Modified Low Density Lipoprotein Enhances the Secretion of Bile Salt-stimulated Cholesterol Esterase by Human Monocyte-Macrophages

SPECIES-SPECIFIC DIFFERENCE IN MACROPHAGE CHOLESTERYL ESTER HYDROLASE*

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Reverse transcriptase-polymerase chain reaction was used to study the biosynthesis of two different cholesteryl ester hydrolases by human and mouse macrophages. Oligonucleotide primers for bile salt-stimulated cholesterol esterase yielded positive reactions with RNA isolated from human peripheral blood monocytes, monocyte-derived macrophages, the human monocyte THP-1 cells, and phorbol ester-induced THP-1 macrophages. In contrast, oligonucleotide primers for hormone-sensitive lipase yielded positive reactions only with RNA isolated from non-differentiated human THP-1 monocytes and peripheral blood monocytes, but not those obtained from differentiated THP-1 macrophages or monocyte-derived macrophages. Thus, while human monocytes were capable of synthesizing both enzymes, human macrophages synthesized only bile salt-stimulated cholesterol esterase and not the hormone-sensitive lipase. The synthesis of bile salt-stimulated cholesterol esterase by human macrophages was confirmed by detection of bile salt-stimulated cholesteryl ester hydrolytic activity in conditioned media of differentiated THP-1 cells and human peripheral blood monocyte-derived macrophages. Moreover, incubating human macrophages with oxidized low density lipoprotein (LDL) or acetylated LDL increased bile salt-stimulated cholesterol esterase activity in the conditioned media of these cells. These results with human macrophages were contrasted with results of studies with mouse macrophages, which showed the presence of hormone-sensitive lipase mRNA but not the bile salt-stimulated cholesterol esterase mRNA. Taken together, these results demonstrated species-specific differences in expression of cholesteryl ester hydrolytic enzymes in macrophages. The expression of bile salt-stimulated cholesterol esterase by human macrophages, in a process inducible by modified LDL, suggests a role of this protein in atherogenesis.

A major characteristic of atherosclerosis is the accumulation of lipid-laden foam cells in the arterial wall. The foam cells are derived mainly from macrophages that have endocytosed modified lipoproteins through the scavenger receptor-mediated pathways (1). The cytoplasmic lipid droplets are mostly cholesteryl esters, which exist in a dynamic equilibrium with unesterified cholesterol and undergo continuous hydrolysis and re-esterification (1). In the presence of exogenous cholesterol acceptors such as high density lipoproteins, the equilibrium favors cholesteryl ester hydrolysis with a net efflux of cholesterol from cells. In such circumstances, very little amount of cholesterol is stored in the macrophages. However, in the absence of extracellular cholesterol acceptors, or when acceptor concentration is low, equilibrium of the cholesteryl ester cycle favors esterification and cholesteryl esters accumulate in the cytosol.

Cholesteryl ester can also accumulate in macrophages due to different levels of the esterification and de-esterification enzymes. Macrophages with high neutral cholesteryl ester hydrolytic activity were shown to accumulate less cholesteryl ester in response to β-migrating very low density lipoproteins in comparison with macrophages with low cholesteryl ester hydrolytic activities (2). This difference in intracellular cholesteryl ester metabolism has been suggested to play a role in dictating susceptibility to atherosclerosis (3). Moreover, increased intracellular accumulation of free cholesterol at the expense of cholesteryl ester and in the absence of adequate supply of cholesterol acceptor may also lead to cell necrosis (4). The lipid-rich debris of foam cell necrosis contributes directly to the lipid core of advanced atheroma (5).

In addition to the neutral lipids derived from necrosis of macrophage foam cells, direct arterial deposition of LDL1 also contributes to the lipid core of the atherosclerotic plaque (6, 7). However, cholesterol in LDL, as well as cholesterol stored as lipid droplets in macrophage foam cells, are mainly esterified cholesterol, whereas lipids in advanced atherosclerosis are mostly unesterified cholesterol (8, 9). Therefore, it is likely that the cholesteryl esters are converted to free cholesterol in the extracellular matrix before cholesterol monohydrates crystallize in the atherosclerotic plaque.

Despite the obvious significance of cholesteryl ester hydrolytic enzymes in atherogenesis, the identity of the cholesteryl ester hydrolyase in macrophages and in the extracellular matrix remains obscure. Earlier studies demonstrated the activation of cholesteryl ester hydrolysis in adenosine 3′,5′-cyclic monophosphate-stimulated murine macrophages (10). These results led to the suggestion that the enzyme responsible for cholesteryl ester hydrolysis in macrophages is similar to the hor-

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† The abbreviations used are: LDL, low density lipoprotein(s); RT, reverse transcriptase; PCR, polymerase chain reaction; PMA, phorbol 12-myristate 13-acetate; bp, base pair(s); HSL, hormone-sensitive lipase.

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Cholesterol Esterase Expression in Human Macrophages

TABLE I

| Identification                                      | Sequence             | Ref      |
|----------------------------------------------------|----------------------|----------|
| Human cholesterol esterase (exons 2 and 7)          | 5'-AGCACCATACGGGTATGAAGA | 24, 25   |
|                                                    | 3'-AGGTATCGCCCGCGTACC  |          |
| Human cholesterol esterase (exons 8 and 11)         | 5'-CAGCAGCAGGGCAGAAG  | 24       |
|                                                    | 3'-TGAGCAGGCTCCTGAGAAC | 1606–1624|
| Mouse cholesterol esterase                          | 5'-GGGACGCTGAGCCTACA  | 216–233  |
|                                                    | 3'-ATGGCTACGCTGGTGGT  | 577–593  |
| Human hormone-sensitive lipase                     | 5'-CCTACCTGTGCTGCTGCCAC | 969–888  |
|                                                    | 3'-CCAGAAGATGTCGGAGCACA | 2245–2264|
| Mouse hormone-sensitive lipase                      | 5'-GCCTGTGACAGAGACAC  | 1515–1532|
|                                                    | 3'-CGCGTCGCGCTGCTTTC  | 1906–1923|

Monocyte-senstive lipase (HSL) found in steroidogenic tissues (11). In support of this hypothesis, HSL mRNA was found to be present in mouse macrophages (12). However, HSL mRNA could not be demonstrated in human macrophages, even with the sensitive reverse transcription-polymerase chain reaction amplification technique (13). These results suggest that another cholesterol ester hydrolase may be responsible for cholesteryl ester metabolism in human macrophages and arterial wall. An enzyme capable of cholesteryl ester hydrolysis is the bile salt-stimulated cholesterol esterase (14). Although this protein is synthesized predominantly in the pancreas and lactating mammary glands in a form that is secreted from the cells (14), a significant amount of intracellular enzyme was also detectable in cells expressing the bile salt-stimulated cholesterol esterase mRNA (15). In view of the recent observations that the pancreatic type of cholesterol esterase (the bile salt-stimulated lipase/cholesterol esterase) is present in human serum (16), and that its serum activity may participate in lipoprotein remodeling (16, 17), this study was undertaken to determine if the bile salt-stimulated cholesterol esterase is expressed in macrophages.

EXPERIMENTAL PROCEDURES

Materials — The human monocyte cell line THP-1, the HT29 intestinal cells, and the mouse macrophage line J774 were purchased from American Type Culture Collection (Rockville, MD). Tissue culture media and reagents were obtained from Fisher Chemical Co. (Cincinnati, OH). Fetal bovine serum was purchased from Hyclone Laboratories (Logan, UT). The C57/B1/6N and C3H/HeN mice were purchased from Harlan Bioproducts (Indianapolis, IN). Reverse transcriptase and Taq DNA polymerase were purchased from Life Technologies, Inc. The cholesteryl [14C]oleate was obtained from NEN Life Science Products. RNA-Stat-60 was obtained from Tel-Test “B,” Inc. (Friendswood, TX). Phorbol 12-myristate 13-acetate (PMA) was obtained from Sigma. Ultrafree-4 centrifugal filter devices were purchased from Millipore (Bedford, MA). Other high quality reagents and chemicals were obtained from either Fisher or Sigma.

Lipoprotein Preparation — Human LDL, d = 1.02–1.05 g/ml, was isolated by centrifugation for 18 h at 59,000 rpm in a Beckman 60 Ti rotor, washed by recentrifugation at d = 1.05 g/ml, and dialyzed against 150 mM NaCl and 1 mM EDTA before storing at 4 °C until use (18). Oxidized LDL was prepared by dialysis of the LDL stock solution against phosphate-buffered saline without EDTA, followed by incubation with 10 μM copper sulfate for 18 h at 37 °C (19). Acetylation of LDL was carried out by sequential addition of acetic anhydride (20). The modified lipoproteins were dialyzed against 150 mM NaCl, 1 mM EDTA and characterized by agarose gel electrophoresis before use.

Cell Culture — Stock cultures of THP-1 cells were grown in RPMI 1640 medium supplemented with 2 mM glutamine, 50 mM Hepes, 5 mM 2-mercaptoethanol, and 10% fetal bovine serum. The HepG2 and HT29 cells were maintained in RPMI 1640 with 2 mM glutamine and 10% fetal bovine serum. Differentiation of the THP-1 monocytic cells to macrophages was induced by plating 5 × 10⁶ cells in 100-mm culture dishes with RPMI 1640 medium containing 1 × 10⁻⁹ M PMA. After a 24-h incubation period, nonadherent cells were removed by washing with phosphate-buffered saline. For experiments, the THP-1 cells were incubated with RPMI 1640, in the presence or absence of 100 μM oxidized LDL, acetylated LDL, or normal human LDL.

Human monocytes were isolated from the peripheral blood of healthy donors using the Ficoll-Hypaque gradient method (21). The separated mononuclear cells were washed three times with phosphate-buffered saline and then plated in 75-cm² flasks at a density of 1 × 10⁶ cells/dish. Nonadherent cells were removed after 3 h of incubation, and fresh RPMI 1640 medium with 10% serum was added to the adhering cell culture. Medium was changed every 3 days, and the cells were used as monocyte-derived macrophages after 9 days in culture. Phenotypic characterization of the cells revealed greater than 90% of the cells were macrophages.

Mouse resident macrophages were harvested by lavage of peritoneal cavity of C57BL/6N mice or C3H/HeN mice with phosphate-buffered saline. The mouse peritoneal macrophages were cultured in 100-mm Petri dishes at a density of 1 × 10⁶ cells/plate with RPMI 1640 medium containing 10% fetal bovine serum. After overnight incubation, nonadherent cells were removed by washing with phosphate-buffered saline.

Reverse Transcriptase-Polymerase Chain Reaction Amplification of RNA — Total cellular RNA was prepared using the single-step guanidinium thiocyanate-phenol-chloroform extraction method with RNA-stat 60 (22). Mouse pancreatic RNA was isolated using the same procedure as described previously for isolation of rat pancreatic RNA (23). One μg of the RNA was used as template for cDNA synthesis by reverse transcriptase with oligo(dT) primers. One-tenth of the resulting cDNA in 1 μl was utilized for PCR amplification. The oligonucleotide primers used for PCR amplification were synthesized on an Applied Biosystems DNA synthesizer based on sequences derived from human pancreatic cholesterol esterase (24, 25), human HSL (26), mouse cholesterol esterase (27), and mouse HSL (28). The exact sequences of these primers are shown in Table 1. Amplification reactions for human cholesterol esterase were carried out for 35 cycles with denaturation at 95 °C for 30 s, annealing at 60 °C for 50 s, and extension at 72 °C for 90 s. The extension time was increased to 10 min for the final cycle. The RT-PCR amplification with human hormone-sensitive lipase primers was carried out using an identical procedure, except the annealing reaction was performed at 62.5 °C. Amplification of mouse cholesterol esterase mRNA was carried out for 35 cycles with denaturation at 95 °C for 30 s, annealing at 55 °C for 50 s, and extension at 72 °C for 90 s. Increasing to 10 min of extension time for the final cycle. The PCR amplification for mouse HSL mRNA was performed exactly as described (22).

Cholesterol Esterase Assay — Conditioned media from human monocytes and macrophages incubated for 1 day with or without lipopolysaccharide were collected and concentrated to 1 ml by centrifugation through an Ultrafree-4 centrifugal filter. An aliquot of each sample (usually 140 μl) was used to measure cholesterol esterase activity, based on the hydrolysis of cholesteryl [14C]oleate in the presence of 33 μM cholate, as described previously (28).

RESULTS

Expression of Cholesterol Ester Hydrolytic Enzymes in Human Monocyte-Macrophages — The RNA isolated from THP-1 cells, PMA-treated THP-1, peripheral blood monocytes, and monocyte-derived macrophages were used for RT-PCR to study cholesterol esterase gene expression in human monocytes and macrophages. Since previous studies by other investigators have reported the presence of a cholesterol esterase-like pseudogene in the human genome that can be transcribed into mRNA (29, 30), and that the major difference between the
authentic cholesterol esterase gene and the pseudogene is the deletion of exon 2 through exon 7 in the latter sequence, PCR amplification was performed initially with oligonucleotide primers corresponding to sequences in exon 2 and exon 7 of the cholesterol esterase gene. An RT-PCR product of 579 bp was consistently observed with RNA isolated from non-differentiating THP-1 cells, PMA-induced THP-1 macrophages, human peripheral blood monocytes, monocyte-derived macrophages, and HepG2 cells (Fig. 1A). The identity of the RT-PCR product as cholesterol esterase cDNA was further confirmed by hybridization of the amplified DNA with a cholesterol esterase cDNA probe corresponding to nucleotides 270–681 of human cholesterol esterase mRNA (Fig. 1B). Moreover, a 555-bp RT-PCR product was also observed with oligonucleotide primers corresponding to exons 8 and 11 of the human cholesterol esterase gene (data not shown). This 555-bp amplification product also hybridized to a cholesterol esterase cDNA probe corresponding to nucleotides 681–1400 of the cholesterol esterase mRNA (data not shown). However, no RT-PCR product was observed when the reaction was performed in the absence of cellular RNA (Fig. 1A). The observation of similar RT-PCR products with RNA from human monocyte-derived macrophages and HepG2, a human hepatoma cell line that has been shown previously to synthesize the bile salt-stimulated cholesterol esterase (31), suggested that human monocyte-macrophages are capable of synthesizing a cholesterol esterase that is similar to the enzyme in pancreas.

The pancreatic type cholesterol esterase expressed in pancreas, liver, and lactating mammary glands is a secretory protein (32). Therefore, additional experiments were performed to determine if its synthesis in human macrophages also resulted in its secretion from the cells. Conditioned media from PMA-stimulated THP-1 cells and monocyte-derived macrophages were isolated for cholesterol esterase assay. The results showed the conditioned medium displayed significant bile salt-dependent cholesteryl-[14C]oleate hydrolysis (Fig. 2). Cholesteryl [3H]oleate hydrolysis was not observed when the enzyme assay was performed with deoxycholate instead of cholate or in the absence of bile salt (data not shown). Cholesteryl ester hydrolytic activity in the conditioned medium was also reduced by approximately 70–75% when the assay was performed after immunoprecipitation with antibodies against rat pancreatic cholesterol esterase (33). The latter observations provided additional support to indicate that the cholesterol esterase secreted by human macrophages is similar, if not identical, to the pancreatic cholesterol esterase (14). Interestingly, bile salt-stimulated cholesterol esterase activity in the conditioned medium was increased 9–12-fold after incubating the monocyte-derived macrophages and PMA-treated THP-1 cells with oxidized LDL, compared with cells incubated without lipoproteins (Fig. 2). Acetylated LDL increased bile salt-stimulated cholesterol esterase secretion by these cells by approximately

FIG. 1. Detection of cholesterol esterase mRNA in human monocytes and macrophages with exons 2 and 7 primers. RNA from THP-1 cells and monocyte/macrophage from human peripheral blood and HepG2 cells were reverse transcribed and amplified by PCR with primers specific for human cholesterol esterase. The reaction products were subjected to 1% agarose gel electrophoresis. The gel was stained with ethidium bromide to identify the reaction products (A) and transferred to nitrocellulose for hybridization with a human cholesterol esterase cDNA probe (B). The expected 579-bp product was identified with RNA isolated from THP-1 (lane 1), PMA-induced THP-1 macrophages, human peripheral blood monocytes, monocyte-derived macrophages, and HepG2 cells (Fig. 1A). The identity of the RT-PCR product as cholesterol esterase cDNA was further confirmed by hybridization of the amplified DNA with a cholesterol esterase cDNA probe corresponding to nucleotides 270–681 of human cholesterol esterase mRNA (Fig. 1B). Moreover, a 555-bp RT-PCR product was also observed with oligonucleotide primers corresponding to exons 8 and 11 of the human cholesterol esterase gene (data not shown). This 555-bp amplification product also hybridized to a cholesterol esterase cDNA probe corresponding to nucleotides 681–1400 of the cholesterol esterase mRNA (data not shown). However, no RT-PCR product was observed when the reaction was performed in the absence of cellular RNA (Fig. 1A). The observation of similar RT-PCR products with RNA from human monocyte-derived macrophages and HepG2, a human hepatoma cell line that has been shown previously to synthesize the bile salt-stimulated cholesterol esterase (31), suggested that human monocyte-macrophages are capable of synthesizing a cholesterol esterase that is similar to the enzyme in pancreas.

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FIG. 2. Effect of modified LDL on cholesterol esterase secretion by PMA-treated THP-1 cells (A) and human peripheral blood monocyte-derived macrophages (B). The macrophages were incubated with the indicated lipoproteins at a concentration of 100 μg/ml. The conditioned medium was removed for determination of cholesteryl-[14C]oleate hydrolytic activity in the presence of cholate. Results represent average of triplicate experiments ± S.E.
The RNA isolated from THP-1 cells, human peripheral blood monocytes, and macrophages were also used for RT-PCR with primers for HSL cDNA. A primer set that overlaps exons 1–6 of the HSL gene amplified a reaction product of 1296 bp using RNA isolated from non-differentiating THP-1 monocytes, human peripheral blood monocytes, and the human colon adenocarcinoma cell line HT29 (Fig. 3). The HT29 cells have been shown previously to display HSL mRNA (34). In contrast, no signal was observed when amplification was performed with RNA isolated from PMA-activated THP-1 cells or from monocyte-derived macrophages (Fig. 3). The latter result was consistent with results reported by Contreras and Lasunci´on (13) showing the absence of HSL mRNA in human monocyte-derived macrophages.

Expression of Cholesterol Ester Hydrolytic Enzymes in Mouse Macrophages—Hormone-sensitive lipase has been reported to be present in mouse macrophages (11–13). However, the potential for expression of the pancreatic type cholesterol esterase in mouse macrophages has not been explored. Therefore, RNA was isolated from mouse peritoneal macrophages for RT-PCR amplification with cholesterol esterase or hormone-sensitive lipase primers and was reverse transcribed and amplified by PCR with primers specific for human hormone-sensitive lipase. The reaction products were analyzed by ethidium bromide staining after electrophoresis in 1% agarose gel. The lane on the left shows the 1-kilobase pair molecular size markers.

FIG. 3. Detection of hormone-sensitive lipase mRNA in human monocyte-macrophages. The RNA isolated from THP-1 cells (lane 1), PMA-induced THP-1 cells (lane 2), human peripheral blood monocytes (lane 3), human monocyte-derived macrophages (lane 4), and HT29 cells (lane 5) were reverse transcribed and amplified by PCR with primers specific for human hormone-sensitive lipase. The reaction products were analyzed by ethidium bromide staining after electrophoresis in 1% agarose gel. The lane on the left shows the 1-kilobase pair molecular size markers.

10- and 6.5-fold, respectively (Fig. 2, A and B). Normal LDL has no effect on bile salt-stimulated cholesterol esterase synthesis and secretion by PMA-treated THP-1 cells (Fig. 2A), but increased cholesterol esterase synthesis and secretion by monocyte-derived macrophages approximately 4-fold (Fig. 2B). The latter observation may be related to the ability of the macrophage to catalyze LDL oxidation or due to differences between THP-1 cells and normal human macrophages.

The results presented in this report revealed striking differences between human and mouse macrophages in synthesis of cholesterol ester hydrolytic enzymes. Consistent with results reported by other investigators, mouse peritoneal macrophages, but not human macrophages, express HSL (12, 13). The inability to detect HSL mRNA in human macrophages was not due to the assay conditions used nor the primers selected for RT-PCR. Using the identical procedure, HSL mRNA was detectable in non-differentiating THP-1 cells and in peripheral blood monocytes. Although the mechanism and the rationale for down-regulation of HSL expression after monocyte-to-macrophage differentiation still remain unknown, these results suggested the presence of another enzyme(s) in human macrophages responsible for hydrolysis of the stored cholesteryl esters in these cells.

One cholesterol ester hydrolytic enzyme synthesized by human macrophages is identical to the bile salt-stimulated cholesterol esterase, previously thought to be synthesized only in pancreas and in lactating mammary glands (35). This conclusion was based on results of several studies. First, RT-PCR amplification of human macrophage RNA with two different sets of oligonucleotide primers, overlapping exons 2–7 and 8–11 of the human cholesterol esterase gene (24, 25, 30), indicating that the products were derived from the authentic cholesterol esterase gene instead of the cholesterol esterase-like pseudogene (30). The results of the two PCR products encompassed the entire coding region of the mature human cholesterol esterase (24, 25). Second, bile salt-stimulated cholesterol esterase activity was also detected in conditioned medium of human macrophages. Thus, these results documented the ability of human macrophages to synthesize an enzymatically active and mature form of the bile salt-stimulated cholesterol esterase. The macrophage enzyme is identical to the pancreatic protein and is significantly different from the 46-kDa variant protein found in tumor cells (36). These results are in striking contrast to results obtained with mouse macrophages, which we failed to show the presence of cholesterol esterase mRNA. Taken together, these results suggest species difference in expression of the cholesterol esterase. Whereas mouse macrophages synthesize HSL and not the pancreatic type cholesterol esterase, human macrophages appear to synthesize the bile salt-stimulated cholesterol esterase instead of hormone-sensitive lipase.

The identification of pancreatic type cholesterol esterase mRNA in human macrophages adds to a growing list of cell types that are capable of synthesizing this protein. Although the bile salt-stimulated cholesterol esterase was originally discovered in the pancreas and in the pancreatic juice (37–39), the cholesterol esterase was also found to be an abundant protein synthesized and secreted into the milk by lactating mammary glands of many species (39). Recently, this protein was found to be synthesized by the liver, including the HepG2 human hepatoma cell line (28, 31, 41). The mRNA for bile salt-stimulated cholesterol esterase was also present in human eosinophils (42). Bile salt-stimulated cholesterol esterase activity, which could be inhibited by antibodies specific for the pancreatic enzyme, was also reported to be present in human serum and in the aorta (16, 17). These results suggested that bile salt-stimulated cholesterol esterase may have a systemic origin and participates in systemic lipid metabolism, in addition to its role for nutrient digestion and transport in the gastrointestinal tract (43).

Species differences in tissue expression of the bile salt-stimulated cholesterol esterase gene have been reported previously (44). While the pancreas of all species examined to date were...
capable of cholesterol esterase biosynthesis, the bile salt-stimulated type cholesterol esterase mRNA was present only in rabbit intestine and not the intestine of rat, human, or mouse (41, 44). Cholesterol esterase mRNA was reported to be present in some but not all rat liver tested (41, 44), and was absent in the rat hepatoma Fuf5AH cells (15). However, the cholesterol esterase mRNA was detectable in the HepG2 human hepatoma cells (Ref. 31 and current study). Lactating mammary glands from human, dog, cat, gorilla, goat, and mouse were reported to synthesize the bile salt-stimulated cholesterol esterase (27). In contrast, rat and cow mammary glands lack cholesterol esterase mRNA. Cholesterol esterase enzyme activity was also undetectable in rat, cow, and pig milk (40). In the current study, we showed that human macrophages were capable of cholesterol esterase biosynthesis while mouse macrophages were lacking in the cholesterol esterase mRNA. These results suggest that the tissue-specific regulatory sequences in the cholesterol esterase gene may be quite different among various species. Comparison of the available 5’-flanking sequences of the rat (45), human (30), and mouse (Refs. 27 and 43, and data not shown) cholesterol esterase genes revealed significant differences in nucleotide sequences. However, direct expression studies with chimeric reporter gene constructs will be necessary to elucidate the factors involved with species differences in tissue-specific expression of the cholesterol esterase gene.

The bile salt-stimulated cholesterol esterase synthesized by human macrophages is secreted from the cells; thereby, it is unlikely to play a role in intracellular cholesterol metabolism. Thus, the enzyme that participates in the futile cholesteryl ester cycle of human macrophages remains to be determined. A possible candidate is the neutral cholesteryl ester hydrolase present in macrophages of various species remains unknown.

The physiological significance of bile salt-stimulated cholesterol esterase biosynthesis by human macrophages also remains speculative. Results showing the presence of the cholesterol esterase in human aorta (17), and its increased secretion by oxidized LDL-stimulated human macrophages (Fig. 3), suggested its possible role in atherosogenesis. Previously, in an in vitro study, Shamir et al. (17) showed the ability of the cholesterol esterase to hydrolyze lysophosphatidylcholine in oxidized LDL. Because lysophosphatidylcholine has been implicated as a contributing factor in atherosclerosis (50), the oxidized LDL-induced cholesterol esterase secretion by human macrophages may be a protective response against the atherogenic effects of the modified lipoproteins (17). However, it is equally possible that the bile salt-stimulated cholesterol esterase secreted by human macrophages may hydrolyze lipoprotein-associated cholesteryl esters and lipid-rich debris deposited on the arterial wall, thereby increasing cholesterol monohydrate formation and promotes atherosclerosis. Thus, advanced lesions with high free cholesterol content observed in human atheroma may be related to cholesterol esterase biosynthesis and secretion by the macrophages. In contrast, the relative resistance of the mouse to develop atherosclerosis, with only mild fatty streak lesions after feeding a high fat/high cholesterol diet, may be attributed to the inability of mouse macrophage to synthesize bile salt-stimulated cholesterol esterase and thus the absence of this protein in atheromatous lesions of the vessel wall. The potential role of cholesterol esterase in unesterified cholesterol formation in the arterial wall is also supported by our recent observation that the enzyme can be activated maximally with low circulating bile salt concentrations in the presence of heparin-like molecules, such as those present in the arterial wall.2

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