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Demonstration of low density lipoprotein receptors in mouse teratocarcinoma stem cells and description of a method for producing receptor-deficient mutant mice

(cholesterol biosynthesis/dioleoyl fluorescein labeling/fluorescence-activated cell sorting/differentiation/hypercholesterolemia disease model)

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ABSTRACT ***Familial hypercholesterolemia*, a widespread human genetic disorder implicated in vascular and coronary disease, has had no laboratory animal counterpart that would enable the pathogenesis to be analyzed and drugs to be tested *in vivo*. The primary lesion in some patients is known to occur in the cells' initial handling of the major cholesterol-carrying lipoprotein of plasma. It entails a deficiency in the specific cell surface receptor that binds low density lipoprotein (LDL), with a consequent alteration in the control of cholesterol metabolism. The present study was undertaken to devise a practical scheme for producing, from developmentally versatile mouse teratocarcinoma stem cells, whole-animal models with a comparable genetic lesion. This requires first learning whether the tumor stem cells in culture express LDL receptors, and next establishing a selection or screening procedure to identify receptor-deficient mutants in mutagenized cell cultures. The results show that the teratocarcinoma cells do in fact have specific high-affinity LDL receptors which are similar to those reported for fibroblasts and the parenchymal cells of specialized tissues and different from those of phagocytic cells. Sterols suppressed the otherwise efficient binding, internalization, and degradation of LDL (^{125}I-labeled) by the cells. Acetylation of LDL blocked the binding. Only LDL and not high density lipoprotein (HDL) was bound. After LDL uptake and degradation, the liberated cholesterol led, as expected, to increased cholesteryl ester formation; it also suppressed activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase [HMG CoA reductase; mevalonate: NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34], the rate-limiting step in cholesterol biosynthesis. Cells with LDL receptors were readily visualized by administering a fluorescent derivative of LDL; in the fluorescence microscope, labeling was seen in all cells. Cells with experimentally depressed receptors, yielding little fluorescence, were separable from those with normal fluorescence in the fluorescence-activated cell sorter. Thus, two methods for isolating receptor-deficient cells from mutagenized cultures are now available, either by visual recognition of low-fluorescing or nonfluorescing colonies in culture plates or by electronic cell sorting. Such mutants in an appropriate line of teratocarcinoma cells can then be passaged into blastocysts for full somatic tissue differentiation and germ-line development into mice.**

The stem cells of malignant mouse teratocarcinomas can be stably channeled into normal differentiation if placed in the company of normal early embryo cells by microinjection into blastocysts (1-3). Participation of the tumor-cell lineage in development, jointly with genetically marked host embryo cells, results in an animal with cellular genotypic mosaicism in any or all of its somatic tissues. In addition, germ-line contributions may be made, thereby enabling the tumor genes to be transmitted to all cells of the mosaic animal's F_1 progeny (1, 4, 5).

This developmental versatility of the malignant cells under appropriate circumstances *in vivo*, combined with their ability to grow *in vitro*, has been proposed as a basis for producing at will mice with predetermined mutations (1, 6-8): While in culture, the stem cells may be mutagenized; clones with the desired genetic change may then be obtained through selection or screening procedures, and the cells may be converted via blastocyst injection to the full array of specialized tissues in mice (9, 10).

Among mutations of particular interest would be ones in which the primary biochemical lesion is similar to that in a human genetic disease (11). A pilot example was described in the synthesis, through the teratocarcinoma-cell route, of mosaic mice with "Lesch-Nyhan" cells deficient in hypoxanthine phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) (10). In this and many other human hereditary disorders, the initial lesion—and perhaps much of the biochemical pathway—may in fact be known, but the pathogenesis has heretofore remained largely obscure. Between the primary error and the ultimate clinical syndrome lie undetected cascades involving cell and tissue interactions found only *in vivo* (11). Whole-animal models are thus required to analyze these diseases as well as to test experimental cures; yet such models are available for very few heritable defects.

Familial hypercholesterolemia is one of the most prevalent of human genetic disorders. No animal model exists. The trait, an autosomal dominant with gene dosage effect, is characterized by high blood cholesterol levels in heterozygotes and—more severely—in homozygotes (12, 13). It has been causally implicated in coronary atherosclerosis and myocardial infarction and thus poses a substantial health problem. Elevated plasma levels of the specific cholesterol-carrying lipoprotein known as low density lipoprotein (LDL) are found. Impairments in various sequential steps in the LDL pathway have been attributed to mutant genes. Of special interest is the discovery that gene-encoded cellular receptors are required for binding of LDL before it can be taken into the cell, and that the receptor molecules may be absent or defective in certain mutant individuals (12, 13). The disease has served as a prototype of genetic disorders newly recognized as being due to receptor anomalies.

Useful mouse models would therefore comprise receptor-deficient mutants. The present study was undertaken in order to learn whether such mutations might be placed in mice through the teratocarcinoma-stem-cell system. This requires

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Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein; HMG CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; LPDS, lipoprotein-deficient serum.

first ascertaining whether the tumor stem cells express LDL receptors in culture, as a basis for distinguishing mutant from wild-type cells, and then establishing a means of selecting or screening for receptor-negative mutant clones.

We report here that mouse teratocarcinoma stem cells do express specific high-affinity LDL receptors; that their receptors resemble those of specialized tissue parenchymal cells and are distinct from those of phagocytic cells; and that a fluorescent derivative of LDL, which is readily taken up by the tumor cell receptors, provides a simple means of selecting or screening for receptor-deficient mutants.

MATERIALS AND METHODS

Teratocarcinoma Cell Line. The developmentally multipotential mouse teratocarcinoma cell culture line known as 6050P was used in these experiments. It is a feeder-independent line isolated from the OTT 6050 transplantable tumor (129/Sv *Sl^J C P* strain); its karyotype and other characteristics have been described (9). Monolayer stock cultures were grown in 250-ml flasks in 10 ml of basal "medium A" [Dulbecco's modified Eagle's medium supplemented with glucose (4500 mg/liter), 1% (vol/vol) glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml)] and 10% fetal calf serum, at 37°C and 5% CO_2 in air in a humidified incubator. Unless otherwise stated, experiments were carried out in 60-mm round culture dishes after dissociating the stock cultures in 0.05% trypsin/0.02% EDTA and seeding ("day 0") at 1×10^5 cells per dish in 3 ml of basal medium A/10% fetal calf serum. On day 2, cultures were washed with 3 ml of phosphate-buffered saline; 2 ml of basal medium A/10% calf lipoprotein-deficient serum (LPDS) (see below) was then added. Experiments were initiated on day 4 after 48 hr in the presence of LPDS.

Lipoproteins. Human LDL (density 1.019–1.063 g/ml), high-density lipoprotein (HDL; density 1.125–1.215 g/ml), and LPDS (density < 1.215 g/ml) were obtained from the plasma of healthy individuals and prepared by ultracentrifugation (14). Fetal calf LPDS (density < 1.215 g/ml) was prepared by ultracentrifugation (15). Acetylated LDL (acetyl-LDL) was derived by treatment of LDL with acetic anhydride (16). Reconstituted (r)-[dioleoyl fluorescein]LDL was produced by a reconstitution method in which the endogenous neutral lipids of LDL were removed by heptane extraction and replaced with dioleoyl fluorescein (17), a compound synthesized by T. Y. Shen and associates at Merck. For LDL and acetyl-LDL, the mass ratio of total cholesterol to protein was 1.5 to 1. For r-[dioleoyl fluorescein]LDL, the mass ratio of dioleoyl fluorescein to protein was 2.2 to 1. For HDL, the mass ratio of total cholesterol to protein was 1 to 4. The concentrations of all lipoproteins are expressed in terms of their protein content. LDL and acetyl-LDL were radiolabeled with ^{125}I as described (18).

Assays. The amounts of surface-bound ^{125}I-labeled LDL (^{125}I-LDL) (dextran sulfate-releasable ^{125}I-LDL), internalized ^{125}I-LDL (dextran sulfate-resistant ^{125}I-LDL), and degraded ^{125}I-LDL were measured in intact cell cultures by described methods (19). Degradation activity represents the cell-dependent rate of proteolysis and is expressed as micrograms or nanograms of ^{125}I-labeled trichloroacetic acid-soluble (noniodide) material released into the culture medium per mg of cell protein (18). The total cellular content of ^{125}I-LDL refers to surface-bound ^{125}I-LDL plus internalized ^{125}I-LDL (18). Activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase [HMG CoA reductase; mevalonate:NADP$^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34] was measured by determining the rate of conversion of 3-hydroxy-3-methyl[3-^{14}C]glutaryl CoA (12,000 cpm/nmol) to [^{14}C]mevalonate in detergent-solubilized extracts (14), and enzyme activity is expressed as picomoles of [^{14}C]mevalonate formed per min per mg of soluble protein. The incorporation of [^{14}C]oleate into cholesteryl [^{14}C]oleate by intact cells was measured as described (20), and the data are expressed as nanomoles of cholesteryl [^{14}C]oleate formed per hr per mg of total cell protein. The content of protein was determined by the method of Lowry *et al.* (21) with bovine serum albumin as a standard. All values represent the average of duplicate incubations. [^{14}C]Oleate (58 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels) and Na^{125}I (11–17 mCi/μg) were purchased from Amersham. DL-3-Hydroxy-3-methyl[3-^{14}C]glutaryl coenzyme A (49.5 mCi/mmol) was obtained from New England Nuclear.

Fluorescence Analyses. After incubation of cells with r-[dioleoyl fluorescein]LDL, the medium was removed and each culture was washed six times at 4°C with an albumin-containing buffer (19). The cells were dissociated in trypsin/EDTA for 1.5 min at 37°C, after which the cell suspension was transferred to a plastic tube containing 1 ml of ice-cold 50 mM Tris·HCl, pH 7.4/0.15 M NaCl (buffer A). The cells were centrifuged (2000 rpm, 5 min, 4°C), and the cell pellet was resuspended in 1 ml of ice-cold buffer A. Nonspecific background fluorescence was reduced by illuminating each cell suspension for 2 min at room temperature with a 75-watt Xenon lamp at a distance of 5 cm. The cells were then kept on ice (3–10 min) until they were analyzed in a Becton-Dickinson FACS III fluorescence-activated cell sorter (*laser settings*: 488-nm line, 300 mW of power in the light stabilized mode; *photomultiplier setting*: 500 V; *flow rate*: 10,000 cells per sample analyzed at approximately 200–300 cells per sec through a 50-μm aperture) (22). The light scatter gates were set to exclude small nonfluorescent cell debris and large cell aggregates. The observed fluorescence intensity is expressed on a relative scale which ranged from 1 to 128 units per cell.

For observation and photography in the fluoresence microscope, cells were grown on glass coverslips in petri dishes. On day 1 after seeding, the cells were washed in phosphate-buffered saline and medium A/10% calf LPDS was added. On day 2, this was replaced with medium A/10% calf LPDS/r-[dioleoyl fluorescein]LDL at 10 μg of protein per ml; unlabeled LDL (400 μg of protein per ml) was added to some cultures. After 19 hr at 37°C, the coverslips were washed six times in the albumin-containing buffer (19) and the cells were fixed with 3% paraformaldehyde in 0.2 M sodium phosphate (pH 7.3) for 10 min at room temperature. Coverslips were mounted with glycerol on glass slides and viewed in the phase contrast and epifluorescence modes of a Zeiss Photomicroscope III equipped with a fluorescein filter package (exciter filter, 485/20 nm; chromatic beam splitter, 510 nm; barrier filter, 520–560 nm).

RESULTS

Table 1 presents evidence for LDL receptor activity in mouse teratocarcinoma stem cells by demonstrating inhibitory effects of sterols on handling of labeled LDL. Cells growing in LPDS were incubated at 37°C for 3 hr with ^{125}I-LDL at 15 μg of protein per ml in the absence or presence of a 33-fold excess of unlabeled LDL. The amounts of high-affinity binding, internalization, and degradation of ^{125}I-LDL were calculated by subtracting the values obtained in the presence of excess unlabeled LDL from those observed in its absence (total values) (19). The measurements indicated that each of the three processes was reduced by more than 90% when the cells were subjected to prior incubation with a mixture of 25-hydroxycholesterol and cholesterol. These sterols have previously been shown to suppress specifically the LDL receptors in human fibroblasts, and the data of Table 1 resemble the data reported for human fi-

Table 1. LDL receptor activity in mouse teratocarcinoma stem cells: Suppression by prior incubation with sterols

Prior treatment of cells	^{125}I-LDL, ng/mg protein					
	Surface-bound		Internalized		Degraded	
	Total	High affinity	Total	High affinity	Total	High affinity
None	320	270	480	410	1820	1600
25-Hydroxycholesterol + cholesterol	77	24	67	43	270	96

On day 1, each culture received 3 ml of basal medium A/10% calf LPDS and either 3 μl of ethanol or a mixture of 1 μg of 25-hydroxycholesterol per ml plus 12 μg of cholesterol per ml added in 3 μl of ethanol. The medium was replaced on day 2. On day 3, each culture was fed 2 ml of medium A/10% human LPDS and ^{125}I-LDL (155 cpm/ng of protein) at 15 μg of protein per ml in the absence or presence of unlabeled LDL at 500 μg of protein per ml. After incubation for 3 hr at 37°C, the amounts of surface-bound, internalized, and degraded ^{125}I-LDL were measured. The *total* values were obtained in the absence of unlabeled LDL. The *high-affinity* values represent the difference between the values obtained in the absence and in the presence of unlabeled LDL.

broblasts and other cell types (12, 13). These data indicate that the teratocarcinoma cells possess an LDL receptor that mediates the high-affinity binding, internalization, and degradation of LDL, and that its activity is markedly reduced by incubation of cells with sterols.

To test the specificity of the receptor-mediated uptake and degradation processes, the teratocarcinoma cells were incubated at 37°C with ^{125}I-LDL at 25 μg of protein per ml in the presence of increasing concentrations of unlabeled LDL or of unlabeled HDL, which does not bind to the fibroblast LDL receptor (Fig. 1). Whereas unlabeled LDL competitively inhibited ^{125}I-LDL uptake (Fig. 1*A*) and degradation (Fig. 1*B*) by 50% at approximately 65 μg of protein per ml, unlabeled HDL did not inhibit significantly, even at concentrations as high as 1 mg of protein per ml.

Acetylation of LDL is known to block its ability to bind to the LDL receptor in human fibroblasts and other cell types (16, 23, 24). Fig. 2 shows that the rate of degradation of ^{125}I-acetyl-LDL in the teratocarcinoma cells was reduced by more than 90% when compared with that of ^{125}I-LDL. Moreover, there was no evidence for a saturable binding or uptake process for ^{125}I-acetyl-LDL (data not shown).

When LDL is taken up and degraded by fibroblasts and other cells through the receptor mechanism, the liberated cholesterol suppresses the activity of HMG CoA reductase, the rate-controlling enzyme in the cholesterol biosynthetic pathway. HDL, which is not taken up through the LDL receptor, does not suppress activity of this enzyme (14). Fig. 3 shows that a similar suppression of HMG CoA reductase occurs in the teratocarcinoma stem cells and that it is specific for LDL.

In fibroblasts and other cells, the cholesterol liberated from the receptor-mediated uptake of LDL enhances the rate of cholesteryl ester formation (20). This reaction can be followed by measuring the incorporation of [^{14}C]oleate into cholesteryl [^{14}C]oleate in intact cells. When the mouse teratocarcinoma cells were incubated with LDL, the rate of [^{14}C]oleate incorporation into cholesteryl [^{14}C]oleate was stimulated more than 150-fold (Fig. 4). This stimulation showed saturation at concentrations of LDL that were saturating for the ^{125}I-LDL binding, uptake, and degradation reactions (Fig. 2). Incubation of the cells with HDL at levels as high as 1 mg/ml caused no stimulation of cholesteryl [^{14}C]oleate formation (Fig. 4).

To demonstrate the LDL receptors on teratocarcinoma cells by visual methods, we used a recently developed LDL reconstitution technique to insert a fluorescent molecule into the core of LDL (17). The cholesteryl esters of LDL were extracted with heptane and replaced with a dioleoyl derivative of fluorescein. When the teratocarcinoma stem cells were incubated with the r-[dioleoyl fluorescein]LDL for 19 hr at 37°C, the cells accumulated substantial amounts of fluorescent material localized in discrete spots—probably lysosomes (Fig. 5 *a* and *b*). All cells took up the labeled LDL. Inclusion of excess native LDL in the incubation medium prevented the uptake of the fluorescent LDL (Fig. 5 *c* and *d*), confirming that uptake was occurring through the LDL receptor. To test further the requirement for the LDL receptor, the teratocarcinoma cells were first incu-

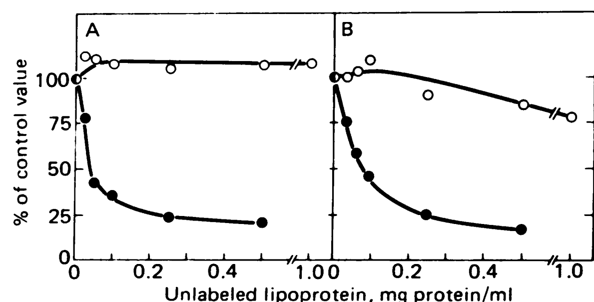

FIG. 1. Specificity of receptor-mediated uptake and breakdown of LDL in mouse teratocarcinoma stem cells shown by effect of unlabeled plasma lipoproteins on cellular content (*A*) and degradation (*B*) of ^{125}I-LDL. On day 4, each culture was fed with 2 ml of basal medium A/10% human LPDS, and ^{125}I-LDL (155 cpm/ng of protein) at 25 μg of protein per ml in the presence of the indicated concentration of unlabeled lipoprotein—either LDL (●) or HDL (○). After 5 hr at 37°C, the total cellular ^{125}I-LDL and total degradation of ^{125}I-LDL were measured; only the unlabeled LDL caused a marked competitive inhibition. The "100% of control values" (◐) for cellular content and degradation of ^{125}I-LDL were 300 and 4970 ng/mg of protein, respectively.

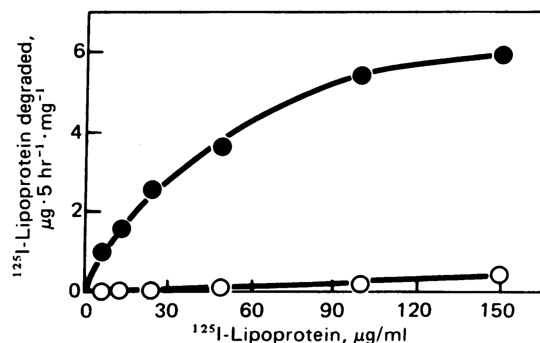

FIG. 2. Saturation curves for degradation of ^{125}I-LDL (●) and ^{125}I-acetyl-LDL (○) by teratocarcinoma stem cells. On day 4, each culture received 2 ml of basal medium A/10% LPDS and the indicated concentration of either ^{125}I-LDL (51 cpm per ng of protein) or ^{125}I-acetyl-LDL (50 cpm per ng of protein). The total ^{125}I-labeled lipoprotein (^{125}I-lipoprotein) degraded was measured after 5 hr at 37°C. Acetylation of LDL blocks its capacity to bind to the LDL receptor and to be subsequently degraded.

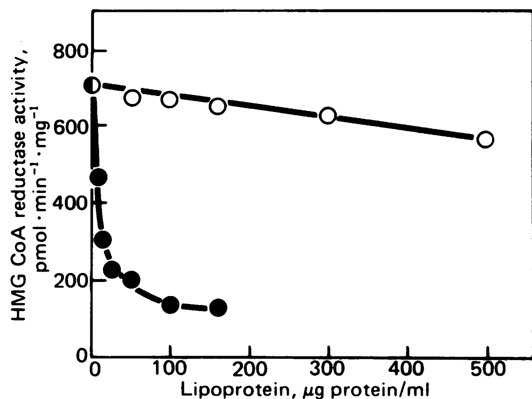

FIG. 3. Suppression of activity of HMG CoA reductase (the rate-limiting step in the cholesterol biosynthetic pathway) in teratocarcinoma stem cells by LDL, but not by HDL. On day 4, each culture was given 2 ml of basal medium A/10% calf LPDS and the indicated concentration of either LDL (●) or HDL (○). After 6 hr at 37°C, the HMG CoA reductase activity was specifically inhibited by the LDL.

bated in the presence of 25-hydroxycholesterol plus cholesterol. Uptake of fluorescent LDL was in fact abolished by these sterols (data not shown).

In order to quantify these visual data, the cells were removed from the dish by trypsinization and passed through a fluorescence-activated cell sorter. Fig. 6 shows the population distribution of fluorescence per cell. In cells incubated with r-[dioleoyl fluorescein]LDL, the mean fluorescence was 65 units per cell. When the incubation was performed in the presence of excess native LDL, the mean fluorescence was reduced to 17 units per cell. In cells that had been incubated with 25-hydroxycholesterol plus cholesterol to reduce the number of LDL receptors, the mean fluorescence in the absence of excess LDL was reduced to 22 units per cell, confirming that the uptake was occurring through the LDL receptor. After incubation with r-[dioleoyl fluorescein]LDL, 37% of the sterol-treated cells had a fluorescence < 15 units per cell, whereas only 1.6% of the untreated cells had fluorescence below this value.

DISCUSSION

The data presented here demonstrate that mouse teratocarcinoma stem cells express LDL receptors that resemble the receptors previously identified in other cell types, including cultured human fibroblasts of fetal or adult origin (12, 13),

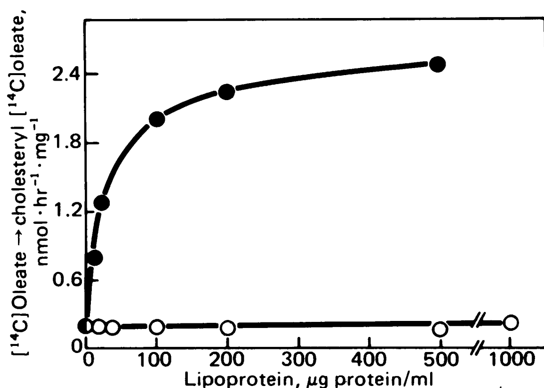

FIG. 4. Stimulation of cholesteryl ester formation in teratocarcinoma stem cells by LDL, but not by HDL. On day 4, each culture received 2 ml of basal medium A/10% calf LPDS and the indicated concentration of either LDL (●) or HDL (○). After 5 hr at 37°C, the culture was pulse-labeled with 0.1 mM [^{14}C]oleate (10,000 cpm/nmol) for 2 hr at 37°C. The formation of cholesteryl [^{14}C]oleate was increased over 150-fold by LDL.

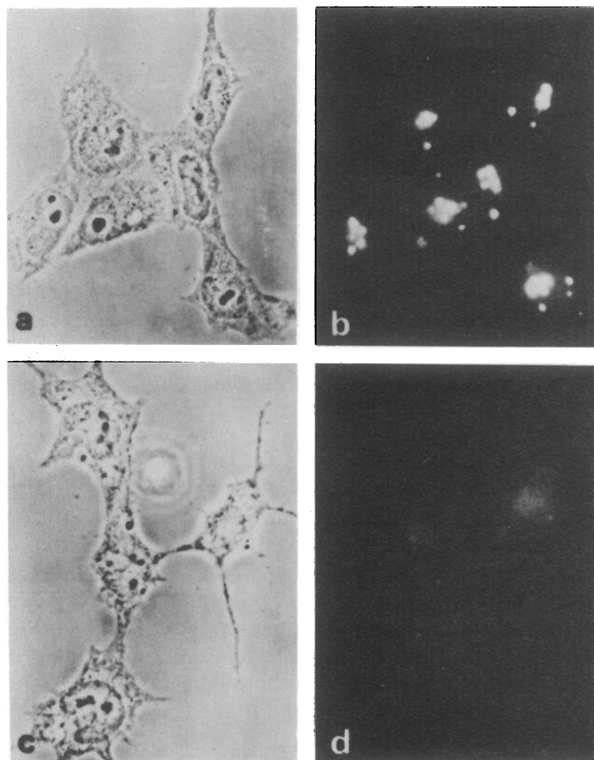

FIG. 5. Teratocarcinoma stem cells incubated with r-[dioleoyl fluorescein]LDL in the absence (*a*, *b*) or presence (*c*, *d*) of excess unlabeled LDL. In each pair of micrographs, the same group of cells is shown with fluorescence (*a*, *c*) and phase contrast (*b*, *d*) illumination. All cells incubated in the fluorescent LDL without excess unlabeled LDL were conspicuously fluorescent; the concentration of fluorescence appears to be lysosomal. Incubation in excess unlabeled native LDL blocked uptake of the fluorescent counterpart, as expected if both enter the cell through the LDL receptor. (×500.)

freshly isolated adult human lymphocytes (25), and cultured mouse adrenal carcinoma cells (26). These receptors enabled the teratocarcinoma cells to bind and internalize ^{125}I-LDL with high affinity and specificity and to degrade the lipoprotein efficiently. The cholesterol liberated from LDL suppressed HMG CoA reductase activity and stimulated cholesterol esterification, indicating that the cells were using the LDL-derived cholesterol to fulfill their sterol requirements.

Of considerable interest was the observation that the teratocarcinoma cells were unable to take up and degrade ^{125}I-acetyl LDL, a modified lipoprotein that does not bind to the LDL receptor (14, 23). Although ^{125}I-acetyl-LDL is not taken up by fibroblasts, or by parenchymal and other cells with comparable native-LDL receptors, it is bound and degraded with high affinity by various phagocytic cells, including peritoneal macrophages, hepatic Kupffer cells, and blood monocytes (23), all of which fail to bind native LDL (23). Thus, the sole LDL receptor type seen to date in mature parenchymal cells is the ontogenetically precocious type, possibly retained from the early totipotent cell stages. The later acetyl-LDL receptor type in phagocytes may be due to another gene.

The expression of LDL receptors in the teratocarcinoma stem cells establishes the possibility that cells bearing mutations in the gene encoding that LDL receptor might be isolated from mutagenized cultures. A practical basis for obtaining such mutants is suggested by the observations that teratocarcinoma cells incubated with r-[dioleoyl fluorescein]LDL took up the lipoprotein through the LDL receptor mechanism, whereas cells in which the LDL receptor had been suppressed by in-

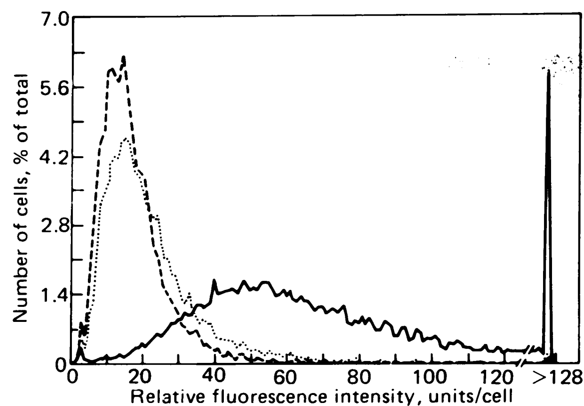

FIG. 6. Distribution of fluorescence in teratocarcinoma stem cells incubated with r-[dioleoyl fluorescein]LDL. On day 1 after subculture, each dish of cells was fed with 3 ml of basal medium A/10% calf LPDS and either 2 μl of ethanol (—, - - -) or a mixture of 25-hydroxycholesterol at 1 μg/ml plus cholesterol at 12 μg/ml added in 2 μl of ethanol (. . .). On day 2, the same medium was renewed, with the addition of r-[dioleoyl fluorescein]LDL at 10 μg of protein per ml in the absence (—, . . .) or presence (- - -) of unlabeled LDL at 400 μg of protein per ml. After 19 hr at 37°C, 10,000 cells from each set of cultures were analyzed in the fluorescence-activated cell sorter. In cultures incubated with r-[dioleoyl fluorescein]LDL alone (—), 6% of the cells had a fluorescence intensity greater than 128 units per cell; this is shown by the sharp peak at the far right of the distribution curve.

cubation with 25-hydroxycholesterol plus cholesterol took up much less fluorescent LDL. These two populations of cells could be separated in the fluorescence-activated cell sorter such that only 1.6% of the untreated (fluorescing) cells would be collected, whereas 37% of the phenotypically receptor-deficient (nonfluorescing) cells would be captured. The next step in this selection scheme will be to mutagenize the teratocarcinoma cells, incubate the cells with r-[dioleoyl fluorescein]LDL, and use the fluorescence-activated cell sorter to isolate a population enriched for cells that have lost LDL receptors. These receptor-deficient cells could be returned to culture, reincubated with fluorescent LDL, and again sorted. After several cycles, only stable variants lacking LDL receptors should be recovered. The technique lends itself to a step-selection procedure whereby heterozygous cells that have lost or altered only one gene for the receptor would be harvested, based on their uptake of half-normal amounts of fluorescent LDL. After purification by repeated cycles of growth and sorting, the heterozygous cells could be subjected to a second cycle of mutagenesis and homozygous deficient cells could then be obtained.

An alternative means of isolating receptor-deficient mutants would be by screening, in the fluorescence and phase contrast microscopes, culture plates seeded at relatively low density after mutagenesis. Low-fluorescing or nonfluorescing colonies could be readily identified and picked. The principle involved in this method and in the preceding method of selection through sorting for low-fluorescing cells is obviously applicable to comparable experiments with other receptor systems.

Teratocarcinoma stem cells have been shown to be capable of conversion to normalcy after injection into embryos at the blastocyst stage (1, 2). There they can contribute to formation of normal somatic tissues in mosaic animals and, through the germ line, to progeny. Therefore, tumor stem cells with LDL receptor mutations could be incorporated into blastocysts to create mosaic mice containing cells that lack the LDL receptor, in a manner analogous to the synthesis of mosaic mice with Lesch–Nyhan-type cells lacking hypoxanthine phosphoribosyltransferase activity (10). Such mice would be of considerable value in analyzing the physiology of the LDL receptor-deficient state at the whole-animal level. If a pure strain of homozygous LDL receptor-deficient mice could be bred from them, the animals would provide the first valid model that mimics genetic hypercholesterolemia in man.

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