Apolipoprotein E Produced by Human Monocyte-derived Macrophages Mediates Cholesterol Efflux That Occurs in the Absence of Added Cholesterol Acceptors

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Human monocyte-derived macrophages can efflux accumulated cholesterol without exogenously added cholesterol acceptors (Kruth, H. S., Skarlatos, S. I., Gaynor, P. M., and Gamble, W. (1994) J. Biol. Chem. 269, 24511–24518). Most of the effluxed cholesterol accumulates in the medium as apolipoprotein E-discoidal lipid particles. In the current study, we determined whether and to what degree cholesterol efflux from human monocyte-macrophages depended on apolipoprotein E secretion. Unexpectedly, 2-week-old differentiated monocyte-macrophages secreted similar amounts of apolipoprotein E without or with cholesterol enrichment. Apolipoprotein E mRNA levels in these macrophages were not increased by cholesterol enrichment and were comparable with levels in HepG2 cells. Without cholesterol enrichment, monocyte-macrophages secreted lipoprotein E with a density >1.21 g/ml. By contrast, cholesterol enrichment of monocyte-macrophages induced the association of apoE with phospholipid and cholesterol to form discoidal particles that floated at densities of 1.08–1.10 g/ml. An anti-apolipoprotein E monoclonal antibody added to the culture medium significantly inhibited cholesterol and phospholipid efflux from the monocyte-macrophages. This showed that apolipoprotein E was required for most of the cholesterol efflux, and that apolipoprotein E did not leave macrophages with lipid but rather associated with lipid after it was secreted. Thus, 1) apolipoprotein E was constitutively secreted by differentiated human monocyte-macrophages, 2) apolipoprotein E only formed discoidal particles following macrophage cholesterol enrichment, 3) apolipoprotein E was necessary for cholesterol efflux to occur in the absence of added cholesterol acceptors and, in addition 4) the level of macrophage unesterified cholesterol was not rate-limiting for this cholesterol efflux, and 5) net phospholipid synthesis occurred in macrophages secondary to apoE-mediated loss of macrophage phospholipid. In conclusion, apolipoprotein E functions in an autocrine pathway that mediates cholesterol efflux from human monocyte-derived macrophages.

Apolipoprotein (apo) E shows an anti-atherogenic effect by facilitating clearance of atherogenic remnant lipoproteins from the plasma (1, 2). Recent studies suggest that apoE may be anti-atherogenic by additional mechanisms. Transgenic expression of apoE restricted to the blood vessel wall (3) or to macrophages (including those in the vessel wall) (4) inhibits development of atherosclerotic lesions in mice, even without altering plasma cholesterol levels. This suggests that expression of apoE locally within the vessel wall is sufficient to produce an anti-atherogenic effect.

One mechanism by which locally produced apoE could be anti-atherogenic is by promoting reverse cholesterol transport from the vessel wall. This could occur through the association of apoE with plasma-derived high density lipoprotein (HDL) which increases HDL’s capacity to carry cholesterol (5, 6). On the other hand, it is possible that apoE could affect reverse cholesterol transport independent of plasma-derived HDL.

We have observed that differentiated human monocyte-macrophages effluxed accumulated cholesterol even when these macrophages are incubated in basal medium without any added serum components or HDL that could function as cholesterol acceptors (7). Most of this effluxed cholesterol accumulates in the medium within apoE-discoidal lipoprotein particles. However, it has not been determined whether and to what degree cholesterol efflux depends on apoE secretion by human monocyte-macrophages. Would this macrophage self-generated cholesterol efflux continue normally if apoE function was impaired?

The cholesterol efflux that occurs from human monocyte-macrophages in the absence of serum or added lipoproteins could be independent of apoE. Some cholesterol effluxed by human monocyte-macrophages is contained within vesicular lipoproteins. These vesicular lipoproteins have a 22,000-Da protein that is not related to apoE (7). In addition, under certain conditions, release of multilamellar lipid particles can be a pathway for cholesterol excretion from macrophages (7–9).

Because of the presence of other possible pathways for macrophage cholesterol excretion, it is not clear to what extent apoE secretion is necessary for cholesterol excretion from human monocyte-macrophages. Also, the relationship between apoE secretion and discoidal particle production in human monocyte-macrophages has not been examined. Thus, the purpose of this study was 1) to determine the importance of apoE in mediating cholesterol efflux from these cells, and 2) to determine the relationship between apoE secretion and production of apoE-discoidal particles.

MATERIALS AND METHODS
Incubation of Monocyte-derived Macrophages with AcLDL and Micocrystalline Cholesterol—Human monocyte-derived macrophages

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1 The abbreviations used are: apo, apolipoprotein; HDL, high density lipoprotein; AcLDL, acetylated low density lipoprotein; ACAT, acyl-CoA:cholesterol acyltransferase; ELISA, enzyme-linked immunosorbent assay; CAPS, 3-(cyclohexylamino)propanesulfonic acid; MOPS, 4-morpholino-propanesulfonic acid.
were cultured and incubated with human acetylated low density lipoprotein (AcLDL) or microcrystalline cholesterol, and analyzed for lipids as described previously (7).

**Density Gradient Analysis of ApoE in Culture Medium**—Following incubations, a total of 12 ml of culture medium was collected from a six-well cluster dish for each condition and centrifuged at 1000 × g for 25 min. This and subsequent procedures were carried out at 4 °C. Centrifugation removed any floating cells and some of the microcrystalline cholesterol that remained after incubations. The supernatant was then passed through low protein-binding polysulfone 0.45-μm (pore size) filters (Gelman Sciences, Ann Arbor, MI) to remove any remaining cells or microcrystalline cholesterol. The filtrate was concentrated to 4 ml in an Amicon (Danvers, MA) stirred cell with a 10,000 molecular weight cutoff cellulose filter. Then, the concentrated filtrates were dialyzed against two changes of 0.15 M NaCl, containing 0.02% sodium azide and 0.1% disodium EDTA (pH 7.4). Recovery of cholesterol and apoE following concentration and dialysis was >80%.

Isoelectric density gradient centrifugation of the filtered and concentrated culture medium was carried out as described previously (7).

**Polyacrylamide Gel Electrophoresis and Immunoblot Analysis of ApoE**—Samples of culture media and media were analyzed on 7.5 cm × 10-cm, 10% acrylamide gels run on a Mini-PROTEAN II cell (Bio-Rad). Samples run on this system were delipidated and concentrated with chloroform/methanol according to the procedure of Wessel and Flugge (10). Then, samples were dissolved in treatment buffer consisting of 62.5 mM Tris-HCl (pH 6.8), 10% glycerol, 5% SDS, 2.5% β-mercaptoethanol, and 0.01% bromphenol blue, and run according to Laemmli (11). Blotting onto nylon membranes was carried out with a Mini Trans-Blot electrophoretic transfer cell (Bio-Rad) with 10 mM 2.5% dithiothreitol, and 0.01% bromphenol blue and run according to Laemmli (11). Blotting and detection were carried out with a chemiluminescence immunoblotting kit (Schleicher & Schuell) using affinity-purified, alkaline phosphatase-conjugated, goat anti-mouse IgG and Linumegen PPD (4-methoxy-4-[3-[3-phosphatethylnyl]spiro][1,2-dioxetane-3,2-

**Northern Blot Analysis of ApoE mRNA**—A full-length apoE cDNA clone was used as a template to prepare sense and anti-sense apoE riboprobes labeled by in vitro transcription with digoxigenin-11-UTP using SP6 and T7 RNA polymerases (Boehringer Mannheim). A riboprobe to detect glyceraldehyde-3-phosphate dehydrogenase mRNA was prepared from a 1.0-kilobase pair cDNA fragment of glyceraldehyde-3-phosphate dehydrogenase attached to a T7 RNA polymerase promoter (Clontech, Palo Alto, CA). The RNA blot was prehybridized for 3 h at 68 °C in 20 ml of prehybridization solution (formulated as specified in the Genius System User's Guide for filter hybridization, Boehringer Mannheim). The blot was hybridized overnight in the prehybridization solution (68 °C) containing probes at 30 ng/ml. Then, chemiluminescence detection of the RNA blot was carried out according to the user's guide provided by Boehringer Mannheim.

### RESULTS

**Monocyte-derived Macrophages Constitutively Secreted ApoE**—Previous investigation has shown that cholesterol accumulation by macrophages can induce synthesis and secretion of apoE (12–14). However, human monocyte-macrophages (2-week-old cultures) incubated with AcLDL (100 μg/ml) for 3 days showed no significant difference in their secretion of apoE compared with monocyte-macrophages that were not incubated with AcLDL (Table I). Monocyte-macrophages incubated with microcrystalline cholesterol (130 nmol/ml) secreted only slightly more apoE (a 15% increase) into the culture medium over a 3-day incubation period than did control monocyte-macrophages. ApoE secretion following enrichment of monocyte-macrophages with cholesterol was also examined (as opposed to apoE secretion during cholesterol enrichment). Monocyte-macrophages incubated with AcLDL (25 μg/ml) for 2 days showed equivalent amounts of apoE secretion during a 4-day postincubation period in RPMI 1640 medium (Table II). During this period of postincubation, cholesterol-enriched macrophages excreted over 3-fold more cholesterol than control macrophages excreted.

It was previously reported that an increase in apoE secretion induced in mouse macrophages by cholesterol enrichment was mediated in part by an increase in apoE mRNA (13, 14). Therefore, we examined whether cholesterol enrichment may have substantially increased apoE mRNA without producing any large increase in apoE secretion. Cholesterol enrichment of monocyte-macrophages did not increase apoE mRNA levels compared with those levels in control monocyte-macrophages (Fig. 1). Furthermore, the apparent level of apoE mRNA in monocyte-macrophages was as high as the level detected in HepG2 cells. Thus, apoE mRNA and apoE secretion were almost equally expressed in the differentiated human monocyte-macrophages. The difference between these results and those of previous studies (12–14) is likely due to the extent of differentiation of the monocyte-macrophages (differentiation in-

### TABLE I

| Incubation condition | Cholesterol | ApoE |
|----------------------|-------------|------|
|                      | Unesterified | Esterified | Total |
| Exp. 1                |             |             |       |
| AcLDL                | 54 ± 1      | 3 ± 1       | 57 ± 2 |
| + AcLDL              | 90 ± 7      | 73 ± 13     | 163 ± 20 |
| Exp. 2                |             |             |       |
| cholesterol          | 54 ± 4      | 3 ± 0       | 57 ± 4  |
| + cholesterol        | 70 ± 2      | 31 ± 5      | 101 ± 3 |

Note: Results are expressed as means ± S.E. of triplicate cultures. Items in brackets indicate statistical significance assessed by Student's t-test. NS, not significant.
**TABLE II**

| Duration of efflux | ApoE in medium | Cholesterol in medium |
|-------------------|----------------|-----------------------|
|                   | -AcLDL | +AcLDL | -AcLDL | +AcLDL |
| days              | µg/mg cell protein | nmol/mg cell protein | µg/mg cell protein | nmol/mg cell protein |
| 1                 | 3.8 ± 0.3 | 3.9 ± 0.4 | 3.2 ± 0.5 | 11.7 ± 0.7 |
| 2                 | 7.3 ± 0.8 | 6.8 ± 0.1 | 3.3 ± 1.2 | 18.5 ± 1.3 |
| 3                 | 9.3 ± 0.7 | 8.5 ± 0.6 | 8.8 ± 1.1 | 28.6 ± 1.0 |
| 4                 | 12.5 ± 1.1 | 12.8 ± 0.5 | 11.3 ± 0.9 | 36.9 ± 3.6 |

**FIG. 1. Comparison of apoE mRNA content of normal and cholesterol-enriched human monocyte-macrophages.** Two-week-old monocyte-macrophage cultures were incubated for 3 days in 2 ml of RPMI 1640 medium either without (-AcLDL) or with 25 µg/ml AcLDL (+ AcLDL) for 2 days. The cellular cholesterol levels of monocyte-macrophage cultures incubated without and with AcLDL for 2 days were, respectively: unesterified cholesterol, 47 ± 1 and 64 ± 1; esterified cholesterol, 2 ± 1 and 81 ± 1 (all in nmol/mg cell protein). Then, cultures were rinsed and incubated in 2 ml of RPMI 1640 medium for the indicated number of days. Following incubations, the apoE and cholesteryl contents of the media were determined. All values are averages ± S.E. of triplicate cultures. ApoE levels in media from monocyte-macrophage cultures incubated in the absence or presence of AcLDL were not significantly different. Cholesterol levels for all efflux time points were greater in media from monocyte-macrophages incubated with AcLDL compared with cultures incubated without AcLDL.

**TABLE III**

| Incubation condition | Cholesterol | Phospholipid |
|----------------------|-------------|--------------|
|                      | Cells | Medium | Cells | Medium |
| 0 days               | 82 ± 2 | 170 ± 1 | 205 ± 11 | 177 ± 8 |
| 2 days AcLDL/4       | 148 ± 7 | 63 ± 4 | 171 ± 11 | 23 ± 1 |
| 2 days RPMI + MOPC21 mAb | 177 ± 11 | 21 ± 3 | 175 ± 4 | 7 ± 1 |

The anti-apoE monoclonal antibody significantly inhibited cholesterol efflux from monocyte-macrophages (Table III). Monocyte-macrophages incubated with a control mouse monoclonal antibody excreted 63 nmol of cholesterol/mg of cell protein into the medium and showed a similar decrease (57 nmol of cholesterol/mg) in their cholesterol content. In contrast, monocyte-macrophages incubated with the anti-apoE monoclonal antibody excreted about one-third the cholesterol that control monocyte-macrophages excreted, and showed less decrease in their cholesterol content.

Similar to the effect on cholesterol excretion, monocyte-macrophages incubated with the anti-apoE monoclonal antibody excreted about one-third the phospholipid that control monocyte-macrophages excreted. Although control monocyte-macrophages excreted 23 nmol of phospholipid/mg of cell protein, they showed no decrease in their phospholipid content. This could have only occurred through synthesis by the macrophages of the same amount of phospholipid lost from the cells. Incubation of monocyte-macrophages with the control antibody did not affect cholesterol and phospholipid levels in the cells and medium.

**Exogenous ApoE Further Stimulated Monocyte-derived Macrophage Cholesterol Excretion**—Next, we determined whether the amount of apoE produced by monocyte-macrophages was rate-limiting for cholesterol efflux from these cells. Monocyte-macrophage cultures were first enriched with cholesterol by a 2-day incubation with AcLDL. Then, cholesterol content during a 3-day period of efflux into basal medium was monitored in the absence and presence of 10 µg/ml recombinant human apoE. This amount of apoE was more than double the concentration of apoE that we had observed in the medium of macrophages following 3 days of efflux. During the 3 days of efflux, macrophages decreased their cholesterol content by 47 nmol of cholesterol/mg of cell protein compared with a decrease of 75 nmol of cholesterol/mg of cell protein, in the presence of added apoE (Table IV). Thus, how much apoE that the macrophages secreted into the medium was rate-limiting with respect to decreasing macrophage cholesterol content. Additional exogenous apoE decreased the cholesterol content of macrophages beyond the decrease in cholesterol content that occurred with the level of apoE secreted by macrophages.

**Macrophage Unesterified Cholesterol Content Was Rate-limiting for Cholesterol Efflux Only in the Presence of Additional ApoE**—It was next determined whether monocyte-macrophage...
unesterified cholesterol content was rate-limiting for the cholesterol efflux mediated by macrophage-secreted apoE. Monocyte-macrophages were first enriched with cholesteryl ester by incubation with AcLDL. Then, the cholesterol content of macrophages was monitored during efflux into basal medium in the presence and absence of the acyl-CoA:cholesterol acyltransferase (ACAT) inhibitor, S58-035. Cellular cholesteryl ester is continually being hydrolyzed and re-esterified (19). Incubation of macrophages during the efflux period with an ACAT inhibitor decreased cellular cholesteryl esters and increased cellular unesterified cholesterol. This was because cholesteryl esters hydrolyzed during this period could not undergo re-esterification and, thus, accumulated in the cells as unesterified cholesterol (Table V, Experiment 1). The decrease in cholesteryl ester content of the mono-ocyte-macrophages compared with controls was balanced by an equivalent increase in unesterified cholesterol content. Although more unesterified cholesterol was available for potential efflux in the monocyte-macrophages treated with ACAT inhibitor, the total cholesterol content of these macrophages did not decrease more than the total cholesterol content of control macrophages incubated without the ACAT inhibitor (Table V, Experiment 1). This showed that the macrophage level of unesterified cholesterol was not rate-limiting for cholesterol efflux from the macrophages.

However, a second experiment showed that this was because secretion of apoE by the macrophages treated with ACAT was not sufficient to remove the accumulating unesterified cholesterol (Table V, Experiment 2). Although much less cholesterol accumulated in monocyte-macrophages incubated with AcLDL in experiment 2, nevertheless, ACAT inhibition still failed to enhance cholesterol efflux from the macrophages. However, in the presence of added exogenous apoE, ACAT inhibition promoted a greater decrease in the cholesterol content of the macrophages than occurred without the ACAT inhibitor.

**Table IV**

| Incubation condition | Cholesterol |   |   |
|----------------------|-------------|---|---|
|                      | Total       | Unesterified | Esterified |
| nmol/mg cell protein |             |              |            |
| 0 days               | 102 ± 2     | 101 ± 1      | 1 ± 1      |
| 2 days AcLDL         | 217 ± 5     | 117 ± 3      | 100 ± 3    |
| 2 days AcLDL/3 days  | 170 ± 1     | 82 ± 1       | 88 ± 1     |
| RPMI                 | 142 ± 3     | 81 ± 1       | 61 ± 4     |

**DISCUSSION**

The goal of this investigation was to learn about the function of apoE in mediating monocyte-derived macrophage cholesterol efflux that occurs in the absence of added cholesterol acceptors such as HDL or apolipoproteins. We have found that 1) apoE produced by monocyte-macrophages mediates most of the cholesterol efflux from the macrophages; 2) cholesterol regulates the association of apoE with phospholipid; 3) apoE associated with lipid after apoE was secreted from macrophages (not during secretion); 4) the level of macrophage unesterified cholesterol was not rate-limiting for cholesterol efflux, but the level of secreted apoE was rate-limiting; and 5) net phospholipid synthesis occurred in macrophages secondary to apoE-mediated loss of macrophage phospholipid (net phospholipid synthesis was not due to cholesterol enrichment).

Previously, it was shown that apoE secretion from mouse peritoneal macrophages does not decrease the cholesterol content of these cells, although apoE-containing discoidal particles could be produced by these macrophages (25, 26). Hara and Yokoyama (27) concluded that this was because mouse peritoneal macrophages do not secrete sufficient levels of apoE to stimulate significant cholesterol efflux. They showed that cholesterol efflux does occur from mouse peritoneal macrophages when sufficient exogenous apoE is added to the culture media. Expression of the human apoE gene in J774 macrophages (a
Effect of an ACAT inhibitor on monocyte-macrophage cholesterol content during efflux

Two-week-old monocyte-macrophage cultures were incubated for 2 days in 2 ml of RPMI 1640 medium with 25 μg/ml AcLDL. Then, cultures were rinsed and incubated for 2 days in 2 ml of RPMI 1640 medium in the absence or presence of 4 μg/ml S58–035 ACAT inhibitor, and additionally in Experiment 2 without or with added recombinant human apoE (10 μg/ml). The different degrees of macrophage cholesterol enrichment induced in Experiments 1 and 2 reflect variability that we observed between different lots of AcLDL. Following incubations, the cholesterol and protein contents of the macrophages were determined. All values are the averages ± S.E. of triplicate cultures. Items in brackets indicate statistical significance assessed by Student’s t test.

| Incubation condition | Cholesterol | Esterified cholesterol |
|----------------------|-------------|------------------------|
|                      | Total       | Unesterified           | Esterified | %          |
|                      | nmol/mg cell protein | | | |
| **Exp. 1**           |             |                        |            |            |
| 0 day                | 83 ± 5      | 80 ± 5                 | 3 ± 1      | 4 ± 1      |
| 2 days AcLDL         | 198 ± 3     | 99 ± 1                 | 99 ± 3     | 50 ± 1     |
| 2 days AcLDL/2 days RPMI (no addition) | 187 ± 7 | (NS) 131 ± 5 | 42 ± 8 | 24 ± 3 |
| 2 days AcLDL/2 days RPMI + ACAT inhibitor | 173 ± 12 | (NS) 100 ± 2 | 6 ± 2 | 6 ± 2 |
| **Exp. 2**           |             |                        |            |            |
| 0 day                | 77 ± 2      | 77 ± 3                 | 0 ± 1      | 0 ± 1      |
| 2 days AcLDL         | 118 ± 3     | 85 ± 3                 | 33 ± 2     | 28 ± 2     |
| 2 days AcLDL/2 days RPMI (no addition) | 106 ± 5 | (NS) 76 ± 2 | 30 ± 3 | 28 ± 2 |
| 2 days AcLDL/2 days RPMI + ACAT inhibitor | 106 ± 1 | (NS) 100 ± 2 | 6 ± 2 | 6 ± 2 |
| 2 days AcLDL/2 days RPMI + apoE | 92 ± 3 | (p = 0.03) 72 ± 3 | 20 ± 0 | 22 ± 1 |
| 2 days AcLDL/2 days RPMI + ACAT inhibitor + apoE | 79 ± 3 | (p = 0.03) 74 ± 2 | 5 ± 2 | 6 ± 2 |

FIG. 2. Density gradient analysis of apoE from media of normal and cholesterol-enriched monocyte-macrophages. Two-week-old monocyte-macrophage cultures were incubated for 3 days in 2 ml of RPMI 1640 medium either without (a) or with 130 nmol/ml microcrystalline cholesterol (b). Then, media were concentrated and subjected to isopycnic density gradient centrifugation. apoE was determined in gradient fractions by ELISA. apoE floated to density 1.08–1.10 g/ml only when macrophages were incubated with cholesterol.

FIG. 3. Western blot analysis of media and cellular apoE from normal and cholesterol-enriched macrophage cultures. Two-week-old monocyte-macrophage cultures were incubated for 3 days in 2 ml of RPMI 1640 medium either without (a and c) or with 130 nmol/ml microcrystalline cholesterol (b and d). Equivalent portions of media or cells were solubilized and electrophoresed under reducing conditions according to Laemmli. The gel was blotted onto a nylon membrane, probed with an anti-apoE mouse monoclonal antibody, and visualized by chemiluminescence as described under “Materials and Methods.” The arrows indicate higher and lower molecular weight isoforms of apoE. Cells contained predominantly a lower molecular weight isoform of apoE with a small amount of higher molecular weight isoform. Media contained only the higher molecular weight isoform of apoE.

mouse macrophage cell line that does not express its endogenous apoE gene) stimulated efflux of radiolabeled cholesterol in the presence of cAMP or an ACAT inhibitor (28). However, the level of efflux was not sufficient to decrease the cholesterol content of these cells. In contrast to these earlier studies with other types of macrophages, we found that apoE secreted from human macrophages did mediate cholesterol efflux from these cells and also decreased their cholesterol content. This was shown from results in which an anti-apoE antibody decreased (about two-thirds) the efflux of cholesterol from monocyte-macrophages. In our earlier work (7), we showed that a minor portion of cholesterol released from monocyte-macrophages was present in vesicles that contained a 22,000-Da protein unrelated to apoE. It is possible that residual cholesterol efflux that was not blocked by the anti-apoE antibody occurred in these vesicles.

 Previous studies have shown that cholesterol accumulation by macrophages can induce the synthesis and secretion of apoE (12–14). Therefore, we thought that cholesterol enrichment of the monocyte-macrophages would induce secretion of apoE that would then be available to form apoE-lipid discoidal particles. However, this turned out not to be the case. The monocyte-macrophages in our study constitutively secreted apoE essentially unaffected by cholesterol accumulation in the macrophages. Although apoE was constitutively secreted, it became lipid-rich and floated to density 1.08–1.10 g/ml only when macrophages had accumulated cholesterol. Thus, cholesterol did not stimulate formation of apoE-discoidal particles by stimulating apoE secretion; instead, cholesterol induced the complexing of apoE with phospholipid and cholesterol. This finding is consistent with earlier proposals that cholesterol alters the physical chemical properties of the plasma membrane in a way
that favors interaction of amphipathic apolipoproteins with phospholipid (29).

The fact that anti-apoE antibody added to the medium blocked cholesterol and phospholipid efflux is consistent with the idea that apoE did not leave the macrophage with lipid (otherwise, the antibody should not have blocked lipid efflux). The finding suggests that apoE picked up lipid after the apoE was released from the macrophage (30). This was considered an unlikely possibility for apoE-transfected L cells because, when incubated with the transfected L cells, exogenously added apoE did not become associated with lipid (21).

Net phospholipid synthesis occurred during cholesterol efflux from cholesterol-enriched human monocyte-macrophages. This was shown by the fact that the phospholipid content of monocyte-macrophages did not decrease, while phospholipid accumulated in culture medium during efflux. Cholesterol stimulation of net phospholipid synthesis in mouse macrophages has been observed (31, 32). However, cholesterol could not have directly stimulated net phospholipid synthesis in the human monocyte-macrophages as appears to be the case in mouse macrophages. This was because the content of macrophage phospholipid did not increase in the presence of the anti-apoE monoclonal antibody, although this antibody inhibited phospholipid excretion. Thus, removal of phospholipid from the macrophage (rather than cholesterol enrichment of the macrophage) must have stimulated net phospholipid synthesis. If cholesterol had directly stimulated phospholipid synthesis, then in the presence of the anti-apoE monoclonal antibody, macrophage phospholipid content should have increased an amount equivalent to the decrease in phospholipid excretion (16 nmol of phospholipid/mg of cell protein, calculated from data in Table III). However, this did not occur.

The level of macrophage unesterified cholesterol was not rate-limiting for cholesterol efflux that occurred in basal medium without serum or added lipoproteins. The lack of effect of ACAT described for this in vitro situation may not apply to the in vivo situation within the blood vessel wall. ApoE concentrations could be higher in the tissue spaces of the blood vessel wall compared with the apoE concentrations achieved in cell culture where a monolayer of monocyte-macrophages secretes apoE into a large volume of culture fluid relative to the number of cells. At higher apoE concentrations produced by adding exogenous apoE to the culture medium, the level of macrophage unesterified cholesterol was rate-limiting for cholesterol efflux. With added apoE, inhibition of ACAT did stimulate a greater decrease in the cholesterol content of monocyte-macrophages compared with the decrease that occurred without added apoE. This was similar to what has been reported previously for plasma HDL-mediated cholesterol efflux from macrophages (33). We attempted to decrease the volume of culture medium to determine the effect on apoE secretion and cholesterol efflux. However, decreasing the volume of culture medium resulted in detachment of monocyte-macrophages from the culture dish.

The accessibility of all regions of atherosclerotic lesions to sufficient levels of plasma-derived HDL could be a factor that limits reverse cholesterol transport from lesions. Given that apoE secretion by human monocyte-macrophages can mediate cholesterol efflux from these cells in vitro, apoE secretion by these cells in human atherosclerotic lesions has the potential to mediate cholesterol efflux in lesions. The significance of cholesterol efflux from macrophages mediated by production of its own nascent HDL is that this efflux would not depend on the availability of plasma HDL. In conclusion, apoE has anti-atherogenic potential not only by accelerating removal of atherogenic lipoproteins from the blood, but also by removing cholesterol from monocyte-macrophages through an autocrine mechanism of cellular cholesterol efflux.

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