Low density lipoprotein-induced growth of U937 cells: a novel method to determine the receptor binding of low density lipoprotein

Johan Frostegård,* Anders Hamsten,* Magnus Gidlund,† and Jan Nilsson*

Department of Medicine and King Gustaf V Research Institute,* and Department of Immunology,† Karolinska Institute, Stockholm, Sweden

Abstract U937 is a monocytic cell-line originally derived from a histiocytic lymphoma. In serum-free medium the growth of U937 cells was stimulated by addition of low density lipoprotein (LDL). Methylation of LDL impaired its ability to be taken up in U937 cells as well as the capacity to stimulate the growth of these cells. Pretreatment of U937 cells with a monoclonal antibody against the LDL receptor was also found to completely block the growth-promoting effect of LDL. Exposure of U937 cells to liposomes with a lipid composition similar to that of LDL did not stimulate the growth rate. These findings demonstrate that growth of U937 cells under serum-free conditions is related to the amount of LDL ingested by the cells and that this uptake is mediated by binding of LDL to the LDL receptor. To determine if LDL-induced growth of U937 cells can be used to identify LDL with decreased binding to the LDL receptor, U937 cells were incubated with LDL isolated from a patient with familial defective apolipoprotein B-100 and from subjects with various lipoprotein phenotypes. LDL containing defective apolipoprotein B-100 was found to be less than half as effective as LDL from normolipidemic controls in stimulating growth of U937 cells. LDL isolated from patients with hyperlipoproteinemia type IIa and IV did not differ from normal LDL in their ability to promote growth of U937 cells. ▨ The present results suggest that LDL-induced growth of U937 cells may be used as an assay to identify defective receptor binding of LDL. — Frostegård, J., A. Hamsten, M. Gidlund, and J. Nilsson. Low density lipoprotein-induced growth of U937 cells: a novel method to determine the receptor binding of low density lipoprotein. *J. Lipid Res.* 1990. **31:** 37–44.

Supplementary key words cholesterol • cell growth • atherosclerosis • familial hypercholesterolemia

Cellular uptake of low density lipoprotein (LDL) is mediated by binding of its protein, apolipoprotein B-100 (apoB) to the LDL receptor (1). Studies on patients with familial hypercholesterolemia (FH) and on Watanabe heritable hyperlipidemic rabbits have demonstrated that defective receptor binding of apoB leads to hypercholesterolemia and to premature development of atherosclerosis (2). In patients with FH, hypercholesterolemia and arterial accumulation of lipids occur as a result of a mutation in the genes coding the LDL receptor (2). In view of these findings it is reasonable to assume that abnormalities in the structure of apoB would also lead to hypercholesterolemia and possibly premature atherosclerosis. Indeed, Innerarity and co-workers (3) recently identified a family where hypercholesterolemia was linked to the presence of an apoB molecule with defective binding properties.

A number of different techniques have been applied in order to provide evidence for the existence of functional abnormalities in the apoB molecule. Studies of restriction fragment length polymorphisms (RFLPs) of apoB have demonstrated several gene variants. Talmud and co-workers (4) reported a significantly higher serum cholesterol level in subjects who were homozygous for the X2 allele of the XbaI polymorphism. Recently, it was also indicated that individuals with this genetic polymorphism have a decreased catabolic rate of LDL (5). Other workers have been able to identify genetic polymorphisms of apoB using monoclonal antibodies, but it has not been possible to demonstrate any connection between a specific immunophenotype and hypercholesterolemia or coronary heart disease (6, 7).

An inherent problem with techniques such as RFLP and immunophenotyping is that they give no information on the apoB function. The most accurate method to determine the receptor-mediated binding of LDL is ^{125}I-labeling of LDL particles and analysis of their binding to cultured fibroblasts as described by Goldstein and Brown (8). However, this method is difficult to apply in clinical studies. When searching for an alternative method to specifically determine the binding properties of LDL, we took advantage of the fact that most cell types require cholesterol for de novo membrane synthesis in order to

proliferate (9). U937 is a monocytic cell line derived from a histiocytic lymphoma and has been used as a model for studies of monocyte/macrophage function (10). These cells have been shown to lack the qualities required for endogenous cholesterol synthesis, and thus cultivation of the cells in a medium supplemented with delipidated serum results in depletion of cellular cholesterol content and arrest of cell growth within 48 h (11). We here confirm this finding and report that the growth rate of U937 cells is dependent on the binding of LDL particles to the LDL receptor and that the growth response of these cells can be used to determine the binding properties of LDL.

METHODS

Human subjects

Plasma for isolation of LDL was obtained from two healthy normolipoproteinemic male subjects and from five male patients with type IIa and four with type IV hyperlipoproteinemia. All nine patients had suffered their first myocardial infarction before the age of 45. They were not taking medications known to affect plasma lipids. Plasma from a patient with familial defective apoB-100 was kindly supplied by Dr. N. Myant, Hammersmith Hospital, London. This patient had a substitution of A/L for G/L in the codon for apoB-100 residue 3500 in her apoB gene. The mutation was identified by amplifying a 285-kb segment of the apoB gene that included codon 3500. The mixture containing the amplified DNA was applied to a nylon membrane by slot blotting and the membrane was incubated with radioactive 13-base synthetic oligonucleotides complementary to either the wild-type or the mutant amplified segment. The radioactive bands were detected by autoradiography of the washed membranes (personal communication, J. Gallagher, N. B. Myant, G. R. Thompson, S. E. Humphries, and A. M. Dunning).

Materials

Medium RPMI 1640 and fetal calf serum (FCS) were purchased from GIBCO BRL, Glasgow, Scotland. Culture flasks and dishes were from Costar, Cambridge, MA. The monoclonal LDL receptor antibody C7,[³H]thymidine and ^{125}I came from Amersham, England. Phosphatidylcholine, cholesterol, bovine serum albumine, trypsin, soybean trypsin inhibitor, and gentamycin sulfate came from Sigma Chemical Co., St. Louis, MO.

Lipoprotein phenotyping

Lipoprotein phenotyping was achieved through a combination of preparative ultracentrifugation and precipitation of apoB-containing lipoproteins followed by lipid analyses of very low density lipoproteins (VLDL), low density lipoprotein (LDL), and high density lipoproteins (HDL) (12). Agarose gel lipoprotein electrophoresis was also performed on whole serum and on the top (VLDL) and bottom (LDL + HDL) fractions obtained after ultracentrifugation at density 1.006 kg/l (13). Hyperlipoproteinemias were defined according to the WHO classification (14) with cutoff limits set to the 90th percentiles of VLDL triglyceride and LDL cholesterol values in an age- and sex-matched random control population.

Preparation of LDL

Blood was drawn, after overnight fasting, into Vacutainer tubes containing Na_2EDTA (1 mg/ml) and chilled to + 1°C. Plasma was recovered by means of low-speed centrifugation (400 g). VLDL were subsequently floated to the top through ultracentrifugation of 5 ml of plasma (40,000 rpm, 16 h, + 1°C) at density 1.006 kg/l in a Beckman 50.3 Ti rotor. The infranatant was adjusted to density 1.21 kg/l with sodium bromide and a 4-ml sample was transferred to a cellulose nitrate centrifuge tube (Ultraclear tubes, Beckman, capacity 13.4 ml). Two ml of density 1.10, 3 ml of density 1.05, 3 ml of density 1.03, and 1 ml of density 1.01 kg/l sodium chloride solutions were layered above to form a density gradient (15). All salt solutions contained 0.02 % sodium azide and 0.01 % EDTA. The tubes were then centrifuged (Beckman L8-55 ultracentrifuge) at 40,000 rpm for 16 h in a Beckman SW 40 Ti rotor at + 1°C. After the centrifugation, LDL in the density range 1.025–1.050 kg/l was recovered with a fraction collector (FRAC 200, Pharmacia, Uppsala, Sweden) coupled to a peristaltic pump by puncturing the bottoms of the tubes and upward displacement of the gradients, using Maxidens FC-43 (inert, water-immiscible liquid of d 1.9 kg/l, Nyegaard A/S, Oslo, Norway).

Total protein content of the LDL preparation was determined by the technique of Lowry et al. (16) and cholesterol was determined by means of an enzymatic method (17).

Preparation of liposomes

Liposomes were prepared according to Piran and Morin (18). Phosphatidylcholine and cholesterol were mixed in a 4:1 molarity proportion, in order to obtain a concentration similar to that in the LDL preparation, and dissolved in ethanol. The solution was rapidly injected from a 1-ml syringe through a 0.38-mm needle under the surface of phosphate buffer in a 25 Erlenmeyer flask with a bottom diameter of 30–40 mm. The suspension was concentrated to 2 ml, using an Amicon ultrafiltration device with a 43-mm diameter XM-100 A membrane, with rapid stirring under a N_2 pressure of 1000 kPa for 60 sec. This procedure was performed three times to eliminate ethanol. The cholesterol concentration was then determined by an enzymatic method (17).

Methylation of LDL

Methylation of LDL was performed according to Weisgraber, Innerarity, and Mahley (19). LDL (2.4 mg) was dialyzed against LDL buffer (0.9% NaCl/0.01% EDTA; pH 7.4) for 24 h and then diluted with this buffer to a final volume of 2 ml. The LDL preparation was mixed with 0.5 ml of sodium borohydrate (1 M, pH 9.0) and shaken vigorously. The solution was mixed with 0.5 mg of sodium borohydrate, and 1 μl of 17.5% formaldehyde was subsequently added at 6-min intervals for 90 min. Thirty min after the first addition of sodium borohydrate, another 0.5 mg of sodium borohydrate was added to the preparation. After the final addition of formaldehyde, methylated LDL was dialyzed overnight against the LDL buffer, filtered through a 0.45-μm filter, and stored at 4°C.

Iodination of LDL

Iodination of LDL and methylated LDL was performed essentially as described by McFarlane (20). Purified LDL was dialyzed against the LDL buffer at 4°C overnight. [125]I was mixed with glycine buffer (1.0 M, pH 10.0) and added to the LDL solution (2–6 mg LDL in 2 ml of LDL buffer) together with 0.2 ml of 0.0033 M ICl. The preparation was shaken vigorously and put on ice for 5 min. Unbound ^{125}I was removed by eluation in LDL buffer on a Pharmacia PD-10 column. The ^{125}I-labeled LDL was dialyzed against the LDL buffer overnight, filtered through a 0.45-μm filter, and stored at 4°C. The specific activity of the preparations ranged from 200 to 500 cpm/ng protein.

Cell culture

U937 cells were grown in medium RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS) and 50 μg/ml of gentamycine sulfate and kept in an atmosphere of 5% CO_2 in air. Fresh medium was added twice weekly and the cell density was kept between 2 and 8 × 10^5 cells/ml.

LDL degradation

U937 cells were transferred to serum-free RPMI 1640 medium for 24 h. They were then centrifuged and resuspended in fresh medium at a concentration of 1.0 × 10^6 cells/ml, and 2 ml of the cell suspension was subsequently added to 2.5-ml polystyrene tubes. The cells were then incubated with 5 μg/ml of ^{125}I-labeled LDL or ^{125}I-labeled methylated LDL for 5 h at 37°C. The degradation process was arrested by placing the tubes on ice. After centrifugation at 1000 g for 4 min, the supernatant was removed and mixed with 100 μl of bovine serum albumin (10 mg/ml) and 100 μl of 50% trichloroacetic acid. The precipitated protein was removed by centrifugation for 2 min at 1200 g. Five % $AgNO_3$ (0.5 ml) was then added to the supernatant to precipitate free iodide. After centrifugation at 12,000 g for 5 min, the trichloroacetic acid-soluble, noniodide ^{125}I was determined in an LKB gamma counter.

Assay of DNA synthesis

U937 cells were transferred to serum-free RPMI 1640 medium for 24 h and resuspended in fresh medium at a concentration of 2 × 10^5 cells per ml. The cells were then seeded out in 96-well plates (100 μl cell suspension per well) and incubated with 2 μCi/ml of [³H]thymidine for 24 h at 37°C. After the incubation, DNA was precipitated in glass-fiber filters by means of an automatic cell harvester and the amount of incorporated ³H was determined in a liquid scintillation spectrometer.

RESULTS

U937 cells had a limited ability to grow under serum-free conditions. The average increase in cell number over a 48-h period was 10%, whereas serum-free culture for longer periods resulted in cell death and a decrease in the total cell number. In serum-supplemented medium, U937 cells proliferated rapidly with an average increase in cell number over a 48-h period of about 100%. Contrarily, U937 cells grown in lipoprotein-deficient serum showed only a limited increase in cell number over a 48-h period (**Fig 1**), indicating either that lipoproteins are major growth factors for U937 cells or they contain an essential
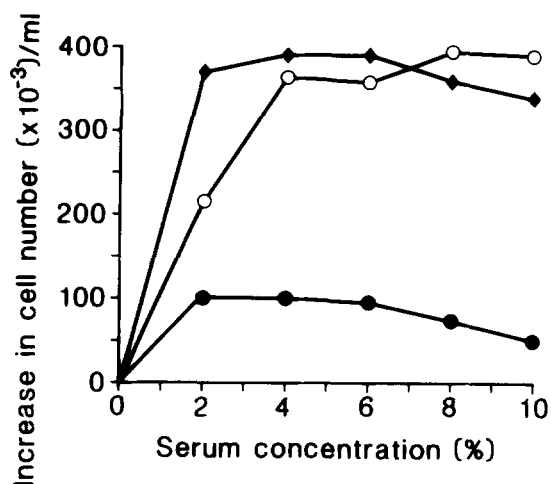

Fig. 1. The effect of serum and lipoprotein-deficient serum on U937 cell growth. Serum-starved U937 cells (1.5 × 10^5) were seeded out in 1 ml of medium RPMI 1640 and allowed to grow for 48 h in the indicated concentrations of fetal calf serum (♦), lipoprotein-deficient human serum (●), or lipoprotein-deficient (○) human serum with addition of 0.15 mmol/l of LDL. The cells were then scraped off with the help of a rubber policeman and the final cell number was determined in an electronic cell counter. Each value represents the mean of triplicate cultures (SD < 10%).

nutrient for U937 cells. Supplementation of the lipoprotein-deficient serum with 0.15 mmol/l of LDL completely restored the growth-promoting effect. Addition of purified LDL to the U937 cells that had been serum-starved for 24 h and then incubated in serum-free RPMI 1640 medium resulted in a dose-dependent and saturable stimulation of cell growth (**Fig. 2**). A considerably smaller growth-stimulatory effect was observed in cultures that had not been serum-starved prior to incubation with LDL. The increase in cell number in cultures stimulated with LDL continued for up to 72 h (Fig. 2). Likewise, signs of a toxic effect of LDL were sometimes present in cultures incubated with the highest concentrations of LDL.

Experiments analyzing uptake and degradation of ^{125}I-labeled LDL and methylated LDL, performed in the presence or absence of a 20-fold excess of unlabeled LDL and methylated LDL, respectively, demonstrated a specific and saturable degradation of native LDL but none of methylated LDL (**Fig. 3**). The cells were also found to lack the ability to take up and degrade LDL modified by oxidation with copper ions (data not shown). In **Fig. 4** the effects of native and methylated LDL on U937 cell growth are compared. In this experiment a maximal stimulation of cell proliferation was obtained already after addition of 5 μg/ml of LDL (concentration is given as the protein value to facilitate comparison with the binding experiments). However, in other experiments, an LDL concentration of 10 μg/ml was sometimes required for optimal cell growth. Addition of methylated LDL did not essentially affect the growth rate, except at the highest concentration where a minute stimulation of cell growth occurred. Notably, this effect was much less pronounced
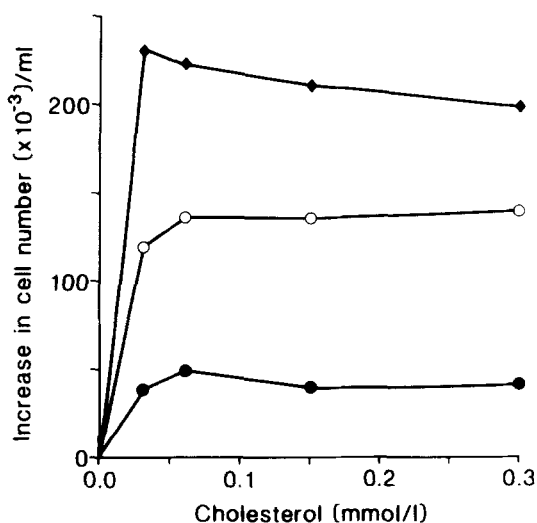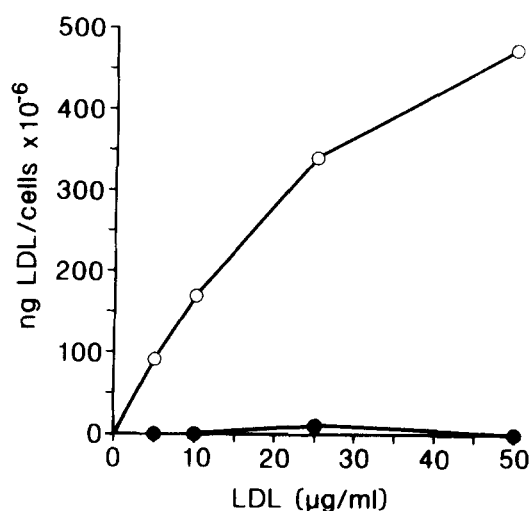

Fig. 3. Degradation of native and methylated LDL in U937 cells. Serum-starved U937 cells (2 × 10⁶cells/ml) were incubated with 5 μg/ml of ^{125}I-labeled LDL (O) or ^{125}I-labeled methylated LDL (●) in the presence or absence of a 20-fold excess of unlabeled LDL or methylated LDL for 5 h at 37°C. The trichloracetic acid-soluble, noniodide ^{125}I was then determined as a measure of LDL degradation. The specific degradation was calculated by subtracting the binding in the presence of an excess of unlabeled lipoprotein from the binding in the absence of an excess of the respective lipoprotein. Each value represents the mean of triplicate cultures (SD < 40).

than that obtained with the lowest concentration of native LDL and could be due to incomplete methylation of a small fraction of the LDL particles. As can be seen in Fig. 3, much higher concentrations of LDL were needed to saturate cell surface binding than for maximal stimulation of cell growth, suggesting that not all of the surface receptors for LDL are engaged in supplying LDL to the cells at a maximal growth rate. The marked increase in cell number that occurs in cultures of U937 cells grown in LDL-supplemented serum-free medium during 48 h is not coupled to a concomitant increase in the rate of DNA synthesis during this period (**Table 1**).

If LDL-induced growth of U937 cells is going to be accepted as a valid test for the binding properties of LDL, it is important to demonstrate that the increased rate of cell growth is due to and proportional to the specific receptor-mediated uptake of LDL particles. A preparation of liposomes with a lipid composition similar to that of LDL did not stimulate the growth of U937 cells (**Fig. 5**). In another set of experiments, U937 cells were preincubated for 2 h with various concentrations of the C7 monoclonal antibody against the LDL receptor. They were then transferred to a medium containing both LDL and the LDL receptor antibody (**Fig. 6**). Addition of the antibody resulted in a dose-dependent inhibition of the LDL-induced cell growth, whereas an unspecific control antibody was ineffective. We also studied whether the growth-promoting effect of LDL could be removed by

Fig. 2. Effect of LDL on growth of U937 cells. Serum-starved U937 cells (1.5 × 10⁵) were grown in 1 ml serum-free RPMI 1640 medium with addition of the indicated concentrations of LDL for 24 h (●), 48 h (O), and 72 h (♦). Each value represents the mean of triplicate determinations (SD < 10%).
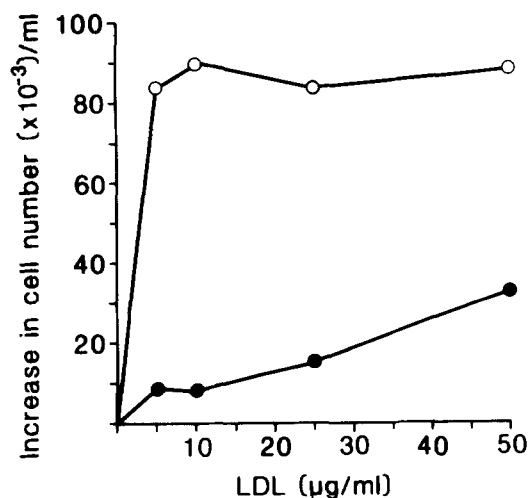
Fig. 4. The effect of native and methylated LDL on U937 cell growth. Serum-starved U937 cells (1.5×10^5 cells/ml) were incubated with various concentrations of LDL (O) or methylated LDL (●) for 48 h. The concentration of LDL is expressed as μg protein/ml to facilitate comparison with the binding experiments. Each value represents the mean of triplicate cultures (SD < 13×10^3).

trypsin digestion. LDL (0.15 mmol/l) was incubated with 50 μg/ml of trypsin with or without 50 μg/ml of soybean trypsin inhibitor for 2 h at 37°C. Trypsinized LDL was found to have only 13.5% ± 6.6 of the growth-promoting effect of normal LDL. In the experiments where a trypsin inhibitor was added together with trypsin, no inhibitory effect on LDL-induced growth occurred.

The following experiments were performed to determine whether LDL-induced growth of U937 cells can be used as an assay for detection of defective apoB-100. LDL (d 1.025–1.050 kg/l) was isolated from two healthy controls, nine post myocardial infarction patients with various lipoprotein phenotypes (for further patient characteristics see **Table 2**), and from one patient with familial defective apoB-100. The isolated LDL was dialyzed against 0.9% NaCl/0.01% EDTA and added to the

culture medium of U937 cells (1.5×10^5 cells/ml) at the cholesterol concentrations of 3, 6, and 9 μmol/l. The cells were then grown in this medium for 72 h and the final cell numbers were determined. Addition of 9 μmol/l of LDL from healthy controls resulted in a mean increase in cell number of 1.65×10^5 cells/ml. All other values were expressed as percent of this number. The growth-promoting effect of LDL from the patient with familial defective apoB-100 was found to be reduced with about 70% as compared with normal LDL (**Fig. 7**). Identical results were obtained in three independent experiments. There was no difference in the growth-promoting capacity of LDL isolated from healthy controls and from patients with hyperlipoproteinemia type IIa and IV.

DISCUSSION

The present findings confirm earlier studies by Esfahani, Scerbo, and Devlin (11) that presence of LDL is a critical requirement for U937 cell growth and that culture of U937 cells in a serum-free medium supplemented with purified human LDL results in a dose- and time-dependent stimulation of cell growth over a 48-h period. As suggested by Esfahani et al. (11), the conspicuous lipoprotein requirement for growth of these cells is probably explained by their inability to synthesize cholesterol. These authors reported that incubation of U937 cells in delipidated serum for 48 h leads to an almost complete removal of intracellular cholesterol (11). LDL is bound to U937 cells by specific cell surface receptors. On the other hand, the cells have no receptors for the modified forms of LDL
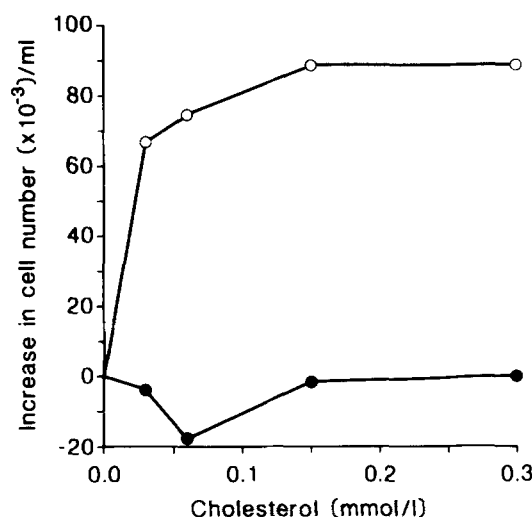
TABLE 1. Coupling between DNA synthesis and cell growth in U937 cells exposed to LDL

Time	Cell Number		DNA Synthesis	
	Control	LDL	Control	LDL
	$\times 10^{-3}$		cpm/cell $\times 10^{-3}$	
24 h	25 ± 6.7	63 ± 9.6	1170 ± 54	963 ± 56
48 h	14 ± 9.8	150 ± 2.5	948 ± 76	682 ± 38

Serum-starved U937 cells were grown for 24 or 48 h with or without addition of 0.15 mmol/l of LDL. For cell counting, the cells were grown in 12-multiwell plates. For determination of DNA synthesis, cells were seeded out in 96-multiwell plates. The cells were then exposed to 2 μCi/ml of [³H]thymidine during the time periods 0–24 h or 24–48 h. Cell numbers are given as means of triplicate cultures and DNA synthesis as means of 12 cultures.

Fig. 5. The effect of LDL and liposomes on the growth of U937 cells. Serum-starved U937 cells (1.5×10^5 cells/ml) were grown for 38 h in the presence of various concentrations of cholesterol given as LDL (O) or liposomes (●). All values represent the mean of triplicate cultures (SD < 10×10^3).
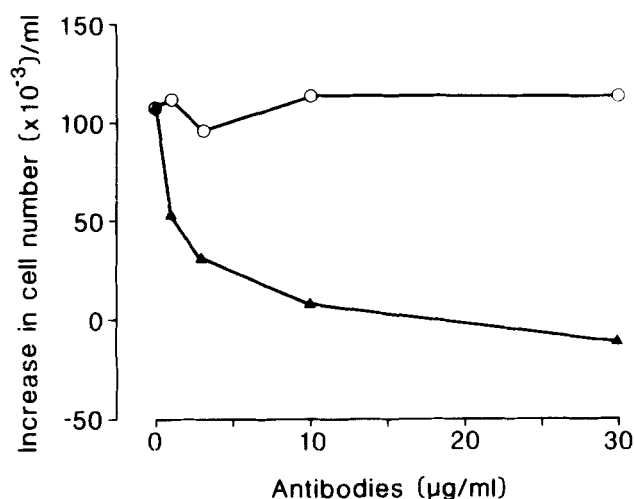
Fig. 6. The effect of an LDL-receptor antibody on the growth of U937 cells. Serum-starved U937 cells (1.5 × 10⁵ cells/ml) were exposed to the indicated concentrations of the monoclonal LDL-receptor antibody C7 (▲) or an unspecific control IgG (○) for 2 h at 37°C. They were then exposed to a medium containing an identical concentration of the antibody and with addition of 0.15 mmol/l of LDL for 48 h. Each value represents the mean of triplicate cultures (SD < 12 × 10³).

known to bind to the scavenger receptor on macrophages. Consequently, growth of these cells is only supported by native LDL and not by methylated or oxidized LDL. Rouis and co-workers (21) have demonstrated that U937 cells, when exposed to phorbol esters, differentiate into macrophage-like cells with fewer LDL receptors and a decreased rate of cell proliferation. This process is analogous to the maturation of monocyte precursors into differentiated monocytes/macrophages in vivo (22).

There is a close relationship between degradation of LDL and rate of cell proliferation in U937 cells. Theoretically, the growth rate may be influenced both by the protein and by the cholesterol supplied by LDL. However, the findings that a 24-h period of serum starvation both depletes the intracellular cholesterol stores and enhances the LDL-induced growth response suggest that the cholesterol content of the LDL particles constitutes the critical factor. Several other studies also support the notion that the supply of cholesterol for de novo synthesis of membranes is a rate-limiting factor in the regulation of cell proliferation. In most cells there is a coupling between steroid synthesis and cell proliferation (9). Furthermore, if cellular steroid synthesis is blocked by inhibition of the enzyme HMG-CoA reductase, both DNA synthesis and cell proliferation is arrested (23). In view of these findings it seems clear that the growth-promoting effect of LDL on U937 cells is dependent on its cholesterol content and not the protein content. The cholesterol/protein ratio of LDL particles isolated from different individuals may vary considerably. Accordingly, when the growth-promoting effects of LDL from different individuals are compared, it is important to adjust the amount of LDL added to the culture medium so that equal molarities of LDL cholesterol are obtained.

Modified LDL, which is not degraded by U937 cells and does not bind to LDL receptors, was unable to support the growth of these cells. This suggests a coupling between receptor-mediator uptake of LDL and stimulation of cell growth. However, the notion that cholesterol is supplied to the cells solely through a receptor-mediated uptake of LDL stems mainly from the experiments using a monoclonal antibody against the LDL receptor. Pre-

TABLE 2. Characteristics of patients and normolipidemic controls

Patient	Age	Cholesterol			Triglycerides		
		VLDL	LDL	HDL	VLDL	LDL	HDL
	yr			*mmol/l*			
HLP IIa patients							
A	41	0.54	5.76	1.20	0.98	0.34	0.12
B	50	0.66	6.49	1.26	1.23	0.44	0.10
C	49	0.67	6.13	1.29	1.04	0.29	0.12
D	44	0.69	5.77	1.42	1.20	0.58	0.16
E	43	1.20	7.34	1.07	1.57	0.77	0.17
HLP IV patients							
F	39	0.89	3.76	0.78	2.27	0.39	0.15
G	48	1.24	5.05	1.06	3.30	0.39	0.14
H	48	1.61	5.11	1.14	2.42	0.72	0.21
I	51	1.29	4.51	0.96	7.69	0.56	0.17
NLP controls							
J	35	0.60	3.36	1.68	0.42	0.22	0.15
K	28	0.32	3.59	1.81	0.78	0.24	0.15

Lipoprotein phenotyping of hyperlipoproteinemic (HLP) patients and normolipidemic (NLP) controls was based on preparative ultracentrifugation (12) and agarose gel electrophoresis (13); hyperlipoproteinemias were defined according to the WHO classification (14). The cutoff limit were set to the 90th percentiles of VLDL triglyceride (1.65 m mol/l) and LDL cholesterol (5.35 mmol/l) values in an age- and sex-matched random control population.
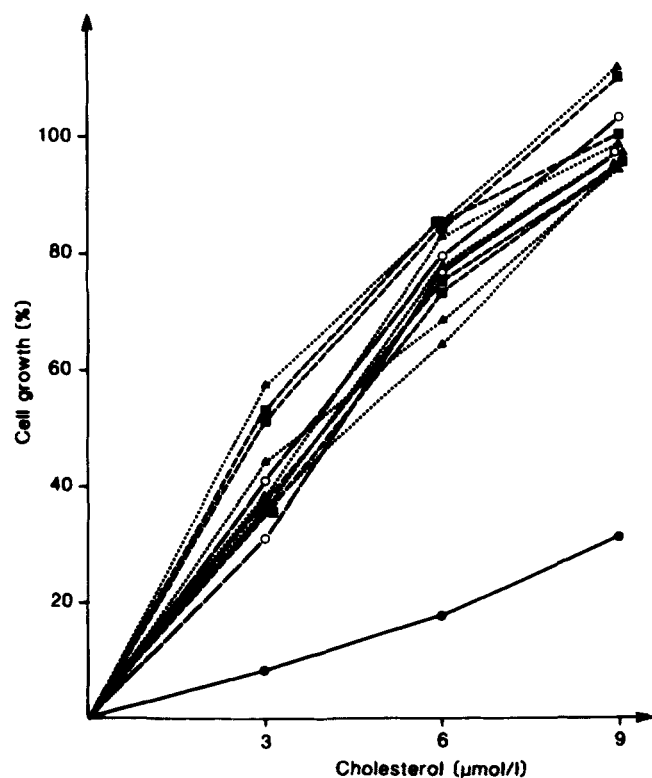
Fig. 7. The effect of LDL from individuals with various lipoprotein phenotypes and from an individual with familial defective apoB-100 on the growth of U937 cells. Serum-starved U937 cells (1.5×10^5 cells/ml) were grown for 48 h in serum-free medium with addition of various concentrations of LDL isolated from five patients with hyperlipoproteinemia type IIa (▲), four patients with hyperlipoproteinemia type IV (■), one patient with familial defective apoB-100 (●), and two healthy controls (○). The values are expressed as percent of the mean increase in cell number obtained with 0.009 mmol/l of LDL cholesterol from healthy controls. Each value represents the mean of triplicate samples (SD < 15 %).

treatment of U937 cells with the C7 LDL receptor antibody resulted in extinction of the LDL-induced cell growth. Indeed, at the highest concentrations of the antibody, the cell number was even lower than in untreated controls. Since the inhibitory effect of the antibody was saturable, it seems unlikely that the inhibition was due to a toxic effect on the cells. Instead, the finding that cultures pretreated with the antibody showed lower numbers than control cultures may depend on the presence of a minor amount of LDL remaining from earlier culture of the cells in serum-containing medium. The residual LDL may thus give rise to an LDL-dependent increase in cell growth, even without addition of new LDL. The growth-stimulatory effect of this LDL would also be blocked by addition of the LDL receptor antibody, however.

The finding that liposomes with a lipid composition similar to that of LDL were unable to initiate growth of U937 cells further disputes the possibility that cholesterol can be taken up in the cells by a non-receptor-mediated mechanism. Incubation of serum-starved U937 cells in LDL-supplemented medium for 48 h resulted in a marked increase in cell number without a concomitant increase in the rate of DNA synthesis. Hence, cell division is not preceded by DNA synthesis during this period, suggesting that the role of cholesterol in regulating U937 cell growth is that of a rate-limiting nutrient rather than a classical growth factor.

From the results discussed above it can be concluded that the growth rate of U937 cells correlates to the amount of cholesterol taken up by the cells and that the uptake of cholesterol is dependent on receptor-mediated uptake of LDL particles. This led us to assume that exposure of U937 cells to LDL with a decreased binding affinity for the LDL receptor would lead to decreased uptake of cholesterol and hence a decreased stimulation of cell proliferation. In that case, LDL-induced growth of U937 cells could be used to identify individuals with functionally defective LDL particles.

In a preliminary study the ability of the U937 assay to detect defective apoB-100 was tested on LDL isolated from one patient with defective apoB-100, two healthy controls, and nine patients with different lipoprotein phenotypes. LDL containing defective apoB-100 was found to have less than half the growth-promoting effect of normal LDL. LDL isolated from patients with hyperlipoproteinemia type IIa and IV did not differ from normal LDL in its ability to promote the growth of U937 cells, indicating that the lipid composition had a limited influence on the receptor binding of LDL in these experiments.

The role of defective LDL receptors for development of hypercholesterolemia in FH patients is well recognized. However, since most patients with hypercholesterolemia do not have FH, factors other than defective LDL receptors must be involved in the majority of hypercholesterolemic patients. Recently, interest has focused on the role of defective binding properties of the LDL particle as a possible causative factor. Vega and Grundy (24) investigated 15 nonFH patients with moderate hypercholesterolemia and found that five of these subjects had LDL particles with decreased affinity for the LDL receptor. In one of these patients the decreased binding affinity was explained by a genetic abnormality of the apoB protein (3). Others have demonstrated that LDL particles with an abnormal lipid composition have a decreased binding affinity for the LDL receptor (25). To evaluate the putative role of the binding properties of LDL particles for development of hypercholesterolemia and coronary atherosclerosis in larger clinical studies, reliable and simple assays are required. The present results suggest that analysis of LDL-induced growth of U937 cells may provide such an assay. ∎

REFERENCES

1. Brown, M., P. Kovanen, and J. Goldstein. 1981. Regulation of plasma cholesterol by lipoprotein receptors. *Science.* **212:** 628–635.
2. Goldstein, J., T. Kita, and M. Brown. 1983. Defective lipoprotein receptors and atherosclerosis. *N. Engl. J. Med.* **309:** 288–296.
3. Innerarity, T., K. Weisgraber, K. Arnold, R. Mahley, R. Krauss, G. Vega, and S. Grundy. 1987. Familial defective apolipoprotein B-100: low density lipoproteins with abnormal receptor binding. *Proc. Natl. Acad. Sci. USA.* **84:** 6919–6923.
4. Talmud, P., N. Barni, A. Kessling, P. Carlsson, C. Darnfors, G. Bjursell, D. Galton, V. Wynn, H. Kirk, M. Hayden, and S. Humphries. 1987. Apolipoprotein B gene variants are involved in the determination of serum cholesterol levels: a study in normo- and hyperlipidaemic individuals. *Atherosclerosis.* **67:** 81–89.
5. Houlston, R., P. Turner, J. Revill, B. Lewis, and S. Humphries. 1988. The fractional catabolic rate of low density lipoprotein in normal individuals is influenced by variation in the apolipoprotein B gene: a preliminary study. *Atherosclerosis.* **71:** 81–85.
6. Tikkanen, M., C. Ehnholm, P. Kovanen, R. Bütler, S. Young, L. Curtiss, and J. Witztum. 1987. Detection of two apolipoprotein B species (apoBc and apoBg) by a monoclonal antibody. *Atherosclerosis.* **65:** 247–256.
7. Duriez, P., N. Vu Dac, M. Koffigan, P. Puchois, C. Demarquilly, C. Fievet, P. Fievet, I. Luyeye, J. Bard, J. Fourrier, N. Slimane, J. Lablanche, M. Bertrand, and J. Fruchart. 1987. Detection of human apolipoprotein B polymorphic species with one monoclonal antibody (BIP 45) against low density lipoprotein. *Atherosclerosis.* **66:** 153–161.
8. Goldstein, J., and M. Brown. 1974. Binding and degradation of low density lipoproteins by cultured fibroblasts: comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J. Biol. Chem.* **249:** 5153–5162.
9. Chen, H. 1984. Role of cholesterol metabolism in cell growth. *Fed. Proc.* **43:** 126–130.
10. Sundström, C., and K. Nilsson. 1976. Establishment and characterization of human histiocytic lymphoma cell-line (U937). *Int. J. Cancer.* **7:** 565–577.
11. Esfahani, M., L. Scerbo, and T. Devlin. 1984. A requirement for cholesterol and its structural features for a human macrophage-like cell line. *J. Cell. Biochem.* **25:** 87–97.
12. Carlson, K. 1973. Lipoprotein fractionation. *J. Clin. Pathol.* **5:** 32–37.
13. Noble, R. P. 1968. Electrophoretic separation of plasma lipoproteins in agarose gel. *J. Lipid Res.* **9:** 693–700.
14. Beaumont, J. L., L. A. Carlson, G. R. Cooper, Z. Feifar, D. S. Fredrickson, and T. Strasser. 1970. Classification of hyperlipidaemias and hyperlipoproteinaemias. *Bull. WHO.* **43:** 891–915.
15. Hatch, F. T., and R. S. Lees. 1968. Practical methods for plasma lipoprotein analysis. *Adv. Lipid Res.* **6:** 1–68.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
17. Roeschlau, P., E. Bernt, and W. Gruber. 1974. Enzymatische bestimmung des Gesamt-Cholesterins im Serum. *Z. Klin. Chem. Klin. Biochem.* **12:** 226–229.
18. Piran, U., and R. J. Morin. 1979. A rapid radioassay procedure for plasma lecithin:cholesterol acyltransferase. *J. Lipid Res.* **20:** 1040–1043.
19. Weisgraber, K. H., T. L. Innerarity, and R. W. Mahley. 1978. Role of lysine residues of plasma lipoproteins in high affinity binding to cell surface receptors on human fibroblasts. *J. Biol. Chem.* **253:** 9053–9062.
20. McFarlane, A. S. 1956. Labelling of plasma proteins with radioactive iodine. *Biochem. J.* **62:** 135–143.
21. Rouis, M., M. Goldstein, P. Thomopoulos, M. Berthelier, C. Hervy, and U. Testa. 1984. Phorbol esters inhibit the binding of low density lipoproteins (LDL) to U937 monocytelike cells. *J. Cell. Physiol.* **121:** 540–546.
22. Harris, P., and P. Ralph. 1985. Human leukemic models of myelomonocytic development: a review of the HL-60 and U937 cell lines. *J. Leukocyte Biol.* **37:** 407–422.
23. Brown, M. S., and J. L. Goldstein. 1980. Multivalent feedback regulation of HMG-CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J. Lipid Res.* **21:** 505–517.
24. Vega, G., and S. Grundy. 1986. In vivo evidence for reduced binding of low density lipoproteins to receptors as a cause of primary moderate hypercholesterolemia. *J. Clin. Invest.* **78:** 1410–1414.
25. Kleinman, Y., Y. Oschry, and S. Eisenberg. 1987. Abnormal regulation of LDL receptor activity and abnormal cellular metabolism of hypertriglyceridemic low density lipoprotein: normalization with bezafibrate therapy. *Eur. J. Clin. Invest.* **17:** 538–543.