The Role of Lipoprotein Lipase in the Metabolism of Triglyceride-rich Lipoproteins by Macrophages*

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Peter Lindqvist†, Ann-Margret Ostlund-Lindqvist‡, Joseph L. Witzum*, Daniel Steinberg, and J. Alick Little||

From the Department of Medicine, Division of Metabolic Disease, University of California, San Diego, La Jolla, California 92039 and the Lipid Research Clinic, University of Toronto and St. Michael's Hospital, Toronto, Ontario, Canada

We have previously shown that cultured macrophages secrete lipoprotein lipase (LPL) into the culture medium. The purpose of these experiments was to determine the role of LPL in the uptake of very low density lipoproteins (VLDL). Both J774 cells and mouse peritoneal macrophages took up and degraded normotriglyceridemic VLDL in a saturable manner. Uptake of VLDL was effectively competed for by rabbit β-VLDL, human VLDL, but not by native LDL or acetylLDL. LPL activity increased saturable uptake and degradation of the C-II-deficient lipoprotein occurred. However, addition of apo-C-II enhanced saturable uptake.

Normal VLDL also promoted cellular accumulation of both triglycerides and cholesterol esters. Accumulation of triglyceride occurred by uptake of intact VLDL particles, by uptake of a triglyceride-depleted particle produced by the action of LPL, and by the direct uptake of free fatty acids generated by the activity of LPL. In turn, the latter process was influenced by the amount of albumin in the medium capable of being an acceptor for free fatty acids. Finally, we have shown that when albumin is present in the medium, the macrophage is capable of mobilizing most of its stored triglyceride over 24 h, even as its cholesterol ester content remains constant. These studies may be relevant to the ability of VLDL to promote macrophage accumulation of cholesterol ester, even as triglyceride accumulation is minimized.

Macrophages have been implicated as precursors of the foam cells observed in atheromatous lesions (1). Because the predominant lipid found in the atheromatous lesion is cholesterol ester much attention has been focused on the uptake of cholesterol-rich lipoproteins by macrophages (2-5). On the other hand, because triglyceride is not a major constituent of the atheroma, little attention has been paid to the possibility that triglyceride-rich lipoproteins could make a significant contribution to the lipid accumulation that occurs in the arterial wall. Yet, both triglycerides and cholesterol ester accumulate in macrophages in hypertriglyceridemic states (6) and as the hypertriglyceridemia resolves, these cells are left with a residual cholesterol ester enrichment (6). Furthermore, remnant of triglyceride-rich lipoproteins with LPL leads to production of remnants that can cause cholesterol ester accumulation in cultured fibroblasts and smooth muscle cells (7, 8). Studies in this laboratory have shown that cultured macrophages constitutively secrete LPL into the culture medium (9, 10). Thus, it is possible that remnants of triglyceride-rich lipoproteins, generated by the action of macrophage LPL, would also cause cholesterol ester accumulation in these cells. These studies were undertaken to define the role of LPL in the uptake and metabolism of VLDL by cultured macrophages.

EXPERIMENTAL PROCEDURES

Materials

Female Swiss-Webster mice (25–30 g) were obtained from Simonsen Laboratories, Gilroy CA. Na23HPO4 was purchased from Amersham Corp. Oleic acid, cholesterol oleate, cholesterol, tristearin, bovine serum albumin, (Cohn fraction V), and free fatty acid-poor albumin were purchased from Sigma. Fetal calf serum (Imune Scientific Sales Co., Foum Valley, CA) was heat-inactivated at 56 °C for 30 min before use. Plastic Petri dishes were obtained from Lux Scientific Corp., Newbury Park, CA. Silica gel plates (0.2 mm) were purchased from Analtech. All other chemicals used were of analytical grade.

Methods

Lipoproteins—Human VLDL (d < 1.006) was isolated by preparative ultracentrifugation from pooled plasma obtained from young healthy fasting normolipidemic donors. Each VLDL preparation was washed once by centrifugation through a layer of d = 1.006 saline containing 0.01% EDTA. Human LDL (d 1.019–1.063) and HDL (d 1.063–1.21) were isolated by standard techniques (11). VLDL was also isolated from one subject homozygous for apo-C-II deficiency (12). VLDL was also isolated from male guinea pigs consuming Wayne chow and from rabbits fed 2% cholesterol (13). The latter consisted of greater than 90% β-VLDL as judged by Pevikon block electrophoresis (13) and is referred to subsequently as “β-VLDL.” The lipid and protein compositions of the VLDL preparations were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 4–

1 The abbreviations used are: LPL, lipoprotein lipase; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; BSA, bovine serum albumin; FFA, free fatty acids; apo-, apolipoprotein.

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TABLE I

Composition of various VLDL preparations

| Lipoprotein preparation | Triglyceride/Cholesterol ratio | Protein content | Apoproteins |
|-------------------------|-------------------------------|-----------------|-------------|
| Human VLDL              | 3.8:4.2                       | 1.0:1.2         | B, C, E     |
| Guinea pig VLDL         | 9:1                            | 0.5             | B, E, C     |
| C-II-deficient human VLDL | 3.4                          | 0.5             | B, E, C (C-II deficient) 10% albumin present |
| Rabbit β-VLDL           | 0.2                            | 4.4             | 80% apo-B, B, trace C |

20% gradient. Acetylated LDL was prepared as previously described (3). Human apo-C-II was isolated as previously described (14) and highly purified LPL was prepared from bovine milk (15). Bovine serum albumin was complexed with oleic acid according to the procedure described by Kohout et al. (16). The protein concentration of the lipoproteins and of the cells was determined by a modification of the Lowry method (17).

VLDL was iodinated by the iodine monochloride method as previously described (18) and specific activities varied from 50 to 250 Cpm/ng of protein. More than 90% of the radioactivity was precipitable by 10% trichloroacetic acid from 10 to 20% was extracted by chloroform:methanol (2:1, v/v) or ethanol:diethyl ether (3:1, v/v). In a typical iodinated VLDL preparation ~60% of total radioactivity was in apoB, 20% in apo-C, and less than 10% in apo-E. In the case of the C-II-deficient VLDL, approximately 10-15% of radioactivity was also found in a protein band comigrating with human albumin as determined on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. 125I-Albumin was prepared by the same method (18).

Preparation of Cultured Macrophages—Monolayer cultures of the J774 macrophage-like cell line (clone G8) were obtained from Drs. Helen Plutner and Jay Unkelless of the Rockefeller University and maintained in α-minimum Eagle’s medium containing gentamicin sulfate (50 μg/ml) and fungizone (0.25 μg/ml) (medium A), to which was added 5% heat-inactivated newborn calf serum. Before each experiment cells were washed twice with medium A (9).

Resident mouse peritoneal macrophages were harvested from normal unstimulated mice using phosphate-buffered saline as previously described (19). The peritoneal washings from 18 to 30 mice were pooled and the cells collected by centrifugation (600 × g for 10 min at room temperature). Cells were resuspended in 12 to 20 ml of medium A containing 15% (v/v) heat-inactivated fetal calf serum. Aliquots (2 ml) of this cell suspension were dispensed into 60-mm dishes and then incubated in a humidified CO₂ (5%) incubator at 37°C. After 1 h of incubation, nonadherent cells were removed by washing the monolayers twice with 2-ml portions of medium A. The monolayers were then incubated for 18 h in 2 ml of medium A containing 15% heat-inactivated fetal calf serum. After this incubation, the cells were washed twice more with 2 ml of medium A and then used for the experiments.

Analysis of Cell Culture Experiments—After each experiment, the medium was removed and each cell monolayer (35-mm or 60-mm dish) washed twice at 4°C with 2 ml of PBS containing 0.5 mM magnesium and 1 mM calcium followed by two washes with the same buffer containing 0.2% bovine serum albumin and, finally, by two additional washes with 2 ml of phosphate-buffered saline. Cells were then harvested by one of the following procedures.

In experiments in which iodinated lipoproteins were used, cells were disrupted in 0.25 ml of 0.25 M NaOH and cell-associated radioactivity and protein content were determined on aliquots as previously described (20). Data are expressed as micrograms of cell-associated protein per mg of cell protein and are not corrected for extent of lipid labeling. Trichloroacetic acid-soluble noniodide degradation products were determined from aliquots of the medium as previously described (29) and expressed as micrograms of protein degraded per mg of cell protein. In experiments in which the lipid content of cells was also determined, cells from three 60-mm dishes were harvested in a total volume of 1.0 ml of distilled water using a rubber policeman. The cell suspension was sonicated for 10 s and aliquots were taken for total cell protein determination and for lipid analysis as described below.

Lipid Extraction and Analysis—For determination of lipid content of cells, sonicated cell suspensions prepared as described above were extracted with 24 volumes of chloroform:methanol (1:1, v/v) for 15 h at 4°C. For the determination of the lipid content of the incubation medium, 1 ml of medium was mixed with 12 ml of methanol and 6 ml of chloroform for 13 h at 4°C. The precipitated protein was then pelleted by centrifugation at room temperature and the supernatant transferred to another extraction tube. Chloroform (6 ml) was then added to make the final chloroform:methanol ratio, 1:1 (v/v). The organic phase was then washed with water, taken to dryness in a vacuum oven under reduced pressure, redissolved in chloroform:methanol (2:1, v/v), and separated into lipid classes by TLC on silica gel plates developed in hexane:diethyl ether:acetic acid (80:20:1, v/v/v) (21). The mass of individual lipids was quantified by a charring method (22). Standards used for the lipid analysis were TLC-purified oleic acid, triolein, free cholesterol, and cholesteryl oleate. The recovery for the entire extraction and lipid analysis using reference standards was 105 ± 8% for free fatty acid, 98 ± 15% for triglyceride, and 103 ± 12% for cholesteryl ester, respectively (mean values of six samples at three different concentrations ± S.D.). Triglyceride and cholesterol content of lipoproteins was determined by enzymatic methods using Boehringer Mannheim kits (126012 and 124087).

RESULTS

Uptake and Degradation of Human 125I-VLDL by J774 Macrophages—In view of the well established requirement for albumin (or other acceptor of free fatty acids) to optimize lipoprotein lipase activity, uptake and catabolism by macrophages of normotriglyceridemic VLDL was studied at different levels of serum albumin in the medium (Fig. 1). In the presence of 4% albumin, a concentration that should ensure that acceptor is not rate limiting, degradation of VLDL protein to trichloroacetic acid-soluble products was a biphasic function of VLDL concentration, implying the operation of a specific saturable process. In the presence of 0.5% albumin, the shape of the concentration dependence curve was also biphagic but rates were somewhat lower at every concentration. In the complete absence of albumin, rates of degradation

FIG. 1. Cellular uptake and degradation of human 125I-VLDL (125I-h-VLDL) in J774 cells in the absence and presence of albumin in the medium. Each 35-mm dish received 1.5 ml of medium A containing 0% (A), 0.5% (●), or 4% (○) bovine serum albumin and the indicated concentration of human 125I-VLDL. After incubation for 6 h at 37°C the amount of noniodide 125I-labeled acid-soluble material in the media (upper) and the cellular content of human 125I-VLDL lower were determined in duplicate dishes.
at low VLDL concentrations (up to 10 µg/ml) were lower still but the curve showed a paradoxical sudden change of slope at 10 µg/ml. We attribute this to the release of FFA in the absence of an effective acceptor. When incubations in the absence of albumin were extended to 20–24 h there was again a plateauing of the curve relating degradation to VLDL concentrations followed by a sharp increase in the slope at higher concentrations. There was significant cell toxicity and cell death at the high concentrations. Unless otherwise specified, subsequent experiments were carried out in the presence of albumin.

Saturability of the uptake and degradation of normotriglyceremic human 125I-VLDL were also shown by competition with unlabeled VLDL (Fig. 2). Interestingly, β-VLDL prepared from plasma of a cholesterol-fed rabbit also competed effectively. In several such experiments this rabbit β-VLDL, in which 80% of the protein was apo-E, competed at least as effectively as normal human VLDL. Acetylated LDL, which is recognized by a specific macrophage receptor (3), failed to compete even at a 10-fold excess; normal human LDL was a poor competitor.

Role of Lipoprotein Lipase—The increase in rate of degradation of VLDL protein in the presence of acceptor albumin described above (Fig. 1) suggested that LPL-catalyzed modification of VLDL enhanced cellular uptake and degradation. In preliminary experiments we added purified bovine milk LPL to incubations containing 125I-VLDL and J774 macrophages. There was visible clearing of the incubation medium due to rapid hydrolysis of VLDL triglyceride and saturable cellular uptake and degradation of VLDL protein was clearly enhanced (data not shown). To determine if VLDL is a substrate for the macrophage LPL activity, we incubated guinea pig VLDL with macrophages for 24 h and serially measured the amount of VLDL triglyceride and protein mass (d < 1.019) remaining in the culture medium. There was a progressive decrease in VLDL triglyceride content so that by 24 h only 50% of the triglyceride mass remained, and the triglyceride:protein ratio of the VLDL fraction remaining had decreased from 12 to 9. However, guinea pig VLDL contain only small amounts of apo-C and are, therefore, a poor substrate for LPL. Addition of purified apo-C-II led to more rapid hydrolysis and as shown in Table II, when added at a concentra-

![Graph](http://www.jbc.org/)

**FIG. 2.** Competition of various lipoproteins for the degradation of human 125I-VLDL (125I-F.H.-VLDL) by J774 cells. Each 35-mm dish received 1 ml of medium A containing 2.5% bovine serum albumin, 15 µg of human 125I-VLDL, and the indicated concentration of unlabeled lipoprotein. After incubation for 5 h at 37 °C the amount of 125I-labeled acid-soluble material was determined. Unlabeled lipoproteins added were: β-VLDL from cholesterol-fed rabbit (●), human VLDL (A), human LDL (O), and human acetyl-LDL (△). Each point represents the mean value of triplicate determinations.

**TABLE II**

| Guinea pig VLDL | Apo-C-II added | Guinea pig 125I-VLDL protein degraded as percentage of control value |
|-----------------|----------------|---------------------------------------------------------------|
| µg/ml           |                |                                                               |
| 50              | 0              | 100                                                           |
| 50              | 1              | 138                                                           |
| 50              | 2.5            | 165                                                           |
| 50              | 5              | 146                                                           |
| 50              | 10             | 160                                                           |

Each 35-mm dish received 1.5 ml of medium A containing 2.5% BSA, guinea pig VLDL, and human apolipoprotein C-II as indicated above. After incubation for 4.5 h at 37 °C the amount of 125I-trichloroacetic acid-soluble noniodide degradation products in the medium were determined. The amount of guinea pig VLDL protein degraded in the absence of added apo-C-II was 0.26 µg/mg of cell protein.

![Graph](http://www.jbc.org/)

**FIG. 3.** Cellular lipid content and protein degradation in mouse peritoneal macrophages incubated with apolipoprotein C-II-deficient VLDL. Apo-C-II (10% of total VLDL protein) was preincubated with C-II-deficient VLDL for 10 h at 4 °C. Media A containing 2.5% BSA and the indicated concentrations of 125I-apo-C-II-deficient VLDL were added to cells in the presence (C) or absence (○) of apo-C-II. After 22 h of incubation at 37 °C the medium was replaced with fresh medium of identical composition. After another 18 h of incubation at 37 °C the amount of 125I-labeled acid-soluble material (upper left) and of triglycerides (lower left) in the medium and the cellular content of cholesteryl esters (upper right) and triglycerides (lower right) were determined. Each point is the average of duplicate determinations.

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chyomicrons also failed to be substrate for the macrophage LPL, but addition of C-II led to dramatic hydrolysis of triglyceride, clearing of medium turbidity, release of FFA, and enhanced uptake of lipoprotein with increased triglyceride and cholesteryl ester accumulation.

Lipid Accumulation—Preliminary studies showed that prolonged incubation of macrophages with normal VLDL caused microscopically evident accumulation of lipid droplets. This was quantified in incubations of J774 macrophages with VLDL in the presence or in the absence of BSA (Fig. 4). In the absence of albumin in the medium, there was a marked increase in cellular triglyceride accumulation, which could not be accounted for on the basis of uptake of intact VLDL. In the absence of albumin, hydrolysis of VLDL triglyceride did occur, though at a suboptimal rate. In this case, in the absence of albumin to act as an acceptor, the FFA released were presumably taken up directly by the cell and incorporated into cellular triglyceride (also see Fig. 6). The presence of BSA again enhanced VLDL protein degradation and also caused a greater depletion of triglycerides from the medium. Yet, unexpectedly, incorporation of triglyceride content was much less in the presence of BSA (1- to 2-fold) than in its absence (10-fold). This apparent paradox was explained by the large increase in FFA accumulating in the medium. Evidently, the FFA generated by the action of LPL were trapped by the medium BSA and prevented from entering the cell. This was consistent with the marked increase in cell cholesteryl ester content in the presence of BSA (Fig. 4F).

J774 cells are rapidly growing and their storage and utilization of lipids is likely to be different than that in a non-dividing cell, such as the resident mouse peritoneal macrophage. Therefore, we studied the effect of normal VLDL on cellular lipid accumulation in mouse peritoneal cells in the presence of 2.5% BSA in the medium. As shown in Fig. 5, after 36 h of incubation with only 25 or 50 µg of VLDL protein per ml, there was an approximately 10-fold increase in triglyceride content and a similar 10-fold increase in cholesteryl ester content. Since the ratio of triglyceride to cholesteryl ester in the VLDL used was about 4:1, the approximately equal increments in the two lipids in the cells could not be explained by random uptake of intact VLDL particles. Selective uptake of smaller triglyceride-poor VLDL in the original preparations might account for this. However, in view of the FFA accumulation shown in the experiments of Fig. 4, it is more likely due to generation and uptake of remnants or selective efflux of triglyceride fatty acids after uptake.

To further define the role of LPL in cellular lipid accumulation, we incubated apo-C-II-deficient VLDL with mouse peritoneal macrophages in the presence and absence of added

![FIG. 4. Cellular lipid content and degradation of human 125I-VLDL (125I-h-VLDL) by J774 cells and lipid content of the cell medium as a function of the concentration of the VLDL in the cell medium. Each 60-mm dish received 2 ml of medium A (●) or medium A containing 2.5% bovine serum albumin (O) and the indicated concentration of human 125I-VLDL. After incubation for 5 h at 37°C the amount of 125I-labeled trichloroacetic acid-soluble material (A), triglycerides (B), and free fatty acids (C) in the culture medium as well as the cellular content of triglyceride (D), free fatty acid (E), and cholesteryl ester (F) were determined in triplicate dishes.](http://www.jbc.org/Downloaded from http://www.jbc.org/)

![FIG. 5. Cellular lipid content of mouse peritoneal macrophages incubated with normal human VLDL. Each 60-mm dish received 2 ml of medium containing 2.5% BSA and the indicated concentration of human 125I-VLDL (125I-h-VLDL). After 18 h of incubation at 37°C the medium was replaced with fresh medium of identical composition. After another 18 h of incubation the cellular lipid content was determined. The mouse cells incubated with human 125I-VLDL at concentrations of 25 µg/ml and 50 µg/ml degraded a total of 29.8 and 44.5 µg of 125I-protein/mg of cell protein, respectively.](http://www.jbc.org/Downloaded from http://www.jbc.org/)
Effect of FFA in the Medium on Triglyceride Accumulation in Cells—To determine if FFA bound to BSA could enter the macrophage and promote cellular triglyceride accumulation, J774 cells were incubated with media containing albumin loaded with FFA. A marked increase in cellular triglyceride content over time occurred with FFA-rich albumin; FFA-poor albumin caused no such increase (Fig. 6). When J774 cells were incubated with VLDL in the absence of albumin there was the usual marked triglyceride accumulation, but when VLDL were incubated in the presence of FFA-poor BSA, there was much less triglyceride accumulation. One explanation for this latter finding is that the FFA-poor albumin in the medium bound and retained much of the FFA released during VLDL triglyceride hydrolysis leaving a triglyceride-poor VLDL particle to be taken up by the cells. Alternatively, the FFA-poor albumin in the medium might act as an acceptor for FFA released from the cells. When VLDL were incubated together with BSA already preloaded with FFA, triglyceride

 apo-C-II. In the absence of C-II, despite the absence of any hydrolysis of VLDL in the medium, apo-C-II-deficient VLDL caused net cellular accumulation of triglyceride and cholesteryl ester. In this case, the increment in cellular triglyceride content could be accounted for entirely by uptake of intact VLDL particles (cell associated (data not shown) plus triglyceride delivered by particles degraded). When hydrolysis was promoted by addition of apo-C-II to the medium, VLDL protein degradation was increased and cholesteryl ester content increased 35% above that seen in the absence of added apo-C-II (Fig. 3).

Fig. 6. Triglyceride accumulation in J774 cells incubated with FFA-loaded albumin. Each 60-mm dish received 2 ml of medium A with the additions indicated below and after indicated time of incubation at 37°C the cellular triglyceride content was determined: ▲, 2.1% FFA-poor BSA; ○, 2.1% FFA-poor BSA plus 50 μg/ml of human VLDL; △, 2.1% oleic acid-loaded BSA (3.6 mol/mol of BSA); ◆, 2.1% oleic acid-loaded BSA plus 50 μg/ml of human VLDL; ○, 50 μg/ml of human VLDL alone. Each point is the average of duplicate determinations.

Fig. 7. Effect of albumin in the medium on changes in the cellular content of triglycerides and cholesteryl ester in mouse peritoneal macrophages previously loaded with lipids by incubation with VLDL. After an initial incubation for 18 h at 37°C with 2 ml of medium containing 2.5% BSA and 50 μg/ml of human VLDL, the medium was replaced with fresh medium of identical composition. After incubation for an additional 18 h at 37°C the medium was removed and the cells were washed twice with 2 ml of medium A. The cells were then incubated with 2 ml of fresh medium A containing no BSA (○) or medium A containing 2.5% BSA (●) for the indicated times at 37°C. Cellular cholesteryl ester content (upper panel) and triglyceride content (lower panel) were determined and expressed as a percentage of the initial values. The control value for cellular cholesteryl ester content and triglyceride content was 32 and 70 μg/ml of cell protein, respectively. Each point represents the average of duplicate determinations.

Fig. 8. Changes in cholesteryl ester and triglyceride content of mouse peritoneal macrophages incubated with varying concentrations of HDL in the absence (A) or presence (B) of albumin in the medium. Cells were first loaded with lipids by incubation with human VLDL as described in the legend of Fig. 7. Fresh medium in the absence (A) or presence (B) of 2.5% BSA and containing the indicated concentrations of HDL was then added. Cells were incubated for 24 h and then cellular free cholesterol (△), cholesteryl ester (●), and triglyceride (○) content were determined and expressed as a percentage of the initial values. The initial values at the end of the loading phase (i.e. the initial values presented in the graph) were 20, 37, and 48 μg/ml of cell protein for triglyceride, free cholesterol, and cholesteryl ester, respectively.
accumulation in the cells was somewhat greater than that in the presence of the FFA-loaded BSA alone but less than that seen when VLDL were incubated in the absence of any BSA. Thus, the capacity of the medium to serve as an acceptor for FFA can be an additional factor determining the net accumulation of triglycerides in the cells.

**Effect of BSA in the Medium on Efflux of Lipids from Cells**

To test the possibility that BSA was promoting efflux of fatty acids from macrophages, the following experiments were performed. Mouse peritoneal macrophages were loaded with lipids by incubation with VLDL for a total of 36 hours. The medium was then removed and fresh medium was added with or without 2.5% BSA (Fig. 7). Over the ensuing 24 h, there was no loss of cellular cholesteryl ester or triglyceride in cells incubated in the absence of BSA. In cells incubated in the presence of BSA there was a 70% decrease in cellular triglyceride content but no change in cholesteryl ester content. Ho et al. (25) have previously shown that HDL, but not BSA, will cause efflux of cholesterol from mouse peritoneal macrophages. To determine whether HDL could effect triglyceride efflux, we loaded mouse macrophages as described above and then incubated them with media containing increasing amounts of HDL in the presence or absence of BSA (Fig. 8). In confirmation of the previous report (25) HDL caused a large loss of cellular cholesteryl esters and a smaller change in free cholesterol but no change in cellular triglycerides. In contrast, BSA caused a marked loss of cellular triglycerides, which was not further enhanced by HDL.

To determine if the efflux of cellular triglyceride was dependent on lysosomal lipase activity, mouse peritoneal macrophages previously loaded with triglycerides by incubation with FFA-rich albumin were incubated for 24 h in media containing 2.5% BSA in the absence or in the presence of 20 μM chloroquine (4). As shown in Fig. 9, chloroquine had little or no effect on mobilization of triglycerides from the cells, suggesting that an extralysosomal lipase must be involved.

**DISCUSSION**

We (9, 10) and others (26, 27) have previously shown that cultured macrophages constitutively secrete LPL into the culture medium. The present studies show that human and guinea pig VLDL are substrates for this LPL. This was most clearly demonstrated in experiments in which apo-C-II-deficient VLDL were the substrate. In the absence of added apo-C-II very little hydrolysis of triglycerides occurred, but when CII was added hydrolysis was rapid. In data presented elsewhere we also show that chylomicrons are substrates for this LPL activity (24).

The protein moiety of normal VLDL was taken up and degraded by macrophages in a saturable manner. Uptake of normotriglyceridemic human VLDL was competed for by unlabeled human VLDL and also by rabbit β-VLDL, but not by human LDL or human acetyl LDL. In this report we also demonstrate that the LPL activity secreted by the macrophage accelerates the saturable uptake of VLDL. Four types of experiments support this conclusion: First, addition of bovine milk LPL accelerated the saturable uptake and degradation of VLDL protein. Second, guinea pig VLDL, which is a poor substrate for LPL because of low levels of LPL activators (23), was taken up and degraded only one-third as rapidly as normal human VLDL, but its uptake was increased 50% by addition of apo-C-II. Third, the addition of BSA to the medium enhanced triglyceride lipolysis and increased saturable degradation of VLDL protein. Fourth, when apo-C-II was added to human C-II-deficient VLDL, the lipoprotein's uptake and degradation was significantly increased. Thus, macrophage LPL clearly accelerates saturable uptake and degradation of VLDL protein. However, the experiments with C-II-deficient VLDL showed that hydrolysis of VLDL triglyceride was not an absolute prerequisite for saturable uptake. The enhancement of uptake of VLDL protein produced by LPL is most likely due to enhanced uptake of a triglyceride-poor VLDL remnant, previously shown to be taken up more rapidly by fibroblasts and smooth muscle cells (7, 8). It is possible that LPL bound to lipoproteins could serve as an independent recognition marker for cellular uptake but this is less likely in view of our preliminary finding that bovine milk LPL added to cultures containing [3H]-BSA caused no acceleration of LDL uptake (data not shown).

We have also shown, by mass measurements, that VLDL from normolipidemic individuals caused net triglyceride accumulation in cultured macrophages and, to a lesser extent, cholesteryl ester accumulation. This was particularly true for the resident mouse peritoneal macrophage, which is a quiescent cell. However, even the J774 cells, which are rapidly growing, showed a net accumulation of triglycerides. Gianurco et al. observed triglyceride accumulation in mouse peritoneal macrophages incubated with VLDL from hypertriglyceridemic subjects but not with VLDL from normal subjects (28). Although different experimental conditions were used, the reasons for the difference in findings are not yet clear. Traber and Kayden have reported that long term exposure of human monocyte macrophages to human VLDL will cause enhanced cellular cholesterol accumulation (29).

The present studies demonstrate that under appropriate conditions the macrophage is capable of mobilizing most of its stored triglycerides over 24 h. Although we have not definitely shown that this is caused by efflux of FFA, the fact that this mobilization only occurs when an FFA acceptor, such as BSA, is in the medium strongly supports this view. Moreover, the mobilization cannot be attributed to lysosomal activity (Fig. 9). Only adipocytes have previously been shown to have this ability to mobilize FFA (30). In the following discussion of the multiple pathways operating reference is made to the scheme shown in Fig. 10. Mobilization of FFA is designated as pathway 5.

Like other cells, the macrophage can take up FFA and store them in the form of triglycerides (Fig. 10, pathway 4). This was demonstrated directly using FFA-albumin complexes. In addition, FFA generated by the action of LPL are also taken up in the absence of albumin. In fact the presence of albumin...
cholesteryl esters due to the preferential re-release of triglycerides when LPL activity is deficient, as in the diabetic lipemia syndrome. Yet, as the hypertriglyceridemia resolves the macrophages show greater relative enrichment in cholesterol, presumably because triglyceride efflux exceeds cholesterol efflux (6).

The present studies disclose new pathways of potential pathophysiologic significance in the generation of foam cells. It remains to evaluate their relative contributions under in vivo conditions.

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P Lindqvist, A M Ostlund-Lindqvist, J L Witztum, D Steinberg and J A Little

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