Stable overexpression of human macrophage cholesteryl ester hydrolase results in enhanced free cholesterol efflux from human THP1 macrophages

Bin Zhao,1 Jingmei Song,1 Richard W. St. Clair,2 and Shobha Ghosh1
1Department of Internal Medicine, Virginia Commonwealth University, Richmond, Virginia; and 2Department of Pathology, Section on Comparative Medicine, Wake Forest University School of Medicine, Winston-Salem, North Carolina

Submitted 1 June 2006; accepted in final form 8 September 2006

Zhao B, Song J, St. Clair RW, Ghosh S. Stable overexpression of human macrophage cholesteryl ester hydrolase results in enhanced free cholesterol efflux from human THP1 macrophages. Am J Physiol Cell Physiol 292: C405–C412, 2007. First published September 13, 2006; doi:10.1152/ajpcell.00306.2006.—Reduction of the lipid burden of atherosclerotic lesion-associated macrophage foam cells is a logical strategy to reduce the plaque volume. Since extracellular cholesterol acceptor-mediated cholesterol efflux is the only recognized mechanism of cholesterol removal from foam cells and this process is rate limited at the level of intracellular cholesterol ester hydrolysis, a reaction catalyzed by neutral cholesteryl ester hydrolase (CEH), we examined the hypothesis that CEH overexpression in the human macrophage monocyte/macrophage cell line THP1 results in increased cholesterol efflux, as well as decreased cellular cholesterol ester accumulation. We generated THP1-CEH cells with stable integration of human macrophage CEH cDNA driven by the cytomegalovirus promoter. Compared with wild-type THP1 cells (THP1-WT), THP1-CEH cells showed increased CEH mRNA expression and increased CEH activity. Efflux of free or unesterified cholesterol by acetylated LDL-loaded THP1-CEH cells to ApoA-I by an ABCA1-dependent pathway or to HDL by an ABCG1-dependent pathway was significantly higher than that in THP1-WT cells. In addition, THP1-CEH cells accumulated significantly lower amount of esterified cholesterol. CEH overexpression, therefore, not only enhances cholesterol efflux but also reduces cellular accumulation of cholesteryl esters. Taken together, these data provide evidence for evaluating CEH expression in human macrophages as a potential target for attenuation of foam cell formation and regression of atherosclerotic plaques.

PRESENCE OF CHOLESTERYL ESTER (CE)-laden macrophage foam cells is one of the most prominent characteristics of an atherosclerotic lesion. In advanced lesions, these foam cells constitute the lipid core of the plaque, where in addition to accumulating the lipids from atherogenic lipoproteins, these cells also secrete proinflammatory mediators such as cytokines, chemokines, and proteases (27). Thus, besides contributing to the thrombogenic lipid core, these foam cells also enhance the infiltration of inflammatory cells and local inflammation at the plaque site, resulting in increased vulnerability of the plaque. Reducing the lipid burden of the macrophage foam cells therefore represents a logical strategy to not only reduce the plaque volume, but also to increase the stability of vulnerable plaques.

Foam cell formation occurs when the balance between the cholesterol uptake and the efflux pathways is perturbed. Unregulated uptake of modified lipoproteins via the scavenger receptors (SR-A and CD36) is the major uptake pathway (2). Lipoprotein-associated CEs are first hydrolyzed in the lysosomes with the resulting free cholesterol (FC) being transferred out of the lysosome. The FC content of cells is maintained under tight control to prevent excessive enrichment of the plasma membranes with FC that can alter membrane function (1). Two processes working in tandem are involved in maintaining the homeostatic balance between intracellular FC and CEs; esterification of excess FC by acyl CoA:cholesterol acyltransferase-1 (ACAT1) and hydrolysis of CE by a neutral cholesteryl ester hydrolase (CEH). This “futility” cycle of cholesterol esterification and hydrolysis is known as the cholesterol ester cycle and serves to maintain appropriate cellular FC concentration (3). Exogenous cholesterol acceptor-mediated removal of cellular FC is the only recognized mechanism for cholesterol efflux. In HDL, FC is esterified by lecithin:cholesterol acyltransferase (LCAT), and the resulting CEs are delivered to the liver for ultimate removal as biliary cholesterol or bile acids. This directional movement of FC from macrophage foam cells or other peripheral tissues to the liver is known as reverse cholesterol transport (RCT). Since it is FC and not CE that is effluxed to exogenous acceptors, the obligatory first step in RCT is, therefore, the hydrolysis of CE to release FC, a reaction catalyzed by a neutral CEH.

Excess CE accumulation and development of foam cells occurs when the FC efflux is not on pace with the scavenger receptor-mediated uptake of modified lipoproteins. Nakata (33, 34) and colleagues first described fat accumulation and lesion formation in artery wall by experimental obstruction of vasa vasorum, providing direct evidence for the importance of RCT in vascular lesion formation. However, the controversy surrounding the identity of CEH in macrophages has been largely the reason for the lack of studies evaluating RCT as a potential therapeutic target despite the observed inverse correlation between the level of CEH expression and susceptibility to atherosclerosis in various animal species (18, 29, 46) and identification of CEH-mediated hydrolysis as the rate-limiting step in RCT (39, 31). Several investigators attributed the CEH activity in murine macrophages to hormone-sensitive lipase (HSL), an enzyme characterized from adipose tissue and shown to hydrolyze CE in addition to its physiological substrate, triacylglycerol (14, 41, 22, 7). However, CEH activity in peritoneal macrophages from HSL knockout animals was not reduced (37), and human macrophages do not express HSL (5, 26),

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
indicating that another enzyme, distinct from HSL, mediates CEH activity in macrophages.

We have reported earlier the cloning and characterization of human macrophage CEH (10) and demonstrated its role in mobilizing cellular CE in LDL receptor-deficient Chinese hamster ovary cells (AC-29) overexpressing ACAT1 (13). While this cell line provided a system where reproducible and consistent accumulation of CE occurred as a result of ACAT1 overexpression and direct effects of CEH overexpression on CE mobilization could be examined, it lacked the dynamics of CE metabolism that exists in macrophages. While Chinese hamster ovary cells do express low levels of scavenger receptors, CD36 and SR-A (38), these cells do not express ABCG1 (9), the major cholesterol transporter involved in cellular cholesterol efflux. Therefore, to demonstrate the physiological role of CEH in reducing the lipid burden of human macrophage foam cells, where all the mechanisms relevant to foam cell formation in vivo are operational (e.g., scavenger receptor-mediated unregulated modified lipoprotein uptake, intracellular CE accumulation not due to high ACAT1 levels but as a result of increased uptake of lipoproteins, and ABC transporter-mediated cholesterol efflux), it is imperative to show enhanced cholesterol efflux from human macrophages or cell line such as THP1, following CEH overexpression to relevant cholesterol acceptors such as ApoA-I or HDL or serum. In the present study we examined the hypothesis that enhanced expression of CEH in human macrophage cell line THP1 [by stable integration of CE cDNA driven by the cytomegalovirus (CMV) promoter into the cellular genome of these cells] will increase FC efflux to extracellular cholesterol acceptors and result in attenuated cholesterol accumulation in these cells. The results presented herein support our hypothesis and demonstrate that enhancing the expression of macrophage CEH may represent a viable mechanism for reducing the lipid burden of plaque-associated macrophage foam cells.

MATERIALS AND METHODS

THP1 cells were purchased from American Type Culture Collection (ATCC, Rockville, MD). ThermoScript RT-PCR system, cell culture media, fetal bovine serum, and cell culture reagents were obtained from Invitrogen (Carlsbad, CA). Phorbol 12-myristate 13-acetate (PMA) was obtained from Sigma (St. Louis, MO). The RNeasy kit and Effectene were from Qiagen (Valencia, CA). TaqMan Universal PCR Master Mix was from Applied Biosystems (Foster City, CA). Lipoproteins [acetylated (Ac)-LDL, HDL, and ApoA-I] were purchased from Intracel (Frederick, MD). All other reagents and chemicals were purchased from Fisher Chemical (Cincinnati, OH).

Stable Integration of CEH into THP1 Cells

THP1 cells were maintained in RPMI medium supplemented with 10% FBS. Freshly split cells (1 × 10⁶ cells) were nucleofected with expression vector pCMV-CEH containing full-length CEH cDNA (13) using Nucleofector (Amaza Biosystems, Koeln, Germany). The cell specific kit (VCA-1003) optimized for THP1 cells was used according to the manufacturer’s instructions. In brief, 1 × 10⁶ cells in 100 μl of Nucleofector solution were mixed with 0.5 μg of pCMV-CEH expression vector and nucleofected using the program U-01 for high cell survival. After addition of normal growth medium, cells were transferred to a 12-well plate. This procedure was shown to result in >50% transfection efficiency. Two days after nucleofection, cells from 3 wells were pooled, the growth medium was replaced with medium supplemented with Geneticin (800 μg/ml), and cells were transferred to a 25-cm² tissue culture flask. Medium was changed every third day. After 2 wk, the concentration of Geneticin was reduced to 500 μg/ml and medium changed every week. Cell viability was monitored by trypan blue exclusion. Three months later, the viability was >98%, indicative of stable integration of the expression vector, making the cells resistant to Geneticin. These cells were labeled THP1-CEH to indicate stable integration of CEH in THP1 cells, and they were maintained in medium supplemented with Geneticin (500 μg/ml). The “parent” THP1 cells will be referred to as wild-type THP1 (THP1-WT) cells.

Genomic DNA PCR

Genomic DNA was extracted from 5 × 10⁷ cells by digestion with proteinase K (0.6 μg/ml) at 58°C overnight, followed by phenol extraction and ethanol precipitation (4). PCR amplification was performed in a standard 25-μl PCR reaction containing 1.5 mM MgCl₂, 200 μM dNTPs, 300 pM of each primer, 1 μg of genomic DNA, and 40 U/ml of Taq DNA polymerase (Invitrogen). The thermal cycle consisted of a preincubation step of 2 min at 94°C to ensure complete denaturation of the DNA, followed by 35 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 2 min, and final extension of 7 min at 72°C. The sequences of the primers used were: forward primer 5'-ATGCAAAGTGCTGGGGAAGTTC-3' and reverse primer 5'-TTCCACGAAACAGAAGTGAGGGC-3', corresponding to nucleotides 133-155 and 792-814 of the human macrophage CEH sequence. All PCR reactions were carried out in a Gene Amp PCR System 2700 (Applied Biosystems). PCR products were separated on 1% agarose gels stained with ethidium bromide.

Determination of Cell Viability After Lipid Loading

THP1-WT and THP1-CEH cells (1 × 10⁶ cells/well) were plated in 24-well culture plates in the presence of 100 nM PMA for 3 days. Cells were then loaded with Ac-LDL (25 μg/ml) for 48 h and equilibrated with serum-free medium for an additional 24 h. One set of THP1-CEH cells were maintained in the absence and the other in the presence of Geneticin. At the end of the equilibration period, cell viability was determined using Alamar Blue.

Measurement of CEH mRNA Levels by Real-Time PCR

Total RNA was extracted with the RNeasy Mini Kit. Five micrograms of total RNA were reverse transcribed with Thermoscript RT-PCR System (Invitrogen), and first strand cDNA was used to perform real-time PCR using Stratagene Mx3000p real-time PCR system with TaqMan Gene Expression Assays for human CEH (Applied Biosystems). CEH expression was calculated as absolute copy number from a standard curve as described earlier (48).

CEH Activity in THP1-WT and THP1-CEH Cells

Cells (2 × 10⁶ cells/well) were plated in 6-well culture plates in the presence of 100 nM PMA. Medium was replaced every 2 days and cells harvested after 4 days in homogenizing buffer containing protease inhibitors (12). The cell suspension was sonicated using Branson Sonifier fitted with a microtip and the cell lysates assayed for CEH activity as described earlier (13). The substrate, cholesterol-[1-¹⁴C]olate, was dissolved in acetone.

Measurement of Cholesterol Efflux

Cells (1 × 10⁶ cells/well) were plated in 24-well culture plates in the presence of 100 nM PMA for 3 days. The intracellular FC and EC pools were labeled with [³H]cholesterol by incubating the cells for 48 h with growth medium containing 1 μCi/ml [³H]cholesterol according to the method of Mahlberg et al. (28). The cells were then washed and incubated with serum-free medium for 24 h to allow all pools of cholesterol to equilibrate. Total lipids were extracted from representative cells, and specific radioactivity of cellular FC and EC
was determined to ensure equilibration of the [3H]cholesterol label within these two forms of cellular cholesterol. Following equilibration, free cholesterol efflux was initiated by the replacement of the medium with serum-free medium alone or media containing ApoA-I (25 μg/ml), HDL (25 μg/ml), or 10% FBS. ApoA-I was dissolved in 5 M guanidine HCl (500 μg/ml), and just prior to addition to the culture medium, the solvent was exchanged for PBS using Zeba desalting spin columns (Pierce, Rockford, IL), thus ensuring the addition of the monomeric form of ApoA-I as the extracellular cholesterol acceptor. To measure the efflux of FC from cells, an aliquot of the medium was withdrawn at the indicated times and centrifuged for 10 min at 14,000 g to remove cell debris, if any. The radioactivity associated with the supernatant fluid was determined by liquid scintillation counting. At the end of 24 h, cells were digested in 1 N NaOH and an aliquot was taken to determine the cell-associated radioactivity. Total incorporation of cholesterol was calculated as the sum of the cell-associated radioactivity and radioactivity present in the total medium at 24 h. Efflux of [3H]FC from the cells into the medium at any given time was calculated as percent of the total [3H]cholesterol incorporated in the cells. Data are expressed as means ± SD for three independent experiments.

**Determination of Cholesterol and Cholesteryl Ester Mass**

Total lipids were extracted from parallel sets of cells (as described above without [3H]cholesterol) with 2-propanol containing sitosterol as an internal standard. The lipid extract was analyzed for total cholesterol and FC content by gas-liquid chromatography by the procedure of Ishikawa et al. (19), as modified by Klansek et al. (23). Esterified cholesterol (EC) mass was determined as the difference above without [3H]cholesterol) with 2-propanol containing stigmasterol as an internal standard. The lipid extract was analyzed for total cholesterol and FC content by gas-liquid chromatography by the procedure of Ishikawa et al. (19), as modified by Klansek et al. (23). Esterified cholesterol (EC) mass was determined as the difference between total and FC. Following lipid extraction, cells were solubilized in 1 N NaOH and total cellular DNA was determined by a fluorometric assay using Hoechst dye (24).

**RESULTS**

**Generation and Characterization of THP1-CEH Cells**

Cells with stable integration of vector coding for CEH and neomycin were selected by acquired resistance to Geneticin. Stable integration of the CEH coding region was determined by PCR amplification of a 681-bp product from genomic DNA using CEH cDNA-specific primers. As shown in Fig. 1, these primers amplified the specific amplicon from the plasmid pCMV-CEH (positive control); no amplification products were obtained from the empty vector (negative control). PCR products were separated on 1% agarose gel and stained with ethidium bromide. The expected 681-bp PCR product was amplified from THP1-CEH genomic DNA, and this band was absent from DNA from THP1 cells.

**Effect of CEH Overexpression on Cellular Cholesterol Efflux**

Hydrolysis of cellular CE is considered the rate-limiting step in FC efflux from macrophages (39, 31). FC is subsequently effluxed by multiple pathways, depending on the presence of appropriate exogenous cholesterol acceptor. To examine the effect of stable expression of CEH on FC efflux, cholesterol efflux by the various efflux pathways, namely aqueous diffusion (independent of a cellular transporter), ABCA1-mediated efflux (to lipid-free ApoA-I), ABCG1/G4-mediated efflux (to HDL), and total efflux (to FBS) from Ac-LDL-loaded THP1 WT and THP1-CEH cells, was compared. Initial studies were performed to compare total cholesterol efflux to FBS from THP1-CEH macrophages grown in the presence or absence of Geneticin. No difference was observed in total efflux control of the corresponding THP1-WT cells (Fig. 2). Consistent with the stable integration of CEH cDNA in THP1-CEH cells, a significant increase in CEH mRNA was observed compared with THP1-WT cells and this expression level did not change with lipid loading. There was no significant difference in the mRNA levels of the scavenger receptor CD36 in THP1-CEH cells compared with THP1-WT cells, and only a modest increase was observed after lipid loading. SR-A mRNA levels were significantly higher in THP1-CEH cells and were not affected by lipid loading. Significantly higher mRNA levels of cholesterol transporters ABCA1 and ABCG1 were observed in THP1-CEH cells, which were further increased by Ac-LDL loading.

In accordance with the increase in CEH mRNA, a 1.6- to 2-fold increase in CEH activity was seen in THP1-CEH cell lysates (Fig. 3), demonstrating overexpression of functional CEH in THP1-CEH cells.
in the presence of Geneticin (101 ± 4.21%, n = 3) compared with in the absence of Geneticin (100%). Therefore, Geneticin was included in the culture medium for all efflux studies.

**Transporter-independent efflux.** A very small amount of FC desorbs from the plasma membrane and accounts for the transporter-independent efflux. To measure cholesterol efflux by this pathway, no exogenous cholesterol acceptor was added to the culture medium. As shown in Fig. 4, a time-dependent increase in cholesterol efflux was observed from both cell types. Overexpression of CEH in THP1-CEH cells resulted in an almost twofold increase in cholesterol efflux by this pathway at any given time. It should be pointed out that, although no exogenous cholesterol acceptor was added to determine the transporter-independent efflux via aqueous diffusion, once de-

---

### Fig. 2. Gene expression in THP1-WT and THP1-CEH cells.

Cells (2 × 10^6 cells/well) were plated in 35-mm tissue culture plates in the presence of 100 nM PMA. After 4 days, cells were incubated in medium containing either 1% FBS, 1% BSA, and no acetylated (Ac)LDL (A: nonloaded cells) or 25 μg/ml Ac-LDL (B: AcLDL-loaded cells). Total RNA was isolated after 48 h and mRNA levels determined by real-time PCR. mRNA levels in THP1-CEH cells are expressed as %control of the corresponding levels in THP1-WT cells. Data shown are means ± SD; n = 3.
sorbed from the plasma membrane, effluxed cholesterol may readily associate with ApoE secreted by macrophages (17).

Efflux to ApoA-I. ABCA1-mediated efflux was determined by including lipid-free ApoA-I as the extracellular cholesterol acceptor. A time-dependent increase in cholesterol efflux was observed from both cell types (Fig. 5). Overexpression of CEH in THP1-CEH cells resulted in an almost twofold increase in cholesterol efflux by this pathway at all time points.

Efflux to HDL. The bulk of cholesterol efflux from macrophage foam cells occurs via ABCG1, and to determine the effect of CEH overexpression on this pathway, HDL was added as the exogenous cholesterol acceptor. A time-dependent increase in efflux was observed from both cell types (Fig. 6), and CEH overexpression in THP1-CEH led to almost a twofold increase in cholesterol efflux by this pathway at all time points.

Total efflux to serum. Cholesterol effluxed to serum in the culture medium represents the total efflux from the cells. A time-dependent increase in cholesterol efflux was obtained from both cell types (Fig. 7). Consistent with the efflux to ApoA-I or HDL, total cholesterol efflux to serum was almost twofold higher from THP1-CEH cells at all time points.

Differences in the Accumulation of Cholesterol in THP1-WT and THP1-CEH Cells

To determine whether overexpression of CEH leads to changes in cholesterol accumulation in THP1-CEH cells, cellular total and esterified cholesterol mass was determined in cells loaded with Ac-LDL. As seen from Fig. 8, compared with THP1-WT cells, THP1-CEH cells accumulated significantly lower total and esterified cholesterol following 2-day loading with Ac-LDL. These data suggest that CEH overexpression results in lower cellular cholesterol accumulation in THP1-CEH cells. As expected based on cholesterol efflux data (Figs. 4–7), the intracellular levels of total and esterified cholesterol in both cell types decreased when efflux was initiated by HDL.
DISCUSSION

The present study provides direct evidence that enhancing intracellular CE hydrolysis by stable overexpression of CEH in the human monocyte/macrophage cell line THP1 leads to significantly increased FC efflux to extracellular cholesterol acceptors. Furthermore, increased CEH expression also resulted in decreased cellular CE accumulation following lipid loading with Ac-LDL. Reduction in the lipid burden of lesion-associated foam cells is central to plaque regression, as well as increasing the stability of the vulnerable plaques, and the data presented here identify CEH as a potential target to achieve this goal.

Multiple pathways exist for the efflux of cellular FC. Efflux of FC via aqueous diffusion that is independent of a cellular transporter occurs with all cell types but is inefficient. The ATP-binding cassette transporter A1 (ABCA1) mediates efflux of both FC and phospholipid to lipid-free ApoA-I (47). ABCG1 and ABCG4 stimulate FC efflux to both smaller and larger subclasses of HDL (HDL3 and HDL2, respectively), but not to lipid-poor ApoA-I (44). Under physiological conditions, where all extracellular cholesterol acceptors are present, these pathways act in concert, and the ABC transporters do not act independently of each other. Gelissen et al. (9) have recently demonstrated that ABCA1-mediated lipid efflux transforms ApoA-I into an efficient substrate for ABCG1-dependent cholesterol efflux, and ABCA1 and ABCG1 may therefore act in series to mediate lipid efflux from macrophages.

Consistent with its role in catalyzing the obligatory first step (CE hydrolysis) in cholesterol efflux and RCT, stable overexpression of CEH in THP1-CEH cells led to an increase in FC efflux to exogenous lipid-free ApoA-I (ABCA1-mediated transport) and to exogenous HDL (ABCG1-mediated transport). Highest efflux was obtained when 10% serum (which also contains phospholipids that act as a “sink” for effluxed FC) was used as the exogenous acceptor. The data presented here show that, while the FC generated by CEH-mediated hydrolysis of CE is effluxed by all known pathways without any apparent preference, the percent FC efflux increased corresponding to the relative importance of these different efflux pathways: aqueous diffusion or transporter independent < ABCA1-mediated < ABCG1-mediated < total (to serum). THP1 cells produce and secrete ApoE, and the FC effluxed via aqueous diffusion (in the absence of an added acceptor) may readily associate with the ApoE secreted by macrophages (17).

Although low levels of CEH or inefficient intracellular CE hydrolysis has been recognized as a reason for increased cholesterol accumulation in macrophages and CE hydrolysis is considered the rate-limiting step in the process of RCT, there has not been a direct demonstration of increased cholesterol efflux by overexpression of CEH in macrophages. Effects of adeno-virus-mediated transient overexpression of another enzyme, HSL, which can also hydrolyze cholesterol esters, was described by Okazaki et al. (36). Paradoxically, overexpression of HSL in THP1 macrophages resulted in greater elimination of cellular CE in the absence of HDL than in the presence of HDL (36), indicating that FC generated by HSL-mediated hydrolysis is not effluxed by a physiologically recognized pathway (to HDL). In contrast, CEH overexpression in THP1-
CEH macrophages described here enhanced FC efflux to all known cholesterol acceptors, indicating that CEH-mediated hydrolysis releases FC such that it is available to all known efflux pathways.

It is important to note that overexpression of CEH resulted in enhanced expression of cholesterol transporters ABCA1 and ABCG1, conceivably positioning the cell to efflux the increased FC generated by CEH-mediated hydrolysis. A similar increase in ABCA1 mRNA levels by overexpression of HSL, in the absence of an increased FC content of the cells, was also observed by Okazaki et al. (36). CEH-mediated hydrolysis generates cholesterol and fatty acids, both of which in their oxidized forms can potentially induce the expression of ABCA1 and ABCG1 via activation of PPARγ and LXRα, respectively (32, 40). Furthermore, fatty acids can directly affect gene transcription (20). Although the total FC content was not increased in THP1-CEH cells, it is tempting to speculate that FC released by CEH might be directed to a distinct “regulatory pool” from where it can affect gene transcription (45, 6). Signaling pathways operational in macrophages are known to affect RNA stability (8, 25, 30), and the role of increased mRNA stability as a potential mechanism underlying the observed changes in the mRNA levels cannot be ruled out.

It is noteworthy that THP1-CEH cells showed significantly lower accumulation of total and esterified cholesterol despite higher mRNA levels of SR-A and CD36. These data are consistent with earlier studies that demonstrate an inverse correlation between cellular CE accumulation and endogenous cholesteryl ester hydrolytic activity in macrophages (18). Furthermore, stimulation of intracellular cholesterol ester hydrolysis by hormone treatment decreases cholesterol accumulation in macrophages (43, 35). On the other hand, macrophages with reduced expression of endogenous cholesterol ester hydrolytic activity (16, 18, 29, 46) and manipulations that reduce intracellular cholesteryl ester hydrolytic activity enhance CE accumulation (42). Thus our data provide direct evidence for the concept that intracellular CE hydrolysis inversely correlates to cellular CE accumulation.

In comparison with murine macrophages such as mouse peritoneal macrophages, J774A.1 or RAW cells, human macrophages, including THP1 macrophages, are reportedly resistant to cholesterol efflux, and the presumed defect is lower endogenous CEH activity (15). An inverse relationship has been described for endogenous CEH activity in macrophages and susceptibility to atherosclerosis (16, 18, 29, 46). The present study confirms the role of CEH in intracellular CE hydrolysis and subsequent efflux of FC to physiologically relevant cholesterol acceptors, such as ApoA-I, HDL, and serum in the human monocyte/macrophage cell line THP1. While future studies with macrophage-specific expression of human macrophage CEH cDNA in ather-susceptible mice such as LDLR−/− mice would allow us to examine the direct effects of CEH overexpression on the development of atherosclerosis, it remains to be seen whether the level of CEH expression in human blood-derived macrophages inversely relates to development of heart disease.

ACKNOWLEDGMENTS

This work was supported by research grant HL-069946 from the National Heart, Lung, and Blood Institute to S. Ghosh.

REFERENCES

C412

CEH ENHANCES CHOLESTEROL EFFLUX FROM HUMAN MACROPHAGES