BINDING, INTERNALIZATION, AND HYDROLYSIS OF LOW DENSITY LIPOPROTEIN IN LONG-TERM LYMPHOID CELL LINES FROM A NORMAL SUBJECT AND A PATIENT WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA*

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Studies in cultured human fibroblasts have defined a metabolic pathway by which these cells are able to take up and utilize cholesterol contained in low density lipoprotein (LDL),¹ the major cholesterol-carrying protein in human plasma (reviewed in references 1 and 2). The rate-controlling step in this LDL pathway involves the binding of the lipoprotein to a high affinity cell surface receptor (3-5). The bound LDL is then internalized by a process resembling adsorptive endocytosis and the lipoprotein is delivered to the lysosomes of the cell where its protein and cholesteryl ester components are hydrolyzed (4, 6-8). The liberated unesterified cholesterol then enters the nonlysosomal cellular compartment where it is utilized by the cell for membrane synthesis and where it exerts three regulatory actions: (a) it suppresses 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), the rate-controlling enzyme in cholesterol biosynthesis, causing a reduction in the rate of endogenous synthesis of cholesterol (9); (b) it activates an acyl CoA:cholesterol acyltransferase that re-esterifies a portion of the lipoprotein-derived cholesterol, apparently to facilitate cholesterol storage (10, 11); and (c) it suppresses the synthesis of LDL receptor molecules themselves, thus limiting LDL uptake and preventing an overaccumulation of cholesterol by the cell (12).

The critical importance of the initial component of this pathway, namely the cell surface LDL receptor, is emphasized by the finding that fibroblasts from patients with the receptor-negative form of homozygous familial hypercholesterolemia (FH), which lack functional LDL receptors, are unable to take up the lipoprotein with high affinity (3-5, 13-16). As a result, these mutant cells are not

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¹ Abbreviations used in this paper: FH, familial hypercholesterolemia; HDL, high density lipoprotein; HMG CoA reductase; 3-hydroxy-3-methylglutaryl coenzyme A reductase; LDL, low density lipoprotein; LPDS, lipoprotein-deficient serum.
able to use LDL cholesterol for membrane synthesis, nor do they show suppression of HMG CoA reductase activity or stimulation of cholesterol esterification in the presence of LDL (reviewed in references 1 and 2).

Recently, Kayden et al. have reported that cultured human lymphocytes appear to respond to LDL in a manner similar to that of fibroblasts (17). Using long-term established lymphoid cell lines from normal human subjects, these investigators demonstrated that human LDL, but not high density lipoprotein (HDL), caused a suppression of HMG CoA reductase activity and stimulation of cholesterol esterification. These effects occurred at LDL concentrations similar to those at which LDL binds to its receptor in fibroblasts. As further evidence for the involvement of the LDL receptor in lymphocytes, these investigators found that lymphocytes from homozygotes with FH failed to show suppression of HMG CoA reductase activity or stimulation of cholesteryl ester formation when incubated with LDL (17).

The present studies were designed to determine whether cultured lymphocytes do in fact possess LDL receptors and whether these receptors are absent in lymphocytes from a homozygote with FH. In order to answer these questions, we have developed an assay for $^{125}$I-LDL binding that can be applied to cells in suspension such as lymphocytes. The results indicate that normal lymphocytes possess LDL receptors and that such receptors are absent in the homozygote lymphocytes.

Materials and Methods

**Materials.** $[^{125}$I$]$sodium (carrier free in 0.05 N NaOH) was purchased from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y. $[^{3}$H$(G)]$concanavalin A (60 Ci/mmol) was obtained from New England Nuclear, Boston, Mass. $[1,2-^{3}$H$]$cholesterol (43Ci/mmol) was purchased from Amersham/Searle Corp., Arlington Heights, Ill. RPMI 1640 medium (catalogue no. 187G), L-glutamine (100x), and Dulbecco’s phosphate-buffered saline (catalogue no. 419) were purchased from Grand Island Biological Co., Grand Island, N. Y. Fetal calf serum was purchased from Flow Laboratories, Inc., Rockville, Md. Bovine serum albumin (once crystallized, catalogue no. A 4378) was purchased from Sigma Chemical Co., St. Louis, Mo. Polyethylene microfuge tubes were obtained from Beckman Instruments, Inc., Fullerton, Calif. Thin-layer chromatographic materials and reagents for assays were obtained from sources as previously reported (9, 18).

**Cells.** Normal and FH homozygote lymphoid cell lines were established in culture by Kayden et al. (17) using a modification of the method described by Beratis and Hirschhorn (19). The homozygote lymphoid line was derived from patient O. C. (17). Biochemical studies of cultured skin fibroblasts from O. C. indicate that she has the receptor-negative form of homozygous FH (13). The lymphoid lines were maintained in a humidified incubator (5% CO$_2$) at 37°C in 250-ml flasks containing RPMI 1640 medium supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), 1% glutamine, and 20% (vol/vol) fetal calf serum. Fresh growth medium was added every other day so as to maintain the lymphocytes at a concentration of about 1.5 x 10$^6$ cells/ml. No differences were detected in either the morphological characteristics or growth rates of the normal and homozygote cell strains. For experimental studies, cells from stock cultures were collected by centrifugation (1,700 rpm, 5 min, 24°C), washed twice with 8 ml of Dulbecco’s phosphate-buffered saline, and resuspended in either buffer A or growth medium as indicated below. Cell viability was determined by exclusion of erythrosin B. Under all conditions, at least 90-95% of the cells were viable.

**Lipoproteins.** Human LDL (density 1.019-1.063 g/ml), human HDL (density 1.085-1.215 g/ml), and human lipoprotein-deficient serum (LPDS) (density > 1.215 g/ml) were obtained from the plasma of healthy subjects and prepared by differential ultracentrifugation (9). Lipoprotein-deficient fetal calf serum was prepared as previously described (18). The concentration of LDL and HDL is expressed in terms of their protein content. The mass ratio of total cholesterol to protein in
LDL and HDL was 1.6:1 and 1:3, respectively. $^{125}$I-LDL was prepared as previously described (3). $[^3H]$cholesteryl linoleate-labeled LDL was prepared by the method of Goodman (20), isolated and purified by silicic acid:celite column chromatography (21) and stored at room temperature in benzene. $[^3H]$cholesteryl linoleate-labeled LDL was prepared as previously described (6).

**Surface Binding of $^{125}$I-LDL by Intact Lymphocytes at 4°C.** Cells were seeded at a concentration of about 50 x $10^6$ cells/280 ml flask in 50 ml of growth medium containing 10% calf LPDS (1 x $10^6$ cells/ml). After incubation for 36 h at 37°C, the cells from two flasks were harvested by centrifugation (1,700 rpm, 10 min, 4°C), washed once in 10 ml of buffer A (Dulbecco's phosphate-buffered saline containing 50 μM CaCl$_2$, and 20 mg/ml bovine serum albumin), and resuspended in ice-cold buffer A at a final concentration of about 65 x $10^6$ cells/ml. The binding assays were carried out in 400-μl polyethylene microfuge tubes that had been precoated with 1% Siliclad (Clay-Adams, Inc., Parsippany, N. J.). Just before its use, the $^{125}$I-LDL was diluted to the appropriate concentration with buffer A and passed through a Millipore filter (0.45 μm). Incubations were conducted in a 4°C cold room as described in the figure legends, and the total amount of $^{125}$I-LDL bound to the cells was determined using the following standard washing procedure: At the completion of the binding assay, each tube was centrifuged in a Beckman Microfuge Model 152 (12,000 rpm, 2 min, 4°C). The supernate was discarded, and the top of the cell pellet was rinsed with 300 μl of buffer A and recentrifuged immediately (12,000 rpm, 1 min, 4°C). The cell pellet was then suspended in 75 μl of buffer A, a 65-μl aliquot of this suspension was layered onto 300 μl of 100% fetal calf serum contained in a siliconized 400-μl microfuge tube, and each tube was then centrifuged (12,000 rpm, 5 min, 4°C) in a modification of the method described by Kulczycki et al. (22). The entire supernate was removed, and the bottom of the tube containing the cell pellet was cut off with a razor blade and placed in a plastic tube for radioactivity determination in a well-type gamma counter. After counting, the tip of the microfuge tube containing the cell pellet was transferred to a glass tube containing 0.5 ml of 0.1 N NaOH and the cell pellet was allowed to dissolve by overnight incubation at 37°C, after which an aliquot was removed for protein determination.

**Cellular Accumulation and Proteolytic Degradation of $^{125}$I-LDL by Intact Lymphocytes at 37°C.** Cells were seeded at a concentration of about 2 x $10^6$ cells/30 ml flask in 2 ml of growth medium containing 10% human LPDS (1 x $10^6$ cells/ml). After the cells were incubated for 36 h at 37°C, $^{131}$I-LDL was added to each flask as indicated and incubations were continued at 37°C. After the indicated interval, the cells and medium were separated by centrifugation (1,700 rpm, 5 min, 4°C). The medium was then treated with TCA to a final concentration of 10% (vol/vol), the soluble fraction was treated with hydrogen peroxide and then extracted with chloroform to remove free iodine (23), and an aliquot of the aqueous phase was counted to determine the amount of $^{131}$I-labeled acid-soluble material formed by the cells and released to the medium (4). A blank value due to the presence of small amounts of acid-soluble material (<0.01% of total added radioactivity) in the $^{131}$I-LDL preparation was routinely determined at the appropriate LDL concentration by incubation in growth medium containing no cells (4). To determine the total cellular content of $^{131}$I-LDL (i.e., the $^{131}$I-LDL contained both at the receptor site on the cell surface and within the cell), after removal of the incubation medium the cell pellet from each flask was washed with 5 ml of buffer B (Dulbecco's phosphate-buffered saline containing 5 mg/ml bovine serum albumin) and collected by centrifugation (1,700 rpm, 5 min, 4°C). After this procedure was repeated three times, each cell pellet was resuspended in 100 μl of buffer B. A 75-μl aliquot of this suspension was layered onto 300 μl of 100% fetal calf serum contained in a siliconized 400 μl microfuge tube and each tube was then centrifuged (12,000 rpm, 5 min, 4°C). The entire supernate was removed, and the bottom of the tube containing the cell pellet was cut off with a razor blade, counted directly for radioactivity, and then used for determination of protein content after the pellet was dissolved in NaOH as described above.

**Hydrolysis of $[^3H]$Cholesteryl Linoleate-Labeled LDL by Intact Lymphocytes.** Cells were seeded and incubated in the same manner as described for measurement of $^{131}$I-LDL protein accumulation and degradation. After incubation for 36 h at 37°C, the cells were exposed to $[^3H]$cholesteryl linoleate-labeled LDL as indicated and incubations were continued at 37°C. After the indicated time interval, the cells were collected by centrifugation (1,700 rpm, 5 min, 4°C). The cells were washed exactly as described above for determination of the cellular accumulation of $^{131}$I-LDL. The final cell pellet, after centrifugation through 100% fetal calf serum, was resuspended in 200 μl of water. An aliquot (10 μl) was removed for determination of protein content and the remaining suspension was extracted with 20 volumes of chloroform:methanol (2:1) after the addition of an
Measurement of Cellular Content of Free and Esterified Cholesterol. Lymphocytes in suspension culture were incubated with LDL in growth medium supplemented with 20% calf LPDS. After the indicated interval the cells and medium were separated by centrifugation (1,700 rpm, 5 min, 4°C). The cells were washed four times with 5 ml of ice-cold buffer C (50 mM Tris-chloride, pH 7.4; 150 mM NaCl; and 2 mg/ml of bovine serum albumin), after which each cell pellet was suspended in 500 μl of buffer C and was layered onto 700 μl of 100% calf LPDS contained in a siliconized 1.5 ml microfuge tube. The tube was subjected to centrifugation (11,000 rpm, 5 min, 4°C) and the resulting cell pellet was washed once more in 5 ml of buffer containing 50 mM Tris-chloride, pH 7.4 and 150 mM NaCl. The final cell pellet was resuspended in 200 μl of water. An aliquot (10 μl) was removed for determination of protein content and the remaining suspension was extracted with 20 volumes of chloroform:methanol (2:1) for steroid measurement. The free and esterified cholesterol fractions were separated on silicic acid:celite columns, and the cholesterol content in each fraction was measured by gas liquid chromatography as previously described for fibroblasts (21).

Other. The protein concentration of extracts and intact cells was determined by a modification of the method of Lowry et al. (24), using bovine serum albumin as a standard. In all figures and tables, each value represents the average of duplicate assays or incubations.

Results

When normal lymphocytes were incubated for 36 h in the absence of lipoproteins and then exposed to 125I-LDL (10 μg protein/ml) at 4°C, there was a rapid binding of the lipoprotein to the cells with equilibrium achieved by 30 min (Fig. 1 A). In the presence of a 25-fold excess of unlabeled LDL, the binding of 125I-LDL was reduced by 90%, indicating that the unlabeled LDL was competing with the 125I-LDL for a limited number of binding sites. In the same experiment, the total binding of 125I-LDL to lymphocytes derived from an FH homozygote was less than 10% of the total binding observed in the normal cells (Fig. 1 B). Moreover, there was no evidence of competition by excess unlabeled LDL, indicating that the homozygote cells were completely lacking in saturable LDL-binding sites.

Further evidence for this deficiency in LDL binding in the homozygote cells was obtained from studies of the saturation kinetics of 125I-LDL binding at 4°C (Fig. 2). In the homozygote cells, as the concentration of 125I-LDL was increased the amount of cell-bound 125I-LDL increased in a linear fashion indicating the absence of high affinity binding sites. In contrast, the normal lymphocytes exhibited a two component binding curve, a high affinity component that appeared to reach saturation at about 25 μg protein/ml and a linear, apparently nonsaturable component that paralleled the nonsaturable component in the homozygote cells. Assuming that the protein component of LDL accounts for 20% of the total molecular weight of the LDL particle (~3 × 10^6) (25), we calculated that at 4°C about 1,800-3,600 particles of LDL were bound to each normal cell at saturation. The nature and significance of the nonsaturable linear component of the 125I-LDL-binding curve in both the normal and homozygote cells is not yet clear, but it is possible that this component reflects the trapping of droplets of extracellular fluid that are not completely removed by the wash procedure. Although unlabeled LDL competed with 125I-LDL for binding to the surface receptor site in normal cells, HDL did not compete effectively (Fig. 3).

As previously described for the LDL receptor in human fibroblasts (4), binding
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**Fig. 1.** Time-course of $^{125}$I-LDL binding at 4°C in normal (A) (●, ▲) and FH homozygote (B) (▲, △) lymphocytes. After incubation for 36 h at 37°C in medium containing 10% calf LPDS, cells were harvested, washed, and resuspended in ice-cold buffer A at a final concentration of about $65 \times 10^6$ cells/ml. Each binding assay was conducted in 100 μl of buffer A containing $4 \times 10^6$ intact cells ($320-450 \mu g$ of total cell protein) and $10 \mu g$ protein/ml of $^{125}$I-LDL (252 cpm/ng) in the presence (●, ▲) and absence (▲, △) of 250 μg protein/ml of native LDL. After incubation at 4°C for the indicated time, the total amount of $^{125}$I-LDL bound to the cells was determined as described in the Materials and Methods.

**Fig. 2.** $^{125}$I-LDL binding at 4°C in normal (●) and homozygote (▲) lymphocytes as a function of $^{125}$I-LDL concentration. After incubation for 36 h at 37°C in medium containing 10% calf LPDS, cells were harvested, washed, and resuspended in ice-cold buffer A at a final concentration of about $65 \times 10^6$ cells/ml. Each binding assay was conducted in 100 μl of buffer A containing $5 \times 10^6$ intact cells (500-800 μg of total cell protein) and the indicated concentration of $^{125}$I-LDL (exp. A, 193 cpm/ng; and exp. B, 164 cpm/ng). After incubation at 4°C for 15 min, the total amount of $^{125}$I-LDL bound to the cells was determined as described in the Materials and Methods.

of $^{125}$I-LDL to the lymphocyte receptor required Ca++. In the presence of 100 μM EDTA and no added Ca++, high affinity $^{125}$I-LDL binding was not detectable, but this binding was restored completely by the addition of 150 μM Ca++ (data not shown).
FIG. 3. Effect of serum lipoprotein fractions on ^251-LDL binding to normal lymphocytes at 4°C. After incubation for 36 h at 37°C in medium containing 10% calf LPDS, normal lymphocytes were harvested, washed, and resuspended in ice-cold buffer A at a final concentration of about 65 x 10⁶ cells/ml. Each binding assay was conducted in 100 µl of buffer A containing 4.5 x 10⁶ intact cells (400-490 µg of total cell protein); the indicated concentration of either unlabeled HDL (A) or unlabeled LDL (B); and 10 µg protein/ml of ^251-LDL (123 cpm/ng). The cells were incubated at 4°C for 30 min, after which the total amount of ^251-LDL bound to the cells was determined as described in the Materials and Methods.

In contrast to their lack of LDL-binding sites, the homozygote lymphocytes bound the same amount of [3H]concanavalin A as did normal lymphocytes at 4°C (Table I). In both the normal and homozygote cells, the [3H]concanavalin A binding was inhibited by α-methyl-d-mannopyranoside.

When normal lymphocytes were incubated with ^125I-LDL at 37°C, the cellular content of lipoprotein reached a steady state within 1 h (Fig. 4A). As with cultured fibroblasts, this constant cellular content appeared to result from a dynamic equilibrium in which the rate of uptake of the lipoprotein was equal to its rate of degradation (4). Thus, during this steady state period, ^125I-labeled acid-soluble degradative products continued to be released into the culture medium at a rate that was linear with time for at least 6 h (Fig. 4A). That this uptake and degradation required binding of LDL to the high affinity surface receptor was indicated by the observation that the homozygote lymphocytes, which lacked cell surface binding, did not take up or degrade the lipoprotein at a detectable rate when it was present at 10 µg protein/ml (Fig. 4B).

Further evidence for the participation of the surface receptor in the uptake process came from studies of the saturation kinetics for the uptake and degradation of ^125I-LDL at 37°C (Fig. 5). In the normal cells the steady state cellular content of ^125I-LDL rose sharply as the concentration of ^125I-LDL was increased, until at 25-50 µg/ml when saturation appeared to be reached. Above this concentration, the cellular content of ^125I-LDL rose in a gradual linear fashion with increasing concentration of ^125I-LDL in the medium. In the homozygote cells the initial high affinity component of the saturation curve was absent, but the gradual linear slope was present. As with the apparently nonsaturable component of the 4°C binding curve, the nature of the linear component of the uptake curve at 37°C is not known. Examination of the rate of degradation of ^125I-LDL as a function of ^125I-LDL concentration indicated that only the initial high affinity component of the uptake curve provided substrate for the degradative process. Thus, in the normal cells degradation reached a maximum rate at
Table 1

Binding of \(^{3}H\)Concanavalin A at 4°C in Normal and Homozygote Lymphocytes

| Cell strain               | Concentration of \(^{3}H\)Concanavalin A in medium | \(^{3}H\)Concanavalin A bound to cells |
|---------------------------|-----------------------------------------------|-----------------------------------|
|                           | \(\mu g/ml\)                                   | \(ng/mg\) protein                  |
| Normal                    |                                               |                                   |
|                           | 1                                             | 22                                |
|                           | 5                                             | 89                                |
|                           | 25                                            | 443                               |
| Familial hypercholesterolemia homozygote | 1                                             | 15                                |
|                           | 5                                             | 77                                |
|                           | 25                                            | 406                               |

Lymphocytes were cultured, harvested, washed, and resuspended in cold buffer A exactly as described for the \(^{125}I\)-LDL-binding studies. Each binding assay contained the following in a final vol of 100 \(\mu l\) of buffer A: 4 \(\times\) \(10^6\) intact cells (360–480 \(\mu g\) of total cell protein), \(^{3}H\)concanavalin A (44 cpm/ng) at the indicated concentration, and 0.2 M \(\alpha\)-methyl-D-mannopyranoside (\(\alpha\)-MMP) as indicated. After incubation at 4°C for 30 min, the total amount of \(^{3}H\)concanavalin A bound to the cells was determined as described in the Materials and Methods for \(^{125}I\)-LDL binding except that the final pellet obtained after centrifugation through fetal calf serum was dissolved in 1 ml of 10% Triton X-100 and counted in Triton-toluene scintillation solution in a liquid scintillation counter.

50 \(\mu g\) protein/ml of \(^{125}I\)-LDL, a concentration at which the uptake process was saturated. Moreover, in the homozygote cells no degradation of \(^{125}I\)-LDL was detectable at concentrations of LDL up to 200 \(\mu g\) protein/ml.

Just as the normal lymphocytes hydrolyzed the protein component of LDL, so too did they hydrolyze the cholesterol ester component, as indicated by the hydrolysis of \(^{3}H\)cholesteryl linoleate-labeled LDL (Fig. 6). Again, the rate of such hydrolysis was limited by the rate of uptake of the lipoprotein, as indicated by the observation that this rate reached a maximum at an LDL concentration of about 50 \(\mu g\) protein/ml. Moreover, in the homozygote cells the rate of hydrolysis paralleled the rate of the nonsaturable uptake process. That the hydrolysis of the protein and cholesteryl esters of LDL occurred in lysosomes of lymphocytes, as previously shown for fibroblasts (6–8), was indicated by a study of the optimum pH for such hydrolysis in cell-free extracts of lymphocytes. Maximal rates of hydrolysis for both the protein and cholesteryl ester components of LDL occurred at about pH 4 (data not shown).

Table II shows that, as in human fibroblasts (21), the net result of the addition of LDL to normal lymphocytes was a sixfold accumulation of esterified cholesterol, whereas in the homozygote cells no such accumulation of esterified cholesterol was detected. In both the normal and homozygote cells, the free cholesterol content was somewhat higher after growth in the presence of LDL. The mechanism for the slight increase in free cholesterol in the homozygote cells
is not known, but it presumably occurs through a mechanism not involving the LDL receptor.

Discussion

The studies reported in this paper, coupled with the previous observations of Kayden et al. (17), indicate that long-term established human lymphoid cells are able to utilize the LDL pathway to obtain cholesterol and regulate their cellular sterol homeostasis. That the cell surface LDL receptor in cultured lymphocytes is the same as that previously described for human fibroblasts (1-5, 15) is suggested by the observation that this receptor is absent in both lymphocytes and fibroblasts obtained from the same patient with the homozygous form of FH.

Because of the small number of high affinity LDL receptors in normal cells, demonstration of their absence in the homozygote lymphocytes necessitated the development of a binding assay in which a high proportion of the observed binding of 125I-LDL represented binding to the LDL receptor. Initially, this proved difficult because 125I-LDL tended to become trapped in the cell pellet in an amount that was proportional to its concentration in the incubation medium. In a large number of preliminary experiments in which lymphocytes were washed by repeated resuspension and centrifugation in a variety of buffers, we could not obtain reproducible results. Moreover, when these techniques were used the amount of specific or displaceable 125I-LDL binding was always small in
cellular content of \(^{125}\text{I}-\text{LDL}\) in the steady state (A) and its rate of proteolytic degradation (B) at 37°C as a function of \(^{125}\text{I}-\text{LDL}\) concentration in normal (●) and homozygote (▲) lymphocytes. Cells were seeded at a concentration of 2 × 10\(^6\) cells/30 ml flask in 2 ml of growth medium containing 10% human LPDS (1 × 10\(^6\) cells/ml). After the cells had incubated for 36 h at 37°C, the indicated concentration of \(^{125}\text{I}-\text{LDL}\) (36 cpm/ng) was added to each flask. The incubations were continued at 37°C for a further 5 h, after which the medium and cells from each flask were separated by centrifugation, and the content of \(^{125}\text{I}-\text{LDL}\) in the medium (B) and the cellular content of \(^{125}\text{I}-\text{LDL}\) (A) were determined as described in the Materials and Methods. The average content of cellular protein was 290 μg/flask for the normal cells and 390 μg/flask for the homozygote cells.

Comparison with the nondisplaceable (i.e., nonsaturable) binding component. However, when we adopted a modification of the procedure used by Kulczycki et al. in which the washed lymphocytes are sedimented through 100% serum (22), we were able to reduce the nonspecific blank value to low proportions. The \(^{125}\text{I}-\text{LDL}\)-binding assay was also improved considerably when the stock \(^{125}\text{I}-\text{LDL}\) solution was diluted in the presence of high concentrations of albumin (20 mg/ml) and passed through a Millipore filter just before use. This procedure appeared to eliminate aggregates of \(^{125}\text{I}-\text{LDL}\) that had a stubborn tendency to spin down with cells at 4°C.

That the \(^{125}\text{I}-\text{LDL}\)-binding assay as finally developed did in fact measure the binding of \(^{125}\text{I}-\text{LDL}\) to the physiologically important LDL receptor was indicated by several findings: (a) the LDL receptor was saturated at an LDL concentration of 25–50 μg protein/ml, the concentration range at which LDL maximally suppressed HMG CoA reductase activity and maximally stimulated cholesterol esterification in lymphocytes (17); (b) the LDL receptor displayed a specificity toward LDL in comparison with HDL and this correlated with the greater ability of LDL to suppress HMG CoA reductase and activate cholesterol esterification (17); and (c) the LDL receptor was absent in lymphocytes from a homozygote with FH in whom previous studies have shown an absence of LDL-mediated effects on cholesterol synthesis and cholesteryl ester formation (17).

As with human fibroblasts, binding of LDL to its receptor was followed by internalization and degradation of the lipoprotein. This was indicated by the fact that normal lymphocytes, which bound \(^{125}\text{I}-\text{LDL}\) at the high affinity receptor site, also hydrolyzed both the protein and cholesteryl ester components by a process that achieved a maximal rate at the concentration of LDL that saturated
Fig. 6. Effect of LDL concentration on the rate of hydrolysis of [3H]cholesteryl linoleate-labeled LDL by normal (○) and homozygote (▲) lymphocytes at 37°C. Cells were seeded at a concentration of 2 × 10⁶ cells/30 ml flask in 2 ml of growth medium containing 10% human LPDS (1 × 10⁶ cells/ml). After the cells had incubated for 36 h at 37°C, the indicated concentration of [3H]cholesteryl linoleate-labeled LDL (18,400 cpm/nmol of cholesteryl linoleate) was added to each flask. The incubations were continued at 37°C for 5 h, after which the medium and cells from each flask were separated by centrifugation and the cellular content of free [3H]cholesterol was determined as described in the Materials and Methods. The average content of cellular protein was 258 μg/flask for the normal cells and 315 μg/flask for homozygote cells.

Table II

| Concentration of LDL in medium (μg protein/ml) | Normal cells | FH homozygote cells |
|---------------------------------------------|--------------|---------------------|
|                                             | Free  | Esterified | Free  | Esterified |
| 0                                           | 23    | 1.8        | 24    | 1.4        |
| 12.5                                        | 35    | 3.4        | 36    | 1.8        |
| 25                                          | 35    | 9.4        | 34    | 1.8        |
| 150                                         | 35    | 11.5       | 30    | 1.6        |

Cells were seeded at a concentration of 3 × 10⁶ cells/30 ml flask in 4 ml of growth medium containing 20% calf LPDS (7.5 × 10⁶ cells/ml). After the cells had incubated for 36 h at 37°C, the indicated concentration of LDL was added to each flask. The incubations were continued for 24 h, after which the cellular content of free and esterified cholesterol was determined as described in the Materials and Methods.

the LDL receptor. Moreover, the homozygote cells, which lacked the receptor, did not show high affinity hydrolysis of LDL protein and cholesteryl esters.

Summary

Long-term established human lymphoid cells were shown to possess high affinity cell surface receptors for low density lipoprotein (LDL), the major
cholesterol-carrying protein in human plasma. Binding of LDL to these receptors was followed by internalization of the lipoprotein and hydrolysis of its protein and cholesteryl ester components. Cultured lymphocytes from a patient with the homozygous form of familial hypercholesterolemia lacked cell surface LDL receptors and therefore failed to take up and degrade the lipoprotein with high affinity. Cultured human lymphocytes should prove useful for further studies of: (a) the relation between cholesterol metabolism and cellular function and (b) the mechanism by which LDL binding at the cell surface leads to internalization of the lipoprotein.

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