Lipoprotein Lipase Reduces Secretion of Apolipoprotein E from Macrophages*

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Madhuri Lucas‡, Per-Henrik Iverius‡, Dudley K. Strickland§, and Theodore Mazzone¶

From the ‡Departments of Medicine and Biochemistry, Rush Medical College, Chicago, Illinois 60612, the §Veteran’s Affairs Medical Center and Department of Internal Medicine, University of Utah, Salt Lake City, Utah 84117, and the ¶Biochemistry Laboratory, American Red Cross, Rockville, Maryland 20855

Macrophages are a significant source of lipoprotein lipase (LPL) and apolipoprotein E (apo E) in the developing arterial wall lesion, and each of these proteins can importantly modulate lipid and lipoprotein metabolism by arterial wall cells. LPL and apo E share a number of cell surface binding sites, including proteoglycans, and we have previously shown that proteoglycans are important for modulating net secretion of apoprotein E from macrophages. We therefore evaluated a potential role for LPL in modulating net secretion of macrophage-derived apo E. In pulse-chase experiments, addition of LPL during the chase period produced a decrease in secretion of apoprotein E from human monocyte-derived macrophages, from the human monocytic THP1 cell line, and from J774 cells transfected to constitutively express a human apo E cDNA. LPL similarly reduced apo E secretion when it was prebound to the macrophage cell surface at 4 °C. A native LPL particle was required to modulate apo E secretion; addition of monomers and aggregates did not produce the same effect. Depletion of cell surface proteoglycans by a 72-h incubation in 4-methylumbelliferyl-β-D-xyloside did not attenuate the ability of LPL to reduce apo E secretion. However, addition of receptor-associated protein attenuated the effect of LPL on apo E secretion. Although LPL could mediate removal of exogenously added apo E from the culture medium, detailed pulse-chase analysis suggested that it primarily prevented release of newly synthesized apo E from the cell layer. Cholesterol loading of cells or antibodies to the low density lipoprotein receptor attenuated LPL effects on apo E secretion. We postulate that LPL sequesters endogenously synthesized apo E at the cell surface by a low density lipoprotein receptor-dependent mechanism. Such post-translational regulation of macrophage apo E secretion by LPL could significantly influence apo E accumulation in arterial vessel wall lesions.

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† To whom correspondence should be addressed: Rush Medical College, 1653 W. Congress Pkwy., Chicago, IL 60612. Tel.: 312-942-6163; Fax: 312-563-2096.

1 The abbreviations used are: apo E, apolipoprotein E; LPL, lipoprotein lipase; 4DX, 4-methylumbelliferyl-β-D-xyloside; LRP; low density lipoprotein receptor-related protein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; RAP, receptor-associated protein; LDLR, low density lipoprotein receptor.

These cells, therefore, can be a significant source of each of these proteins in the arterial wall. Both proteins have important roles in systemic lipoprotein metabolism (3, 4) as well as in lipidoprotein metabolism at the level of the individual cell (5–14). Regional accumulation of each of these proteins has been detected at sites of atherosclerotic lesion development (15); apo E has been found predominantly on the surface of macrophages and in the matrix surrounding macrophages in atherosclerotic lesions, whereas LPL is also associated with arterial smooth muscle cells. apo E expression in the vessel wall has been shown to be important for modulating vessel wall cholesterol homeostasis (16, 17). In fact, macrophage-specific expression of apo E in apo E-null mice has been shown to protect against atherosclerotic lesion development even in the presence of high levels of circulating atherogenic lipoproteins (17). As noted above, LPL also accumulates in diseased vessel wall. A number of cell surface binding sites for LPL have been identified (11, 18, 19), including the LRP, plasma membrane-associated proteoglycans, and a 116-kDa nonproteoglycan binding site on endothelial cells. LPL accumulates in the extracellular matrix and has been shown to directly bind apoprotein B (6). LPL bound to cell surface or extracellular matrix sites, therefore, has been implicated in enhancing cellular uptake of lipoproteins, including LDL and triglyceride-rich lipoproteins (4). apo E also binds to proteoglycans and to the LRP (13, 14).

In a recent report, it was found that preincubation of endothelial-derived matrix with apoprotein E did not reduce the binding of subsequently added LPL to this matrix (10). This observation suggested that apoprotein E and LPL may bind to distinct proteoglycan species in the subendothelial matrix. In addition, apo E reduced LPL-mediated retention of LDL in the subendothelial matrix in a dose-dependent manner.

Expression of macrophage apo E is modulated transcriptionally by cholesterol and by cytokines (20, 21). There is also significant post-transcriptional regulation of macrophage apo E secretion, and we have recently shown that one important site for post-translational regulation of macrophage apo E secretion is at the pericellular proteoglycan matrix (22). A substantial portion of the apo E synthesized by the macrophage is retained in the pericellular proteoglycan matrix and is rapidly returned to the cell for degradation (22). In view of this observation and previous reports regarding the interaction between pericellular proteoglycans and LPL, we formally evaluated a role for LPL in modulating the secretion/metabolism of endogenously produced apo E in the macrophage. For these studies, we utilized a macrophage cell model in which apo E is constitutively synthesized and evaluated the effect of exogenously added LPL.

EXPERIMENTAL PROCEDURES

Materials—4-Methylumbelliferyl-β-D-xyloside was obtained from Sigma. Lipoprotein lipase was isolated from bovine milk as described.

13000 This paper is available on line at http://www-jbc.stanford.edu/jbc/
FIG. 1. Effect of LPL on apo E secretion. Cells were plated and grown as described under "Experimental Procedures." After a 45-min pulse period, cells were chased for 45 min with the concentration of LPL shown. Values shown are the apo E dpm recovered from the medium at the end of the chase and represent means of duplicate samples.

Previously (23). Human recombinant RAP was prepared as described (24). Acetylated LDL was prepared by previously described method (20). Human apo E isolated from plasma was purchased from Alpha Biomedical (Bellevue, WA) and iodinated using Iodobeads (Pierce) according to the manufacturer's instructions. Unbound iodine was removed by chromatography on a PD-10 column followed by dialysis. Polyclonal rabbit anti-LDLR antisera was prepared against LDLR protein isolated from bovine adrenal glands as described previously (25). Monoclonal antibody IgGC7 was prepared from mouse ascites by standard techniques. This antibody has been previously characterized (25). Monoclonal antibody IgGC7 was prepared from mouse ascites by standard techniques. This antibody has been previously characterized (25). Monoclonal antibody IgGC7 was prepared from mouse ascites by standard techniques. This antibody has been previously characterized (25). Monoclonal antibody IgGC7 was prepared from mouse ascites by standard techniques. This antibody has been previously characterized (25).

Cell Culture—J774 cells were stably transfected to express a wild type human apo E cDNA as described previously in detail (26). This cell line constitutively expresses a human apo E cDNA (E3 isoform) and secretes 900 ng of apo E/mg of cell protein in 24 h, an amount similar to that produced by mature cholesterol-loaded human monocyte-derived macrophages in culture. Cells were maintained in 400 μg/ml selection agent G418 (Genetecin, Sigma) until 1 week prior to the initiation of experiments. Cells were grown in 10% fetal bovine serum in Dulbecco's modified Eagle's medium until the start of the described experimental incubations. apo E-expressing J774 cells were used for all experiments unless indicated otherwise. For studies using J774 cells not secreting apo E, the same parental J774 cell line transfected only with a neomycin-resistance construct was used. These cells were maintained in G418 exactly as for apo E-secreting cells. Human monocytes were purified by elutriation. Cells were >90% monocytic (as determined by differential counts of Wright-stained smears) and grown as described previously (21). The THP1 human monocytic cell line was obtained from the American Type Culture Collection and grown as described previously (21).

Isolation and Characterization of Monomeric/Aggregated LPL—Bovine LPL (1 mg), isolated as described previously (23), was diluted in 300 ml of 0.15 M NaCl, 1 mM EDTA, 10 mM sodium phosphate buffer (pH 7.5) containing 0.1 mg/ml ovalbumin and incubated at 37 °C for 30 min in this dilute solution. After the incubation, the sample was chilled on ice and passed through a 1-ml heparin-agarose column (HiTrap Hepa-rin, Pharmacia Biotech Inc.) using a peristaltic pump at flow rate of 2.5 ml/min. The column, submerged in an ice bath and attached to a Pharmacia fast protein liquid chromatography system, was washed with 20 ml of 0.15 M NaCl, 1 mM EDTA, 10 mM sodium phosphate buffer (pH 7.5) at a flow rate of 1 ml/min and then eluted with a 15-ml gradient of 0.15–1.5 M NaCl in the same buffer at a flow rate of 0.25 ml/min while 0.25-ml fractions were collected and immediately chilled on ice. The effluent was monitored by absorbance at 280 nm. As described previously for human LPL (27), the protein emerged in a low affinity active peak and a high affinity active peak with material of heterogeneous heparin affinity in between. Fractions containing the inactive monomeric and aggregated material preceding the high affinity active peak were pooled, dialyzed against 50% (v/v) glycerol, 10 mM sodium phosphate (pH 7.5), and stored at −20 °C. The yield of inactive monomeric and aggregated enzyme (0.36 mg) was determined by absorbance at 280 nm.

Quantitation of apo E Synthesis and Secretion—2–3 x 10^6 cells were plated into 35-mm wells and grown for 48–72 h. All media used during the following procedures were warmed to 37 °C before use. Pulse me-
dium contained 100 μCi/ml [35S]methionine with 1–2 μCi/ml unlabeled methionine added to methionine-free Dulbecco's modified Eagle's medium. Pulse and chase times were as indicated in the figure legends. Chase medium contained 500 μCi/ml unlabeled methionine. At the end of the chase period, apo E secreted into the medium and apo E that remained associated with the cell was quantitatively immunoprecipitated and analyzed by SDS-polyacrylamide gel electrophoresis as described previously in detail (26). All immunoprecipitations from cell lysates and media were performed starting with equal numbers of total trichloroacetic acid-precipitable counts. Therefore, the disintegrations per minute in secreted or cell-associated apo E are normalized for total labeled secreted protein or total labeled cell-associated protein, respectively, in each experiment. For most experiments, the apo E bands were excised from the gel after they were localized by autoradiography. The dried gel piece from each sample was cut out and, after the gel was rehydrated with 3% glycerol, the backing paper and glycerol solution were removed and the gel slices were digested in 0.5 ml of 30% hydrogen peroxide. After 48–72 h at 60 °C, scintillant was added and the samples were counted against an external standard so that counting efficiency could be used to calculate the disintegrations per minute incorporated into apo E for each sample. For some experiments, apo E radioactivity on SDS-polyacrylamide gel electrophoresis gels was quantitated using the Storm PhosphorImager (Molecular Dynamics), and results are ex-

RESULTS

The effect of LPL on the net medium accumulation of apo E from J774 macrophages transfected to constitutively express a human apo E cDNA, as a function of LPL concentration, is shown in Fig. 1. Cells were incubated for 45 min with labeled methionine and chased for an additional 45 min with vehicle or
LPL at the indicated concentrations. Addition of LPL at 10 μg/ml led to a 39% reduction of apo E content, and 20 μg/ml produced a 49% reduction. The LPL effect was maximal at 20 μg/ml, and higher doses of LPL, up to 80 μg/ml, had no additional effect on apo E levels. A final LPL concentration of 40 μg/ml was used for all subsequent experiments.

Similar results were obtained in experiments using human monocyte-derived macrophages. Elutriated human monocytes (>90% pure) were allowed to differentiate to a macrophage phenotype for 4 days in culture before being placed in 0.2% bovine serum albumin for an overnight incubation. Cultures were then pulse-labeled with methionine for 45 min followed by a 45-min chase at 37 °C with vehicle or LPL (40 μg/ml). apo E immunoprecipitated from the medium (quantitated by PhosphorImager) showed a 43% reduction in the presence of LPL.

In Table I, suggested that a nonproteoglycan, RAP-sensitive binding site was involved in LPL sequestration of apo E. This result, in conjunction with the results shown in Fig. 1, indicated that proteoglycan depletion did not enhance LPL-mediated sequestration of apo E compared with addition of LPL alone. This result, in conjunction with the results shown in Table I, suggested that a nonproteoglycan, RAP-sensitive binding site was involved in LPL sequestration of apo E.

Because LPL can bind to several cell surface sites, the observed effect on apo E when LPL was present in the medium during the 37 °C chase could represent the effect of LPL bound at the cell surface or LPL in the culture medium. The next experiment was performed to address this issue. For the 4 °C bars in Fig. 2, LPL was prebound to cells at 4 °C for 60 min immediately following a 45-min pulse labeling period. Following this 4 °C incubation, cells were extensively washed and incubated for an additional 45 min at 37 °C with no added LPL.

For the 37 °C bars, LPL was added only during a 45-min chase at 37 °C. apo E was then immunoprecipitated from chase medium for each experimental incubation. As shown, apo E medium content was reduced to similar levels by LPL whether it was present in the chase medium or prebound to the cell surface at 4 °C.

Both apo E and LPL bind to cell surface proteoglycans (14, 18, 22). The results above suggest that these ligands do not compete for the same saturable proteoglycan binding sites, because LPL does not displace apo E from the cell layer. LPL binding of the newly synthesized apo E particle could, therefore, increase the binding capacity for apo E in the pericellular proteoglycan matrix. We investigated the potential involvement of cellular proteoglycans by depleting cell surface proteoglycans using a 72-h preincubation in βDX (Table I). βDX substitutes for the core protein moiety of proteoglycans during their synthesis and substantially reduces their appearance at the cell surface (22). We have previously shown (22) that cell surface proteoglycans retain a large portion of newly synthesized apo E in the pericellular matrix so that preincubation in βDX alone led to a large increase in the release of apo E into the medium (26,159 ± 162 versus 12,512 ± 112 dpm). In cells not preincubated in βDX, LPL addition reduced medium apo E content as previously observed. In proteoglycan-depleted cells, LPL retained its ability to reduce apo E levels (from 26,159 ± 162 to 6,164 ± 79 dpm). This result indicated that proteoglycan binding sites are not necessary for LPL modulation of apo E expression.

LPL can bind to additional cell surface nonproteoglycan sites (11, 18, 19, 28), and we utilized RAP to gain further insight into important cell surface sites. This protein has been shown to bind to multiple members of the LDL receptor family including the LRP, LDLR, VLDL receptor, and gp 330/megalin and to inhibit their ligand binding properties. As shown in Table II, addition of RAP enhanced apo E release from the cell layer into the medium. Furthermore, RAP significantly interfered with LPL-mediated sequestration of apo E.

In Fig. 3, we investigated the requirement for the addition of native LPL for its effect on medium apo E accumulation. Prior to addition to cells, inactive monomeric and aggregated LPL was prepared as described under “Experimental Procedures,” native LPL was excluded from the cultures labeled “aggregated/monomeric” LPL. Addition of native LPL produced the expected result on medium apo E content. Monomeric/aggregated LPL, however, not only failed to decrease medium apo E level but actually increased it by 40%.

**Table II**

| Secreted Apo E | dpm       |
|---------------|-----------|
| Control       | 10,221 ± 832 |
| RAP           | 18,807 ± 703 |
| LPL           | 5,213 ± 442  |
| LPL + RAP     | 10,421 ± 601 |

**Fig. 3. Comparison of the effect of native and aggregated/monomeric LPL on macrophage apo E secretion.**

Cells were plated and grown as described under “Experimental Procedures,” pulsed for 45 min at 37 °C with labeled methionine, and incubated for 60 min at 4 °C with vehicle, native LPL (40 μg/ml), or monomeric/aggregated LPL (40 μg/ml) in Dulbecco’s modified Eagle’s medium. The medium was then removed and the cells were washed once with Dulbecco’s modified Eagle’s medium and chased for an additional 45 min with no addition at 37 °C. Cells and medium were then harvested and the results analyzed using the Storm PhosphorImager. Values are expressed as fold change with native LPL or aggregated/monomeric LPL compared with control, and are the mean ± S.D. from triplicate samples. Preparation of monomeric/aggregated LPL and its characterization are described under “Experimental Procedures.”
The reduction we observed in medium apo E content in the presence of LPL could be related to LPL-mediated reduction of apo E release from cells or LPL-mediated enhancement of apo E reuptake from the medium. Therefore, we next evaluated the effect of LPL on the removal of exogenously added apo E (Table III). Delipidated apo E, isolated from human plasma lipoproteins, was iodinated and added to apo E-secreting and nonsecreting J774 cells with or without LPL. After 120 min at 4 °C, labeled apo E remaining in the medium was recovered by trichloroacetic acid precipitation. Total apo E radioactivity added to the cell cultures equaled 53,360 cpm. In the absence of LPL, apo E-secreting J774 macrophages sequestered very little exogenous apo E (52,839 ± 734 dpm remaining), whereas nonsecreting cells may have sequestered a small amount (44,223 ± 6,917 remaining). Addition of LPL stimulated removal of apo E only from apo E-secreting cells (23,489 ± 2,428 remaining). This observation suggested that synthesis of endogenous apo E was necessary for LPL-mediated uptake of exogenously added apo E. This could implicate a cell surface pool of apo E or the lipid component of the endogenously synthesized apo E particle as important for LPL-mediated uptake (see “Discussion”). These results indicated that LPL could reduce apo E in the medium by enhancing its reuptake.

To further investigate this issue, we studied cells after a 20-min pulse with labeled methionine followed by multiple chase times. For the experiment shown in Fig. 4, LPL was added at time 0 (immediately following a 20-min pulse period). By 15 min, apo E in the medium from LPL-treated cells was already lower than that in control medium (6,288 ± 667 versus 9,588 ± 2,432 dpm). The difference in medium apo E between LPL-treated and control cultures also continues to increase through the 45-min chase period. Fig. 4B shows the results of immunoprecipitating apo E from the cell layer. apo E in the cell layer tends to be higher in LPL-treated cells; however, the magnitude of the difference between LPL-treated and control cultures appears to change very little after 15 min. The above results suggested that LPL prevents release of apo E from the cell layer and that the apo E retained by the cells is subject to degradation.

To gain further insight into the physiologic parameters modulating LPL-mediated sequestration of macrophage-derived apo E secretion.

### Table III

**Uptake of exogenous apo E by apo E-secreting and nonsecreting J774 cells after addition of LPL**

Apo E secreting and nonsecreting J774 cells were plated at 1.5 × 10⁶ cells in 35-mm wells as described under Experimental Procedures. After 48 h cells were washed twice with 0.2% bovine serum albumin in Dulbecco's modified Eagle's medium at 4 °C and incubated for an additional 120 min at 4 °C with 0.1 μg/ml ¹²⁵I-apo E (equal to 53,360 cpm) in 0.2% bovine serum albumin in Dulbecco's modified Eagle's medium. At that time, the medium was recovered for trichloroacetic acid precipitation of ¹²⁵I-apo E. Values shown are mean ± S.D. for triplicate samples.

|                      | apo E remaining in the medium (cpm) | Reduction with LPL (%) |
|----------------------|-------------------------------------|------------------------|
| **apo E-secreting cells** |                                     |                        |
| No addition          | 52,839 ± 734                        | 66                     |
| + LPL                | 23,489 ± 2428                       |                        |
| **Nonsecreting cells** |                                     |                        |
| No addition          | 44,223 ± 917                        | 1                      |
| + LPL                | 43,668 ± 977                        |                        |

**Fig. 4.** Turnover analysis of secreted and cellular apo E after addition of LPL. Cultures were pulse-labeled for 20 min. At that time some cultures were harvested (0 time), and the balance were chased with vehicle or LPL (40 μg/ml) for the indicated times. apo E was immunoprecipitated from media (A) and cell lysates (B) as described under “Experimental Procedures.” The apo E bands were excised from the gel, and radioactivity in apo E was measured. Values shown are mean ± S.D. from triplicate samples.
LPL Modulates Macrophage apo E Secretion

**TABLE IV**

| Cholesterol loading inhibits LPL-mediated sequestration of apo E |
| --------------------------------------------------------------- |
| Cells were plated at 1.5 × 10⁶ cells in 35-mm wells as described under “Experimental Procedures.” 24 h later the medium was changed to 5% fetal calf serum in Dulbecco’s modified Eagle’s medium with or without acetylated LDL at 50 μg/ml. After 48 h the cells were washed and placed in 0.2% bovine serum albumin in Dulbecco’s modified Eagle’s medium for an additional 18 h to allow internalization of residual acetylated LDL and equilibration of cell cholesterol. After harvesting selected wells for measurement of cellular cholesterol and protein, the balance of the wells were pulse-labeled with [35S] methionine at 37 °C for 45 min and chased at 37 °C for 45 min with no addition or with LPL at 40 μg/ml as indicated. apo E radioactivity was immunoprecipitated as described under “Experimental Procedures” and quantitated using the Storm imaging system. The mean ± S.D. of triplicate samples is shown.

| Medium apo E | Reduction with LPL |
|--------------|--------------------|
| phosphorimaging units | % |
| No cholesterol loading | |
| No addition | 6.4 ± 0.4 |
| LPL | 2.7 ± 0.2 |
| Cholesterol-loaded cells | |
| No addition | 4.9 ± 0.4 |
| LPL | 4.8 ± 0.3 |

**TABLE V**

Antibody to LDL receptor inhibits LPL-mediated sequestration of apo E in THP1 cells

3 × 10⁵ THP1 cells in 35-mm wells were differentiated to a macrophage phenotype by the addition of 12-0-tetradecanoylphorbol-13-ace-tate for 48 h. At that time cells were pulse-labeled for 45 min alone or in the presence of rabbit non-immune serum or a rabbit antiserum to the LDL receptor (each at 10 μl/ml). Cell layers were then washed twice with ice-cold phosphate-buffered saline and incubated for an additional 60 min at 4 °C with these same additions. LPL (40 μg/ml) was added as indicated. Thereafter, fresh chase medium at 37 °C without further additions was added. After 45 min, media were harvested for immunoprecipitation of apo E as described under “Experimental Procedures.” Values shown are expressed as -fold change compared with control and represent the mean ± S.D. from triplicate samples.

| Secreted apo E | -fold change |
|----------------|-------------|
| Control | 1.00 ± 0.08 |
| LPL | 0.60 ± 0.07 |
| Nonimmune serum + LPL | 0.55 ± 0.01 |
| LDLR antiserum + LPL | 1.04 ± 0.09 |

apo E, we evaluated the effect of cellular cholesterol loading on this process using the transfected J774 cell model. Cholesterol loading was accomplished by a 48-h preincubation in acetylated LDL. This preincubation was followed by an 18-h equilibration period in serum-free medium to allow for the internalization of remaining acetylated LDL and equilibration of cell cholesterol. Cells preincubated with acetylated LDL contained 56.1 and 12.8 μg/mg free and esterified cholesterol, respectively (average of duplicate determinations), compared with 23.3 μg/mg free cholesterol and no detectable cholesterol ester in cells grown without acetylated LDL. As shown in Table IV, cholesterol loading of J774 cells completely abolished the effect of LPL on apo E secretion.

We next investigated the potential involvement of specific cell surface binding sites for the LPL effect. The results shown in Table I indicate that proteoglycans on the cell surface are not likely involved. The results in Table II suggest the potential involvement of a member of the LDL receptor gene family (e.g. LRP, VLDL receptor, or LDLR). Of these, only the LDLR would be expected to behave in a sterol-suppressible fashion, as is demonstrated in Table IV. Therefore, we focused on this receptor as important in mediating LPL effects in our experiments. As shown in Tables V and VI, polyclonal or monoclonal antibodies to the LDLR abrogated LPL-mediated reduction of apo E secretion by THP1 cells and transfected J774 cells.

**TABLE VI**

Polyclonal and monoclonal antibody to LDL receptor inhibits LPL-mediated sequestration of apo E in J774 cells

Cells were grown and labeled for 45 min as described under “Experimental Procedures.” After labeling cells were washed and incubated at 4°C for 1 h in phosphate-buffered saline containing vehicle alone, LPL (40 μg/ml), LPL plus IgG7 (100 μg/ml), or LPL plus 10 μl of a rabbit polyclonal antiserum to the LDL receptor. After 60 min cells were chased for 45 min at 37 °C in fresh medium with no additions. apo E was immunoprecipitated and quantitated using the Storm imaging system. Results are shown as -fold change compared with control and represent the mean ± S.D. of triplicate samples.

| Secreted apo E | -fold change |
|----------------|-------------|
| Control | 1.00 ± 0.02 |
| LPL | 0.30 ± 0.03 |
| IgG7 + LPL | 0.79 ± 0.18 |
| LDLR antiserum + LPL | 1.02 ± 0.02 |

**DISCUSSION**

The data presented indicate that native LPL reduces the secretion of newly synthesized apo E in the macrophage in an LDLR-dependent manner. We postulate as an explanation for our findings that native LPL can directly interact with the endogenously synthesized apo E-lipid particle and act as a bridge to sequester it at the cell surface. apo E that is not secreted in the presence of LPL is thereby returned to the cell for degradation. LPL and apo E may bind to unique cell surface proteoglycan species, since LPL does not displace apo E from the cell layer. Binding to different proteoglycan species would allow for alternative fates in the extracellular matrix for these two proteins. In addition, binding of LPL and apo E to unique proteoglycan species is consistent with previous observations that apo E and LDL act in a complementary fashion to enhance binding of proteoglycans to triglyceride-rich lipoproteins (29). This would not likely occur if LPL and apo E were competing for binding to the same proteoglycan sites.

The data shown in Fig. 3 indicate that native LPL is required for LPL-mediated reduction of macrophage apo E secretion. Addition of LPL monomers and aggregates prepared by column chromatography actually increased macrophage apo E secretion. We believe that the altered properties of aggregated/monomeric LPL in our experiments are related primarily to loss of critical secondary structure for a bridging function and not to loss of enzyme catalytic activity. Our experiments conducted with native LPL were performed in serum-free medium without a source of apolipoprotein CII. The latter is a critical cofactor for LPL activity that can be supplied by serum or triglyceride-rich lipoproteins. Because of the absence of apolipoprotein CII in our experimental system, there was likely little LPL enzymatic activity in any of our experiments. Additional insight into the contribution of enzymatic activity will require extensive analysis of LPL structural mutants. Using such an analysis, the bridge and catalytic functions for LPL-induced metabolism of VLDL have been separated (30). In these studies it was determined that although catalytic function was not important for the ”bridging” function of LPL for VLDL, intact folding of the catalytic loop must be maintained for such function. Studies using inhibitors of LPL activity that produce covalent modification of the enzyme active site may also produce changes in critical secondary structure for bridging and therefore cannot substitute for mutational analysis.

The data shown in Table III suggest that the lipid portion of the apo E-lipid particle may, in fact, be responsible for LPL-mediated effects on apo E secretion in the macrophage. The uptake of exogenously added delipidated apo E to nonexpressing J774 cells was not enhanced by the addition of LPL. However, LPL did enhance the uptake of exogenous apo E by apo
E-secreting J774 cells. As an explanation for this observation, we speculate that the exogenously added apo E is able to rapidly exchange onto an apo E-lipid particle (produced by the apo E-secreting J774 cells) and thereby be removed by LPL binding to the lipid portion of this particle. Uptake of exogenously added apo E cannot proceed in nonsecreting J774 cells because these cells do not produce the apo E-lipid particles required for this exchange. Thus, our data predict that an intact lipid binding domain of native LPL is required for its reduction of apo E secretion by the macrophage. This domain may be irreversibly altered during preparation of aggregated/monomeric LPL.

Reduction of medium apo E by LPL could be related to reduced release of apo E from the cell layer, enhanced reuptake of apo E after secretion, or both. LPL can mediate the removal of exogenously added apo E (Table III). The turnover study of apo E after secretion, or both. LPL can mediate the removal of exogenously added apo E cannot proceed in nonsecreting J774 cells (apo E-secreting J774 cells) and thereby be removed by LPL rapidly exchange onto an apo E-lipid particle (produced by the LDL receptor expression, and therefore the local accumulation, of apo E at any tissue site where macrophages are the major source of apo E, e.g. the atherosclerotic vessel wall lesion (15, 32). Because macrophage expression of apo E has been shown to be anti-atherogenic (16, 17), increased LPL could have deleterious effects on the cholesterol homeostasis of the vessel wall. This is consistent with observations in intact mice indicating that high macrophage lipoprotein lipase expression and secretion are associated with enhanced susceptibility to atherosclerosis (33). It is also of interest that the regulation of macrophage apo E and LPL expression are different. For example, it has been recently demonstrated that oxidant stress (e.g. by addition of hydrogen peroxide) enhances macrophage LPL mRNA levels and LPL production (34). We have been unable to detect any effect of hydrogen peroxide on macrophage apo E mRNA levels. Platelet-derived growth factor appears to have a major role in modulating the expression of macrophage LPL (35), but such a regulatory effect for apo E has not been demonstrated.

Cholesterol loading of macrophages enhances macrophage apo E synthesis and secretion predominantly by stimulating apo E gene transcription (20). The data in this report indicate that cholesterol loading will also suppress the LPL-mediated degradation of endogenously synthesized apo E in the macrophage. Cholesterol loading will, therefore, function synergistically at transcriptional and post-translational loci to enhance the net secretion of apo E by macrophage foam cells in the arterial wall.

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REFERENCES

1. Basu, S. K., Brown, M. S., Ho, Y. K., Havel, R. J., and Goldstein, J. L. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 11657–11662
2. Chait, A., Iverius, P.-H., and Brunzell, J. D. (1982) J. Clin. Invest. 69, 490–493
3. Mahley, R. W. (1988) Science 240, 622–630
4. Goldberg, J. J. (1990) J. Lipid Res. 31, 795–797
5. Mazzone, T., and Reardon, C. (1994) J. Lipid Res. 35, 1345–1353
6. Choi, S. Y., Sivaram, P., Walker, D. E., Curtiss, L. K., Gretch, D. G., Sturley, S. L., Attie, A. D., Deckelbaum, R. J., and Goldberg, I. J. (1995) J. Biol. Chem. 270, 8081–8086
7. Nykjer, A., Nielsen, M., Lookene, A., Meyer, N., Roigaard, H., Etzerodt, M., Reissigel, U., Oliveroena, G., and Glemann, J. (1994) J. Biol. Chem. 269, 3174–31755
8. Williams, K. J., Fleiss, G. M., Petrie, K. A., Snyder, M. L., Brocia, R. W., and Swenson, T. L. (1992) J. Biol. Chem. 267, 13294–13292
9. Takahashi, S., Suzuki, M., Oda, Y., Oda, K., Tamai, T., Miyabe, S., Yamamoto, T., and Nakai, T. (1995) J. Biol. Chem. 270, 15747–15754
10. Saxena, U., Ferguson, E., and Binaigui, C. L. (1993) J. Biol. Chem. 268, 14812–14819
11. Williams, S. E., Inoue, I., Tran, H., Fry, G. L., Pladet, M. W., Iverius, P.-H., Lalouel, J.-M., Chappell, D. A., and Strickland, D. K. (1994) J. Biol. Chem. 269, 8653–8658
12. Chappell, D. A., Inoue, I., Fry, G. L., Pladet, M. W., Bowen, S. L., Iverius, P.-H., Lalouel, J.-M., and Strickland, D. K. (1994) J. Biol. Chem. 269, 18001–18006
13. Ji, Z.-S., Fazio, S., Lee, Y.-L., and Mahley, R. W. (1994) J. Biol. Chem. 269, 2764–2772
14. Ji, Z.-S., Brecht, W. J., Miranda, R. D., Hussain, M. M., Innerarity, T. L., and Mahley, R. W. (1993) J. Biol. Chem. 268, 10160–10167
15. Oberan, K. D., Deeb, S. S., Ferguson, M., McDonald, T. O., Allen, M. D., Alpers, C. P., and Chait, A. (1994) Am. J. Pathol. 144, 538–548
16. Shimano, H., Ohuga, J., Shimada, M., Namba, Y., Gotoda, T., Harada, K., Katoaki, M., Yazaki, Y., and Yamada, N. (1995) J. Clin. Invest. 95, 449–476
17. Bellotta, S., Mahley, R. W., Sanan, D. A., Murata, J., Newland, D. L., Taylor, J. M., and Pittas, R. E. (1995) J. Clin. Invest. 96, 2170–2179
18. Saxena, U., Klein, M. G., and Goldberg, I. J. (1991) J. Biol. Chem. 266, 17614–17621
19. Stins, M. F., Sivaram, P., Sasaki, A., and Goldberg, I. J. (1993) J. Lipid Res. 34, 1853–1861
20. Mazzone, T., Basheeruddin, K., and Poulos, C. (1989) J. Biol. Chem. 30, 1055–1064
21. Duan, H., Li, Z., and Mazzone, T. (1995) J. Clin. Invest. 96, 915–922
22. Luo, M., and Mazzone, T. (1996) J. Biol. Chem. 271, 13444–13460
23. Iverius, P.-H., and Ostlund-Lindquist, A. M. (1976) J. Biol. Chem. 251, 7791–7795
24. Williams, S. E., Ashcom, J. D., Argarvies, W. S., and Strickland, D. K. (1992) J. Biol. Chem. 267, 9095–9040
25. Mazzone, T., Basheeruddin, K., Ping, L., Frazer, S., and Getz, G. S. (1989) J. Biol. Chem. 264, 1787–1792
26. Mazzone, T., Pastelnickas, L., and Reardon, C. A. (1992) J. Biol. Chem. 267, 1081–1087
27. Hata, A., Ridinger, D. N., Sutherland, S. D., Mami, M., Kong, L. K., Shuhua, J., Lobbiers, A., Guy-Grand, B., Bassevant, A., Iverius, P.-H., Wilson, D. E., and Lalouel, J. M. (1992) J. Biol. Chem. 267, 20159–20139
28. Sivaram, P., Vanni-Reyes, T., and Goldberg, I. J. (1996) J. Biol. Chem. 271, 15261–15266
29. van Bartinge, H. H. J., de Jong, H., Ekerelos, D. W., and de Bruin, T. W. A. (1996) J. Biol. Chem. 37, 754–767
30. Salini, S., Jing-Yi, L., Mims, M. P., Zeigmond, E., Smith, L. C., and Chan, L. P. (1996) J. Biol. Chem. 271, 10155–10160
31. Medhi, D. J., Bowen, S. L., Fry, G. L., Ruben, S., Andracki, M., Inoue, I., Lalouel, J.-M., Strickland, D. K., and Chappell, D. A. (1996) J. Biol. Chem. 271, 17073–17080
32. Rosenfeld, M. E., Butler, S., Ordl, V. A., Lipton, B. A., Dyer, S. A., Curtiss, L. K., Palinski, W., and Witztum, J. L. (1993) Arteriosclerosis. Thromb. 13, 1382–1389
33. Renier, G., Skamene, E., DeSanctis, J. B., and Radzioch, D. (1993) Arteriosclerosis. Thromb. 13, 190–196
34. Renier, G., Desfaits, A.-C., Lambert, A., and Mikhail, R. (1996) J. Lipid Res. 37, 799–809
35. Inaba, T., Kawanuma, M., Gotoda, T., Harada, K., Shimada, M., Ohuga, J., Shimano, H., Akamura, Y., Yazaki, Y., and Yamada, N. (1995) Arteriosclerosis. Thromb. Vasc. Biol. 15, 522–528