The metabolism of native and malondialdehyde-altered low density lipoproteins by human monocyte-macrophages

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Abstract We have recently shown that cultured human monocyte-macrophages degraded ^{125}I-labeled low density lipoprotein (^{125}I-native-LDL) by a saturable high-affinity process with maximal velocity at 25–50 μg protein/ml (Fogelman et al., 1980. *Proc. Nat. Acad. Sci. USA.* **77:** 2214–2218). We now describe studies of the binding of ^{125}I-native-LDL at 4°C and the effects of chloroquine, Ca^{2+} concentration, and reductive methylation on high-affinity ^{125}I-native-LDL degradation that indicate that native-LDL is processed by the monocyte-macrophages via the classic LDL receptor pathway. The high-affinity degradation of ^{125}I-native-LDL increased substantially when monocyte-macrophages were exposed to the lipoprotein deficient-fraction of serum (LPDS) for periods as brief as 4 hours, and was 25-fold greater than that of lymphocytes. Freshly isolated monocytes that had never been exposed to LPDS also demonstrated high-affinity degradation of ^{125}I-native-LDL. When these monocytes were cultured for 7 days in a medium containing native-LDL at a concentration (186 μg protein/ml) greatly in excess of that apparently needed to saturate the high-affinity process, there was more than a 10-fold increase in ^{125}I-native-LDL high-affinity degradation. LDL modified by treatment with malondialdehyde was processed by a second high-affinity cell surface receptor that appears identical to the "scavenger" receptor that processes acetylated LDL (Goldstein, et al., 1979, *Proc. Nat. Acad. Sci. USA.* **76:** 333–337).—**Shechter, I., A. M. Fogelman, M. E. Haberland, J. Seager, M. Hokom, and P. A. Edwards.** The metabolism of native and malondialdehyde-altered low density lipoproteins by human monocyte-macrophages. *J. Lipid Res.* 1981. **22:** 63–71.

Supplementary key words receptors · atherosclerosis · platelets · familial hypercholesterolemia · cholesteryl esters

In a previous communication we demonstrated that cultured human monocyte-macrophages degraded substantial amounts of native-LDL and LDL modified by treatment with malondialdehyde (MDA-LDL) via high-affinity processes, but only the MDA-LDL produced cholesteryl ester accumulation (1). Recently, Goldstein et al. reported that "Several types of macrophages, including peritoneal macrophages from the mouse, rat and dog, macrophages derived from human blood monocytes, and Kupffer cells from the guinea pig, express very low numbers of cell-surface LDL receptors of the type that normally transport cholesterol into nonmacrophage cells such as cultured fibroblasts and lymphocytes . . . As a result of their low numbers of LDL receptors, macrophages do not accumulate large amounts of cholesterol when incubated with these normal lipoproteins." (See page 1839 in reference 2.) Subsequently, we carried out experiments to determine if the degradation of native-LDL that we had observed in human monocyte-macrophages (1) actually occurred via the classic LDL receptor pathway (3).

We report here that the metabolism of native-LDL by human monocyte macrophages is similar to the metabolism of native-LDL by the classic LDL receptor pathway of cultured human fibroblasts and that the activity actually increases when monocytes develop into macrophages in the presence of LDL levels far in excess of those apparently required to saturate the classic high-affinity LDL receptor. In addition, we provide further evidence that the metabolism of MDA-LDL involves a high-affinity cell surface receptor which is distinctly different from the classic LDL receptor.

Abbreviations: LDL, low density lipoprotein; MDA-LDL, LDL modified by malondialdehyde; acetyl-LDL, LDL modified by acetylation with acetic anhydride; HDL, high density lipoprotein; LPDS, lipoprotein-deficient fraction of serum.

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EXPERIMENTAL PROCEDURES

Materials

Na ^{125}I (carrier free in 0.01 N NaOH, 16 Ci/mg) was from Schwartz/Mann. Phosphate-buffered saline, serumless medium (Cat. No. 320-1630), and Dulbecco's modified Eagle's medium (Cat. No. 430-1600) were from Grand Island Biological Co., Grand Island, NY. Normal serum albumin (human) 25%, U.S.P. was from Cutter Biological, Berkeley, CA. Ficoll-Paque was from Pharmacia, Piscataway, NJ. Malonaldehyde bis(dimethyl acetal) (Cat. No. 10,838-3) was from Aldrich Chemical Co., Milwaukee WI. Tissue culture dishes (Cat. No. 3001) were from Falcon, Oxnard, CA and Nalgene Teflon FEP containers (Cat. No. 1600-0001) were from Scientific Products, Irvine, CA. Glass-distilled solvents for gas–liquid chromatography were from Burdick and Jackson Laboratories, Muskegon, MI. All other equipment and supplies were from sources previously reported (4, 5).

Subjects

Normal subjects were recruited from the staff and student body at UCLA. No one received drugs that might have affected serum cholesterol levels or lipid metabolism. All had normal hematocrits, white blood cell and differential counts, serum cholesterol, triglycerides, and normal values on a multiphasic screening panel that included calcium, phosphorus, glucose, blood urea nitrogen, uric acid, total protein, albumin, total bilirubin, alkaline phosphatase, lactic dehydrogenase, and glutamic oxalacetic transaminase activity. Informed consent was obtained in writing from each person.

Collection of blood and separation of cells

Blood was taken after a 12–14 hr fast and the monocytes and lymphocytes were separated as previously described (method B in reference 4), except that the cells were injected into the loading chamber with the pump on at a flow rate of 5.2 ml/min and Ficoll-Paque was used instead of Ficoll-Hypaque.

Classification and viability of cells

The cells were classified by their morphology on Wright-stained smears, reaction with α-naphthyl butyrate esterase (lipase) stain, and ability to ingest latex particles and heat-killed *Candida albicans* as described previously (4). The ability of the cells to exclude supravital stain was determined as previously described (6). The purity of the lymphocytes exceeded 99.5% and that of the monocytes 95%. More than 95% of the cells were viable at the end of the incubations.

Cell culture

After separation the cells were suspended in autologous serum (10 to 50%) supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml) in either serumless medium (medium A) or in Dulbecco's modified Eagle's medium supplemented with $NaHCO_3$ (24 mM), Hepes (10 mM), insulin (8 μg/ml), and glucose (2 mg/ml) (medium B). One-ml samples of the cell suspension (10^6 cells) were transferred to 35 × 10 mm plastic tissue culture dishes and incubated at 37°C in a humidified incubator with 5% CO_2. The day after the cells were plated and every 72 hr thereafter, the medium was aspirated and replaced with fresh medium of the same composition.

Preparation of the lipoproteins

Lipoproteins (LDL, d = 1.019–1.063 g/ml; HDL, d = 1.063–1.21 g/ml) and the lipoprotein-deficient fraction of serum (d > 1.21 g/ml) (LPDS) were prepared as previously described (5, 6). For experiments in which the efflux of cholesterol from the cells into the medium was to be determined, LPDS was extracted with diethyl ether as previously described (5, 6). The cholesterol content of the solvent-extracted LPDS was <200 ng/ml. LDL was radioiodinated by the method of McFarlane (7) as modified by Bilheimer, Eisenberg, and Levy (8). The concentration of all lipoprotein fractions is given in terms of protein content unless otherwise stated. MDA-LDL and LDL modified by acetylation with acetic anhydride (acetyl-LDL) were prepared as described previously (1). These lipoproteins were then dialyzed for 16 hr at 4°C with two changes against 10^3 volumes of 0.01M phosphate, 0.15M NaCl, and 0.01% EDTA at pH 7.4. LDL was modified by reductive methylation as described by Weisgraber, Innerarity, and Mahley (9). All lipoproteins were filtered through a 0.45 μm filter just prior to addition to the cells.

Assays

The binding and total cellular content of ^{125}I-native-LDL and ^{125}I-MDA-LDL was measured at 4°C as described by Goldstein et al. (10, 11). The proteolytic degradation of ^{125}I-native-LDL, ^{125}I-MDA-LDL, and ^{125}I-acetyl-LDL was measured by assaying the amount of ^{125}I-labeled trichloracetic acid-soluble (noniodide) material formed by the cells and excreted into the culture medium as described by Goldstein and Brown (12). Corrections were made for the small amounts (<0.01% of total radioactivity added) of ^{125}I-labeled acid-soluble material that was found in parallel incubations without cells.

The free and esterified cholesterol contents of the cells were determined as described previously (1).

The protein content was determined by the method of Lowry et al. (13). Serum cholesterol and triglyceride determinations and statistical analyses were carried out by methods cited previously (4).

RESULTS

The free cholesterol content of monocyte-macrophages increased with time of incubation in medium containing 10% autologous serum, whereas cholesteryl ester content did not change appreciably after the first few days (data not shown). In other experiments it was determined that the cholesteryl ester content of the cells did not increase when the concentration of autologous serum in the incubation medium was varied from 10% to 50%.

The experiment shown in **Fig. 1** demonstrates that even high concentrations of LDL, twice those to which the cells had been exposed in vivo as circulating monocytes, failed to produce cholesteryl ester accumulation after 15 days of incubation. The experiment in Fig. 1 also confirms the effectiveness of the washing procedure: the free and esterified cholesterol contents of cells that had never been exposed to the supplemental LDL did not differ from those of cells that had only been exposed to the supplemental LDL at 4°C. The LDL:HDL ratio (LDL cholesterol:HDL cholesterol) to which the cells were exposed in vivo as monocytes was approximately 2:1, whereas the ratio was approximately 8:1 in the experiment described in Fig. 1 (data not shown). In other experiments not shown here, the LDL:HDL ratio was varied from as low as 1.7:1 to as high as 10.2:1 at a concentration of 1600 μg LDL protein/ml. The highest cholesteryl ester content found in these incubations that varied from 3 to 19 days was 3.8 μg/mg cell protein. It was concluded that the cholesteryl ester content of the cells was not a simple function of the LDL:HDL ratio.

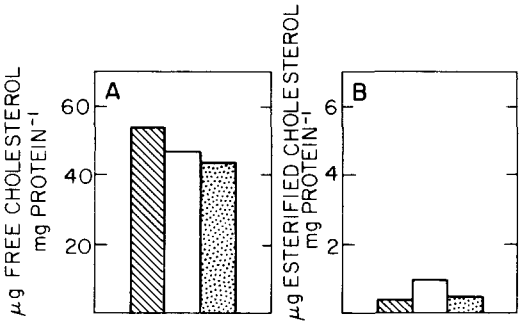

Fig. 1. The effects of LDL supplementation on the free and esterified cholesterol contents of human monocyte-macrophages. Normal human monocytes were cultured in 1 ml of 50% autologous serum in medium A. LDL was added to some of the dishes (solid bars) to give a final concentration of 1,606 μg protein/ml (twice that to which the cells had been exposed as monocytes in vivo). A volume of normal saline (80 μl) equivalent to the volume of LDL was added to control dishes which were otherwise identical (cross hatched bars). After 15 days of culture some of the control dishes that had not been exposed to the supplemental LDL were cooled to 4°C for 30 min and 1,606 μg of LDL protein/ml was added (stippled bars). After 4 hr the cells were washed three times in the dishes with phosphate-buffered saline containing 1% bovine serum albumin. The cells were harvested and their free (Panel A) and esterified (Panel B) cholesterol contents determined.

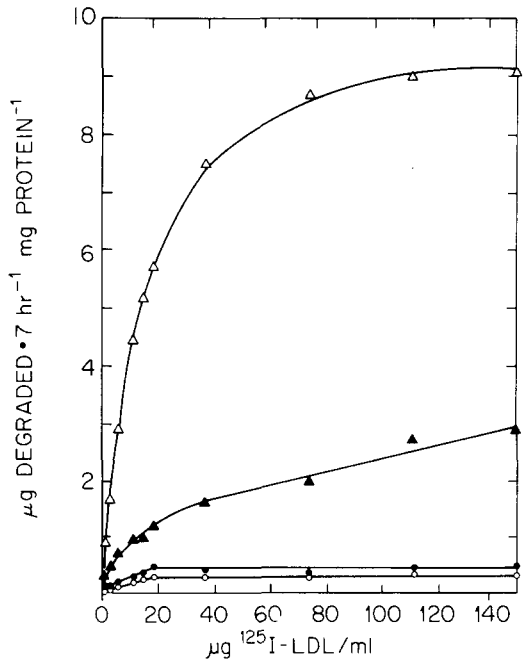

Fig. 2. ^{125}I-native-LDL degradation by human monocytes and lymphocytes. Normal monocytes (triangles) and lymphocytes (circles) were prepared and incubated in 1 ml of medium A with 20 mg/ml bovine serum albumin fortified with penicillin, 100 units/ml, and streptomycin, 100 μg/ml (closed symbols) or in the same medium containing 20 mg/ml of solvent-extracted LPDS (open symbols) instead of bovine serum albumin. After 18 hr of incubation, fresh medium of the same composition and ^{125}I-native-LDL (267 cpm/ng protein) was added to give the protein concentrations shown on the abscissa. After 7 hr at 37°C the content of ^{125}I-labeled acid-soluble material in the media was determined.

Uptake and degradation of LDL

The failure of LDL to increase the cholesteryl ester content of macrophages in vitro has been attributed to the relatively low rates at which these cells take up and degrade native-LDL (2). The experiments shown in **Fig. 2** demonstrate that human monocyte-macrophages degraded LDL by a saturable, high-affinity process with maximal velocity at 25–50 μg protein/ml. The experiments shown in **Table 1** provide further evidence that the initial portions of the curves shown in Fig. 2 actually represent high-affinity degradation.

^{125}I-native-LDL high-affinity degradation by lympocytes incubated with or without LPDS was only 4% or 22%, respectively, of that obtained with monocyte-macrophages incubated in the same medium (Table 1).

TABLE 1. ^{125}I-Native-LDL degradation by human monocytes and lymphocytes

Cell Type	Incubation Medium	^{125}I-Native-LDL Degradation		
		^{125}I-Native-LDL, 8 μg/ml (a)	^{125}I-Native-LDL, 8 μg/ml + Nonradioactive Native-LDL, 280 μg/ml (b)	High-Affinity Degradation (a–b)
		μg degraded·7 hr^{-1} mg protein^{-1}		
Monocytes	A + BSA	0.68	0.10	0.58
	A + LPDS	3.35	0.28	3.07
Lymphocytes	A + BSA	0.13	0.00	0.13
	A + LPDS	0.13	0.01	0.12

Some of the cells from the experiment described in Fig. 2 were incubated in duplicate in 1 ml of medium A with 20 mg/ml bovine serum albumin (BSA) or in the same medium containing 20 mg/ml of solvent-extracted LPDS instead of BSA. After 18 hours incubation, ^{125}I-native-LDL, 8 μg/ml (267 cpm/ng protein) was added in the presence or absence of a great excess of nonradioactive LDL (280 μg/ml) and the content of ^{125}I-labeled acid-soluble material in the medium was determined 7 hr later.

The usual assay for ^{125}I-native-LDL degradation is conducted in the presence of LPDS (12). Bilheimer et al. (14) found that the differences between freshly isolated normal and heterozygous familial hypercholesterolemic mixed mononuclear cells were best shown when the assay was performed in the presence of 30% LPDS. In order to test whether these conditions might induce high-affinity ^{125}I-native-LDL degradation by human monocyte-macrophages during a 4-hour assay, cells were cultured for 7 days in 30% autologous serum in medium A. The cells were then washed and the degradation of ^{125}I-native-LDL was measured in medium A supplemented with either 20 mg/ml of bovine serum albumin or 20 mg/ml of LPDS. A Lineweaver-Burk plot of the data revealed that the apparent "K_m's" were not different but the apparent "V_{max}" was approximately 50% higher in the assay carried out in the presence of LPDS (data not shown). In other experiments it was determined that the results of the assays were independent of the presence or absence of bovine serum albumin.

Johnson, Mei, and Cohn (15) demonstrated that human monocyte-macrophages secreted enzymes into the culture medium. In order to determine if the degradation of ^{125}I-native-LDL was due to the release of proteolytic enzymes from the cells into the medium, the culture medium from monocyte-macrophages was collected and incubated with ^{125}I-native-LDL. The results showed that the degradation was not due to extracellular proteolysis.

Because dramatic changes in cellular metabolism have been observed to occur when monocytes are converted to macrophages in vitro (16, 17), we studied the effects on ^{125}I-native-LDL degradation of converting monocytes into macrophages. The freshly isolated monocytes were incubated on a surface to which they could not adhere, Teflon FEP (18). The degradation of ^{125}I-native-LDL was determined and compared to that of cells that were allowed to adhere in plastic culture dishes and maintained in culture in 30% autologous serum for 7 days. As shown in **Fig. 3,** freshly isolated human monocytes degraded ^{125}I-native-LDL by a saturable high-affinity process even when the assay was carried out in the absence of LPDS. Following conversion of monocytes into macrophages in vitro, there was more than an order of magnitude increase in the high-affinity degradation of ^{125}I-native-LDL. This increase occurred even though the cells had been cultured in an incubation medium containing LDL at a concentration (186 μg protein/ml) far in excess of that apparently needed to saturate the high-affinity process (25–50 μg/ml).

Binding and metabolism of native-LDL and MDA-LDL

The binding of ^{125}I-native-LDL and ^{125}I-MDA-LDL at 4°C occurred by saturable high-affinity processes (**Fig. 4**). The degradation of ^{125}I-native-LDL and ^{125}I-MDA-LDL was inhibited by chloroquine, a known inhibitor of lysosomal function (19) (**Fig. 5**). It can be concluded that both native-LDL and MDA-LDL are bound to high-affinity cell surface sites, internalized, and delivered to lysosomes where the lipoproteins are degraded. As was the case for human fibroblasts (12), the degradation of ^{125}I-native-LDL was more readily inhibited by non-radioactive LDL than by HDL (data not shown).

Inhibition of ^{125}I-native-LDL high-affinity degrada-

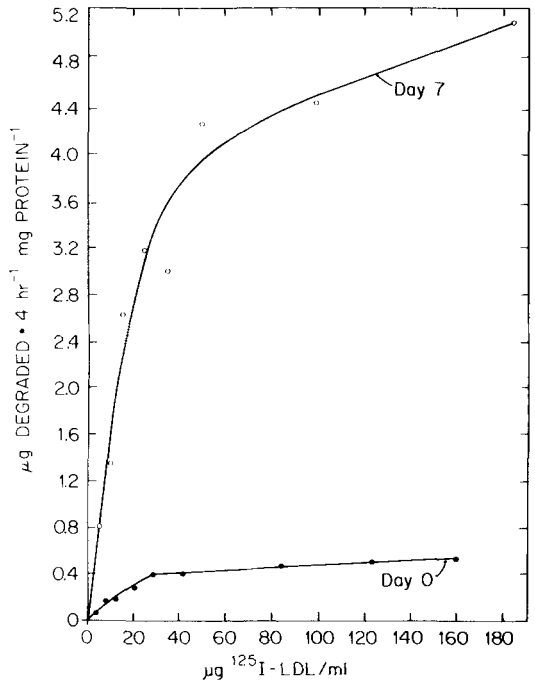

Fig. 3. ^{125}I-Native-LDL degradation by human monocytes and macrophages. Normal monocytes were prepared and some were incubated on a surface to which they could not adhere (Teflon FEP) in medium A containing 20 mg/ml of bovine serum albumin and the concentrations of ^{125}I-native-LDL protein (88 cpm/ng) shown on the abscissa (Day 0, closed symbols). After 4 hr at 37°C the incubation mixture was centrifuged at 2,500 *g* for 10 min at 4°C and the content of ^{125}I-labeled acid-soluble material in the medium was determined. The remainder of the monocytes were cultured in 30% autologous serum in medium A (LDL concentration 186 μg protein/ml) in plastic petri dishes. After 7 days the medium was removed, the cells washed twice with 1.5 ml of medium A and 1 ml of medium A containing 20 mg/ml of bovine serum albumin and the concentration of ^{125}I-native-LDL protein (77 cpm/ng) shown on the abscissa (Day 7, open symbols) was added. After 4 hr at 37°C the content of ^{125}I-labeled acid-soluble material in the medium was determined.

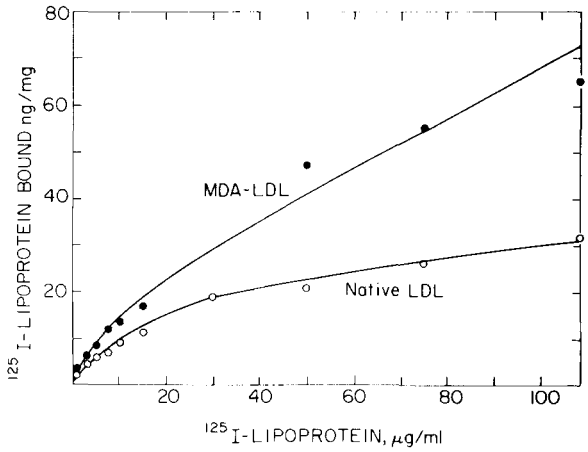

Fig. 4. Binding of ^{125}I-native-LDL (Native-LDL, open circles) and ^{125}I-MDA-LDL (MDA-LDL, closed circles) by normal human monocyte-macrophages at 4°C. Normal human monocytes were cultured in 1 ml of 30% autologous serum in medium B. After 13 days the medium was removed and replaced with 2 ml of ice-cold medium B (without bicarbonate) and the cells were placed at 4°C. After 30 min the medium was removed and 1 ml of ice-cold medium B (without bicarbonate) supplemented with LPDS, 5 mg/ml, was added together with the concentration of ^{125}I-native-LDL (112 cpm/ng) or ^{125}I-MDA-LDL (112 cpm/ng) shown on the abscissa. The monolayers were incubated at 4°C for 2 hr, washed, and the amount of ^{125}I-native-LDL or ^{125}I-MDA-LDL bound to the cells was determined in duplicate dishes.

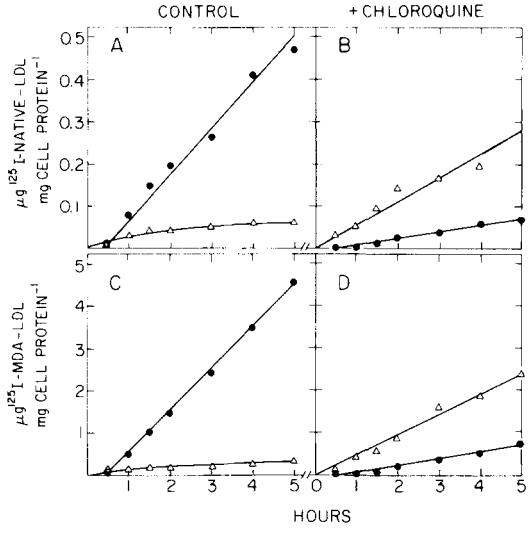

Fig. 5. Effect of chloroquine on time course of accumulation (triangles) and degradation (circles) of ^{125}I-native-LDL (panels A and B) and ^{125}I-MDA-LDL (panels C and D). Normal monocytes were cultured in 1 ml of 30% autologous serum in medium B. After 7 days the medium was removed, the cells were washed twice with 1.5 ml medium B (without bicarbonate) and 1 ml of medium B containing 5 mg/ml of LPDS and 15 μg of ^{125}I-native-LDL (91 cpm/ng) (panels A and B) or 15 μg of ^{125}I-MDA-LDL (91 cpm/ng) (panels C and D) was added with (panels B and D) or without (panels A and C) 75 μM chloroquine. After incubation at 37°C for the indicated interval, the amount of ^{125}I-labeled acid-soluble material in the medium (circles) and the amount of ^{125}I-lipoprotein in the cells (triangles) was determined in duplicate dishes.

tion by 2 mM EDTA (in medium with 1.29 mM Ca^{2+} ion and 0.58 mM Mg^{2+} ion) was reversed by addition of 1.6 mM Ca^{2+} ion (**Table 2**). The inclusion of the same concentration of EDTA did not inhibit the high-affinity degradation of ^{125}I-MDA-LDL (Table 2), indicating that the metabolism of ^{125}I-MDA-LDL is not dependent on Ca^{2+} ion concentration.

Weisgraber, Innerarity, and Mahley (9) demonstrated that LDL in which lysine residues had been modified by reductive methylation failed to bind to the LDL receptor on human fibroblasts. Similarly, as shown in **Fig. 6,** non-radioactive native-LDL modified by reductive methylation was unable to inhibit the high-affinity degradation of ^{125}I-native-LDL by human monocyte-macrophages. The high-affinity degradation of ^{125}I-MDA-LDL was not affected by the addition of nonradioactive native-LDL modified by reductive methylation (Fig. 6). Indeed, ^{125}I-LDL altered by re-

TABLE 2. Effect of Ca^{2+} ion on ^{125}I-native-LDL and ^{125}I-MDA-LDL degradation

Additions	^{125}I-Lipoprotein Degradation					
	^{125}I-Native-LDL, 25 μg/ml (a)	^{125}I-Native-LDL, 25 μg/ml + Nonradioactive-Native-LDL, 517 μg/ml (b)	High Affinity (a–b)	^{125}I-MDA-LDL, 16 μg/ml (c)	^{125}I-MDA-LDL, 16 μg/ml + Nonradioactive MDA-LDL, 389 μg/ml (d)	High Affinity (c–d)
	μg degraded·4 hr^{-1} mg protein^{-1}					
None	1.21	0.34	0.87	3.98	0.35	3.63
EDTA (2.0 μmol)	0.24	0.22	0.02	3.90	0.34	3.56
EDTA (2.0 μmol) + Ca^{2+} (1.6 μmol)	1.13	0.35	0.78	3.94	0.30	3.64

Normal monocytes were cultured in 1 ml of 30% autologous serum in medium B for 9 days and then changed to 1 ml of 10% autologous serum in medium B and cultured for an additional 5 days, as described in Experimental Procedures. On day 14, the medium was removed and the cells were washed twice with 1.5 ml of medium B (without bicarbonate). To the cells was added 1 ml of medium B containing either ^{125}I-native-LDL, 25 μg protein/ml (115 cpm/ng protein) with or without 517 μg of nonradioactive native-LDL, or ^{125}I-MDA-LDL, 16 μg protein/ml (103 cpm/ng protein) with or without 389 μg of nonradioactive MDA-LDL. In addition, EDTA (2.0 μmol) with or without Ca^{2+} ion (1.6 μmol) was added where indicated. After 4 hr the content of ^{125}I-labeled acid-soluble material in the medium was determined.

ductive methylation itself was not degraded by a high-affinity process (**Fig. 7**).

In our previous report (1), we found that acetyl-LDL did not inhibit the degradation of ^{125}I-native-LDL (see Fig. 5 in reference 1). The acetyl-LDL used in these experiments had an electrophoretic mobility intermediate between native-LDL and MDA-LDL.[5] Nonradioactive acetyl-LDL with the same electrophoretic mobility as MDA-LDL (1) inhibited the degradation of ^{125}I-MDA-LDL (**Fig. 8,** Panel A). Conversely, nonradioactive MDA-LDL inhibited the degradation of ^{125}I-acetyl-LDL (Fig. 8, Panel B). Moreover, fucoidin in a concentration of 50 μg/ml in-

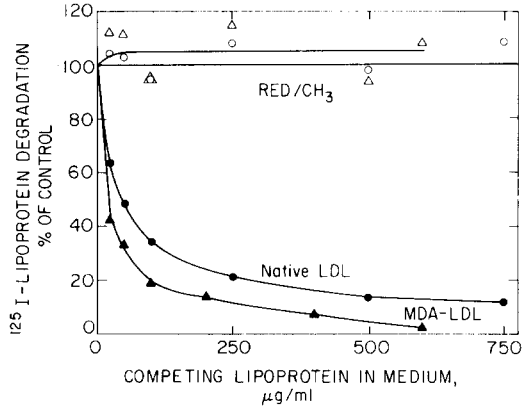

Fig. 6. Ability of nonradioactive native-LDL (●) and native LDL modified by reductive methylation (RED/CH_3) (○) to inhibit the degradation of ^{125}I-native-LDL and ability of nonradioactive MDA-LDL (▲) and native-LDL modified by reductive methylation (RED/CH_3) (△) to inhibit the degradation of ^{125}I-MDA-LDL. Normal human monocytes were cultured in 1 ml of 30% autologous serum in medium B. On day 13 the medium was removed, the cells washed twice with 1.5 ml medium B (without bicarbonate) and 1 ml of medium B containing either ^{125}I-native-LDL, 15 μg protein/ml (66 cpm/ng protein) or ^{125}I-MDA-LDL, 10 μg protein/ml (66 cpm/ng protein) and the nonradioactive lipoproteins at the concentrations shown on the abscissa was added. After 5 hr of incubation at 37°C, the content of ^{125}I-labeled acid-soluble material in the medium was determined. The 100% value for the degradation of ^{125}I-native-LDL in the absence of competing lipoproteins was 0.61 μg·5 hr^{-1} mg protein^{-1} and for ^{125}I-MDA-LDL, 4.0 μg·5 hr^{-1} mg protein^{-1}.

[5] Shechter, I., A. M. Fogelman, M. E. Haberland, J. Seager, M. Hokom, and P. A. Edwards. Unpublished observations.

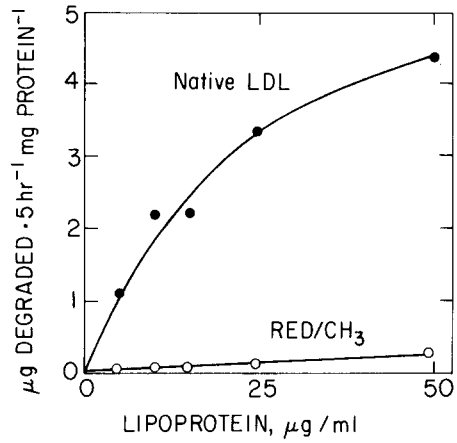

Fig. 7. Degradation of ^{125}I-native-LDL (●) and ^{125}I-native-LDL modified by reductive methylation (RED/CH_3) (○) by human monocyte-macrophages. Normal human monocytes were cultured in 1 ml of 30% autologous serum in medium B. On day 7 the medium was removed, the cells washed twice with 1.5 ml of medium B (without bicarbonate) and 1 ml of medium B containing either ^{125}I-native-LDL (58 cpm/ng/protein) or ^{125}I-native-LDL modified by reductive methylation (45 cpm/ng/protein) at the concentrations shown on the abscissa was added. After incubation for 5 hr at 37°C, the content of ^{125}I-labeled acid-soluble material in the medium was determined.

hibited the high-affinity degradation of both ^{125}I-MDA-LDL and ^{125}I-acetyl-LDL. These data suggest that MDA-LDL is processed by the same "scavenger" receptor that processes acetyl-LDL (10).

In our preliminary studies (1), we demonstrated that after only a 3-day incubation with MDA-LDL (500 μg protein/ml) the cholesteryl ester content of the cells increased to 17 μg/mg cell protein (26% of total cellular cholesterol), whereas the cholesteryl ester content of cells incubated with the same concentration of native-LDL remained low (1.0 μg/mg cell protein or 3% of total cellular cholesterol). Since the hallmark of the arterial foam cell is a cholesteryl ester content equal to or in excess of 50% of the total cellular cholesterol (20), the effect of longer periods of incubation with MDA-LDL was determined. The results given in **Table 3** demonstrate that such levels of cholesteryl ester accumulation were readily obtained after 12 days of incubation with MDA-LDL but not with native-LDL.

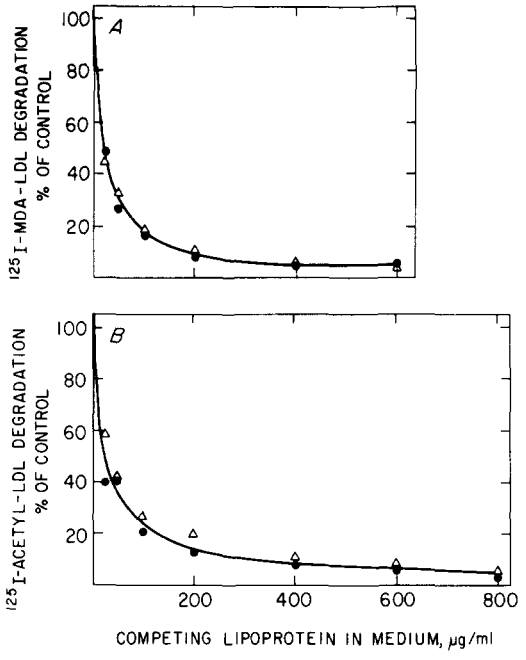

Fig. 8. Ability of nonradioactive acetyl-LDL and MDA-LDL to inhibit the degradation of ^{125}I-MDA-LDL or ^{125}I-acetyl-LDL. Normal monocytes were cultured in 1 ml of 30% autologous serum in medium B. On day 15 the medium was removed, the cells were washed twice with 1.5 ml of medium B (without bicarbonate) and 1 ml of medium B containing ^{125}I-MDA-LDL, 10 μg protein/ml (97 cpm/ng protein) and nonradioactive acetyl-LDL (●) or MDA-LDL (△) at the concentrations indicated on the abscissa was added (Panel A). In other experiments, ^{125}I-acetyl-LDL, 10 μg protein/ml (99 cpm/ng protein) was substituted for ^{125}I-MDA-LDL (Panel B). After 5 hr at 37°C the content of ^{125}I-labeled acid-soluble material in the medium was determined. The 100% value for the degradation of ^{125}I-MDA-LDL in the absence of competing lipoproteins was 2.6 μg·5 hr^{-1} mg protein^{-1}. The 100% value for the degradation of ^{125}I-acetyl-LDL in the absence of competing lipoproteins was 2.2 μg·5 hr^{-1} mg protein^{-1}.

TABLE 3. Free and esterified cholesterol contents of human monocyte-macrophages incubated with native-LDL or MDA-LDL

Lipoprotein	Free Cholesterol	Esterified Cholesterol
	μg/mg cell protein	
Native-LDL	61.5 ± 4.4[a,c]	3.7 ± 0.3[a,d]
MDA-LDL	67.5 ± 1.5[b,c]	76.0 ± 3.2[b,d]

[a] = $P < 0.001$.
[b] = $P < 0.005$.
[c] = $P < 0.05$.
[d] = $P < 0.001$.

Normal human monocyte-macrophages were cultured in 1 ml of 30% autologous serum in medium B, as described in Experimental Procedures. After 7 days the medium was changed to 10% autologous serum in medium B containing 500 μg protein/ml of either native-LDL or MDA-LDL. After 12 days of incubation with the supplemental lipoproteins, the cells were washed in the dishes, harvested, and their free and esterified cholesterol contents determined. The values given are the mean ± 1 S.D., n = 4.

DISCUSSION

There is increasing evidence that the foam cells in the atherosclerotic reaction are macrophages which are derived from blood-borne monocytes and/or smooth muscle cells which have taken on macrophage properties (21–23). We would have predicted that human monocyte-macrophages would have accumulated substantial amounts of cholesteryl esters when incubated in the presence of high concentrations of LDL in a medium with a high LDL:HDL ratio. However, such an accumulation was not observed (Fig. 1). In contrast, when the cells were incubated in the presence of the same concentration of MDA-LDL, the cholesteryl ester content of the cells accounted for more than 50% of the total cellular cholesterol (Table 3).

Unlike the case for mouse peritoneal macrophages (2, 10), the failure to accumulate cholesteryl esters in human monocyte-macrophages cannot be ascribed to a low rate of uptake and degradation of native-LDL.

In the presence of LPDS, the degradation of ^{125}I-native-LDL increased 50% during the usual 4-hour assay. Presumably the increased degradation was due to an increase in the number of LDL receptors which may have been induced in part in response to cholesterol efflux (5, 24).

Human monocytes to not divide in culture, but, as previously described by Johnson, Mei, and Cohn (15) and confirmed by our own observations, these cells markedly increase in size as they convert into macrophages as a function of both time in culture and of concentration of autologous serum in the medium.[5] The need for cholesterol for membrane synthesis under these conditions may have provided a stimulus

for increased native-LDL receptor activity (Fig. 3). Another stimulus under these conditions may have come from the inclusion of insulin in the incubation media (25). Either or both of these factors may account for the different results of other investigators who have primarily studied mature macrophages from lower animals (2, 10).

The following evidence strongly indicates that native-LDL is processed by human monocyte-macrophages via the classic LDL receptor pathway, whereas MDA-LDL is processed by a high-affinity cell surface receptor that is distinctly different from the classic LDL receptor and is probably the same as the "scavenger" receptor that processes acetyl-LDL (10): i) the binding of ^{125}I-native-LDL and ^{125}I-MDA-LDL at 4°C each appeared to have a saturable high-affinity component with maximal binding at 25 and 10 μg protein/ml, respectively (Fig. 4); ii) ^{125}I-native-LDL was degraded by a saturable high-affinity process with maximal velocity at 25–50 μg protein/ml (Fig. 2 and Table 1); iii) ^{125}I-MDA-LDL was degraded by a saturable high-affinity process with maximal velocity at 10–15 μg/protein/ml (1); iv) chloroquine, a known inhibitor of lysosomal function, prevented the degradation of ^{125}I-native-LDL and ^{125}I-MDA-LDL and resulted in the accumulation of these lipoproteins within the cells (Fig. 5); v) native-LDL failed to inhibit the degradation of ^{125}I-MDA-LDL (1); vi) MDA-LDL failed to inhibit the degradation of ^{125}I-native-LDL (1); vii) sodium heparin inhibited the high-affinity degradation of ^{125}I-native-LDL but not the high-affinity degradation of ^{125}I-MDA-LDL (1); viii) LDL more readily inhibited the degradation of ^{125}I-native-LDL than HDL; ix) the addition of the metal chelator EDTA abolished the high-affinity degradation of ^{125}I-native-LDL but not ^{125}I-MDA-LDL, and the effect of EDTA on the high-affinity degradation of ^{125}I-native-LDL was reversed with Ca^{2+} ion (Table 2); x) reductive methylation of nonradioactive native-LDL prevented the nonradioactive lipoprotein from inhibiting the degradation of ^{125}I-native-LDL (Fig. 6); xi) ^{125}I-native-LDL modified by reductive methylation was no longer degraded by a high-affinity process (Fig. 7); xii) normal human fibroblasts demonstrated high-affinity degradation of ^{125}I-native-LDL but not of ^{125}I-MDA-LDL (1); xiii) nonradioactive acetyl-LDL inhibited the high-affinity degradation of ^{125}I-MDA-LDL (Fig. 8, Panel A); xiv) nonradioactive MDA-LDL inhibited the high-affinity degradation of ^{125}I-acetyl-LDL (Fig. 8, Panel B); xv) fucoidin inhibited the high-affinity degradation of ^{125}I-MDA-LDL.

The evidence presented above clearly demonstrates that under the culture conditions that we have employed, human monocyte-macrophages possess both an active LDL receptor pathway (3) and an active "scavenger" receptor pathway (10). At equivalent or nearly equivalent protein concentrations, MDA-LDL was degraded 7- to 10-fold faster than native-LDL (Figs. 5 and 6). From the data presented here we cannot exclude the possibility that the two receptors are located on different cells.

Goldstein et al. (10) have demonstrated that acetyl-LDL produced cholesteryl ester accumulation in mouse peritoneal macrophages. There is no known mechanism for the production of acetyl-LDL in vivo. However, malondialdehyde is released as a fragmentation product in the metabolism of arachidonic acid by blood platelets (26–28) and also may be generated during lipid peroxidation (29, 30). Based on the experiments reported here and those previously reported (1), we hypothesize that native-LDL may have to be modified in vivo before it can cause cholesteryl ester accumulation in the cells of the arterial wall. Furthermore, we hypothesize that malondialdehyde released from blood platelets and/or produced by lipid peroxidation at the site of arterial injury may modify native-LDL by forming Schiff base bonds with the ϵ-amino groups of lysines in LDL (31).

The evidence presented in this report indicates that MDA-LDL could cause cholesteryl ester accumulation of the degree found in arterial foam cells (Table 3). Whether sufficient malondialdehyde is generated in vivo to produce MDA-LDL, remains to be determined. The fact that MDA-LDL is taken up by a cell surface receptor that is distinctly different from the classic LDL receptor may explain one mechanism for the accumulation of cholesteryl esters in the cells of the atherosclerotic reaction of normals and of receptor negative homozygous familial hypercholesterolemics. It has been recently demonstrated that when the cholesterol content of blood platelets was increased by incubation with cholesterol-loaded liposomes, there was an increased conversion of arachidonic acid to thromboxane B_2 (the stable endproduct of thromboxane A_2) (32). Since 1 mole of malondialdehyde is produced for every mole of thromboxane A_2 synthesized by blood platelets from arachidonic acid (26–28), an increase in malondialdehyde formation would also be predicted under these conditions. Platelets from persons with Type IIa hypercholesterolemia have been reported to release substantially more malondialdehyde than normal (33). These two observations (32, 33), taken together with those previously reported by us (1) as well as those reported here, may explain in part the accelerated atherosclerosis of familial hypercholesterolemia.

We thank Dr. David B. Weinstein for helpful discussions. This research was supported in part by USPHS Grants HL 25590, HL 19063, 1T32 HL 07412, RR 865, and a grant

from the American Heart Association, Greater Los Angeles Affiliate (649-PS-1).

Manuscript received 16 May 1980 and in revised form 24 July 1980.

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