Apolipoprotein A-I (apoA-I) overexpression inhibits atherogenesis in mice, and apolipoprotein E (apoE) secreted by foam cell macrophages may exert antiatherogenic effects within the arterial wall. We hypothesized that interaction between apoA-I and apoE contributed to the antiatherogenic properties of apoA-I, and therefore investigated whether apoA-I stimulated secretion of apoE by foam cell macrophages. Cholesterol enrichment of primary murine and human macrophages increased spontaneous apoE secretion 2-fold, as quantified by Western blot and chemiluminescence detection. Human apoA-I caused a further marked increase of apoE secretion from both murine (3.8-fold, p < 0.01) and human (3.2-fold, p = 0.01) foam cells in a time- and concentration-dependent manner, and this increase was confirmed by immunoprecipitation of [35S]methionine-labeled macrophage apoE. The protein synthesis inhibitor cycloheximide, but not the transcription inhibitor actinomycin D, markedly inhibited apoE secretion to 70.5% (p < 0.01) and completely suppressed apoE secretion beyond 4 h. Pretreatment of macrophages with Pronase inhibited initial apoA-I-mediated apoE secretion by 70.5 ± 6.5% at 2 h, but by 8 h apoA-I-induced apoE secretion was the same in Pronase-treated and non-pretreated cells. Non-apolipoprotein-mediated cholesterol efflux induced by trimethyl-β-cyclodextrin did not enhance apoE secretion, whereas phospholipid vesicles inducing the same degree of cholesterol efflux substantially enhanced apoE secretion, and apoA-I and phospholipid vesicles in combination demonstrated additive induction of apoE secretion. We conclude that apoA-I concurrently stimulates apoE secretion and cholesterol efflux from foam cell macrophages and that lipoprotein-derived apoA-I may enhance local secretion and accumulation of apoE in atherosclerotic lesions.

Apolipoprotein A-I (apoA-I, \( M_f 28,000 \)) is the major protein component of HDL, and there is increasing evidence that it contributes to a direct antiatherogenic effect of this lipoprotein. For example, mice transgenic for human apoA-I have decreased susceptibility to atherosclerosis (1, 2). Several lines of evidence implicate lipid-poor apoA-I as a particularly important mediator of cholesterol efflux \textit{in vivo}. There are increased concentrations of lipid-poor apoA-I particles in interstitial fluid and lymphatic fluid (3–5); pure apoA-I mediates cholesterol efflux \textit{in vitro}, especially from cholesterol-enriched cells (6–8); and preβ-migrating, lipid-poor HDL species are the major initial acceptors of cellular cholesterol in human plasma (9). ApoA-I has also been identified within atherosclerotic lesions by immunohistochemistry (10).

Apolipoprotein E (apoE, \( M_f 34,000 \)) has major roles in the hepatic clearance of triglyceride-rich lipoproteins, and is commonly isolated from VLDL and HDL fractions, and less commonly from LDL fractions, of human plasma (11–15). ApoA-I is synthesized by the liver and cells of the central nervous system but not by peripheral cells in the arterial wall such as macrophages (16), whereas apoE is also secreted by macrophages, and both synthesis and secretion increase in response to cholesterol accumulation by these cells (17–19). \textit{In vitro}, apoE secreted by macrophages is at least in part associated with cell-derived phospholipid and cholesterol (17, 20, 21). Secretion of apoE by macrophages may thus contribute to spontaneous clearance of cholesterol from macrophages (22), as well as reduce hyperlipidemia and atherosclerosis in apoE knockout (apoE KO) mice transplanted with apoE-secreting macrophages (23, 24). ApoE protein and mRNA are abundant within human atherosclerotic lesions, especially in regions rich in monocyte-derived macrophage foam cells (25). In transgenic mice matched for plasma lipoprotein concentrations, secretion of apoE in the artery wall is associated with a reduction in the extent of atherosclerosis (26, 27). In apoE KO mice, apoE expression by bone marrow transplanted macrophages alters apoE-I distribution and serum HDL concentration (28), and apoE-deficient macrophages increase atherosclerosis in apoE KO mice indicating local arterial apoE secretion may be antiatherogenic (29). Interestingly, very recent studies have suggested that local macrophage apoE secretion may be proatherogenic (30) or antiatherogenic (31). In addition to inducing local homozgyous apolipoprotein E knockout mice; MPM, mouse peritoneal macrophages; HMDM, human monocyte-derived macrophages; AcLDL, acetylated low density lipoprotein; LPDS, lipoprotein-deficient serum; FC, unesterified cholesterol; CE, cholesteryl ester; PLV, phospholipid vesicles; hpiCD, hydroxypropyl-β-cyclodextrin; tmjICD, trimethyl-β-cyclodextrin; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; AU, arbitrary chemiluminescence units; PBS, phosphate-buffered saline; DMEM, Dulbecco's modified Eagle's medium; PAGE, polyacrylamide gel electrophoresis; BSA, bovine serum albumin.
cholesterol efflux (26), apoE secreted by macrophages may have anti-inflammatory and anti-proliferative activity (32, 33).

ApoA-I and apoE are both proposed to have important lipid transfer activities in the central nervous system, and apoE is locally secreted during nerve degeneration and repair (34, 35). Importantly, Boyles et al. (36) demonstrated that apoA-I, apoE, and monocyte-derived foam cell macrophages were all present during remyelination of damaged nerves. A co-operative transfer of lipid from macrophages by apoA-I and its delivery to the remyelinating nerve via apoE was suggested, but a direct mechanism linking exogenous apoA-I and the secretion of apoE was not demonstrated. Similarly, a possible interaction between exogenous apoA-I and macrophage-secreted apoE has recently been hypothesized to inhibit atherogenesis in the vessel wall in vivo (37).

We here demonstrate that there is a unifying mechanism for the co-localization and interaction of exogenous apoA-I and macrophage-secreted apoE, namely that apoA-I stimulates the secretion of apoE from foam cell macrophages. Primary murine and human macrophages secrete increased quantities of apoE in response to cholesterol loading, but, additionally, there is a further marked increase in secretion of apoE in response to incubation with human apoA-I. The apoE secreted in response to apoA-I is only partially derived from a preformed cell surface pool, most secreted apoE requiring de novo protein synthesis. The direct antiatherogenic effects of apoA-I may thus be augmented by the biological activities of locally secreted apoE.

**EXPERIMENTAL PROCEDURES**

**Reagents—**Dulbecco’s modified Eagle’s medium (DMEM), RPMI 1640, and L-glutamine were supplied by Trace Biosciences, and phosphate-buffered saline (PBS) and penicillin/streptomycin by Sigma. Dr. George Setlow’s yeast culture (5) containing <24 h ex vivo) and human serum were kindly provided by the New South Wales Red Cross Blood Transfusion Service, Sydney, Australia. All solvents were high-performance liquid chromatography (HPLC) grade (Mallinckrodt). Monoclonal mouse anti-human antibodies to human apoE were obtained from Roche Molecular Biochemicals, polyclonal goat antibodies to human apoE were obtained from Fitzgerald, and polyclonal rabbit anti-mouse antibodies to mouse apoE were obtained from BioDesign (Kennewick, ME). Secondary species-specific horseradish peroxidase-linked antibodies, nitrocellulose membranes (0.45 μm), and enhanced chemiluminescence reagents and film were obtained from Amersham Scientific (Australia). Phospholipid vesicles (PLV) were prepared from phosphatidyicholine (Sigma P6-385) by sonication as described (38), and hydroxypropyl-β-cyclodextrin (hpβCD) and trimethyl-β-cyclodextrin (tmβCD) were obtained from Aldrich Chemicals. Pronase, heparinase, cycloheximide, and actinomycin D were supplied by Sigma. [35S]Methionine was supplied by ICN Chemicals.

**LDL Preparation—**Human LDL (1.02<d<1.05) and lipoprotein-deficient serum (LPDS, d<1.25 g/ml) were isolated from healthy, fasting volunteers in the presence of EDTA, aprotinin, and soybean trypsin inhibitor (all reagents from Sigma) by discontinuous density-gradient ultracentrifugation, dialyzed, filtered, and stored at 4 °C under N2 in EDTA and chloramphenicol as described previously (39).

**Preparation of Human Apolipoprotein A-I—**Purified human apoA-I was isolated by FPLC, delipidated, lyophilized, and stored at −20 °C prior to reconstitution as described previously (40, 41). The purity of each preparation was confirmed on SDS-PAGE by detection of a single band of molecular mass 29 kDa, and confirmed by Western blotting to cross-react with antibodies to human or murine apoE.

**LDL Acetylation—**LDL was acetylated (AcLDL), excess reagents removed by dialysis against Chelex-100-treated PBS containing chloramphenicol (0.1 mg/ml), and acetylation assessed using non-denaturing agarose gel electrophoresis on 1% Universal Agarose gels (Ciba-Corning) in Tris-barbitone buffer (pH 8.6) at 90 V for 45 min, as described (9). The LDL band was visualized with Fat Red 7B stain. A relative electrophoretic mobility of >5, using native LDL as a reference, was routinely obtained.

**Isolation and Culture of Macrophages—**Homozygous apoE-deficient (apoE KO)-C57BL/6J mice and QS mice were provided by the Biological Facility of the Heart Research Institute. ApoE KO mice (males and females; 6–8 weeks old) were derived from initial breeding pairs supplied by Jackson Laboratories (Bar Harbor, ME; Ref. 42). Resident mouse peritoneal macrophages (MPM) were isolated by lavage (43) and plated at 5–6 × 106 cells/35-mm2 tissue culture wells, incubated at 37 °C for 1–2 h to establish adherence, then washed with prewarmed PBS. Adherent cells were incubated for 24 h with DMEM containing LPDS (10% v/v), final protein concentration 2.5 mg/ml, penicillin G and streptomycin (50 units/ml and 50 mg/ml, respectively), 1% (2 mg/ml), and AcLDL (50 μg of protein/ml) to achieve enrichment with cholesterol (FC) and cholesterol esters (CE) (8, 41, 44, 45).

Human macrophones (HMCs) were isolated from white cell concentrates using centrifugal ultracentrifugation as described (46, 47). Purified macrophones (>95% purity by nonspecific esterase staining) were differentiated by plating at a density of 1.5 × 105 cells/22-mm2 culture dish (Costar) containing 10% v/v culture medium containing 10% (v/v) heat-inactivated whole human serum for 9 days. Following differentiation, the cells were washed and incubated with RPMI 1640 containing 10% LPDS (v/v) and AcLDL (50 μg protein/ml) for 4 days to achieve cellular enrichment with FC and CE.

In specified experiments, cells were pretreated with cycloheximide (2.0 μg/ml), actinomycin D (1.0 μg/ml), heparinase 1 (3 units/ml), and actinomycin D exposures were continued during efflux incubations, whereas Pronase and heparinase were removed and cells washed before incubation with efflux medium. The functional activity of heparinase exposure was separated and confirmed for each experiment by measuring the rate of formation of unsaturated β-ornidols generated during the degradation of heparin, detected by absorbance at 232 nm (48).

**Sterol Efflux and ApoE Secretion from Macrophages—**Macrophages that had been incubated with LDL (or control medium without AcLDL) for 24 h (MFM) or 96 h (HMDM), were washed in warm PBS and incubated for another 24 h in 1.0 ml of efflux medium comprising DMEM (MFM) or RPMI (HMDM) with or without apoA-I (0–25 μg/ml) as described (8, 41, 44, 45). To evaluate physicochemical cholesterol efflux, HMDM were incubated with AcLDL as above and then incubated in efflux medium comprising RPMI with 1.0 mg/ml hpβCD, 1.0 mg/ml tmβCD, or 20–400 μg/ml PLV for 24 h. (In comparative experiments, efflux media containing 1.0 mg/ml bovine serum albumin (BSA), Sigma) resulted in cholesterol efflux, apoE secretion, and cell viability in HMDM was separated and confirmed for each experiment by measuring the rate of formation of unsaturated β-ornidols generated during the degradation of heparin, detected by absorbance at 232 nm (48).

**Analysis of FC and CE—**Aliquots of cell suspensions and of efflux media were placed into separate glass Kimble tubes (Kimble, MA), made up to a final aqueous volume of 1.0 ml with ice-cold PBS containing butyalted hydroxytoluene and EDTA, and the total extracted with methanol (2.5 ml) and then hexane (5 ml) as described (39). 4 ml of the hexane layer was evaporated to dryness, redissolved in mobile phase, and analyzed by HPLC as described below (39).

**Quantitation of Apolipoprotein E in Cell Lysates and Efflux Media by Western Blot—**Aliquots (40 μl) of cell culture medium were mixed with sample buffer (20 μl) containing 26.7 m Tri-HCl (pH 6.8), 2% (w/v) SDS, 133 mM sucrose, 0.01% (w/v) bromphenol blue, 0.67 mM EDTA, reduced with 10 mM dithiothreitol and heated to 100 °C for 5 min. Cell
Apolipoprotein A-I-stimulated Secretion of Apolipoprotein E

Apolipoprotein A-I-stimulated Secretion of Apolipoprotein E from Primary Human Monocyte-derived Macrophages

Apolipoprotein A-I-stimulated Secretion from Primary Human Monocyte-derived Macrophages (HMDM)—Human and murine cells differ in their accumulation, metabolism, and efflux of cholesterol (22, 54–58), and there are significant differences between the amino acid composition of human and rodent apoE (59–61). Consequently, it was important to ensure...
that apoA-I-mediated apoE secretion was applicable to human macrophage foam cells and human apoE.

As with MPM, cholesterol enrichment caused a clear increase in the secretion of apoE by HMDM, and apoA-I caused a further marked increment in the secretion of apoE while simultaneously promoting cholesterol efflux (Fig. 5). In multiple experiments using monocytes isolated from different donors, the mass of apoE secreted by cholesterol-enriched HMDM under control conditions (i.e. without apoA-I) ranged between 0.5–3.0 μg/mg of cell protein/24 h. However, apoA-I further enhanced apoE secretion from cholesterol-enriched cells 2–6-fold (3.2 ± 0.6, mean ± S.E., n = 6 experiments, p = 0.01), regardless of the mass of apoE secreted under control conditions. In contrast, when added to HMDM that had not been cholesterol enriched, there was modest (as seen in Fig. 5) and inconsistent (over multiple experiments) stimulation of apoE secretion and cholesterol efflux by apoA-I.

ApoA-I induced a time- and concentration-dependent secretion of apoE from HMDM. As with MPM, total secretion of apoE was maximal at 5 μg/ml apoA-I and continued to increase over 24 h (Fig. 6). Unlike MPM, however, there was no detectable depletion in cell-associated apoE after exposure of HMDM to apoA-I (data not shown). ApoA-I always induced less percentage of cholesterol efflux from cholesterol-enriched HMDM than from cholesterol-enriched MPM (Fig. 3, MPM 53% efflux versus Fig. 5, HMDM 16.8% efflux), and induced negligible efflux from non-loaded HMDM. This is consistent with the known relative resistance of human macrophages to apoA-I-mediated cholesterol efflux (56, 58, 62, 63), and may explain the lack of depletion of the cell-associated apoE pool in HMDM after exposure to apoA-I (see “Discussion”).

ApoA-I Induces the Secretion of de Novo Synthesized ApoE—In order to establish if apoA-I simply mediated the displacement of preformed, cell surface-associated apoE a series of experiments were undertaken. Cholesterol-enriched HMDM and MPM were metabolically labeled with [35S]methionine during efflux incubation with apoA-I or control medium, and apoE secreted into efflux medium was measured by immunoprecipitation and quantification of 34-kDa bands after SDS-PAGE and phosphorimaging (Fig. 7 after 24 h of efflux). At all time points up to 24 h, apoA-I achieved greater secretion of [35S]methionine-labeled apoE secretion than control medium, and the amount of apoE secreted to apoA-I increased progressively with the duration of incubation. The protein synthesis inhibitor cycloheximide almost completely inhibited secretion of [35S]methionine-labeled apoE secretion compared to control medium. To confirm that protein synthesis was critical for the increment in total apoE in response to apoA-I exposure, and not only relevant to a potentially small pool which was labeled with [35S]methionine, HMDM were incubated with cycloheximide for 1 h prior to efflux (to ensure inhibition of protein synthesis at beginning of incubation with apoA-I), as well as during efflux to apoA-I, and apoE quantified by Western blot (Fig. 8). There was very rapid and marked inhibition of apoE secretion to apoA-I, which, over five independent experiments using both MPM and HMDM, was always significantly inhibited by 4 h (inhibited by 73.1 ± 9.8% at 4 h), and beyond 4 h there was almost complete inhibition of apoE secretion to apoA-I and control medium (data not shown) without significant impairment of cholesterol efflux. Thus, ongoing de novo apoE synthe-
with or without 50 % serum, washed and incubated for 96 h in RPMI containing 10% LPDS with or without 50 μg/ml AcLDL (± AcLDL loading), then washed and incubated for 24 h in RPMI with or without 25 μg/ml apoA-I (± apoA-I).

Concentrations of apoE (panel A) and cholesterol (panel B) in efflux medium were calculated per milligram of cell protein and were determined as described under “Experimental Procedures.” Percentage of cholesterol efflux corresponding to each condition from left to right were: 16.8 ± 0.98%; 8.8 ± 1.8%; 6.52 ± 1.5%, and 7.4 ± 1.5%, respectively.

Apoptosis contributes very substantially to the pool of apoE secreted to apoA-I.

Actinomycin D, a transcription inhibitor, exerted a much more gradual and less significant effect on apoE secretion. In experiments evaluating earlier time points from 2 to 16 h, identical apoA-I-mediated apoE secretion was achieved with and without actinomycin D (data not shown). By 24 h, actinomycin inhibited apoE secretion to apoA-I by only 21.3% (apoA-I alone 361 ± 36.5 ng of apoE/mg of cell protein, apoA-I + actinomycin D 284 ± 20.1 ng of apoE/mg of cell protein). These data indicate that although most apoE released to apoA-I requires active protein synthesis, it is unlikely to be meditated by acute up-regulation of mRNA transcription by apoA-I.

To evaluate the quantitative importance of the cell surface pool of apoE (both total and proteoglycan-bound) to apoA-I-mediated secretion, macrophages were pretreated with Pronase or heparinase, respectively (Fig. 9). As our studies (Figs. 2 and 3) had shown that MPM showed most evident depletion of cell apoE after exposure to apoA-I, we hypothesized that MPM were most likely to demonstrate a significant cell surface pool of apoE, which may be displaced by apoA-I. Heparinase treatment had little effect on total cell-associated apoE and did not interfere with apoA-I-mediated apoE secretion in MPM (Fig. 9, A and C) or HMDM (data not shown). In contrast, Pronase pretreatment depleted total cell-associated apoE by 64.0 ± 6.5% compared with control incubation, and reduced secretion of apoE in response to apoA-I (Fig. 9B) over the first 5 h.

However, by 8 h there was identical apoE secretion to apoA-I media from cells that had or had not been exposed to Pronase. ApoA-I-mediated apoE secretion from HMDM was not significantly different with or without Pronase exposure (data not shown).

Differential Effect of ApoE Secretion upon Basal Cholesterol Efflux and ApoA-I-induced Cholesterol Efflux—Expression of human apoE in murine J774 cells was reported to increase cholesterol efflux to control medium and to HDL (64), suggesting that apoE could play a role in cholesterol efflux induced by other acceptors. We therefore investigated whether the extent of apoA-I-mediated cholesterol efflux was affected by its stimulation of cellular apoE secretion by comparing cholesterol efflux from AcLDL-loaded apoE-secreting C57/BL6 macrophages to that from apoE KO C57/BL6 macrophages. ApoE-secreting MPM spontaneously released more cholesterol to apoA-I-free medium than did apoE KO cells (8.5 ± 0.9% versus 1.3 ± 0.2% cholesterol efflux, respectively; p = 0.005). However, cholesterol efflux to medium containing apoA-I (25 μg/ml) from each of the macrophage types was very similar (48.4 ± 3.5% and 53.3 ± 2.0%, respectively; p = NS). Thus, although our data confirmed that constitutive apoE secretion and cholesterol efflux from macrophages may be closely linked (21, 22), apoA-I-mediated cholesterol efflux, at optimal concentrations,
ApoA-I induces secretion of \[^{35}\text{S}\]methionine-labeled apoE. Differentiated MPM (panel A) and HMDM (panel B) were cholesterol enriched by incubating with 50 \(\mu\text{g/ml}\) AcLDL, washed in methionine-free DMEM, and then incubated for 24 h in methionine-free DMEM containing 50 \(\mu\text{Ci}[^{35}\text{S}\]methionine/ml, with or without 25 \(\mu\text{g/ml}\) apoA-I (\(\pm\) A-I), with or without 2.0 \(\mu\text{g/ml}\) cycloheximide (\(\pm\) Cy) as described under “Experimental Procedures.” \[^{35}\text{S}\]Methionine-labeled apoE secreted into the medium was immunoprecipitated and subjected to SDS-PAGE electrophoresis and the 34-kDa band corresponding to apoE quantified by phosphorimager (photostimulated luminescence, expressed as AU/mg of cell protein, mean \(\pm\) S.D.) from one experiment representative of two. Insets show \[^{35}\text{S}\]methionine 34-kDa bands detected by phosphorimager from each of two cell cultures \(\pm\) apoA-I after 24 h in efflux medium.

Two Different Vehicles of Physicochemical Cholesterol Efflux Exert Differential Effects on ApoE Secretion—Whether apoA-I-mediated stimulation of apoE secretion could be explained by its stimulation of cholesterol efflux per se, independent of any apolipoprotein-specific or receptor-specific effect (9, 65) was investigated by comparing it to the effects of efflux to PLV (66) and \(\beta\)-cyclodextrins (67) (Fig. 10).

As described previously, at low concentrations (1.0 mg/ml) hydroxypropyl-\(\beta\)-cyclodextrin did not induce net cholesterol efflux (41, 67), whereas trimethyl-\(\beta\)-cyclodextrin did induce significant cholesterol release (67). At the low concentrations used, \(\beta\)-cyclodextrin-mediated cholesterol efflux is not associated with significant net mass release of cell phospholipid (67). Despite the clearly different extent of cholesterol release with the two cyclodextrins, apoE secretion was not enhanced by either agent. In contrast, PLV inducing the same cholesterol efflux as trimethyl-\(\beta\)-cyclodextrin caused a marked increase in apoE secretion. It was concluded that cholesterol efflux did not in itself necessarily stimulate apoE secretion from macrophages, and that additional properties of the efflux-inducing agent may contribute to such stimulation.

It is well known that apoA-I induces phospholipid efflux from MPM during cholesterol efflux (7). We have confirmed these observations (68), and very recently, this has been shown to be associated with apoE secretion from THP-1 cells (69). In cholesterol-enriched HMDM, we have recently identified that <5.0 \(\mu\text{g}\) of phospholipid is released/ml of apoA-I-containing efflux medium during 24 h of cholesterol efflux.\(^{3}\) To investigate whether the amount of PL solubilized by apoA-I could completely explain the effect of apoA-I upon apoE secretion from HMDM, we exposed HMDM to 20 \(\mu\text{g/ml}\) PLV (4-fold the mass of phospholipid removed by apoA-I during cholesterol efflux), apoA-I 10–25 \(\mu\text{g/ml}\), or both (without prior generation of apoA-I-phospholipid discs) and quantified apoE secretion. We found an additive interaction of PLV and apoA-I on apoE secretion (Fig. 11) with on average a 2.3-fold (\(p < 0.01\)) greater secretion of apoE achieved by the combination of apoA-I and PLV than either alone. This indicates that there are complementary mechanisms by which PLV and apoA-I mediate apoE secretion, and that apoA-I-mediated apoE secretion is unlikely to be completely explained by apoA-I-mediated solubilization of cell phospholipid.

**DISCUSSION**

To our knowledge, this is among the first mechanistic studies of how one apolipoprotein can stimulate the secretion of another apolipoprotein from macrophages. It provides a mechanism for a direct interaction between lipoprotein-derived apoA-I and the local secretion of apoE in various tissues, in-
cluding atherosclerotic tissue in the vessel wall, and in the central and peripheral nervous system. This process is likely to be most important in sites accessible to apoA-I and normally inaccessible to large whole VLDL or HDL, which are the major carriers of apoE in plasma.

ApoA-I is more likely to achieve proximity to foam cells in atherosclerotic lesions than are larger intact lipoproteins. ApoA-I-containing particles isolated from intima are generally smaller than plasma HDL (3) and as lipid-poor apoA-I is abundant at the more dilute lipoprotein concentrations present in interstitial fluid (4, 5, 70, 71), apoA-I-mediated stimulation of apoE secretion is likely to occur in the vessel wall. Our observations for murine macrophages from two strains of mice (QS and C57/Bl6) and human macrophages derived from multiple monocyte donors suggests this is not a process specific to one apoE phenotype or species, but relevant to all apoE-secreting primary macrophages. Stimulation of apoE secretion by apoA-I was much more marked in cholesterol-enriched cells than in control cells, and cellular cholesterol enrichment is known to increase apoE synthesis (18). Thus, this property of apoA-I can be expected to be most relevant in the presence of cholesterol-rich foam cell macrophages such as those in atherosclerotic lesions. This may also apply during nerve regeneration, where prior increased synthesis of apoE in cholesterol-enriched foam cells would allow optimal stimulation of apoE secretion during cholesterol efflux mediated by apoA-I.

The ability of apoE to associate with HDL species has been characterized using large HDL particles in whole serum (e.g. Refs. 72 and 73). [35S]Methionine labeling of cells can only detect [35S]methionine incorporated into recently synthesized apoE, and cannot detect preformed cellular apoE. Previous studies of HDL and its effects on secretion of [35S]methionine-labeled apoE have provided conflicting results. In one study, HDL decreased apoE synthesis and secretion (18). In another, HDL increased apoE secretion and this could be prevented by...
tetranitromethane modification of HDL without affecting efflux of cholesterol, implying the two processes are quite separate (74), as previously suggested (75). By determining total mass cholesterol efflux and total apoE secretion, we have demonstrated clear net stimulation of apoE secretion by apoA-I. We have also confirmed our observations using [35S]methionine-labeled apoE, indicating that because apoE turnover is rapid, net mass apoE secretion and labeled apoE secretion generate qualitatively similar data. The correlated kinetics of cholesterol efflux and apoE secretion (Figs. 4 and 6) are consistent with a temporal link between the two processes, but as seen with our data using cycloheximide (Figs. 7 and 8) and cyclo-dextrin-mediated cholesterol efflux (Fig. 10), this need not be a mechanistic link.

Our findings with cycloheximide, [35S]methionine metabolic labeling, and Pronase indicate preformed cell surface pools and de novo synthesis during efflux may both contribute to apoE secretion induced by apoA-I. Initially, some apoE secreted to apoA-I from MPM is cell surface-derived and/or Pronase-sensitive, but is not bound to the cell surface by a heparinase-apoA-I receptor or binding protein via which apoE secretion is increased substantially between 8 and 24 h of exposure to apoA-I, murine macrophages, we observed that apoE secretion in these primary murine cells, as distinct from the reported effects in cell lines such as J774 cells (64).

The enhanced secretion of apoE was associated with a clear depletion of cell-associated apoE in MPM but not HMDM, even though apoA-I induced substantial apoE secretion by both cell types. Depletion of cell cholesterol (by HDL or apoA-I) would be expected to decrease apoE synthesis (18, 19). Assuming similar intracellular turnover of apoE in HMDM and MPM, the selective depletion of MPM-associated apoE by apoA-I may be explained by the greater depletion of cholesterol from MPM than HMDM and therefore greater continued apoE synthesis in the latter.

As there is not significant re-uptake of secreted apoE by macrophages (79), our observations with actinomycin D and cycloheximide are most consistent with apoA-I-stimulated apoE secretion being a posttranscriptional regulatory effect (74). Glycosylation to the higher Mr form of apoE occurs at the trans-Golgi network, therefore regulation of the secretion or degradation of high Mr apoE as others (80–82) have observed is likely to occur in a post-Golgi compartment. We postulate that during apoA-I-mediated cholesterol efflux apoA-I induces local alterations of the plasma-membrane to redirect glycosylated apoE away from normal lysosomal or non-lysosomal degradation (83) and toward surface-connected compartments (82, 84), from which it can be secreted into the medium. Whether apoA-I induces a receptor-mediated stimulation of secretion or promotes a physicochemical solubilization of cell surface apoE is the subject of ongoing investigation.

Acknowledgment—We thank Hannah Nicholas for excellent technical assistance.

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