Comparative Binding and Degradation of Lipoprotein(a) and Low Density Lipoprotein by Human Monocyte-derived Macrophages*

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The binding and degradation of equimolar concentrations of lipoprotein(a) (Lp(a)) and low density lipoprotein (LDL) isolated from the same individual were studied in primary cultures of human monocyte-derived macrophages (HMDM). At 4°C, LDL receptor-mediated binding of both Lp(a) and LDL was of low affinity, being 0.8 and 0.23 μM, respectively. Competitive binding studies indicated that the binding of Lp(a) to HMDM was competed 63% by excess LDL. In contrast, the 4°C binding data for the degradation of Lp(a) at 37°C was mainly nonspecific because the amount of Lp(a) processed by the LDL receptor pathway in 5 h was 17% that of LDL. According to pulse-chase experiments, this phenomenon may be accounted for by the facts that less Lp(a) is bound to HMDM at 37°C and that Lp(a) has a lower intrinsic degradation rate and was not due to increased intracellular accumulation or retroendocytosis of the lipoprotein. Degradation of both lipoproteins was primarily lysosomal and only modestly affected by up- or down-regulation of the LDL receptor. The rate of retroendocytosis in HMDM was approximately equal to the degradation rate and appeared to be independent of the type of lipoprotein used, up- or down-regulation of the LDL receptor, or the presence of the lysosomotropic agent chloroquine. Overall, the results indicate that HMDM degrade Lp(a) mainly via a nonspecific pathway with only 25% of total Lp(a) degradation occurring through the LDL receptor pathway. As both 37°C degradation and 4°C binding of LDL are mainly LDL receptor specific, the different metabolic behavior observed at 37°C suggests that the apo(a) undergoes temperature-induced conformational changes on cooling to 4°C that allows better recognition of Lp(a) by the LDL receptor at a temperature lower than the physiological temperature of 37°C. How apo(a) affects these structural changes remains to be established.

Evidence indicating that high plasma levels of Lp(a) place

individuals at risk for cardiovascular disease has led to increased interest in the role played by the LDL receptor in the catabolism of Lp(a) by cultured cells. To date, most studies on the binding and degradation of Lp(a) have been conducted in fibroblasts without coming to a consensus as to the role played by the LDL receptor pathway (1–9). In particular, quantitative comparisons of Lp(a) and LDL have been highly variable. This may have been due to a lack of appreciation in some of the earlier studies of Lp(a) heterogeneity, Lp(a) stability, and the difficulty of obtaining Lp(a) preparations that are free of LDL. For example, some Lp(a) species with large apo(a) polymorphs aggregate in the cold, thus potentially compromising the results of the 4°C binding studies (10, 11). Also, Lp(a) that is isolated in the absence of protease inhibitors may undergo degradation of the apo(a) moiety thereby creating an LDL-like particle (12).

Lacking knowledge of the molecular weight of Lp(a) or Lp(a) protein, comparative studies of Lp(a) and LDL have been made on an equal protein or cholesterol basis or on equal apoB concentration (determined immunochemically) (1–9). When protein is used as the basis of comparison, the molar Lp(a) concentration is actually 2–3-fold lower than that of LDL, whereas when comparisons are made on equal apoB concentrations, Lp(a) levels may actually be up to 2-fold higher than those of LDL because of the reduced affinity of anti-apoB antibodies for Lp(a) relative to LDL (13). Although the variability in the molarity of Lp(a) and LDL may be less when comparisons are made on an equal cholesterol basis, this is also not ideal because the cholesterol content of Lp(a) and LDL is not fixed (10, 14).

In previous studies, we determined the molecular weight of Lp(a) protein from an individual having Lp(a) with a fast apo(a) polymorph (Mf = 251,000) by compositional and ultracentrifugation analysis (10, 14). In the present study we have utilized this well-characterized Lp(a) along with LDL isolated from the same individual in order to determine the role of the LDL receptor pathway in the cellular binding and degradation of these two lipoproteins. In addition, experiments were performed and analyzed on a molar basis. We chose the human monocyte-derived macrophage (HMDM) as a model cell type because it is an important component of atheromatous lesions (15, 16) and is known to possess receptors of high specificity for LDL (17–19) which may, however, not be identical to the LDL receptor present on fibroblasts (20). Our studies show that HMDM degrade Lp(a) at a rate slower than LDL. A preliminary account of these results has been reported (21).

EXPERIMENTAL PROCEDURES

Materials—Sodium [125]Iodide, [1-14C]oleic acid, and [1,2-3H]cholesteryl oleate were purchased from Amersham Corp.; Dulbecco’s modified Eagle’s medium (DMEM) containing 25 mM HEPES, L-glutamine, and 4500 mg/liter glucose, and phosphate-buffered saline...
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minus calcium and magnesium (PBS(CMF)) were from Gibco; Plasmagel was from Cellular Products Inc. (Buffalo, NY); phenylmethylsulfonyl fluoride, soybean trypsin inhibitor, triloine, oleic acid cholesteryl oleate, bovine serum albumin fraction V, and human serum albumin were all purchased from Sigma; Ficoll-Hypaque and PEG 6000 (Sobjioed G-2-65 cm) were from Pharmacia (Uppsala, Sweden). Plastic 12-well tissue culture dishes were from Flow Laboratories (McLean, VA); Heparin USP 1000 units/ml was obtained from LynBioMed (Rosemont, IL).

Preparation of Lp(a) and LDL—Autologous LDL and Lp(a) were isolated from the plasma of two subjects with a fast apo(a) polymorph (M = 281,000 (14)). Prior to donation, all subjects gave informed consent. The molecular mass of the protein moiety of this Lp(a) was determined previously from the molecular mass and chemical composition of Lp(a) (10, 14) and was found to be 927,000 daltons. This molecular weight is also consistent with the presence of 2 mol of apo(a) and 1 mol of apoB/Lp(a) molecule. Because 28.1% of the mass of apo(a) is carbohydrate, the protein portion of 2 mol of apo(a) is equivalent to 404,000 daltons and when added to the mass of apoB results in a mass of 918,000 daltons for the protein moiety of Lp(a).

Lp(a) was purified from the d < 1.21 g/ml total lipoproteins by lysine-Sepharose chromatography. Total lipoproteins in 0.1 M phosphate buffer containing 0.01% Na2-EDTA, 0.01% NaN3, and 1 mM benzamidine, pH 7.4, were passed over a column (20 × 5 cm) containing lysine-Sepharose at 50 ml/h. Following application of the lipoproteins, the column was washed with 50 ml of 0.5 M NaCl, 0.1 M NaHCO3, 1 mM benzamidine, pH 5.5, to remove nonspecifically bound lipoproteins. The column was then eluted with 200 ml of 0.1 M hexanoic acid (EAA) dissolved in 0.1 M phosphate, 1 mM benzamidine, and 0.01% Na2-EDTA and NaNO2, pH 7.4. This heterogeneous Lp(a) fraction was made 7.5% wt % with CsCl and centrifuged for 4 h at 49,000 rpm at 20 °C in the 50.2 Ti rotor in quick seal tubes. The CsCl forms a self-density gradient which allows the separation of Lp(a) species with different apo(a) polymorphs and also serves to remove any contaminating plasma lipoproteins. LDL was isolated from the unbound lipoproteins obtained after lysine-Sepharose chromatography by density gradient centrifugation in 3.75 wt % CsCl-Ti-50.2 rotor, 49,000 rpm at 20 °C for 4 h. Lp(a) and LDL preparations were analyzed by SDS-gradient polyacrylamide gel electrophoresis on 2-16% acrylamide gels (Pharmacia, Uppsala, Sweden). The Lp(a) and LDL (15 g/sample) were incubated with 1% SDS for 5 min at 95 °C. The gels were stained with Coomassie Blue R-250. Both lipoproteins were stored filter sterilized (0.45 μm) in Sarstedt vials, filled to an air space, in 0.15 M NaCl, pH 7.4, containing 0.01% Na2-EDTA and NaN3.

Iodination of Lp(a) and LDL—The radioiodination of Lp(a) and LDL was performed using the iodine monochloride method of McFarlane (22) as modified by Bilheimer et al. (23). The specific radioactivity for both lipoproteins averaged 600 cpm/ng lipoprotein protein. Intact integrity of the radiolabeled Lp(a) and LDL was determined by SDS-polyacrylamide gel electrophoresis (2-16%) as described above. The 125I-labeled Lp(a) and LDL (approximately 100,000 cpm/sample) were transferred to nitrocellulose, and autoradiography was performed with Kodak XAR-5 film. The percent of the radioactive label incorporated into apo(a) and apoB was determined by two different methods. Apo(a) was separated from the parent radiiodinated lipoprotein after reduction of Lp(a) with 50 mM dithiothreitol and counted for radioactivity. After correcting for the radioactivity found in the two apoprotein bands with Coomassie Blue, they were assayed for radioactivity. The specific radioactivity in apo(a) was 58.2 ± 1.6 and 41.8 ± 1.6 in apoB,00.

These numbers correspond well with the distribution of tyrosines in Lp(a) as calculated from their respective amino acid sequences which were 62.3% in apo(a) and 37.7% in apoB,00 and appears to indicate that neither apoprotein is preferentially labeled.

The mock iodination of Lp(a) was carried out using the same conditions employed with the radiiodinations except that for each milligram of lipoprotein protein, 0.6 nmol of cold NaI was used in place of 0.5 nmol of Na125I.

Isolation and Culture of HMDM—Isolation of human monocytes from donors who gave informed consent was carried out according to Fogelman et al. (24) with minor changes. Briefly, freshly drawn human blood (300 ml) was collected into sterile 50-ml plastic centrifuge tubes at a final heparin concentration of 6 units/ml. An additional sample (50 ml) was collected into a citrate/phosphate/dextrose solution at a blood to citrate/phosphate/dextrose solution ratio of 6:1. This sample was collected with 0.5% heparin as an anticoagulant for the preparation of autologous serum and LPDS.

The heparinized blood was mixed with 100 ml of Plasmagel (Cellular Products, Inc.) in a 500-ml Teflon separatory funnel. The red cells were allowed to settle 60-75 min at room temperature at which time they were slowly run out the bottom of the funnel. After removal of the red cells, the preparation of leukocytes, citrate/phosphate/dextrose containing plasma, the hypertonic treatment of the cells, density gradient centrifugation, and preparation of monocytes for culture proceeded as outlined by Fogelman et al. (24). After the cells were counted, they were resuspended in DMEM (20% autologous serum) to a final concentration of 1.0 × 106 cells/ml. 1 ml of cell suspension was plated into each well of Linbro 12-well tissue culture plates (area/well = 4.5 cm2). The cells were placed in a 37 °C humidified-air incubator (95% air, 5% CO2) and allowed to attach for 30 min, after which time they were washed twice with serum-free DMEM to remove any contaminating lymphocytes and platedets and finally overlaid with 1.0 ml of DMEM (20% autologous serum). The medium was replaced on the second and fifth day of culture for 8-day-old cells and the first, fourth, and seventh day for 10-day-old cells.

Twenty-four h prior to the initiation of experiments, the cells were washed twice with serum-free DMEM and placed into DMEM containing 20% autologous LPDS to up-regulate the LDL receptor or into 20% autologous serum to down-regulate the LDL receptor. For the competitive binding and degradation experiments in which 10-day-old cells were used, the preincubation with 20% autologous LPDS was eliminated. We came to this decision after discovering that our schedule of medium changes mimicked up-regulated cells so that LDL-specific binding of either LDL or Lp(a) was unchanged, and LDL-specific degradation of Lp(a) and LDL (a) was only 12.1 and 10.5% lower, respectively, than in the up-regulated cells. The preparation of autologous serum and autologous lipoprotein-deficient serum was carried out as described above.

HMDM were classified and their viability determined as described by Fogelman et al. (25). The cells were >98% monocyte-macrophages by the time the experiments were performed. There was no change in cell viability after either binding or degradation experiments.

4 °C Binding—Lipoprotein binding assays at 4 °C with 8-10-day-old HMDM were carried out essentially according to Innerarity et al. (26). Briefly, macrophage monolayers were washed once with DMEM, then twice with DMEM (0.2% human serum albumin (HSA)) and cooled to 4 °C. The medium was then removed and DMEM (0.2% HSA) containing 125I-labeled lipoprotein with and without unlabeled competitor were incubated with the cells and Lp(a) was incubated with and without unlabeled competitor. The cells were then washed four times with 1.0 ml/well of ice-cold PBS(CMF); the first wash was allowed to sit 10 min before proceeding with the remaining four washes in rapid succession as described by Haberland et al. (27). The cell monolayer was then dissolved with two additions of 0.5 ml of 0.1 M NaOH and counted for radioactivity, and an aliquot was removed to determine cellular protein by the method of Lowry et al. (28) as modified by Markwell et al. (29) using bovine serum albumin as standard.

37 °C Degradation—Degradation experiments were performed essentially as stated by Goldstein et al. (30) on 8-10-day-old HMDM. Briefly, cell monolayers were washed once with DMEM then twice with DMEM (0.2% HSA). DMEM (0.2% HSA) containing various concentrations of either 125I-Lp(a) or 125I-LDL with and without unlabeled competitor was added to the cells, and the cells were returned to the 37 °C incubator for 5 h. Proteolytic degradation of 125I-labeled lipoproteins was measured by assaying the amount of 125I-labeled trichloroacetic acid-soluble (noniodide) material formed by the cells and excreted into the medium. Cell monolayers were then washed, lysed, and protein determined as described for the binding assay.

Retroendocytosis—Retroendocytosis experiments were performed essentially as described by Greenspan et al. (31). Briefly, 8-day-old HMDM were incubated for 24 h in DMEM containing either 20% autologous LPDS or 20% autologous serum. The cells were washed once with DMEM then twice with DMEM (0.2% HSA) containing either 100 nm 125I-Lp(a) or 125I-LDL was added to the cells which were then returned to the 37 °C incubator for 4 h. This period is referred to as the "pulse period." The culture
medium from the pulse period was removed and the amount of $^{125}\text{I}$-labeled trichloroacetic acid-soluble (noniodide) material formed was determined as described by Goldstein et al. (30). The cells were then chilled on ice, washed four times with 1.0 ml/well of ice-cold PBS(CMF), and incubated at 4 °C with heparin (10 mg/ml) with shaking to release the cell surface bound $^{125}\text{I}$-lipoproteins. After 1 h, the heparin containing medium was removed and counted to determine the amount of $^{125}\text{I}$-Lp(a) or $^{125}\text{I}$-LDL released. The cells were washed again several times with ice-cold PBS(CMF), once with DMEM containing 20% LPDS, and warmed to 37 °C before being chased for 2 h with DMEM (20% autologous LPDS) at 37 °C. The chase period culture medium was removed after 2 h, and lipoprotein degradation was determined as described above. The resulting precipitate was washed once with 10% trichloroacetic acid and retroendocytosis was determined as picomoles of trichloroacetic acid-precipitable $^{125}\text{I}$-material released into the chase medium/mg cell protein.

The cells were again washed several times with ice-cold PBS(CMF) and lysed with 0.1 N NaOH. This material was counted to determine the amount of internalized $^{125}\text{I}$-Lp(a) or $^{125}\text{I}$-LDL remaining in the cells after the chase period, and an aliquot was taken for the determination of protein. When employed, chloroquine (100 μM) was dissolved in the culture medium of the pulse and chase periods.

**RESULTS**

4 °C Binding Studies—The 4 °C binding of Lp(a) to 8-day-old up-regulated human monocyte-derived macrophages was compared to that of LDL. All comparisons were made on a molar basis using a molecular weight of 514,000 for the protein moiety of LDL (32), and a molecular weight of 918,000 for the protein moiety of Lp(a) (10, 14). The integrity and purity of the hot and cold ligands as determined by SDS-gradient gel electrophoresis are shown in Fig. 1. Representative binding curves indicate substantial binding of Lp(a) to LDL receptors (see Fig. 2). Total binding for Lp(a) at 100 nM lipoprotein was 0.324 ± 0.126 pmol/mg cell protein ($n = 5$) and 0.388 ± 0.106 pmol/mg cell protein ($n = 5$) for LDL. Specific binding was derived by subtracting the nonspecific binding curve obtained in the presence of a 50-fold molar excess unlabeled LDL) from the total binding curve. Specific binding of Lp(a) at 100 nM lipoprotein as measured in three experiments was 79% that of LDL (0.253 ± 0.055 pmol/mg cell protein for Lp(a) and 0.320 ± 0.103 pmol/mg cell protein for LDL). In contrast to normal human skin fibroblasts which usually exhibit saturable binding with 10–20 nM LDL (5–10 μg/ml) (33), HMDM required more than 10-fold higher LDL concentrations for saturation. Since Lp(a) did not appear to saturate LDL-specific sites at 200 nM, three experiments were carried out using higher concentrations of the ligand up to 400 nM (see Fig. 3). Note that in panels A and B, lipoprotein concentration is plotted on a logarithmic scale in order to generate sigmoidal binding curves that plateau when all the binding sites are occupied or saturated (34). As can be seen, neither specific binding curve has reached saturation although the one for LDL appears to pass through an inflection point. The resulting precipitated $^{125}\text{I}$-lipoprotein ($^{125}\text{I}$-Lp(a) or $^{125}\text{I}$-LDL) was dissolved in the culture medium of the pulse and chase periods.

**Fig. 1.** SDS-gradient polyacrylamide gel electrophoresis (2–16%) of ligands used in this study. Lanes 1 and 2 are $^{125}\text{I}$-labeled LDL and Lp(a), respectively, each containing 100,000 cpm. Lanes 3 and 4 are unlabeled Lp(a) and LDL, respectively, each containing 15 μg of protein. All samples are unreduced.

**Fig. 2.** Total and LDL specific binding of $^{125}\text{I}$-Lp(a) (panel A) and $^{125}\text{I}$-LDL (panel B) to HMDM at 4 °C. Monocytes were cultured for 7 days in DMEM (20% autologous serum) then for 24 h in DMEM (20% autologous LPDS). On day 8, the cells were washed once with DMEM and twice with DMEM (0.2% HSA), then cooled to 4 °C. The medium was removed and either $^{125}\text{I}$-Lp(a) or $^{125}\text{I}$-LDL in DMEM (0.2% HSA) was added at the indicated concentrations, with and without a 50-fold molar excess unlabeled LDL, and incubated for 4 h. Total binding (solid line) and specific binding (C) are plotted. Each data point is the average of triplicate incubations with the indicated standard deviation. Subtraction of nonspecific binding curves from total binding curves represents specific binding (−−−). 100 nM lipoprotein is equivalent to 91.8 μg/ml Lp(a) protein and 51.4 μg/ml LDL protein.

**Fig. 3.** 4 °C specific binding of $^{125}\text{I}$-Lp(a) and $^{125}\text{I}$-LDL to 8-day-old up-regulated HMDM. Macrophages were incubated with the indicated concentrations of radiolabeled lipoprotein with and without a 50-fold molar excess of unlabeled LDL for 4 h. The specific binding was obtained by subtracting nonspecific binding from total binding. Panels A and C refer to $^{125}\text{I}$-Lp(a) and panels B and D to $^{125}\text{I}$-LDL. In panels C and D, the solid line represents a computer-generated curve derived from analysis of the data by non-linear least square regression; the solid circles are the actual data points. 100 nM lipoprotein is equivalent to 91.8 μg/ml Lp(a) protein and 51.4 μg/ml LDL protein.
0.8 μM for Lp(a) and 0.23 μM for LDL. Maximal lipoprotein binding for Lp(a) was 2.23 ± 0.44 pmol/mg cell protein and for LDL 1.05 ± 0.07 pmol/mg cell protein.

4 °C Competitive Binding Assays—The LDL-specific nature of Lp(a) binding to HMDM was confirmed by competitive binding assays performed at 4 °C. As shown in Fig. 4, a 50-fold molar excess of unlabeled LDL was as effective as Lp(a) in inhibiting the binding of 25 nM 125I-Lp(a) to HMDM. In fact, LDL inhibited the binding of 125I-Lp(a) by 61.8 ± 11.9% (n = 5) compared to 63.3 ± 7.3% (n = 4) for Lp(a). Although not as effective as LDL, Lp(a) was nevertheless an efficient competitor for the binding of 25 nM 125I-LDL. Unlabeled LDL inhibited binding of 125I-LDL by 80.3 ± 5.7% (n = 4) while Lp(a) competed with the binding of 125I-LDL by 53.8 ± 7.2% (n = 4). This difference between Lp(a) and LDL for competition with 125I-LDL was significant at p < 0.001.

37 °C Degradation Studies—In contrast to its cell surface binding at 4 °C, Lp(a) was degraded much less efficiently than LDL both in terms of total degradation and LDL-specific degradation. Representative degradation curves from one experiment are shown in Fig. 5 indicating that the rate of degradation of Lp(a) was much lower than that of LDL. Results averaged from five experiments indicate that at 100 nM lipoprotein, the total degradation of Lp(a) was only 31.8% of LDL (1.51 ± 0.47 pmol/mg cell protein for Lp(a) compared to 4.74 ± 1.78 pmol/mg cell protein for LDL). LDL-specific degradation was obtained by subtracting the nonspecific degradation (degradation obtained in the presence of a 50-fold molar excess unlabeled LDL) from the total degradation of either lipoprotein. The LDL-specific degradation of Lp(a) was only 17.3% that of LDL. For Lp(a), the specific degradation was 0.738 ± 0.062 pmol/mg cell protein (n = 3) and 4.26 ± 0.18 pmol/mg cell protein (n = 3) for LDL. Both the total and specific degradation curves for 125I-Lp(a) in HMDM were linear and non-saturable, whereas those of 125I-LDL appeared to saturate albeit at much higher concentrations than have been observed in normal fibroblasts (33).

Formation of Cholesteryl [14C]Esters—The poor degradation of Lp(a) is reflected in its diminished capacity to induce the formation of cholesteryl esters relative to LDL. As shown in Fig. 6, at 100 nM lipoprotein, Lp(a) stimulated only 17% as much cholesterol esterification as did LDL. The dose-response curve for Lp(a) appears to be linear up to 200 nM while the curve for LDL approaches saturation.

37 °C Competitive Degradation Studies—The results obtained from the 37 °C competitive degradation assays are at variance with those obtained from the 4 °C competitive binding assays. As expected, unlabeled LDL was an excellent competitor for the degradation of 125I-LDL (25 nM) in that a 50-fold molar excess inhibited degradation by 78.6 ± 3.6% (n = 6) (Fig. 7). In contrast, LDL was able to inhibit the degradation of 25 nM 125I-Lp(a) by only 26.2 ± 16.8% (n = 8). Interestingly, unlabeled Lp(a) was a poor competitor for both 125I-Lp(a) and 125I-LDL in that a 50-fold molar excess of unlabeled Lp(a) inhibited the degradation of 125I-Lp(a) and 125I-LDL by 26.4 ± 18.5% (n = 8) and 13.2 ± 17.7% (n = 6), respectively.

Degradation with Mock-iodinated Lp(a)–Poor competition

**FIG. 4.** Competition for the binding of 125I-Lp(a) and 125I-LDL by unlabeled Lp(a) or LDL. Monocytes were cultured for 10 days in DMEM (20% autologous serum). The medium was removed and the cells washed once with DMEM, twice with DMEM (0.2% HSA), and then chilled to 4 °C. The cells were incubated for 4 h at 4 °C in the presence of 25 nM 125I-Lp(a) or 125I-LDL in DMEM (0.2% HSA) and a 50-fold molar excess of unlabeled Lp(a) or LDL.

**FIG. 5.** Total and specific degradation of 125I-Lp(a) (panel A) and 125I-LDL (panel B) by HMDM. Monocytes were cultured for 7 days in DMEM (20% autologous serum) and then up-regulated for 24 h in DMEM (20% autologous LPDS). On day 8, the medium was removed, the cells were washed once with DMEM and twice with DMEM (0.2% HSA), and then overlaid with increasing concentrations of either 125I-Lp(a) or 125I-LDL in DMEM (0.2% HSA) with and without a 50-fold molar excess unlabeled LDL. After incubation for 5 h at 37 °C, the amount of 125I-labeled, trichloroacetic acid-soluble, noniodide material in the medium was determined. Total degradation (□) and non-specific degradation (○) are plotted. Each data point is the average of triplicate incubations with the indicated standard deviation. Specific degradation (■) was obtained by subtracting the nonspecific degradation curve from the total degradation curve.

**FIG. 6.** Stimulation of cholesteryl ester formation in HMDM incubated with either Lp(a) or LDL. Monocytes were cultured for 7 days in DMEM (20% autologous serum) and then up-regulated for 24 h in DMEM (20% autologous LPDS). On day 8, the cells were washed twice in serum-free DMEM and overlaid with DMEM containing 0.2 mM [14C]oleate/albumin (7200 dpm/nmol of oleate) and the indicated concentrations of either Lp(a) (○) or LDL (●). After incubation for 17 h at 37 °C, cells were washed and the lipids containing cholesteryl esters were extracted and separated by thin layer chromatography. Each data point is the average of triplicate incubations with the indicated standard deviation.
of cold Lp(a) for $^{125}$I-Lp(a) could result from an inability to saturate all the binding sites on HMDM due to the nonspecific nature of Lp(a) binding at $37^\circ C$ as suggested by the degradation experiments. However, if iodination altered Lp(a) in such a way as to make it recognizable by a different binding site, native Lp(a) might not have the ability to compete. In order to rule out this possibility, we mock iodinated Lp(a) with cold NaI in order to compare its ability to compete for $^{125}$I-Lp(a) with native Lp(a) at a 20-fold molar excess. The results indicated that native Lp(a) inhibited degradation 26% and mock iodinated Lp(a) 50%. This difference was not statistically different suggesting that the poor competition was not due to lipoprotein modification but more likely due to the inability to saturate all binding sites.

Retroendocytosis Experiments—Several pulse-chase experiments were performed in an effort to resolve the discrepancy between the results obtained from the $4^\circ C$ binding and the $37^\circ C$ degradation assays and to determine specifically whether (a) the binding of Lp(a) differs at $37^\circ C$ from that at $4^\circ C$, or (b) Lp(a) binds to macrophages but follows a degradation pathway different from that of LDL. The results showed, that while the total and specific binding of LDL and Lp(a) at $4^\circ C$ were approximately equal, at $37^\circ C$ the binding of Lp(a) was only one-third that of LDL (0.045 ± 0.01 pmol/mg cell protein for Lp(a) and 0.135 ± 0.03 pmol/mg cell protein for LDL) (Table I). Second, the amount of Lp(a) internalized after the pulse was only 39% that of LDL indicating that Lp(a) was not accumulating inside the cell. This also demonstrated that the internalization rate of Lp(a) did not differ significantly from that of LDL because the binding ratio of Lp(a) to LDL was approximately equal to the internalization ratio. Third, the total degradation of Lp(a) during both the pulse and chase was only 25 and 28% that of LDL, respectively. Finally, the total amount of Lp(a) excreted into the medium was only 40% that of LDL indicating that there was no preferential retroendocytosis of Lp(a). The amount excreted was significant and for each lipoprotein represents 22% of the quantity that was internalized during the 4-h pulse. This value was approximately equal to the amount of each lipoprotein that was degraded during the 2-h chase, being 15% for Lp(a) and 22% for LDL. This difference in degradation was highly significant as shown by the paired Student’s t test ($p < 0.001$) indicating that LDL was degraded more efficiently than Lp(a).

The lysosomotropic agent chloroquine was used to determine whether Lp(a) was degraded in lysosomes. In the presence of 100 µM chloroquine, approximately 100% more LDL and Lp(a) were bound (Table II). The percent change refers to the difference between the results presented in Table I and those in Table II. There was also a 200% increase in the amount of Lp(a) and LDL internalized during the pulse period and a 270% increase in the amount of Lp(a) and LDL that remained internalized after the chase period. The total degradation of LDL was drastically decreased by 82% during the pulse when chloroquine was present, whereas that of Lp(a) was decreased by 68%. The intrinsic degradation rate of Lp(a), as measured during the 2-h chase, decreased by 19% in the presence of chloroquine and that of LDL dropped by 29%. When expressed as the amount degraded relative to the amount of internalized lipoprotein, the degradation of both Lp(a) and LDL were drastically reduced from 15 to 4.2% and from 22 to 4.9%, respectively. In addition, the amount of Lp(a) and LDL excreted by retroendocytosis was increased...
132 and 260%, respectively, when chloroquine was present. However, the percentage of either lipoprotein that was subject to retroendocytosis remained basically unchanged.

**Regulation of Degradative Pathway**—To determine whether the degradation of LDL and Lp(a) is influenced by LDL receptor activity in HMDM, half of the cells in three experiments were preincubated for 24 h with 20% autologous serum and the other half with 20% autologous LPDS. Values for the total and specific degradation of both lipoproteins at 100 nM are given in Table III. For cells growing in the presence of an external cholesterol source, the specific degradation rate was decreased 3-fold for LDL and 2.2-fold for Lp(a) indicating that the regulation of LDL receptor activity on HMDM was relatively modest. The total degradation rate of LDL was decreased 2.4-fold and that of Lp(a) 1.7-fold.

We also performed a pulse-chase experiment to determine whether the amount of Lp(a) bound, internalized, and retroendocytosed was dependent upon up- or down-regulation of LDL receptor activity. In the down-regulated cells, the 37 °C heparin releasable binding of Lp(a) was reduced 18% in comparison to the up-regulated cells while LDL was reduced by 53% (compare Table IV to Table I). The amount of internalized Lp(a) and LDL was not significantly changed in the down-regulated cells. The degradation rate of LDL was only 40% that seen in the up-regulated cells during both the pulse and the chase while the Lp(a) degradation rate was reduced by 50% during the pulse and by only 13% during the chase. Lastly, the rate of retroendocytosis for both lipoproteins remained relatively unchanged being 23% for Lp(a) and 24% for LDL compared to 22% for both lipoproteins in the up-regulated cells.

**Table III**

| Effect of up- and down-regulation of LDL receptor activity on the degradation of 100 nM Lp(a) and LDL in HMDM |
|-----------------|-----------------|-----------------|-----------------|
| Lp(a) LDL       | 20% LPDS 20% Serum | 20% LPDS 20% Serum |
|-----------------|-------------------|-------------------|
| Total           | 1.20 ± 0.08       | 0.70 ± 0.11       | 3.95 ± 1.3      | 1.66 ± 0.43    |
| Specific        | 0.56 ± 0.15       | 0.25 ± 0.10       | 3.32 ± 1.2      | 1.10 ± 0.38    |

**Table IV**

| Processing of 100 nM Lp(a) and LDL in down-regulated macrophages |
|-----------------|-----------------|-----------------|
| Lp(a) LDL % change | pmol/mg cell protein |
|-----------------|-------------------|-------------------|
| 37 °C heparin-releasable binding | 0.037 ± 0.012 | 0.063 ± 0.023 | 18 | 53 |
| Internalized    |                   |                   |                 |
| During pulse    | 0.32 ± 0.017     | 0.68 ± 0.16      | 0 | 16 |
| Remaining after chase | 0.21 ± 0.007 | 0.44 ± 0.14      | 5 | 4 |
| Degraded        |                   |                   |                 |
| During pulse    | 0.03 ± 0.075     | 3.00 ± 0.098     | 50 | 60 |
| During chase    | 0.04 ± 0.007     | 0.01 ± 0.022     | 3 | 7 |
| Retroendocytosed during chase | 0.075 ± 0.006 | 0.16 ± 0.003 | 9 | 6 |
| % retroendocytosed during 2-h chase | 23% | 24% | 5 | 9 |
| % degraded during 2-h chase | 13% | 10.5% | -13 | -106 |

*Percent change refers to the difference between the processing in down-regulated cells from that in up-regulated cells (Table I).*

**DISCUSSION**

The results presented here indicate that the LDL receptor pathway of HMDM is only minimally involved in the degradation of Lp(a) when compared on an equimolar basis to LDL. This is exemplified by the fact that unlabeled LDL at a 50-fold molar excess was able to inhibit the degradation of 125I-Lp(a) by only 26.2%. This finding is particularly surprising considering that at 4 °C, LDL was a good competitor for 125I-Lp(a) by reducing its binding by 61.8%. In contrast to the dichotomous behavior of Lp(a), excess LDL competed effectively for 125I-LDL in both 4 °C binding and 37 °C degradation experiments. Several mechanisms could account for these differences: 1) the binding of Lp(a) to HMDM at 37 °C is less than that observed at 4 °C; 2) Lp(a) binding is normal, however, the rate of internalization is less than that of LDL; 3) Lp(a) is bound and internalized normally but degraded slower than LDL; and 4) a greater percentage of Lp(a) than LDL could escape degradation by entering a retroendocytotic pathway.

The pulse-chase experiments designed to investigate these possibilities supported the first mechanism, namely, that at 37 °C, binding was less than at 4 °C. These experiments also ruled out the second hypothesis that was based on the possibility that the internalization rate of Lp(a) was less than that of LDL. In addition, the results partially supported the third mechanism that, in addition to lower binding, Lp(a) also had a lower intrinsic degradation rate when compared to LDL as determined during the 2-h chase period. Thus, both the decreased binding and slower degradation contributed to the overall lower degradation rate of Lp(a) and prevented overt accumulation of Lp(a) inside the cells.

The conclusion that reduced degradation of Lp(a) relative to LDL can be accounted for by lower binding and degradation does not explain why the binding of Lp(a) to the LDL receptor of HMDM was so much better at 4 than at 37 °C. One possibility is that the conformation of these two lipoproteins is differentially affected by temperature. Some Lp(a) species with large apo(a) polymorphs are sensitive to changes in temperature, e.g. they can undergo a reversible cold-induced aggregation (11), a process different from irreversible aggregation caused by foaming, oxidation, proteolysis, or bacterial degradation. It is conceivable that Lp(a) polymorphs that do not aggregate in the cold undergo conformational changes that affect the receptor-binding domain of apoB resulting in better interaction with the LDL receptor when the temperature is lowered to 4 °C.

Our studies showed that the affinity of LDL for the LDL receptor on HMDM is at least two orders of magnitude lower than in fibroblasts indicating that the LDL receptor in these two cells is probably different. In fact, in an experiment designed to compare the efficacy of heparin in releasing LDL versus Lp(a) from the macrophage cell surface at 37 °C, we found that 20 mg/ml heparin was no more effective than PBS in releasing either lipoprotein from their binding sites which is indicative of low affinity binding. This conclusion is in keeping with the findings of Knight and Soutar (36), and Van Lenten and Fogelman (20) who demonstrated that HMDM have receptors with low affinity for LDL. Van Lenten and Fogelman proposed the existence of two classes of LDL recep-
Finally, it should be noted that the retroendocytic pathway in HMDM is highly active since it is, at least for LDL, 3-10-fold higher than that reported in fibroblasts or smooth muscle cells during similar pulse-chase periods (31, 39, 40). No comparisons for Lp(a) can be made since retroendocytosis of Lp(a) has not been studied in these cell lines.

Unlike the classic LDL receptor of fibroblasts, the LDL receptor activity of HMDM is comparatively less responsive to regulation by cellular cholesterol content. In agreement with Fogelman et al. (17) and Knight and Soutar (36), we also observed only a 2-3-fold increase in LDL degradation upon preincubation of HMDM with LPDS. Similarly, down regulation of the LDL receptor of HMDM resulted in decreased degradation of Lp(a). However, based on the low percentage of Lp(a) degraded by the LDL receptor pathway (26%), the observed decrease in degradation appeared to be greater than expected. This decrease may be explained in part by the fact that down-regulation also caused a 30% decrease in nonspecific degradation of Lp(a) for reasons that are not clear at this time.

Low degradation of Lp(a) and an inability to induce formation of cholesteryl esters in comparison to LDL do not appear to support a significant involvement of the scavenger receptor of HMDM in the processing of Lp(a). This conclusion is in keeping with the preliminary results by Haberland et al. (41) that indicate that Lp(a) (same Lp(a) as was used in the present study) modified with malondialdehyde, but not native Lp(a), is taken up by the scavenger receptor of HMDM. These findings, however, are at variance with the preliminary observations of Powell et al. (42), Gavish et al. (43), and Zioncheck et al. (44) who reported that Lp(a) binds to the scavenger receptor. The reason for this discrepancy is not apparent but may be due to differences in experimental conditions.

In summary, our results indicate that HMDM degrade Lp(a) mainly through a nonspecific mechanism(s) with only one-quarter being degraded by the LDL receptor pathway. In quantitative terms, only one-third as much Lp(a) as LDL is degraded when the two lipoproteins are compared on a molar basis: moreover, when LDL-specific degradation is compared, Lp(a) degradation is 6-fold lower than that of LDL. The exact reason for the low LDL-specific degradation of Lp(a) is not known but may be related to temperature-induced conformational changes in Lp(a) (which do not occur in LDL) that permit a better recognition by the LDL receptor of HMDM at 4 than at 37°C.

We would like to stress that these results were obtained with only one species of Lp(a) that was chosen because of its stability and known physical parameters. As more Lp(a) species with different apo(a) polymorphs are characterized, we plan to extend these studies in order to determine the effect of apo(a) polymorphism on the uptake and degradation of Lp(a) by macrophages and other cell lines, i.e. human skin fibroblasts and primary human hepatocytes.

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