Phagocytosis of aggregated lipoprotein by macrophages: Low density lipoprotein receptor-dependent foam-cell formation

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ABSTRACT Low density lipoprotein (LDL) modified by incubation with phospholipase C (PLC-LDL) aggregates in solution and is rapidly taken up and degraded by human and mouse macrophages, producing foam cells *in vitro*. Human, mouse, and rabbit macrophages degraded ^{125}I-labeled PLC-LDL (^{125}I-PLC-LDL) more rapidly than native ^{125}I-labeled LDL (^{125}I-LDL), while nonphagocytic cells such as human fibroblasts and bovine aortic endothelial cells degraded ^{125}I-PLC-LDL more slowly than ^{125}I-LDL. This suggested the mechanism for internalization of PLC-LDL was phagocytosis. When examined by electron microscopy, mouse peritoneal macrophages appeared to be phagocytosing PLC-LDL. The uptake and degradation of ^{125}I-PLC-LDL by human macrophages was inhibited >80% by the monoclonal antibody C7 (IgG2b) produced by hybridoma C7, which blocks the ligand binding domain of the LDL receptor. Similarly, methylation of ^{125}I-LDL (^{125}I-MeLDL) prior to treatment with phospholipase C decreased its subsequent uptake and degradation by human macrophages by >90%. The uptake and degradation of phospholipase C-modified ^{125}I-MeLDL by macrophages could be restored by incubation of the methylated lipoprotein with apoprotein E, a ligand recognized by the LDL receptor. These results indicate that macrophages internalize PLC-LDL by LDL receptor-dependent phagocytosis.

The morphological hallmark of the early cellular lesion of atherosclerosis is the presence of lipid-laden foam cells, predominantly derived from macrophages (1). The mechanisms that lead to accumulation of cholesterol by these cells remain unknown. The major pathway for delivery of exogenous cholesterol to most cells is uptake of low density lipoprotein (LDL) (2). Because LDL receptor activity is tightly coupled to the level of cellular cholesterol, it is likely that a pathway separate from LDL receptor-mediated endocytosis is responsible for the pathological accumulation of cholesterol.

Macrophages are also able to ingest particulate material such as senescent cells and microorganisms by phagocytosis (3). Phagocytosis requires initial binding to specific receptors on the cell surface. After binding, the plasma membrane of the macrophage surrounds the particle by the interaction of ligands distributed circumferentially around the particle with receptors on the surface of the cell (4).

The receptor-mediated endocytosis of lipoproteins has been widely studied as a potential mechanism for foam-cell formation (2, 5). In contrast, the possibility that phagocytosis might mediate the unregulated accumulation of cholesterol by macrophages has received little attention. When LDL is modified by treatment with phospholipase C (PLC-LDL), ≈70% of the phospholipid of LDL undergoes hydrolysis, and aggregation of the lipoprotein occurs. This particulate aggregated lipoprotein is rapidly taken up and degraded by human and mouse macrophages, producing foam cells *in vitro*. Our

results indicate that human monocyte-derived macrophages ingest PLC-LDL by a LDL receptor dependent process and that the mechanism of internalization of PLC-LDL by macrophages is likely to be phagocytosis.

EXPERIMENTAL PROCEDURES

Materials. Minicolumns, Sephadex G-50, and Ficoll-Paque were supplied by Pharmacia. Phospholipase C (type XIII) from *Bacillus cereus* and alkaline phosphatase (type III-S) were obtained from Sigma. The mouse C7 hybridoma cells producing monoclonal antibody (of type IgG2b) that binds to LDL receptors were obtained from the American Type Culture Collection. Monoclonal antibody OKM1 (IgG2b) was provided by Ortho Diagnostics. All other materials were obtained from the indicated sources (6).

Methods. *Macrophages.* Human monocyte-derived macrophages were isolated by density gradient centrifugation by the method of Boyüm (7). The mononuclear cell band was washed twice at 4°C in RPMI 1640 medium and then plated at 3×10^5 cells per 16-mm well in the same medium containing 20% (vol/vol) autologous serum and 100 units of penicillin and 100 μg of streptomycin per ml. Resident mouse peritoneal macrophages were prepared from unstimulated Swiss Webster mice (25–35 g) by the method of Edelson and Cohn (8). After one wash in Dulbecco's modified Eagle's medium, the cells were plated at 1×10^6 cells per 16-mm well in the same medium supplemented with 20% (vol/vol) fetal calf serum and 100 units of penicillin and 100 μg of streptomycin per ml. After a 2-hr incubation, nonadherent cells were removed from the human and mouse macrophages by three washes with serum-free medium. The mouse cells were used for experiments 24 hr after plating, while human cells were incubated for 7–14 days prior to use. Rabbit alveolar macrophages were obtained by pulmonary lavage with phosphate-buffered saline (9) and then were plated and cultured as described for mouse macrophages.

Other cells. Human skin fibroblasts were obtained by punch biopsy from the anterior thighs of healthy volunteers and cultured as described (10). Human arterial smooth muscle cells were isolated from medial explants of aorta by the method of Ross (11) and cultured in Dulbecco's modified Eagle's medium (6). Bovine aortic endothelial cells, cultured as described (12), were a gift of Steven Schwartz, University of Washington. Endothelial cells, fibroblasts, and smooth muscle cells were incubated for 48 hr in 10% (vol/vol) lipoprotein-deficient human serum to up-regulate LDL receptor activity (13) before use in experiments.

Lipoproteins. LDL (density = 1.019–1.063 g/ml) was prepared from human plasma (final concentrations: EDTA, 4

mM; phenylmethylsulfonyl fluoride, 1 μM) by discontinuous density gradient ultracentrifugation by the method of Chung *et al.* (14) as described (6). LDL was iodinated by using Na^{125}I (15) and acetylated by the method of Basu *et al.* (16). Acetylation of LDL was confirmed by electrophoresis in 0.5% agarose gel at pH 8.6 (17). Apoprotein E, prepared by the method of Weisgraber *et al.* (18), was a gift from John Albers, University of Washington. ^{125}I-labeled LDL (^{125}I-LDL; 40.0 mg) was methylated as described (19). Lipoprotein and apoprotein concentrations are expressed as μg of protein per ml.

Modification of LDL by phospholipase C. Phospholipase C (specific activity, 2500 units/ml) was diluted to 100 units/ml with Tris buffer (50 mM Tris·HCl, pH 7.4). After dialysis of the enzyme over 12 hr at 4°C against Tris buffer, LDL (final concentration, 1–3 mg/ml) was incubated with 1 unit (or the indicated final concentration) of phospholipase C per ml of Tris buffer for 1 hr at 37°C in a 12 × 75 mm borosilicate glass tube. The modified lipoprotein was reisolated by passage over a minicolumn (10 cm × 2 cm) packed with Sephadex G-50 with RPMI 1640 medium as the elutant and immediately was used for experiments. Control experiments showed that no phospholipase C activity was present in reisolated PLC-LDL.

Phospholipid hydrolysis. The extent of phospholipid hydrolysis of PLC-LDL was estimated by measuring the appearance of aqueous phosphorous colorimetrically after digestion of the released phosphate polar head group with alkaline phosphatase (20).

Metabolism of lipoproteins by cells. The uptake and degradation of ^{125}I-labeled lipoprotein (^{125}I-lipoprotein; specific activity, 100–300 cpm/ng) was measured as the appearance of trichloroacetic acid-soluble non-^{125}I-radioactivity in the medium of the cells (10). Degradation rates are corrected for cell-free incubations carried out in parallel. Results are expressed as ng of lipoprotein protein degraded per mg of cell protein. The delivery of lipoprotein cholesterol to cells was estimated by measuring the incorporation of [^{14}C]oleate into cholesteryl ester (10). Results are expressed as nmol of cholesteryl [^{14}C]oleate per mg of cell protein.

Other assays. Protein was measured by the method of Lowry *et al* (21) with bovine serum albumin as the standard. The total cholesterol, cholesteryl ester, triglyceride, and phospholipid of PLC-LDL and LDL were measured enzymatically (22). For transmission electron microscopy, mouse peritoneal macrophages were fixed *in situ* with 3% (wt/vol) glutaraldehyde, stained with 1% osmium tetroxide, poststained with uranyl acetate and lead citrate, and examined with a JEOL 100B electron microscope. Nondenaturing gradient gel electrophoresis was carried out as described (23).

All results are means of duplicate determinations unless otherwise stated.

RESULTS

When LDL was incubated with increasing concentrations of phospholipase C, the light scattering of the solution increased dramatically, measured as the increase in optical density at 450 nm (Fig. 1). The change in turbidity of the PLC-LDL solution and extent of phospholipid hydrolysis were related in a linear manner (Fig. 1 *Inset*). Little additional change in turbidity or phospholipid hydrolysis was observed at concentrations of phospholipase C > 1 unit/ml. The lipid composition of PLC-LDL modified by incubation with 1 unit of phospholipase C per ml showed almost no change in total cholesterol, cholesteryl ester, or triglyceride but showed a 70% loss of phospholipid compared with LDL. When examined under nondenaturing conditions on gradient gel electrophoresis, all PLC-LDL entered the running gel, and the modified lipoprotein comigrated with LDL. Therefore, PLC-
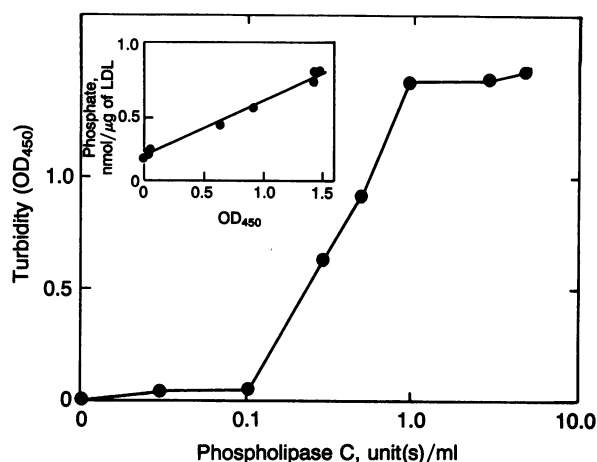

FIG. 1. Dose–response of LDL modification by phospholipase C. ^{125}I-LDL (2 mg/ml) in 50 mM Tris buffer (pH 7.4) was incubated for 1 hr at 37°C with the indicated final concentration of phospholipase C. A 0.1-ml aliquot was combined with 1.25 ml of distilled water, and its turbidity was measured as optical density at 450 nm. An additional 0.1-ml aliquot was removed and extracted with chloroform, and the alkaline phosphatase-releasable inorganic phosphate in the aqueous phase was determined as described. (*Inset*) Relationship between these two measures of phospholipase C activity.

LDL aggregates in a reversible manner, but fusion of individual modified LDL particles does not occur. When PLC-LDL at 5–500 μg/ml was incubated for 5 hr at 37°C, its optical density at 450 nm remained a linear function of concentration. Thus, PLC-LDL does not dissociate to form smaller aggregates or LDL-sized particles when the concentration of the lipoprotein is varied.

On agarose gel electrophoresis, PLC-LDL migrated slightly more rapidly than did LDL, indicating that no major changes had occurred in the charge-to-mass ratio of the modified lipoprotein. In contrast to oxidized LDL (6), the apo B100 of PLC-LDL remained intact on sodium dodecyl sulfate/polyacrylamide gel electrophoresis. PLC-LDL also showed no evidence of lipid peroxidation as assayed by the appearance of material reactive with thiobarbituric acid.

Human monocyte-derived macrophages incubated with ^{125}I-labeled PLC-LDL (^{125}I-PLC-LDL) internalized and degraded the lipoprotein at up to 40 times the rate of ^{125}I-LDL as assessed by the appearance of acid-soluble ^{125}I radioactivity in the medium (Fig. 2A). Similar findings were obtained by using the stimulation of incorporation of [^{14}C]oleic acid into cholesteryl ester as an index of cholesterol delivery to the macrophages (Fig. 2B). At lipoprotein concentrations > 100 μg/ml, ^{125}I-PLC-LDL was degraded more rapidly than ^{125}I-labeled acetylated LDL (^{125}I-AcLDL) by human macrophages (Fig. 2A). The uptake and degradation of ^{125}I-PLC-LDL was nonsaturable at concentrations of lipoprotein up to 1000 μg/ml (data not shown). The nonsaturable uptake of ^{125}I-PLC-LDL is consistent with a mechanism of internalization by macrophages that is not limited by receptor number. Alternatively, the molar concentration of PLC-LDL might be much lower than the protein concentration would suggest. ^{125}I-LDL incubated with heat-inactivated phospholipase C (1 min at 100°C) did not aggregate in solution, and uptake by human macrophages was unchanged relative to native ^{125}I-LDL.

By light microscopy, mouse peritoneal macrophages incubated with 500 μg of PLC-LDL per ml for 12 hr contained many cytoplasmic inclusion bodies (data not shown) resembling the foam cells of atherosclerotic lesions (24). These inclusions stained positive with Oil Red O, suggesting that they were neutral lipid droplets. In contrast, mouse macrophages incubated under identical conditions with LDL or
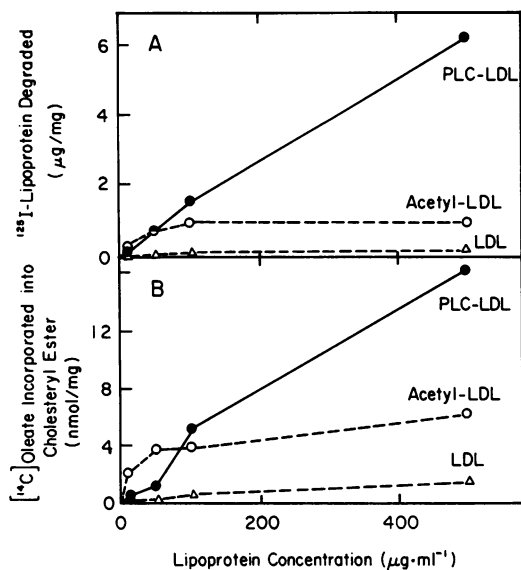
FIG. 2. (A) ^{125}I-lipoprotein degradation by human monocyte-derived macrophages. Human macrophages were washed twice with serum-free medium, and then medium containing ^{125}I-lipoprotein at the indicated concentration was added at 0.5 ml per 16-mm dish. After a 5-hr incubation at 37°C, the medium was removed for determination of acid-soluble ^{125}I-lipoprotein degradation products as described. ^{125}I-PLC-LDL (●) was prepared by incubation of ^{125}I-LDL with phospholipase C (1 unit/ml) as described and then was reisolated by passage over a Sephadex G-50 minicolumn in RPMI 1640 medium. ^{125}I-LDL (△) and ^{125}I-AcLDL (○) were similarly transferred to RPMI 1640 medium by passage over the minicolumn. Lipoprotein concentrations were standardized for protein on the basis of radioactivity. (B) Incorporation of [^{14}C]oleic acid into cholesteryl ester by human monocyte-derived macrophages. Human macrophages were washed twice with serum-free medium; then medium containing lipoprotein at the indicated concentrations, 0.2 mM [^{14}C]oleic acid, and 0.4 mM albumin (essentially fatty acid free) was added (0.5 ml per 16-mm well). The cells were incubated for 5 hr at 37°C, after which they were washed and extracted for determination of cholesteryl [^{14}C]oleate as described. ●, PLC-LDL; ○, AcLDL; △, LDL.

AcLDL contained few inclusions and failed to stain with Oil Red O.

To investigate potential mechanisms responsible for internalization of PLC-LDL, we tested the ability of a variety of cell types to take up and degrade ^{125}I-PLC-LDL. Mouse peritoneal macrophages, human monocyte-derived macrophages, and rabbit alveolar macrophages degraded 6–13 times more ^{125}I-PLC-LDL than native ^{125}I-LDL during a 5-hr incubation with 100 μg of radiolabeled lipoprotein per ml (Table 1). Under identical conditions, human skin fibroblasts, human arterial smooth muscle cells, and bovine aortic endothelial cells degraded little ^{125}I-PLC-LDL relative to ^{125}I-LDL (Table 1). Similar results were obtained by using the incorporation of [^{14}C]oleate into [^{14}C]cholesteryl ester as an index for delivery of lipoprotein cholesterol to the cells (data not shown). Thus, only macrophages internalized and degraded ^{125}I-PLC-LDL more rapidly than ^{125}I-LDL, consistent with phagocytosis as a potential mechanism for the uptake of PLC-LDL by cells. When conditioned medium from human macrophages incubated with PLC-LDL was subsequently incubated with ^{125}I-PLC-LDL in the absence of cells, no ^{125}I-labeled degradation products appeared in the medium. These results indicate that proteases released by the macrophages were not responsible for the increased rate of degradation of ^{125}I-PLC-LDL.

When mouse peritoneal macrophages incubated with 500 μg of PLC-LDL per ml were examined by transmission electron microscopy, particulate material resembling aggre-

Table 1. Cellular uptake and degradation of ^{125}I-LDL, ^{125}I-PLC-LDL, and ^{125}I-AcLDL

Cells	^{125}I-labeled degradation products, ng/mg per 5 hr		
	LDL	PLC-LDL	AcLDL
Macrophages			
Human-monocyte derived	179 ± 32	2280 ± 280	621 ± 151
Mouse peritoneal	853 ± 77	4870 ± 180	7180 ± 930
Rabbit alveolar	224 ± 19	1680 ± 230	197 ± 49
Nonmacrophage cells			
Bovine aortic endothelial	72 ± 32	0 ± 15	168 ± 48
Human skin fibroblasts	601 ± 43	171 ± 17	29 ± 11
Human smooth muscle	788 ± 46	323 ± 21	37 ± 15

The uptake and degradation of ^{125}I-lipoprotein (100 μg/ml) were determined after a 5-hr incubation as described in Fig. 2A. Values are the means ± SD of triplicate determinations.

gated lipoprotein was observed outside the cells (Fig. 3A). No such material was observed when cells were incubated with the same concentration of either LDL or AcLDL (Fig. 3B and C). This particulate material was probably PLC-LDL. In many electron micrographs of mouse macrophages incubated with PLC-LDL, pseudopodia extending from the cell surface surrounded the aggregated material, and similar material was present in cytoplasmic vacuoles (Fig. 3A). Pseudopodia surrounding aggregated material were not observed in mouse macrophages incubated with LDL or AcLDL. Numerous vacuoles containing multivesicular bodies and non-membrane-bound electron-dense cytoplasmic inclusions appeared in the cells after 6–12 hr of incubation with PLC-LDL (Fig. 3A). These vacuoles and cytoplasmic inclusions are probably derived from digested PLC-LDL and resemble those found in foam cells of the atherosclerotic lesion (24, 25). Mouse macrophages incubated with AcLDL contained smaller numbers of similar-appearing vacuoles and cytoplasmic inclusions (Fig. 3C), while macrophages incubated with LDL lacked vacuoles or inclusions (Fig. 3B).

Phagocytosis requires initial binding of particulate material to a cell-surface receptor, followed by internalization (3). Macrophages possess at least two receptors able to interact with lipoproteins, the LDL receptor and the scavenger receptor (5). Human and rabbit macrophages degraded ^{125}I-PLC-LDL more rapidly than ^{125}I-AcLDL, while mouse macrophages degraded ^{125}I-PLC-LDL less rapidly than ^{125}I-AcLDL (Table 1). Bovine aortic endothelial cells, which degraded little ^{125}I-PLC-LDL, rapidly took up and degraded ^{125}I-AcLDL. The ability to dissociate the rates of degradation of ^{125}I-PLC-LDL and ^{125}I-AcLDL by these cell types suggests that the scavenger receptor is unlikely to be involved in the uptake of PLC-LDL.

The C7 hybridoma-produced IgG2b-type monoclonal antibody blocks binding of LDL to its receptor, inhibiting the uptake and degradation of LDL by cells (26). In the presence of the C7 monoclonal antibody, the degradation of both ^{125}I-LDL and ^{125}I-PLC-LDL by human macrophages was inhibited by >80% (Table 2). Under identical conditions, the degradation of ^{125}I-AcLDL was inhibited 14% by C7 monoclonal antibody. Similar results were obtained using the incorporation of [^{14}C]oleate into cholesteryl ester as an index for delivery of lipoprotein cholesterol to the cells (data not shown). An irrelevant IgG monoclonal antibody OKM1, which recognizes a component of the cell-surface complex mediating neutrophil and monocyte adhesion (27), had little effect on the rates of degradation of either ^{125}I-LDL or ^{125}I-PLC-LDL by human macrophages (Table 2). Thus, it is likely that ^{125}I-PLC-LDL was interacting with the cell surface of human macrophages via the LDL receptor.
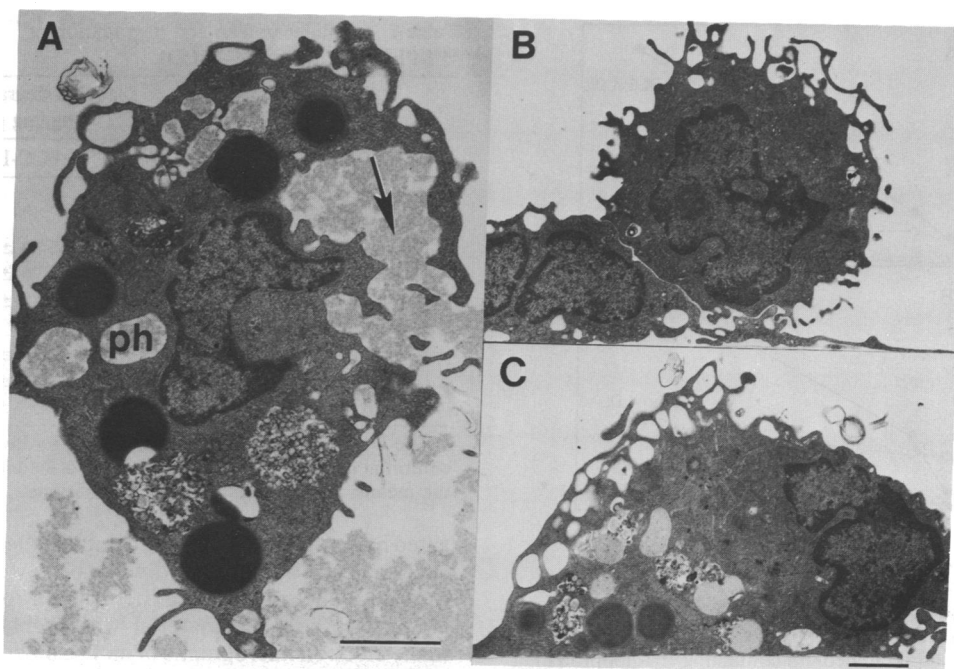
FIG. 3.   Electron micrograph of mouse peritoneal macrophage phagocytosing PLC-LDL. Mouse macrophages were incubated for 12 hr with 500 μg of PLC-LDL per ml (*A*), 500 μg of LDL per ml (*B*), or 500 μg of AcLDL per ml (*C*) and then fixed and prepared for electron microscopy as described. The large vacuoles (ph) observed in macrophages incubated with PLC-LDL probably represent phagosomes containing recently ingested PLC-LDL. Note the resemblance of the particulate material in the large vacuoles to aggregated lipoprotein outside the cells (arrow). (Bar = 2 μm).

When the lysine residues of apolipoprotein B100 of LDL are reductively methylated, recognition of the lipoprotein by the LDL receptor on fibroblasts is lost (19). Similarly, ^{125}I-labeled methylated LDL (^{125}I-MeLDL) was taken up and degraded at only 26% of the rate of ^{125}I-LDL by human macrophages (Table 2). When ^{125}I-MeLDL was treated with phospholipase C under standard conditions, it was taken up and degraded at only 9% of the rate of ^{125}I-PLC-LDL (Table 2). MeLDL and native LDL incubated with phospholipase C aggregated to about the same extent, measured as the change in optical density at 450 nm (0.870 and 0.960, respectively). This suggests that the loss of macrophage degradation of phospholipase C-modified ^{125}I-MeLDL could not be attrib-

Table 2.   Effect of anti-LDL receptor monoclonal antibody (IgG2b type) produced by hybridoma C7 and lipoprotein methylation on the rate of degradation of ^{125}I-PLC-LDL by human monocyte-derived macrophages

Condition	^{125}I-labeled degradation products, ng/mg per 5 hr	
	LDL	PLC-LDL
Antibody		
None	12.5	35.3
C7 (IgG2b)	1.1	5.2
OKM1	11.1	31.9
Lipoprotein		
Control	9.3	27.3
Methylated	2.4	2.5
Methylated + apolipoprotein E	7.4	17.7

The uptake and degradation of ^{125}I-lipoprotein (10 μg/ml) by human macrophages were determined after a 5-hr incubation as described in Fig. 2*A*. ^{125}I-lipoprotein was incubated for 30 min at 37°C with C7 monoclonal antibody (IgG2b) (in 0.5 ml of C7 hybridoma supernatant) or OKM1 (final concentration, 4 μg/ml) prior to addition to the macrophages. ^{125}I-LDL was methylated prior to modification with phospholipase C. Apolipoprotein E was added to ^{125}I-lipoprotein at a final concentration of 4 μg/ml and incubated for 60 min at 37°C prior to addition to the macrophages.

uted to reduced aggregation. Moreover, aggregation alone does not appear adequate to stimulate phagocytosis.

Apolipoprotein E can also serve as a ligand recognized by the LDL receptor (28, 29). When ^{125}I-MeLDL was preincubated with apolipoprotein E, the degradation of the methylated lipoprotein by human macrophages was increased 3-fold (Table 2). Similarly, the uptake and degradation of phospholipase C-treated ^{125}I-MeLDL was increased 7-fold by prior incubation with apolipoprotein E (Table 2).

DISCUSSION

Several lines of evidence indicate that macrophages internalize PLC-LDL by a phagocytic-like process. First, LDL that has been treated with phospholipase C demonstrates a marked increase in turbidity, resulting from aggregation of PLC-LDL. By definition, phagocytosis involves ingestion of particulate material. Second, only human, mouse, and rabbit macrophages degraded ^{125}I-PLC-LDL more rapidly than native ^{125}I-LDL. In contrast, human fibroblasts, human smooth muscle cells, and bovine aortic endothelial cells degraded ^{125}I-PLC-LDL more slowly than ^{125}I-LDL. Finally, mouse macrophages incubated with PLC-LDL appeared to be phagocytosing aggregated lipoprotein when examined by electron microscopy.

To test directly the role of the LDL receptor in the phagocytosis of PLC-LDL and subsequent foam-cell formation, we examined the effect of the C7 monoclonal antibody on the degradation of ^{125}I-PLC-LDL by human macrophages. In the presence of C7 monoclonal antibody, which blocks the ligand-recognition domain of the LDL receptor (26), the degradation of ^{125}I-LDL by human macrophages was inhibited by >80%. Similarly, C7 monoclonal antibody inhibited the degradation of ^{125}I-PLC-LDL by >90%, indicating that the LDL receptor plays an important part in the phagocytosis of PLC-LDL by these cells.

When ^{125}I-LDL was methylated prior to treatment with phospholipase C, the ability of human macrophages to degrade the lipoprotein was inhibited by >70%. Because

methylation of apolipoprotein B100 blocks the binding of LDL to the LDL receptor (19), these results are consistent with a role for apoprotein B100 as the ligand of PLC-LDL. Apolipoprotein E, which also is able to bind to the LDL receptor (28, 29), restored the degradation of phospholipase C-modified ^{125}I-MeLDL by human macrophages to almost the same rate seen with ^{125}I-PLC-LDL. The ability to block the degradation of ^{125}I-PLC-LDL by methylation and then restore the uptake of the phospholipase C-modified ^{125}I-MeLDL by preincubation with apolipoprotein E suggests that the ligand mediating the binding of PLC-LDL to the LDL receptor during phagocytosis is likely to be apolipoprotein B100.

These results suggest a model for the internalization of PLC-LDL by macrophages. PLC-LDL undergoes hydrolysis of $\approx$70% of its phospholipid. The loss of the hydrophilic portion of the phospholipid forces the modified lipoprotein to aggregate, forming particulate PLC-LDL containing multiple copies of apolipoprotein B100. The apolipoprotein B100 mediates the attachment of PLC-LDL to the surface of macrophages by binding to multiple LDL receptors. Like human skin fibroblasts, human and mouse macrophages possess significant numbers of LDL receptors (30). Macrophages then internalize the PLC-LDL by phagocytosis. Phagocytosis probably occurs by a zipper-like mechanism (3, 4), with progressive apposition of the surface membrane around the lipoprotein aggregates by the interaction of apolipoprotein B100 with the LDL receptor.

The results reported here suggest that any mechanism that induces aggregation of LDL might promote phagocytosis of the lipoprotein by a receptor-dependent process. LDL incubated with mast cell granules (31) or insoluble proteoglycan–LDL complexes (32–34) could be taken up by this mechanism. Recently, extracellular forms of phospholipases have been reported (35), suggesting that the mechanism of aggregation that we have described might occur *in vivo*. An alternate possibility is that a source of cholesterol, such as senescent cells or secreted platelet products (36–38), that has acquired an exchangeable ligand recognized by the LDL receptor (such as apolipoprotein E) could be phagocytosed by macrophages in the arterial wall. Alternatively, phagocytosis of aggregated LDL might activate monocytes, enhancing their migration into the arterial wall or altering their secretory function (39).

Nonphagocytic cells that possess LDL receptors failed to degrade PLC-LDL at a significant rate. This suggests that particles above a certain size may not be able to undergo LDL receptor-mediated endocytosis. Macrophages, using cellular mechanisms developed specifically for ingesting particulate material, overcome this size limitation. These cells ingest massive quantities of cholesterol by LDL receptor-dependent phagocytosis of PLC-LDL, rapidly producing foam cells *in vitro*.

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