Degradation of lipoproteins by human monocyte-derived macrophages

Evidence for two distinct processes for the degradation of abnormal very-low-density lipoprotein from subjects with type III hyperlipidaemia

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Human blood monocyte-derived macrophages that had been cultured in medium containing human serum for 7 days degraded the abnormal very-low-density lipoproteins (VLDL) from the plasma of subjects with type III hyperlipoproteinaemia by two distinct saturable processes. One process was stimulated when cells from normal subjects were preincubated with lipoprotein-free medium, was inhibited by excess unlabelled low-density lipoproteins (LDL) and was absent from cells from subjects with homozygous familial hypercholesterolaemia; on these criteria it was identified as an LDL-receptor-dependent process. Degradation by the second process was of equal magnitude in both cell types and was unaffected by excess unlabelled LDL or acetylated LDL. The activity of this process was reduced when the cells were preincubated in lipoprotein-free medium. The abnormal VLDL from the plasma of cholesterol-fed rabbits were also degraded by this process, which was similar to that in mouse peritoneal macrophages mediated by the receptor for VLDL of β -electrophoretic mobility [Goldstein, Ho, Brown, Innerarity & Mahley (1980) *J. Biol. Chem.* **255**, 1839–1848].

Since the uptake and degradation of normal plasma LDL by cultured human cells is regulated and does not lead to accumulation of lipid within cells, there is reason to believe that abnormal or modified lipoproteins must be taken up *in vivo* in order to form the lipid-laden cells that are typically found in atherosclerotic lesions (Fowler *et al.*, 1979). As a result, much attention has been focused recently on the ability of cells, particularly macrophages, to take up and degrade lipoproteins by processes that do not involve the LDL receptor. For example, mouse peritoneal and human monocyte-derived macrophages have been shown to have distinct high-affinity receptors for electronegatively modified LDL (Goldstein *et al.*, 1979; Fogelman *et al.*, 1980), complexes of LDL with dextran sulphate (Basu *et al.*, 1979; Soutar & Knight, 1982) and β -VLDL (Goldstein *et al.*, 1980), an abnormal lipoprotein found in the circulation of animals fed a cholesterol-rich diet

(Mahley, 1978). More recently it has been suggested that incubation of human LDL with cultured rabbit aortic endothelial cells modifies the lipoprotein in such a way that it can be recognized by the acetyl-LDL receptor in mouse peritoneal macrophages (Henriksen *et al.*, 1981).

Human monocyte-derived macrophages, however, are unusual among cultured cells so far studied in that they also express functional LDL receptors when grown in medium containing whole serum (Soutar & Knight, 1982; Traber & Kayden, 1980). The activity of the various lipoprotein receptors in these cells during the culture period reflects, on the one hand, the need for cholesterol for cell membrane synthesis while the cells are growing, and, on the other hand, the development of the phagocytic properties of the cells (Knight & Soutar, 1982a). Thus it is important to exclude a possible role of the LDL receptor in studies of the uptake and degradation of different lipoproteins by normal human monocyte-derived macrophages. This potential problem can be overcome (Soutar & Knight, 1982) by comparing the uptake of a particular lipoprotein by human monocyte-derived macrophages from normal subjects with that by

Abbreviations used: LDL, low-density lipoprotein(s); VLDL, very-low-density lipoprotein(s); β -VLDL, very-low-density lipoprotein of β -electrophoretic mobility; apoE, apolipoprotein E; apoB, apolipoprotein B; FH, familial hypercholesterolaemia.

the same cells isolated from subjects with homozygous familial hypercholesterolaemia (FH) in whom there are no functional LDL receptors (Goldstein & Brown, 1977).

In familial type III hyperlipoproteinaemia, a disease that is accompanied by premature atherosclerosis (Fredrickson *et al.*, 1978), an abnormal cholesterol-rich lipoprotein of β -mobility accumulates in the VLDL fraction in plasma. This lipoprotein is believed to comprise the remnants of metabolism of chylomicrons or VLDL that fail to be cleared from the circulation in the normal way (Chait *et al.*, 1977) due to two independent factors: first the presence in the lipoproteins of a genetic variant of apolipoprotein E (apoE2) (Utermann *et al.*, 1977) that is not recognized by the LDL (apoB,E) receptor (Schneider *et al.*, 1981), and second, overloading of alternative removal mechanisms due to an increased rate of production of triacylglycerol-rich lipoproteins determined by a second genetic factor (Utermann *et al.*, 1979) or by some other metabolic disturbance (Myant, 1981). The abnormal VLDL in the plasma of type III subjects (type III VLDL) has properties in common with the abnormal β -VLDL found in the plasma of animals fed a cholesterol-rich diet (Mahley, 1978), and its uptake by macrophage cells may well be responsible for the massive deposition of cholesterol in the peripheral tissues and arterial wall of type III patients that also occurs in cholesterol-fed animals. Since this abnormal lipoprotein has many of the characteristics of normal remnant lipoproteins (Mjøs *et al.*, 1975), it is important to understand the mechanisms involved in its uptake and degradation by cells. In the present study we have examined the degradation of type III VLDL and rabbit β -VLDL by monocyte-derived macrophages from normal subjects and from patients with homozygous FH, and by macrophages from the peritoneal cavity of mice.

Methods

Isolation and maintenance of cells

Culture medium was adjusted to pH 7.4 and contained 100 units of penicillin/ml and 100 μ g of streptomycin/ml. All incubations were performed at 37°C in an atmosphere of CO₂/air (1:19) in a humidified incubator.

Human mononuclear cells were isolated from freshly drawn blood of five normal donors and from the buffy coat obtained during plasma exchange (Thompson *et al.*, 1975) of two FH patients (homozygous, type IIa receptor-defective) by methods described previously (Knight & Soutar, 1982b). Cells were seeded at a density of approx. 3×10^6 cells/well in Linbro Multidishes (Flow Laboratories, Irvine, Ayrshire, Scotland, U.K.),

containing 1 ml of RPMI-1640 medium (Gibco Europe) with 10% (v/v) foetal-calf serum. [Each Multidish contains 12 wells of diameter 24 mm and depth 17 mm.] After incubation for 1 h at 37°C, non-adherent cells were removed by washing twice with RPMI-1640 medium. Adherent cells (approx. 20% of those seeded) were maintained in RPMI-1640 medium containing 20% (v/v) fresh serum from normal donors. Normal cells always received autologous serum. On day 3 after seeding, cells were washed and fresh medium containing the appropriate serum (20%, v/v) was added. Experiments were performed on day 7 or 8 after seeding. As described previously (Knight & Soutar, 1982a), no differences were observed in the development of cells from normal subjects compared with those from FH subjects prepared by the two methods, except in LDL-receptor activity; cells from a normal subject undergoing plasma exchange behaved in culture identically with normal cells obtained as described above. In order to reduce the likelihood of variability between cell lines due to the cholesterol content of the medium, cells from normal and FH subjects were maintained in medium containing normal serum. No significant differences have been observed in the development of cells from FH subjects in medium containing normal or autologous serum (A. K. Soutar & B. L. Knight, unpublished work).

Peritoneal macrophages were obtained from unstimulated CFLP mice, essentially by the method described by Cohn & Benson (1965). Dulbecco's modified Eagle's medium (DME) containing 5 units of heparin/ml was injected into the peritoneal cavity and as much as possible withdrawn with a wide-gauge needle. Macrophages were collected by centrifugation at 400g for 10 min and the pellet resuspended in DME medium containing 10% foetal-calf serum. Macrophages were dispensed at a concentration of 1×10^6 cells/well into 24mm-diameter Multidish wells containing a final volume of 1 ml of DME medium with 10% foetal-calf serum, and incubated at 37°C for 4 h. Non-adherent cells were removed by washing with DME medium. Adherent cells were incubated in 1 ml of DME medium containing 10% foetal-calf serum or lipoprotein-deficient human serum (5 mg of protein/ml) and the experiments were performed the next day. Each well then contained approx. 60 μ g of cell protein.

Lipoproteins

LDL [density (ρ) = 1.019–1.055 g/ml] was isolated from fresh plasma from normal healthy donors, labelled with Na¹²⁵I (carrier-free; Amersham International) and acetylated with acetic anhydride as described previously (Soutar & Knight, 1982). Lipoproteins of $\rho < 1.006$ g/ml (type III

VLDL) were isolated from the plasma of three subjects with familial type III hyperlipoproteinemia [apoE phenotype E2/E2 determined by the isoelectric-focusing method described by Warnick *et al.* (1979)] by centrifugation of plasma at background density for 16 h at 8°C at 53000 rev./min in a Beckman 60 Ti rotor. The lipoproteins were washed once by centrifugation at ρ 1.006 g/ml for 16 h at 8°C at 43000 rev./min in a Beckman SW50.1 rotor. Lipoproteins of ρ < 1.006 g/ml (rabbit β -VLDL) were isolated from the serum of cholesterol-fed rabbits by the same procedures. New Zealand White rabbits were fed a commercially available cholesterol-rich diet (standard rabbit diet with 2% cholesterol added; Special Diet Services Ltd., Witham, Essex, U.K.) for 4–6 weeks, which resulted in serum cholesterol concentrations of 1500–2000 mg/100 ml. Type III VLDL and rabbit β -VLDL were labelled with ^{125}I essentially as described for LDL (Soutar & Knight, 1982). The compositions of the type III VLDL and rabbit β -VLDL are shown in Table 1. The triacylglycerol content of lipoprotein solutions was determined with a commercially available kit (DOW Triglycerides Reagent Kit; Uniscience Ltd., Cambridge, U.K.). Free and total cholesterol were determined enzymically as described by Johnson (1979). Protein and tetramethylurea-soluble-protein concentrations were determined using the method of Kane *et al.* (1975).

All lipoproteins were stored sterile at 4°C and were dialysed for 2 h against 100 vol. of Dulbecco's phosphate-buffered saline immediately before each experiment.

Experimental procedure

Human cells to be preincubated in non-auto-logous serum were washed twice with RPMI-1640 medium before the addition of fresh medium (1 ml/well) containing lipoprotein-deficient serum (5 mg of protein/ml of medium) or the alternative whole serum (20%, v/v); preincubation was normally for 18–20 h. Immediately before the experi-

ments, human monocyte-derived macrophages and mouse peritoneal macrophages were washed twice with 1 ml of medium (RPMI-1640 for human cells; DME for mouse cells) and medium containing lipoprotein-deficient serum (2.5 mg of protein/ml), and lipoproteins, labelled and unlabelled as indicated, were added to give a final volume of 0.5 ml/well. The lipoprotein solutions did not account for more than 15% of the total volume. The cells were incubated for 4 h at 37°C, after which lipoprotein degradation was determined from the amount of trichloroacetic acid-soluble, non-iodide radioactivity in the medium, as previously described (Soutar & Knight, 1982).

All values (mean values for duplicate wells) were corrected for apparent degradation of labelled lipoproteins in equivalent dishes containing no cells, and expressed as ng of lipoprotein degraded/4 h per mg of cell protein. After a brief lag (approx. 30 min) the rate of degradation of lipoproteins was constant throughout the 4 h incubations. Although macrophages are known to secrete enzymes into the medium, non-cellular degradation of lipoproteins in medium that had been in contact with cells for 18 h was negligible, showing that essentially all the degradation by cells incubated for 4 h in fresh medium was intracellular.

The incorporation of [^{14}C]oleate into cellular cholesteryl esters was determined essentially as described by Goldstein *et al.* (1980). Mouse peritoneal macrophage monolayers were washed and incubated for 6 h in medium containing 10% foetal-calf serum to which was added 0.2 mM-[1- ^{14}C]oleate (Amersham International; sp. radioactivity 750 Ci/mol) complexed to albumin (sp. radioactivity = 12 d.p.m./pmol) and different lipoproteins. Monocyte-derived macrophages, from normal and FH subjects, that had been maintained in medium containing 20% normal serum for 7 days were washed and incubated for 6 h with [^{14}C]oleate and lipoproteins in medium containing either normal human serum (20%, v/v) or no serum. Cellular cholesteryl esters were extracted as described by Goldstein *et al.* (1980), and incorpora-

Table 1. Composition of type III VLDL and rabbit β -VLDL

Abbreviation used: TMU = 1,1,3,3-tetramethylurea.

	[Triacylglycerol] (mg/ml)	[Cholesterol] (mg/ml)	Esterified cholesterol (% of total)	[Protein]* (mg/ml)	TMU-soluble protein (% of total)
Type III VLDL†	4.96 ± 1.06	2.67 ± 0.96	53 ± 9	1.0	63 ± 7
Rabbit β -VLDL‡	1.64 ± 0.68	9.42 ± 2.6	72 ± 9	1.0	55 ± 6

* Values for lipid composition are normalized to lipoprotein solutions containing 1 mg of protein/ml.

† Values are means ± S.E.M. for four separate preparations of type III VLDL from three different patients.

‡ Values are means ± S.E.M. for four separate preparations of rabbit β -VLDL obtained from pooled serum samples from two or three rabbits on each occasion.

tion of [^{14}C]oleate expressed as nmol of cholesteryl [^{14}C]oleate/6h per mg of protein. The free (non-esterified) and esterified cholesterol content of cells was determined by g.l.c., with 5α -cholestane as internal standard (Goldstein *et al.*, 1980).

Results

Blood monocytes from normal and FH subjects were maintained for 7 days in medium containing 20% (v/v) autologous serum and then incubated in lipoprotein-free medium to which was added different concentrations of ^{125}I -labelled lipoproteins of $\rho < 1.006\text{ g/ml}$ isolated from the plasma of a subject with type III hyperlipoproteinaemia (type III VLDL); degradation of the labelled lipoprotein was determined from the amount of acid-soluble non-iodide radioactivity in the medium at the end of a 4h incubation period (Fig. 1a). Cells from both normal subjects (normal cells) and subjects with homozygous FH (FH cells) degraded type III VLDL by processes that were essentially saturated with a concentration of $20\mu\text{g}$ of protein/ml. However, at each concentration, normal cells degraded at least twice as much type III VLDL as did FH cells. In five separate experiments, with preparations of labelled lipoproteins from one of three type III patients and cells from two different FH subjects or five different normal subjects, maintained in medium containing normal serum, cells from normal subjects degraded $106.2 \pm 11.1\text{ ng}$

of lipoprotein protein/mg of cell protein in 4h, whereas cells from FH subjects degraded $56.0 \pm 7.1\text{ ng/mg}$ of cell protein. We have shown previously that normal human monocyte-derived macrophages express LDL receptors to some extent even when incubated in medium containing normal serum. Thus the difference between normal and FH cells suggested that at least part of the degradation of type III VLDL observed with normal cells was mediated by the LDL receptor and should be influenced by the content of LDL in the medium in which the cells had been maintained. Indeed, normal monocyte-derived macrophages preincubated for 24h in medium containing 20% (v/v) FH serum, when the LDL receptor is fully repressed (Soutar & Knight, 1982), degraded only half as much type III VLDL as did cells preincubated in lipoprotein-free medium (Fig. 1b). However, degradation of type III VLDL by normal cells in which the LDL receptor was fully repressed was not directly proportional to the concentration of ^{125}I -labelled lipoprotein in the medium, but showed evidence of saturability (Fig. 1b); degradation of type III VLDL by normal cells under these conditions was of similar magnitude to that by FH cells (Figs. 1a and 1b). Half-maximum degradation of type III VLDL by normal cells preincubated in FH serum and by FH cells occurred with the same concentration of lipoprotein in the medium ($13.3\mu\text{g}$ of protein/ml), suggesting that the process was the same in each cell type.

Fig. 1. Degradation of type III VLDL by monocyte-derived macrophages from normal and FH subjects (a) Cells from normal (○) and FH (●) subjects were maintained in culture for 7 days in medium containing normal human serum (20%, v/v). On the day of the experiment the cells were washed and incubated for 4h in medium containing lipoprotein-deficient serum to which was added ^{125}I -labelled type III VLDL (sp. radioactivity 100 c.p.m./mg of protein) to give the concentration indicated. (b) Cells from a normal subject were maintained in culture for 7 days in medium containing 20% (v/v) normal human serum, then washed and preincubated for 20h in either fresh medium containing lipoprotein-deficient serum (5 mg of protein/ml) (Δ) or fresh medium containing FH serum (20%, v/v) ($\blacktriangle$). After preincubation, the cells were washed and incubated with ^{125}I -labelled type III VLDL as described above. Each point is the mean of values obtained in two experiments, which did not differ by more than 10%.

Monocyte-derived macrophages, from normal and FH subjects, that had been maintained in medium containing 20% (v/v) normal human serum were also able to degrade lipoproteins of $\rho < 1.006 \text{ g/ml}$ isolated from the serum of cholesterol-fed rabbits (rabbit β -VLDL). Comparison of the degradation of this lipoprotein by normal and FH cells suggested that rabbit β -VLDL was also degraded by two saturable processes in normal cells, one of which was dependent on the LDL receptor. As expected, therefore, preincubation of normal cells in lipoprotein-deficient medium increased the amount of rabbit β -VLDL degraded at each concentration of lipoprotein in the medium (Fig. 2a). However, somewhat surprisingly, preincubation in medium containing lipoprotein-

deficient serum (lipoprotein-free medium) reduced the amount of rabbit β -VLDL degraded by FH cells (Fig. 2b). A similar reduction in the degradation of type III VLDL by FH cells was found when they were preincubated in lipoprotein-free medium. In three separate experiments with cells from FH subjects, the degradation of type III VLDL and β -VLDL by cells preincubated in medium containing LPDS was $66 \pm 10\%$ of that by cells maintained in medium containing normal serum.

These results show that preincubation of monocyte-derived macrophages from FH subjects in lipoprotein-free medium results in a reduction in the activity of the non-LDL-receptor-mediated saturable processes by which rabbit β -VLDL and type III VLDL are degraded. It is likely that similar reductions in these processes also occur in normal cells when they are preincubated in lipoprotein-free medium. As shown in Fig. 1, total degradation of type III VLDL was only approx. 2-fold greater in normal cells that had been preincubated in lipoprotein-free medium than in cells that had been preincubated with medium containing FH serum, whereas we have shown previously that the difference between the degradation of LDL by LDL-receptor-mediated processes by cells under these same conditions is at least 5-fold (Soutar & Knight, 1982). Half-maximal degradation of rabbit β -VLDL by FH cells, and therefore via the non-LDL-receptor-mediated process in normal human monocyte-derived macrophages, occurred with a concentration of approx. $6.5 \mu\text{g}$ of protein/ml.

Mouse macrophages, isolated from the peritoneal cavities of mice and maintained for 24h in medium containing 10% (v/v) foetal-calf serum, were also able to degrade type III VLDL and rabbit β -VLDL by processes that were saturated with low concentrations of lipoprotein in the medium (Figs. 3 and 4a); maximum degradation occurred with $15\text{--}20 \mu\text{g}$ of lipoprotein/ml. In contrast, degradation of LDL by mouse cells under these conditions showed no evidence of saturability with concentrations of LDL as high as $50 \mu\text{g}$ of lipoprotein protein/ml (Fig. 4b). Thus the LDL receptor was fully repressed in these cells, and degradation of rabbit β -VLDL and type III VLDL was by saturable non-LDL-receptor-mediated processes; in this respect, mouse cells incubated in medium containing 10% foetal-calf serum behaved similarly to monocyte-derived macrophages from FH subjects, which lack the LDL receptor, and cells from normal subjects in which the LDL receptor was fully repressed. Half-maximal degradation by mouse macrophages of rabbit β -VLDL occurred with approx. $5.5 \mu\text{g}$ of protein/ml and of type III VLDL with approx. $13 \mu\text{g}$ of protein/ml. When mouse peritoneal macrophages were preincubated for 24h in medium containing no lipopro-

Fig. 2. Degradation of rabbit β -VLDL by monocyte-derived macrophages from normal and FH subjects. Cells from normal (a) and FH subjects (b) were preincubated for 20h in fresh medium containing lipoprotein-deficient serum (○) or maintained in the same medium for 20h (●). After the preincubation the cells were washed and incubated for 4h at 37°C in medium containing lipoprotein-deficient serum to which was added ^{125}I -labelled rabbit β -VLDL (sp. radioactivity 164 c.p.m./ng of protein) to give the concentration indicated. Each point is the mean of values obtained in two experiments, which did not differ by more than 10%.

Fig. 3. Degradation of rabbit β -VLDL by mouse peritoneal macrophages

Freshly isolated mouse peritoneal macrophages were cultured for 4 h in medium containing 10% (v/v) foetal-calf serum and then preincubated for 20 h in the same medium (●) or in medium containing lipoprotein-deficient human serum (5 mg of protein/ml) (○). After the preincubation the cells were washed and incubated for 4 h at 37°C in fresh medium containing lipoprotein-deficient serum to which was added the indicated concentration of 125 I-labelled rabbit β -VLDL (sp. radioactivity 215 c.p.m./ng of protein). Each point is the mean of values obtained in two experiments, which did not differ by more than 10%.

teins, degradation of type III VLDL and rabbit β -VLDL were stimulated approx. 2-fold; a saturable process for the degradation of LDL was also apparent in these cells, with maximum degradation occurring with 40–50 μ g of LDL protein/ml. As with human cells, the difference between the amount of type III VLDL and rabbit β -VLDL degraded by mouse cells in which the LDL receptor was either induced or repressed was much smaller than the difference between the amounts of LDL degraded, suggesting that the non-LDL-receptor-mediated saturable processes for the degradation of type III VLDL and rabbit β -VLDL were less active in cells that had been preincubated in lipoprotein-free medium.

Comparison of the degradation of each lipoprotein by the different cell types in several different experiments showed that monocyte-derived macrophages from FH subjects degraded slightly less type III VLDL than rabbit β -VLDL (Table 2); these amounts were similar to those degraded by normal human cells in which the LDL receptor was not induced. In contrast, mouse peritoneal macrophages maintained in medium containing 10% (v/v) foetal-calf serum, conditions under which the LDL receptor was not induced, degraded approx.

Fig. 4. Degradation of type III VLDL and LDL by mouse peritoneal macrophages: effect of preincubation with lipoprotein-deficient serum

Mouse peritoneal macrophages were cultured for 4 h in medium containing 10% (v/v) foetal-calf serum and then either washed and preincubated for 20 h in medium containing lipoprotein-deficient human serum (5 mg of protein/ml) (○) or maintained in the same medium (●). After the preincubation the cells were washed and then incubated for 4 h at 37°C in medium containing lipoprotein-deficient serum to which was added either (a) 125 I-labelled type III VLDL (sp. radioactivity 75 c.p.m./ng of protein) or (b) 125 I-labelled LDL (sp. radioactivity 50 c.p.m./ng of protein). Each point is the mean of values obtained in two experiments, which did not differ by more than 10%.

five times as much rabbit β -VLDL as type III VLDL (Table 2). This difference suggested that the processes for the degradation of type III VLDL and rabbit β -VLDL in the human cells had differ-

Table 2. Degradation of rabbit β -VLDL and type III VLDL by non-LDL-receptor-mediated processes

Human monocyte-derived macrophages were cultured for 7 days in medium containing 20% (v/v) autologous serum. Mouse peritoneal macrophages were cultured for 24h in medium containing 10% (v/v) foetal-calf serum. Immediately before the experiment the cells were washed and incubated for 4h in medium containing lipoprotein-deficient serum to which the concentration of ^{125}I -labelled lipoprotein indicated in the Table was added. Values shown are means $\pm$ S.E.M., with the number of separate experiments with different cells and different lipoproteins given in parentheses after the corresponding values.

Cell type	[Protein] ($\mu\text{g/ml}$) Lipoprotein	Lipoprotein degradation (ng/4h per mg of cell protein)		
		50	5	
			Type III VLDL	Rabbit β -VLDL
Mouse macrophages		1393 $\pm$ 47 (3)	133 $\pm$ 21 (3)	766 (2)
FH monocyte-derived macrophages		100 $\pm$ 22 (4)	40 $\pm$ 2 (3)	84 $\pm$ 31 (3)

Fig. 5. Effect of unlabelled lipoproteins on the degradation of ^{125}I -labelled LDL by monocyte-derived macrophages from normal subjects

Cells were cultured for 7 days in medium containing 20% (v/v) autologous serum, then washed and incubated for 4h at 37°C in medium containing lipoprotein-deficient serum to which was added ^{125}I -labelled LDL (50 μg of protein/ml, sp. radioactivity 60c.p.m./ng of protein) and different concentrations of unlabelled LDL (○) or unlabelled type III VLDL (□). Each point is the mean of values obtained in two experiments which did not differ by more than 10%.

labelled lipoprotein together with different concentrations of unlabelled lipoprotein as indicated in the Figures. Unlabelled type III VLDL and LDL both inhibited the degradation of ^{125}I -labelled LDL by normal cells (Fig. 5); the maximum inhibitory effect of type III VLDL was identical with the maximum effect of LDL, confirming that type III VLDL was taken up by the LDL receptor in these cells. In contrast with this, LDL and type III VLDL were not equally effective in inhibiting the degradation of ^{125}I -labelled type III VLDL by normal cells (Fig. 6a). With a concentration of 100 μg of protein/ml, at which its effect was maximum, unlabelled LDL was approximately half as effective as type III VLDL in inhibiting the degradation of type III VLDL. Presumably LDL effectively competed with type III VLDL for the LDL-receptor-mediated process, but was unable to compete for the second saturable process. This was confirmed by the observation that unlabelled LDL had a negligible effect on the degradation of ^{125}I -labelled type III VLDL by FH cells (Fig. 6b).

Comparison of the effects of adding unlabelled type III VLDL on the degradation of ^{125}I -labelled type III VLDL and ^{125}I -labelled rabbit β -VLDL confirmed that the processes in the human cells for the degradation of these lipoproteins were not identical with those in the mouse peritoneal macrophages. Unlabelled type III VLDL inhibited the degradation of ^{125}I -labelled type III VLDL (Fig. 7a) and ^{125}I -labelled rabbit β -VLDL (Fig. 7b) by FH cells to the same extent, suggesting that in human cells the non-LDL-receptor-mediated processes for the degradation of type III VLDL and rabbit β -VLDL were the same, and that there was no obvious difference in its recognition of the two lipoproteins. However, in mouse peritoneal macrophages, although unlabelled type III VLDL had a marked inhibitory effect, similar to that in FH

ent properties from those in mouse peritoneal macrophages.

Studies on the ability of various unlabelled lipoproteins to inhibit the degradation of either labelled LDL or labelled type III VLDL confirmed that there were two distinct processes for the degradation of type III VLDL in normal human monocyte-derived macrophages. Cells were maintained in medium containing autologous serum (20%, v/v) for 7–8 days, and then incubated for 4h in medium containing lipoprotein-deficient serum to which was added a fixed concentration of

Fig. 6. Effect of addition of unlabelled lipoproteins on the degradation of ^{125}I -labelled type III VLDL by monocyte-derived macrophages from normal and FH subjects

Cells from normal (a) and FH (b) subjects were maintained in culture for 7 days, then washed and incubated for 4 h at 37°C in medium containing lipoprotein-deficient serum to which was added ^{125}I -labelled type III VLDL ($15\mu\text{g}$ of protein/ml, sp. radioactivity 100 c.p.m./ng of protein) together with different concentrations of unlabelled type III VLDL ($\square$, $\blacksquare$) or unlabelled LDL ($\circ$, $\bullet$). Degradation of type III VLDL in the absence of unlabelled lipoproteins (control values) was 197 ng/mg of protein by normal cells and 107 ng/mg of protein by FH cells. Each point is the mean of values obtained in two experiments, which did not differ by more than 10%.

Fig. 7. Effect of addition of unlabelled type III VLDL on the degradation of ^{125}I -labelled type III VLDL and ^{125}I -labelled rabbit β -VLDL by mouse peritoneal macrophages and by monocyte-derived macrophages from FH subjects

Mouse peritoneal macrophages ($\circ$) were cultured for 24 h in medium containing 10% (v/v) foetal-calf serum. FH cells ($\bullet$) were cultured for 7 days in medium containing 20% (v/v) normal human serum. On the day of the experiment, the cells were washed and then incubated for 4 h at 37°C in medium containing lipoprotein-deficient serum to which was added either (a) ^{125}I -labelled type III VLDL ($4.84\mu\text{g}$ of protein/ml, sp. radioactivity 47 c.p.m./ng of protein) or (b) ^{125}I -labelled rabbit β -VLDL ($4.88\mu\text{g}$ of protein/ml, sp. radioactivity 167 c.p.m./ng of protein) together with different concentrations of unlabelled type III VLDL. In the absence of unlabelled lipoproteins (100% values) mouse cells degraded 163 ng of type III VLDL and 893 ng of rabbit β -VLDL per mg of cell protein, and FH cells degraded 36.2 ng of type III VLDL and 38.1 ng of rabbit β -VLDL per mg of cell protein. Each point is the mean of values obtained in two experiments, which did not differ by more than 10%.

cells, on the degradation of ^{125}I -labelled type III VLDL (Fig. 7a), it had much less effect on the degradation of ^{125}I -labelled rabbit β -VLDL (Fig. 7b). On the other hand, unlabelled rabbit β -VLDL (a 10-fold excess over the concentration of labelled

lipoprotein) inhibited the degradation by mouse cells of both ^{125}I -labelled type III VLDL and ^{125}I -labelled rabbit β -VLDL by approx. 70–80% (results not shown). Thus, although it is probable that in mouse cells, as in human, degradation of rabbit

Table 3. *Incorporation of [14 C]oleate into cellular cholesteryl esters*

Human cells were cultured for 7 days in medium containing 20% (v/v) normal human serum. Mouse peritoneal macrophages were cultured for 24 h in medium containing 10% foetal-calf serum. Immediately before the experiment, cells were either washed and incubated for 6 h in medium containing no serum (– serum) or incubated for a further 6 h in the same medium (+ serum) to which was added the amount of lipoprotein indicated in the Table and [14 C]oleate complexed with albumin. Values shown are means $\pm$ S.E.M. for three separate experiments with different lipoprotein preparations for human cells, and are the means for two experiments with mouse peritoneal cells.

Incorporation of [14 C]oleate into cholesteryl esters (μ mol/6 h per mg of protein)					
Addition to medium	Human monocyte-derived macrophages				Mouse peritoneal macrophages
	Medium + serum		Medium – serum		Medium + serum
	Normal	FH	Normal	FH	
None	0.48 $\pm$ 0.13	0.88 $\pm$ 0.52	1.51 $\pm$ 0.7	2.55 $\pm$ 0.55	0.35
LDL (50 μ g/ml)	0.82 $\pm$ 0.10	1.04 $\pm$ 0.63	3.4 $\pm$ 1.8	5.44 $\pm$ 2.32	0.21
Rabbit β -VLDL (75 μ g/ml)	4.79 $\pm$ 0.98	3.42 $\pm$ 1.30	11.37 $\pm$ 2.15	11.98 $\pm$ 2.60	5.42
Type III VLDL (75 μ g/ml)	0.44 $\pm$ 0.06	0.89 $\pm$ 0.34	1.89 $\pm$ 0.43	2.92 $\pm$ 1.30	0.38
(155 μ g/ml)	0.42 $\pm$ 0.16	0.83 $\pm$ 0.32	1.63 $\pm$ 0.97	3.55 $\pm$ 1.81	n.d.

β -VLDL and type III VLDL occurs by the same process, it appears that mouse cells do not recognize the human type III VLDL as readily as rabbit β -VLDL. Since LDL receptors were not induced in these cells, unlabelled LDL had little effect on the degradation of either [125 I]-labelled lipoproteins by mouse or FH cells. The addition of unlabelled acetyl-LDL had little or no effect on the degradation of any of the labelled lipoproteins by mouse or human cells.

In an attempt to determine whether degradation of type III VLDL and rabbit β -VLDL via the LDL-receptor-mediated or non-LDL-receptor-mediated processes leads to accumulation of cholesteryl esters in human monocyte-derived macrophages, incorporation of [14 C]oleate into cellular cholesteryl esters was determined. As shown in Table 3, rabbit β -VLDL effectively stimulated the incorporation of oleate into cellular cholesteryl esters by both normal and FH cells to the same extent, suggesting that uptake and degradation via the non-LDL-receptor-mediated process was responsible, and confirming that degradation of the lipoprotein by this process was intracellular. However, at an equivalent or higher protein concentration, type III VLDL had little effect on [14]oleate incorporation. Incubation of normal and FH cells with LDL resulted in small, but similar, stimulation of oleate incorporation, suggesting that uptake of lipoproteins via the LDL receptor in these cells does not lead to significant cholesterol accumulation. Similar results were obtained with two preparations of mouse peritoneal macrophages.

One possibility for the apparent lack of accumulation of cholesteryl esters when human monocyte-derived macrophages are incubated with type III

VLDL is that lipoprotein lipase, known to be released from these cells in culture (Mahoney *et al.*, 1982; Chait *et al.*, 1982), may hydrolyse the triacylglycerol content of the lipoprotein, thereby diluting the specific radioactivity of the [14 C]oleate. To investigate this point, the absolute amount of cholesterol and cholesteryl ester present in FH monocyte-derived macrophages was determined after they had been incubated for 18 h in medium containing no serum to which nothing, LDL (50 μ g of protein/ml), rabbit β -VLDL (75 μ g of protein/ml) or type III VLDL (75 μ g of protein/ml) was added. Cells incubated in medium containing no lipoproteins contained $16.9 \pm 4.4 \mu$ g of cholesteryl ester/mg of cell protein. Incubation of the cells with either LDL or type III VLDL increased this amount significantly ($P < 0.05$) to values of 39.8 ± 2.8 (LDL) and 45.2 ± 2.5 (type III VLDL), whereas incubation with rabbit β -VLDL resulted in the accumulation of $75.5 \pm 0.35 \mu$ g of cholesteryl ester/mg of cell protein. These data suggest that some dilution of [14 C]oleate may occur when the cells are incubated with triacylglycerol-rich lipoproteins, but confirm that incubation of cells with rabbit β -VLDL results in greater accumulation of cholesteryl ester than does incubation with LDL or type III VLDL.

Discussion

In the present study we have shown that monocyte-derived macrophages from human blood that have been cultured for several days are able to take up and degrade type III VLDL by a saturable process that is distinct from the LDL receptor, since it is present in cells from FH subjects and is

not subject to competition by an excess of unlabelled LDL. Normal cells in which the LDL receptor is partially or fully induced degrade more type III VLDL than do either normal cells in which the LDL receptor is fully repressed or FH cells, suggesting that degradation also results from uptake by the LDL receptor. The abnormal VLDL found in the plasma of cholesterol-fed rabbits was also degraded by both these processes in normal cells and by the non-LDL-receptor-mediated saturable process in FH cells; competition studies with unlabelled lipoproteins suggested that the same non-LDL-receptor-mediated saturable process was involved. In terms of apolipoprotein concentration, the apparent affinity of this process for rabbit β -VLDL was similar to that for type III VLDL, and maximum degradation of the two lipoproteins occurred at similar rates. However, since we do not have accurately determined values for lipoprotein particle size or weight, it is not possible to compare numbers of particles taken up. The lipid-to-protein ratio of the two lipoproteins was similar, but rabbit β -VLDL contained a greater proportion of its lipid as cholesterol than did type III VLDL. Thus the degradation of equal amounts of lipoprotein protein would not result in equivalent uptake of cholesterol into cells, which may explain why degradation of rabbit β -VLDL leads to greater accumulation of cellular cholesteryl esters than type III VLDL in the short term. For example, from the data shown in Tables 1 and 2, it can be calculated that with the same concentration of lipoprotein in the medium, degradation of β -VLDL results in the cellular uptake of almost eight times as much cholesterol as does degradation of type III VLDL by the non-LDL-receptor-mediated process.

A receptor-mediated process for uptake of β -VLDL from cholesterol-fed animals that results in cholesterol accumulation within the cells has previously been demonstrated in mouse peritoneal macrophages (Goldstein *et al.*, 1980; Mahley *et al.*, 1980). The non-LDL-receptor-mediated process in human monocyte-derived macrophages has properties in common with this. However, they differ in that we found that the mouse cells degrade much more rabbit β -VLDL than type III VLDL, and have an apparently greater affinity for rabbit β -VLDL than for type III VLDL, whereas the process in the human cells did not appear to discriminate between the two lipoproteins. Whether this represents a real difference between the receptor-mediated process in the two cell types or whether it represents a minor species difference in specificity of the same receptor is not clear. Indeed, the specific requirements for uptake of a lipoprotein by this ' β -VLDL' receptor are not known. Studies in mouse peritoneal macrophages by others (Goldstein *et al.*, 1980; Mahley *et al.*, 1980) have shown

that apoE or apoB in lipoproteins other than β -VLDL are not recognized, whereas our own studies with human cells have demonstrated that apoB in LDL is not recognized. Since type III VLDL and rabbit β -VLDL are thought to contain at least some lipoproteins of intestinal origin (Mahley, 1978; Myant, 1981), they contain apoB48, the genetic variant of apoB synthesized by intestinal cells in animals (Wu & Windmueller, 1981) and found in human chylomicrons (Kane *et al.*, 1980).

Fainaru *et al.* (1982) have recently demonstrated that only a high- M_r fraction of the β -VLDL from cholesterol-fed dogs leads to accumulation of cholesteryl esters in mouse peritoneal macrophages and that this fraction is probably of intestinal origin. A similar population of particles was isolated by these authors from human type III VLDL which stimulated cholesteryl ester accumulation to a small extent. However, it is clear that neither the LDL-receptor-mediated process nor the non-LDL-receptor-mediated process in human monocyte-derived macrophages results in the accumulation of cellular cholesteryl esters to the same extent as does degradation of acetyl-LDL (Knight *et al.*, 1983), at least in cultured cells during a short incubation period. Further studies are needed to define the specificity of recognition of lipoproteins by the non-LDL-receptor-mediated process and to determine whether uptake of normal lipoproteins by this pathway can lead to cholesteryl ester accumulation under different circumstances. There is no obvious explanation for the reduction in activity of the non-LDL-receptor-mediated process in human and mouse cells that have been preincubated in medium containing lipoprotein-deficient serum. Since this work was completed, Van Lenten *et al.* (1983) have shown that degradation of β -VLDL by normal human monocyte-derived macrophages is increased when cells are incubated in lipoprotein-deficient serum, but, since this would increase LDL-receptor activity, it is not clear that their methodology distinguishes adequately between the two receptors in normal cells. Fogelman *et al.* (1981) have suggested that changes taking place in cellular processes when cells are exposed to lipoprotein-deficient serum may represent non-physiological control mechanisms or changes due to unrelated changes in cholesterol homeostasis.

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