Degradation by cultured monocyte-derived macrophages from normal and familial hypercholesterolaemic subjects of modified and unmodified low-density lipoproteins

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Human blood monocyte-derived macrophages that had been cultured for 7 days in the presence of 20% whole human serum exhibited saturable degradation of low-density lipoprotein (LDL). This degradation could be abolished by pre-incubating the cells with a high concentration of LDL in the medium and increased by pre-incubating the cells in medium containing lipoprotein-deficient serum. Cells obtained from the blood of homozygous familial-hypercholesterolaemic (FH) patients only exhibited a low rate of non-saturable degradation of LDL, even when pre-incubated without lipoproteins. Thus the saturable degradation of LDL by normal cells was mediated by the LDL receptors that are defective in FH patients and little LDL was taken up and degraded through any of the other endocytotic processes present in macrophages. Degradation by normal cells pre-incubated with lipoprotein-deficient serum had a higher apparent affinity for LDL than that of cells maintained in whole serum, which suggests that incubation with lipoprotein-deficient serum may not only induce the formation of LDL receptors but may also have a direct effect on the receptors themselves. Monocyte-derived macrophages from normal and FH subjects showed similar saturable degradation of acetylated LDL and also of LDL complexed with dextran sulphate. Maximal degradation of each was in the same range as the degradation of unmodified LDL by normal cells, and was not increased if the cells were pre-incubated with lipoprotein-deficient serum.

The lipid-laden foam cells that are typically found in atherosclerotic lesions in the arterial wall are believed to be derived, at least in part, from tissue macrophages that have become engorged with cholesteryl ester-rich lipoproteins (Fowler *et al.*, 1979). Tissue macrophages themselves are derived from circulating blood monocytes. Human blood monocytes, when cultured *in vitro* in the presence of autologous serum, develop many of the properties of tissue macrophages (Gordon & Cohn, 1973) and thus provide a ready source of human phagocytic cells.

Macrophages isolated from the peritoneal cavity of mice do not take up normal LDL, but have been shown to have distinct high-affinity receptors that recognize LDL that has been modified by acetyla-

tion (Goldstein *et al.*, 1979), complexes of LDL with dextran sulphate (Basu *et al.*, 1979) and β -VLDL (Goldstein *et al.*, 1980), an abnormal lipoprotein obtained from the blood of animals fed a high-cholesterol diet (Mahley, 1978). By means of these receptors, mouse peritoneal macrophages will accumulate large quantities of cholesteryl esters when incubated with the abnormal or modified lipoproteins and as a result take on the appearance of foam cells (Brown *et al.*, 1980). Human macrophages derived *in vitro* from circulating blood monocytes (monocyte-derived macrophages) have been less extensively studied, but have been shown to have high-affinity receptors that recognize acetylated LDL (Goldstein *et al.*, 1979), as well as LDL modified by treatment with malonaldehyde (Fogelman *et al.*, 1980). However, unlike mouse peritoneal macrophages, human monocyte-derived macrophages have been shown to take up and degrade normal human LDL by a high-affinity process (Traber & Kayden, 1980; Shechter *et al.*, 1981;

Abbreviations used: apoB, apolipoprotein B; FH, familial hypercholesterolaemia; LDL, low-density lipoprotein; β -VLDL, very-low-density lipoprotein with β -mobility.

Knight & Soutar, 1982). Although this process has many properties in common with the LDL receptor of human skin fibroblasts (Goldstein & Brown, 1977), it is active in cells that have been cultured in the presence of levels of lipoprotein cholesterol that would be expected to repress fully the LDL receptor in skin fibroblasts (Traber & Kayden, 1980; Shechter *et al.*, 1981). Thus the possibility remains that the uptake of LDL by such cells is dependent on some recognition site distinct from the LDL receptor.

One way to establish without doubt whether any process for the degradation of a lipoprotein is dependent on uptake via the LDL receptor is to compare the process in cells from normal subjects with that in cells in which the LDL receptor is totally inactive due to a genetic defect (Goldstein & Brown, 1977). Since macrophages from FH subjects would be expected to degrade lipoproteins by processes neither mediated by, nor influenced in any way by, the LDL receptor, such a comparison provides information not only on the role of the LDL receptor in degradation of LDL by normal cells, but also on the extent to which LDL-receptor activity in normal cells influences the degradation of modified LDL or abnormal lipoproteins whose uptake is generally believed to be LDL-receptor-independent. In this study we have determined the ability of monocyte-derived macrophages obtained from normolipidaemic subjects and FH subjects to degrade normal human LDL, acetylated LDL and complexes of LDL and dextran sulphate.

Experimental

Isolation and maintenance of cells

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

Mononuclear cells were isolated from freshly drawn blood of normal donors and from the buffy coat obtained during plasma exchange (Thompson *et al.*, 1975) of homozygous FH patients (type IIa; receptor-defective). Whole blood from normal donors, to which was added disodium EDTA to give a final concentration of 1 mM, was centrifuged at 1000 g for 20 min and the plasma was replaced with an equal volume of buffer [Dulbecco's phosphate-buffered saline without Ca²⁺ and Mg²⁺ (Gibco Europe), to which was added 1 mM-disodium EDTA] in which the cells were resuspended. The cell suspension was layered on to Ficoll-Hypaque and centrifuged at 400 g for 45 min at 18°C (Boyum, 1968; Johnson *et al.*, 1977). The band containing the mononuclear cells was removed, diluted with 3 vol. of phosphate-buffered saline with 1 mM-disodium

EDTA and centrifuged at 800 g for 10 min. The cells were washed twice with Dulbecco's phosphate-buffered saline without Ca²⁺ and Mg²⁺ (Gibco Europe), containing 1 mM-disodium EDTA, and once with RPMI-1640 medium (Gibco Europe), by resuspending and centrifuging at 100 g for 10 min. The final pellet was resuspended in RPMI-1640 medium containing 10% (v/v) fetal-calf serum and dispensed so that each Multi-dish well (diameter 24 mm) contained approx. 3×10^6 mononuclear cells in a final volume of 1 ml of RPMI-1640 medium with 10% (v/v) fetal-calf serum. They were incubated for 1 h at 37°C and non-adherent cells (mainly lymphocytes) were removed by washing twice with RPMI-1640 medium. The adherent cells (approx. 20% of the cells added) were then incubated at 37°C in 1 ml of RPMI-1640 medium containing 20% (v/v) autologous serum. On day 3 after seeding, any residual lymphocytes had become detached. The cells were washed once with RPMI-1640 medium and fresh medium containing 20% autologous serum was added. The buffy coat from FH patients was decanted into a fresh container and diluted to 100 ml with buffer [Dulbecco's phosphate-buffered saline without Ca²⁺ and Mg²⁺, to which was added 1 mM-disodium EDTA]. The cell suspension was layered on to Ficoll-Hypaque and the mononuclear cells were isolated and cultured exactly as described above for cells from normal donors.

Experiments were performed 7–9 days after seeding. At this time approx. 50% of the original adherent cells remained attached, each of which had increased in size and assumed features characteristic of cultured macrophages (Johnson *et al.*, 1977). There was no observable differences between cultures from normal and FH subjects. Each exhibited a 5-fold increase in the protein/DNA ratio during the period of culture, a 3-fold increase in the specific activities of the lysosomal enzymes *N*-acetyl-β-glucosaminidase and acid phosphatase and a capacity for phagocytosis, as indicated by the release of lysosomal enzymes during incubation with opsonized zymosan (B. L. Knight & A. K. Soutar, unpublished work).

Peritoneal macrophages were obtained from CBA/Ht mice as described by Cohn & Benson (1965). They were dispensed into 24 mm Multi-dish wells (approx. 10⁶ cells per well) and incubated for 24 h at 37°C in 1 ml of Dulbecco's modified Eagle's medium containing 10% (v/v) fetal-calf serum.

Isolation and labelling of lipoproteins

Plasma from normal donors was obtained from freshly drawn blood containing 1 mM-sodium EDTA. LDL (ρ 1.019–1.055 g/ml) was isolated from plasma by differential density centrifugation by the methods described by Havel *et al.* (1955). LDL was subjected to a second centrifugation at ρ

1.063 g/ml in the Beckman SW 50.1 rotor for 16 h at 43 000 rev./min, which resulted in a final background density for the isolated LDL of 1.055 g/ml. The LDL was dialysed for 3 h against at least four changes of 50–100 vol. of 0.15 M-NaCl/1 mM-disodium EDTA/0.01% NaN_3 , adjusted to pH 7.4, to remove the bulk of the KBr.

The dialysed LDL was labelled with Na^{125}I immediately after isolation by using the method of McFarlane (1958) essentially as described by Bilheimer *et al.* (1975). The newly-labelled lipoprotein was separated from the reaction products by chromatography on Sephadex G-25 equilibrated with 0.15 M-NaCl/1 mM-disodium EDTA, adjusted to pH 7.4, containing 0.01% (w/v) NaN_3 and then dialysed overnight against the same solution without azide to remove residual non-bound iodide. Labelled LDL was then mixed with unlabelled LDL to give a specific radioactivity of approx. 60 c.p.m./ng of protein, sterilized by passage through a Millipore filter (0.45 μm pore diam.) and stored at 4°C for up to 2 weeks, during which time no changes in the properties of the lipoproteins could be detected.

^{125}I -Labelled LDL and LDL were acetylated by treatment with acetic anhydride as described by Basu *et al.* (1976). Excess reagents were removed by chromatography on Sephadex G-25 equilibrated with 0.15 M-NaCl/1 mM-disodium EDTA, adjusted to pH 7.4, followed by dialysis overnight against the same solution. Acetylated LDL was sterilized by passage through a Millipore filter (0.45 μm) and stored at 4°C for up to 2 weeks, during which time no changes in the lipoproteins could be detected. The ^{125}I -labelled acetylated LDL showed no ability to bind to the high-affinity LDL receptor on normal human skin fibroblasts, and the unlabelled acetylated LDL showed no ability to compete with ^{125}I -labelled LDL for the high-affinity binding sites. Before each experiment the ^{125}I -labelled LDL and ^{125}I -labelled acetylated LDL were dialysed for 2 h against Dulbecco's phosphate-buffered saline. The specific radioactivity of the labelled lipoproteins was then determined, using the method of Lowry *et al.* (1951) to assay the protein concentration, with bovine serum albumin as standard.

Lipoprotein-deficient serum

Lipoprotein-deficient serum was prepared by ultracentrifugation from the infranatant solution obtained after isolation of LDL from normal donors as described previously (Knight & Soutar, 1982).

Experimental procedure

For experiments in which the effect of pre-incubation of cells in non-autologous serum or

lipoprotein-deficient serum was to be tested, cells were washed twice on day 7 with RPMI-1640 medium before the addition of fresh medium (1 ml per well) containing lipoprotein-deficient serum (final concentration of 5 mg of protein/ml) or 20% (v/v) of the non-autologous whole serum; experiments were normally carried out 18 h later. For all other experiments not involving pre-incubation, the cells were maintained from day 3 until the day of the experiment in the same medium [RPMI-1640, containing 20% (v/v) autologous serum].

On the day of the experiment, the cells were washed twice with RPMI-1640 medium and medium containing lipoprotein-deficient serum (final concentration 2.5 mg of protein/ml) was added. Radioactive and non-radioactive lipoproteins or medium were added as required to give a final volume of 0.5 ml of medium per well. The lipoprotein solutions did not account for more than 15% of the final volume. Cells were incubated for 4 h at 37°C. At the end of the incubation the medium was removed and the macrophages washed twice with 0.5 M-NaCl/50 mM-Tris/HCl (pH 7.4) before being dissolved in 0.1 M-NaOH for the assay of protein (Lowry *et al.*, 1951). Radioactive acid-soluble, non-iodide products of lipoprotein catabolism were assayed in samples of medium after precipitation with 10% (w/v) trichloroacetic acid, oxidation with H_2O_2 and two extractions with chloroform (Goldstein & Brown, 1974). Values were corrected for breakdown that occurred in medium alone to give the amount of lipoprotein degraded by the cells. After a brief lag (approx. 30 min) the rate of degradation of lipoproteins was constant throughout the 4 h incubations.

Materials

All constituents of tissue culture medium were obtained from Gibco Europe, Paisley, Scotland, U.K., and Linbro Multi-Dishes (12 mm $\times$ 24 mm) from Flow Laboratories, Irvine, Scotland, U.K. Ficoll-Hypaque and dextran sulphate (sodium salt; mol.wt. approx. 500 000) were from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Na^{125}I (carrier-free) was obtained from Amersham International, Amersham, Bucks., U.K.

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; degradation of lipoprotein during the succeeding 4 h was determined from the appearance of acid-soluble non-iodide radioactivity in the medium. Degradation of LDL (Fig. 1) by normal cells increased

Fig. 1. Degradation of LDL by monocyte-derived macrophages from normal and FH subjects

Cells from normal (●) and FH (■) subjects were cultured for 7 days in medium containing 20% autologous serum. Immediately before the experiments, cells were washed and then incubated for 4 h at 37°C in medium containing human lipoprotein-deficient serum to which was added different concentrations of ^{125}I -labelled normal human LDL (specific activity approx. 60 c.p.m./ng of protein).

rapidly as the concentration of lipoprotein in the medium was increased in the range 0–50 μg of protein/ml, and then progressively less steeply until the rate of increase was constant (insert to Fig. 1). The shape of the curve suggested that the cells degraded LDL by both a saturable and a non-saturable process. The saturable process accounted for the bulk of the degradation at low concentrations of LDL, but was essentially saturated with 100 μg of LDL protein per ml. Monocyte-derived macrophages from an FH subject did not exhibit saturable degradation, in that the amount of LDL degraded was directly proportional to the concentration of lipoprotein in the medium throughout the concentration range; degradation of LDL by the cells from FH subjects was similar to degradation by normal cells via the non-saturable process.

There was a complicated relationship between the degradation of complexes of LDL with dextran sulphate and their concentration in the medium, but the curves appeared to be identical for both types of cells (Fig. 2a). Maximum degradation occurred at 15 μg of LDL protein/ml with a dextran sulphate/

LDL protein ratio of 1:1 (w/w). Both cell types degraded acetylated LDL rapidly at low concentrations by apparently similar processes (Fig. 2b). As the concentration of acetylated LDL in the medium was increased, degradation increased in a hyperbolic manner, to reach a maximum when the concentration of lipoprotein in the medium was approx. 20 μg of protein/ml. Any non-saturable degradation of the abnormal lipoproteins was negligible at the concentrations required for maximal degradation via the saturable processes.

Degradation of each type of lipoprotein by monocyte-derived macrophages isolated from the blood of each of five normal subjects and the two available FH homozygotes was determined in several separate experiments and the results are summarized in Table 1. Degradation of LDL by normal cells at a concentration of 50 μg of LDL protein/ml, which represents over 80% of the maximal saturable degradation with only a small contribution from the non-saturable process, was almost five times higher than that obtained with cells from the FH patients. Although cells from FH patients appeared to degrade more acetylated LDL and LDL–dextran sulphate complexes than normal cells, there was no statistically significant difference between the two groups. The maximum rate of degradation of acetylated LDL and LDL–dextran sulphate complexes by normal cells was not greatly different from the maximum rate of degradation of unmodified LDL via the saturable process. These data are in contrast with those obtained with mouse peritoneal macrophages, as shown in Table 1. The mean rates of degradation by three different preparations of mouse peritoneal macrophages of LDL, acetylated LDL and LDL–dextran sulphate complexes at the same concentrations as those used for the human monocyte-derived macrophages is also shown in Table 1. The concentrations of acetylated LDL and LDL–dextran sulphate complexes were such that the rates of degradation by the mouse cells were maximum; the process for the degradation of normal human LDL by mouse peritoneal macrophages is essentially non-saturable (A. K. Soutar & B. L. Knight, unpublished work). The data in Table 1 show that mouse peritoneal macrophages degraded twice as much LDL as normal human monocyte-derived macrophages, but up to 10 times as much acetylated LDL or LDL–dextran sulphate complex as either normal or mutant cells.

The difference in degradation between normal cells and those from FH homozygotes indicated that the saturable degradation of LDL was mediated by the LDL receptor, even though the cells had been incubated in medium containing 20% (v/v) whole serum. In human fibroblasts, this concentration of serum represses the expression of LDL receptors (Goldstein & Brown, 1977). To check that the

Fig. 2. Degradation of acetylated LDL and LDL-dextran sulphate complexes by monocyte-derived macrophages from normal and FH subjects

Cells from normal (●) and FH (■) subjects were cultured for 7 days in medium containing 20% autologous serum. Immediately before the experiments, cells were washed and then incubated for 4 h at 37°C in medium containing human lipoprotein-deficient serum to which was added different concentrations of ^{125}I -labelled lipoprotein: (a) a mixture of LDL (sp. radioactivity approx. 60 c.p.m./ng of protein) and dextran sulphate in a constant ratio of 1.0 μg of dextran sulphate/ μg of LDL protein; (b) acetylated LDL (sp. radioactivity approx. 60 c.p.m./ng of protein).

Table 1. Degradation of ^{125}I -labelled lipoproteins by monocyte-derived macrophages from normal and FH subjects and by mouse peritoneal macrophages

Human monocyte-derived macrophages were cultured for 7 days in medium containing 20% (v/v) autologous serum. Mouse peritoneal macrophages were cultured for 24 h in medium containing 10% (v/v) fetal-calf serum. Immediately before the experiment, all cells were washed and then incubated for 4 h in medium containing lipoprotein-deficient serum to which the concentration of ^{125}I -labelled lipoprotein indicated in the Table was added. The specific radioactivity of ^{125}I -labelled LDL and acetylated LDL was approx. 60 c.p.m./ng of protein. Values are means $\pm$ S.E.M., with the number of different experiments given in parentheses after the corresponding values. The data were obtained from several different experiments with cells from two FH and five normal subjects. Abbreviation used: N.S., not significant.

	Degradation (ng/mg of cell protein)		
	Normal cells	FH cells	Mouse cells
LDL (50 μg of protein/ml)	501 $\pm$ 53 (7)	112 $\pm$ 22 (7)*	998 $\pm$ 223 (5)
Acetylated LDL (15 μg of protein/ml)	828 $\pm$ 114 (7)	1274 $\pm$ 274 (7) N.S.	4783 $\pm$ 623 (3)
LDL-dextran sulphate (20 μg of protein/ml)	464 $\pm$ 162 (4)	712 $\pm$ 222 (5) N.S.	5897 $\pm$ 647 (5)

* Significant difference between normal and FH cells ($P < 0.05$, t test).

receptor in monocyte-derived macrophages was in fact under metabolic control, we examined the effect of pre-incubating normal cells in medium containing 10% (v/v) lipoprotein-deficient serum or in medium containing serum (20%, v/v) from FH patients ('hypercholesterolaemic medium'). Replacing normal serum with serum from FH patients increased the

concentration of LDL protein in the medium from approx. 130 $\mu\text{g}/\text{ml}$ to approx. 330 $\mu\text{g}/\text{ml}$. Cells that had been pre-incubated in hypercholesterolaemic medium, unlike cells maintained in normal medium, did not show any evidence of saturable degradation of LDL (Fig. 3b). This loss of saturable degradation could be attributed to the higher concentration of

LDL in the pre-incubation medium, since the same effect was observed when normal cells were pre-incubated in medium containing normal serum to which normal LDL had been added. Conversely, pre-incubation of normal cells in medium with lipoprotein-deficient serum increased the subsequent degradation of LDL at all concentrations tested (Fig. 3*b*). Thus the saturable receptor-mediated degradation of LDL by monocyte-derived macrophages was repressed by high concentrations of LDL and induced in the absence of LDL. At least 6 h pre-incubation in medium with 10% lipoprotein-deficient serum was required before any induction of receptors could be observed, and no further induction occurred after 16 h pre-incubation. The lack of saturable degradation in cells from FH patients ('FH cells') did not result from the use of autologous serum during culture, since pre-incubation in medium containing normal or lipoprotein-deficient serum did not alter the amount of LDL degraded by the cells (Fig. 3*a*).

The increase in LDL degradation brought about by pre-incubation of normal cells with lipoprotein-deficient serum was most marked if measured with low concentrations of LDL (Fig. 3*b*). This suggested that the apparent affinity of the saturable process for LDL could be affected by the conditions of incubation, which was confirmed by calculating the amount of LDL degraded through this process from results such as those shown in Fig. 3(*b*). The values for the rate of degradation of LDL by normal cells pre-incubated in medium containing FH serum represented non-LDL-receptor-mediated degradation at each concentration of LDL. Double-reciprocal plots of saturable degradation versus the concentration of LDL in the medium were both linear and showed that the apparent affinity of the LDL-receptor-mediated process for LDL was greater in cells pre-incubated in medium containing lipoprotein-deficient serum (half-maximum degradation with approx. $3.5 \mu\text{g}$ of protein/ml) than in cells maintained in medium containing normal serum (half-maximum degradation with approx. $30 \mu\text{g}$ of protein/ml). Virtually identical results were obtained in three separate experiments using cells from different donors.

Pre-incubation of FH cells or normal cells in medium containing lipoprotein-deficient serum did not increase the subsequent rates of degradation of either acetylated LDL or complexes of LDL with dextran sulphate.

To determine whether or not the specificity of the acetylated LDL receptor in normal cells was identical with that in FH cells, the ability of unlabelled lipoproteins to compete for the degradative process for labelled acetylated LDL was determined. The effect of increasing concentrations of unlabelled LDL or acetylated LDL on the

Fig. 3. Effect of pre-incubation of monocyte-derived macrophages from normal and FH subjects in medium containing human lipoprotein-deficient serum, normal serum and serum from FH subjects on degradation of human ^{125}I -labelled LDL

Cells from FH (*a*) and normal (*b*) subjects were cultured for 6 days in medium containing 20% autologous serum, and then incubated for 18 h in medium containing 20% normal serum (○), 20% FH serum (△) or human lipoprotein-deficient serum (2.5 mg of protein/ml; ●). Immediately before the experiment, the cells were washed and incubated for 4 h in medium containing lipoprotein-deficient serum to which ^{125}I -labelled LDL was added at different concentrations. In (*b*), ···· represents the degradation of LDL via the LDL receptor in cells pre-incubated in medium containing lipoprotein-deficient serum, whereas ---- represents the degradation of LDL via the LDL receptor in cells pre-incubated in medium containing normal serum. These values were calculated by subtracting the values for the rate of degradation by normal cells pre-incubated in medium containing serum from FH subjects from the values for rates of degradation by cells pre-incubated in medium containing lipoprotein-deficient serum or normal serum. The specific radioactivity of ^{125}I -labelled LDL was approx. 60 c.p.m./ng of protein.

degradation of ^{125}I -labelled acetylated LDL is shown in Fig. 4. Identical results were obtained for normal and FH cells, which confirmed the lack of influence of the LDL receptor on the degradation of acetyl-

Fig. 4. Effect of addition of unlabelled lipoproteins on the degradation of ^{125}I -labelled acetylated LDL by monocyte-derived macrophages from normal and FH subjects

Cells from normal (O, Δ) and FH (●, ▲) subjects were cultured for 7 days in medium containing 20% (v/v) autologous serum. Immediately before the experiment, the cells were washed and then incubated for 4 h in medium containing lipoprotein-deficient serum (2.5 mg/ml) to which was added ^{125}I -labelled acetylated LDL (15 μg of protein/ml) together with different concentrations of unlabelled acetylated LDL (Δ, ▲) or unlabelled LDL (O, ●). The specific radioactivity of the ^{125}I -labelled acetylated LDL was approx. 60 c.p.m./ng of protein. Degradation of ^{125}I -labelled acetylated LDL in the absence of unlabelled lipoproteins (control values) was 853 ng/mg of cell protein by normal cells and 1528 ng/mg of cell protein by cells from FH subjects.

ated LDL. Surprisingly, unlabelled LDL competed to some extent for the degradation of ^{125}I -labelled acetylated LDL but much less effectively than acetylated LDL. Since this inhibition occurred in both normal and FH cells equally, it could not have involved the LDL receptor.

Discussion

In this study we have shown that human monocyte-derived macrophages isolated from normolipoproteinaemic individuals and maintained for several days in medium containing 20% normal autologous serum degraded normal human LDL by a high-affinity process that had many of the characteristics of the LDL-receptor-mediated degradation found in human skin fibroblasts (Brown & Goldstein, 1977). The most important evidence in favour of the involvement of the LDL receptor in this process is that it was not present in cells isolated from homozy-

gous FH subjects. In addition, it could be further induced in normal cells by pre-incubation of the cells in medium containing no lipoproteins and was fully repressed in cells pre-incubated with high concentrations of LDL. Traber & Kayden (1980) and Shechter *et al.* (1981) have suggested previously that the LDL receptor is active in human monocyte-derived macrophages, but have not demonstrated its absence from homozygous FH cells.

Human monocyte-derived macrophages differ from skin fibroblasts, in which the LDL receptor was first described (Brown & Goldstein, 1977), in that the LDL receptor is active in cells that have been exposed to relatively high concentrations of lipoproteins. This has enabled us to observe a property of the LDL receptor that has not been noted previously, namely that its apparent affinity for LDL is dependent on the cellular environment. Monocyte-derived macrophages maintained in normal serum required a concentration of LDL in the medium of approx. 30 μg of protein/ml to achieve half-maximum degradation, whereas for cells pre-incubated in lipoprotein-free medium the concentration of LDL required was 3.5 μg of protein/ml. The data of others confirm this observation indirectly, since Traber & Kayden (1980) found that with human monocyte-derived macrophages pre-incubated in medium without serum, half-maximum degradation occurred with a concentration of LDL in the medium of 14 μg of protein/ml, whereas Fogelman *et al.* (1980) found that an LDL concentration of 40 μg of protein/ml was required to attain the same by monocyte-derived macrophages grown in 30% autologous serum. Thus pre-incubation of human monocyte-derived macrophages in the presence of lipoprotein-deficient serum not only induces the formation of LDL receptors, but may have a direct effect on the receptor itself, possibly by modifying the properties of the cell membrane.

We have also shown in this study that monocyte-derived macrophages from both normolipoproteinaemic and FH subjects are able to degrade both acetylated LDL and complexes of LDL with dextran sulphate. The maximum rate of degradation of these two lipoproteins by FH cells was at least as great as that by normal cells, and the apparent affinities of the degradative processes for each lipoprotein were identical in the two types of cells. Thus these data provide direct evidence that the acetylated LDL receptor that has been observed by others in human monocyte-derived macrophages (Goldstein *et al.*, 1980; Shechter *et al.*, 1981) is distinct from the LDL receptor. Furthermore, it is not influenced by uptake and degradation of lipoproteins by LDL-receptor-mediated processes in normal cells.

It has been postulated by Goldstein *et al.* (1979) and Fogelman *et al.* (1980) that the degradation of

lipoproteins via the acetylated LDL receptor in phagocytic cells may represent a pathway whereby LDL is degraded *in vivo* by non-LDL-receptor-mediated processes. Such a pathway must be of particular importance in homozygous FH subjects in whom, on average, five times as much LDL is degraded per day as in normal subjects (Bilheimer *et al.*, 1979). However, in this study we have shown that FH monocyte-derived macrophages are unable to degrade acetylated LDL at a rate significantly greater than that in normal cells, even when they have been maintained in medium containing FH serum. Nor does there appear to be any other mechanism in monocyte-derived macrophages from FH patients to enable them to degrade large quantities of LDL, modified or otherwise. Thus it is not possible to explain the enhanced non-LDL-receptor-mediated degradation of LDL in FH subjects by a change in the properties of phagocytic cells.

One point that emerges from this study, as well as those of other authors (Goldstein *et al.*, 1980; Fogelman *et al.*, 1980), is that resident mouse peritoneal macrophages and human blood monocyte-derived macrophages do not have identical properties. First, mouse peritoneal macrophages do not appear to have functional LDL receptors that recognize human LDL (Goldstein *et al.*, 1979), although the possibility has not been excluded that this failure to take up human LDL results from a species specificity for LDL similar to that found with rat fibroblasts (Drevon *et al.*, 1981; Innerarity *et al.*, 1980). Secondly, mouse peritoneal macrophages have a much greater ability to degrade modified or abnormal lipoproteins than have human monocyte-derived macrophages from either normal or FH subjects when the data are expressed in terms of cell protein.

Both macrophages isolated from the peritoneal cavity of mice and monocyte-derived macrophages developed in culture from human blood monocytes (Gordon & Cohn, 1973) have been claimed to represent tissue macrophages; yet clearly neither of these cell types are human tissue macrophages. Thus it is difficult to determine which of these cell types provides a better model for the human tissues that are responsible for non-LDL-receptor-mediated degradation *in vivo*, if indeed tissue macrophages are the main site of this process.

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