Endothelial Lipase Modulates Susceptibility to Atherosclerosis in Apolipoprotein-E-deficient Mice*

Apolipoprotein-E-deficient Mice*  

Received for publication, June 8, 2004, and in revised form, July 16, 2004  
Published, JBC Papers in Press, August 9, 2004, DOI 10.1074/jbc.M406360200

Tatsuro Ishida‡§, Sungshin Y. Choi‡, Ramendra K. Kundu‡, Josh Spin‡, Tomoya Yamashita§, Ken-ichi Hirata§, Yoko Kojima§, Mitsuhiro Yokoyama§, Allen D. Cooper¶, and Thomas Quertermous‡‡

From the ‡Donald W. Reynolds Cardiovascular Clinical Research Center, Divisions of Cardiovascular Medicine and Gastroenterology, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, California 94305, the §Division of Cardiovascular and Respiratory Medicine, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan, and the ¶Research Institute, Palo Alto Medical Foundation, Palo Alto, California 94301

Endothelial lipase (EL) expression correlates inversely with circulating high density lipoprotein (HDL) cholesterol levels in genetic mouse models, and human genetic variation in this locus has been linked to differences in HDL cholesterol levels. These data suggest a role for EL in the development of atherosclerotic vascular disease. To investigate this possibility, LIPG-null alleles were bred onto the apoE knockout background, and the homozygous double knockout animals were characterized. Both apoE knockout and double knockout mice had low HDL cholesterol levels when compared with wild-type mice, but the HDL cholesterol levels of the double knockout mice were higher than those of apoE knockout mice. Atherogenic very low density lipoprotein and intermediate density lipoprotein/low density lipoprotein cholesterol levels of the double knockout mice were also greater than those of the apoE knockout animals. Despite this lipid profile, there was a significant ~70% decrease in atherosclerotic disease area in double knockout mice on a regular diet. Immunohistochemistry and protein blot studies revealed increased EL expression in the atherosclerotic aortas of the apoE knockout animals. An observed decrease in macrophage content in vessels lacking EL correlated with ex vivo vascular monocyte adhesion assays, suggesting that this protein can modulate monocyte adhesion and infiltration into diseased tissues. These data suggest that EL may have indirect atherogenic actions in vivo through its effect on circulating HDL cholesterol and direct atherogenic actions through vascular wall processes such as monocyte recruitment and cholesterol uptake.

It is known that members of the highly conserved lipase family of lipid metabolic enzymes affect vascular disease through regulation of high density lipoprotein cholesterol (HDL-C) levels, as well as through local actions in the vessel wall. Regulation of HDL-C level is important since serum HDL level is widely accepted as an inverse correlate of coronary heart disease risk (1, 2). Lipases have been shown to regulate HDL-C through effects on HDL particle size and metabolism (3). Further, lipoprotein lipase and hepatic lipase perform functions in the vessel wall that are independent of their catalytic activity. It is well known that lipases can form a molecular bridge between endothelial cells and lipoproteins or circulating macrophages through interaction with heparan sulfate proteoglycans (4). This nonenzymatic action of lipases can increase cellular lipoprotein uptake and monocyte adhesion. For instance, the local expression of lipoprotein lipase in the vascular wall has been implicated in the local trapping of lipid and acceleration of atherosclerosis through this bridging function (5).

To further investigate the genetic basis of HDL-C level and the modulation of atherosclerosis by lipases, recent studies have focused on a new member of the lipase gene family termed endothelial lipase (EL) (gene nomenclature LIPG, locus link ID. 16891) (6, 7). EL has been shown to be expressed by vascular cells, including endothelial cells (6, 7), and to be highly regulated in these cells in vitro and in vivo (8). Recent gene manipulation and antibody blocking experiments have established EL to be a primary determinant of HDL levels in the mouse (9–11). Further, association-based human genetic studies have provided evidence that variation in the LIPG locus is linked to differences in circulating HDL-C levels (10, 12, 13). Recently, it has been shown that EL directly mediates cellular lipoprotein uptake (14, 15).

Because of its significant effect on HDL levels and the documented association of other lipase family members with atherosclerosis, it seemed possible that EL might also play a role in atherogenesis. Here we report studies confirming this hypothesis by showing that apoE knockout animals, which also are targeted in the LIPG locus, have decreased atherosclerosis.

**Experimental Procedures**

Animal Preparation—Homozygous apoE−/− mice with C57BL/6J background were obtained from the Jackson Laboratory (Bar Harbor, Maine) and bred to EL knockout (LIPC−/−) mice on a C57BL/6J background to generate LIPC−/−, apoE−/− double knockout mice (9). PCR was performed for genotyping the apoE locus (16), and Southern
Fig. 1. Quantitation of atherosclerotic lesions in the aortic sinus of different genetic models. A, representative photographs of Sudan III-stained aortic root sections from apoE−/− and LIPG−/−, apoE−/− mice fed normal chow for 12 weeks. Sections were taken at the same level of the aortic valves (original magnification ×40). B, quantitative analysis of atherosclerotic lesion size in male and female apoE−/− and double knockout mice. The total lesion area of five sections in the aortic root from each mouse was quantified morphometrically as described under “Experimental Procedures.” After 12 weeks on normal chow, the atherosclerotic lesion areas were significantly attenuated in LIPG−/−, apoE−/− when compared with apoE−/− mice (*, p < 0.001). C, representative photographs of Sudan III-stained aortic root sections from apoE−/− and LIPG−/−, apoE−/− mice fed a high fat diet for 12 weeks. Sections were taken at the same level of the aortic valves (original magnification ×40). D, quantitative analysis of atherosclerotic lesion size in male and female apoE−/− and double knockout mice. After 12 weeks on a high fat diet, the atherosclerotic lesion areas were significantly attenuated in male double knockout animals when compared with apoE−/− mice (*, p < 0.001).

blot was performed for genotyping the LIPG locus (9). Between 14 and 16 mice were included in each experimental subgroup. ApoE−/− mice and LIPG−/−, apoE−/− mice were weaned at 4 weeks of age onto a high fat diet (0.15% cholesterol, 21% milk fat, 19.5% casein) or normal chow diet and maintained on the diet for 12 weeks. Wild-type (WT) and LIPG−/− mice were used as control groups. All animal experiments were conducted according to the Guidelines for Animal Experiments at the Stanford University School of Medicine.

Histological Analysis of Atherosclerotic Lesions—The apoE−/− and LIPG−/−, apoE−/− mice were euthanized at the age of 16 weeks (12 weeks on the chow diet or high fat diet), and the atherosclerotic lesions were analyzed as described previously (17). The aortic samples were fixed in 10% buffered formalin phosphate, embedded in OCT compound, and sectioned (10 μm thickness). Five consecutive sections, spanning 550 μm of the aortic root, were collected from each mouse and stained with Sudan III or Masson’s trichrome. For quantitative analysis of atherosclerosis, the total lesion area of five sections from each mouse was measured with the NIH Image 1.61 software by modifying the method reported previously (18). The amount of aortic lesion formation in each animal was measured as the percentage of the lesion area per total area of the aorta (17).

Analysis of Plasma Lipids—Following an overnight fast, whole blood was collected by cardiac puncture into tubes containing 0.3 mg of EDTA. Plasma was collected by centrifugation at 8000 × g for 5 min at 4 °C. Total plasma cholesterol, triglyceride (TG), and phospholipid (PL) levels were measured as described (9). Separation of plasma lipoproteins by ultracentrifuge was performed using an anti-CD3 antibody and an anti–smooth muscle actin (DAKO) antibody, respectively. Quantitative analysis was measured as a percentage of the positive-stained area in the total atherosclerotic lesion area as described previously (17). Western blots and immunohistochemical analysis for EL in the vessel wall were performed using an anti-mouse EL antibody (generous gift from Dr. Daniel J. Rader, University of Pennsylvania, Philadelphia, PA).

Ex Vivo Adhesion Assay—Ex vivo adhesion assays were performed as described (20). Briefly, the murine thoracic aorta was isolated and fixed in an agarose-coated culture plate. Fluorescein-labeled cultured mouse monocyte/macrophage cells, WEHI78/24 or MH-S cell lines, were plated onto the aortic strip, and the plate was gently shaken for 30 min. Some experiments were performed in the presence of heparin (1–5 units/ml) (Sigma). After washing the tissue to remove unbound cells, the number of binding cells per high power field, 10 arbitrary views per one tissue, was counted with fluorescence microscopy.

Statistical Analysis—Data are expressed as mean ± S.E. An unpaired Student t test was used to detect significant differences when two groups were compared. One-way analysis of variance was used to compare the differences among three or four groups, with Bonferroni’s test for post hoc analysis. Repeated measures analysis of variance was used to compare lipid fraction results obtained from FPLC. p values less than 0.05 were considered statistically significant.

RESULTS

Atherosclerosis Is Attenuated in Animals with Functional EL Deletion—Male and female apoE−/− and LIPG−/−, apoE−/− mice were evaluated for development of atherosclerosis on normal chow and high fat dietary challenge. After 12 weeks on normal chow, at the age of 16 weeks, atherosclerotic lesion formation was evaluated by Sudan III staining of sections at the level of the aortic valve (Fig. 1). The extent of disease was quantified as the total lesion area determined by planimetry. Interestingly, the atherosclerotic lesions were markedly attenuated in double knockout mice when compared with apoE−/− mice. For animals on a normal chow diet, lesion size was significantly decreased by 71% in males (p < 0.001) and by 67% in females (p < 0.001) when double knockout mice were com-
pared with apoE−/− mice (Fig. 1, A and B). Feeding mice with a high fat diet for 12 weeks accelerated atherosclerotic lesion formation in both apoE−/− and LIPG−/−, apoE−/− mice (Fig. 1, C and D). As was the case with normal chow, formation of atherosclerosis was attenuated in male double knockout mice when compared with apoE−/− mice. Quantitative analysis showed a significant 24% decrease (p < 0.001) in the lesion area of male LIPG−/−, apoE−/− mice when compared with apoE−/− mice (Fig. 1D). However, the difference was smaller and not statistically significant between female apoE−/− and double knockout mice.

**Table I**

| Diet      | Gender | WT (n = 8) | LIPG−/− (n = 8) | p value | apoE−/− (n = 8) | LIPG−/−, apoE−/− (n = 8) | p value |
|-----------|--------|------------|----------------|---------|----------------|--------------------------|---------|
| Cholesterol (mg/dl) |        |            |                |         |                |                          |         |
| NC        | M      | 58.9 ± 4.3 | 157.5 ± 11.1   | <0.001  | 549.1 ± 46.9   | 621.8 ± 37.5             | NS      |
|           | HF     | 177.1 ± 13.6 | 246.9 ± 19.5  | <0.001  | 736.6 ± 56.7   | 1047.7 ± 117.0           | <0.001  |
| NC        | F      | 55.2 ± 7.8 | 147.0 ± 19.9   | <0.001  | 409.2 ± 18.0   | 468.5 ± 34.8             | NS      |
|           | HF     | 80.7 ± 5.9 | 157.4 ± 11.5   | <0.01   | 540.3 ± 48.0   | 699.8 ± 149.0            | NS      |
| Triglyceride (mg/dl) |        |            |                |         |                |                          |         |
| NC        | M      | 38.9 ± 2.2 | 50.9 ± 4.2     | <0.05   | 113.5 ± 10.5   | 141.2 ± 11.8             | <0.05   |
|           | HF     | 54.0 ± 4.4 | 54.3 ± 2.8     | NS      | 101.0 ± 9.7    | 169.5 ± 12.0             | <0.05   |
| NC        | F      | 39.8 ± 1.6 | 60.1 ± 14.2    | <0.05   | 88.0 ± 5.6     | 77.3 ± 11.5              | NS      |
|           | HF     | 54.1 ± 4.4 | 55.8 ± 7.6     | NS      | 60.4 ± 4.1     | 83.4 ± 6.3               | <0.05   |
| Phospholipid (mg/dl) |        |            |                |         |                |                          |         |
| NC        | M      | 157.8 ± 3.7 | 256.0 ± 3.3    | <0.001  | 220.0 ± 10.3   | 280.1 ± 7.2              | <0.05   |
|           | HF     | 204.2 ± 8.3 | 232.3 ± 6.2    | <0.001  | 245.1 ± 9.2    | 405.0 ± 10.7             | <0.01   |
| NC        | F      | 128.2 ± 4.7 | 223.4 ± 6.7    | <0.001  | 197.1 ± 6.6    | 252.9 ± 8.2              | <0.05   |
|           | HF     | 142.8 ± 4.0 | 205.3 ± 6.4    | <0.01   | 194.0 ± 5.3    | 222.1 ± 9.9              | <0.05   |

By FPLC analysis, only a minimal increase in HDL was seen in male LIPG−/− mice over WT was confirmed in these studies for male and female animals on both diets (Table I) (9). The male LIPG−/−, apoE−/− mice on a high fat diet had a significantly higher cholesterol level than either single knockout alone, and female double knockout mice on high fat had higher but not significantly increased cholesterol levels when compared with either single knockout strain. There was no difference between cholesterol levels in the LIPG−/−, apoE−/− mice and the apoE−/− animals on regular diet.

TG levels were increased in female LIPG−/− animals on normal chow, as reported previously, and male animals also showed a significant increase in these experiments (Table I) (9). These differences disappeared on a high fat diet. Plasma TG levels were increased over WT in male and female apoE−/− animals on a regular diet, as expected. TG levels for the double knockout female mice on high fat were significantly higher than control and single knockout female mice but the same as seen on a normal chow diet. A different pattern was seen in male mice on the high fat diet. There was a decrease in serum TG in the apoE−/− animals, whereas TG levels in the LIPG−/− animals remained unchanged. On this atherogenic diet, there was no significant difference in TG levels between control, apoE−/−, and LIPG−/− male animals. Interestingly, the LIPG−/−, apoE−/− male animals on high fat continued to have significantly elevated TG levels.

Plasma PL levels tended to be higher in LIPG−/− animals. Both male and female LIPG−/− animals showed a significantly greater than 50% increase in plasma PL levels when compared with WT mice (Table I). LIPG−/−, apoE−/− animals showed modest but significant increases in PL levels when compared with LIPG−/− animals. These changes are likely due to the loss of the phospholipase activity of EL.

Fasting plasma from the different mouse lines was fractionated by gel filtration chromatography (FPLC) and cholesterol measured in individual fractions (Fig. 2, A–D). For both the WT and LIPG−/− lines, the majority of plasma cholesterol was found in the HDL fractions. The previously described 2-fold increase in cholesterol measured in HDL fractions was again observed for LIPG−/− animals when compared with WT animals on normal chow (Fig. 2, A and B) (9). This increase was less apparent for those animals on the high fat diet but was still significant in females. Also, significant increases in IDL/LDL cholesterol were found in LIPG−/− animals when compared with WT, for female mice on normal chow and both male and female mice on a high fat diet (Fig. 2, C and D). The cholesterol concentration in the HDL fractions was similar for the apoE−/− and LIPG−/−, apoE−/− animals, and in all cases, it was significantly lower than in the other two genotype groups. By FPLC analysis, only a minimal increase in HDL was seen in apoE−/− knockout mice also missing EL. By contrast, in the apoE−/− and LIPG−/−, apoE−/− animals, most of the cholesterol was distributed among larger lipoproteins in the VLDL and IDL/LDL range. Examining the VLDL fractions, male double knockout animals had significantly greater VLDL cholesterol than apoE−/− animals, on both chow and high fat diet, and female double knockout animals had greater mean VLDL cholesterol levels that approached significance.

To further characterize lipid metabolism in these genetic mouse models, lipoprotein fractions from pooled mouse plasma were separated by ultracentrifugation, and the cholesterol, TG, and PL content of each fraction was determined by biochemical assays. These studies also showed that LIPG−/− mice had 2-fold higher HDL-C than WT mice (Table II). HDL-C was found to be markedly decreased with loss of apoE, but this method found that EL deficiency partially restored the decreased HDL levels in apoE−/− mice. Also, a significant increase in LDL-C was observed in LIPG−/− when compared with WT. A prominent increase in VLDL/LDL triglyceride was observed in LIPG−/−, apoE−/− animals when compared with apoE−/− animals, consistent with the FPLC data showing that loss of EL was associated with increased TG-rich lipoproteins. HDL phospholipid was markedly increased in both the WT and apoE−/− mice missing EL. A smaller but significant increase in LDL phospholipid was observed in LIPG−/− animals when
FIG. 2. Cholesterol levels in lipoprotein fractions obtained by FPLC. Individual animals were analyzed separately, and data are expressed as mean ± S.E. The right panels show a magnified HDL range (fractions 27–30). Statistical significance (p < 0.05) is indicated by an asterisk for comparisons of VLDL cholesterol values between LIPG−/−, apoE−/− and apoE−/− animals, a double asterisk for comparisons of IDL/LDL cholesterol values between LIPG−/− and wild-type animals, and a single dagger for comparisons of HDL cholesterol between LIPG−/− and wild-type animals. WT indicates WT C57Bl/6.

TABLE II
Lipid and lipoprotein profile in apoE−/−, LIPG−/−, LIPG−/−, apoE−/−, and wild-type mice on normal chow

The HDL, LDL, and VLDL fractions were obtained from 1.5 ml pooled plasma from 2–3 male mice by ultracentrifugation. The cholesterol (chol), TG, and PL levels in each lipoprotein fraction were determined by biochemical assay. A total of 16–23 mice were included in each group. Data are shown as mean ± S.E. (mg/dl). The abbreviations are as follows: WT, wild-type C57Bl/6; NS, not significant.

|               | WT (n = 5) | LIPG−/− (n = 5) | p value | apoE−/− (n = 8) | LIPG−/−, apoE−/− (n = 8) | p value |
|---------------|------------|----------------|---------|-----------------|-------------------------|---------|
| Cholesterol (mg/dl) |            |                |         |                 |                         |         |
| HDL-chol      | 67.2 ± 2.0 | 113.7 ± 3.1    | <0.0001 | 17.5 ± 2.6      | 39.7 ± 4.7              | <0.001  |
| LDL-chol      | 12.3 ± 1.0 | 22.2 ± 2.5     | <0.05   | 146.7 ± 9.7     | 152.8 ± 10.0            | NS      |
| VLDL-chol     | 7.0 ± 0.3  | 8.6 ± 1.3      | NS      | 448.5 ± 35.3    | 393.9 ± 20.7            | NS      |
| Triglyceride (mg/dl) |          |                |         |                 |                         |         |
| HDL-TG        | 2.4 ± 0.4  | 3.9 ± 0.3      | <0.05   | 1.4 ± 0.2       | 1.7 ± 0.2               | NS      |
| LDL-TG        | 8.4 ± 1.1  | 6.6 ± 0.3      | NS      | 5.4 ± 0.6       | 9.7 ± 1.7               | <0.05   |
| VLDL-TG       | 27.0 ± 1.6 | 34.5 ± 3.9     | NS      | 77.1 ± 12.3     | 139.9 ± 12.4            | <0.01   |
| Phospholipid (mg/dl) |        |                |         |                 |                         |         |
| HDL-PL        | 156.2 ± 3.4| 248.4 ± 3.4    | <0.0001 | 46.3 ± 3.9      | 95.5 ± 12.5             | <0.01   |
| LDL-PL        | 13.3 ± 2.1 | 23.7 ± 2.8     | <0.05   | 66.5 ± 6.3      | 88.4 ± 8.8              | 0.066   |
| VLDL-PL       | 15.3 ± 1.3 | 34.5 ± 3.9     | 0.063   | 175.1 ± 6.7     | 183.5 ± 8.2             | NS      |
compared with WT, and there was a nonsignificant increase in LDL phospholipid in LIPG−/−, apoE−/− animals when compared with apoE−/− animals. These data suggest that HDL phospholipid is the most specific substrate for EL, and as a result of EL action, HDL catabolism produces a decrease in HDL-C levels. Moreover, EL may have weak enzymatic action on apoB-containing lipoproteins such as LDL.

EL Expression in the Vascular Wall May Mediate Local Effects on Atherosclerosis—Since the lipoprotein profile in apoE−/− animals did not provide a clear mechanism for the observed decrease in atherosclerosis and because other lipases have been shown to have local effects in the vessel wall, we investigated vascular wall differences in the single and double knockout apoE mice. First, to determine whether EL was expressed in the apoE model atherosclerotic lesions, immunohistochemistry was performed using an antibody against mouse EL. In large vessels of WT mice, EL was predominantly expressed by luminal endothelial cells, although expression was also detected in the adventitia (Fig. 3, a and b). In apoE−/− mice, expression of EL was detected in the atherosclerotic plaque as well as endothelial cells (Fig. 3, d and e). Most of the EL-positive area was rich in macrophages, suggesting that EL-positive cells in the plaque were mainly infiltrating macrophages (Fig. 3, g and h). Smooth muscle α-actin staining was barely detectable in the EL-positive area of the atheroma (data not shown). Specificity of the staining for EL was confirmed by immunohistological analysis of atherosclerotic lesions from LIPG−/−, apoE−/− mice. These studies suggested reduced macrophage infiltration when compared with apoE−/− animals (Fig. 3, i and j).

To compare EL protein levels in normal versus atherosclerotic vessels, homogenates of aortic tissue from WT and apoE−/− animals on normal chow or high fat diet were analyzed. Tissue samples were subjected to Western blotting using the same antibody as employed for immunohistochemistry (Fig. 4). This quantitative assay revealed that EL expression was increased by 2.1-fold in aortas from apoE−/− mice when compared with aortas from WT mice. Feeding mice with a high fat diet resulted in a modest (−10%) increase in EL expression, in both the WT and the apoE−/− mice.

These results in the apoE−/− mouse model of atherosclerosis are consistent with a previous study that documented EL expression in infiltrating macrophages and smooth muscle cells in advanced lesions of atherosclerosis in postmortem human coronary arteries (21). Thus, the degree and extent of EL expression appears to be increased early in human atherosclerotic vascular disease.

To examine whether lesions in the apoE−/− and LIPG−/−, apoE−/− mice had different cellular composition, we performed immunohistochemical staining of atherosclerotic areas with antibodies chosen to specifically label different cell types. An antibody to MOMA-2 was employed to evaluate the area containing infiltrating macrophages. The percentage of the

---

**Fig. 3.** Immunohistochemical analysis of EL expression in the mouse aortic sinus. EL immunoreactivity was detected in the endothelial lining in nonatherosclerotic WT (a and b) and atherosclerotic (apoE−/−) aorta (d and e). In addition, there was staining associated with cells infiltrating the atherosclerotic lesions (d and e). Experiments with anti-MOMA2 (g and h) revealed staining that mainly overlapped with EL staining (d and e versus g and h). Atherosclerotic lesions of LIPG−/−, apoE−/− mice were negative for EL expression (i) but positive for MOMA-2 (j). Panels c and f were negative controls in which the primary antibodies were replaced with nonspecific rabbit or rat immunoglobulins. Original magnifications were as follows: ×400 (a, c, d, f, g, i, and j) and ×1000 (b, e, and h).

**Fig. 4.** Analysis of EL expression in mouse aortic tissue. Whole tissue homogenates of aortas from WT or apoE−/− mice, on a normal chow (NC) or high fat diet (HF), were employed for Western blotting. The membrane was probed with an anti-mouse EL polyclonal antibody. Relative expression levels are shown as the percentage of WT mice on normal chow. *, p < 0.05 versus corresponding WT mice.

Endothelial Lipase Modulates Atherosclerosis

---

45089
FIG. 5. Macrophage content in atherosclerotic lesions in the aortic root. A, immunostaining for MOMA-2 was employed to quantify the relative area occupied by macrophages in representative sections of atherosclerotic lesions in the apoE−/− and double knockout animals fed normal chow (a, b, e, and f) or high fat diet (c, d, g, and h) for 12 weeks. Original magnifications were as follows: ×40 (a−d), ×200 (e and f), or ×400 (g and h). B, quantitation of the lesion area stained with MOMA-2 reveals significantly less accumulation of macrophages in lesions of apoE−/− animals when compared with the double knockouts. *, p < 0.05 versus apoE−/− mice.

FIG. 6. Monocyte/macrophage binding is attenuated in LIPG−/−, apoE−/− mice. Mouse aortic strips were isolated from WT, apoE−/−, and LIPG−/−, apoE−/− mice and incubated with WEHI78/24 cells. A, representative images for the adhesion assay are shown as follows: wild-type (a), apoE−/− (b), and LIPG−/−, apoE−/− (c) mice. B, the graph represents the average adherent cell number in 10 random ×200 fields of each aortic strip, showing that the number of adherent cells was significantly lower in LIPG−/−, apoE−/− mice than in apoE−/− mice (p < 0.05, n = 8). Heparin treatment (5 units/ml) significantly diminished the augmented cell adhesion to apoE−/− aorta, suggesting the involvement of EL through heparan sulfate proteoglycans. NS, not significant.

MOMA-2-stained area in the aortic sinus lesions was significantly smaller in LIPG−/−, apoE−/− mice, consistent with overall fewer macrophages in these lesions (Fig. 5). There was no significant difference in T-cell or smooth muscle cell composition of the lesions in the apoE−/− and the LIPG−/−, apoE−/− mice, as evaluated with immunohistochemistry with antibodies to CD3 and smooth muscle α-actin respectively (data not shown).

To investigate the mechanism for the observed difference in macrophage composition of lesions between EL-expressing and non-EL-expressing apoE mice, aortic strips were isolated from LIPG−/−, apoE−/− and apoE−/− mice and incubated with cultured mouse monocyte/macrophage cells (the WEHI78/24 cell line). An ex vivo adhesion assay revealed that monocyte/macrophage binding to LIPG−/−, apoE−/− aortic strips was significantly less than to strips from apoE−/− mice (Fig. 6, A and B). Similar results were obtained when MH-S monocyte cells were used (data not shown). Heparin treatment significantly diminished the augmented cell adhesion to aortic strips from apoE−/− mice but not to those from LIPG−/−, apoE−/− mice, suggesting that up-regulation of EL in the aorta may in part mediate the cell adhesion through heparan sulfate proteoglycans (Fig 6B). These findings support the measured decrease in macrophage composition of EL-negative lesions and provide a possible mechanism by which EL might act directly to impact the development of vascular wall disease. Whether EL might modulate monocyte adherence and transit into the vessel wall through local lipid metabolism or through a direct bridging function will require further investigation.

DISCUSSION

To initiate studies investigating EL involvement in vascular disease, the previously described congenic strain of mice carrying a 129SvJ-null LIPG allele in the C57Bl/6 background was bred to a strain homozygous for null apoE alleles in the C57Bl/6 background (9, 16, 22, 23). This model has been extensively characterized, both in terms of the altered lipoprotein and lipid profiles and in terms of the predisposition to atherosclerosis. Homozygous apoE knockout animals have a markedly elevated serum cholesterol with increased VLDL and IDL/LDL cholesterol and reduced HDL-C. As a result of this highly atherogenic lipoprotein profile, apoE−/− animals develop spontaneously complex atherosclerotic lesions by 15 weeks of age that are similar to those in humans. The atherogenic potential of a broad range of lipid metabolism and inflammatory mediator genes has been investigated by evaluation of disease development in the apoE−/− model (24).

It was anticipated that LIPG−/−, apoE−/− animals might
Endothelial Lipase Modulates Atherosclerosis

45091

show increased HDL-C levels and a related decrease in the rate and extent of atherosclerotic disease when compared with the apoE−/− animals. Interestingly, the LIPG−/−, apoE−/− mice showed only a modest increase in HDL-C levels. There was also an increase in VLDL cholesterol in LIPG−/−, apoE−/− animals, and this increase was significant for the males. The increase in serum TG level was consistent with the increase in VLDL cholesterol in LIPG−/−, apoE−/− animals. The mechanism for this increase in VLDL is unclear, but the observation is consistent with the recent study that demonstrated a role for EL in the metabolism of apoB-containing lipoprotein particles such as VLDL and LDL (25). In this case, loss of EL in addition to apoE would result in further prolonged half-life of VLDL, accounting for the increases noted in the double knockout animals. A previous study has indicated that EL has some weak triglyceride lipase activity, particularly when lipoprotein substrates are abundant, as found in apoE−/− mice on a high fat diet (26). It is possible that EL triglyceride lipase activity accounted for part of the difference in TG level. Consistent with this hypothesis is the observation that LIPG−/− animals on a high fat diet showed increased LDL/IDL levels when compared with WT animals. Although loss of EL does not impair the metabolism of apoB-containing particles in the setting of a normal diet, challenge with high fat provides evidence for a decreased ability to clear LDL/IDL particles.

The lipid profile of the LIPG−/−, apoE−/− animals showed an increase in antiatherogenic HDL and an increase in atherogenic VLDL/LDL. This balanced increase in lipoprotein particle size was attributed to a decrease in HDL particle size in EL-null animals. A previous study has indicated that EL mediates binding and uptake of plasma lipoproteins via a process that is independent of its enzymatic activity, requires cellular heparan sulfate proteoglycans, and is regulated by ligand clustering (14, 15). As a first step to investigate possible direct vascular wall functions of EL, we have documented increased EL expression in the diseased aorta of apoE knockout animals, and validated this observation in human atherosclerosis (21). Through studies investigating the cell-specific expression of EL in atherosclerotic plaque, we have shown that infiltrating macrophages are primarily responsible for this increased expression. Interestingly, these experiments also identified a decreased relative macrophage content in the combined EL− and apoE-negative lesions. Follow-up studies have documented a related decrease in monocyte adhesion to vascular wall tissue missing EL. Increased monocyte adhesion was documented in vitro and ex vivo using EL-overexpressing COS7 cells and EL-overexpressing vessels, and the EL-mediated adhesions were inhibited by heparin and heparanase I, suggesting that up-regulation of EL may enhance monocyte adhesion to the vessel wall by interaction with heparan sulfate proteoglycans (30). Taken together, these data strongly suggest a primary role for EL in mediating monocyte adhesion and uptake into the vessel wall in the setting of atherosclerotic disease.

Comparative studies performed with other lipases have provided results similar to those reported here for EL. Mice lacking both hepatic lipase and apoE had increased total plasma cholesterol, with the increase in cholesterol mainly due to VLDL and only a very modest increase in HDL cholesterol (28, 31). Despite this increase in plasma cholesterol, hepatic lipase deficiency in apoE−/− mice resulted in significantly decreased aortic plaque sizes in female mice fed normal chow. The improved capacity to promote cholesterol efflux, together with the modest increase in HDL, were thought to overcome the increase in atherogenic lipoproteins. Such observations, in conjunction with those reported here for EL, argue that this family of proteins can modulate atherosclerosis through local vascular wall disease-related processes as well as regulation of circulating lipid levels.

Acknowledgment—We thank Dan Rader for thoughtful discussions and for providing the anti-mouse EL antibody.

REFERENCES

1. Gordon, D. J., and Rifkind, B. M. (1989) N. Engl. J. Med. 321, 1311–1316
2. Assmann, G., Schulte, H., von Eckardstein, A., and Huang, Y. (1996) Athero-

sclerosis 124, Suppl. I, S11–S20
3. Jin, W., Marchadier, D., and Rader, D. J. (2002) Trends Endocrinol. Metab. 13, 174–178
4. Goldberg, I. J., and Merkel, M. (2001) Front. Biosci. 6, D388–D405
5. Bahador, V. R., Faxo, S., Cleaves, L. A., Carter, K. J., Semenovich, C. F., and

Linton, M. F. (1999) J. Clin. Invest. 103, 1697–1705
6. Hirata, K., Dichiè, H. L., Cioffi, J. A., Choi, S. Y., Leeper, N. J., Quintana, L.

Kronmal, G. S., Cooper, A. D., and Quertermous, T. (1999) J. Biol. Chem. 274, 1470–14715
7. Jaye, M., Lynch, K. J., Krawiec, J., Marchadier, D., Maugeais, C., Doan, K.

South, V., Amin, D., Perrone, M., and Rader, D. J. (1999) Nat. Genet. 21, 424–428
8. Hirata, K., Ishida, T., Matsuhashi, H., Tsao, P. S., and Quertermous, T. (2000)

Biochem. Biophys. Res. Commun. 272, 90–93
9. Ishida, T., Choi, S., Kondo, R. K., Hirata, K., Rubin, E. M., Cooper, A. D., and

Quertermous, T. (2003) J. Clin. Invest. 111, 347–355
10. Ma, K., Cinglirongl, M., Otvos, J. D., Ballantyne, C. M., Marrian, A. J.,

and Chan, L. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 2748–2753
11. Jin, W., Miller, J. S., Breed, U., Glick, J. M., and Rader, D. J. (2003) J. Clin.

Invest. 111, 357–362
12. Yamakawa-Kobayashi, K., Yanagi, H., Endo, K., Arinami, T., and Hamaguchi,

H. (2003) Hum. Genet. 113, 311–315
13. Paradis, M. E., Couture, P., Boisse, Y., Despres, J. P., Perusse, L., Bouchard, C.

Vohl, M. C., and Lamarre, B. (2003) J. Lipid Res. 44, 1902–1908
14. Strauss, J. G., Zimmermann, R., Hrzenjak, A., Zhou, Y., Krakty, D., Levak-

Franke, S., Kostner, G. M., Zechar, R., and Frank, S. (2002) Biochem. J. 368, 69–79
15. Fuki, I. V., Blanchard, N., Jin, W., Marchadier, D. H., Miller, J. S., Glick, J. M.,

and Rader, D. J. (2003) J. Biol. Chem. 278, 34331–34338
16. Pfeffer, J. A., Zhang, S. H., Hagaman, J. R., Oliver, P. M., and Maeda, N.

(1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4471–4475
17. Ozaki, M., Kawashima, S., Yamashita, T., Ohashi, Y., Rikitake, Y., Inoue, N.,

Hirata, K. I., Hayashi, Y., Itoh, H., and Yokoyama, M. (2001) Hypertension 37, 322–327
18. Paijen, B., Morrow, A. J., Holmes, P. A., Mitchell, D., and Williams, R. A. (1987)

Atherosclerosis 66, 231–240
19. Eberz, T. J., and Brewer, H. B., Jr. (1977) Clin. Chem. 23, 2089–2098
20. Tsao, P. S., Niebauer, J., Buitrago, R., Lin, P. S., Wang, B. Y., Cooke, J. F.,

Chen, Y. D., and Reaven, G. M. (1998) Atherosclerosis 14170–14175
21. Anzumi, H., Hirata, K., Ishida, T., Kojima, Y., Rikitake, Y., Takeuchi, S., Inoue,

N., Kawashima, S., Hayashi, Y., Itoh, H., Quertermous, T., and Yokoyama, M. (2003) Cardiolo-

g. 58, 657–654
22. Plump, A. S., Smith, J. D., Hayek, T., Aalto-Selkala, K., Walsh, A., Verstuyft,

J. G., Rubin, E. M., and Breslow, J. L. (1992) Cell 71, 343–353
23. Zhang, H. S., Reddick, R. L., Pfeffer, J. A., and Maeda, N. (1992) Science 258, 468–471
24. Plump, A. S., and Breslow, J. L. (1995) *Annu. Rev. Nutr.* **15**, 495–518
25. Broedl, U. C., Maugeais, C., Millar, J. S., Jin, W., Moore, R. E., Fuki, I. V., Marchadier, D., Glick, J. M., and Rader, D. J. (2004) *Circ. Res.* **94**, 1554–1561
26. McCoy, M. G., Sun, G. S., Marchadier, D., Maugeais, C., Glick, J. M., and Rader, D. J. (2002) *J. Lipid Res.* **43**, 921–929
27. Maugeais, C., Tietge, U. J., Broedl, U. C., Marchadier, D., Cain, W., McCoy, M. G., Lund-Katz, S., Glick, J. M., and Rader, D. J. (2003) *Circulation* **108**, 2121–2126
28. Singaraja, R. R., Fievet, C., Castro, G., James, E. R., Hennuyer, N., Cle, S. M., Bisada, N., Chey, J. C., Fruchart, J. C., McManus, B. M., Staels, B., and Hayden, M. R. (2002) *J. Clin. Invest.* **110**, 35–42
29. Gauster, M., Osokliva, O. V., Innerlohinger, J., Glatter, O., Knipping, G., and Frank, S. (2004) *Biochem. J.* **382**, 75–82
30. Shimokawa, Y., Hirata, K., Kojima, Y., Iwai, K., Ishida, T., Hirase, T., Inoue, N., Kawashima, S., and Yokoyama, M. (2003) *Circ J.* **67**, Suppl. 1, 220
31. Mezdour, H., Jones, R., Dengremont, C., Castro, G., and Maeda, N. (1997) *J. Biol. Chem.* **272**, 13570–13575
Endothelial Lipase Modulates Susceptibility to Atherosclerosis in Apolipoprotein-E-deficient Mice
Tatsuro Ishida, Sungshin Y. Choi, Ramendra K. Kundu, Josh Spin, Tomoya Yamashita, Ken-ichi Hirata, Yoko Kojima, Mitsuhiro Yokoyama, Allen D. Cooper and Thomas Quertermous

J. Biol. Chem. 2004, 279:45085-45092.
doi: 10.1074/jbc.M406360200 originally published online August 9, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M406360200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 30 references, 13 of which can be accessed free at http://www.jbc.org/content/279/43/45085.full.html#ref-list-1