Role of Macrophage-Derived Lipoprotein Lipase in Lipoprotein Metabolism and Atherosclerosis

Miranda Van Eck, Robert Zimmermann, Pieter H.E. Groot, Rudolf Zechner, Theo J.C. Van Berkel

Abstract—Lipoprotein lipase (LPL) synthesis by macrophages is upregulated in early atherogenesis, implicating the possible involvement of LPL in plaque formation. However, it is still unclear whether macrophage-derived LPL displays a proatherosclerotic or an antiatherosclerotic role in atherosclerotic lesion development. In this study, the role of macrophage-derived LPL on lipid metabolism and atherosclerosis was assessed in vivo by transplantation of LPL-deficient (LPL−/−) and wild-type (LPL+/+) bone marrow into C57BL/6 mice. Eight weeks after bone marrow transplantation (BMT), serum cholesterol levels in LPL−/−→C57BL/6 mice were reduced by 8% compared with those in LPL+/+→C57BL/6 mice (P<0.05, n=16), whereas triglycerides were increased by 33% (P<0.05, n=16). Feeding the mice a high-cholesterol diet increased serum cholesterol levels in LPL−/−→C57BL/6 and LPL+/+→C57BL/6 mice 5-fold and 9-fold, respectively, resulting in a difference of ≈50% (P<0.01) after 3 months on the diet. No effects on triglyceride levels were observed under these conditions. Furthermore, serum apolipoprotein E levels were reduced by 50% in the LPL−/−→C57BL/6 mice compared with controls under both dietary conditions. After 3 months on a high-cholesterol diet, the atherosclerotic lesion area in LPL−/−→C57BL/6 mice was reduced by 52% compared with controls. It can be concluded that macrophage-derived LPL plays a significant role in the regulation of serum cholesterol, apolipoprotein E, and atherogenesis, suggesting that specific blockade of macrophage LPL production may be beneficial for decreasing atherosclerotic lesion development. (Arterioscler Thromb Vasc Biol. 2000;20:e53-e62.)

Key Words: atherosclerosis ■ transplantation ■ blood cells ■ enzymes ■ lipids

Lipoprotein lipase (LPL) is a 60-kD glycoprotein that plays a central role in plasma triglyceride metabolism by hydrolysis of triglyceride-rich chylomicrons and VLDL.1–3 LPL is bound to the capillary endothelium by interactions with heparan sulfate proteoglycans and is present in organs with a high demand for free fatty acids (FFAs), like the heart, skeletal muscle, and adipose tissue.5,5 Small amounts of LPL are also present in other types of tissues, including the adrenals, brain, lung, and spleen.4 Furthermore, in atherosclerotic lesions, LPL is produced by monocytes, macrophages, and smooth muscle cells.8–9 Foam cells in atherosclerotic lesions are mainly derived from macrophages, and LPL secretion by this cell type may thus have a direct effect on the formation of atherosclerotic lesions. Several functions for LPL in the atherosclerotic process have been postulated, including a proatherosclerotic or an antiatherosclerotic role. Previously, it has been demonstrated that pharmaceutical interventions intended to increase LPL expression protect against atherosclerosis due to elevations of HDL, indicating that LPL displays an antiatherogenic function.10–12 Furthermore, overexpression of LPL in the liver is highly effective in normalizing the atherosclerotic lipoprotein profiles of both apoE-deficient and LDL receptor–deficient mice13,14 and protects wild-type mice against diet-induced hyperlipidemia.15 Alternatively, LPL may induce the retention and/or uptake of atherogenic lipoproteins by cells in the arterial wall, thereby promoting the atherosclerotic process.16,17 Furthermore, LPL has been demonstrated to increase the retention of LDL and VLDL by proteoglycans of the subendothelial matrix in the arterial wall,18–20 increase the permeability of the endothelium by formation of lipolysis products,21 and facilitate proteoglycan-mediated monocyte adhesion to the endothelium.22

This potentially proatherosclerotic function of LPL is supported by the findings of Renier et al,23 who demonstrated that the susceptibility to atherosclerosis in inbred mouse strains is associated with high LPL expression, whereas resistance is associated with low LPL levels. Analysis of the effects of homozygous LPL deficiency on lipoprotein metabolism or atherosclerosis in mice is not possible, because LPL deficiency leads to death of the animals shortly after birth.23,24 In heterozygotes, however, both triglycerides and cholesterol
are only slightly increased compared with corresponding levels in wild-type animals.23,24 Recently, Semenkovich et al26 demonstrated that feeding the heterozygous LPL-deficient mouse an atherogenic diet results in a more profound dyslipidemia due to an increase in VLDL triglycerides and VLDL and LDL cholesterol. Despite this increase in fasting lipids, no effect on atherosclerotic lesion area could be demonstrated in these animals, indicating that the detrimental effects of dyslipidemia may be influenced by the positive effects of decreased LPL expression in the vascular wall. The role of LPL in atherogenesis is thus still in dispute, although it is most likely that its role may depend on the tissue in which it is expressed. In the arterial wall, LPL may be atherogenic, whereas in muscle and adipose tissue it may be protective.

To elucidate the role of macrophage-derived LPL in atherogenesis, LPL-deficient bone marrow from mice expressing LPL exclusively in muscle was transplanted into irradiated C57BL/6 mice. Using this technique, we created chimeric animals that were deficient in LPL in cells derived from the transplanted stem cells, including monocytes and macrophages. Our results indicate that the absence of macrophage-derived LPL profoundly influences serum cholesterol and apoE levels, whereas atherosclerotic lesion formation is reduced by 52%.

Methods

Animals

Transgenic knockout mice expressing LPL exclusively in muscle (L0-MCK) were generated by breeding transgenic mice, containing the muscle-specific creatine kinase promoter driving a human LPL minigene, onto the LPL knockout background as described earlier.27-29 Littermates with normal LPL expression were used as controls (L2-MCK). All mice were hybrids of the C57BL/6 and CBA strains. C57BL/6NCrlBR mice, purchased from Broekman Institute BV, Someren, The Netherlands, were used as recipients for bone marrow transplantation (BMT).

All mice used for BMT experiments were housed in sterilized, filter-top cages and given free access to food and water. Animals were maintained on sterilized regular chop (SRM-A) containing 5.7% (wt/wt) fat (Hope Farms) or were fed a high-cholesterol diet that contained 15% (wt/wt) fat, 1% (wt/wt) cholesterol, and 0.5% (wt/wt) cholic acid formulated according to Nishina et al.30 Drinking water was supplied with antibiotics (83 mg/L ciprofloxacin and 67 mg/L polymyxin B sulfate) and 6.5 g/L sugar.

A cocktail of ketamine, fentanyl, droperidol, and fluanisone (10 µL/g mouse) was used to anesthetize the mice before all lethal experiments. Animal procedures were performed at the Sylvius Laboratories of the Leiden/Amsterdam Center for Drug Research in accordance with national laws. All experimental protocols were approved by the Ethics Committee for Animal Experiments of the Leiden University Medical Center on an automated analyzer and according to the recommendations of the International Federation of Clinical Chemistry.31

Determination of Lipolytic Enzyme Activity

To determine plasma LPL activity, blood was drawn 5 months after BMT after an overnight fast both before and after an intravenous bolus injection of heparin (100 U/kg). Lipolytic activity was measured in several tissues, including heart muscle, straight femoral muscle, brain, spleen, lung, and liver 5 months after BMT, as described previously.27-29 In brief, tissue specimens of 100 mg were incubated in Dulbecco’s modified Eagle’s medium/2% bovine serum albumin (BSA) and 2 U/mL heparin (Leo Pharmaceutical Products BV) for 1 hour at 37°C after the tissue was minced with scissors. The lipolytic activity of the tissue supernatant as well as that in preheparin and postheparin plasma was measured by using a radio-labeled triolein emulsion as described by Zechner.32 In brief, the substrate consisted of a radio-labeled triolein emulsion prepared by sonication (4× 1 minute on ice at 100 W) of a mixture of 50 µCi glycerol-3[H]oleate[9,10(α)-H]oleate, 4 mg of unlabeled glycerol trioleate, 0.1 mol/L Tris-HCl (pH 8.6), 0.1% Triton X-100, 2% BSA, and 2 mL of heat-inactivated human serum (a source of apoC-II, an LPL activator). Subsequently, LPL-containing samples (10 to 100 µL) were added to 0.2 mL of substrate and incubated for 30 minutes at 37°C. The reaction was stopped by addition of 3.25 mL of a mixture of 4% paraformaldehyde/methanol-heptane (1:1.29:1.37 vol/vol) and 1 mL of 0.1 mol/L K2CO3/H2BO3 (pH 10.5). FFAs were extracted by vortexing this mixture for 15 seconds, phases were separated by centrifugation at 3000 rpm for 4°C for 20 minutes, and 1 mL of the upper phase was counted for radioactivity. The lipolytic activity was calculated from the amount of FFAs released per tissue weight per hour. Liver lipase activity was determined in the presence or absence of 1 mol/L NaCl to differentiate between LPL and hepatic lipase activity. LPL activity was calculated as the portion of total lipase activity inhibited by 1 mol/L NaCl.

VLDL Isolation and Labeling

VLDL was isolated from sera of fasted, healthy volunteers by discontinuous KBr gradient ultracentrifugation at 250 000g for 18 hours, as described by Redgrave et al.34 The fraction of d<1.006 g/mL was isolated and dialyzed against PBS/1 mmol/L EDTA. VLDL was labeled with 125I at pH 10.0 according to McFarlane,35 modified as described earlier.36

Isolation and Culture of Murine Peritoneal Macrophages

Thioglycollate-elicited macrophages were harvested from control LPL+/+→C57BL/6 and LPL+/−→C57BL/6 mice and plated in 24-well plates at a density of 0.5×106 cells/500 µL in Dulbecco’s modified Eagles medium, supplemented with 10% (wt/vol) bovine calf serum, 2 mmol/L L-glutamine, 100 µg/mL streptomycin, and 100 IU/mL penicillin. After 4 hours, nonadhering cells were removed by washing. After 2 days in culture, the cells were washed and incubated with 10 µg/mL 125I-VLDL in Dulbecco’s modified

Liver Function Test

A liver function test was performed by analysis of alanine aminotransferase (ALT) enzyme activity in serum after a 1-month feeding with the high-cholesterol diet N (3 months after BMT). The analysis was performed by the Central Clinical Chemical Laboratory of the Leiden University Medical Center on an automated analyzer and according to the recommendations of the International Federation of Clinical Chemistry.31

Serum Cholesterol, Triglyceride, and ApoE Analysis

After an overnight fast, ~100 µL of blood was drawn from each mouse by tail bleeding. The concentrations of total cholesterol and triglycerides in serum were determined by using enzymatic procedures (Boehringer Mannheim). Precipath (standardized serum, Boehringer Mannheim) was used as an internal standard. The distribution of cholesterol and triglycerides among the different lipoproteins in serum was determined by loading 30 µL of serum from each mouse onto a Superose 6 column (3.2×30 mm; Smart-system, Pharmacia). Serum was fractionated at a constant flow rate of 50 µL/min with PBS. Total cholesterol and triglyceride contents in the effluent were determined enzymatically. ApoE was measured with a sandwich ELISA specific for mouse apoE, as described earlier.31 Pooled sera from C57BL/6 mice, with a known apoE level, was used as the standard.
Eagle’s medium/2% BSA for 3 hours at 37°C. At the indicated times, cells were washed and lysed in 0.1 mol/L NaOH, and cell protein content was determined according to Lowry et al.37 Cell-associated radioactivity and degradation products of VLDL in the supernatant were determined.

The effect of LPL deficiency on macrophage apoE secretion was analyzed in thioglycollate-elicited macrophages from LPL−/− and LPL+/+ littermates. After 24 hours in culture in Dulbecco’s modified Eagle’s medium/2% BSA, apoE secretion in the medium was measured with a sandwich ELISA for mouse apoE as described previously.31 Furthermore, the lipolytic activity of the supernatant was measured by using a radiolabeled triolein emulsion as described by Zechner.33 The lipolytic activity was calculated from the amount of FFAs (μmol) released per milligram of cell protein per minute.

Histological Analysis of Hearts and Aortas for Atherosclerosis

To analyze the development of atherosclerosis, transplanted mice were killed after 3 months of feeding with a high-cholesterol diet (15% fat, 1% cholesterol, and 0.5% cholic acid). Hearts and aortas were perfusion-fixed, and atherosclerotic lesion area in oil red O-stained cryostat sections of the aortic root was quantified, as described previously.31,38 Mean lesion area was calculated (in μm²) from 10 sections, starting at the appearance of the tricuspid valves as described previously.31,38

Immunocytochemistry

Macrophages were detected in atherosclerotic lesions by immunocytochemical staining. MOMA-2 macrophage marker. Formaldehyde-fixed, cryostat sections were washed with washing buffer (100 mmol/L Tris, 150 mmol/L NaCl, pH 7.5) and incubated for 1 hour in blocking buffer (100 mmol/L Tris, 150 mmol/L NaCl, 1% block reagent for ELISA, and 5% normal goat serum, pH 7.5). Subsequently, sections were incubated with rabbit anti-mouse MOMA-2 antiserum (dilution 1:10 in blocking buffer) for 1 hour at room temperature and overnight at 4°C. After being washed, the sections were exposed for 1 hour to goat anti-rat IgG conjugated to alkaline phosphatase (Sigma Immuno Chemicals, dilution 1:200 in blocking buffer). Thereafter, sections were extensively washed with washing buffer, and MOMA-2-positive macrophages were visualized by incubation with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium alkaline phosphatase substrate (Sigma Chemicals) in a 0.2 mol/L Tris-HCl/10 mmol/L MgCl₂ buffer, pH 9.6.

The presence of LPL in atherosclerotic lesions was assessed immunohistochemically with a rabbit anti-human LPL polyclonal antibody (kindly provided by Dr S. Vilaro, University of Barcelona, Barcelona, Spain). Formaldehyde-fixed, cryostat sections were washed with PBS and subsequently incubated for 0.5 hour with 0.3% H₂O₂ and for 1 hour with blocking buffer (PBS, 1% BSA, and 10% normal goat serum, pH 7.4). Subsequently, the sections were incubated with rabbit anti-human LPL polyclonal antibody (1:25 in blocking buffer) for 1 hour at room temperature and overnight at 4°C. After being washed, the sections were exposed for 2 hours to biotin α-rabbit immunoglobulin (Amersham, dilution 1:200 in blocking buffer), washed again, and exposed for 1 hour to biotinylated streptavidin, conjugated to horseradish peroxidase (Amersham, dilution 1:200 in blocking buffer). Thereafter the sections were extensively washed with PBS, and LPL-positive lesion areas were visualized by incubation with 3,3′-diaminobenzidine (Sigma Chemicals) as the horseradish peroxidase substrate in 0.05 mol/L Tris-HCl (pH 7.4), 7% sucrose, and 0.03% H₂O₂.

Statistical Analysis

Statistically significant differences among the means of populations in repeated measurements of cholesterol and triglyceride levels were tested in time by ANOVA with the Student-Newman-Keuls post test (Instat Graphpad software). Individual comparisons were made by using unpaired Student’s t test. A P value of <0.05 was regarded as significant.

Results

Effect of BMT on Serum Cholesterol and Triglyceride Levels in Chow-Fed Mice

To assess the role of macrophage-derived LPL in atherosclerosis, C57BL/6 mice were transplanted with LPL-deficient bone marrow, giving rise to animals that were deficient for LPL in all cell types of hematopoietic origin. The effect of transplantation on serum cholesterol and triglyceride levels was evaluated (Figures IA and IB). At 8 weeks after BMT, total cholesterol levels in LPL−/−→C57BL/6 mice were reduced by 8% (P<0.05, n=16) compared with LPL+/+→C57BL/6 mice (Figure IA). As shown in Figure IB, serum triglyceride levels were increased by 33% (P<0.05, n=16) in the LPL−/−→C57BL/6 animals at 8 weeks after BMT. The cholesterol and triglyceride distribution among the different lipoprotein fractions was analyzed by liquid chromatography 8 weeks after BMT (Figures IIA and IIB). Analysis of the cholesterol distribution in serum revealed that it had not significantly changed by 8 weeks after BMT on a normal chow diet (Figure IIA). The increase in serum triglyceride levels in LPL−/−→C57BL/6 mice 8 weeks after BMT seemed to be due to an increase in VLDL triglycerides, although no statistically significant difference was achieved (Figure IIB).

Effect of BMT on Serum ApoE Levels

Macrophages in the atherosclerotic lesion produce LPL as well as apoE.7,8,39 Because cholesterol homeostasis in mac-
TABLE I. Effect of Macrophage LPL Deficiency on LPL Activity and ApoE Secretion Into the Medium

<table>
<thead>
<tr>
<th>Mouse Type</th>
<th>LPL Activity, (\mu\text{m}^3\text{mg}^{-1}) Protein</th>
<th>ApoE, ng · mg Protein (-1·24\text{h}^{-1})</th>
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<tr>
<td>LPL+/+</td>
<td>97.8 ± 28.4</td>
<td>1863 ± 147</td>
</tr>
<tr>
<td>LPL−/−</td>
<td>8.4 ± 2.56*</td>
<td>950 ± 120*</td>
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**Thioglycollate-elicited LPL+/+ and LPL−/− macrophages** were isolated from control L2-MCK and transgenic knockout L0-NMCK mice, respectively. After 24 hours in culture in Dulbecco’s modified Eagle’s medium/2% BSA, apoE secretion was measured with a sandwich ELISA specific for mouse apoE. LPL activity was measured as described in “Methods.” Values are mean ± SEM of 5 mice.

*Significant difference at \(P<0.05\) compared with macrophages from control mice.

Figure II. Distribution of serum cholesterol and triglycerides among different lipoprotein fractions in C57BL/6 mice transplanted with LPL+/+ or LPL−/− bone marrow 8 weeks after BMT. Blood samples were drawn by tail bleeding after an overnight fast. A 30-μL aliquot of serum from each mouse was loaded onto a Pharmacia Smart column and fractions were collected. Fractions 3 to 7 represent VLDL and chylomicrons; fractions 8 to 14, LDL; and fractions 15 to 19, HDL. The distribution of cholesterol (A) and triglycerides (B) among different lipoproteins in LPL+/+→C57BL/6 (○) and LPL−/−→C57BL/6 (●) mice is shown. Values are mean of 8 mice. For clarity, SEM is shown only for fractions containing the top of the VLDL, LDL, and HDL peaks. No significant differences were observed.

Effect of BMT on Plasma and Tissue Lipase Activity

Analysis of the lipolytic activity of preheparin and postheparin plasma (Table II) revealed that macrophage LPL production did not contribute significantly to the lipolytic activity located either in plasma or on the endothelial surface. Furthermore, tissue LPL activity was determined in cardiac muscle, skeletal muscle, brain, spleen, lung, and liver. As depicted in Figure III, heparin-releasable lipase activity was significantly reduced in organs containing high amounts of macrophages, like the lungs (10-fold, \(P=0.04\)) and spleen (2-fold, \(P=0.03\)). Unexpectedly, heparin-releasable lipase activity in cardiac muscle was also found to be reduced by 69% (\(P=0.0009\)) in the mice transplanted with LPL-deficient bone marrow compared with controls. Compared with control C57BL/6 mice, irradiation and the BMT procedure itself did not influence either the postheparin plasma or the tissue LPL activity (data not shown).

![Figure III. Effect of BMT of LPL-deficient bone marrow to C57BL/6 mice on LPL activity in multiple organs. The heparin-releasable lipolytic activity of cardiac muscle, skeletal muscle, brain, spleen, lung, and liver was measured 5 months after transplantation of LPL+/+ (hatched bars) or LPL−/− (closed bars) bone marrow by using a Triton X-100 substrate–based assay. Values represent mean ± SEM of 6 mice. Significant difference of *\(P<0.05\), **\(P<0.001\), vs LPL+/+→C57BL/6 mice. Compared with control C57BL/6 mice, irradiation and BMT did not influence tissue LPL activity (data not shown).](image-url)
Effect of BMT on Association and Degradation of Iodinated Human VLDL by Macrophages

The association and degradation of iodinated human VLDL by macrophages isolated from control transplanted animals was compared with the activity of macrophages from animals transplanted with LPL-deficient bone marrow. From the data shown in Figure IVA, it is evident that the association, including binding and uptake, was significantly higher (P = 0.04, n = 3) in control macrophages compared with macrophages that lacked LPL. The association of 125I-VLDL with control macrophages after 3 hours of incubation was 888 ± 138 ng/mg cell protein compared with 584 ± 119 ng/mg cell protein in macrophages from LPL−/−→C57BL/6 animals. No effect was observed on the degradation of iodinated human VLDL, indicating that macrophage-derived LPL is important for the association of VLDL but not for its degradation (Figure IVB).

Effect of BMT on the Response to a High-Cholesterol Diet and Atherosclerosis

To analyze the effect of the introduction of LPL-deficient macrophages in C57BL/6 mice on atherosclerosis, the mice were challenged with a high-cholesterol diet. As shown in Figure VA, after 8 weeks on this diet LPL−/−→C57BL/6 mice and control LPL+/+→C57BL/6 animals responded with a 5-fold and a 9-fold increase in serum cholesterol levels, respectively, thereby increasing the difference in serum cholesterol levels between the groups to 52% (P < 0.01). On feeding the mice a high-cholesterol diet, serum triglyceride levels were slightly reduced in both groups.

However, no significant difference in serum triglyceride levels could be demonstrated between LPL−/−→C57BL/6 and LPL+/+→C57BL/6 mice under these conditions (Figure VB).

Fractionation of serum lipoproteins by liquid chromatography revealed that the increase in cholesterol levels after feeding the mice a high-cholesterol diet was associated with an increase in cholesterol in VLDL and LDL, whereas HDL cholesterol was unchanged (Figure VIA). The difference in response to the high-cholesterol diet between LPL−/−→C57BL/6 and LPL+/+→C57BL/6 animals was due to a difference in the increase in VLDL and LDL levels. Under these dietary conditions, no significant differences in triglyceride levels in VLDL between LPL−/−→C57BL/6 mice and LPL+/+→C57BL/6 animals were found (Figure VIB). The combined increase in VLDL cholesterol and reduction in VLDL triglycerides indicates that feeding the mice a high-cholesterol diet induces both an increase in VLDL and LDL levels and replacement of triglycerides in VLDL by cholesterol esters.

Analysis of serum apoE levels after the mice were fed the high-cholesterol diet for 2 and 8 weeks revealed that the diet induced a large increase in serum apoE levels in both groups of mice (Figure VII). However, the reduction in serum apoE levels in mice transplanted with LPL-deficient bone marrow,
Effect of macrophage-derived LPL on serum apoE levels after feeding with a high-cholesterol diet. After transplantation of C57BL/6 mice with LPL+/+ or LPL−/− bone marrow after 2 months on a high-cholesterol diet, blood samples were drawn by tail bleeding after an overnight fast. A 30-μL aliquot of serum from each mouse was loaded onto a Pharmacia Smart column and fractions were collected. Fractions 3 to 7 represent VLDL and chylomicrons; fractions 8 to 14, LDL; and fractions 15 to 19, HDL. The distribution of cholesterol (A) and triglycerides (B) among different lipoprotein fractions in C57BL/6 mice transplanted with LPL+/+ or LPL−/− bone marrow after 2 months on a high-cholesterol diet (38% reduction; P<0.05 vs LPL−/−) bone marrow after 2 months on a high-cholesterol diet. Blood samples were drawn by tail bleeding after an overnight fast. A 30-μL aliquot of serum from each mouse was loaded onto a Pharmacia Smart column and fractions were collected. Fractions 3 to 7 represent VLDL and chylomicrons; fractions 8 to 14, LDL; and fractions 15 to 19, HDL. The distribution of cholesterol (A) and triglycerides (B) among different lipoproteins in LPL+/+→C57BL/6 (○) and LPL−/−→C57BL/6 (●) mice is shown. Values are mean of 8 mice. For clarity, SEM is shown only for fractions containing the top of the VLDL, LDL, and HDL peaks. Significant difference of *P<0.05 vs LPL+/+→C57BL/6 mice.

as observed on the chow diet at 8 weeks after BMT, also remained during the period when the mice were fed the high-cholesterol diet (38% reduction; P<0.05, n=16).

After 3 months on the high-cholesterol diet, the hearts and aortas were perfused, fixed, and examined histologically. Quantification of atherosclerotic lesion area demonstrated that transplantation of LPL-deficient bone marrow into C57BL/6 mice reduced the susceptibility of these mice to diet-induced atherosclerosis (Figure VIII). The mean atherosclerotic lesion area in LPL−/−→C57BL/6 mice (6898±1560 μm², n=13) was 52% (P<0.01) smaller than that found in LPL+/+→C57BL/6 mice (14,652±2441 μm², n=13). Representative photomicrographs of lipid-rich atherosclerotic lesions are shown in Figure IX. Staining of the atherosclerotic lesion area for MOMA-2, a macrophage marker, revealed that in both transplantation groups the atherosclerotic lesion area consisted primarily of lipid-laden macrophages (Figures XA and XB).

The extent of reconstitution of arterial wall macrophages by LPL−/− macrophages after BMT is important when analyzing the effects on atherosclerosis. Therefore, localization of the LPL protein in atherosclerotic lesions was determined by using an antibody against human LPL that cross-reacts with murine LPL (kindly provided by Dr S. Vilaro, University of Barcelona, Barcelona, Spain). In mice transplanted with LPL+/+ bone marrow, LPL was abundantly present in macrophage-rich regions of atherosclerotic lesions (Figure XC) as well as the smooth muscle cells in the aortic root. In mice transplanted with LPL−/− bone marrow, however, the macrophage-rich lesion area did not stain for LPL (Figure XD), whereas some staining of the smooth muscle cells of the aortic root was still visible.

Effect of BMT on Liver Function

To exclude the possibility that the observed effects on serum cholesterol levels were caused by liver damage as a result of the BMT procedure and the subsequent consumption of an atherogenic diet, serum ALAT activity was measured and the liver was analyzed histologically. As indicated in Table III, no significant difference in serum ALAT activity was observed between both groups of transplanted mice and control C57BL/6 mice of the same age. These data indicate that the liver parenchyma and biliary system were not seriously damaged by the BMT procedure and subsequent feeding with the atherogenic diet at 3 months after BMT and 1 month of feeding with the high-cholesterol diet. Histological analysis of the livers after 3 months of feeding the high-cholesterol diet revealed no histological evidence of hepatocyte necrosis or fibrosis.
Discussion

In the present study, we investigated the in vivo role of macrophage-derived LPL in lipoprotein metabolism and atherogenesis by using BMT. BMT provides a powerful technique to produce an animal model that lacks LPL specifically in all types of macrophages. Transplantation of LPL-deficient hematopoietic stem cells into lethally irradiated C57BL/6 mice resulted in a significant reduction in

Figure IX. Photomicrographs of atherosclerotic lesions in cross sections of aortic root from C57BL/6 mice transplanted with LPL+/+ or LPL−/− bone marrow. Sections were stained with oil red O to visualize lipid-rich lesions and counterstained with hematoxylin. Representative cross sections of aortic root from C57BL/6 animals transplanted with either LPL+/+ (A) or LPL−/− (B) bone marrow are shown. Magnification ×40.

Figure X. Immunohistochemical detection of macrophages and LPL protein in cross sections of aortic root from C57BL/6 mice transplanted with LPL+/+ (A, C) or LPL−/− (B, D) bone marrow. After 3 months on a high-cholesterol diet, the transplanted mice were killed and the hearts perfused and fixed with neutral-buffered formalin. Sections of aortic root were stained with antibodies against MOMA-2 to visualize macrophages (A, B) and with antibodies against human LPL (C, D) to visualize LPL in the lesions. Magnification ×400.
tissue lipase activity in organs containing high amounts of macrophages, like the spleen and lungs, indicating that these tissue macrophages were successfully replaced by cells of donor origin.

Macrophage LPL deficiency induced a significant reduction in serum cholesterol levels on both a chow diet and a high-cholesterol diet. Fractionation of serum lipoproteins revealed that this decrease in cholesterol levels was confined to the VLDL- and LDL-size fractions, while no significant effect on HDL cholesterol could be demonstrated. Thus, the reduction in tissue LPL activity due to the absence of LPL production by macrophages is not critical for the regulation of serum HDL levels in mice. These results are consistent with those of Coleman et al,25 who demonstrated that LPL activity has a limited role in determining HDL cholesterol levels in mice. Surprisingly, VLDL and LDL cholesterol levels were reduced in the absence of macrophage-derived LPL. In contrast, heterozygous LPL deficiency in all LPL-expressing organs is associated with an increase in VLDL and LDL cholesterol levels.36 These contradictory results support the hypothesis that the physiological function of LPL depends on the tissue in which it is expressed. It is unlikely that the observed effects on serum cholesterol levels were caused by liver damage as a result of the BMT procedure and the subsequent consumption of a high-cholesterol diet, because no effect on serum ALAT activity was observed, indicating that the liver parenchyma and biliary system were not damaged. Furthermore, no histological evidence of hepatocyte necrosis or fibrosis was found. Analysis of postheparin lipolytic activity in the plasma of transplanted mice revealed that macrophage LPL deficiency did not significantly affect the activity of the LPL pool located on the endothelial surface of the vascular tree. Thus, the effect of macrophage LPL deficiency on cholesterol levels in the circulation also appears not to be mediated by effects on the functional LPL pool on the endothelial surface. Another mechanism that can be postulated that may explain the observed reduction in cholesterol levels as induced by macrophage LPL deficiency might be reduced VLDL production by the liver. This alternative mechanism is currently under investigation. Although the exact mechanism of the cholesterol lowering remains to be determined, our data clearly provide evidence that macrophage LPL production significantly affects the metabolism of lipoproteins in the circulation.

Despite the decrease in serum cholesterol levels, serum triglyceride levels were increased 8 weeks after BMT in LPL−/−→C57BL/6 mice, probably due to reduced triglyceride hydrolysis in the periphery. Feeding the mice a high-cholesterol diet reduced triglyceride levels again, which is consistent with data from Semenkovich et al26 and LeBoeuf et al,40 who demonstrated that increased dietary fat decreases serum triglycerides in mice.

Strikingly, reconstitution of C57BL/6 mice with LPL-deficient macrophages also resulted in a sustained decrease in circulating apoE levels. Although the liver is the major source of apoE synthesis, macrophages in different organs are also active in secreting large quantities of apoE.41–43 Recently, we31 and others44,45 demonstrated, using the technique of BMT, that introduction of apoE-producing macrophages in apoE-deficient mice reduced the severe hypercholesterolemia in these mice, indicating the important role of macrophage-derived apoE in an apoE-deficient background. However, we46 and Fazio et al47 also showed that no effect on serum apoE concentration was observed in macrophage-specific apoE knockouts, suggesting that apoE production by macrophages cannot influence serum apoE levels in the presence of normal liver apoE production. Therefore, it is most likely that the 50% reduction in serum apoE concentration in LPL−/−→C57BL/6 mice was not caused by any direct effects of macrophage LPL deficiency on macrophage apoE synthesis but was a consequence of the observed reduction in serum cholesterol levels.

Because local production of apoE by macrophages may influence the process of foam cell formation, the effect of macrophage LPL deficiency on macrophage apoE production was analyzed in vitro. This study revealed that LPL-deficient macrophages were less active in secreting apoE into the medium than were wild-type macrophages, indicating that apoE secretion is influenced by endogenous LPL production. Recently, Lucas et al48 demonstrated that addition of exogenous LPL to mouse J774 cells transfected with human apoE cDNA prevented the release of newly synthesized apoE by these cells. Thus, both absence of endogenous LPL and addition of exogenous LPL reduce macrophage apoE secretion.

Transplantation of LPL-deficient bone marrow into C57BL/6 mice results in the replacement of all tissue macrophages, including those of the arterial wall that are involved in fatty streak formation. To analyze the effect of macrophage LPL deficiency on atherosclerosis susceptibility, the transplanted mice were challenged with a high-cholesterol diet containing cholate, a diet that has been extensively used to evaluate atherosclerotic lesion development in resistant mouse models.49–51 After 3 months of feeding this diet, atherosclerotic lesion development was reduced by 52% in C57BL/6 mice transplanted with LPL-deficient bone marrow compared with control transplanted animals. Cholesterol accumulation in macrophages and therefore, foam cell formation depends on the balance between cholesterol influx and cholesterol efflux. The following factors will influence the susceptibility to diet-induced atherosclerosis in C57BL/6 mice transplanted with LPL-deficient bone marrow: (1) decreased influx of cholesterol due to decreased exposure to serum cholesterol (antitherogenic), (2) decreased influx of cholesterol due to reduced uptake of atherogenic lipoproteins (antitherogenic), and (3) decreased efflux of cholesterol due to reduced apoE synthesis by macrophages (proatherogenic).

By performing immunohistochemistry, we showed that reconstitution of C57BL/6 mice with LPL-deficient macrophages resulted in an almost complete absence of LPL in fatty

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<tr>
<th>Mouse Type</th>
<th>n</th>
<th>ALAT Activity, U/L</th>
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<tr>
<td>LPL+/+→C57BL/6</td>
<td>16</td>
<td>29.3±8.5</td>
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<tr>
<td>LPL−/−→C57BL/6</td>
<td>16</td>
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<td>25.3±11.8</td>
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streak lesions. Thus, most of the LPL present in fatty streak lesions was derived from local synthesis by macrophages, rather than from the plasma compartment. Atherosclerotic lesion area quantification showed that absence of macrophage LPL production in the arterial wall resulted in reduced susceptibility to diet-induced atherosclerosis, indicating that the observed decreased influx of VLDL cholesterol in macrophages of LPL−/− C57BL/6 mice is the major determinant of the effect of LPL on atherosclerotic lesion development. Furthermore, the lowered apoE production was apparently still sufficient for cholesterol release from macrophages.

While our manuscript was under review, a relevant article describing the transplantation of fetal liver cells from LPL-deficient mice to C57BL/6 animals was published by Babae et al. In accordance with our data, they showed that macrophage LPL deficiency resulted in a 55% reduction in mean atherosclerotic lesion area, whereas no effect on serum lipid levels was observed. In summary, we have shown that macrophage LPL deficiency decreases serum cholesterol concentrations, circulating apoE levels, and diet-induced atherosclerosis in C57BL/6 mice, suggesting that specific blockade of macrophage LPL production may be beneficial for lowering atherosclerotic lesion formation.

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