Carboxyl Ester Lipase Expression in Macrophages Increases Cholesteryl Ester Accumulation and Promotes Atherosclerosis*

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Ahmer Kodvawala, Amy B. Ghering, W. Sean Davidson, and David Y. Hui

From the Department of Pathology and Laboratory Medicine, Genome Research Institute, University of Cincinnati College of Medicine, Cincinnati, Ohio 45237

Carboxyl ester lipase (CEL, also called cholesterol esterase or bile salt-dependent lipase) is a lipolytic enzyme capable of hydrolyzing cholesteryl esters, triacylglycerols, and phospholipids in a trihydroxy bile salt-dependent manner but hydrolyzes ceramides and lysophospholipids via bile salt-independent mechanisms. Although CEL is synthesized predominantly in the pancreas, a low level of CEL expression was reported in human macrophages. This study used transgenic mice with macrophage CEL expression at levels comparable with that observed in human macrophages to explore the functional role and physiological significance of macrophage CEL expression. Peritoneal macrophages from CEL transgenic mice displayed a 4-fold increase in [3H]oleate incorporation into cholesteryl [3H]oleate compared with CEL-negative macrophages when the cells were incubated under basal conditions in vitro. When challenged with acetylated low density lipoprotein, cholesteryl ester accumulation was 2.5-fold higher in macrophages expressing the CEL transgene. The differences in cholesteryl ester accumulation were attributed to the lower levels of ceramide and lysophosphatidylcholine in CEL-expressing cells than in CEL-negative cells. CEL transgenic mice bred to an atherosclerosis susceptible apoE<sup>−/−</sup> background displayed an approximate 4-fold higher atherosclerotic lesion area than apoE<sup>−/−</sup> mice without the CEL transgene when both were fed a high fat/cholesterol diet. Plasma levels of the atherogenic lysophosphatidylcholine were lower in the CEL transgenic mice, but plasma cholesterol level and lipoprotein profile were similar between the two groups. These studies documented that CEL expression in macrophages is pro-atherogenic and that the mechanism is because of its hydrolysis of ceramide and lysophosphatidylcholine in promoting cholesteryl esterification and decreasing cholesteryl efflux.

The pancreatic type carboxyl ester lipase (CEL),<sup>2</sup> also called cholesterol esterase or bile salt-dependent lipase, is a 74-kDa molecular mass lipolytic enzyme capable of liberating fatty acyl chains from cholesteryl esters, triacylglycerols, phospholipids, and lysophospholipids through a serine esterase mechanism (1, 2). Additionally, CEL is also a lipoprotein lipase, capable of releasing fatty acyl groups from amide linkages such as those present in ceramides (3, 4). The conformation of CEL stimulates that its hydrolytic activity on cholesteryl esters, triacylglycerols, and phospholipids is dependent on the presence of bile salt (1, 2, 5, 6). However, CEL hydrolysis of lysophosphatidylcholine and ceramide does not have an absolute requirement for bile salt (2, 3).

The CEL is synthesized predominantly in the pancreas, and is also found to be present in abundance in human milk. Accordingly, early investigations on its physiological functions have focused primarily in the digestive tract, where CEL is postulated to play a role in lipid nutrient absorption. Our recently completed study revealed that CEL plays a significant role in catalyzing cholesteryl ester absorption from the intestinal lumen (7) and promoting large chylomicron assembly and secretion from enterocytes in the digestive tract (8). Interestingly, CEL-mediated cholesteryl ester absorption is dependent on its cholesteryl ester hydrolytic activity, whereas its modulation of large chylomicron production is unrelated to its cholesteryl ester hydrolytic activity but dependent on its hydrolysis of ceramide generated during the lipid absorption process (8).

In addition to its presence in the digestive tract and its participation in the intestinal lipid absorption and transport pathway, CEL is also found to be present in human plasma and in the vessel wall (9). We have shown previously that CEL is synthesized by human macrophages and endothelial cells (10, 11). The presence of CEL in the vasculature, and its increased synthesis by vascular cells after incubation with oxidized LDL (10, 11), suggested a potential role of CEL in modulation of atherosclerosis. Cell culture studies have shown that CEL induces vascular smooth muscle cell proliferation (12), suggesting its contributory role toward atherosclerosis. In contrast, other in vitro studies showed that CEL reduces lysophospholipid content in oxidized LDL (9). The latter studies suggested that CEL expression in the vessel wall may be protective against atherosclerosis. Whether the presence of CEL in the vessel wall is protective or contributory to the atherosclerotic lesion development has not been clarified.

The paucity of information regarding the physiological role of the vascular CEL in atherosclerosis is because of species-specific differences in macrophage CEL expression. Unlike the human macrophages, mouse macrophages do not synthesize CEL (10). To circumvent this problem, we have produced transgenic mice expressing CEL in the vessel wall to evaluate its role in atherosclerosis. Results of this study showed that CEL expression in macrophages promotes cholesteryl ester synthesis and accumulation in response to modified LDL and increases atherosclerosis lesions in apoE-null mice.

**EXPERIMENTAL PROCEDURES**

Production and Screening of Transgenic Mice—The sheep visna virus long terminal repeat (LTR) was used as promoter to drive macrophage
CEL expression in transgenic mice. The LTR-apoE gene construct containing the visna virus LTR upstream of the human apoE3 gene was obtained from Dr. John Taylor (Gladstone Institute, San Francisco, CA) (13). A 2-kb cDNA fragment containing the entire coding sequence of rat CEL, without the polyadenylation signal was obtained by SmaI digestion of the plasmid pSVL-CEL (14) and inserted into the unique Ball site located in the beginning of exon 2 of apoE to replace the coding exons and subsequent introns in the LTR-apoE chimeric gene. The resulting chimeric construct contains from the 5' to 3' direction: the visna virus LTR, the noncoding exon 1 sequence and intron 1 sequence of the human apoE gene, and the entire coding sequence of rat CEL. A schematic depicting the approach for construction of the LTR-CEL chimeric gene is presented in Fig. 1. The sequence of the chimeric gene was confirmed by dyeoxy chain termination sequence analysis. The sequence predicts that the entire native rat CEL protein, with no additional residue from apoE, will be produced from the LTR promoter. The LTR-CEL chimeric gene was separated from vector sequence by digestion with ScaI and SalI, and then purified by agarose gel electrophoresis. Four thousand copies of the chimeric gene were microinjected into the pronuclei of fertilized mouse eggs obtained from superovulated female C57BL/6 mice. The injected eggs were surgically transferred to oviducts of surrogate females. Offspring were screened for integration of the transgenic through PCR amplification of tail DNA with the upstream primer sequence: 5' - GTGTTGACTCTGGTGACACTTT-3' and the downstream primer sequence: 5' - TGGCTGTAGACATCGTTTCTTG-3' to yield a 131-bp product that overlaps the nucleotide sequence between residues 148 and 279 of rat CEL CDNA. Transgenic founder lines were maintained and propagated. The expression profile of the CEL transgene in the transgenic mice was determined by reverse transcriptase-PCR amplification of RNA isolated from various tissues using the above CEL primers, with GAPDH primers (forward: 5' - CATCAAGAAGGT-GGTGAAGC-3' and reverse: 5' - GAGCTTGACAAAGTTGTC-3') for amplification of GAPDH mRNA as control. Peritoneal macrophages were obtained from the progeny and used to determine CEL expression. Two lines were shown to display macrophage CEL expression levels similar to that observed in human monocyte-derived macrophages (10).

**Cholesterol Ester Hydrolytic Activity in Mouse Macrophages**—Wild type C57BL/6 and CEL transgenic mice were injected intraperitoneally with 4% thioglycolate solution, and macrophages were harvested 3 days later by lavage of the peritoneal cavity with phosphate-buffered saline. The mouse peritoneal macrophages were cultured in 6-well plates at a density of 2 x 10^6 cells/well with Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Non-adherent cells were removed after 2 h by exhaustive washing and macrophages attached to the dishes were then incubated with 200 µg/ml [3H]cholesterol oleate-containing acLDL overnight at 37 °C. The cells were washed twice with phosphate-buffered saline and then equilibrated in serum-free media for 6 h. One set of wells was used to measure the total amount of [3H]cholesterol loaded into the macrophages. Equivalent wells were incubated with or without apolip-A-I in serum-free media for 6 h to induce cholesterol efflux. The amount of [3H]cholesterol transferred from the cells to apolip-A-I in the incubation media was quantified by determining the radioactivity level in the media. Each treatment was performed in triplicates and the data are presented as percentage of total radiolabel loaded into the macrophages.

**Effects of CEL Transgenic Expression on Plasma Lipids**—The impact of macrophage CEL expression on plasma lipid levels and atherosclerosis was evaluated by crossing the CEL-transgenic mice with the atherosclerosis-susceptible apoE-null mice. Female apoE-/- mice with or without macrophage-specific CEL transgenic expression were fed the Western-type high fat/cholesterol diet (TD88137, Harlan Teklad), which contains 21% fat, 0.15% cholesterol, and 19.5% casein by weight with no sodium cholate (19). After 8 weeks, fasting plasma was collected from the animals. Cholesterol distribution among the various classes of lipoproteins was analyzed by subjecting 150 µl of plasma to fast phase liquid chromatography gel filtration on two Superose 6 HR columns in tandem (20), as described previously (21). Each fraction (0.5 ml) was collected for cholesterol determination by colorimetric assay (Wako Chemicals, Richmond, VA).

Total lysophosphatidylcholine concentration in plasma was assayed enzymatically as described previously by Kishimoto et al. (22). Briefly, 8 µl of each plasma sample was preincubated for 5 min in 240 µl of reagent A (0.1 x Tris-HCl, pH 8.0, 0.01% Triton X-100, 1 mM CaCl_2, 3 mM N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylenediamine sodium dihydride; 10 kilozl/Liter peroxydase, 0.1 kilozl/liter glycophosphorylcholine phosphodiesterase, 1 kilozl/liter choline oxidase) in 96-well plates. The initial absorbance at 600 nm was recorded prior to starting the reaction with the addition of 80 µl of reagent B (0.1 M Tris-HCl, pH 8.0, 0.01% Triton X-100, 5 mM 4-aminoantipyrine, and 1 kilozl/liter phospholipase B). The incubation was continued for 30
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RESULTS

Human monocyte-derived macrophages but not mouse macrophages express low level of CEL, detectable by reverse transcriptase-PCR amplification of cellular RNA and trihydroxy bile salt-specific enhancement of cholesteryl ester hydrolytic activity in the conditioned media (10). The physiological role of the macrophage-derived CEL is unknown. Therefore, this study generated transgenic mouse lines with macrophage expression of a rat CEL cDNA to address this issue. The visna virus LTR, which has previously been shown to promote low levels of human apoE expression in transgenic mouse macrophages (13), was used to direct CEL expression in macrophages. The insertion of the rat CEL cDNA into the 5'-untranslated region located in exon 2 of the human apoE disrupts apoE coding and effectively replaces expression of the apoE gene with a chimeric gene that encodes the rat CEL protein (Fig. 1A). Polymerase chain termination reaction amplification of tail DNA identified CEL transgenic mice (Fig. 1B), and reverse transcriptase-PCR amplification of RNA obtained from various tissues of these transgenic animals showed CEL expression in brain, heart, kidney, lung, and peritoneal macrophages (Fig. 1C). The expression profile of the CEL transgene is consistent with visna virus LTR-directed transgenic expression reported previously (25).

Peritoneal macrophages isolated from CEL transgenic mice showed elevated levels of cholate-induced cholesteryl [3H]oleate hydrolytic activity in comparison with macrophages isolated from their nontransgenic littermates (Fig. 2A). When the assays were performed with deoxycholate instead of sodium cholate, only low levels of cholesteryl ester hydrolytic activity were detected in macrophages from either control or CEL transgenic mice (Fig. 2B). Importantly, the increase in cholesteryl ester hydrolytic activity in the CEL transgenic macrophages was not observed when the assays were performed with deoxycholate instead of cholate (Fig. 2). The specific induction of cholesteryl ester hydrolytic activity with the trihydroxy bile salt deoxycholate confirmed the expression of the pancreatic type bile salt-stimulated CEL in macrophages of the transgenic mice. A transgenic mouse line in which their peritoneal macrophages displayed similar levels of cholate-specific cholesteryl ester hydrolytic activity as that observed previously in human macrophages (10) was selected for expansion and additional experimentation.

Initial experiments to examine the impact of CEL expression on macrophage lipid metabolism compared cholesterol esterification activity between wild type and CEL transgenic mice. As expected, significantly higher levels of [3H]oleate incorporation into cholesteryl [3H]oleate was observed with both wild type and CEL transgenic mouse macrophages when the cells were incubated for 3 h with acLDL in comparison to cells incubated without added lipoproteins. However, no difference in cholesterol esterification activity was observed between wild type and CEL transgenic macrophages under these experimental conditions, with both types of cells displaying similar levels of cholesteryl [3H]oleate accumulation (Fig. 3A). In contrast, the level of cholesteryl [3H]oleate accumulated in CEL transgenic macrophages after 48 h incubation with acLDL was only marginally higher than that observed in macrophages from nontransgenic wild type littermates (Fig. 3D). Importantly, when the total amount of cholesteryl ester accumulation was measured, incubation with 200 μg/ml of acLDL for 48 h

FIGURE 1. Schematic diagram of the LTR-CEL chimeric gene construct. Panel A shows the approach used for construction of the LTR-CEL chimeric gene construct. A 2-kb SmaI CEL cDNA fragment was inserted into the Ball-digested LTR-apoE chimeric gene replacing exons 2–4 and their intervening introns of the apoE gene. The open box represents the LTR promoter, solid boxes and lines represent apoE exons and introns, respectively, and the hatched box represents CEL cDNA. Panel B shows PCR amplification of the transgene from tail DNA obtained from the transgenic mice (TG) with no signal from DNA obtained from wild type mice (WT). Panel C shows results of reverse transcriptase-PCR amplification using CEL transgene and GAPDH primers on RNA obtained from aorta (A), brain (B), heart (H), kidney (K), liver (L), lung (Lu), and peritoneal macrophages (M) of the CEL transgenic (CEL tg) mice, along with macrosphages from wild type mice (WT, M).

FIGURE 2. Cholesteryl ester hydrolytic activity in mouse macrophages. Peritoneal macrophages were obtained from wild type and CEL transgenic (CEL tg) mice and cultured in 6-well plates at a density of 2 × 10⁶ cells/well. Conditioned media from the macrophages were concentrated to 300 μl. Cholesteryl ester hydrolytic activity was measured from 140 μl of the concentrate based on the ability to convert cholesteryl [3H]oleate to [3H]oleate in the presence of 33 μm sodium cholate (panel A) or sodium deoxycholate (panel B). Note the difference in scales for cholesteryl [3H]oleate hydrolytic activity in the two graphs.

min at 37 °C and the final absorbance at 600 nm was recorded. Total LPC concentration in each sample was calculated by comparing the change in absorbance between the standard and samples after subtraction of the background absorbance recorded prior to initiation of the reaction. The accuracy of this method was confirmed by comparing concentrations determined by this enzymatic assay with high performance liquid chromatography analysis of the pooled sample.

Atherosclerosis Studies—Female apoE−/− mice with or without the CEL transgene were maintained on the Western-type high fat/cholesterol diet for 8 weeks and then sacrificed. The heart and aorta were perfused with phosphate-buffered saline and then formalin fixed with a 4% neutral formalin solution. Fat tissues were carefully trimmed. The upper heart section was incubated in phosphate-buffered saline containing 4% neutral formalin solution. Fat tissues were carefully trimmed. The upper heart section was incubated in phosphate-buffered saline containing 30% sucrose for 18 h, embedded in OCT medium, frozen, and sectioned with a cryostat at 10-μm intervals throughout the aortic sinus and the aortic arch as described (19, 23). The sections were stained with oil red O and lesions were quantified according to the procedure of Paigen et al. (24).
resulted in a 2.5-fold higher cholesteryl ester mass in the CEL transgenic macrophages compared with that observed in the wild type macrophages (Fig. 4).

The difference in cholesteryl ester accumulation between wild type and CEL transgenic macrophages was not related to uptake and degradation of acLDL because incubation with various concentrations of 125I-acLDL at 37 °C revealed no difference in the amount of 125I-acLDL internalized and degraded by macrophages isolated from wild type and CEL transgenic mice (data not shown). Additionally, the ACAT-specific inhibitor PD138142-0000 (Parke-Davis, Ann Arbor, MI), which has no effect on CEL activity in vitro (data not shown), was capable of inhibiting cholesteryl ester accumulation in both wild type and CEL transgenic macrophages in a concentration-dependent manner (Fig. 5). The latter observation indicated that ACAT was primarily responsible for cholesteryl esterification in both wild type and CEL transgenic macrophages, and CEL expressed in macrophages does not participate directly in intracellular cholesteryl esterification.

We also considered the possibility that CEL transgenic macrophages accumulated more cholesteryl esters than wild type cells in response to acLDL challenge because of their failure to hydrolyze cholesteryl esters associated with the incoming acLDL. To test this possibility, wild type and CEL transgenic macrophages were incubated with [3H]cholesterol-containing acLDL. The amounts of non-esterified and esterified [3H]cholesterol accumulated in the cells were determined after 30 min. Results showed the presence of higher levels of both non-esterified and esterified [3H]cholesterol in CEL transgenic macrophages than in wild type macrophages (Fig. 6). The increased levels of both free and esterified [3H]cholesterol in CEL transgenic macrophages, despite similar levels of acLDL uptake and degradation, suggested a more active futile cycle for cholesteryl esterification and de-esterification in macrophages expressing CEL. These observations also implied reduced cholesterol efflux capability in CEL transgenic macrophages. This possibility was examined directly by pre-loading the cells with [3H]cholesterol and then measured cholesterol efflux to apoA-I added to the incubation media. The results clearly demonstrated that significantly less [3H]cholesterol was transferred to the extracellular acceptor from CEL transgenic macrophages than from wild type cells (Fig. 7).

One mechanism by which CEL expression may influence ACAT-mediated cholesteryl esterification and apoA-I-mediated cholesterol efflux is via its ability to hydrolyze lysophospholipids. Previous studies have shown that LPC reduces the cholesterol level in the endoplasmic reticulum (26), thus reducing substrate availability for ACAT esterification. Accordingly, we compared LPC content in wild type and CEL transgenic macrophages after a 48-h incubation with acLDL. Results showed an approximate 40% reduction of LPC level in CEL transgenic macrophages compared with that observed in wild type macrophages (Fig. 8). These results are consistent with the interpretation that CEL expressed in macrophages is capable of LPC hydrolysis.

Carboxyl ester lipase is also a lipoamidase, capable of hydrolyzing ceramides even under low or no bile salt conditions (3, 4). In view of previous reports showing that intracellular sphingolipid content can influence cholesterol esterification activity and cholesteryl ester accumulation (27), particularly ceramide inhibition of ACAT activity and promotion of cholesterol efflux has been noted in cultured cells (28, 29), we ascertained the possibility that increased cholesteryl ester accumulation in the macrophages of CEL transgenic mice may be related to
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FIGURE 6. Hydrolysis and accumulation of acLDL-associated [3H]cholesteryl oleate. Peritoneal macrophages obtained from wild-type and CEL transgenic (CEL tg) mice were incubated with 7 μg/ml [3H]cholesteryl oleate-containing acLDL for 30 min at 37 °C. Intracellular lipids were extracted for thin layer chromatography separation in petroleum ether:ethyl ether:acetic acid (300:60:1, v/v/v) solvent. The bands corresponding to free cholesterol (A) and cholesteryl esters (B) were scraped and quantified by liquid scintillation counting.

FIGURE 7. Cholesterol efflux from mouse peritoneal macrophages. Peritoneal macrophages isolated from wild type (closed symbols) and CEL transgenic (open symbols) mice were incubated overnight at 37 °C with 200 μg/ml [3H]cholesteryl oleate-containing acLDL followed by a 6-h equilibration period with serum-free media. Cholesterol efflux was initiated by the addition of apoA-I at the concentrations as indicated. The amount of [3H]cholesterol excreted to the acceptor apoA-I in the incubation media was quantified by liquid scintillation counting. The results represent the mean ± S.D. from triplicate determinations. * indicates significant difference from the wild type group at p < 0.05.

FIGURE 8. Lyso-PC content in mouse macrophages. Peritoneal macrophages obtained from wild type and CEL transgenic mice were incubated with 200 μg/ml acLDL for 48 h at 37 °C. Lyso-PC concentration was determined by the enzymatic method. * denotes difference at p < 0.01 (n = 4).

FIGURE 9. Intracellular ceramide content in mouse macrophages. Peritoneal macrophages obtained from wild type and CEL transgenic (CEL tg) mice and incubated with 200 μg/ml acLDL for 48 h at 37 °C. Intracellular ceramide content was determined from cell lysates based on the amount of [32P]ATP incorporated into ceramide in the presence of diacylglycerol kinase. * denotes difference at p < 0.05 (n = 4).

alteration in ceramide concentrations in the cells. Accordingly, extracts were prepared from wild type and CEL transgenic macrophages after their incubation with 200 μg/ml acLDL for 48 h at 37 °C. Results revealed that acLDL-induced ceramide accumulation was ~2-fold lower in the CEL transgenic macrophages compared with that in wild type macrophages (Fig. 9).

The results showing increased cholesteryl ester accumulation in CEL transgenic macrophages compared with cells from nontransgenic wild type mice suggested that macrophage expression of CEL may promote atherosclerosis. To test this hypothesis, we cross-bred the CEL transgenic mice with the atherosclerosis-susceptible apoE−/− mice. The progenies were fed a Western-type high fat/cholesterol diet (no cholate added) to facilitate atherogenesis (19). Although apoE−/− mice with or without macrophage CEL transgenic expression displayed similar plasma cholesterol level and lipoprotein profile (Fig. 10), plasma LPC concentration was 3-fold lower in apoE−/− mice expressing the CEL transgene than in the nontransgenic apoE−/− mice (Fig. 11). Importantly, a significant increase of atherosclerosis lesions was observed in apoE−/− mice with CEL expression in macrophages compared with apoE−/− mice without the CEL transgene (Fig. 12).

DISCUSSION

Carboxyl ester lipase is an avid cholesteryl ester hydrolase. One of its functions in the gastrointestinal tract is to catalyze cholesteryl ester hydrolysis to facilitate its absorption (7, 30). This enzyme is also synthesized in human macrophages but not in mouse macrophages (10). The current study used macrophages from CEL transgenic mice to show that CEL expression in macrophages actually increased instead of decreased acLDL-induced cholesteryl ester accumulation, implying that the role of CEL in macrophages is probably not related to its cholesteryl ester hydrolytic activity. This hypothesis is consistent with previous reports showing that CEL cDNA did not promote intracellular cholesteryl ester hydrolysis when transfected into hepatoma cells (31). The inability of the CEL transgene to decrease cholesteryl ester accumulation in macrophages is most likely because of the low level or absence of trihydroxy bile salts in the cytosolic compartment where cholesteryl ester droplets accumulate in macrophages. Thus, in this environment, CEL cannot function as a cholesteryl ester hydrolase (1, 2, 5, 6) and the cells continue to accumulate cholesteryl esters in response to acLDL.

The data of the current study revealed that macrophages expressing the CEL transgene actually displayed increased cholesterol esterifica-

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Results of the current study also revealed a pathophysiological role of CEL in macrophages. Its reduction of ceramide and LPC levels in acLDL-treated macrophages may also be related to its ability to hydrolyze LPC, thereby decreasing its inhibition of cholesterol distribution to the endoplasmic reticulum (26) and promoting cholesterol efflux.

It is interesting to note that the difference between wild type and CEL transgenic macrophages in cholesteryl ester accumulation was less obvious when the assays were performed by measuring [3H]oleate incorporation into cholesteryl [3H]olate in the presence of acLDL. However, the total amount of acLDL-induced cholesteryl ester accumulation remained notably different between wild type and CEL transgenic macrophages. These latter observations indicated that under cholesterol-enriched conditions, CEL may facilitate overall intracellular lipid turnover by supplying increasing amounts of endogenously derived fatty acids to compete with the exogenously supplied [3H]oleate as substrates for cholesterol esterification. This interpretation is consistent with previous experiments demonstrating that CEL cDNA transfection into Chinese hamster ovary cells increased intracellular phospholipid turnover rates (34). In view of previous reports documenting a direct effect of ceramide in inhibiting phospholipid biosynthesis (35–37), the mechanism by which CEL increases phospholipid turnover may also be related to the reduction of steady state levels of ceramide in acLDL-treated macrophages. Taken together, these observations suggested that CEL may function as a lipoamidase and a lysophospholipase to promote intracellular lipid trafficking between different membrane compartments.

Results of the current study also revealed a pathophysiological role of CEL in macrophages. Its reduction of ceramide and LPC levels in macrophages that led to increased cholesteryl ester accumulation in response to atherogenic lipoproteins also resulted in increased athero-

comparison to macrophages lacking CEL expression. These observations suggested that CEL participates either directly as a cholesterol esterification enzyme in macrophages or indirectly via substrate delivery to an ACAT-sensitive pool. The possibility that CEL may serve as a cholesterol esterification enzyme was suggested previously from in vitro studies demonstrating that the incubation of CEL with cholesterol, cholesteryl esters, and fatty acids favors the de-esterification reaction in the presence of trihydroxy bile salt, but the reaction equilibrium favors the cholesterol esterification direction in the absence of trihydroxy bile salt (32). However, in the current study, we showed that cholesterol accumulation in CEL transgenic macrophages was inhibited by specific ACAT inhibitors. Thus, the increased cholesteryl ester accumulation in these cells is unlikely because of the cholesterol esterification activity of CEL and probably an indirect effect because of other enzymatic activities of CEL that may influence cholesterol delivery to the ACAT-sensitive pool.

Previous studies have shown that preincubation of macrophages with ceramide decreased whole cell cholesterol esterification activity by −50% (27). The mechanism appears to be related to ceramide induction of endogenous sphingomyelin synthesis (27) and/or ceramide trapping of cholesterol in endosomal compartments (33), both of which would affect intracellular cholesterol trafficking and limit cholesterol access-

FIGURE 10. Cholesterol distribution among plasma lipoproteins in mice. Plasma was obtained from apoE−/− (apoE null) or apoE−/− mice with macrophage-specific CEL transgene (open triangles). An aliquot (150 µl) of the plasma from each animal was applied to two Superose 6 FPLC columns connected in tandem. Fractions of 0.5 ml were collected and used for enzymatic measurement of total cholesterol concentrations. A representative profile from one apoE−/− mouse and one apoE−/− CEL-tg mouse was shown. No difference in profile was observed with 3 different animals from each group.

FIGURE 11. Plasma lysophosphatidylcholine concentrations in mice. Plasma was obtained from apoE−/− (apoE null) or apoE−/− mice with the CEL transgene (apoE null; CEL tg). Total lysophosphatidylcholine concentration in plasma was measured enzymatically. * denotes significance difference from the group without the CEL transgene at p < 0.05 (n = 4).

FIGURE 12. Atherosclerotic lesions in mice. Female apoE−/− (apoE null) mice and apoE−/− mice with the CEL transgene (apoE null; CEL tg) were fed a Western-type high fat/cholesterol diet for 8 weeks. Perfused heart and aorta were sectioned at 10-µm intervals throughout the aortic sinus and the aortic arch. Atherosclerotic lesions were identified based on areas stained positively with oil red O. Representative histological staining of aortic sections from apoE−/− mice with or without the CEL transgene are shown. Lesion areas were quantified from 10 sections obtained from 4 mice in each group. * denotes significant difference from the nontransgenic group at p < 0.001 (n = 4).
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sclerosis lesion size in vivo. Interestingly, macrophage expression of CEL in vivo also resulted in decreased plasma LPC level in apoE−/− mice. This decrease can be attributed to a direct effect of CEL on hydrolysis of LPC in atherogenic lipoproteins (9). However, despite the decrease of this pro-atherogenic constituent (38), atherosclerosis was exacerbated in the CEL transgenic mice compared with their nontransgenic apoE−/− littermates (Fig. 10). These observations implied that the increased LPC level is not a prerequisite of atherogenesis and the increased atherosclerosis observed in apoE−/− mice compared with their wild type counterparts is not because of the elevated levels of LPC in the apoE−/− mice. In fact, results of the current study suggest that LPC may be anti-atherogenic under selected circumstances, such as in hypercholesterolemia where the cholesterol is esterified in macrophages to induce foam cells. Reports showing LPC promotion of cholesterol efflux from macrophage foam cells (39) and the ability of lipoprotein-associated phospholipase A2 to reduce LDL degradation and foam cell formation in vitro (40) are supportive of this hypothesis.

In summary, our data documented that macrophage expression of CEL is pro-atherogenic, favoring cholesteryl ester accumulation and foam cell formation to increase atherosclerosis lesions in mice. The mechanism by which CEL expression in macrophages promotes atherosclerosis is likely because of its ability to promote intracellular cholesterol esterification by reducing ceramide and LPC levels in cholesterol-loaded cells and lowering LPC levels in the circulation. These results, along with previous in vitro studies demonstrating CEL promotion of smooth muscle cell proliferation (12), offer a new therapeutic target to reduce the severity of vascular occlusive disease.

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REFERENCES

1. Wang, C. S., and Hartsuck, J. A. (1993) Biochim. Biophys. Acta 1166, 1–19
2. Hui, D. Y. (1996) Biochim. Biophys. Acta 1303, 1–14
3. Hui, D. Y., Hayakawa, K., and Ozumi, J. (1993) Biochem. J. 291, 65–69
4. Nyberg, L., Farooqi, A., Blackberg, L., Duan, R. D., Nilsson, A., and Hernell, O. (1998) J. Ped. Gastroenterol. Nutr. 27, 560–567
5. Rudd, E. A., and Brockman, H. L. (1984) in Lipases (Borgstrom, B., and Brockman, H. L., eds) pp. 185–204, Elsevier Science Publishers, New York
6. Jacobson, P. W., Wiesenfeld, P. W., Gallo, L. L., Tate, R. L., and Osborne, J. C. (1990) J. Biol. Chem. 265, 515–521
7. Howles, P. N., Carter, C. P., and Hui, D. Y. (1996) J. Biol. Chem. 271, 7196–7202
8. Kirby, R. J., Zheng, S., Tso, P., Howles, P. N., and Hui, D. Y. (2002) J. Biol. Chem. 277, 4104–4109
9. Shamir, R., Johnson, E. J., Morlock-Fitzpatrick, K., Zolfaghari, R., Li, L., Mas, E., Lambardo, D., Morel, D. W., and Fisher, E. A. (1996) J. Clin. Investig. 97, 1696–1704
10. Li, F., and Hui, D. Y. (1997) J. Biol. Chem. 272, 28666–28671
11. Li, F., and Hui, D. Y. (1998) Biochem. J. 329, 675–679
12. Auge, N., Rebar, O., Lepetit-Thevenin, J., Bruneau, N., Thiers, J.-C., Mas, E., Lambardo, D., Negre-Salvayre, A., and Verine, A. (2003) Circulation 108, 86–91
13. Bellosta, S., Mahley, R. W., Sanan, D. A., Murata, J., Newland, D. L., Taylor, J. M., and Pitas, R. E. (1995) J. Clin. Investig. 96, 2170–2179
14. DiPersio, L. P., Fontaine, R. N., and Hui, D. Y. (1990) J. Biol. Chem. 265, 16801–16806
15. Camulli, A. E., Linke, M. J., Brockman, H. L., and Hui, D. Y. (1989) Biochim. Biophys. Acta 1005, 177–182
16. Innerarity, T. L., Pitas, R. E., and Mahley, R. W. (1986) Methods Enzymol. 129, 542–566
17. Goldstein, J. L., Ho, Y. K., Basu, S. K., and Brown, M. S. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 333–337
18. Schneider, E., and Kennedy, E. (1973) J. Biol. Chem. 248, 3739–3748
19. Plump, A. S., Smith, J. D., Hayek, T., Aalto-Setala, K., Walsh, A., Verstuyft, J. G., Rubin, E. M., and Breslow, J. L. (1992) Cell 71, 343–353
20. delSilva, H. V., Mas-Oliva, J., Taylor, J. M., and Mahley, R. W. (1994) J. Lipid Res. 35, 1297–1310
21. Du, H., Heur, M., Duannu, M., Grabowski, G. A., Hui, D. Y., Witte, D. P., and Misra, J. (2001) J. Lipid Res. 42, 489–500
22. Kishimoto, T., Soda, Y., Matsuyama, Y., and Mizuno, K. (2002) Clin. Biochem. 35, 411–416
23. Schreier, S. A., Lystig, T. C., Vick, C. M., and Leboeuf, R. C. (2003) Atherosclerosis 171, 49–55
24. Paigen, R., Morrow, A., Holmes, P. A., Mitchell, D., and Williams, R. A. (1987) Atherosclerosis 68, 231–240
25. Hopkins, P. C. R., Huang, Y., McGuire, J. G., and Pitas, R. E. (2002) J. Lipid Res. 43, 1881–1889
26. Lange, Y., Ye, J., and Steck, T. L. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 11664–11667
27. Okebu, A. K., Xu, X.-X., Shiratori, Y., and Tabas, I. (1994) J. Lipid Res. 35, 644–655
28. Ridgway, N. D. (1995) Biochim. Biophys. Acta 1256, 39–46
29. Witting, S. R., Maiorano, J. N., and Davidson, W. S. (2003) J. Biol. Chem. 278, 40121–40127
30. Weng, W., Li, L., van Beersum, A. M., Potter, S. H., Harrison, E. H., Blaner, W. S., Breslow, J. L., and Fisher, E. A. (1999) Biochemistry 38, 4143–4149
31. Zolfaghari, R., Glick, J. M., and Fisher, E. A. (1993) J. Biol. Chem. 268, 13532–13538
32. Kyger, E. M., Riley, D. J. S., Spilburg, C. A., and Lange, L. G. (1999) Biochemistry 28, 3853–3858
33. Puri, V., Jefferson, R. J., Singh, R. D., Wheatley, C. L., Marks, D. L., and Pagano, R. E. (2003) J. Biol. Chem. 278, 20961–20970
34. Le Petit-Thevenin, J., Bruneau, N., Nobili, O., Lombardo, D., and Verine, A. (1998) Biochim. Biophys. Acta 1393, 307–316
35. Allan, D. (2000) Biochem. J. 345, 603–610
36. Ramos, B., Mouedden, M. E., Claro, E., and Jackowski, S. (2002) Mol. Pharmacol. 62, 1068–1075
37. Bodenmee, J., Pelled, D., Riebling, C., Trajkovic, S., and Furter, A. H. (2002) FASEB J. 16, 1814–1816
38. Quinn, M. T., Parthasarathy, S., and Steinberg, D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2805–2809
39. Hara, S., Shike, T., Takasu, N., and Mizui, T. (1997) Arterioscler. Thromb. Vasc. Biol. 17, 1258–1266
40. Turunen, P., Jalkanen, J., Heikura, T., Puhakka, H., Karppi, J., Nyyssonen, K., and Yla-Herttuala, S. (2004) J. Lipid Res. 45, 1633–1639