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Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition

(cell surface receptors/denatured proteins/cultured cells/lysosomes/familial hypercholesterolemia)

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ABSTRACT **Resident mouse peritoneal macrophages were shown to take up and degrade acetylated ^{125}I-labeled low density lipoprotein (^{125}I-acetyl-LDL) *in vitro* at rates that were 20-fold greater than those for the uptake and degradation of ^{125}I-LDL. The uptake of ^{125}I-acetyl-LDL and its subsequent degradation in lysosomes were attributable to a high-affinity, trypsin-sensitive, surface binding site that recognized acetyl-LDL but not native LDL. When ^{125}I-acetyl-LDL was bound to this site at 4°C and the macrophages were subsequently warmed to 37°C, 75% of the cell-bound radioactivity was degraded to mono[^{125}I]iodotyrosine within 1 hr. The macrophage binding site also recognized maleylated LDL, maleylated albumin, and two sulfated polysaccharides (fucoidin and dextran sulfate) indicating that negative charges were important in the binding reaction. A similar binding site was present on rat peritoneal macrophages, guinea pig Kupffer cells, and cultured human monocytes but not on human lymphocytes or fibroblasts, mouse L cells or Y-1 adrenal cells, or Chinese hamster ovary cells. Uptake and degradation of acetyl-LDL via this binding site stimulated cholesterol esterification 100-fold and produced a 38-fold increase in the cellular content of cholesterol in mouse peritoneal macrophages. Although the physiologic significance, if any, of this macrophage uptake mechanism is not yet known, we hypothesize that it may mediate the degradation of denatured LDL in the body and thus serve as a "backup" mechanism for the previously described receptor-mediated degradation of native LDL that occurs in parenchymal cells. Such a scavenger pathway might account for the widespread deposition of LDL-derived cholesteryl esters in macrophages of patients with familial hypercholesterolemia in whom the parenchymal cell pathway for LDL degradation is blocked, owing to a genetic deficiency of receptors for native LDL.**

Studies in cultured human fibroblasts and blood lymphocytes have defined a receptor-mediated pathway by which cells take up and degrade low density lipoprotein (LDL), the major cholesterol-transport protein in human plasma (1, 2). This LDL uptake pathway is believed to function primarily in extrahepatic parenchymal cells where it serves a dual function: it provides the major physiologic route of LDL degradation in the human body and, at the same time, it supplies cholesterol to cells for the synthesis of membranes and steroid hormones.

Patients with familial hypercholesterolemia (FH) have a mutation in the gene specifying the LDL receptor (1, 2). In these patients, LDL degradation through the physiologic pathway is blocked, and each LDL particle survives in the circulation 2–3 times longer than it does in normal subjects (3–5). As a result, the lipoprotein accumulates to abnormally high levels in plasma. Eventually the LDL is degraded through an alternate pathway. Inasmuch as the excess LDL-cholesterol becomes deposited in nonparenchymal cells throughout the body, including hepatic Kupffer cells and macrophages of the spleen, kidney, bone marrow, skin, tendons, and other organs (2, 6, 7), we have postulated that the alternate pathway for LDL degradation is expressed primarily in such scavenger cells (2, 5). Because this scavenger mechanism acts on plasma LDL that has circulated for an abnormally long time *in vivo*, it may recognize some form of LDL that has become denatured or chemically modified. The clearance of denatured proteins *in vivo* by macrophages is well known (8, 9).

As a first step in a search for the postulated macrophage system mediating the uptake and degradation of modified or denatured LDL, we have used as a probe ^{125}I-labeled LDL (^{125}I-LDL) that has been extensively acetylated *in vitro* (^{125}I-acetyl-LDL). The rationale for this choice was based on previous studies showing that acetylation of LDL altered the lipoprotein to the extent that it was no longer recognized by the LDL receptor in fibroblasts (10). As a model cell system for studying the metabolism of ^{125}I-acetyl-LDL, we have used resident mouse peritoneal macrophages isolated by the techniques developed by Cohn and coworkers (11, 12). We demonstrate that these cells possess a high-affinity binding site that recognizes acetyl-LDL but not native LDL. This binding site, which so far appears to occur only on macrophages, mediates the rapid uptake and degradation of acetyl-LDL, producing massive cholesterol deposition in these cells.

METHODS

Mouse Peritoneal Macrophages. Female NCS mice (25–30 g) were obtained from The Rockefeller University. Peritoneal cells were harvested from unstimulated mice in Dulbecco's phosphate-buffered saline (cat. no. 310-4190, GIBCO) (11, 12). The fluid from 12–20 mice (6–10 × 10^6 cells per mouse) was pooled, and the cells were collected by centrifugation (400 × *g*, 10 min, 24°) and washed once with 30 ml of Dulbecco's modified Eagle's medium (henceforth referred to as medium). The cells were resuspended in medium A (medium containing 20% fetal calf serum and 100 units of penicillin and 100 μg of streptomycin per ml) at 2 × 10^6 cells per ml. Aliquots (1 ml) were dispensed into 35 × 10-mm plastic petri dishes and incubated in a humidified CO_2 (5%) incubator at 37°C. After 2 hr each dish was washed three times with 2 ml of medium without serum. Unless otherwise stated, the medium was immediately replaced with 1 ml of medium B (medium containing 10% human lipoprotein-deficient serum), and the experiment was initiated. Each dish of adherent macrophages contained about 30% of the total number of cells originally plated (30–40 μg of cell protein).

Other Cells. Human fibroblasts, mouse L cells, mouse Y-1 adrenal cells, and Chinese hamster ovary cells (CHO-K1) were

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Abbreviations: FH, familial hypercholesterolemia; LDL, low density lipoprotein.

grown in monolayer in either Eagle's minimum essential medium (13) or Ham's F-12 medium containing 10% fetal calf serum. Human lymphoblasts (1) were grown in suspension in RPMI 1640 medium containing 10% fetal calf serum. Rat peritoneal macrophages were isolated as described for mouse macrophages. Monolayers of guinea pig Kupffer cells were prepared by Thomas Rogoff by a modification of the method of Garvey (14). Human mononuclear cells were isolated from peripheral blood of a healthy subject by the method of Boyum (15), and the monocytes and lymphocytes were separated by differential adherence to plastic (16). The monocytes (adherent cells) and lymphocytes (nonadherent cells) were incubated separately for 5 days in RPMI 1640 medium containing 10% autologous human serum (16).

Lipoproteins. LDL (ρ, 1.019–1.063 g/ml) and lipoprotein-deficient serum ($\rho > 1.215$ g/ml) were isolated from plasma of individual subjects by differential ultracentrifugation (10). The concentration of LDL is given in terms of its protein content.

Chemical Modification of Proteins. LDL and human serum albumin (Cohn fraction V) were acetylated with repeated additions of acetic anhydride (17) as described (10). Acetic anhydride was added at 40-fold molar excess with regard to total lysines in LDL. LDL and albumin were maleylated by repeated additions of maleic anhydride (18). All of the acetylated and maleylated proteins had enhanced mobility on electrophoresis in agarose gel at pH 8.6 (see figure 1 in ref. 10). The particle size of acetyl-LDL was similar to that of LDL by electron microscopy after negative staining with uranyl acetate. LDL, acetyl-LDL, and maleyl-albumin were labeled with ^{125}I as described (10).

Assays. The total cellular content of ^{125}I-LDL and ^{125}I-acetyl-LDL in mouse macrophage monolayers was measured at either 4 or 37°C as described for ^{125}I-LDL in fibroblasts (13) except for three modifications: (*i*) the volume of each of the six washes was 2 ml rather than 3 ml; (*ii*) the heparin-release step was omitted; and (*iii*) after the standard wash, the cells were dissolved by incubation at 24°C for at least 1 hr in 0.6 ml of 0.2 M NaOH. The whole sample was assayed to determine the ^{125}I radioactivity associated with the cells, after which an aliquot was used to determine the content of cellular protein (19). The proteolytic degradation of ^{125}I-LDL, ^{125}I-acetyl-LDL, and ^{125}I-labeled maleyl-albumin was measured by assaying the amount of ^{125}I-labeled trichloroacetic acid-soluble (noniodide) material formed by the cells and excreted into the culture medium (20). The incorporation of [^{14}C]oleate into cellular cholesteryl [^{14}C]oleate (21) and the cellular content of free and esterified cholesterol (22) were measured by the referenced methods.

RESULTS

When freshly isolated mouse peritoneal macrophages were incubated with increasing concentrations of ^{125}I-acetyl-LDL at 37°C, the cellular content of radioactivity increased in a saturable fashion (Fig. 1*A*). During the 5-hr incubation, a portion of the added ^{125}I-acetyl-LDL was degraded to trichloroacetic acid-soluble material (Fig. 1*B*). This material was shown by paper chromatography to consist entirely of mono[^{125}I]iodotyrosine. As the ^{125}I-acetyl-LDL concentration increased, the rate of degradation increased hyperbolically in parallel with the saturable component of the cellular uptake process; in both cases, half-maximal values were achieved at an ^{125}I-acetyl-LDL concentration of about 25 μg/ml. In contrast, only small amounts of ^{125}I-LDL became associated with the macrophages (Fig. 1*A*). This uptake showed no evidence of saturation. Moreover, only small amounts of ^{125}I-LDL were degraded by the freshly isolated mouse macrophages (Fig. 1*B*).

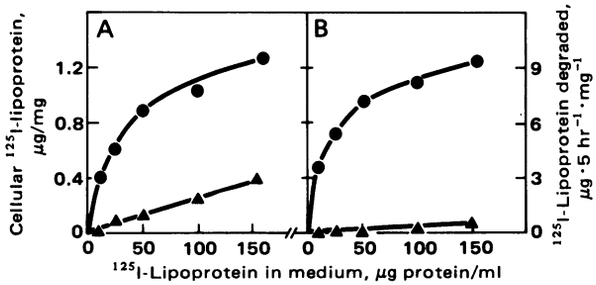

FIG. 1. Accumulation and degradation of ^{125}I-LDL (▲) and ^{125}I-acetyl-LDL (●) by mouse peritoneal macrophages. Each dish received 1 ml of medium B containing the indicated concentration of either ^{125}I-LDL (57,000 cpm/μg of protein) or ^{125}I-acetyl-LDL (50,000 cpm/μg of protein). After incubation for 5 hr at 37°C, the amount of ^{125}I-lipoprotein in the cells (*A*) and the amount of ^{125}I-labeled acid-soluble material in the medium (*B*) were determined in duplicate dishes.

In macrophages incubated with ^{125}I-acetyl-LDL at 37°C for varying lengths of time, the cellular content of radioactivity reached a steady-state plateau after 1 hr (Fig. 2*A*). After this steady-state was reached, acid-soluble radioactivity continued to appear in the medium at a linear rate, reflecting the continuing uptake and degradation of the ^{125}I-acetyl-LDL. After 5 hr, approximately 9 times as much ^{125}I-acetyl-LDL had been degraded as was contained within the cells during the steady-state. In the presence of the lysosomal inhibitor chloroquine (1), degradation was abolished, and the cellular content of ^{125}I-acetyl-LDL increased linearly for 5 hr (Fig. 2*B*).

To measure the surface binding of ^{125}I-acetyl-LDL, we incubated the macrophages with increasing concentrations of ^{125}I-acetyl-LDL at 4°C. The ^{125}I-acetyl-LDL bound to the cells with saturation kinetics (Fig. 3*A*). Maximal binding was achieved within 120 min at both 5 and 50 μg/ml. Two-thirds of the ^{125}I-acetyl LDL bound at 4°C could be released by subsequent incubation of the cells with trypsin (1 mg/ml) at 4°C. As with native LDL in fibroblasts (13), the half-maximal concentration for ^{125}I-acetyl-LDL binding in macrophages at

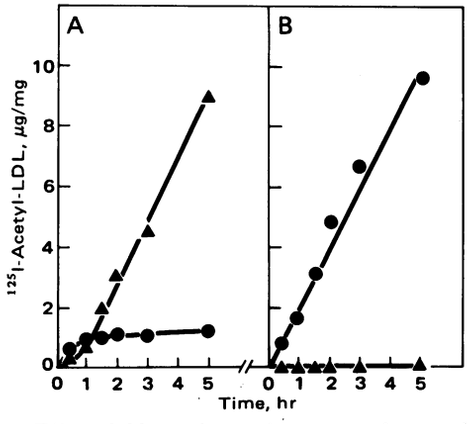

FIG. 2. Effect of chloroquine on time course of accumulation (●) and degradation (▲) of ^{125}I-acetyl-LDL by mouse peritoneal macrophages. The cells were prepared by the standard method except that the monolayers were incubated for 18 hr in 1 ml of medium A prior to the experiment. Each monolayer was then washed with 2 ml of medium, after which 1 ml of medium B containing ^{125}I-acetyl-LDL (10 μg/ml; 98,000 cpm/μg of protein) without (*A*) or with (*B*) 75 μM chloroquine was added. After incubation at 37°C for the indicated interval, the amount of ^{125}I-labeled acid-soluble material in the medium and the amount of ^{125}I-acetyl-LDL in the cells were determined in duplicate dishes.

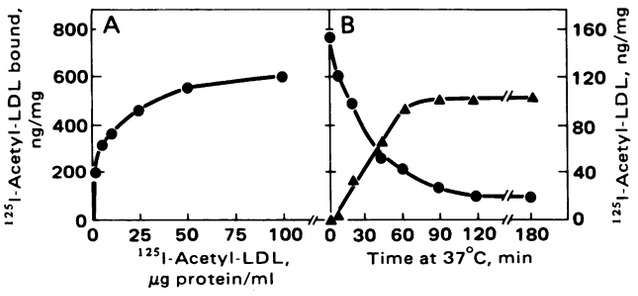

FIG. 3. (*A*) Binding of ^{125}I-acetyl-LDL by mouse peritoneal macrophages at 4°C. Each dish received 1 ml of ice-cold medium B and was placed at 4°C for 30 min, after which the indicated concentration of ^{125}I-acetyl-LDL (734 cpm/ng protein) was added. The monolayers were incubated at 4°C for 2 hr and then washed, and the amount of ^{125}I-acetyl-LDL bound to the cells was determined in duplicate dishes. (*B*) Degradation at 37°C of ^{125}I-LDL previously bound by mouse peritoneal macrophages at 4°C. Each dish was incubated for 2 hr at 4°C with ^{125}I-acetyl-LDL (10 μg/ml; 738 cpm/ng protein) as in *A*, after which each monolayer was washed by the standard procedure. Each dish then received 1 ml of warm medium B containing 20 μg of unlabeled acetyl-LDL per ml, and all the dishes were incubated at 37°C. At the indicated interval, duplicate dishes were rapidly chilled to 4°C, the medium was removed, and its content of ^{125}I-labeled acid-soluble material (▲) was measured. The total amount of ^{125}I-acetyl-LDL that remained associated with the cell (●) was also determined.

4°C (5 μg/ml) was lower than the half-maximal concentration for cellular uptake at 37°C (25 μg/ml, Fig. 1*A*). In another experiment, cells that had bound ^{125}I-acetyl-LDL at 4°C were subsequently warmed to 37°C (Fig. 3*B*). The cell-bound radioactivity declined rapidly. Seventy-five percent of the decline was due to the excretion of acid-soluble radioactivity from the cell, a reaction that reached completion by 60 min. The 25% of cell-bound ^{125}I-acetyl-LDL that was not degraded dissociated from the cell surface soon after warming and appeared in the medium as acid-precipitable material. Such dissociation did not occur at 4°C.

Fig. 4 shows experiments in which macrophages were incubated with ^{125}I-acetyl-LDL in the presence of increasing concentrations of potential competitors for the binding site. LDL failed to compete with the ^{125}I-acetyl-LDL for degradation. On the other hand, unlabeled acetyl-LDL was an effective competitor as was maleyl-LDL (Fig. 4*A*). Acetyl-albumin failed to compete with ^{125}I-acetyl-LDL; however, maleyl-albumin was an effective competitor (Fig. 4*B*).

Inasmuch as acetyl-LDL, maleyl-LDL, and maleyl-albumin have net negative charges, the data suggested that negative charges play a role in the binding reaction. This conclusion was supported by the finding that dextran sulfate (M_r 500,000) and fucoidin, two sulfated polysaccharides, both competed effectively with ^{125}I-acetyl-LDL for degradation. However, poly(D-glutamic acid) (M_r 50,000–100,000), which also has a negative charge, failed to compete (Fig. 4*C*). In each of the experiments of Fig. 4, the amount of cell-bound ^{125}I-acetyl-LDL declined in a manner parallel to that of the degradation rate. Moreover, each of the compounds in Fig. 4 was tested as an inhibitor of ^{125}I-acetyl-LDL binding to the cells at 4°C, and again the results paralleled those shown in Fig. 4. That these inhibitors were acting by binding to the cells and not by binding to ^{125}I-acetyl-LDL was indicated by the finding that prior incubation of cells with fucoidin or dextran sulfate at 4°C followed by washing the cells inhibited the subsequent ^{125}I-acetyl-LDL binding by more than 90%.

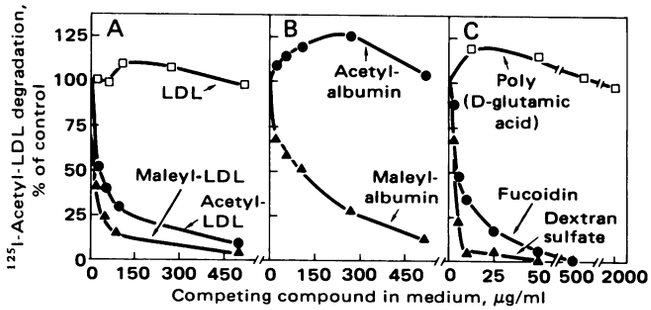

FIG. 4. Ability of various compounds to inhibit the degradation of ^{125}I-acetyl-LDL by mouse peritoneal macrophages. Each dish received 1 ml of medium B containing ^{125}I-acetyl-LDL (25 μg/ml; 60–70 cpm/ng of protein) and the indicated compound. After incubation for 5 hr at 37°C, the amount of ^{125}I-labeled acid-soluble material in the medium was determined. The 100% values for degradation of ^{125}I-acetyl-LDL in the absence of competing compounds were 4600, 4355, and 11,500 ng·5 hr^{-1}·mg of protein^{-1} for *A*, *B*, and *C*, respectively.

Neither dextran (M_r 500,000) nor three sulfated polysaccharides (heparin and chondroitin sulfates A and C) inhibited ^{125}I-acetyl-LDL binding or degradation when tested at concentrations up to 500 μg/ml. Other negatively charged compounds, such as RNA, phosvitin, colominic acid (polysialic acid), and polyphosphates (chain length, 65), were also ineffective inhibitors at 500 μg/ml. Other ineffective inhibitors included: lysozyme, thyroglobulin, fetuin, orosomucoid, and asialo-orosomucoid at concentrations of 500–5000 μg/ml; whole human serum at 30 mg of protein per ml (50% serum); and mannan (from bakers yeast) at 5 mg/ml.

^{125}I-Maleyl-albumin (25 μg/ml) was degraded by the mouse peritoneal macrophages at a rate (4.7 μg·5 hr^{-1}·mg protein^{-1}) similar to that for ^{125}I-acetyl-LDL. The degradation of ^{125}I-maleyl albumin was inhibited by more than 80% by a 20-fold excess of unlabeled maleyl-albumin and acetyl-LDL, confirming that these two substances were taken up through the same binding site. A 20-fold excess of native albumin did not compete for the degradation of ^{125}I-maleyl albumin or ^{125}I-acetyl-LDL.

The amount of high-affinity binding of ^{125}I-acetyl-LDL at 4°C was reduced by 90% when the macrophages were first incubated for 50 min at 37°C with either trypsin (250 μg/ml) or Pronase (10 μg/ml).

High-affinity degradation of ^{125}I-acetyl-LDL occurred not only in mouse peritoneal macrophages but also in rat peritoneal macrophages, guinea pig Kupffer cells, and cultured human blood monocytes (Table 1). These phagocytic cells degraded ^{125}I-acetyl-LDL 3.8–177 times more rapidly than they degraded ^{125}I-LDL. On the other hand, none of the nonmacrophage cells showed significant high-affinity degradation of ^{125}I-acetyl-LDL. In fact, all the nonmacrophage cells tested showed much greater rates of degradation of ^{125}I-LDL than of ^{125}I-acetyl LDL. When the nonmacrophage cells were incubated in the absence of lipoproteins for 48 hr, a condition known to increase LDL receptor activity (1), the rate of degradation of ^{125}I-LDL increased 5- to 15-fold above the values shown in Table I, whereas the degradation of ^{125}I-acetyl-LDL did not change.

In fibroblasts the degradation of LDL releases free cholesterol, and this in turn enhances the rate at which the cells incorporate [^{14}C]oleate into cholesteryl [^{14}C]oleate (21). Whereas LDL did not cause such an effect in macrophages, acetyl-LDL caused a 100-fold increase in the incorporation of [^{14}C]oleate into cholesteryl [^{14}C]oleate (Table 2). This stimulation was inhibited by maleyl-albumin and by fucoidin, indicating that it required uptake for acetyl-LDL through the high-affinity binding site. In the same experiment, acetyl-LDL did not stimulate the incorporation of [^{14}C]oleate into ^{14}C-labeled triglycerides or phospholipids.

Table 1. Ability of various cell types to degrade ^{125}I-acetyl-LDL and ^{125}I-LDL

Cell type	Time in culture	High-affinity degradation, ng·5 hr^{-1} per mg protein		Ratio:
		^{125}I-Acetyl-LDL	^{125}I-LDL	^{125}I-Acetyl-LDL/^{125}I-LDL
Mouse peritoneal macrophages	0	8,780 ± 1,310*	360 ± 120*	24
Rat peritoneal macrophages	1 day	33,700	190	177
Guinea pig Kupffer cells	1 day	4,700	590	8.0
Human monocytes	5 days	1,910	500	3.8
Human lymphocytes	5 days	30	83	0.36
Human fibroblasts	16 generations	15	780	0.02
Human lymphoblasts	>100 generations	13	422	0.03
Mouse L cells	>100 generations	320	1170	0.27
Mouse Y-1 adrenal cells	>100 generations	100	960	0.10
Chinese hamster ovary cells	>100 generations	0	2000	0

Cells were grown in the appropriate medium containing 10% whole serum. For experiments, cells were incubated with 1 or 2 ml of medium containing 10% human lipoprotein-deficient serum and either (*i*) ^{125}I-acetyl-LDL (25 μg/ml; 50–100 cpm/ng) in the absence and presence of 350 μg of unlabeled acetyl-LDL per ml or (*ii*) ^{125}I-LDL (25 μg/ml; 50–100 cpm/ng) in the absence and presence of 350 μg of unlabeled LDL per ml. After 5 hr at 37°C, the amount of ^{125}I-labeled acid-soluble material in the medium was determined in triplicate dishes. High-affinity degradation was calculated by subtracting the values obtained in the presence of unlabeled lipoprotein from those obtained in its absence.
* Mean ± SEM for four experiments carried out on four different days.

The uptake of acetyl-LDL produced a massive increase in the total cholesterol content of the cells after incubation for 4 days (Table 3). This increase was inhibited by maleyl-albumin. Eighty percent of the cholesterol in the macrophages incubated with acetyl-LDL was esterified. In studies to be reported elsewhere, we observed that macrophages incubated with acetyl-LDL developed large numbers of inclusions that stained with Oil Red O and showed birefringence in polarized light, two characteristics of cholesteryl ester-laden macrophages *in vivo*.

The rate of uptake of ^{125}I-acetyl-LDL in macrophages was not increased by incubating the cells for 48 hr in lipoprotein-deficient serum, nor was it suppressed when the cells were incubated for 48 hr in the presence of 25-hydroxycholesterol and cholesterol, two conditions that, respectively, stimulate and suppress the activity of the LDL receptor in fibroblasts and lymphocytes (1, 2). Moreover, prior incubation of the macrophages with unlabeled acetyl-LDL (25 μg/ml) for 72 hr under conditions that caused massive cellular cholesterol deposition (Table 3) did not diminish the rate at which the macrophages took up and degraded ^{125}I-acetyl-LDL, indicating that the acetyl-LDL binding site was not regulated by the cellular cholesterol content.

Table 2. Stimulation of cholesteryl ester formation in mouse peritoneal macrophages incubated with acetyl-LDL

Addition to medium	Incorporation of [^{14}C]oleate into cholesteryl [^{14}C]oleate, nmol/mg protein
None	0.62
LDL, 25 μg/ml	0.83
LDL, 250 μg/ml	0.92
Acetyl-LDL, 25 μg/ml	70.0
Acetyl-LDL, 25 μg/ml + maleyl-albumin, 250 μg/ml	10.6
Acetyl-LDL, 25 μg/ml + fucoidin, 50 μg/ml	2.2

Cell monolayers were prepared by the standard procedure except that 2 ml of medium A containing 5 × 10^6 peritoneal cells was dispensed into 60 × 15-mm plastic petri dishes. After the adherence step (2 hr, 37°C) and the subsequent washes, each dish received 1.5 ml of medium containing 10% fetal calf serum, 0.1 mM [^{14}C]oleate-albumin (9600 cpm/nmol), and the indicated addition. After incubation at 37°C for 24 hr, the monolayers were washed by the standard procedure (13), the cells were scraped from the dish in 1 ml of phosphate-buffered saline, and the cellular content of cholesteryl [^{14}C]oleate was determined. Each value is the mean of duplicate incubations.

DISCUSSION

In the current study, we have used ^{125}I-acetyl-LDL as an initial probe to search for a binding site on macrophages that mediates the uptake and degradation of chemically altered or denatured LDL. The studies disclosed that such a binding site was present on mouse peritoneal macrophages as well as on macrophages from several other tissues and species but not on various other isolated cell types.

The important property of the acetyl-LDL binding site is that it facilitates the rapid uptake of the bound lipoprotein and its delivery to lysosomes. When macrophages bound ^{125}I-acetyl-LDL at 4°C and were then warmed to 37°C, lipoprotein degradation products in the form of mono[^{125}I]iodotyrosine were detectable in the culture medium within 10 min, and all of the lipoprotein that entered the cell was degraded within 60 min. These rapid uptake kinetics, which resemble those of the LDL receptor in fibroblasts (1), suggest that the ^{125}I-acetyl-LDL binding site is functionally adapted to mediate the uptake and degradation of molecules that bind to it. This rapid degradation affects only the molecule that is attached to the binding site and

Table 3. Increase in cellular cholesterol content in mouse peritoneal macrophages incubated with acetyl-LDL

Addition to medium	Total cellular cholesterol content, μg sterol/mg protein*
None	28 (20%)
LDL, 25 μg/ml	33
LDL, 250 μg/ml	55
Acetyl-LDL, 25 μg/ml	1050 (80%)
Acetyl-LDL, 25 μg/ml + maleyl-albumin, 250 μg/ml	83

Cell monolayers were prepared as described in the legend to Table 2. Each dish received 2 ml of medium containing 10% fetal calf serum and the indicated addition (day 0). The cells were incubated for 4 days at 37°C. The medium was replaced with fresh medium of identical composition on days 1 and 3. On day 4 the monolayers were washed by the standard procedure (13), the cells were scraped into 1 ml of phosphate-buffered saline, and the cellular content of cholesterol was determined. Each value is the mean of triplicate incubations.
* The number in parentheses is the proportion of the total cholesterol that was esterified.

is not due to a ligand-mediated stimulation of nonspecific endocytosis. This follows from the observation that unlabeled acetyl-LDL did not accelerate the uptake or degradation of ^{125}I-LDL or of ^{125}I-labeled human serum albumin (data not shown).

The binding site for ^{125}I-acetyl-LDL in macrophages showed high affinity and saturability. In addition to acetyl-LDL, this binding site recognized maleyl-LDL, maleyl-albumin, and at least two negatively charged sulfated polysaccharides, fucoidin and dextran sulfate. These findings suggest that the binding site recognizes, at least in part, regions of multiple negative charge. However, not all negatively charged molecules were bound to this site. Acetyl-albumin, polyphosphates, RNA, phosvitin, and heparin failed to inhibit the binding, uptake, and degradation of ^{125}I-acetyl-LDL.

Recent studies have shown that denatured proteins, such as albumin treated with formaldehyde (8, 23) or nitroguanidine (9), are rapidly cleared from the circulation when injected into mice or rats. Large amounts of these proteins appear in liver Kupffer cells (8, 9). Moreover, *in vitro* studies of rat peritoneal macrophages (24), rat yolk sac (23), and rat Kupffer cells (25) have shown rapid uptake and degradation of ^{125}I-labeled formaldehyde-treated albumin. These studies have led to the postulation that macrophages contain specific binding sites that mediate the uptake of denatured proteins. The present findings with ^{125}I-acetyl-LDL lend support to this concept. Moreover, the formal demonstration of binding activity at 4°C, competition with related substances, and destruction of the binding site with proteolytic enzymes all suggest that, in the case of altered LDL, a discrete receptor molecule may be involved.

The uptake of acetyl-LDL produced a large accumulation of free and esterified cholesterol within the macrophages. Werb and Cohn (26) showed that mouse peritoneal macrophages possess high levels of lysosomal cholesterol esterase activity and that, when macrophages ingested particulate cholesteryl ester–albumin complexes, the cholesteryl esters were hydrolyzed and the free cholesterol was excreted by the cells. Whether such lysosomal hydrolysis and excretion of acetyl-LDL-derived cholesteryl esters occurs is an important question that remains to be answered.

The physiologic significance, if any, of the acetyl-LDL uptake system in macrophages *in vivo* is not yet known. However, the demonstration that uptake of acetyl-LDL through this binding site leads to massive cellular deposition of cholesterol raises the possibility that this receptor may be responsible for the accumulation of LDL-derived cholesteryl esters in macrophages and other scavenger cells that occurs throughout the body in patients with familial hypercholesterolemia. Support for this hypothesis will require the demonstration that native LDL can be converted in the body into a form that is recognized by the acetyl-LDL binding site. Although *in vivo* acetylation of plasma LDL seems unlikely at this point, some chemical or physical alteration of LDL occurring in plasma or interstitial fluid may make it susceptible to recognition by the macrophage binding site.

Note Added in Proof. In experiments to be reported elsewhere, we have observed that ^{125}I-labeled acetyl-LDL injected intravenously into mice is cleared by the liver within 2 min. This *in vivo* hepatic uptake process is blocked by fucoidin and dextran sulfate and presumably occurs in Kupffer cells.

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1. Goldstein, J. L. & Brown, M. S. (1977) *Annu. Rev. Biochem.* **46,** 897–930.
2. Goldstein, J. L. & Brown, M. S. (1978) *Johns Hopkins Med. J.* **143,** 8–16.
3. Simons, L. A., Reichl, D., Myant, N. B. & Mancini, M. (1975) *Atherosclerosis* **21,** 283–298.
4. Bilheimer, D. W., Goldstein, J. L., Grundy, S. M. & Brown, M. S. (1975) *J. Clin. Invest.* **56,** 1420–1430.
5. Goldstein, J. L. & Brown, M. S. (1977) *Metabolism* **26,** 1257–1275.
6. Chomette, G., De Gennes, J. L., Delcourt, A., Hammou, J. C. & Perie, G. (1971) *Ann. Anat. Pathol* **16,** 233–250.
7. Watanabe, T., Tanaka, K. & Yanai, N. (1968) *Acta Pathol. Jpn.* **18,** 319–331.
8. Mego, J. L. & McQueen, J. D. (1965) *Biochim. Biophys. Acta* **100,** 136–143.
9. Buys, C. H. C. M., De Jong, A. S. H., Bouma, J. M. W. & Gruber, M. (1975) *Biochim. Biophys. Acta* **392,** 95–100.
10. Basu, S. K., Goldstein, J. L., Anderson, R. G. W. & Brown, M. S. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3178–3182.
11. Cohn, Z. A. & Benson, B. (1965) *J. Exp. Med.* **121,** 153–169.
12. Edelson, P. J. & Cohn, Z. A. (1976) in *In Vitro Methods in Cell-Mediated and Tumor Immunity,* eds. Bloom, B. R. & David, J. R. (Academic Press, New York), pp. 333–340.
13. Goldstein, J. L., Basu, S. K., Brunschede, G. Y. & Brown, M. S. (1976) *Cell* **7,** 85–95.
14. Garvey, J. S. (1961) *Nature (London)* **191,** 972–974.
15. Boyum, A. (1968) *Scand. J. Clin. Lab. Invest. Suppl.* 21 **97,** 77–89.
16. Johnson, W. D., Mei, B. & Cohn, Z. A. (1977) *J. Exp. Med.* **146,** 1613–1626.
17. Fraenkel-Conrat, H. (1975) *Methods Enzymol.* **4,** 247–269.
18. Glazer, A. N., Delange, R. J. & Sigman, D. S. (1975) *Chemical Modifications of Proteins* (North-Holland, Amsterdam), p.79. Amsterdam), p. 79.
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
20. Goldstein, J. L. & Brown, M. S. (1974) *J. Biol. Chem.* **249,** 5153–5162.
21. Goldstein, J. L., Dana, S. E. & Brown, M. S. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4288–4292.
22. Brown, M. S., Faust, J. R. & Goldstein, J. L. (1975) *J. Clin. Invest.* **55,** 783–793.
23. Moore, A. T., Williams, K. E. & Lloyd, J. B. (1977) *Biochem. J.* **164,** 607–616.
24. Pratten, M. K., Williams, K. E. & Lloyd, J. B. (1977) *Biochem. J.* **168,** 365–372.
25. Nilsson, M. & Berg, T. (1977) *Biochim. Biophys. Acta* **497,** 171–182.
26. Werb, Z. & Cohn, Z. A. (1972) *J. Exp. Med.* **135,** 21–44.