhart, IN):H2O, 1:1) and quickly frozen in O.C.T. 10-μm-thick sections were collected starting with the first and most proximal section of the aorta where the aorta becomes round and the aortic valves distinct. Sections were stained with oil-red O and hematoxylin; the lesion area was determined by measuring the oil-red O stained lesions using a calibrated eyepiece at 200× magnification. The mean lesion area/section/animal was determined for each individual animal.

Statistical Analysis—Significant differences between means were determined using the Mann-Whitney U test for nonparametric analysis.

RESULTS

Gene Expression—To quantitatively assess SR-BI expression and to distinguish expression of the SR-BI transgene from that of the endogenous SR-BI gene, we performed RNase protection assays of SR-BI in differing genetic and dietary environments. Results were normalized densitometrically against the β-actin signal. Neither the SR-BI nor the human apo B transgenes altered the expression of the endogenous SR-BI gene (Fig. 1). The endogenous SR-BI expression levels also were not affected by diet-induced hypercholesterolemia. These results are inconsistent with the previous report (23). A possible explanation is that RNase protection assays, which we performed with total liver RNA, may mask the reciprocal effects on parenchymal and nonparenchymal cells as previously reported. The SR-BI transgenes in this study, consistent with their previous analysis (16), expressed at levels more than 10-fold (high SR-BI transgenics) and 2-fold (low SR-BI transgenics) higher than that of the endogenous SR-BI genes on both the chow and the atherogenic diet.

The RNase protection assays for mouse LDLr analyzed the response of this gene to diet as well as SR-BI genotype. The presence of an SR-BI transgene did not alter the LDLr expression levels, and LDLr expression was down-regulated after the
atherogenic diet treatment in every genotype. This suggests that the atherogenic diet treatment results in increases in hepatocellular cholesterol concentrations of sufficient magnitude to down-regulate LDLr expression. Human apo B transgene induces up-regulation of LDLr with chow diet and higher response to the atherogenic diet compared with wild type mice. Human apo B-containing LDL may block the interaction between mouse apo B and LDLr while not being internalized, resulting in up-regulation of LDLr.

**Plasma Lipid and Apolipoprotein Analyses and FPLC Profiles**—When fed mouse chow, the SR-BI expression level was inversely associated with total, HDL, and non-HDL cholesterol concentrations in apo B transgenic mice (Fig. 2, a–c). Relationship of plasma human apo B levels to genotype was similar to that of cholesterol, whereas plasma mouse apo B levels did not show this trend (Fig. 2, a–e). The atherogenic diet treatment, however, led to dramatic increases in total cholesterol levels in all the transgenic lines studied (Fig. 2f). Notably, non-HDL cholesterol concentrations in each of the three genotypes studied was no longer inversely related to SR-BI expression levels when the animals were fed the atherogenic diet (Fig. 2h). Non-HDL cholesterol levels were similar in low SR-BI/apo B
and apo B transgenic mice, whereas high SR-BI/apo B transgenic mice had significant increases in non-HDL cholesterol concentrations compared with the low SR-BI/apo B mice. There no longer exists any significant difference in human apo B levels among the three genotypes of animals after the atherogenic diet treatment (Fig. 2j). Unlike the changes in non-HDL cholesterol in response to the atherogenic diet, HDL cholesterol levels remained inversely related to SR-BI expression levels in these animals (Figs. 2, f and g). These results were identical in animals after either 4 or 18 weeks on the atherogenic diet. Male mice of each genotype show characteristics in lipid and apolipoprotein analyses comparable with those in female mice (data not shown).

To examine apolipoprotein distribution among lipoprotein fractions, mouse and human apo A and mouse apo A-I concentrations in FPLC fractions were measured in the different groups of animals fed the atherogenic diet (Fig. 3). Apo A-I concentrations in the HDL fractions decreased inversely with SR-BI expression level (Fig. 3b). HDL lipid composition shows no significant differences between low SR-BI/apo B and apo B transgenics (Table I). In contrast, high level expression of SR-BI was associated with the generation of triglyceride-rich, phospholipid depleted HDL. Although there is no significant difference in human apo B concentrations in LDL/IDL fractions between low SR-BI/apo B and apo B transgenics, high SR-BI/apo B mice have higher concentrations of human apo B in these fractions (Fig. 3c). Comparing lipid composition and apo B concentrations of LDL/IDL fractions (Table II) between the different groups of mice revealed no consistent pattern of differences in cholesterol, triglyceride, phospholipid, and apo B content in these particles with SR-BI expression level. These results suggest that these three lines of mice can serve as reagents for exploring the effect of SR-BI expression levels on atherogenesis in a large part, but not entirely, independent of its effect on non-HDL cholesterol.

The effects of SR-BI on HDL subclass distribution was studied by two-dimensional non-denaturing gradient gel electrophoresis (Fig. 4). The size and distribution of pre β-HDL species was not affected by the level of expression of SR-BI, whereas the α HDL fraction, predominantly monodisperse, was influenced by the expression of SR-BI and is markedly decreased in high SR-BI/apo B plasma. Interestingly, the amount of a less prominent but distinct α HDL population of smaller particles was induced by SR-BI, suggesting a dose effect.

The HDL particle size distribution in mice fed the atherogenic diet, as determined by gradient gel electrophoresis of HDL isolated by ultracentrifugation, is in agreement with a previous study (16) and consisted of a monodisperse population of particles. When SR-BI was overexpressed, there was a slight decrease in particle size in low expressors (9.39 ± 0.09 nm (n = 4) versus 9.51 ± 0.03 nm (n = 4) for low SR-BI/apo B and apo B mice, respectively) and the complete absence of large HDL particles with the appearance of a minor population of particles with peak diameter of 7.74 ± 0.15 nm (n = 6) in high expressors.

**Lesion Development**—To evaluate the effect of SR-BI overexpression and associated changes in lipoprotein metabolism on atherogenesis, the various lines of transgenic mice were fed the atherogenic diet for 18 weeks. Consistent with previous studies

### Table I

| Genotype     | UC  | CE  | TG  | PL  |
|--------------|-----|-----|-----|-----|
| m-apo B      | 1.2 | 3.3 | 0.19| 7.7 |
| h-apo B      | 0.81| 1.9 | 0.21| 5.4 |
| m-apo B      | 0.07| 0.35| 0.26| 0.25|

### Table II

| Genotype     | UC  | CE  | TG  | PL  | m-apo B | h-apo B | TG/apo B |
|--------------|-----|-----|-----|-----|---------|---------|-----------|
| m-apo B      | 2.6 | 9.3 | 0.97| 6.9 | 0.44    | 0.33    | 0.029     |
| h-apo B      | 1.7 | 8.1 | 0.95| 0.95| 0.34    | 0.34    | 0.026     |
| m-apo B      | 3.8 | 10.7| 0.97| 6.0 | 0.44    | 0.57    | 0.25      |

Fig. 4. Two-dimensional non-denaturing polyacrylamide gel electrophoresis and antibody blotting of plasma from apo B (a), low SR-BI/apo B (b), and high SR-BI/apo B mice (c) fed the atherogenic diet. Plasma samples of 10–15 mice from each group were combined and subjected to two-dimensional electrophoresis. 20 μl of plasma were electrophoresed on a 0.75% agarose gel (first dimension) and then placed on a 3–16% polyacrylamide gradient gel. Electrophoresis in the second dimension was carried out for 4.5 h. Plasma proteins were transferred to nitrocellulose membranes, and the murine apo AI was visualized with a polyclonal rabbit anti-mouse apo AI antibody. Arrows indicate electrophoretic mobility of lipoproteins in each gel.
The lesion area development after the 18-week atherogenic diet treatment (a), individual plots of the lesion area and HDL cholesterol levels (b), and the lesion area of the mice subgrouped according to the ranges of non-HDL cholesterol levels (c). a, lesion areas in high SR-BI/apo B (solid column), low SR-BI/apo B (shaded column), and apo B (open column) transgenic mice were determined by measuring the oil-red O stained lesions as described under "Materials and Methods." The bar graph represents the mean ± standard deviation from 14 high SR-BI/apo B, 14 apo B, or 15 low SR-BI/apo B female mice. b, lesion area versus plasma HDLc levels of the individual high SR-BI/apo B (solid circles), low SR-BI/apo B (solid triangles), and apo B (open circles) animals after 18-week atherogenic diet treatment. c, animals subgrouped according to the range of their non-HDL cholesterol levels: 250–499, 500–749, and 750–1000 mg/dl. The mean lesion area is represented by solid columns (high SR-BI/apo B), shaded columns (low SR-BI/apo B), or open columns (apo B) with p values shown at the top. The numbers in parentheses show the mean plasma HDLc levels (mg/dl) of the animals. One outlying apo B transgenic mouse whose lesion area (46944 μm²/aorta) was more than 2 standard deviations beyond the other animals of the group was excluded. The exclusion of this animal did not affect the results or statistical significance.

(17, 18), the 18-week atherogenic diet treatment resulted in large fatty streak lesions in the apo B mice (10133 ± 4035 μm²/aorta) (Fig. 5a). The apo B transgenics also containing the low expression SR-BI transgene had significantly smaller lesions (4448 ± 1908 μm²/aorta) than the mice expressing exclusively the human apo B transgene (p < 0.001). This difference in atherogenesis susceptibility between apo B and low SR-BI/apo B mice occurred in the setting of similar non-HDL cholesterol levels, despite the significant decreases in plasma HDL and apo A-I levels in the low SR-BI/apo B transgenics. The individual animal plots of the lesion area and HDL cholesterol levels (Fig. 5b) indicate that low SR-BI/apo B mice develop smaller fatty streak lesions than apo B mice with similar plasma HDLc levels. This observation also is apparent when mice were subgrouped according to their non-HDLc levels (Fig. 5c).

The effect on diet-induced atherogenesis of the high expression SR-BI transgene differed significantly from that of the low expression SR-BI transgene. The lesion area of high SR-BI/apo B transgenics was three times greater than that observed in low SR-BI/apo B animals (p < 0.001). High SR-BI/apo B transgenics also developed larger lesions (14692 ± 7238 μm²/aorta) than those expressing the apo B transgene alone, although this difference did not quite achieve statistical significance (p = 0.06) (Fig. 5c). The effect of the differences in non-HDL cholesterol levels in the high SR-BI/apo B transgenics compared with the low SR-BI/apo B and apo B transgenics on atherogenesis was minimized when lesion area was compared in animals grouped according to similar non-HDL cholesterol concentrations. The lesion area of the high SR-BI/apo B transgenics with lower HDL cholesterol levels was larger than that of low SR-BI/apo B and the apo B transgenics, when comparing animals grouped at three similar levels of non-HDL cholesterol (Fig. 5c).

**DISCUSSION**

Analysis of SR-BI in several in vivo and in vitro studies has provided convincing evidence that SR-BI mediates the selective transport of cholesterol ester into cells, a process intimately linked to how HDL is believed to participate in reverse cholesterol transport (8, 12–16, 24). In the present study we have investigated the relationship between murine atherosogenesis and differing HDL cholesterol and hepatic SR-BI expression levels. The demonstration that the low SR-BI/apo B transgenic mice, despite low HDL cholesterol concentrations, are protected against diet-induced atherogenesis provides in vivo (25–27) support for the hypothesis that HDL-mediated reverse cholesterol transport is likely one mechanism by which HDL participates in inhibiting atherogenesis.

The effects of SR-BI on HDL speciation, size, and composition previously have been reported (12, 24). In our studies, significant differences with regard to HDL lipid composition and particle distribution where noted in the high SR-BI/apo B transgenics compared with the low SR-BI/apo B transgenics and apo B transgenic control animals. The near complete absence of α-migrating HDL supports the hypothesized involvement of these particles in the later steps of reverse cholesterol transport (28) and in agreement with the increased HDL-cholesteryl ester clearance previously observed in the high SR-BI compared with the low SR-BI expressing transgenics (16). Taken together, these findings suggest increased reverse cholesterol transport in the high SR-BI transgenics compared with the two other groups of animals studied.

The finding that the SR-BI/apo B mice have lower non-HDL as well as HDL cholesterol levels compared with apo B transgenics when fed the chow diet agrees with previous SR-BI transgenic studies, suggesting that the selective uptake of cholesterol esters via SR-BI is not restricted to HDL but also includes non-HDL lipoproteins (15, 16, 29–32). Recently, SR-BI transgenics containing an inactive LDLr allele were shown to experience decreases in both HDL as well as non-HDL cholesterol when fed an atherogenic diet (25). This is in contrast to the results of the present study where the non-HDL cholesterol levels in both the low and high SR-BI/apo B transgenics, when fed a similar atherogenic diet, underwent marked increases which eliminated any significant difference between these mice and the apo B animals. FPLC analyses shown in Fig. 3 indicate increases of non-HDLc and human apo B levels in high SR-BI/apo B mice, which are not significant in the plasma lipid analyses shown in Fig. 2. These findings suggest that the high influx of non-HDLc via overexpressed SR-BI may stimulate the secretion of lipoproteins, as well as down-regulate LDLr expression. To what extent this increase of non-HDL lipoproteins contributes to the atherogenic susceptibility in high SR-BI/apo B mice is to be investigated in a future study. A possible explanation for the increase of non-HDLc in SR-BI transgenic...
animals is that non-HDL lipoproteins may be a poor substrate for SR-BI. Because non-HDLc is not taken up as efficiently as HDLc via SR-BI, non-HDL lipoproteins saturate their binding sites in SR-BI when fed the atherogenic diet. This renders the levels of non-HDLc and apo B independent of SR-BI expression. The majority of non-HDL lipoproteins in these animals contain human apo B, which raises the possibility that human apo B containing lipoproteins may be poorer ligands for mouse SR-BI than mouse apo B containing lipoproteins.

The effect of SR-BI on atherogenesis has recently been assessed in two groups of animals: mice containing an SR-BI transgene expressing at more than 10-fold endogenous levels in combination with LDLr knockout alleles (25) and homozygous knockouts for both SR-BI and apo E (24). In these studies dramatic increases and decreases in SR-BI expression resulted in decreases and increases in atherogenesis, respectively. In both studies significant alterations in non-HDL cholesterol as well as HDL cholesterol levels prevented an assessment of the impact of SR-BI on atherogenesis via its ability to participate in the metabolism of HDL. The results from our study of low SR-BI/apo B mice sheds light on the antiatherogenic property of HDL as a key of the reverse cholesterol transport system. Moreover, studying animals with two different overexpression levels of SR-BI reveals the complexity of the protective mechanism of HDL against atherogenesis.

A general observation in studies exploring the effect of transgenes on organismal phenotypes is that there invariably exists a direct relationship between the expression level of the transgene and the magnitude of the transgene effect. Thus, it is surprising that the high SR-BI/apo B transgens fail to be protected from atherogenesis at a level greater or even equal to that of the low SR-BI/apo B transgens. The molecular mechanisms contributing to the atheroprotective properties of HDL have long been thought to be multifactorial (4–7). Studies of the high SR-BI expressor based on the absence of α-migrating particles and their increased HDL cholesterol clearance (16) suggest that these animals have increased reverse cholesterol transport compared with the low SR-BI transgenics, as well as the control animals. Whether the reverse cholesterol transport system, including peripheral cholesterol efflux, is activated in SR-BI overexpressing animals remains to be clarified.

The dramatic effects of high SR-BI expression on HDL and more modest effects on non-HDL lipoproteins suggest that the atherogenesis findings in these animals may be mediated via these lipoprotein changes, thus counteracting any atheroprotective effect of the increase in reverse cholesterol transport. It is possible that the profound decrease in HDL concentration and/or HDL lipid composition in high expressor SR-BI transgenics may lead to a shortage of specific HDL particles with atheroprotective properties. Alterations of LDL/IDL lipoprotein particles and their effect on atherogenesis in the high SR-BI/apo B transgenics may also contribute to the increased diet-induced atherogenic susceptibility of these animals.

By examining a large number of inbred mice differing in SR-BI expression levels, we have derived a perspective on the effect of this molecule on atherogenesis that would have been missed had we examined a smaller number of mice expressing the SR-BI transgene at a single level. Although directly supporting the anti-atherogenic properties of HDL via participation in SR-BI-mediated reverse cholesterol transport, our results suggest that marked overexpression of SR-BI and its impact on HDL and possibly non-HDL lipoproteins may lessen the anti-atherogenic properties normally associated with elevated reverse cholesterol transport. Taken together, these findings suggest that, although activating the reverse cholesterol transport system through increased SR-BI expression is a potential way to reduce atherogenesis, the level of activity may need to be monitored to maximize anti-atherogenic benefits.

REFERENCES

1. Castelli, W. P., Doyle, J. T., Gordon, T., Hames, C. G., Hjortland, M. C., Hulley, S. B., Kagan, A., and Zuckel, W. J. (1977) Circulation 55, 767–772

2. Keys, A. (1980) Lancet 2, 695–696

3. Miller, N. E., La Ville, A., and Crook, D. (1985) Nature 314, 109–111

4. Parthasarathy, S., Barnett, J., and Fong, L. G. (1990) Biochim. Biophys. Acta 1044, 275–283

5. Navab, M., Innes, S. S., Hama, S. Y., Hough, P. G., Ross, L. A., Bork, R. W., Valente, A. J., Berlinder, J. A., Drinkwater, D. C., Laks, H., and Fogelman, A. M. (1991) J. Clin. Invest. 88, 2059–2046

6. Berlinder, J. A., Navah, M., Fogelman, A. M., Frank, J. S., Demer, L. L., Edwards, P. A., Watson, A. D., and Lunis, A. J. (1995) Circulation 91, 2488–2496

7. Griffin, J. H., Kojima, K., Banka, C. L., Curtiss, L. K., and Fernandez, J. A. (1999) J. Clin. Invest. 103, 219–227

8. Acton, S., Rigotti, A., Landschulz, K. T., Xu, S., Hobbs, H. H., and Krieger, M. (1996) Science 271, 518–520

9. Kozarsky, R. P., Donahoe, M. H., Rigotti, A., Ishgal, S. N., Edeleman, E. R., and Krieger, M. (1997) Nature 387, 414–417

10. Babitt, J., Trigatti, B., Rigotti, A., Smart, E. J., Anderson, R. G., Xu, S., and Krieger, M. (1997) J. Biol. Chem. 272, 13242–13249

11. Xu, S., Laccotripe, M., Huang, X., Rigotti, A., Zannis, V. I., and Krieger, M. (1997) J. Biol. Chem. 272, 13240–13249

12. Rigotti, A., Trigatti, B. L., Penman, M., Rayburn, H., Herz, J., and Krieger, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12810–12815

13. Temel, R. E., Trigatti, B., DeMattos, R. B., Azhar, S., Krieger, M., and Williams, D. L. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 13600–13605

14. Varhan, M. L., Rinninger, F., Wang, N., Fairchild-Huntress, V., Dunmore, J. H., Fang, Q., Gosselin, M. L., Dixon, K. L., Deed, J. D., Acton, S. L., Tall, A. R., and Huszar, D. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 4619–4624

15. Wang, N., Arat, T., Ji, Y., Rinninger, F., and Tall, A. R. (1998) J. Biol. Chem. 273, 32920–32926

16. Ueda, Y., Röver, L., Gong, E., Zhang, J., Cooper, P. N., Francone, O., and Rubin, E. M. (1999) J. Biol. Chem. 274, 7163–7171

17. Parcell-Huynh, D. A., Farese, R. V., Jr., Johnson, D. P., Flynn, L. M., Pierotti, V., Newland, D. L., Linton, M. F., Sanan, D. A., and Young, S. G. (1995) J. Clin. Invest. 95, 2246–2257

18. Chen, M. J., Verstuyft, J., Tangirala, R. P., Palinski, W., and Rubin, E. M. (1995) J. Clin. Invest. 96, 1639–1646

19. Nishina, P. M., Verstuyft, J., and Paigen, B. (1990) J. Lipid. Res. 31, 859–869

20. Francone, O. L., Haghpsassand, M., Bennett, J. A., Royer, L., and McNeish, J. (1997) J. Lipid. Res. 38, 114–122

21. Francone, O. L., Royer L. and Haghpsassand, M. (1996) J. Lipid. Res. 37, 1268–1277

22. Yelchade, M., Hamman, R. E., Ishibashi, S., Brown, M. S., and Goldstein, J. L. (1990) Science 250, 1273–1275

23. Fluiiter, K., van der Westhuizen, D. R., and van Berkel, T. J. (1998) J. Biol. Chem. 273, 8434–8438

24. Trigatti, B., Rayburn, H., Vinals, M., Braun, A., Miettinen, H., Penman, M., Hertz, J., Schrenzel, M., Amigo, L., Rigotti, A., and Krieger, M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 9322–9327

25. Arai, T., Wang, N., Benzueso, M., Welch, C., and Tall, A. R. (1999) J. Biol. Chem. 274, 2366–2371

26. Chase, M. B., Santamarina-Fojo, S., Shamburek, R. D., Amar, M. J., Knapper, C. L., Meyn, S. M., Brewer, H. B., Jr. (1998) Circulation 97, I-202

27. Krieger, M., Kozarsky, K. (1999) Curr. Opin. Lipidol. 10, 491–497

28. Fielding, C. J., and Fielding, P. E. (1995) J. Lipid. Res. 36, 2246–2257

29. Calvo, D., Gómez-Coronado, D., Lasuncio, M. A, and Vega, M. A. (1997) Arterioskler. Thromb. Vasc. Biol. 17, 2341–2349

30. Chao, G., Wyne, K. L., and Hobbs, H. (1998) J. Biol. Chem. 273, 31902–31908

31. Swarna Kar, S., Temel, R. E., Connolly, M. A., Azhar, S., and Williams, D. L. (1999) J. Biol. Chem. 274, 29733–29739