Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 317 (2004) 223–229
BBRC
www.elsevier.com/locate/ybbrc

Effect of macrophage-derived apolipoprotein E on hyperlipidemia and atherosclerosis of LDLR-deficient mice

Weibin Shi,[a,c,*] Xuping Wang,[d,e] Jack Wong,[d,e] Catherine C. Hedrick,[b,c] Howard Wong,[d] Lawrence W. Castellani,[d,e] and Aldons J. Lusis[d,e]

[a] *Department of Radiology, University of Virginia, Charlottesville, VA 22908, USA*
[b] *Department of Medicine, University of Virginia, Charlottesville, VA 22908, USA*
[c] *Cardiovascular Research Center, University of Virginia, Charlottesville, VA 22908, USA*
[d] *Department of Medicine, University of California, Los Angeles, CA 90095-1679, USA*
[e] *Department of Microbiology and Molecular Genetics, University of California, Los Angeles, CA 90095-1679, USA*

Received 19 February 2004

Abstract

LDL receptor-deficient ($LDLR^{-/-}$) mice fed a Western diet exhibit severe hyperlipidemia and develop significant atherosclerosis. Apolipoprotein E (apoE) is a multifunctional protein synthesized by hepatocytes and macrophages. We sought to determine effect of macrophage apoE deficiency on severe hyperlipidemia and atherosclerosis. Female $LDLR^{-/-}$ mice were lethally irradiated and reconstituted with bone marrow from either $apoE^{-/-}$ or $apoE^{+/+}$ mice. Four weeks after transplantation, recipient mice were fed a Western diet for 8 weeks. Reconstitution of $LDLR^{-/-}$ mice with $apoE^{-/-}$ bone marrow resulted in a slight reduction in plasma apoE levels and a dramatic reduction in accumulation of apoE and apoB in the aortic wall. Plasma lipid levels were unaffected when mice had mild hyperlipidemia on a chow diet, whereas IDL/LDL cholesterol levels were significantly reduced when mice developed severe hyperlipidemia on the Western diet. The hepatic VLDL production rate of mice on the Western diet was decreased by 46% as determined by injection of Triton WR1339 to block VLDL clearance. Atherosclerotic lesions in the proximal aorta were significantly reduced, partially due to reduction in plasma total cholesterol levels ($r = 0.56$; $P < 0.0001$). Thus, macrophage apoE-deficiency alleviates severe hyperlipidemia by slowing hepatic VLDL production and consequently reduces atherosclerosis in $LDLR^{-/-}$ mice.

Keywords: Atherosclerosis; Macrophages; Apolipoprotein E; Hyperlipidemia; Bone marrow transplantation

Apolipoprotein E (apoE), a 34-kDa glycoprotein, is a structural component of chylomicron remnants, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and some subpopulations of high-density lipoproteins (HDL) [1,2]. ApoE mediates the uptake and degradation of chylomicron and VLDL remnants by acting as a ligand for the LDL receptor and the LDL receptor-related protein [1,3]. ApoE-deficiency ($apoE^{-/-}$) results in severe hypercholesterolemia and diffuse atherosclerotic lesions in human [4] and gene-targeted mice [5,6].

The liver produces the vast majority of plasma apoE, but apoE is also synthesized by macrophages in various organs [7–9]. The macrophage-produced apoE has been proposed to exert an anti-atherosclerotic effect by promoting cholesterol efflux from macrophages [10,11] and reverse cholesterol transport [12]. Bone marrow transplantation has been used to determine the role of macrophage-derived apoE in the development of atherosclerosis. Reconstitution of an atherosclerosis-susceptible mouse strain C57BL/6 (B6) with bone marrow from $apoE^{-/-}$ mice had no influence on plasma lipid levels but markedly increased atherosclerosis [11,13]. In striking contrast, Boisvert and Curtiss [14] reported that elimination of macrophage-derived apoE reduced atherosclerosis in B6 recipient mice. We found that reconstitution of an atherosclerosis-resistant mouse strain C3H/HeJ with $apoE^{-/-}$ bone marrow had no influence on plasma lipid levels and atherosclerosis [15].

* Corresponding author. Fax: 1-434-924-9242.
E-mail address: ws4v@virginia.edu (W. Shi).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.03.037

Wild-type mice develop only mild hyperlipidemia and fatty streak lesions at the proximal aorta. Moreover, an unphysiological cholate-containing high-fat/cholesterol diet has to be used to induce atherosclerosis in the mice. In contrast, LDL receptor knockout ($LDLR^{-/-}$) mice exhibit severe hypercholesterolemia and develop all phases of atherosclerotic lesions seen in humans when the mice are fed a high-fat/cholesterol diet [16]. In the present study, female $LDLR^{-/-}$ mice were transplanted with $apoE^{-/-}$ or $apoE^{+/+}$ bone marrow and examined for the effect of macrophage apoE deficiency on severe hyperlipidemia and atherosclerotic lesion formation. We have now provided evidence that macrophage apoE-deficiency lessens severe hyperlipidemia and reduces atherosclerotic lesion formation in $LDLR^{-/-}$ mice.

Methods

Mice. All mice were obtained from The Jackson Laboratories, Bar Harbor, ME. The mice were fed a standard rodent chow diet containing 4% fat (Ralston-Purina, St. Louis, MO) or an adjusted Western-type diet containing 42% fat, 0.15% cholesterol, and 19.5% casein without sodium cholate (TD 88137, Madison, WI). All procedures were in accordance with current NIH guidelines and approved by our University Animal Research Committees.

Bone marrow transplantation. Female $LDLR^{-/-}$ recipient mice, at 8 weeks of age, were lethally irradiated with a dose of 1100 rads. Donor bone marrow cells were harvested from male $apoE^{-/-}$ or $apoE^{+/+}$ mice by flushing femurs and tibias and prepared as we previously described [17]. Each recipient mouse was injected through the tail veins with 10^7 bone marrow cells in 0.3 ml DMEM.

Four weeks after bone marrow transplantation (BMT), mice were fasted overnight and then bled from retro-orbital veins under isoflurane anesthesia. After centrifugation, the plasma was collected and used for lipid analysis. The cell pellet was lysed with the ACK buffer to remove the red blood cells. DNA was extracted from the remaining cells and used to identify engraftment by detecting the presence of the Y chromosome and the mutant apoE gene [17].

Western blot analysis for ApoE and ApoB. The presence of apoE in plasma and of apoE and apoB in the descending aorta was determined by Western blot analysis. The aorta was washed thoroughly with PBS containing 5 U/ml heparin and 1 mM EDTA through the left ventricle of the heart, cleaned of periadventitial fat and connective tissues, and snap-frozen in liquid nitrogen. The frozen aorta was mechanically broken up, dispersed in lysis buffer containing 10 mM Tris, pH 8, 1 mM EDTA, 2.5% SDS, and 5% mercaptoethanol, and centrifuged at 500*g* for 10 min at 4 °C, and the supernatant was collected and used for detection of apoE and apoB. One microliter of plasma or 10 μg of aorta protein was separated by electrophoresis on 4–12% Tris polyacrylamide gels and electrophoretically transferred onto nitrocellulose membranes. The membranes were incubated with primary antibodies for mouse apoE, apoB (BioDesign International) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Chemicon International) for 1 h and then incubated for 0.5 h with horseradish peroxidase-conjugated secondary antibodies. The signals were detected by the enhanced chemiluminescent detection method according to the manufacturer's instructions (ECL Western blotting, Amersham). The density of the bands was quantified with a densitometer.

Plasma lipid measurements and separation of lipoproteins. Mice were fasted overnight before blood was collected through retro-orbital veins under isoflurane anesthesia. Plasma total cholesterol, HDL cholesterol, and triglyceride were measured with enzymatic assays as previously described [18]. Analysis of cholesterol profiles by fast phase liquid chromatography (FPLC) was performed using Superose 6 (Pharmacia) columns as previously described [18]. Plasma used FPLC analysis was pooled from four individual mice for each group.

Determination of hepatic VLDL production. Hepatic VLDL production was determined in mice that were fed 8 weeks of the Western diet by intravenous injection of Triton WR1339 (Tyloxapol, Sigma Chemical) [19]. Overnight fasted mice (4–5 mice/group) received a tail vein injection of 10 mg Triton WR1339 in 100 μl PBS solution. The VLDL production rate was calculated by the increase in plasma triglyceride levels from baseline to 1 h after Triton WR1339 injection as described by Maugeais et al. [20].

Determination of LPL and HL activity. Overnight fasted mice (4–5 mice/group) received a tail vein injection of 100 U/kg heparin (Sigma). Blood was obtained by retro-orbital bleeding before and at 5 min after injection. LPL and HL activity in plasma was quantitated by a stable, radioactive substrate emulsion assay as described by Nilsson-Ehle and Schotz [21] and expressed as milliunits/ml (1 milliunit = 1 nmol of free fatty acid released per minute).

Aortic lesion analysis. Methods for the quantification of atherosclerotic lesions in the aorta were the same as previously reported by Qiao et al. [22] In brief, the heart and proximal aorta were excised and embedded in OCT compound. Serial 10-μm-thick cryosections from the middle portion of the ventricle to the aortic arch were collected and mounted on poly-D-lysine-coated slides. In the region from the appearance to the disappearance of the aortic valves, every other section was collected. In all other regions, every fifth section was collected. Sections were stained with oil red O and hematoxylin, counterstained with fast green, and lesion areas were counted by light microscopy.

Statistical analysis. All values were expressed as means ± SEM except for atherosclerotic lesion areas, which were expressed as values of individual mice. "*n*" indicated the number of mice. Student's *t* test was used to compare differences between $apoE^{-/-} \rightarrow LDLR^{-/-}$ and $apoE^{+/+} \rightarrow LDLR^{-/-}$ mice in expression levels of apoB and apoE, lipid levels, LPL/HL activity, and atherosclerotic lesions. Regression analysis was performed to assess the association of atherosclerotic lesions with plasma lipid levels. Differences were considered statistically significant at $P < 0.05$.

Results

Reconstitution of recipient bone marrow

Female $LDLR^{-/-}$ mice were lethally irradiated and transplanted with bone marrow from male $apoE^{-/-}$ or $apoE^{+/+}$ mice to determine the effect of macrophage apoE deficiency on hyperlipidemia and atherosclerosis. Because male mice containing XY chromosomes were used as marrow donors for female recipients, we designed primers to amplify a segment of the Y chromosome by PCR. As shown in Fig. 1A, 4 weeks after transplantation, the Y chromosome was detected in the peripheral blood of recipient mice. Also, PCR analysis of the blood DNA showed the presence of the targeted apoE gene (the 245-bp band) in mice reconstituted with $apoE^{-/-}$ bone marrow (Fig. 1B). The faint 155-bp band, denoting the presence of the wild-type $apoE^{+/+}$ gene, was also observed in the mice. In contrast, mice reconstituted with $apoE^{+/+}$ bone marrow exhibited only the 155-bp wild-type band.

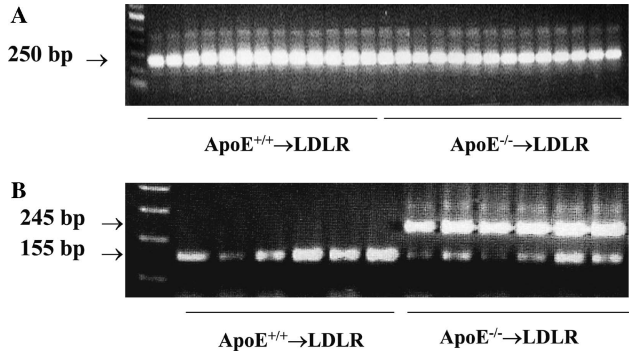

Fig. 1. Reconstitution of female LDLR$^{-/-}$ mice with bone marrow from male apoE$^{-/-}$ or apoE$^{+/+}$ mice. DNA was extracted from the blood of recipient mice 4 weeks after transplantation and amplified by PCRs. Each lane represents an individual mouse. (A) The presence of a 250-bp sequence of the Y chromosome. (B) The presence of the 245-bp apoE knockout band and 155-bp wild-type apoE band.

Effect of macrophage apoE deficiency on apoE and apoB levels in plasma and aortic walls

ApoE in plasma and apoE and apoB in descending aortas were assessed by Western blot analysis after the mice had been fed the Western diet for 8 weeks (Fig. 2). Densitometric scanning of the bands showed that absence of the macrophage-derived apoE resulted in a slight, but statistically significant, reduction in plasma apoE levels [1067 ± 19 (optical density) in apoE$^{-/-}$ → LDLR$^{-/-}$ vs. 1256 ± 18 in apoE$^{+/+}$ → LDLR$^{-/-}$; $P < 0.0001$]. The apoE level within the aortic wall was dramatically reduced in apoE$^{-/-}$ → LDLR$^{-/-}$ mice as compared with apoE$^{+/+}$ → LDLR$^{-/-}$ mice (41 ± 12 vs. 461 ± 29; $P = 0.0009$). To determine whether LDL accumulation in the arterial wall was affected, apoB was assessed by Western blot analysis. The amount of apoB was significantly lower in apoE$^{-/-}$ → LDLR$^{-/-}$ than in apoE$^{+/+}$ → LDLR$^{-/-}$ mice (64 ± 23 vs. 217 ± 38; $P = 0.042$). In contrast, the level of GADPH in the aorta was not significantly different between the two groups (727 ± 52 vs. 636 ± 13; $P = 0.23$).

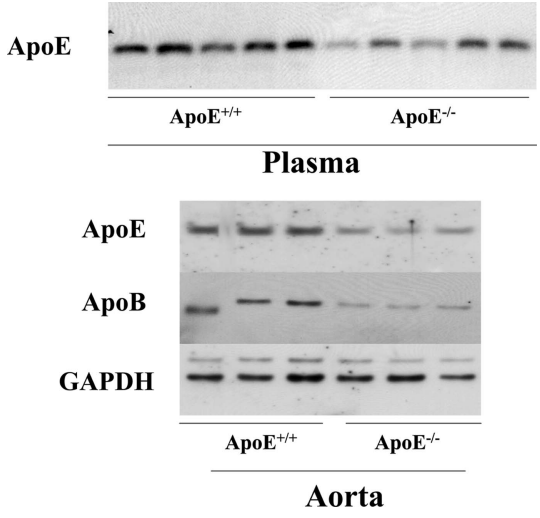

Fig. 2. Western blot analysis of apoE in plasma and of apoE, apoB, and GAPDH in aortic walls of LDLR$^{-/-}$ mice reconstituted with apoE$^{-/-}$ or apoE$^{+/+}$ bone marrow after being fed 8 weeks of the Western diet. One microliter of plasma or 10 μg protein from the descending aorta was electrophoresed on Tris polyacrylamide gels, transferred to nitrocellulose membranes, and probed with antibodies for the proteins. Each lane represents one individual mouse.

Effect of macrophage apoE deficiency on plasma lipid levels

Reconstitution with apoE$^{-/-}$ bone marrow had no significant influence on plasma cholesterol and triglyceride levels of LDLR$^{-/-}$ recipient mice on the chow diet (Fig. 3). Four weeks after transplantation on the chow diet, apoE$^{-/-}$→LDLR$^{-/-}$ ($n = 14$) and apoE$^{+/+}$→ LDLR$^{-/-}$ mice ($n = 13$) had similar plasma levels of total cholesterol, HDL cholesterol, and triglyceride ($P > 0.05$). However, after being fed the Western diet for 8 weeks, the mice reconstituted with apoE$^{-/-}$ bone marrow showed a significantly smaller increase in plasma levels of total cholesterol (879 ± 19 vs. 1129 ± 38 mg/dl) ($P < 0.0001$) and triglyceride (260 ± 6 vs. 337 ± 14 mg/dl) ($P = 0.018$) compared with the mice reconstituted with apoE$^{+/+}$ bone marrow. Since HDL cholesterol levels were similar in the two groups (81 ± 2 vs. 88 ± 3 mg/dl), the difference in plasma total cholesterol levels was primarily due to variation in the level of non-HDL cholesterol.

The distribution of plasma lipoproteins was examined by FPLC when mice were fed either chow or Western diets (Fig. 4). On the chow diet, apoE$^{-/-}$ → LDLR$^{-/-}$ and apoE$^{+/+}$ → LDLR$^{-/-}$ mice had comparable levels of VLDL, LDL, and HDL cholesterol, while on the Western diet the increase in IDL/LDL levels was smaller in apoE$^{-/-}$ → LDLR$^{-/-}$ than in apoE$^{+/+}$ → LDLR$^{-/-}$ mice. The HDL-cholesterol level of mice remained unchanged by the challenge with the Western diet in either group.

Effect on hepatic VLDL production and LPL and HL activities

The rate of hepatic VLDL production and the activity of plasma lipoprotein lipase (LPL) and hepatic lipase (HL) were assessed after the mice were fed 8 weeks of the Western diet (4–5 mice/group). The hepatic VLDL production rate of apoE$^{-/-}$→LDLR$^{-/-}$ mice was significantly lower ($P = 0.0002$) than that of apoE$^{+/+}$ → LDLR$^{-/-}$ mice (186.3 ± 11.0 mg/dl vs. 345.8 ± 15.5 mg/dl; Fig. 5A). Plasma LPL and HL activities were measured before and 5 min after intravenous heparin injection. At baseline, circulating LPL activity was not detectable in both apoE$^{-/-}$ → LDLR$^{-/-}$ and apoE$^{+/+}$ →

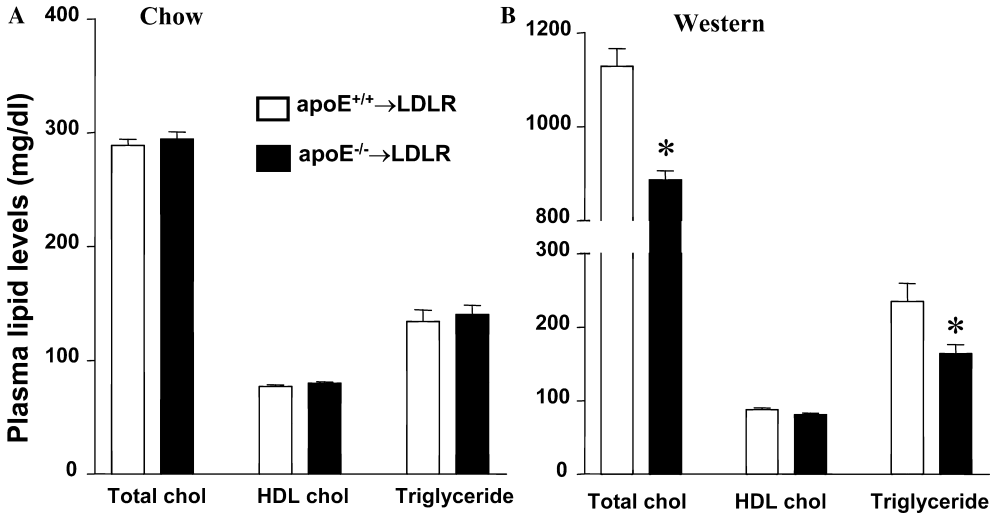

Fig. 3. Plasma cholesterol and triglyceride levels of $LDLR^{-/-}$ mice transplanted with $apoE^{-/-}$ or $apoE^{+/+}$ bone marrow. The mice were bled before (A) and after (B) being fed 8 weeks of the Western diet. Values are means ± SE of 13–14 mice. $^{*}P < 0.05$ versus mice reconstituted with $apoE^{+/+}$ bone marrow.

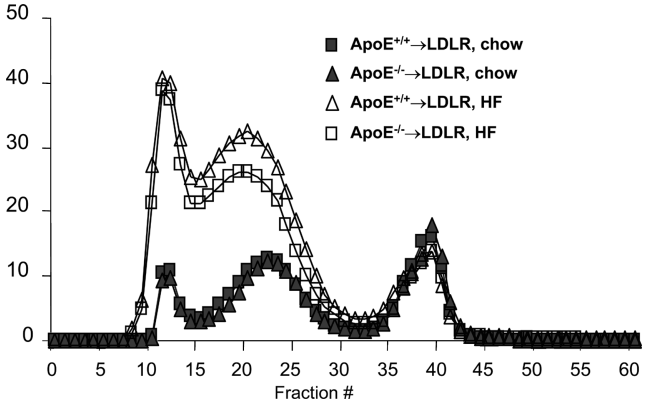

Fig. 4. Distribution of plasma lipoprotein cholesterol in $LDLR^{-/-}$ mice transplanted with $apoE^{-/-}$ or $apoE^{+/+}$ bone marrow. The samples were pooled plasmas from four mice fed either chow or Western diets and separated into 60 fractions by fast phase liquid chromatography (FPLC) using Superose 6 columns. Each fraction was then assayed for cholesterol in triplicate, which was expressed in mg/dl. The three peaks are VLDL, LDL, and HDL cholesterol, respectively.

$LDLR^{-/-}$ mice (Fig. 5B). The injection of heparin resulted in an increase in plasma LPL and HL activities. However, there were no significant differences between the two groups of mice in the LPL or HL activity.

Effect on aortic atherosclerotic lesions

After being fed the Western diet for 8 weeks, the mice were sacrificed and the size of atherosclerotic lesions in the proximal aorta was quantitated by light microscopy. The mean area of aortic lesions in $apoE^{-/-} \rightarrow LDLR^{-/-}$ was $152.4 \pm 12.7 \times 10^3$ μm^2/section ($n = 14$), whereas in $apoE^{+/+} \rightarrow LDLR^{-/-}$ mice the lesion area was $205.2 \pm 15.0 \times 10^3$ μm^2/section ($n = 13$) (Fig. 6). The difference in lesion areas between the two groups was statistically significant ($P = 0.0125$). In order to determine whether the difference in lesion formation could be explained by variations in plasma cholesterol levels, we performed regression analysis using pooled data for the two groups and found that the size of atherosclerotic lesions was positively correlated with the level of plasma total cholesterol ($r = 0.56$ and $P = 3.0 \times 10^{-8}$; Fig. 7).

Discussion

The present study was undertaken to examine the influence of macrophage apoE deficiency on lipoprotein metabolism and atherosclerosis in the $LDLR^{-/-}$ mouse model in which severe hyperlipidemia and atherosclerosis could be induced by feeding of a Western diet. We found that the $LDLR^{-/-}$ mice reconstituted with $apoE^{-/-}$ bone marrow exhibited less severe hyperlipidemia, reduced accumulation of apoE and apoB in aortic walls, and decreased aortic lesion formation as compared with control mice reconstituted with $apoE^{+/+}$ bone marrow.

Bone marrow transplantation leads to replacement of recipient bone marrow and most bone marrow-derived cells, including monocytes/macrophages, with cells of donor origin [23]. In the present study, the reconstitution of bone marrow in female recipients was confirmed by the presence of the Y chromosome from male donor cells. We also confirmed marrow reconstitution by testing the presence of the mutant apoE gene in those mice transplanted with $apoE^{-/-}$ bone marrow. However, these mice also exhibited a faint wild-type apoE band, indicating the existence of host cells in the blood. Previous studies have indicated that most of the residual

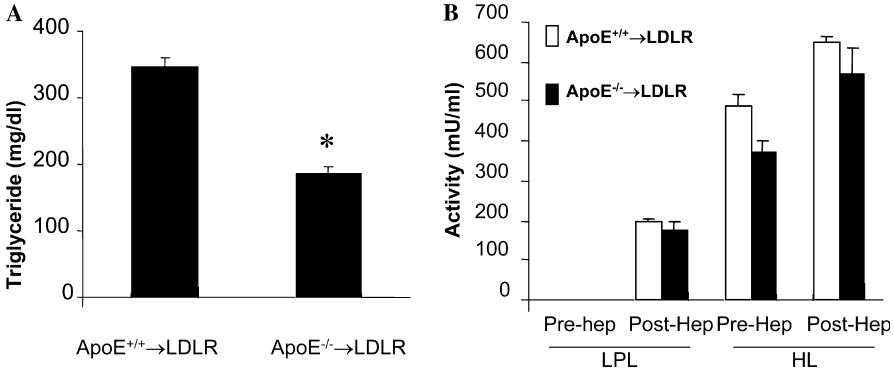

Fig. 5. (A) The hepatic VLDL production rate of LDLR$^{-/-}$ mice transplanted with apoE$^{-/-}$ or apoE$^{+/+}$ bone marrow. Four weeks after transplantation, the mice were started on the Western diet and maintained on the diet for 8 weeks. The production rate was calculated as differences in plasma triglyceride levels between baseline and 1 h after intravenous Triton WR1339 injection. (B) Lipoprotein lipase (LPL) and hepatic lipase (HL) activity before and 5 min after intravenous heparin injection. Values are means ± SE of 4–5 mice. *$P < 0.05$ versus mice reconstituted with apoE$^{+/+}$ bone marrow.

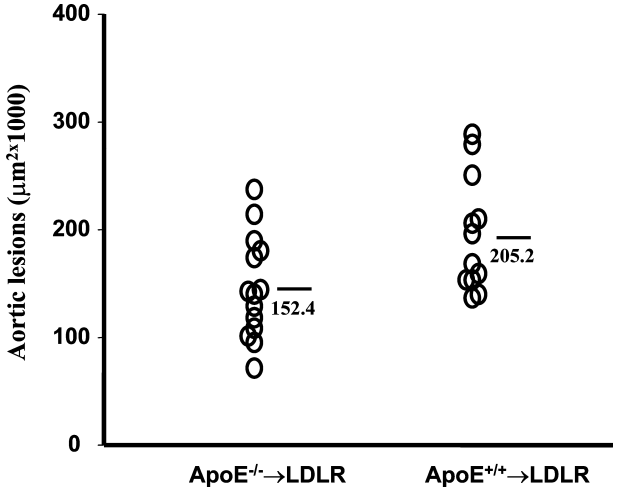

Fig. 6. Atherosclerotic lesion areas in cross-sections of the proximal aorta from LDLR$^{-/-}$ mice transplanted with apoE$^{-/-}$ ($n = 14$) or apoE$^{+/+}$ ($n = 13$) bone marrow. Each point represents a mean lesion area per section of each individual mouse. The horizontal bars represent mean lesion areas of each group. The mice were fed the Western diet for 8 weeks. Cross sections of the aorta were stained with oil red O and hematoxylin and the lipid-stained areas were measured by light microscopy. The difference in lesion areas between the two groups was significant ($P = 0.0125$).

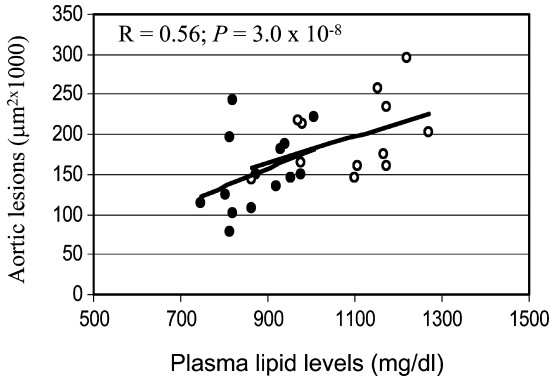

Fig. 7. Correlations between plasma total cholesterol levels and aortic atherosclerotic lesions in LDLR$^{-/-}$ mice transplanted with apoE$^{+/+}$ or apoE$^{-/-}$ bone marrow. The mice were fed the Western diet for 8 weeks. Each point represents an individual value of one mouse. Filled symbols represent mice reconstituted with apoE$^{-/-}$ bone marrow and unfilled symbols represent mice reconstituted with apoE$^{+/+}$ marrow. The correlation coefficient ($r = 0.56$) and significance ($P = 3.0 \times 10^{-8}$) are also shown in the figure.

host cells are radiation-resistant long-living lymphocytes [24]. In this study, we observed that reconstitution with apoE$^{-/-}$ bone marrow resulted in a slight, but statistically significant, reduction in plasma apoE levels of LDLR$^{-/-}$ recipient mice. Similar findings were also observed in wild-type C3H/HeJ mice reconstituted with apoE$^{-/-}$ bone marrow [15]. These data indicate that macrophages are an important source of plasma apoE, although most of the plasma apoE is synthesized by the liver. In the descending aorta, we observed a dramatic reduction of apoE protein in those mice transplanted with apoE$^{-/-}$ bone marrow. The lower plasma apoE probably contributed to the apoE reduction in the arterial wall. In a recent study, Tsukamoto et al. [25] provided evidence that plasma apoE diffused into vessel walls in that expression of the human apoE by the liver but not by macrophages resulted in substantial apoE retention in the arterial wall of apoE$^{-/-}$ mice. The replacement with apoE$^{-/-}$ macrophages probably also contributed to the reduction of apoE in the arterial wall because studies have shown that most apoE expression in the artery wall is from macrophages [13,25].

A major finding of this study is that the absence of macrophage-derived apoE resulted in a significant reduction in the plasma levels of IDL/LDL cholesterol and triglyceride when LDLR$^{-/-}$ mice developed severe hyperlipidemia on the Western diet. Because production of VLDL by the liver is an important determinant of

plasma lipid levels, we examined the hepatic VLDL production rate of mice on the Western diet and found that the rate of hepatic VLDL production in $apoE^{-/-} \rightarrow LDLR^{-/-}$ mice was reduced by 46% compared with control $apoE^{+/+} \rightarrow LDLR^{-/-}$ mice. The reduction in plasma apoE levels probably directly contributed to the decrease in VLDL production of $apoE^{-/-} \rightarrow LDLR^{-/-}$ mice. The replacement of Kupffer cells with transferred $apoE^{-/-}$ cells could reduce the availability of locally produced apoE by these cells to adjacent parenchymal cells in the liver. ApoE has been shown to be an important regulator involved in the synthesis and secretion of VLDL by hepatocytes [26]. Accordingly, our present findings suggest that large amounts of apoE are required to produce large amounts of VLDL in the liver of $LDLR^{-/-}$ mice when fed the Western diet. To determine whether an increased lipolysis of VLDL/LDL contributed to the reduction in plasma lipid levels, plasma lipoprotein lipase and hepatic lipase activity was analyzed. The present finding that $apoE^{-/-} \rightarrow LDLR^{-/-}$ mice had similar activity of lipoprotein lipase and hepatic lipase to $apoE^{+/+} \rightarrow LDLR^{-/-}$ mice does not support this possibility. Several studies have shown that absence of macrophage-derived apoE has no influence on the clearance of VLDL/LDL in the setting of normal hepatic production of apoE [13,27]. Bone marrow transplantation studies using $apoE^{-/-}$ mice have demonstrated that less than 10% of normal levels of plasma apoE is sufficient to maintain lipoprotein clearance [13,28]. Thus, an alteration in lipoprotein clearance is unlikely to be the mechanism for the reduction in plasma lipid levels of $apoE^{-/-} \rightarrow LDLR^{-/-}$ mice.

In this study, FPLC analysis of plasma lipoproteins revealed that the reduced plasma cholesterol level of $apoE^{-/-} \rightarrow LDLR^{-/-}$ mice on the Western diet was primarily due to a decrease in IDL/LDL but not VLDL levels. However, these mice exhibited a 46% reduction in VLDL production rate. This discrepancy can be explained by the fact that the VLDL production rate was assayed after blocking VLDL clearance with Triton, while the lipoprotein profile was analyzed without the interference of VLDL clearance. In mice, VLDL has a very short half-life (few minutes) in plasma and is quickly converted to IDL [29]. Also, as discussed above, the decrease in IDL/LDL was not due to an alteration in LDL clearance.

ApoE has been proposed to promote reverse cholesterol transport from peripheral tissues [12]. However, this conclusion is based on in vitro studies using macrophages. In the present study, we observed a significant reduction in apoB in those aortas that had a lower apoE level. The lower level of plasma IDL/LDL probably contributed to the reduction in apoB accumulation in the aortic wall. However, in a recent study, we found that C3H/HeJ mice also exhibited a dramatic reduction in apoB deposition in the aortic wall despite the fact that there was no alteration in plasma IDL/LDL levels [15]. Although the connections between apoE and apoB reductions are unknown, it is known that apoE binds to cell-surface heparan sulfate proteoglycans in vitro [30] and is colocalized with apoB and proteoglycans in atherosclerotic lesions [31]. Thus, the binding of apoB-containing lipoproteins to the extracellular matrix could be reduced due to the decreased availability of apoE in the vessel wall. Also, Tangirala et al. [32] provided evidence that apoE has anti-oxidant effects in vivo, since overexpression of human apoE by the liver of aged $LDLR^{-/-}$ mice markedly reduced levels of isoprostanes in urine, plasma, and aorta. Thus, in those vessels with a lower level of apoE, the accumulated LDL may be readily oxidized and then taken up by endothelial cells or macrophages.

We found that reconstitution with apoE null bone marrow resulted in a significant reduction in atherosclerotic lesions of $LDLR^{-/-}$ mice. The lower plasma cholesterol level, at least partially, contributed to the reduction in lesion formation because in these mice the size of aortic lesions was statistically correlated with the level of plasma total cholesterol. Probably more significant was the reduced accumulation of pro-atherogenic lipoproteins in the vessel wall of the mice transplanted with $apoE^{-/-}$ bone marrow. The trapped LDL undergoes oxidative modification to become oxidized LDL, which contributes to the initiation and progression of atherosclerosis. In a recent study, Fazio et al. [33] reported that $LDLR^{-/-}$ mice reconstituted with $apoE^{-/-}LDLR^{-/-}$ bone marrow developed larger atherosclerotic lesions than those reconstituted with $apoE^{+/+}LDLR^{-/-}$. The reason for this discrepancy in atherosclerotic lesion formation is unknown. The major difference between Fazio et al.'s and our studies is that $LDLR^{-/-}$ mice were transplanted with bone marrow cells containing $LDLR^{+/+}$ in our study while in Fazio et al.'s study, mice were transplanted with bone marrow cells containing $LDLR^{-/-}$. Also, the plasma total cholesterol level of $LDLR^{-/-}$ mice (treated: 724 ± 86 vs. control: 789 ± 76 mg/dl) reported by Fazio et al. was much lower than the level in our study. Thus, the higher cholesterol level of $LDLR^{-/-}$ mice could overwhelm the protective effect of apoE on atherosclerosis in our study.

In summary, our current study examined the physiologic effect of native apoE expression by macrophages on cholesterol metabolism and atherogenesis of $LDLR^{-/-}$ mice that developed severe hyperlipidemia and advanced atherosclerosis on the Western diet. Our results indicate that macrophage apoE-deficiency lessens severe hyperlipidemia by slowing hepatic VLDL production and reduces the accumulation of apoB in the arterial wall and the development of atherosclerotic lesion in the mice.

Acknowledgments

This work was supported by the Partners' Fund Award from the Cardiovascular Research Center and the Dean's R&D award from the University of Virginia School of Medicine, NIH Grant HL30568, and the Laubisch Fund from UCLA.

References

[1] R.W. Mahley, Apolipoprotein E: cholesterol transport protein with expanding role in cell biology, Science 240 (1988) 622–630.
[2] L. Krimbou, M. Tremblay, J. Davignon, J.S. Cohn, Characterization of human plasma apolipoprotein E-containing lipoproteins in the high density lipoprotein size range: focus on pre-beta1-LpE, pre-beta2-LpE, and alpha-LpE, J. Lipid Res. 38 (1997) 35–48.
[3] U. Beisiegel, Receptors for triglyceride-rich lipoproteins and their role in lipoprotein metabolism, Curr. Opin. Lipidol. 6 (1995) 117–122.
[4] G. Ghiselli, E.J. Schaefer, P. Gascon, H.B. Brewer Jr., Type III hyperlipoproteinemia associated with apolipoprotein E deficiency, Science 214 (1981) 1239–1241.
[5] A.S. Plump, J.D. Smith, T. Hayek, K. Aalto-Setala, A. Walsh, J.G. Verstuyft, E.M. Rubin, J.L. Breslow, Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells, Cell 71 (1992) 343–353.
[6] S.H. Zhang, R.L. Reddick, J.A. Piedrahita, N. Maeda, Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E, Science 258 (1992) 468–471.
[7] V.I. Zannis, F.S. Cole, C.L. Jackson, D.M. Kurnit, S.K. Karathanasis, Distribution of apolipoprotein A-I, C-II, C-III, and E mRNA in fetal human tissues: time-dependent induction of apolipoprotein E mRNA by cultures of human monocyte-macrophages, Biochemistry 24 (1985) 4450–4455.
[8] D.L. Williams, P.A. Dawson, T.C. Newman, L.L. Rudel, Synthesis of apolipoprotein E by peripheral tissues: potential functions in reverse cholesterol transport and cellular cholesterol metabolism, Ann. NY Acad. Sci. 454 (1985) 222–229.
[9] D.M. Driscoll, G.S. Getz, Extrahepatic synthesis of apolipoprotein E, J. Lipid Res. 25 (1984) 1368–1379.
[10] T. Mazzone, C. Reardon, Expression of heterologous human apolipoprotein E by J774 macrophages enhances cholesterol efflux to HDL, J. Lipid Res. 35 (1994) 1345–1353.
[11] M. Van Eck, N. Herijgers, M. Vidgeon-Hart, N.J. Pearce, P.M. Hoogerbrugge, P.H. Groot, T.J. Van Berkel, Accelerated atherosclerosis in C57Bl/6 mice transplanted with ApoE-deficient bone marrow, Atherosclerosis 150 (2000) 71–80.
[12] S.K. Basu, J.L. Goldstein, M.S. Brown, Independent ways for secretion of cholesterol and apolipoprotein E by macrophages, Science 219 (1983) 871–873.
[13] S. Fazio, V.R. Babaev, A.B. Murray, A.H. Hasty, K.J. Carter, L.A. Gleaves, J.B. Atkinson, M.F. Linton, Increased atherosclerosis in mice reconstituted with apolipoprotein E null macrophages, Proc. Natl. Acad. Sci. USA 94 (1997) 4647–4652.
[14] W.A. Boisvert, L.K. Curtiss, Elimination of macrophage-specific apolipoprotein E reduces diet-induced atherosclerosis in C57BL/6J male mice, J. Lipid Res. 40 (1999) 806–813.
[15] W. Shi, X. Wang, K. Tangchitpiyanond, J. Wong, Y. Shi, A.J. Lusis, Atherosclerosis in C3H/HeJ mice reconstituted with apolipoprotein E-null bone marrow, Arterioscler. Thromb. Vasc. Biol. 22 (2002) 650–655.
[16] S. Ishibashi, J.L. Goldstein, M.S. Brown, J. Herz, D.K. Burns, Massive xanthomatosis and atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice, J. Clin. Invest. 93 (1994) 1885–1893.
[17] W. Shi, X. Wang, N.J. Wang, V.Z. Sun, X. Wang, A.J. Lusis, Effect of macrophage-derived apolipoprotein E on established atherosclerosis in apolipoprotein E-deficient mice, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 2261–2266.
[18] C.C. Hedrick, L.W. Castellani, C.H. Warden, D.L. Puppione, A.J. Lusis, Influence of mouse apolipoprotein A-II on plasma lipoproteins in transgenic mice, J. Biol. Chem. 268 (1993) 20676–20682.
[19] S. Otway, D.S. Robinson, The use of a non-ionic detergent (Triton WR 1339) to determine rates of triglyceride entry into the circulation of the rat under different physiological conditions, J. Physiol. 190 (1967) 321–332.
[20] C. Maugeais, U.J.F. Tietge, K. Tsukamoto, J.M. Glick, D.J. Rader, Hepatic apolipoprotein E expression promotes very low density lipoprotein–apolipoprotein B production in vivo in mice, J. Lipid Res. 41 (2000) 1673–1679.
[21] P. Nilsson-Ehle, M.C. Schotz, A stable, radioactive substrate emulsion for assay of lipoprotein lipase, J. Lipid Res. 17 (1976) 536–541.
[22] J.-H. Qiao, P.-Z. Xie, M.C. Fishbein, J. Kreuzer, T.A. Drake, L.L. Demer, A.J. Lusis, Pathology of atheromatous lesions in inbred and genetically engineered mice: genetic determination of arterial calcification, Arterioscler. Thromb. 14 (1994) 1480–1497.
[23] D.L. Longo, M.L. Davis, Early appearance of donor-type antigen-presenting cells in the thymuses of 1200 R radiation-induced bone marrow chimeras correlates with self-recognition of donor I region gene products, J. Immunol. 130 (1983) 2525–2527.
[24] A.M. Yeager, C. Shinn, D.M. Pardoll, Lymphoid reconstitution after transplantation of congenic hematopoietic cells in busulfan-treated mice, Blood 78 (1991) 3312–3316.
[25] K. Tsukamoto, R. Tangirala, S.H. Chun, E. Puré, D.J. Rader, Rapid regression of atherosclerosis induced by liver-directed gene transfer of apoe in ApoE-deficient mice, Arterioscler. Thromb. Vasc. Biol. 19 (1999) 2162–2170.
[26] F. Kuipers, M.C. Jong, Y. Lin, M. Eck, R. Havinga, V. Bloks, H.J. Verkade, M.H. Hofker, H. Moshage, T.J. Berkel, R.J. Vonk, L.M. Havekes, Impaired secretion of very low density lipoprotein-triglycerides by apolipoprotein E-deficient mouse hepatocytes, J. Clin. Invest. 100 (1997) 2915–2922.
[27] M.F. Linton, J.B. Atkinson, S. Fazio, Prevention of atherosclerosis in apolipoprotein E-deficient mice by bone marrow transplantation, Science 267 (1995) 1034–1037.
[28] A.H. Hasty, M.F. Linton, L.L. Swift, S. Fazio, Determination of the lower threshold of apolipoprotein E resulting in remnant lipoprotein clearance, J. Lipid Res. 40 (1999) 1529–1538.
[29] M. Van Eck, N. Herijgers, J. Yates, N.J. Pearce, P.M. Hoogerbrugge, P.H. Groot, T.J. Van Berkel, Bone marrow transplantation in apolipoprotein E-deficient mice. Effect of ApoE gene dosage on serum lipid concentrations, (beta)VLDL catabolism, and atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 17 (1997) 3117–3126.
[30] Z.S. Ji, S.J. Lauer, S. Fazio, A. Bensadoun, J.M. Taylor, R.W. Mahley, Enhanced binding and uptake of remnant lipoproteins by hepatic lipase-secreting hepatoma cells in culture, J. Biol. Chem. 269 (1994) 13429–13436.
[31] K.D. O'Brien, K.L. Olin, C.E. Alpers, W. Chiu, M. Ferguson, K. Hudkins, T. Wight, Comparison of apolipoprotein and proteoglycan deposits in human coronary atherosclerotic plaques: colocalization of biglycan with apolipoproteins, Circulation 98 (1998) 519–527.
[32] R.K. Tangirala, D. Praticó, G.A. FitzGerald, S. Chun, K. Tsukamoto, C. Maugeais, D.C. Usher, E. Pure, D.J. Rader, Reduction of isoprostanes and regression of advanced atherosclerosis by apolipoprotein E, J. Biol. Chem. 276 (2001) 261–266.
[33] S. Fazio, V.R. Babaev, M.E. Burleigh, A.S. Major, A.H. Hasty, M.F. Linton, Physiological expression of macrophage apoE in the artery wall reduces atherosclerosis in severely hyperlipidemic mice, J. Lipid Res. 43 (2002) 1602–1609.