Cell-surface Expression of an Amino-terminal Fragment of Apolipoprotein B Increases Lipoprotein Lipase Binding to Cells*

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Previous studies (Sivaram, P., Choi, S. Y., Curtiss, L. K., and Goldberg, I. J. (1994) J. Biol. Chem. 269, 9409-9412) from this laboratory showed that the NH2-terminal region of apoB (NTAB) has binding domains for lipoprotein lipase (LPL). LPL binding to endothelial cells, we hypothesize, involves interaction both with heparan sulfate proteoglycans and with a protein that has homology to NTAB. To test whether cell-surface NTAB would increase the amount and affinity of LPL binding to cells, we produced stable Chinese hamster ovary cell lines that have NTAB anchored to the cell surface. A cDNA encoding the amino-terminal 17% of apoB (apoB17) was fused to a cDNA coding for the last 37 amino acids of decay-accelerating factor (DAF), which contains the signal for glycosylphosphatidylinositol anchor attachment. The fused construct was sequence-verified and cloned into expression vector pCMV5. The pCMV5-apoB17-DAF plasmid was cotransfected with a neomycin resistance gene into wild-type (WT) cells and mutant heparan sulfate proteoglycan-deficient Chinese hamster ovary cells (745 cells), and stable cell lines were established. Expression of apoB17 on the cell surface was confirmed by the release of apoB17 by phosphatidylinositol-specific phospholipase C. LPL binding to WT and apoB17-DAF-transfected cells was determined. Using 0.8-6 μg of LPL, 1.3-2.2-fold more LPL associated with apoB17-DAF WT cells compared with WT cells; apoB17-DAF also increased LPL binding to 745 cells. After heparinase treatment, LPL binding to apoB17-DAF cells was still greater than to treated WT cells. This increased binding to apoB17-DAF cells was almost abolished by treatment of cells with phosphatidylinositol-specific phospholipase C or anti-apoB monoclonal antibody. LPL dissociated from WT cells with k-1 = 2.55 × 10^-2 min^-1, whereas LPL dissociated more slowly from apoB17-DAF-containing cells with k-1 = 1.08 × 10^-2 min^-1. Furthermore, almost 95% of the LPL on WT cells was dissociated by 1 M NaCl, while only 65% of the LPL dissociated from apoB17-DAF cells at the same high salt concentration. Similarly, in high salt, more LPL remained associated with apoB17-DAF cells than with nontransfected 745 cells. These data show that NTAB on cell surfaces can function as a LPL-binding protein. Moreover, they demonstrate that LPL association with cells can be increased by simultaneously binding to both proteoglycan and non-proteoglycan binding sites.

Lipoprotein lipase (LPL) hydrolyzes triglycerides in the circulating plasma lipoproteins, chylomicrons, and very low density lipoproteins. Although LPL is synthesized by adipocytes, myocytes, and neurons, its primary site of action is at the luminal side of the endothelium. It is widely believed that LPL binds to endothelial cells via an electrostatic interaction with heparan sulfate proteoglycans (HSPG) (2-5). Earlier studies from this laboratory suggested that LPL binding to endothelial cells also involves a 116-kDa non-proteoglycan LPL-binding protein (6, 7). The 116-kDa LPL-binding protein has homology to the NH2-terminal region of apoB (NTAB) (8), the major structural protein of LDL, very low density lipoproteins, and chylomicrons (9). Furthermore, we have recently demonstrated that endothelial cells synthesize a full-length apoB protein and process it to generate the 116-kDa LPL-binding fragment (10).

Several lines of evidence suggested that LPL interacts with NTAB. Ligand blotting of apoB fragments generated by thrombin digestion of LDL showed that labeled LPL bound specifically to NTAB. In addition, LPL bound to NH2-terminal fragments obtained from apoB-transfected CHO cells (8). LPL binding to endothelial cells was inhibited by antibodies to NH2-terminal regions of apoB, but not by antibodies that recognize epitopes closer to the carboxyl terminus of apoB. Based on these data, it was hypothesized that NTAB mediates LPL binding to cells and may confer specificity to LPL binding to endothelial cells.

The amino-terminal region of apoB is relatively hydrophilic. Models of LDL suggest that NTAB extends away from the lipid core (9). Similarly, NTAB on the surface of a cell would not be expected to be buried within the cellular phospholipid bilayer and thus should be available to interact with LPL. Cells that overexpress truncated apoB fragments have been generated by other investigators. These cells, however, either secrete the small NH2-terminal fragments of apoB or degrade them intracellularly (11-13). Thus, these cells cannot be used to assess cell-surface actions of NTAB. Decay-accelerating factor (DAF) is a 70-kDa protein that contains sequences for glycosylphosphatidylinositol (GPI) attachment in the carboxy-terminal region (14). To test our hypothesis that NTAB can function as a LPL-binding protein, we generated several CHO cell lines that overexpress NTAB as a fusion protein with a 37-amino acid peptide of DAF such that apoB was expressed as a GPI-anchored membrane protein. Our results show that NTAB present on cell surfaces mediates high affinity LPL binding.

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1 The abbreviations used are: LPL, lipoprotein lipase; HSPG, heparan sulfate proteoglycan(s); NTAB, NH2-terminal region of apoB; LDL, low density lipoprotein; CHO, Chinese hamster ovary; DAF, decay-accelerating factor; GPI, glycosylphosphatidylinositol; BSA, bovine serum albumin; DMEM, Dulbecco’s modified Eagle’s medium; PIP, phosphatidylinositol-specific phospholipase C; mAb, monoclonal antibody; WT, wild-type; PAGE, polyacrylamide gel electrophoresis.
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**MATERIALS AND METHODS**

Na<sup>125</sup>I was obtained from Amersham Corp. 48-well plates were from Falcon (Oxrand, CA). Bovine serum albumin (BSA), heparin, heparinase, and heparitinase were from Sigma. Dulbecco's modified Eagle's medium/Coon's F-12 medium (DMEM/F-12; 1:1) was from Life Technologies, Inc. Prepackaged PD-10 (Pharmacia Biotech Inc) heparin-agarose and H. Ginsberg (Columbia University). All the CHO cells were maintained by trypsinization and plated in 75-cm<sup>2</sup> culture flasks (1–2 × 10<sup>6</sup> cells/flask). Confluent cells were incubated for 24 h at 4°C in the absence or presence of a 50-fold excess of unlabelled LPL. After 2 h, the cells were washed, and the cell-surface-associated LPL was released with DMEM/F-12/BSA (3%) containing 100 units/ml heparin for 30 min at 4°C as described previously (21). Specific binding was defined as the difference between binding in the absence or presence of unlabelled ligand.

**RESULTS**

**Expression of apoB17-DAF in Transfected Cells**—The apoB17-DAF construct was cotransfected with the Neo<sup>+</sup> gene

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**FIG. 1. pCMV5-apoB17-DAF construct.** The sequence including the last 37 amino acids of DAF, which contains the signal for GPI anchor attachment, was released from pUC19 as a 480-base pair fragment with XmnI/EcoRV and ligated in frame to the blunted MluI site of the COOH terminus of apoB17 DNA in pCMV5. The pCMV5-apoB17-DAF construct was verified by sequencing. CMV, cytomegalovirus; Kb, kilobases.
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into WT CHO and mutant CHO 745 cells. Stable transfecants were then selected in G418. To assess the expression of apoB17 and apoB17-DAF, transfected cells and conditioned media were collected and immunoprecipitated with anti-apoB polyclonal antibodies. Immunoprecipitates were analyzed by SDS-PAGE and Western blotting (Fig. 2A). Nontransfected cells (WT CHO) did not express apoB (lane 1). ApoB17-transfected cells secreted apoB17 into the media (lane 2), although a portion of apoB that probably represents intracellular apoB17 was still associated with the cells. ApoB17-DAF-transfected WT and 745 cells expressed apoB17 as a cell-associated protein (lanes 3 and 4), and no apoB17 was detected in the media. These results show that in apoB17-DAF cells, apoB17 was cell-associated.

We next tested whether cell-associated apoB17-DAF was on the cell surface and was anchored by a GPI anchor. ApoB17-DAF WT and 745 cells were incubated with 1 unit/ml PIPLC for 1 h. PIPLC-released proteins (Media) and cell extracts (Cells) were immunoprecipitated using an anti-apoB polyclonal antibody and analyzed by SDS-PAGE and Western blotting as described under “Materials and Methods.” Lanes 3 and 4 are immunoprecipitates from PIPLC-treated apoB17-DAF WT and 745 cells.

Binding of 125I-LPL to WT CHO and apoB17-transfected Cells—We next tested whether LPL binding to apoB17-containing cells was increased. WT CHO and apoB17-DAF cells were incubated with 0.05–6 μg of 125I-LPL in the presence or absence of a 50-fold excess of unlabeled LPL at 4°C for 2 h. Specific binding was defined as the difference between binding in the presence and absence of unlabeled LPL. At LPL concentrations of 0.8–6 μg/ml, the amount of LPL (5.2–57.1 ng/10^6 cells) associated with apoB17-DAF WT cells was 33–135% higher than the amount of LPL (3.9–26.5 ng/10^6 cells) associated with WT CHO cells (Fig. 3A). 2.8–25 ng of 125I-LPL/10^6 cells specifically bound to the apoB17-DAF 745 cells when 0.2–6 μg/ml LPL was included in the medium (Fig. 3B). In contrast, CHO 745 cells, which have neither HSPG nor apoB, had no specific binding (Fig. 3B). For this reason, the 745 cells were not used in subsequent experiments. Specific binding to apoB was estimated in two ways: 1) as the amount of LPL on apoB17-DAF WT cells minus the amount of LPL on WT cells and 2) as the specific binding of LPL to apoB17-DAF 745 cells. These two estimates gave comparable results, i.e. at 6 μg/ml LPL, 25 ng of LPL/10^6 cells bound to apoB17-DAF 745 cells, while the difference in LPL binding between apoB17-DAF WT cells and WT cells was 30.6 ng/10^6 cells. LPL binding to apoB17-transfected cells was also assessed. ApoB17 cells bound the same amount of LPL as WT cells (data not shown). Therefore, cell-surface apoB led to a significant increase in the amount of LPL that associated with WT and HSPG-deficient cells.

Effects of Heparinase, Heparin, PIPLC, and mAb19 on LPL Binding to WT and apoB17-DAF-transfected Cells—To further confirm that the increased binding to the apoB17-DAF cells was due to the apoB and not to an alteration in cell-surface HSPG, confluent monolayers of cells were treated with 1 unit/ml heparinase and 0.1 unit/ml heparitinase or with 1 unit/ml PIPLC at 37°C for 1 h. Cells were washed and incubated with 1.5 μg/ml 125I-LPL at 4°C for 2 h, and the total amount of heparin-releasable cell-surface LPL was assayed as described under “Materials and Methods.” ApoB17-DAF WT cells bound 14.7 ng/10^6 cells (48%) more LPL than WT CHO cells (45.6 ± 1.9 versus 30.9 ± 0.6 ng/10^6 cells) (Fig. 4A). After heparinase/heparitinase treatment, apoB17-DAF WT cells still bound 17.2 ng/10^6 cells (103%) more 125I-LPL than WT cells. The increased binding was comparable to the difference in LPL binding between untreated apoB17-DAF WT and WT cells, suggesting that the increased binding was not due to increased HSPG. Pretreatment with PIPLC did not affect 125I-LPL binding to WT cells, but decreased 125I-LPL binding to apoB17-DAF WT cells by 44%, making the amount of LPL binding to these cells almost comparable to that of WT cells.

The effect of anti-apoB mAb19, which recognizes NTAB, on LPL binding to cells was also assessed. Cells were incubated with a 1:500 dilution of mAb19 ascites at 4°C for 2 h, and 125I-LPL binding to cells was determined as described “Materials and Methods.” mAb19 did not affect 125I-LPL binding to WT cells, but decreased 125I-LPL binding to apoB17-DAF cells.
by 30%. A control experiment was also performed using monoclonal antibody 47, which binds to a region of apoB not within apoB17. This antibody did not affect LPL association with the apoB17-DAF cells (data not shown). The results suggest that NTAB on cell surfaces can function as a LPL-binding protein.

A similar series of experiments were performed using apoB17-DAF CHO 745 cells. As expected, heparinase treatment had no effect on LPL binding to the cells (Fig. 4B). Since a greater percent of the LPL binding to these cells should have been via NTAB, not surprisingly, the effects of PIPLC and anti-apoB antibodies were more marked. Both treatments reduced total (specific plus nonspecific) binding to the cells by −50%. Therefore, a greater percent of LPL association with the cells was due to NTAB in HSPG-deficient cells versus apoB17-DAF WT cells.

Previous studies with basic fibroblast growth factor, which has both proteoglycan and non-proteoglycan receptors, showed that binding to HSPG was more sensitive to heparin than binding to a non-HSPG receptor (22). We tested the effect of heparin on 125I-LPL binding to cells to determine if this was also true for LPL. In the presence of 1 μg/ml heparin, binding of 125I-LPL to all three cell types was almost abolished (Fig. 4A and B). LPL binding to cells was then assessed using heparin concentrations of 0.1–10 μg/ml. 125I-LPL binding was similar between control and transfected cells at every concentration (data not shown). Thus, it appears that unlike basic fibroblast growth factor binding, LPL binding to both HSPG and NTAB is heparin-sensitive.

**Dissociation of 125I-LPL Binding to Cells**—We assessed the dissociation of LPL bound to WT and transfected cells. Cells were incubated with 1.5 μg/ml 125I-LPL at 4 °C for 2 h. Then cells were washed and incubated with fresh medium alone (C) or with medium containing a 50-fold excess of unlabeled LPL (●) at 4 °C for the indicated times. The amount of radioactivity remaining on WT (A), apoB17-DAF WT (B), and apoB17-DAF 745 (C) cells was determined as described under “Materials and Methods.” Data are means ± S.D. from triplicate experiments. For some values, the error bars are within the data points. Regression analysis of the data (solid line) was performed before the plateau. Initial dissociation rates were determined from the regression lines.
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**TABLE I**

| Cells          | Total binding | Surface | Intracellular | Degradation |
|----------------|---------------|---------|---------------|-------------|
|               | % of initial binding |        |               |             |
| WT            | 35.7 ± 0.6     | 7.37 ± 0.4 | 11.2 ± 0.88  | 3.8 ± 1.11  |
| ApoB17-DAF WT | 52.75 ± 1.9    | 12.3 ± 1.7 | 13.6 ± 1.03  | 3.79 ± 0.13 |
| ApoB17-DAF 745| 26.56 ± 1.57   | 7.3 ± 0.36 | 4.4 ± 0.24   | 0.77 ± 0.04 |

**Fig. 6. Dissociation of 125I-LPL from WT and apoB17-DAF CHO cells by NaCl.** Confluent cultures of cells were incubated with 1.5 μg/ml 125I-LPL cells at 4°C for 2 h. The cells were washed with DMEM/BSA (0.3%) three times, followed by different concentrations of NaCl twice. After NaCl washing, the amount of radioactivity remaining on WT (●), apoB17-DAF WT (○), and apoB17-DAF 745 (□) cells was determined as described under "Materials and Methods." Data are means ± S.D. from triplicate experiments. For some values, the S.D. bars are within the data points.

**TABLE II**

| Cells          | Total binding | Surface | Intracellular | Degradation |
|----------------|---------------|---------|---------------|-------------|
| apoB17-DAF WT  | 52.75 ± 1.9    | 12.3 ± 1.7 | 13.6 ± 1.03  | 3.79 ± 0.13 |
| ApoB17-DAF 745| 26.56 ± 1.57   | 7.3 ± 0.36 | 4.4 ± 0.24   | 0.77 ± 0.04 |

**Discussion**

Our experiments show that NTAB on the surface of cells can mediate the association of LPL with the cells and that the LPL-NTAB association is more stable than the LPL-HSPG association. To show this, we first produced an apoB17 DNA fragment that was fused to the 37 amino acids of DAF. When transfected into CHO cells, apoB17-DAF was expressed on the cell surface and released by treatment of cells with PIPICL, which specifically cleaves the GPI anchor. ApoB17-DAF-transfected WT and HSPG-deficient cells were then used to test the hypothesis that NTAB mediates LPL binding.

Cell-surface NTAB increased the amount, the affinity, and the stability of LPL associated with the surface of CHO cells. Cells expressing NTAB bound up to 2.2-fold more LPL than WT cells. Several experiments were done to prove that the increased binding was due to NTAB. 1) The increased binding was abolished by PIPICL treatment. 2) Heparinase had no significant effect on the increased binding. 3) The increased binding was inhibited by mAb19, which recognizes the amino-terminal region of apoB. LPL-apoB binding was of a higher affinity than LPL-HSPG binding. Binding to cell-surface HSPG in WT cells was rapidly reversible (k<sub>-1</sub> for LPL binding to HSPG = 2.55 × 10<sup>-2</sup> min<sup>-1</sup>). In contrast, LPL dissociation from apoB-containing cells was much slower (k<sub>-1</sub> = 1.08 × 10<sup>-2</sup> min<sup>-1</sup>). This suggests that LPL associates more tightly with apoB.

Previous studies with basic fibroblast growth factor showed differential sensitivity of high and low affinity binding sites to heparin (22). We, however, failed to detect such differences at
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hemin concentrations ranging from as low as 0.1 to 10 μg/ml. A very likely explanation for this is that both LPL-HSPG and LPL-apoB interactions are sensitive to heparin. LPL has several putative heparin-binding domains (23–25), one of which may be involved in apoB binding, thus making it sensitive to heparin. NTAB by itself also binds heparin (6, 8). Therefore, it is not surprising that heparin prevents LPL from binding to apoB.

A second method to show the relative stability of the LPL-NTAB and LPL-HSPG interactions was used. LPL purification commonly includes an affinity chromatography step in which the LPL is dissociated from heparin using high concentrations of NaCl (17). Therefore, we tested whether a similar concentration of NaCl would affect LPL-apoB association. At 1 m NaCl, most of the LPL-HSPG interaction was abolished, while significant association of LPL with apoB persisted. This was also found in experiments using apoB17-DAF 745 cells, which do not express HSPG. Thus, although not differentially sensitive to heparin, LPL-NTAB association was more resistant to salt.

Although NTAB provided high affinity binding for LPL, it did not increase LPL degradation. Both WT and apoB17-DAF cells degraded only a small portion of LPL, <10%. Most of this LPL degradation was via a HSPG-mediated pathway. ApoB17-DAF 745 cells, which do not express HSPG, show similar amounts of LPL as WT cells, but degraded much less LPL.

Several studies have examined the metabolic fate of LPL in adipocytes (26, 27), heart (29), and liver (30, 31). Cultured adipocytes and heart cells internalize and degrade LPL in the lysosomal compartment (26, 27, 29). Similarly, when isolated rat livers are perfused with LPL-containing media, LPL is taken up and degraded (31). Cultured endothelial cells, however, do not degrade LPL significantly compared with the above-mentioned cell types (21, 32). It was hypothesized that endothelial cells internalize LPL, store it in a non-lysosomal acidic compartment (endocytic vesicle), and then recycle the LPL either to the medium or the cell surface (21). Based on the present study showing that apoB-expressing CHO cells degrade only a small portion of LPL and our recent observation that endothelial cells have NTAB (6, 8), we hypothesize that apoB on the surface of endothelial cells provides a high affinity LPL-binding site that stabilizes LPL activity and delays its release into the bloodstream and eventual catabolism in the liver (33).

A number of proteins that bind to cell-surface HSPG also have a second non-proteoglycan receptor. They include basic fibroblast growth factor (34, 35), interleukin-8 (36), tissue factor pathway inhibitor (37), thrombin (38), thrombospondin (39), and angiopoietin-1 (40–42). These proteins bind to HSPG with low affinity, but with high capacity. This initial binding is often necessary for the subsequent binding to the higher affinity non-proteoglycan receptor. Such a scenario is to some extent also true for LPL. LDL receptor-related protein can bind LPL and mediate its uptake and degradation (43, 44). This pathway is operative in both hepatic and several non-hepatic cells that express LDL receptor-related protein (40, 43–45). Endothelial cells, on the other hand, do not express LDL receptor-related protein (28).

Previous results from this laboratory showed that endothelial cells contain a 116-kDa LPL-binding protein that has sequence homology to NTAB (8). By employing reverse transcription-polymerase chain reaction and metabolic labeling, we further demonstrated that endothelial cells synthesize apoB mRNA and protein (10). Our studies also showed that the 116-kDa LPL-binding NTAB is generated by proteolysis of full-length apoB. Thus, NTAB is in a position to interact with LPL on the endothelial cell surface. We postulate, based on our current data, that NTAB increases the affinity of LPL binding to cells. Moreover, a dual molecular interaction of LPL with both HSPG and NTAB leads to the most stable association of LPL with cells. In the turbulent in vivo environment along the luminal endothelial surface, maintenance of high concentrations of LPL might require such dual interactions.