Apolipoprotein A-I promotes cholesterol release and apolipoprotein E recruitment from THP-1 macrophage-like foam cells

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Abstract **Apolipoprotein E (apoE) is synthesized and secreted by arterial macrophages while apolipoprotein A-I (apoA-I) is present in surrounding interstitial fluids. Both apolipoproteins play important roles in macrophage cholesterol homeostasis by forming lipid complexes (nascent-HDL) with cellular phospholipids (PL) and cholesterol (UC) thereby promoting cholesterol efflux. In this study, we evaluated the relative contributions of apoA-I and endogenously produced apoE in mediating the recruitment of cellular cholesterol. THP-1 human monocytes were differentiated (300 nM phorbol dibutyrate) into macrophages and macrophage-foam cells were generated by cholesterol loading with acetylated LDL (50 μg protein/ml). ApoA-I (10 μg/ml) depleted macrophage-foam cell cholesteryl esters by 50% in 24 h. This reduction was accompanied by a significant increase in the UC/PL mole ratio of nascent HDL (UC/PL = 0.80 ± 0.15) in the medium compared to complexes isolated from macrophages (UC/PL = 0.59 ± 0.08). Significantly more (70%) nascent-HDL were formed in incubations of apoA-I with macrophage-foam cells than with macrophages. Medium apoE accumulation paralleled the assembly of apoA-I containing nascent HDL where 2- and 4-fold increases were observed with macrophages and macrophage-foam cells, respectively, compared to incubations in the absence of apoA-I. Despite the increase in medium apoE accumulation, a majority (85%) of particles (11, 9, and 7.4 nm in diameter) from macrophages and macrophage-foam cells possessed apoA-I without apoE. ApoA-I plus apoE particles (13–16 nm) were also formed along with a small quantity of apoE-only particles (19–20 nm).** **The predominance of apoA-I only particles indicates, however, that the assembly of apoA-I-containing nascent-HDL represents a major metabolic pathway of cellular cholesterol recruitment compared to the endogenous production of apoE.—**Bielicki, J. K., M. R. McCall, and T. M. Forte. **Apolipoprotein A-I promotes cholesterol release and apolipoprotein E recruitment from THP-1 macrophage-like foam cells.** ***J. Lipid Res.*** **1999.** 40: **85–92.**

Supplementary key words macrophage-foam cells • cholesterol recruitment • sphingomyelin • apolipoprotein A-I • apolipoprotein E • nascent-HDL assembly

Elevated plasma concentrations of high density lipoproteins (HDL) are inversely correlated with the development of atherosclerosis (1–3). The protective effects of HDL are attributed, in part, to its ability to promote the efflux of cholesterol from cells of the artery wall and mediate the transport of cholesterol to the liver for catabolism (4–6).

Apolipoprotein A-I (apoA-I), the major structural protein of HDL, consists of 243 amino acids arranged in a series of amphipathic alpha-helices that bind lipid (7). In lipid-free form, apoA-I is able to recruit cellular phospholipid and cholesterol for the assembly of nascent HDL particles (8–11). Recently, Yancey et al. (12) demonstrated that lipid-free apoA-I was more efficient than plasma HDL at promoting the efflux of cholesterol from macrophage-foam cells, indicating that the recruitment of cholesterol by apoA-I represents a major metabolic pathway of cellular cholesterol efflux.

ApoA-I belongs to a class of exchangeable apolipoproteins that, under the appropriate conditions, can desorb from the surface of HDL particles into the aqueous phase (13). Lipid-free apoA-I can also be generated by the hydrolysis of triglyceride-rich lipoproteins and by the action of cholesteryl ester transfer protein (CETP) on HDL (14–16). The concentration of lipid-free apoA-I in normolipidemic human plasma has been estimated to be approximately 8% (75 μg/ml) of the total plasma apoA-I pool (17). Because of its small size relative to plasma HDL, apoA-I is thought to enter the subendothelial space of the artery wall wherein it promotes the removal of excess cholesterol from cells. Numerous studies reporting on the efflux of cellular cholesterol agree that the concentrations

Abbreviations: HDL, high density lipoprotein; apo, apolipoprotein; UC, unesterified cholesterol; PL, phospholipid; SM, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; and PE, phosphatidylethanolamine.

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of lipid-free apoA-I required for maximal recruitment of cholesterol are far below the estimated concentrations of lipid-free apoA-I found in vivo (8–12). Indeed, it is more likely that cellular factors, such as cell-type and membrane cholesterol content, limit the extent of lipid recruitment mediated by apoA-I (8, 18, 19).

Macrophages and macrophage-foam cells, but not monocytes, synthesize and secrete apolipoprotein E (20–24). The secretion of apolipoprotein E (apoE) from macrophages has been found to enhance HDL_3-mediated cholesterol efflux (25, 26). Recently, Kruth et al. (27) demonstrated that the endogenous production of apoE can deplete macrophages of their cholesteryl ester stores. However, unloading of macrophage cholesterol by the endogenous production of apoE required several days, suggesting that this mechanism may represent a quantitatively minor pathway compared to cholesterol efflux mediated by apoA-I.

In this study, we investigated whether lipid-free apoA-I was able to recruit cellular phospholipid and cholesterol from the human monocytic cell line, THP-1, or whether such lipid recruitment was dependent on a macrophage phenotype. We also assessed the relative importance of lipid-free apoA-I and endogenously produced apoE in mediating the recruitment of cellular lipids from macrophages and macrophage-foam cells. We found that apoA-I recruited considerably more sphingomyelin from macrophages, compared to monocytes, suggesting that specific metabolic pathways of cellular lipid recruitment may be activated upon differentiation of monocytes to macrophages. Incubations of macrophages and macrophage-foam cells with apoA-I yielded a predominance of apoA-I without apoE nascent HDL particles. Thus, apoA-I was the principal mediator of cellular cholesterol efflux compared to the endogenous production of apoE.

MATERIALS AND METHODS

Cell culture

The THP-1 human monocytic cell-line was maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum and 5×10^{-5} M β-mercaptoethanol (BME). Cells were kept at 37°C in a humidified atmosphere of 95% air / 5% CO_2. For experiments, cells were grown to a density of 1×10^6 cells / ml in T-175 flasks (final volume = 60 ml / flask). To differentiate monocytes into macrophages, 300 nM phorbol dibutyrate was added to the growth medium and cells incubated for 6 days. Cholesterol-loaded macrophages (hereafter referred to as macrophage-foam cells) were prepared by including acetylated LDL (50 μg protein / ml) in the differentiation medium. The concentration of acetylated LDL used in the present study was previously shown by Banka et al. (23) to produce maximal cholesteryl ester accumulation in differentiated THP-1 macrophages.

Incubations of THP-1 cells with lipid-free apolipoprotein A-I

Monocyte suspension cultures, macrophages, and macrophage-foam cells were rinsed twice with 10 ml / flask of Hank's balanced salt solution (HBSS) followed by an extended 2-h rinse with HBSS containing bovine serum albumin (2 mg / ml). After this albumin rinse, cells were washed once with serum-free RPMI-1640 medium. This final rinse was decanted and one group of cells received serum-free RPMI-1640 medium (20 ml / flask), and the other group received serum-free medium containing 10 μg / ml of lipid-free apoA-I. Conditioned medium from cells was harvested after 24 h of incubation.

Isolation of apolipoprotein-containing nascent HDL from conditioned medium

Cells and cell-debris in conditioned medium were pelleted by low speed centrifugation (500 *g*). Medium supernatant was collected and gentamicin sulfate (50 μg / ml), phenyl methylsulfonal fluoride (3.5 mM), and EDTA (2.7 mM) were added. This medium was concentrated 100-fold using a Minitan concentrating system fitted with a 10,000 MW cutoff membrane. Lipidated apoA-I complexes (d 1.063–1.21 g / ml), referred to as nascent-HDL throughout this paper, were isolated from concentrated conditioned medium by ultracentrifugation as described (9, 10), and samples were dialyzed into 0.15 M NaCl / 2.7 mM EDTA (pH 7.4).

Physical / chemical properties of nascent HDL assembly products

Protein was measured by the method of Markwell et al. (28) using bovine serum albumin as a standard. Cholesterol was quantified enzymatically by the method of Sale et al. (29) and phospholipid by measuring inorganic phosphorus (30). The phospholipid subclass distribution of nascent HDL was determined by thin-layer chromatography using silica gel H plates and a solvent system of chloroform–methanol–acetic acid–water 100:60:16:8. SDS-PAGE was performed to evaluate the relative mass ratios of apoA-I and apoE associated with nascent HDL (31); gels were stained with Coomassie R-250, and the mass distributions of apolipoproteins were determined by densitometric scanning.

Nascent-HDL particle size was evaluated by nondenaturing gradient gel electrophoresis (NGGE); the relative distribution of particle mass was determined by Coomassie G-250 staining and densitometry (32). The association of apoA-I and apoE with specific nascent HDL subpopulations was evaluated by NGGE followed by Western Blot analysis using monoclonal antibodies (Chemicon International Inc., Temecula, CA) specific for apoA-I or apoE. ApoA-I primary antibody was detected using a biotinylated anti-IgG secondary antibody and an avidin-peroxidase color development system (Vector Lab., Burlingame, CA), and apoE primary antibody using a peroxidase-labeled anti-IgG linked to a sensitive chemiluminescence detection system (NEN Life Sciences, Boston, MA). As the sensitivity of these two detection methods differ considerably, the relative distribution of apoA-I versus-E containing nascent-HDL does not accurately reflect mass but, rather, qualitatively identifies apolipoprotein-specific nascent-HDL subpopulations. The mass of apoE in concentrated (Minitan) conditioned medium was quantified by ELISA (33).

Quantification of cellular cholesterol

Cellular cholesterol concentrations were quantified before and after incubations with apoA-I to determine extent of cholesterol recruitment. Lipids were extracted from cells by the method of Bligh and Dyer (34). Unesterified cholesterol in lipid extracts was quantified enzymatically (29); total cholesterol was similarly quantified after cholesteryl esterase treatment. Cholesteryl esters were calculated from the difference between total and unesterified cholesterol and multiplying by a conversion factor (1.68). Cellular protein was dissolved in NaOH (0.3 N) and protein was measured by the method of Markwell (28). Results were expressed as μg cholesteryl ester / mg cell protein.

RESULTS

Reduction in macrophage cholesteryl esters by apoA-I

Figure 1 shows the concentrations of cellular cholesteryl esters (A) and unesterified cholesterol (B) in differentiated THP-1 macrophages and macrophage-foam cells. Incubation of macrophage-foam cells with apoA-I produced a 50% reduction in cellular cholesteryl esters by 24 h; whereas, little or no reductions in cellular cholesteryl esters were observed in the absence of apoA-I during the time-course of these experiments. The cellular concentrations of unesterified cholesterol were unchanged by the acetylated LDL treatment and remained constant throughout the efflux period.

Lipidation of apoA-I by monocytes, macrophages, and macrophage-foam cells

To determine whether reductions in macrophage cholesteryl esters were accompanied by an increase in the mass of apoA-I-forming lipid complexes, the percentage of medium apoA-I associating with membrane lipids and floating as nascent HDL (d 1.063–1.21 g/ml) was quantified. The formation of lipid complexes in the media from macrophages and monocytes was also examined for comparative purposes. **Figure 2** shows that monocytes and macrophages lipidated apoA-I to approximately the same extent (7.5% and 9.0%, respectively). In contrast, cholesterol loading of macrophages with acetylated LDL increased the degree of apoA-I lipid complex formation by 70%. The latter observation is consistent with those of Bielicki et al. (8) and Yancey et al. (12) demonstrating that cholesterol enrichment of cells enhances the efflux of cellular phospholipid to apoA-I and facilitates the assembly of nascent HDL particles.

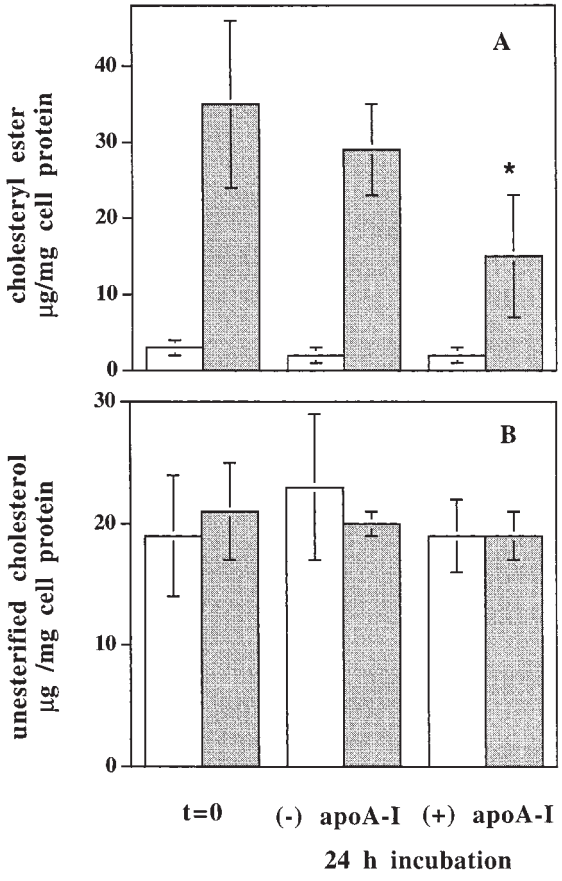

Fig. 1. Reductions in macrophage cholesteryl esters by apoA-I. THP-1 monocytes were differentiated into macrophages (300 nm phorbol dibutyrate) and cholesterol-loaded with acetylated LDL (50 μg protein/ml) as described in Methods. Shown are the cellular concentrations of cholesteryl esters (A) and unesterified cholesterol (B) before (t = 0) and after incubations (24 h) with either serum-free medium (− apoA-I) or medium plus apoA-I (10 μg/ml). Open bars are macrophages, and shaded bars are macrophage-foam cells. Values are means ± SD; n = 4, *$P < 0.05$ compared to cholesteryl ester concentrations at (t = 0) and at t = 24 h with serum-free medium.

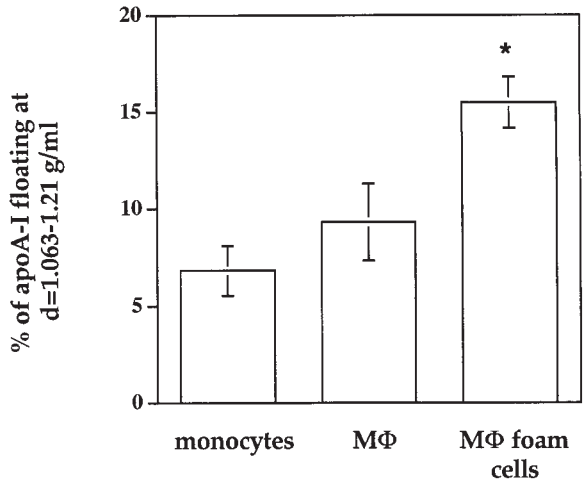

Fig. 2. Nascent HDL formation following incubations of THP-1 monocytes, macrophages, and macrophage-foam cells with apoA-I. THP-1 monocytes were differentiated into macrophages (300 nm phorbol dibutyrate) and cholesterol-loaded with acetylated LDL (50 μg protein/ml). Cells were incubated with lipid-free apoA-I (10 μg/ml) for 24 h. Conditioned medium was harvested and nascent HDL (d 1.063–1.21 g/ml) was isolated by ultracentrifugation. The percentage of medium apoA-I floating in the nascent-HDL fraction was quantified by radial immunodiffusion. Values are means ± SD, n = 5; *$P < 0.05$ compared to monocytes and macrophages.

Chemical composition of nascent HDL isolated from monocytes, macrophages, and macrophage-foam cells

The net reduction of cellular cholesteryl esters from macrophage-foam cells suggests that, in addition to an increase in nascent HDL formation, the cholesterol content of these particles may be elevated. This was indeed the case; **Table 1** shows that there was a significant increase in UC content and UC/PL ratio ($P < 0.02$) of complexes isolated from macrophage-foam cells compared to macrophages and monocytes. This increase in nascent HDL cholesterol paralleled reductions in macrophage cholesteryl esters (Fig. 1) suggesting that the cholesterol released from cells was quantitatively recovered in the nascent HDL density interval (d 1.063–1.21 g/ml). No differences were observed between monocytes and control macrophages with regards to protein/lipid and UC/PL mole ra-

TABLE 1. Chemical composition of nascent HDL complexes isolated from conditioned medium of THP-1 monocytes, macrophages, and macrophage-foam cells

Cell Type	n	Protein	UC	PL	UC/PL Mole Ratio
		% weight distribution			
Monocytes	3	45 ± 4	12 ± 1	43 ± 4	0.59 ± 0.07
Macrophages	6	47 ± 4	12 ± 2	41 ± 4	0.59 ± 0.06
Macrophage-foam cells	7	44 ± 2	16 ± 2[a]	40 ± 3	0.80 ± 0.15[a]

[a] $P < 0.02$ compared to monocytes and macrophages (Student's unpaired *t*-test).

tios of nascent HDL consistent with the similar degree of apoA-I lipid complex formation obtained with these two cellular phenotypes (Fig. 2).

Phospholipid subclass distribution of nascent HDL obtained from monocytes and macrophages

Table 2 shows that there were major differences in phospholipid composition of apoA-I-containing nascent HDL isolated from THP-1 monocytes and macrophages. Nascent HDL obtained from macrophages and macrophage-foam cells had approximately 3.5-fold greater sphingomyelin than that of monocytes. However, cholesterol-loading of macrophages did not alter the phospholipid subclass distribution of nascent HDL, suggesting that increased cholesterol recruitment, per se, was not associated with preferential phospholipid recruitment. The observation that monocyte-derived nascent HDL have a different phospholipid composition from that of macrophages and macrophage-foam cells suggests that differentiation of THP-1 monocytes into macrophages changes the cellular phospholipid pool that is availaible to apoA-I for nascent HDL assembly and cellular cholesterol efflux.

Particle size distribution of nascent HDL subpopulations determined by nondenaturing gradient gel electrophoresis

We have previously shown, using Chinese hamster ovary (CHO) cell cultures, that apoA-I forms nascent HDL complexes where the larger particles (11 and 9 nm) are relatively enriched in cholesterol and the small (7.4 nm) particles are cholesterol-poor (9, 10). To determine whether the increase in cholesterol recruitment from macrophage-foam cells was related, in part, to the assembly of larger-sized nascent HDL, the particle size distribution of apoA-I lipid complexes obtained from monocytes and macrophages was evaluated. **Figure 3A** shows that monocytes incubated with apoA-I produce approximately equal proportions of 11, 9, and 7.4 nm sized particles; however, the particle size distribution was skewed to larger particles with macrophages (Figs. 3B and C). The most pronounced change was seen with macrophage-foam cells where there was a relative predominance of 10.5, 13, and 16 nm sized particles and corresponding reductions in the 7.4 and 9.0 nm subpopulations. These results suggest that nascent HDL particle size distribution is influenced by cellular phenotype where an abundance of larger sized lipid complexes, enriched in cholesterol, are obtained from macrophage-foam cells.

TABLE 2. Distribution of phospholipid subclasses associated with nascent HDL complexes isolated from THP-1 monocytes, macrophages and macrophage-foam cells

Cell Type	n	SM	PC	PE
		% weight distribution		
Monocytes	3	8 ± 1	63 ± 3	29 ± 3
Macrophages	4	29 ± 8[a]	50 ± 3[a]	21 ± 5
Macrophage-foam cells	4	26 ± 8[a]	52 ± 4[a]	22 ± 6

[a] $P < 0.02$ compared to monocytes (Student's unpaired *t*-test).

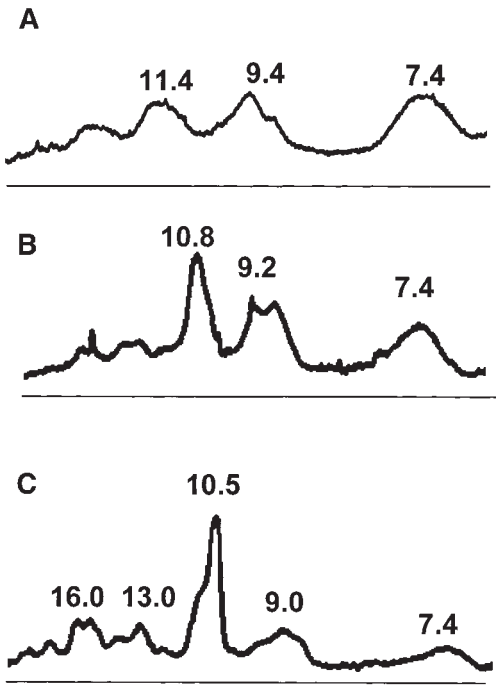

Fig. 3. Particle size distribution of nascent HDL assembly products determined by nondenaturing gradient gel electrophoresis. Nascent HDL (d 1.063–1.21 g/ml) were isolated from conditioned medium of THP-1 monocytes (A), macrophages (B), and macrophage-foam cells (C) which had been incubated with lipid-free apoA-I (10 μg/ml) for 24 h. Shown is a densitometric scan of a representative Coomassie G-250-stained gel. Protein loads were 12 μg/well. Numbers above the peaks are particle diameters in nanometers.

ApoA-I versus apoE assembly products

Differentiated THP-1 macrophages are known to synthesize and secrete apoE (23, 24); whereas, THP-1 monocytes do not. However, it is not known whether macrophage apoE secretion alters the mass and/or particle size distribution of apoA-I nascent HDL particles. Therefore, THP-1 monocyte-derived macrophages and macrophage-foam cells were used to determine the relative mass of apoE and apoA-I associated with nascent HDL complexes. SDS-PAGE analysis (**Fig. 4**) shows that apoA-I was the major apolipoprotein (90%) associated with nascent HDL, and that a smaller proportion (10%) of the apolipoprotein mass was attributed to apoE. No differences were ob-

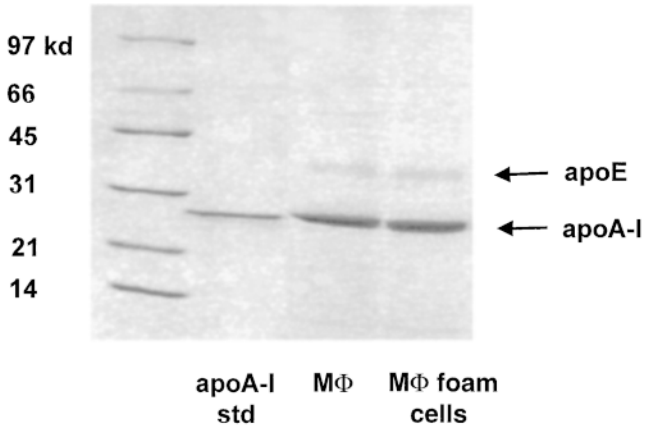

Fig. 4. Distribution of apoA-I and apoE mass on nascent HDL determined by SDS-PAGE. Nascent HDL assembly products were isolated from conditioned medium obtained from macrophages and macrophage-foam cells that had been incubated with lipid-free apoA-I (10 μg/ml) for 24 h. Shown is a representative Coomassie R-250-stained SDS-polyacrylamide gel. Lane 1 contains molecular weight standards; lane 2, lipid free-apoA-I (no cells); lane 3, nascent HDL obtained from macrophages; lane 4, nascent HDL from macrophage-foam cells. Protein loads of nascent-HDL were 5 μg/well for both macrophages and macrophage-foam cells.

served between macrophages and macrophage-foam cells regarding the relative mass ratios of apoA-I and apoE associated with nascent HDL. As apoA-I lipidation was higher (70%) with the macrophage-foam cells (Fig. 2), it is likely that relatively more apoE accumulated in the medium of these cells compared to macrophages thus normalizing the apolipoprotein mass ratios.

The increase in the relative proportions of larger-sized nascent HDL in the medium from macrophages, compared to monocytes (Fig. 3), suggests the assembly of apoE-containing nascent HDL. To test this hypothesis, nascent HDL from macrophage and macrophage-foam cell cultures were subjected to nondenaturing gradient gel electrophoresis followed by Western blot analysis (**Fig. 5**). Three distinct patterns of immunoreactivity toward monoclonal antibodies against apoA-I and apoE were observed with both phenotypes. The 11, 9, and 7.4 nm sized particles were found to possess apoA-I only; whereas, particles banding at 19–20 nm contained only apoE. Particles in the range of 13–16 nm reacted with antibodies to both apoA-I and apoE, suggesting colocalization of these apolipoproteins on similar particles. This latter observation was verified by heparin-Sepharose chromatography where apoE-containing particles that bound to the column possessed apoA-I immunoreactivity (data not shown); thus apoA-I with apoE particles were likely formed. More detailed analyses of particles accumulating in serum-free medium in the absence of apoA-I revealed, however, the assembly of 16–19 nm sized particles, indicating that some apoE-only particles may also have been formed within this size interval (data not shown).

Quantification of apoE accumulating in conditioned medium

As discussed above (Fig. 4), the relative mass ratios of apoA-I and E associated with nascent HDL obtained from macrophages and macrophage-foam cells were similar despite an increase in apoA-I lipidation by the latter (Fig. 2). For this reason, we hypothesized that apoA-I may enhance the extracellular accumulation of apoE from macrophage-foam cells compared to macrophages. Quantification of apoE mass secreted into serum-free medium (**Fig. 6**) revealed that cholesterol-loading of THP-1 macrophages had little effect on medium apoE concentrations consis-

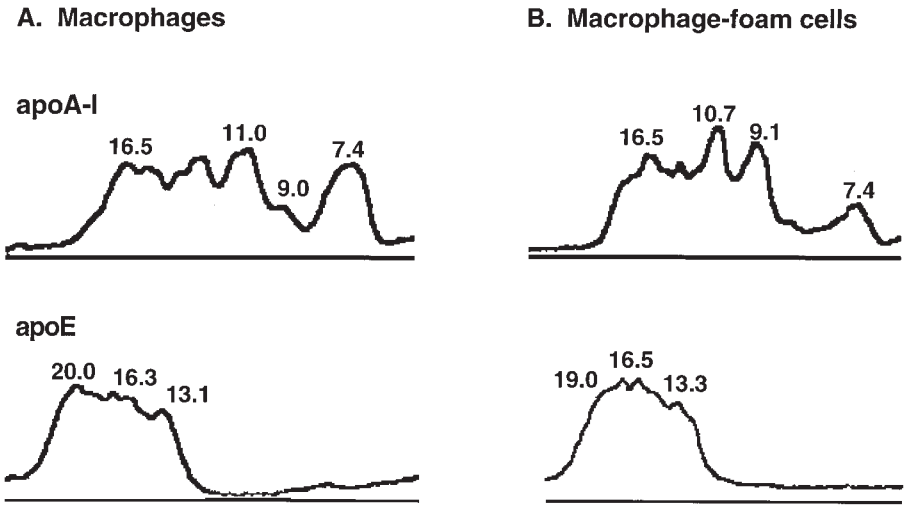

Fig. 5. Distribution of apoA-I- and apoE-specific nascent HDL subpopulations determined by Western blot analysis of nondenaturing gradient gels. Nascent HDL assembly products were isolated from conditioned medium obtained from macrophages (A) and macrophage-foam cells (B) that had been incubated with lipid-free apoA-I (10 μg/ml) for 24 h. Western blot analysis of nondenaturing gradients gels was performed using monoclonal antibodies specific for apoA-I (top scans) and apoE (bottom scans). Shown are densitometric scans of developed blots. Numbers over peaks are particle diameters in nanometers.

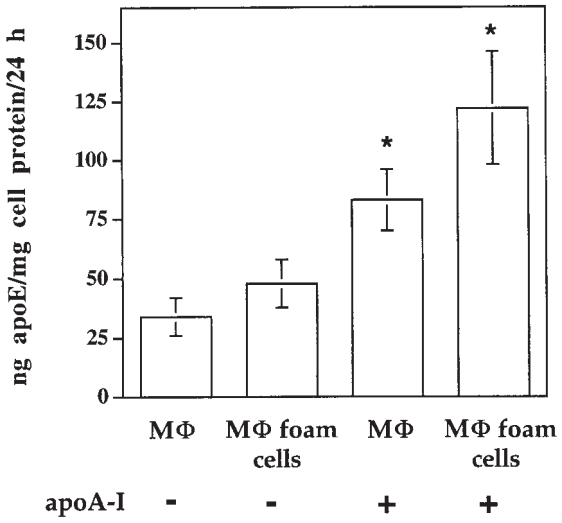

Fig. 6. Quantification of medium apoE mass in conditioned medium from macrophages and macrophage-foam cells. THP-1 macrophages and macrophage-foam cells were incubated with serum-free medium or medium containing lipid-free apoA-I (10 μg/ml) for 24 h. The concentration of apoE in conditioned medium was quantified by ELISA. Values are means ± SEM, n = 6, $*P < 0.05$ compared to incubations without apoA-I.

tent with the results of Banka et al. (23). However, when macrophages and macrophage-foam cells were incubated with apoA-I for 24 h, 2- and 4-fold increases in the extracellular accumulation of apoE were observed, respectively (Fig. 6). This increase in medium apoE mass obtained with macrophage-foam cells, compared to macrophages, paralleled the increase in apoA-I lipid complex formation that was observed with cholesterol loading of these cells (Fig. 2), suggesting that apoE lipidation may also be elevated. In a preliminary study we found that, in the presence of apoA-I, most (>90%) of the medium apoE was in the form of lipidated particles (data not shown). Thus, the lipidation of both apoA-I and apoE is increased in cultures of macrophage-foam cells, compared to macrophages, thereby providing an explanation for the similar apoA-I/E mass ratios of particles isolated from the medium of these two cell phenotypes.

DISCUSSION

Relative roles of apoA-I and macrophage produced apoE in mediating cholesterol recruitment

From the early observation of Basu, Goldstein, and Brown (35) that cholesterol-loaded macrophages secrete apoE, it has been suggested that apoE plays a role in reverse cholesterol transport. Kruth et al. (27) and Zhang, Gaynor, and Kruth (36) later showed that apoE secreted from human monocyte-derived macrophages could mediate the efflux of cellular cholesterol and reduce macrophage cholesteryl ester stores, but this mechanism was slow, requiring several days of incubation. Recent studies with lipid-free apoA-I have also demonstrated cholesterol removal from macrophage-foam cells (11, 12). Because of the high circulating concentration of lipid-free apoA-I, it is conceivable that this apolipoprotein may have a significant role in cholesterol removal from macrophage-foam cells in vivo. The key observation of the present study is that extracellular apoA-I had a greater capacity to remove cholesterol from THP-1 macrophage-foam cells than endogenously produced apoE. Nascent HDL obtained from incubations of apoA-I with macrophages and macrophage-foam cells were mostly apoA-I without E particles with diameters of 11, 9, and 7.4 nm (Fig. 3). These observations strongly suggest that cellular cholesterol efflux mediated by apoA-I is largely a distinct metabolic pathway for reversing macrophage-foam cell phenotype.

In addition to the major subpopulations of apoA-I-only nascent HDL (11, 9 and 7.4 nm sized particles), a small number of relatively larger-sized (13–20 nm) particles were found to possess apoE, and the proportion of these particles increased in response to cholesterol-loading of macrophages. These latter observations are consistent with those of Kruth et al. (27) demonstrating that the endogenous production of apoE can mediate the efflux of cholesterol from human monocyte-derived macrophage-foam cells. Although we have found that apoA-I was the major driving force for cholesterol recruitment from THP-1 macrophage-foam cells, we cannot exclude the possibility that, within the subendothelial space of the artery wall, conditions may exist where the endogenous production of apoE plays a more prominent role in unloading macrophage cholesteryl ester stores. As shown in this study, the presence of apoA-I was associated with an increase in the extracellular accumulation of apoE, suggesting that there may be some interaction between these apolipoproteins in promoting the efflux of excess cholesterol from macrophage-foam cells.

Stimulation of macrophage apoE secretion by apoA-I

The synthesis and secretion of apoE can be regulated by transcriptional as well as post-translational mechanisms. It is known that the differentiation of THP-1 monocytes into macrophages stimulates apoE transcription to maximal levels by 48 h (24). In our system, THP-1 cells were differentiated for 6 days. Thus, it is unlikely that apoA-I stimulated apoE secretion by activating transcriptional mechanisms in the present study. Moreover, Zhang et al. (36) recently found that cholesterol enrichment of human monocyte-derived macrophages with acetylated LDL had no effect on cellular apoE mRNA levels and did not alter the mass of apoE secreted into the extracellular medium. We have obtained similar results with THP-1 macrophages where, in the absence of an extracellular acceptor, cholesterol loading has little effect on the extracellular accumulation of apoE. These observations are consistent with the growing body of evidence that post-translational mechanisms are more important for modulating net output of apoE from macrophages. Indeed, it has been shown that a large pool of newly synthesized apoE is diverted to lysosomes for degradation (37), and that the presence of HDL in the extracellular medium can rescue apoE from degra-

dation (38). In the present study, incubations of macrophages and macrophage-foam cells with apoA-I increased medium apoE concentrations by 2- and 4-fold, respectively, suggesting that the assembly of apoA-I containing nascent HDL may play a role in modulating post-translation secretion of apoE from macrophages. In support of this hypothesis, nondenaturing gradient gel electrophoresis and Western blot analysis identified subpopulations of particles that were composed of both apoA-I and E, suggesting colipidation of these apolipoproteins and/or binding of newly secreted, lipid-poor apoE to apoA-I nascent HDL particles. Ongoing investigations have revealed that most (approx. 90%) of medium apoE is in the form of lipidated particles when apoA-I is present during incubations of macrophage and macrophage-foam cells, whereas, only 10% of the medium apoE is lipidated in the absence of apoA-I (data not shown). Thus, medium apoE accumulation appears to be largely dependent on the formation of apoA-I-containing nascent-HDL.

Recruitment of phospholipid subclasses from THP-1 monocytes and macrophages

An important finding in the present study was that differentiation of monocytes into macrophages was associated with an increase in the availability of cellular sphingomyelin for recruitment by apoA-I. This observation suggests that upon differentiation of monocytes into macrophages there is a fundamental change in the phospholipid subclass distribution of cellular membranes. We have found, in preliminary experiments, that the sphingomyelin content of cellular membranes is 2-fold greater in macrophages compared to monocytes: % weight distribution of SM, PC, PI/PS, PE was 11, 40, 20, 29 for macrophages versus 5, 43, 17, 35 for monocytes, respectively. Whether this increase in membrane sphingomyelin occurs at the level of the plasma membrane and/or within a specific subcellular compartment is unknown at this time. The present study, however, does indicate that cholesterol enrichment of macrophages with acetylated LDL did not alter the distribution of phospholipid subclasses recruited by apoA-I. These findings suggest that specific metabolic pathways of cellular cholesterol recruitment mediated by apoA-I may be "turned-on" upon differentiation of monocytes into macrophages. With cholesterol enrichment of cells, this pathway may be more active thereby facilitating the assembly of nascent HDL particles. This suggestion is supported by our finding that cholesterol enrichment of THP-1 macrophages (i.e., macrophage-foam cells) resulted in an increase in apoA-I lipidation that was accompanied by reductions in cellular cholesteryl ester stores.

Physiological significance

Because lipid-free apoA-I is relatively high in plasma, its distribution in the interstitial space is likely to be equally elevated because of its small molecular radius thus providing a mechanism(s) for apoA-I's antiatherogenic function. The present study suggests that apoA-I may have a direct role in preventing foam cell formation by stimulating the removal of excess cholesterol from macrophages. We have also found, using the THP-1 model, that exogenous addition of apoA-I to cultures of macrophages and macrophage-foam cells enhances the extracellular accumulation of apoE. The ability of apoA-I to enhance the extracellular accumulation of apoE-containing nascent-HDL suggests a coordinate role for both apoA-I and apoE in promoting the unloading of macrophage cholesteryl ester stores. However, in our model, apoA-I pre se has a greater capacity for recruiting cholesterol than apoE. Although speculative, apoA-I may be the driving force for reversing the macrophage-foam cell phenotype.

ApoA-I may also have an indirect role in preventing macrophage-foam cell accumulation. Retention of LDL in the artery wall is thought to be a prerequisite to its oxidation and subsequent induction of inflammatory mechanisms which promote monocyte infiltration into subendothelial spaces and the formation of macrophage-foam cells. Recent reports by Saxena, Ferguson, and Bisgaier (39) and Auerback et al. (40) indicate that apoE-containing plasma HDL may serve a beneficial role by displacing atherogenic lipoproteins, such as LDL and oxidized LDL, from the subendothelial cell matrix. Our observation that apoA-I enhances the net output of apoE from macrophages and macrophage-foam cells suggests that, in addition to direct anti-atherogenic properties of apoA-I, the assembly of apoA-I/apoE nascent HDL particles in the subendothelial space may serve to "flush" the artery wall of atherogenic lipoproteins. Such a mechanism, if occurring in vivo, may reduce the amount of oxidized LDL in the artery wall thereby limiting the subsequent induction of inflammatory events that promote atherogenesis.

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