Expression of the Acetyl Low Density Lipoprotein Receptor by Rabbit Fibroblasts and Smooth Muscle Cells

UP-REGULATION BY PHORBOL ESTERS*

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The acetyl low density lipoprotein (LDL), or scavenger receptor, which binds modified forms of LDL, was thought to be expressed only on macrophages and endothelial cells. We demonstrate that rabbit fibroblasts and smooth muscle cells bind, internalize, and degrade acetoacetylated LDL, a ligand for the acetyl LDL receptor. Degradation is specific in that unlabeled acetoacetylated LDL and fucoidin, a known competitor for binding to the acetyl LDL receptor, are effective competitors, while native LDL is not. The acetyl LDL receptor on these cells is readily regulated. Higher levels of degradation are observed in cells preincubated with serum than in cells preincubated with plasma. This up-regulation of the acetyl LDL receptor is most likely due to the presence of platelet secretory products in serum since secretion products derived from thrombin-stimulated platelets also cause an increase in degradation. In addition, preincubation of rabbit fibroblasts with phorbol esters results in a 16-20-fold increase in specific degradation. These results indicate that rabbit fibroblasts and smooth muscle cells express the acetyl LDL receptor and that increased receptor expression appears to be mediated through activation of the protein kinase C pathway.

Modification of the lysine residues of apolipoprotein B of low density lipoprotein (LDL) by acetylation or acetoacetylation (AcAc) prevents the binding of LDL to the LDL receptor (1, 2). However, this modified LDL binds with high affinity to the acetyl LDL, or scavenger, receptor (1-3). Modified LDL that binds to the acetyl LDL receptor is internalized and delivered to lysosomes, where the protein and cholesteryl esters are degraded. The free cholesterol is then transferred to the cytoplasm, where it is re-esterified (4). This pathway of receptor-mediated endocytosis is similar to the LDL receptor pathway. However, in contrast to the LDL receptor expression of the acetyl LDL receptor is not down-regulated by an increase in intracellular cholesterol (1). For this reason, incubation of macrophages with high levels of acetyl LDL leads to cholesterol accumulation and makes the macrophages resemble foam cells found in atherosclerotic lesions (1, 5). While acetyl LDL and AcAc LDL do not occur naturally, malondialdehyde-modified LDL (6) and oxidized LDL (7), two other ligands for the acetyl LDL receptor, may be generated in vivo (8, 9). These modified forms of LDL have been postulated to contribute to the accumulation of cholesteryl esters in foam cells of atherosclerotic lesions (10, 11).

The acetyl LDL receptor is expressed on macrophages (1) and endothelial cells both in vivo (12, 13) and in vitro (14), but its expression has not been previously demonstrated on fibroblasts and smooth muscle cells. In this study we have investigated the expression of the acetyl LDL receptor by rabbit fibroblasts and smooth muscle cells. We found that these cells degrade AcAc LDL and that degradation is increased by preincubating the cells with serum-containing medium, platelet secretory products, or phorbol esters. Increased degradation appears to be mediated through activation of protein kinase C.

EXPERIMENTAL PROCEDURES

Materials—Fetal bovine serum was obtained from HyClone (Logan, UT). Dulbecco’s modified Eagle’s medium (DMEM), Dulbecco’s phosphate-buffered saline (PBS), penicillin, and streptomycin were obtained from GIBCO. Sodium [35S]iodide was purchased from Amerham Corp. The fluorescent probe 1,1'-dioctadecyl-3,3',3',3'-tetramethylindocarbocyanine perchlorate (DiI) was purchased from Molecular Probes, Inc. (Eugene, OR). Fresh human platelet concentrates were obtained from the Peninsula Blood Bank (San Mateo, CA). Hirudin, benzamidine, thrombin, and phosphor esters (phorbol 12-myristate 13-acetate, phosphor 12,13-didecanote, and 4a phosphor 12,13-didecanote) were purchased from Sigma, New Zealand White (NZW) rabbits were obtained from Hazelton Research Products, Inc. (Denver, PA). Fucoidin was purchased from ICN Pharmaceuticals (Plainview, NY). Nylon mesh for filtering cells (Nitex, 10 μm) was obtained from Teko, Inc. (Elmsford, NY).

Lipoproteins—Rabbit blood was drawn either with or without anticoagulant (EDTA, 1 mg/ml) for obtaining plasma or serum, respectively. Serum was obtained by centrifuging the blood after it had clotted at room temperature. Rabbit plasma was obtained by centrifuging (2500 X g) the anticoagulant-containing blood for 30 min. Platelet secretory products, or phorbol esters (phorbol 12-myristate 13-acetate, phosphor 12,13-didecanote, and 4a phosphor 12,13-didecanote) were obtained from Sigma, New Zealand White (NZW) rabbits were obtained from Hazelton Research Products, Inc. (Denver, PA). Fucoidin was purchased from ICN Pharmaceuticals (Plainview, NY). Nylon mesh for filtering cells (Nitex, 10 μm) was obtained from Teko, Inc. (Elmsford, NY).

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* The abbreviations used are: LDL, low density lipoprotein; AcAc, acetoacetylated; Dil, 1,1'-dioctadecyl-3,3',3',3'-tetramethylindocarbocyanine perchlorate; DMEM, Dulbecco’s modified Eagle’s medium; FACS, fluorescence-activated cell sorter; NZW, New Zealand White; PBS, phosphate-buffered saline; WHHL, Watanabe heritable hyperlipidemic.

The acetyl low density lipoprotein (LDL), or scavenger, receptor, which binds modified forms of I.D.I., was thought to be expressed only on macrophages and endothelial cells. We demonstrate that rabbit fibroblasts and smooth muscle cells bind, internalize, and degrade acetoacetylated LDL, a ligand for the acetyl LDL receptor. Degradation is specific in that unlabeled acetoacetylated LDL and fucoidin, a known competitor for binding to the acetyl LDL receptor, are effective competitors, while native LDL is not. The acetyl LDL receptor on these cells is readily regulated. Higher levels of degradation are observed in cells preincubated with serum than in cells preincubated with plasma. This up-regulation of the acetyl LDL receptor is most likely due to the presence of platelet secretory products in serum since secretion products derived from thrombin-stimulated platelets also cause an increase in degradation. In addition, preincubation of rabbit fibroblasts with phorbol esters results in a 16-20-fold increase in specific degradation. These results indicate that rabbit fibroblasts and smooth muscle cells express the acetyl LDL receptor and that increased receptor expression appears to be mediated through activation of the protein kinase C pathway.

Modification of the lysine residues of apolipoprotein B of low density lipoprotein (LDL) by acetylation or acetoacetylation (AcAc) prevents the binding of LDL to the LDL receptor. However, this modified LDL binds with high affinity to the acetyl LDL, or scavenger, receptor. Modified LDL that binds to the acetyl LDL receptor is internalized and delivered to lysosomes, where the protein and cholesteryl esters are degraded. The free cholesterol is then transferred to the cytoplasm, where it is re-esterified. This pathway of receptor-mediated endocytosis is similar to the LDL receptor pathway. However, in contrast to the LDL receptor expression of the acetyl LDL receptor is not down-regulated by an increase in intracellular cholesterol. For this reason, incubation of macrophages with high levels of acetyl LDL leads to cholesterol accumulation and makes the macrophages resemble foam cells found in atherosclerotic lesions. While acetyl LDL and AcAc LDL do not occur naturally, malondialdehyde-modified LDL and oxidized LDL, two other ligands for the acetyl LDL receptor, may be generated in vivo and in vitro, but its expression has not been previously demonstrated on fibroblasts and smooth muscle cells. In this study we have investigated the expression of the acetyl LDL receptor by rabbit fibroblasts and smooth muscle cells. We found that these cells degrade AcAc LDL and that degradation is increased by preincubating the cells with serum-containing medium, platelet secretory products, or phorbol esters. Increased degradation appears to be mediated through activation of protein kinase C.
DI as described (16, 17), and the $^{125}$I-labeled LDL and Dil-labeled LDL were acetocetylated as described (2).

Platelet Secretory Products—Secretory products were derived from platelets activated with thrombin as described below. Ten units of fresh human platelet concentrate were centrifuged at 1,000 rpm (200 × g) for 25 min at room temperature to remove erythrocytes. The supernatant was then spun at 2,500 rpm (1,300 × g) for 25 min at room temperature to pellet it. The pellet was washed twice by suspension in 8 volumes of buffer (123 mM NaCl, 33 mM dextrose, 13 mM sodium citrate, 20 mM Tris, pH 7.3) and centrifugation (1,300 × g). The pellet was then resuspended in 8 volumes of ETS buffer (10 mM EDTA, 150 mM NaCl, 10 mM Tris, pH 7.4) and pelleted by centrifugation (1,300 × g) for 25 min at room temperature. After resuspension of the pellet in 50 ml of ETS buffer, thrombin (0.5 unit/ml) was added and the platelet suspension was incubated for 5 min at room temperature. Immediately after this incubation period, heparin (1.0 unit/ml) was added to inhibit thrombin, and the suspension was centrifuged at 10,000 × g for 15 min at 4°C. The pellet was discarded, and sodium chloride and benzamidine were added to the supernatant (final concentration of 0.35 M and 10 mM, respectively). Aliquots of the supernatant were frozen at −70°C until used.

Cell Culture Experiments—Human fibroblasts were cultured as previously described (17). The rabbit cell lines have been described (18). The NZW rabbit fibroblast cell lines NF1 and N2F1 were established in our laboratory, as were the Watanabe heritable hyperlipidemic (WHHL) rabbit fibroblast cell line WFB1 and the NZW rabbit smooth muscle cell line NSMC. The rabbit skin fibroblast line (RABs) was obtained from the American Type Culture Collection (Rockville, MD). The NZW rabbit smooth muscle cell lines SMC2 and SMC3 were provided by Drs. Lisa Minor and George Rothblat (Medical College of Pennsylvania, Philadelphia, PA). Cells were routinely grown in DMEM containing 10% heat-inactivated fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). The medium was routinely changed 48 h before an experiment. The cells were then incubated for 48 h at 37°C in DMEM containing either 10% fetal bovine serum, 10% human lipoprotein-deficient serum, 10% rabbit serum, or 10% rabbit lipoprotein-deficient serum. An example of this is shown in Fig. 2. This apparent regulation of receptor expression was consistently observed, although the magnitude was variable (Table I). As a control, experiments were also performed with DiI-labeled LDL. As

![Photomicrographs showing the uptake of fluorescently labeled AcAc LDL by rabbit fibroblasts and smooth muscle cells.](image)

**FIG. 1.** Photomicrographs showing the uptake of fluorescently labeled AcAc LDL by rabbit fibroblasts and smooth muscle cells. The cells were incubated for 5 h at 37°C with Dilabeled AcAc LDL (5 μg/ml). Representative fields were selected for fluorescence micrographs. A, New Zealand White rabbit fibroblasts; B, Watanabe heritable hyperlipidemic rabbit fibroblasts; C, New Zealand White rabbit smooth muscle cells.

![FACS analysis of rabbit fibroblasts following incubation at 37°C with Dilabeled AcAc LDL.](image)

**FIG. 2.** FACS analysis of rabbit fibroblasts following incubation at 37°C with Dilabeled AcAc LDL. New Zealand White rabbit fibroblasts (NF1) were grown for 7 days in DMEM containing 10% fetal bovine serum (FBS) or changed for the final 48 h to DMEM containing 10% human lipoprotein-deficient serum (LPDS). The cells were then incubated with Dilabeled AcAc LDL (5 μg/ml) for 16 h before removal from the dishes for FACS analysis. The mean relative fluorescence intensity of the cells preincubated with lipoprotein-deficient serum (solid line) or fetal bovine serum (broken line) was 1.4 or 3.6, respectively. The mean fluorescence intensity of control cells not incubated with lipoprotein was 0.45.

**RESULTS**

We have previously demonstrated that cells that internalize and degrade Dil-labeled lipoproteins become intensely fluorescent because the fluorescent probe Dil remains in the cells (21). The fluorescence intensity (Dil accumulation) is therefore proportional to the amount of lipoprotein internalized and degraded. To determine whether fibroblasts or smooth muscle cells bind and internalize chemically modified lipoproteins, initial studies were performed with lipoproteins labeled with Dil. Rabbit fibroblasts and smooth muscle cells were incubated for 5 h at 37°C with Dilabeled AcAc LDL (5 μg/ml) and then viewed under the fluorescence microscope. These studies clearly demonstrated uptake of the chemically modified LDL by the rabbit cells (Fig. 1). As expected, when control experiments were conducted with human fibroblasts, no uptake of Dilabeled AcAc LDL was seen (data not shown). Fibroblasts from both normal rabbits and from WHHL rabbits, which lack normal LDL receptors, internalized Dilabeled AcAc LDL.

From FACS analysis it was apparent that the rabbit fibroblasts preincubated with fetal bovine serum internalized more Dilabeled AcAc LDL (i.e. became more highly fluorescent) than those preincubated with human lipoprotein-deficient serum. An example of this is shown in Fig. 2. This apparent regulation of receptor expression was consistently observed, although the magnitude was variable (Table I). As a control, experiments were also performed with DiI-labeled LDL. As
TABLE I

Effect of preincubation of rabbit fibroblasts with either fetal bovine serum or human lipoprotein-deficient serum on the uptake of Dil-labeled acetoacetylated low density lipoproteins

Cells were grown, incubated with Dil-labeled AcAc LDL or Dil-labeled LDL, and subjected to FACS analysis as described in the legend to Fig. 2. FBS, fetal bovine serum; LPDS, lipoprotein-deficient serum.

| Cells* | LPDS | FBS | Ratio |
|--------|------|-----|-------|
| NFbl   | 3.5  | 6.2 | 1.8   |
| NFbl   | 0.9  | 3.1 | 3.4   |
| WFbl   | 2.8  | 3.4 | 1.2   |

*Cells (NFbl) were either from NZW rabbits (NFbl) or from WHHL rabbits (WFbl), which lack normal LDL receptors. The data on the NZW fibroblasts were obtained with the same cell line at two different times.

TABLE II

Effect of preincubation of New Zealand White rabbit fibroblasts with either serum or plasma on the degradation of 125I-labeled acetoacetylated low density lipoproteins

| Preincubation* | 125I-AcAc LDL degraded | µg/mg cell protein/16 h |
|----------------|-------------------------|------------------------|
| Human lipoprotein-deficient serum | 0.27 | |
| Fetal bovine serum | 0.73 | |
| Rabbit | | |
| Lipoprotein-deficient serum | 0.27 | |
| Plasma | 0.53 | |
| Serum 1 | 0.53 | |
| Serum 2 | 0.93 | |

*Cells (NFbl) were preincubated for 48 h as indicated (see “Experimental Procedures” for a description of the preparation of the plasma and sera used). The cells were then washed with DMEM and incubated for 16 h with DMEM containing 125I-labeled AcAc LDL (5 µg/ml). See “Experimental Procedures” for the method of quantitating lipoprotein degradation products in the medium and of deriving lipoprotein-deficient sera from plasma.

expected, uptake of Dil-labeled LDL was decreased following preincubation with fetal bovine serum, and fibroblasts from WHHL rabbits, which lack normal LDL receptors, failed to internalize Dil-labeled LDL (Table I).

In the experiments described above, the rabbit cells were preincubated with either human lipoprotein-deficient serum or with fetal bovine serum. To ensure that the modulation of receptor expression observed was not an artifact caused by species differences, the effect of rabbit plasma and serum on AcAc LDL metabolism was studied. As shown in Table II, preincubation of the cells with rabbit lipoprotein-deficient serum or rabbit plasma resulted in levels of 125I-labeled AcAc LDL degradation comparable to those obtained when the cells were incubated with human lipoprotein-deficient serum. In addition, the increase in degradation caused by preincubation with rabbit serum was similar to that seen when the cells were preincubated with fetal bovine serum.

To ensure that the metabolism of AcAc LDL was not restricted to the specific cell lines initially studied, the ability of several other rabbit fibroblast and smooth muscle cell lines to degrade 125I-labeled AcAc LDL was determined. As shown in Table III, all the rabbit fibroblast and smooth muscle cell lines were capable of degrading AcAc LDL, and in all cases degradation was increased in cells preincubated with fetal bovine serum. Since human fibroblasts have been reported to lack acetyl LDL receptors, they were used as a control. As expected, human fibroblasts preincubated with fetal bovine serum degraded very little 125I-labeled AcAc LDL (less than 0.1 µg/mg cell protein/16 h).

Competition studies performed at 37 °C demonstrated that the degradation was mediated through uptake via the acetyl LDL receptor. As shown in Fig. 3, unlabeled AcAc LDL or fucoidin (a known competitor for binding to the acetyl LDL receptor), but not native LDL, competed for the degradation of 125I-labeled AcAc LDL by WHHL fibroblasts. Similar results were obtained when this experiment was performed using lipoprotein-deficient sera from plasma.

TABLE III

Degradation of 125I-labeled acetoacetylated low density lipoproteins by rabbit fibroblast and smooth muscle cell lines preincubated with either fetal bovine serum or human lipoprotein-deficient serum

| Cell lines* | LPDS | FBS | Ratio |
|-------------|------|-----|-------|
| Fibroblasts | | | |
| NFblp5 | 0.30 | 0.57 | 1.9 |
| NFblp11 | 0.27 | 0.73 | 2.7 |
| N2Fbl | 0.10 | 0.25 | 2.5 |
| RAB9 | 0.89 | 0.99 | 1.1 |
| WFBlp4 | 0.69 | 1.1 | 1.6 |
| WFBlp10 | 0.54 | 1.4 | 2.6 |
| Smooth muscle cells | | | |
| NSMC | 0.15 | 0.33 | 2.2 |
| SMC2 | 0.26 | 0.43 | 1.6 |
| SMCC | 0.25 | 0.36 | 1.4 |

*Fibroblasts from both NZW rabbits (NFbl, N2Fbl, and RAB9) and WHHL rabbits (WFbl) were used. The NFbl and WFbl fibroblasts were tested at different passage numbers.
using fibroblasts and smooth muscle cells from New Zealand White rabbits (data not shown).

Since one difference between plasma and serum is their content of platelet secretory products, we activated human platelets with thrombin and tested the ability of a supernatant released by low speed centrifugation to stimulate the degradation of AcAc LDL by rabbit fibroblasts. As shown in Fig. 4, platelet secretory products stimulated the degradation of AcAc LDL in a dose-dependent manner. Thrombin itself, when incubated with WHHL fibroblasts, did not affect the amount of AcAc LDL degraded (data not shown). The factor(s) released from thrombin-activated platelets that stimulate the degradation of 125I-labeled AcAc LDL by fibroblasts is unknown and is the subject of current studies.

In subsequent experiments designed to determine the mechanism of regulation of acetyl LDL receptor expression, we found that phorbol esters are potent stimulators of the degradation of 125I-labeled AcAc LDL in rabbit fibroblasts. As shown in Fig. 5, preincubation of WHHL rabbit fibroblasts with phorbol myristate acetate or phorbol didecanoate (both of which activate protein kinase C) stimulated the degradation of AcAc LDL in these cells by 16-20-fold above the level in untreated controls. In contrast, preincubation with 4a-phorbol didecanoate (which does not activate protein kinase C) did not stimulate the degradation of AcAc LDL (Fig. 5). Preincubation of human fibroblasts with phorbol esters did not stimulate the degradation of AcAc LDL (data not shown).

**DISCUSSION**

The acetyl LDL receptor was originally described on mouse and rat peritoneal macrophages, Kupffer cells, and cultured human monocytes (1) and was subsequently shown to be expressed by endothelial cells both in vivo (12, 13) and in vitro (14). We now report that rabbit fibroblasts and smooth muscle cells also express the acetyl LDL receptor and that it can be up-regulated by preincubation of the cells with serum, by supplementation of medium with platelet secretory products, or by phorbol ester-induced activation of protein kinase C.

In the present study, LDL was modified by acetoacetylation. Both unmodified AcAc LDL and fucoidin, but not native LDL, inhibited the degradation of 125I-labeled AcAc LDL. These criteria are essentially those used originally to define the acetyl LDL receptor (1, 4). These data, however, do not demonstrate that the receptor present on the rabbit fibroblasts and smooth muscle cells is identical to the acetyl LDL receptor that is expressed by macrophages and endothelial cells. In fact, Krieger and colleagues (22-24) have recently purified, cloned, and sequenced two forms of the scavenger (acetyl LDL) receptor. The receptor, purified from bovine lung membranes, is a 220-kDa protein that is a trimer of 77-kDa subunits (23). Two forms of the receptor have been cloned from a bovine lung cDNA library and their sequences deduced (23, 24). The receptors are identical except that one form has a cysteine-rich carboxyl-terminal domain, whereas in the other form this domain is replaced by a 6-residue carboxyl terminus. When expressed in COS cells, both forms of the receptor bind acetyl LDL. The similar structure of these receptors suggests that they may exist as mixed trimers (23).

High molecular mass (>260 kDa) acetyl LDL-binding proteins have also been purified by Via et al. (25) and Kelley et al. (26) from a mouse peritoneal macrophage cell line (P388D1) and from rabbit carrageenan-induced granulomas, respectively.

While expression of the LDL receptor by cells is readily regulated by the cholesterol content of the cells, the acetyl LDL receptor is resistant to regulation by intracellular cholesterol (4). The macrophage acetyl LDL receptor is clearly not down-regulated by cholesterol, since incubation of macrophages with acetyl LDL or AcAc LDL leads to cholesteryl ester accumulation and foam cell formation. In the current study, when rabbit smooth muscle cells and fibroblasts were preincubated with fetal bovine serum, LDL receptor expression was decreased, as expected; however, expression of the acetyl LDL receptor was enhanced. It is likely that the increase in acetyl LDL receptor expression observed was unrelated to the cholesterol status of the cells. The increased...
expression of the receptor in the cells preincubated with serum is more likely to be due to the presence of platelet-secretory products in the serum, since secretory products from thrombin-stimulated platelets also enhanced receptor expression.

Activated platelets secrete numerous bioactive compounds.

In the present study, enhanced degradation of AcAc LDL was induced by preincubation of fibrillar platelets with platelet-secretory products; however, it has been reported that human monocyte-derived macrophages preincubated with platelet-secretory products degrade less modified LDL than controls (27). The differences in these results could be due to a difference in the regulation of the receptor in the two cell types or to a difference in the experimental design. Aviram (27) preincubated the cells with pure serotonin, fibrinogen, fibronectin, or platelet-derived growth factor, whereas our experiments were performed using a mixture of products released from thrombin-stimulated platelets. In addition, it has been reported that platelets secrete a glycoprotein that inhibits the degradation of AcAc LDL by macrophages (28). The inhibitory factor blocks the binding of AcAc LDL to the acetyl LDL receptor and therefore must be present in the medium at the same time as the modified LDL to compete for binding and degradation. In the current study the cells were washed, following preincubation with the platelet supernatant, before addition of the 125I-labeled AcAc LDL. The inhibitory protein was not present during the degradation assay. These data suggest that platelets secrete several factors that affect the metabolism of modified LDL.

Alteration of acetyl LDL receptor expression has also been demonstrated upon differentiation of monocytes to macrophages. Human monocytes that become adherent to tissue culture dishes and attain macrophage-like characteristics undergo a 6-fold increase in acetyl LDL receptor expression in 6–10 days (29). This increase in acetyl LDL receptor expression is inhibited by lipopolysaccharide (30), while dexamethasone stimulates receptor activity slightly (31).

In a recent study Via et al. (32) reported a dramatic increase in acetyl LDL receptor expression in the human monocyte cell line THP-1 when the cells were induced to differentiate to macrophages by incubation with phorbol esters, which activate protein kinase C. This protein kinase C-mediated differentiation is also associated with increased secretion of lipoprotein lipase and apolipoprotein F (33) due to activation of transcription of their respective genes. Therefore, in these cells the phorbol esters induce the expression of a number of properties associated with differentiated macrophages.

In the present study, phorbol esters (which activate protein kinase C) increased the degradation of acetyl LDL by rabbit fibroblasts by 16–20-fold, i.e. to the level observed by Via et al. (32) in the phorbol ester-treated THP-1 cells. Protein kinase C is normally activated by diacylglycerols produced by agonist-induced hydrolysis of inositol phospholipids (34, 35). These data suggest that under specific physiological conditions, acetyl LDL receptor expression in cells might be modulated over a wide range by bioactive agonists that activate protein kinase C.

In atherosclerotic lesions, cholesteryl ester-rich lipid inclusions are found in both macrophages and smooth muscle cells. The mechanism by which the smooth muscle cells accumulate lipid in vivo has not been established. Since smooth muscle cells can express the acetyl LDL receptor in vitro, it is possible that cholesteryl ester accumulation in smooth muscle cells in vivo results from the uptake of modified forms of LDL. The environment of the atherosclerotic lesion may contribute to the up-regulation of acetyl LDL receptor expression in that smooth muscle cells, macrophages, and aggregating platelets can exist in close proximity. Macrophages are known to secrete numerous bioactive substances, and we have demonstrated that platelets secrete products that can regulate acetyl LDL receptor expression. In addition, extracellular lipid often accumulates in the core of advanced atherosclerotic lesions as a result of cell death. It is possible that diacylglycerols in this lipid could activate protein kinase C and up-regulate acetyl LDL receptor expression in smooth muscle cells.

There is, in fact, evidence that suggests that the acetyl LDL receptor can be expressed by smooth muscle cells in atherosclerotic lesions and that this receptor expression may be regulated. We reported that macrophage-derived foam cells that migrated from the explants expressed acetyl LDL receptors in that study. However, Jaakkola et al. (36) reported uptake of D1-labeled acetyl LDL by smooth muscle cells released from collagenase-treated rabbit atheroma. They found that many smooth muscle cells, which contained lipid inclusions, internalized D1-labeled acetyl LDL. However, in agreement with our study of explants, they found that most of the proliferating smooth muscle cells did not take up D1-labeled AcAc LDL. The data obtained in the present study, in conjunction with the data from Jaakkola et al. (36), clearly demonstrate that fibroblasts and smooth muscle cells can express the acetyl LDL receptor and that receptor expression can be regulated.

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