Macrophage Apolipoprotein E Reduces Atherosclerosis and Prevents Premature Death in Apolipoprotein E and Scavenger Receptor–Class BI Double-Knockout Mice

Hong Yu, Wenwu Zhang, Patricia G. Yancey, Mark J. Koury, Youmin Zhang, Sergio Fazio, MacRae F. Linton

Objective—Mice null for both apolipoprotein (apo)E and scavenger receptor (SR)-BI (DKO) develop severe hypercholesterolemia, occlusive coronary atherosclerosis, myocardial infarction, and premature death. The current study examines the ability of macrophage apoE to improve the dyslipidemia, reduce atherosclerosis, and rescue the lethal phenotype of DKO mice.

Methods and Results—Initially, bone marrow transplantation (BMT) was unsuccessful, because the DKO mice died from a rapidly fatal anemia 3 to 5 days after lethal irradiation. Therefore, probucol was used to rescue the DKO mice during BMT and was discontinued 2 weeks after BMT, allowing successful reconstitution with donor marrow. Twelve male apoE<sup>−/−</sup>SR-BI<sup>−/−</sup> mice fed 0.5% probucol in a chow diet were lethally irradiated and transplanted with either wild-type (WT) or DKO bone marrow. Two weeks after BMT, apoE was detected in serum in WT→DKO mice, and mean serum cholesterol levels were reduced by 70% versus DKO→DKO mice. Lipoprotein profiles and HDL subpopulations in WT→DKO mice were similar to apoE<sup>+/+</sup>SR-BI<sup>+/+</sup>→DKO mice and resembled those of SR-BI<sup>−/−</sup> mice. In WT→DKO mice, aortic atherosclerosis was reduced by 88% to 90% versus DKO→DKO mice. Furthermore, the DKO→DKO mice died after 8 weeks after BMT, whereas WT→DKO mice exhibited a life span >40 weeks after BMT.

Conclusions—Macrophage apoE is able to rescue the lethal phenotype of apoE<sup>−/−</sup>SR-BI<sup>−/−</sup> mice by improving the dyslipidemia and dramatically reducing atherosclerotic lesion development. (Arterioscler Thromb Vasc Biol. 2006;26: 150-156.)

Key Words: macrophages ■ apolipoprotein E ■ SR-BI ■ cholesterol ■ atherosclerosis

Mice deficient in both apolipoprotein (apo) E and scavenger receptor–class (SR)-BI (DKO) have severe dyslipidemia, develop early occlusive atherosclerotic coronary artery disease, and die prematurely at 6 to 8 weeks of age with evidence of myocardial infarction.<sup>1</sup> The dyslipidemia in DKO mice is characterized by increased serum cholesterol levels (800 to 1000 mg/dL) because of accumulation of apoB containing remnant lipoproteins and large, free cholesterol (FC)–enriched, high-density lipoprotein (HDL) particles.<sup>2</sup> The accelerated atherosclerosis and lethal phenotype of DKO mice is disproportionate to the degree of hypercholesterolemia given that apoE<sup>+/−</sup> and low-density lipoprotein (LDL) receptor–deficient (LDLR<sup>−/−</sup>) mice fed Western type diets develop atherosclerosis more slowly despite having comparable or higher levels of serum cholesterol.<sup>1,4,5</sup> Thus, dual deficiency of apoE and SR-BI may accelerate the development of atherosclerosis by producing a toxic dyslipidemia with accumulation of both apoB containing remnant lipoproteins and large, dysfunctional HDL particles.

ApoE is a crucial ligand for the hepatic clearance of remnant lipoproteins. The absence of apoE leads to the accumulation of sphingomyelin-enriched remnants, which, compared with wild-type (WT) particles, induces more cholesterol accumulation in macrophages.<sup>4</sup> ApoE also functions in HDL metabolism by participating in HDL formation, maturation, and hepatic uptake.<sup>5</sup> ApoE deficiency results in impairment of HDL maturation and decreased lecithin-cholesterol acyltransferase activity.<sup>6</sup> SR-BI plays a key role in HDL metabolism by mediating selective uptake of cholesterol esters by the liver.<sup>7,8</sup> Mice deficient in SR-BI accumulate abnormally large free cholesterol–enriched HDL.

Most of the apoE in plasma is derived from liver, and a small fraction of plasma apoE is secreted by macrophages. Previously, we reported that the small amount of apoE produced by macrophages is adequate to correct the dyslipidemia and to prevent atherosclerosis in apoE<sup>−/−</sup> mice.<sup>9</sup> In addition, macrophage apoE expression is able to significantly impact the abnormal profile of HDL cholesterol in apoE<sup>−/−</sup>...
mice. Surprisingly, reconstitution of macrophage apoE expression in apoE−/− LDLR−/− mice results in increased serum levels of apoE but no changes in serum lipids or lipoproteins, demonstrating in vivo a requirement for hepatic apoE in the clearance of remnant lipoproteins when the LDLR is absent. Furthermore, our recent studies using hypomorphic apoE mice demonstrated that hepatocyte apoE is more efficient in promoting the clearance of remnant lipoproteins than extrahepatic apoE. Studies have also demonstrated that hepatic apoE and SR-BI are coordinately regulated with deletion of 1 protein enhancing the expression of the other suggesting intersecting functions of the 2 proteins. Arai et al demonstrated a separate impact of hepatic apoE compared with apoE on HDL in mediating the uptake of HDL cholesterol and that the hepatic selective uptake of HDL cholesteryl esters is decreased in apoE−/− mice despite increased hepatic SR-BI. Furthermore, studies have recently suggested a role for hepatic SR-BI in the clearance of remnant lipoproteins.

Zhang et al have recently reported that low-level expression of hepatic apoE from the transgenic hypomorphic apoE allele is capable of promoting lipoprotein clearance and rescuing the lethal phenotype of DKO mice. However, given the possibility of intersecting roles of hepatic apoE and SR-BI in HDL cholesterol metabolism and studies demonstrating the greater efficiency of hepatic apoE versus extrahepatic apoE in promoting uptake of remnant lipoproteins, it is uncertain whether extrahepatic apoE is capable of correcting the toxic dyslipidemia present in apoE−/− SR-BI−/− mice. We report that macrophage apoE promotes dramatic reductions in serum cholesterol levels, improves HDL subpopulations, provides extensive protection from atherosclerotic lesion development, and prevents premature death in DKO mice.

### Methods

#### Mice and Genotyping

C57BL/6, apoE−/− (C57BL/6 genetic background), and SR-BI−/− (mixed C57BL/6/SI129 genetic background) mice were obtained from Jackson Laboratories. SR-BI−/− mice were backcrossed to C57BL/6 background. ApoE−/−SR-BI−/− mice on the C57BL/6 background were produced and genotyped as described previously. ApoE−/− SR-BI−/− mice were generated from heterozygous mating pairs or mating female apoE−/−SR-BI−/− with apoE−/−SR-BI−/− males maintained on 0.5% (wt/wt) probucol. All of the mice were housed in microisolator cages on a rodent chow diet as described by Braun et al. All of the mice were housed in microisolator cages on a rodent chow diet either with or without 0.5% probucol supplementation. Animal care and experimental procedures were performed in accordance with the regulations of the Institutional Animal Care and Usage Committee of Vanderbilt University.

#### Lethal Irradiation and Bone Marrow Transplantation

Seven-week-old apoE−/−SR-BI−/− recipient mice maintained on 0.5% probucol in chow diet were lethally irradiated (9 Gy; Cesium γ source), and 8×10^6 donor bone marrow cells were injected as previously described.

#### Lipid and Lipoprotein Analyses

Blood samples were collected by retro-orbital venous plexus puncture after overnight fasting at 2-week intervals. Serum or HDL cholesterol and triglyceride levels were determined enzymatically using Cholesterol Reagent and Triglycerides GPO Reagent kits (Raichem). Serum from 4 mice per group were randomly chosen for FPLC on a Superose 6 column (Pharmacia) using the Waters 600E system as described.

### 2D Gel Electrophoresis

The HDL subpopulations were analyzed by 2D nondenaturing agarose-polyacrylamide gel electrophoresis. Briefly, in the first dimension, 2 μL of fresh plasma lipoproteins were separated on 0.7% agarose gel. Individual agarose strips were transferred to the second dimension, 16% nondenaturing polyacrylamide gradient gels and separated in the second dimension. Lipoproteins were transferred to 0.2 μm nitrocellulose membranes (BA83 Scheicher and Schuell), and 2D patterns of apoAI-containing lipoproteins were detected using a phosphoimaging device (Molecular Imager FX System, Bio-Rad) after immunolocalization by rabbit anti-mouse apoAI primary and 125I-labeled anti-rabbit secondary antibodies.

#### Quantification of Atherosclerotic Lesions

Eight weeks after bone marrow transplantation (BMT), the extent of atherosclerosis was examined both in oil red O–stained cross-sections of the proximal aorta (15 alternate 10-μm cryosections) and by en face analysis with quantitation using the KS300 imaging system as described previously.

#### Statistical Analysis

The Mann–Whitney test was used to measure the statistical differences in lesion area. Data are expressed as mean±SEM. The other results were analyzed by 2-tailed Student t test. A value of P<0.05 was considered to be statistically significant.

### Results

Initial efforts to rescue the DKO mice by BMT were unsuccessful because of hypersensitivity to lethal irradiation, as the DKO recipient mice died within 3 to 5 days, irrespective of the genotype of donor marrow. In contrast, death because of sepsis related to neutropenia usually occurs ~2 weeks after lethal irradiation in mice. In DKO mice, ~80% of plasma cholesterol is unesterified, leading to marked accumulation of FC in red blood cells (RBCs), resulting in deformed RBCs, anemia, and dramatic reticulocytosis. Therefore, we considered the possibility that the severe anemia causes the hypersensitivity to lethal radiation. Examination of blood counts 1 and 3 days after lethal irradiation of the DKO mice revealed a precipitous decline in hematocrit as early as 2 weeks after transplantation but

B. Braun et al have reported previously that treatment of DKO mice with probucol, an antioxidant and lipid-lowering agent, lowers serum cholesterol levels, corrects the anemia, and rescues the lethal phenotype of DKO mice. Therefore, we examined the ability of probucol to temporarily rescue the DKO mice during BMT. Seven-week–old male DKO recipient mice (n=12 in each group) fed a chow diet containing probucol were lethally irradiated and transplanted with bone marrow cells from WT or DKO donor mice. The mice survived the transplantation period, indicating that probucol was successful in rescuing the mice from premature death because of radiation. Probucol treatment was withdrawn 2 weeks after BMT. ApoE was detectable in the serum of WT→DKO mice as early as 2 weeks after transplantation but
not in the DKO→DKO mice (Figure I, available online at http://atvb.ahajournals.org), indicating the successful reconstitution of recipient mice with donor bone marrow cells. Four weeks after BMT (2 weeks after the discontinuation of probucol), serum apoE levels in WT→DKO were ~10% of normal serum apoE levels in WT mice.

At baseline, the DKO recipient mice maintained on 0.5% probucol had mean serum cholesterol levels of 452±20 mg/dL. Coincident with the appearance of apoE in the serum 2 weeks after BMT, serum cholesterol levels in WT→DKO mice decreased by 70% compared with DKO→DKO mice (135±16 mg/dL versus 457±16 mg/dL, respectively). The serum cholesterol levels remained low in the WT→DKO mice (Table) and were similar to those of SR-BI−/− mice fed a chow diet (data not shown). In contrast, the mean serum cholesterol level in the DKO→DKO mice increased to 722±30 mg/dL by 6 weeks after BMT. Eight weeks after BMT, the serum cholesterol levels in the DKO→DKO mice were similar to the levels of untreated (no probucol) 8-week-old DKO mice. The introduction of apoE into DKO mice had a marked effect on serum-free cholesterol (FC) levels with serum FC being reduced by 71% as early as 2 weeks after BMT and accounting for 52% of the total cholesterol at 8 weeks after BMT. In contrast, the serum FC in DKO→DKO mice increased dramatically 8 weeks after BMT 748±27 versus 232±21 mg/dL at baseline, accounting for 77% of the total serum cholesterol. Similarly, serum triglyceride levels were significantly reduced in WT→DKO mice as early as 4 weeks after BMT but were increased in the DKO→DKO mice (Table).

The reductions in serum cholesterol levels of WT→DKO mice were because of decreases in particles that were of very LDL (VLDL), intermediate density lipoprotein (IDL), and LDL size (Figure 1). These decreases in lipoprotein cholesterol were evident at 2 weeks after BMT and were more pronounced at 4 weeks after BMT (Figure 1A). Furthermore, the probucol-induced decreases in cholesterol of the VLDL-, IDL-, LDL-, and HDL-sized particles were restored gradually after probucol was removed from the diet of DKO→DKO mice 2 weeks after BMT (Figure 2B), and the cholesterol in VLDL-IDL-sized particles was similar to that in the FPLC profiles of untreated DKO (no probucol) mice by 8-weeks after BMT (Figure 1B). After 6 weeks without probucol (8 weeks after BMT), the lipoprotein profile of WT→DKO mice was stable (Figure 1C) and approached that of SR-BI−/− mice. In addition, we examined whether macrophage SR-BI expression might be contributing to the changes in serum lipids and lipoprotein profiles seen in the WT→DKO mice. The changes in serum lipid levels and lipoprotein profiles in the apoE−/−SR-BI−/−→DKO mice and apoE−/−SR-BI+/+→DKO mice (data not shown) were similar to the changes seen in the WT→DKO mice and DKO→DKO mice, respectively, showing that macrophage SR-BI does not contribute to the correction of the abnormal lipoprotein profile in DKO mice.

Using 2D gel electrophoresis, we investigated the effect of macrophage apoE expression on apoAI-containing HDL subpopulations in the plasma of WT→DKO mice (Figure 2). ApoAI-containing HDL subpopulation profiles are also shown for plasma from WT, SR-BI−/−, DKO (no probucol treatment), and DKO→DKO mice (Figure 2). The majority of apoAI (80% to 90%) in plasma of WT mice had α mobility, and relatively small amounts of pre-β migrating (pre-B1 and pre-B2) HDL particles containing apoAI were detected. Compared with WT mice, SR-BI−/− mice had an accumulation of large HDL migrating between pre-β and α without changes in pre-β HDL. The HDL subpopulation profiles of DKO mice were strikingly different from those of WT mice, with the accumulation of extremely large particles migrating between pre-β and α mobility. After transplantation, the HDL subpopulations of WT→DKO mice were very similar to those of SR-BI−/− mice. In contrast, there was no significant difference between the HDL subpopulation profiles of DKO→DKO mice and DKO mice. Examination of the effects of macrophage SR-BI on HDL subpopulations showed that the distribution of apoAI-containing particles was similar in WT→DKO mice compared with apoE−/−SR-BI−/−→DKO mice, and DKO→DKO mice compared with apoE−/−SR-BI+/+→DKO mice (data not shown). Thus, the introduction of macrophage SR-BI expression in DKO mice does not promote significant changes in HDL subpopulations, a finding consistent with our previous studies in apoE−/− mice.

Consistent with the changes in HDL subpopulations, the introduction of macrophage apoE into DKO mice markedly affected HDL composition (Table I, available online at http://atvb.ahajournals.org). Compared with HDLs from the
other 3 mouse strains, DKO HDL had elevated cholesterol with the bulk of this increase being FC, which represented 30% of the HDL mass compared with only 5% FC in WT HDL. The FC/PL ratio of DKO HDL was 1.1 compared with 0.2, 0.3, and 0.64, respectively, for WT, apoE⁻/⁻, and SR-BI⁻/⁻ HDL. Eight weeks after BMT, the introduction of the macrophage apoE decreased the HDL FC/PL ratio (0.4) to a level intermediate between WT and SR-BI⁻/⁻ HDL. Similar trends were observed for the TC/protein and FC/protein ratios (Table 1).

The influence of macrophage apoE on atherosclerotic lesion development in DKO mice was examined 8 weeks after BMT (6 weeks off probucol), because the DKO→DKO mice begin to die around this time. The WT→DKO mice were dramatically protected from atherosclerosis with only small fatty streak lesions in the proximal aorta (Figure 3A) compared with large, complicated lesions in DKO→DKO mice (Figure 3B). Quantitative analysis of cross-sections of the proximal aorta demonstrated an 88% decrease in mean atherosclerotic lesion area in WT→DKO mice (n=6) versus DKO→DKO mice (n=8; 83.4±14.7×10⁶ versus 677.7±73.5×10⁵ μm²; mean±SEM; P=0.0007; Figure 4A). End face analysis of pinned-out aortas revealed a 90% decrease in atherosclerotic lesion area in WT→DKO mice compared with DKO→DKO mice (Figure 3C and 4B).

Another goal of the current study was to determine whether expression of macrophage apoE rescued the lethal phenotype of apoE⁻/⁻ SR-BI⁻/⁻ mice. Figure 5 shows the survival curves for WT→DKO mice and DKO→DKO controls. The average survival for the DKO→DKO control mice (solid line) was 8 weeks after BMT (6 weeks after removal of probucol). In contrast, the life span of the WT→DKO mice (dashed line) was dramatically prolonged. All of the WT→DKO mice had a life span of >40 weeks after BMT, and the majority of mice were still alive after 59 weeks. Thus, macrophage apoE is capable of rescuing the lethal phenotype of DKO mice.

Discussion

Combined deficiency of apoE and SR-BI in the mouse results in severe hypercholesterolemia because of accumulation of VLDL and large HDL particles, occlusive coronary atherosclerosis, and premature death from myocardial infarction. We examined the ability of macrophage apoE introduced by BMT to improve the dyslipidemia and prevent atherosclerosis and premature death in DKO mice. After the transplantation of DKO mice with either WT or apoE⁺/⁺SR-BI⁻/⁻ bone marrow, macrophage apoE expression promoted dramatic improvements in serum lipid and lipoprotein levels and in HDL composition and subpopulations resulting in lipid and lipoprotein profiles similar to those seen in SR-BI⁻/⁻ mice. The improved dyslipidemia in the WT→DKO mice was associated with dramatic reductions in the extent of atherosclerosis and improved survival compared with the DKO→DKO mice. Thus, macrophage apoE is capable of rescuing the lethal phenotype in mice deficient in both apoE and SR-BI by improving the dyslipidemia, reducing atherosclerosis, and preventing premature death.

The DKO mice have been reported to have anemia characterized by abnormal RBC morphology, increased membrane cholesterol, autophagolysosome inclusions, and a dramatic reticulocytosis.²³ We found that the DKO mice showed an increased sensitivity to lethal radiation resulting in death by 3 to 5 days. Lethal irradiation was accompanied by a dramatic drop in hematocrit (data not shown), indicating that lethal irradiation impairs the ability of DKO bone marrow to sustain RBC turnover resulting in a rapidly fatal anemia. Based on the work by Braun et al²² demonstrating that probucol, an antioxidant, lowers cholesterol, corrects the anemia, and rescues the lethal phenotype of DKO mice, we examined the ability of probucol to temporarily rescue the DKO mice during BMT. Indeed, we found that treatment...
with probucol rescued the DKO recipient mice from accelerated death after lethal irradiation, allowing time for reconstitution with donor bone marrow.

Based on the knowledge that macrophage apoE is able to correct the dyslipidemia in apoE<sup>−/−</sup> mice<sup>9</sup> and recent evidence that low-level expression of hepatic apoE from the transgenic hypomorph apoE allele is capable of correcting the dyslipidemia of DKO mice<sup>19</sup>, one might predict that introduction of macrophage apoE into DKO mice would improve the dyslipidemia in DKO mice by providing a ligand for the clearance of remnant lipoproteins. However, this was not a forgone conclusion, given data demonstrating a functional difference in hepatic apoE and extrahepatic apoE in remnant lipoprotein clearance in vivo.<sup>20</sup> Hepatocyte apoE is more efficient in promoting the clearance of remnant lipoproteins than extrahepatic apoE in the hypomorphic apoE mice.<sup>13</sup> In addition, hepatic expression of apoE is required for remnant lipoprotein clearance in the absence of the LDLR in vivo, because macrophage apoE does not promote remnant lipoprotein clearance in apoE<sup>−/−</sup>LDLR<sup>−/−</sup> mice.<sup>11,12</sup> SR-BI has been implicated in remnant lipoprotein clearance based on in vitro studies<sup>17</sup> and in vivo overexpression studies.<sup>16,18</sup> However the physiological relevance of SR-BI in remnant lipoprotein metabolism remains to be elucidated. The current results demonstrate that macrophage apoE is able to efficiently promote resolution of the VLDL-IDL cholesterol accumulation found in DKO mice (Figure 1) despite the absence of hepatocyte SR-BI expression, indicating that, unlike the LDLR<sup>11,12</sup> SR-BI does not play a critical role in extrahepatic apoE-mediated remnant lipoprotein clearance in the absence of hepatic apoE expression. Furthermore, macrophage SR-BI expression did not contribute to changes in plasma lipids, lipoproteins, or HDL subpopulations, because these parameters did not differ in WT→DKO or apoE<sup>−/−</sup>SRI<sup>−/−</sup>→DKO mice.

The DKO mice have marked accumulation of abnormally large HDL particles that are enriched in FC.<sup>2</sup> Both SR-BI and apoE operate in HDL metabolism, and deletion of either protein results in the formation of HDL particles with dysfunctional properties and accelerated atherosclerosis. Deletion of apoE results in reduced HDL cholesterol, with the particles having reduced apoAI but increased apoAIV content<sup>19</sup> and reduced capacity to accept cholesterol.<sup>26</sup> ApoE deficiency also results in impaired maturation of HDL because of decreased lecithin-cholesterol acyltransferase activity.<sup>4</sup> In contrast, deletion of SR-BI increases HDL cholesterol and results in the formation of large, FC-enriched HDL<sup>2</sup> that induces abnormal RBCs.<sup>22</sup> The studies of Trigatti et al<sup>22</sup> have demonstrated that combined deficiency of SR-BI and apoE results in a much more marked increase in HDL FC than that seen with single deletion of SR-BI, with the HDL particles being abnormally large and similar to lipoprotein X (LpX).<sup>22,27</sup> ApoAI, which makes up ~60% of the HDL protein content, is thought to be largely responsible for the antiatherogenic effects of HDL.<sup>28</sup> Analysis of the apoAI-containing lipoprotein particles from DKO mice by 2D gel

![Figure 2. 2D separation of HDL subpopulations in plasma samples of WT, SR-BI<sup>−/−</sup>, DKO mice and DKO mice transplanted with WT or DKO bone marrow. ApoAI-containing lipoproteins were recognized by immunolocalization of mouse apoAI. The mobilities of α and pre-β migrating particles (first dimension) are indicated.](http://atvb.ahajournals.org/)

![Figure 3. Representative atherosclerotic lesions in crosssections of proximal aortas stained with oil-red O from WT→DKO (A) or DKO→DKO mice (B) at 8 weeks after BMT. Representative en face aortas from WT→DKO mice compared with DKO→DKO mice (C).](http://atvb.ahajournals.org/)

![Figure 4. Macrophage apoE dramatically protects WT→DKO mice from developing atherosclerosis. Quantitation of the mean cross-sectional lesion area of proximal aortas from WT→DKO mice compared with DKO→DKO mice (A). Percentage lesion area in the aorta by en face analysis for each mouse and the mean (bar) for each group (B).](http://atvb.ahajournals.org/)
electrophoresis also revealed the presence of markedly enlarged apoAI-containing particles that migrate between pre-β and α electrophoretic mobility (Figure 2). Because the combined deficiency of SR-BI and apoE results in the formation of abnormally enlarged and FC-enriched HDL, it is probable that these particles are severely dysfunctional compared with the HDL from single knockout mice and that these particles also contribute significantly to the accelerated atherosclerosis development in DKO mice. Consistent with this concept, the present studies demonstrate that expression of macrophage apoE in DKO mice markedly reduces atherosclerosis while reducing the accumulation of the markedly enlarged, FC-enriched, apoAI-containing HDL particles and producing an HDL composition and subpopulation profile similar to that in SR-BI−/− mice.

The formation of extremely abnormal HDL particles with double deficiency of SR-BI and apoE compared with single deletion of SR-BI and the reduced accumulation of extremely large HDL with the introduction of extrahepatic apoE also substantiate a role of apoE in clearance of HDL. Studies have shown that deletion of apoE reduces the selective uptake of HDL cholesteryl ester despite increased hepatic expression of SR-BI, and the reduced selective uptake was attributed to the absence of apoE on HDL and in the liver.15 Consistent also with a role of apoE in HDL clearance is the marked accumulation of apoE on HDL in SR-BI−/− mice.14 It is also worth noting that recent studies of Zhang et al.19 demonstrated reduced accumulation of abnormally large HDL particles when low levels of hepatic apoE were expressed in DKO mice. Given the formation of extremely abnormal HDL particles with dual deficiency of the 2 proteins compared with single deletion of SR-BI, it is probable that apoE is able to mediate clearance of HDL independent of SR-BI. Because apoE is a ligand for LDLR-related protein, possible mechanisms include holoparticle uptake of apoE containing HDL and/or selective uptake via LDLR-related protein.29

In addition to the toxic dyslipidemia because of the impact of dual deficiency of apoE and SR-BI on metabolism of both HDL and remnant lipoproteins, it is likely that the macro-

phage deficiency of both apoE and SR-BI also contributes significantly to the dramatically accelerated occlusive coronary atherosclerosis in DKO mice. Macrophage expression of both apoE10 and SR-BI11 have been implicated in promoting cholesterol efflux. Furthermore, in macrophages expressing apoE, increased expression of SR-BI has been reported to reduce the expression of apoE and cholesterol efflux,32 suggesting a connection between these 2 pathways. Deficiency of macrophage apoE has been shown to increase atherosclerosis in a number of murine models.12,33,34 Similarly, macrophage SR-BI deficiency has been shown to promote atherosclerosis in apoE−/− and LDLR−/− mice.21,35 Thus, it seems likely that macrophage deficiency of both apoE and SR-BI also contributes to the rapid development of occlusive coronary heart disease through accelerated foam cell formation. However, the dramatic improvement in dyslipidemia seen in the WT→DKO mice prevents one from drawing any conclusions regarding the relative contribution of macrophage deficiency of both apoE and SR-BI to the accelerated atherosclerosis in DKO mice. Additional studies will be required to define the macrophage-specific contributions of dual deficiency of apoE and SR-BI on macrophage function and cholesterol homeostasis.

Acknowledgments

This work was supported by National Institutes of Health grants HL53989, HL64505 (to M.F.L.), HL57986, DK59637 (to S.F.), and DK59637 (Lipid, Lipoprotein, and Atherosclerosis Core of the Vanderbilt Mouse Metabolic Phenotype Centers), and by an American Heart Association Grant-in-Aid from the Southeast Affiliate (to M.F.L.). Special thanks to Drs. June Liu, Yan Ru Su, and Vladimir Babaev for expert advice and Prapapom Kopsombut technical assistance.

References


Macrophage Apolipoprotein E Reduces Atherosclerosis and Prevents Premature Death in Apolipoprotein E and Scavenger Receptor –Class BI Double-Knockout Mice
Hong Yu, Wenwu Zhang, Patricia G. Yancey, Mark J. Koury, Youmin Zhang, Sergio Fazio and MacRae F. Linton

Arterioscler Thromb Vasc Biol. 2006;26:150-156; originally published online November 3, 2005;
doi: 10.1161/01.ATV.0000194096.89476.73

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/26/1/150

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2005/11/08/01.ATV.0000194096.89476.73.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
Methods:

**Determination of apoE Expression by Western Blotting**

For detection of apoE in serum, 3 µL of serum was separated on 4-12% NuPAGE Bis-Tris Gel (Invitrogen) and transferred to nitrocellulose membranes. ApoE was detected using a polyclonal rabbit anti-mouse apoE antibody (Biodesign), a goat anti-rabbit antibody conjugated with horseradish peroxidase (Sigma) and enhanced chemiluminescence detection (ECL Western blotting, Amersham). The same blot was reprobed using rabbit anti-mouse apoAI (Biodesign).

**HDL Composition**

HDL was isolated from pooled plasma samples. First the apoB containing lipoproteins were precipitated by the addition of 20% polyethylene glycol (PEG) in 200mM glycine (pH= 10.0, plasma/PEG ratio, 1.0:0.04, vol/vol)\(^{1,2}\). After dialysis of the HDL containing supernatent against 0.9%NaCl, 0.3mM EDTA, the HDL was isolated at denisty < 1.21g/ml by ultracentrifugation as described\(^3\). HDL protein was determined by the method of Lowry\(^4\) HDL phospholipid (PL) was measured using Wako Phospholipids B enzymatic kit (Wako Laboratory Chemicals, USA).

**Figure I**

Immunoblot of serum apoE in DKO mice after transplantation. Lanes 1 to 3: WT mouse serum dilutions (1:5, 1:10, 1:20, respectively); Lane 4 to 6: WT→DKO undiluted mouse serum at 2, 4, 10 weeks post-BMT; Lane 7 to 8: DKO→DKO undiluted mouse serum at 2, 6 weeks post-BMT, lane 10: DKO undiluted mouse serum. ApoAI was probed as the loading control.
References:


Table I. Composition of HDL isolated from WT, ApoE<sup>−/−</sup>, SR-BI<sup>−/−</sup>, ApoE<sup>−/−</sup>SR-BI<sup>−/−</sup>, and WT→DKO mice.

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>%FC ± SE</th>
<th>%TC ± SE</th>
<th>%PL ± SE</th>
<th>%Protein ± SE</th>
<th>%TG ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>4.7 ± 0.7</td>
<td>18.7 ± 0.6</td>
<td>28.8 ± 1.6</td>
<td>50.9 ± 1.7</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>8.3 ± 1.0*</td>
<td>19.3 ± 1.5</td>
<td>28.4 ± 2.4</td>
<td>49.7 ± 2.0</td>
<td>2.6 ± 0.6</td>
</tr>
<tr>
<td>SR-BI&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>18.7 ± 0.5*</td>
<td>29.4 ± 1.9*</td>
<td>29.1 ± 1.6</td>
<td>39.5 ± 1.8*</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>ApoE&lt;sup&gt;−/−&lt;/sup&gt;SR-BI&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>29.6 ± 3.5*</td>
<td>35.1 ± 1.9*</td>
<td>27.9 ± 2.7</td>
<td>34.6 ± 4.2*</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>WT→DKO</td>
<td>11.2 ± 4.5</td>
<td>27.8 ± 5.1</td>
<td>27.8 ± 1.9</td>
<td>39.8 ± 3.9*</td>
<td>4.7 ± 0.6</td>
</tr>
</tbody>
</table>