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Amphotericin B selection of mutant Chinese hamster cells with defects in the receptor-mediated endocytosis of low density lipoprotein and cholesterol biosynthesis

(polyene antibiotic/receptor-dependent killing/cholesterol auxotrophs)

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ABSTRACT **This paper describes a rapid and efficient two-step procedure for the isolation of mutant cells with defects in receptor-mediated endocytosis. The procedure takes advantage of two fungal metabolites, compactin (ML236B), a potent inhibitor of cholesterol biosynthesis, and amphotericin B, a polyene antibiotic that forms toxic complexes with sterols in membranes. Mutagen-treated Chinese hamster ovary cells were preincubated overnight in a medium containing mevalonate, low density lipoprotein (LDL), and compactin (Mev/LDL/Com). At the end of the preincubation period, wild-type cells were cholesterol replete while mutant cells that could not utilize the cholesterol in LDL were cholesterol deficient. Subsequent incubation with amphotericin B for 6 hr killed most of the wild-type cells. After a second round of Mev/LDL/Com–amphotericin B selection, endocytosis-defective clones appeared at a frequency of $\approx 2.6 \times 10^{-5}$. Some of these clones expressed LDL receptor-defective phenotypes and fell into one of two previously defined classes of mutation. Sensitivity of the mutants to infection by vesicular stomatitis virus suggested that the mutations do not disrupt the coated pit–coated vesicle pathway of endocytosis. Minor modifications in the Mev/LDL/Com–amphotericin B selection permit the isolation of cholesterol auxotrophs and might allow the isolation of conditional-lethal mutations. Because LDL can be coupled to ligands that bind to receptors other than the LDL receptor, Mev/LDL/Com–amphotericin B selection may permit the isolation of mutant cells with defects that specifically disrupt other endocytic pathways.**

Receptor-mediated endocytosis is a process responsible for the cellular uptake of a wide variety of physiologically active macromolecules, including transport proteins, viruses, and peptide hormones (1). These macromolecules bind to specific cell surface receptors with high affinity, are internalized through coated pits and coated vesicles, and in many cases are subsequently degraded in lysosomes. The lysosomal hydrolysis of the cholesteryl esters in the core of plasma low density lipoprotein (LDL) supplies mammalian cells with cholesterol which, in combination with endogenously synthesized cholesterol, serves as a substrate for membrane synthesis. Cholesterol is also a negative feedback regulator of LDL receptors and 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, the enzyme that converts HMG-CoA to mevalonate in the rate-controlling step of cholesterol biosynthesis. Mevalonate is a precursor both for sterol and essential isoprenoid (e.g., dolichol and ubiquinone) synthesis (2).

In the current studies, we report a rapid and simple approach for isolating mutant Chinese hamster ovary (CHO) cells with defects in the receptor-mediated endocytosis of LDL. The selection protocol takes advantage of two fungal metabolites, compactin and amphotericin B. Amphotericin B is a polyene antibiotic that forms toxic complexes with the cholesterol (or analogous sterols) in normal but not cholesterol-deficient cellular membranes (refs. 3 and 4; unpublished data). The current studies are based on the previous use of polyene antibiotics to isolate mutants in sterol biosynthesis (5–8). Compactin is a potent competitive inhibitor of HMG-CoA reductase (9). When CHO cells are incubated with compactin, they become cholesterol deficient and eventually (2–5 days) die (ref. 10; unpublished data). When CHO cells are incubated with compactin and LDL, only those that can utilize the cholesterol in LDL are sensitive to amphotericin B. Those that cannot are cholesterol deficient and, therefore, resistant to amphotericin B. Using LDL-dependent amphotericin B toxicity, we have isolated mutant clones of CHO cells that are markedly deficient in LDL receptor activity.

MATERIALS AND METHODS

Materials. Human and newborn calf lipoprotein-deficient sera and human LDL were prepared by ultracentrifugation (11). ^{125}I-Labeled LDL (^{125}I-LDL) and LDL reconstituted with the trioleoyl glycerol triolein (r-[tg]LDL) were prepared as described (11, 12). The stock of vesicular stomatitis virus (VSV, Glascow strain) was a gift from H. Lodish. All other reagents and supplies were obtained as described (refs. 11–13; also unpublished data).

Cells. Parental CHO-K1 cells and subclones of the LDL receptor-deficient mutant strains 14-1a1 (14-2) and 11-1a1 (11-2) were maintained in culture as described (13). Stock cultures were grown in medium A [Ham's F-12 medium containing penicillin (100 units/ml), streptomycin (100 μg/ml), and glutamine (2 mM)] supplemented with 10% (vol/vol) newborn calf serum. The parental CHO strain was subcloned prior to mutagenesis. Cells were treated with the mutagen ethyl methanesulfonate in 10% newborn calf serum as described (13). All incubations were at 37°C in 5% CO_2/95% air unless otherwise noted.

Two-Step Isolation of Mutant Clones. *Step I: Preincubation to deplete mutant cells of cholesterol.* Mutagen-treated or control parental CHO cells were seeded into 100-mm Petri dishes (10^6 cells per dish) in medium A supplemented with 3% (vol/vol) newborn calf lipoprotein-deficient serum and allowed to grow for 2 days to induce high levels of LDL receptors and HMG-CoA reductase. Each dish was then re-fed with 7 ml of medium

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Abbreviations: LDL, low density lipoprotein; HMG, 3-hydroxy-3-methylglutaryl; r-[tg]LDL, LDL reconstituted with the trioleoyl glycerol triolein; VSV, vesicular stomatitis virus; Mev/LDL/Com, medium A containing newborn calf lipoprotein-deficient serum (3%), mevalonate (0.25 mM), LDL (30 μg of protein/ml), and compactin (5 μM).

A supplemented with 3% newborn calf lipoprotein-deficient serum, 0.25 mM mevalonate, LDL (30 μg of protein per ml), and 5 μM compactin (Mev/LDL/Com). The low concentration of mevalonate was added to insure synthesis of all essential nonsteroidal isoprenoids (ref. 2; unpublished data).

Step II: Incubation with amphotericin B to kill cholesterol-replete cells. After preincubation with Mev/LDL/Com for 24 hr, the cells were washed once with phosphate-buffered saline and refed with 1% newborn calf lipoprotein-deficient serum containing 300 μg of amphotericin B per ml. After incubation for 7–8 hr, the cells were washed three times and cultured in 3% newborn calf lipoprotein-deficient serum. Colonies were isolated and cloned or fixed and stained as described (13).

Assays. The amounts of high-affinity ^{125}I-LDL surface binding, internalization, and degradation were measured in medium B (medium A supplemented with 5% human lipoprotein-deficient serum) at 37°C or 4°C as described (13). The protein concentration of cells and lipoproteins and the inhibition of protein synthesis (incorporation of [^{3}H]leucine) by the toxic lectin ricin were measured as described (11). The sensitivity of cell strains to infection by VSV was measured by plaque assay (14). The sensitivity of clones to amphotericin B was determined by a microtiter plate assay (unpublished data).

RESULTS

The cholesterol biosynthesis inhibitor compactin can induce resistance to amphotericin B in CHO cells, and the provision of an exogenous source of cholesterol can abolish this compactin-conferred resistance (unpublished data). In the experiment shown in Fig. 1, cells were preincubated overnight with the indicated additions and then incubated for 6.5 hr in the absence or presence of amphotericin B. When cells were preincubated in the absence of compactin (Fig. 1, column I), they were sensitive to amphotericin B. Preincubation with 5 μM compactin induced amphotericin B resistance (Fig. 1, column II, third row), which was abolished by the addition of LDL (≥5 μg of protein per ml) to the preincubation medium (column II, rows 4–6). Thus, when parental cells were preincubated with compactin, the toxicity of amphotericin B became LDL-dependent.

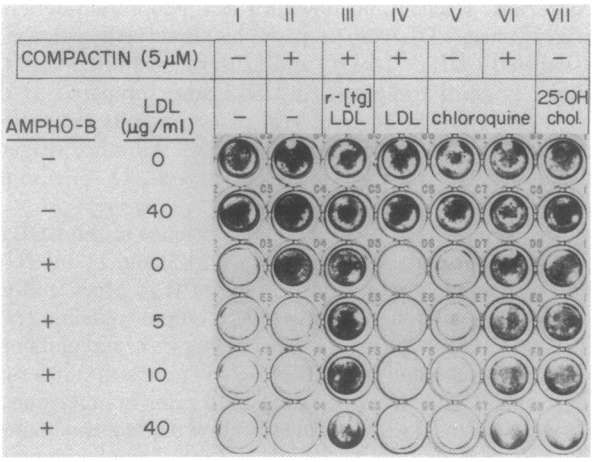

FIG. 1. LDL-dependent amphotericin B (AMPHO-B) killing of CHO cells: Effects of inhibitors of the LDL pathway. On day 0, CHO cells were seeded at a concentration of 32,000 cells per well in 0.2 ml of 3% newborn calf lipoprotein-deficient serum into the wells of a microtiter plate. On day 1, the medium was replaced with 0.2 ml of 3% newborn calf lipoprotein-deficient serum containing 0.25 mM mevalonate, the indicated additions of compactin and LDL, and one of the following additions: no additions (columns I and II), r-[tg]LDL at 400 μg of protein per ml (column III), LDL at 400 μg of protein per ml (column IV), 90 μM chloroquine (columns V and VI), or 25-hydroxycholesterol at 10 μg/ml (column VII). On day 2 (19 hr later), the cells were washed and incubated for 6.5 hr with 0.1 ml of 1% newborn calf lipoprotein-deficient serum in the absence (upper two rows) or presence (lower four rows) of 300 μg of amphotericin B per ml. Afterwards the cells were washed, fixed, and stained with crystal violet.

The reconstituted LDL r-[tg]LDL cannot deliver cholesterol to cells but can bind with normal affinity to LDL receptors (12). When a large excess of r-[tg]LDL was added to the preincubation medium to block LDL receptors, LDL-dependent amphotericin B toxicity was inhibited (Fig. 1, column III). The addition of excess native LDL did not interfere with amphotericin B toxicity (Fig. 1, column IV). In a separate experiment (data not shown), the previously isolated LDL receptor-defective clone 14-2 (11, 13) was markedly resistant to LDL-dependent amphotericin B toxicity. Thus, LDL receptor activity was essential for LDL-dependent amphotericin B toxicity. Preincubation of CHO cells with the lysosomotropic drug chloroquine (Fig. 1, columns V and VI) did not alter the effects of amphotericin B in cells preincubated in the absence of LDL, but it did prevent LDL-dependent amphotericin B toxicity (column VI). These results suggest that lysosomal degradation of LDL was required for LDL-dependent amphotericin B killing.

25-Hydroxycholesterol inhibits cholesterol biosynthesis by suppressing the activity of HMG-CoA reductase and inhibits cellular uptake of LDL by suppressing LDL receptor activity (13). The addition of 25-hydroxycholesterol to the preincubation medium induced amphotericin B resistance (Fig. 1, column VII). This resistance could only be overcome by the addition of very large amounts (40 μg of protein per ml) of LDL, presumably because LDL uptake was inhibited by the suppression of receptor activity.

These results suggested that LDL-dependent amphotericin B toxicity requires an intact LDL pathway of endocytosis and, thus, could be used to isolate CHO cells defective in virtually any step of the pathway. Therefore, cells were subjected to the two-step selection protocol of preincubation in Mev/LDL/Com for 24 hr, followed by exposure to amphotericin B (300 μg/ml) for 6.5 hr. Only a few colonies of parental CHO cells could survive one round of this selection (Table 1), and no parental colonies survived two rounds of selection (survival frequency, $<3 \times 10^{-7}$). In contrast, even after two rounds of selection, there was a high frequency of survival, 2.6×10^{-5}, when the cells had been treated with the mutagen ethyl methanesulfonate.

Nine clones derived from six independent stocks of mutagen-treated CHO cells were isolated by using Mev/LDL/Com–

Table 1. Isolation of CHO cells resistant to Mev/LDL/Com–amphotericin B: Dependence on mutagenesis with ethyl methanesulfonate

Rounds of selection	Colonies surviving treatment, no. per 10^6 cells: Without EtMes	With EtMes
1	6	182
2	0	26

Parental CHO cells and CHO cells treated with ethyl methanesulfonate (EtMes) were seeded into 100-mm plastic dishes at a concentration of 10^6 cells per dish, subjected to either one or two rounds of Mev/LDL/Com–amphotericin B selection, and subsequently fixed and stained. The data from two independent experiments were pooled. The total numbers of cells subjected to selection were: no EtMes, one round—9×10^6; no EtMes, two rounds—3×10^6; with EtMes, one round—9×10^6; with EtMes, two rounds—14×10^6.

amphotericin B selection and were analyzed in detail. One clone (320-12a-1) expressed a wild-type phenotype. Seven of the clones were sensitive to amphotericin B when preincubated in the absence of compactin but were resistant after preincubation in Mev/LDL/Com (Table 2). All of these clones were markedly defective in binding, uptake, and degradation of ^{125}I-LDL at 37°C (Table 2). To compare directly the LDL receptor binding activity of the parental CHO cells and the clones when endocytosis was inhibited (1), the surface binding of ^{125}I-LDL was measured at 4°C. All of the clones examined exhibited markedly reduced levels of ^{125}I-LDL binding at 4°C (data not shown). To determine if the reduction in LDL processing was due to an alteration in the affinity of the LDL receptor, we measured the saturation kinetics for ^{125}I-LDL degradation by parental CHO cells and clone 320-7a-1 (Fig. 2). Clone 320-7a-1 did not significantly degrade ^{125}I-LDL at lipoprotein concentrations as high as 80 μg of protein per ml.

Table 2. Characteristics of Mev/LDL/Com–amphotericin B-resistant CHO clones*

Clone designation	Sensitivity to amphotericin B† after additions: Mev	Mev/LDL/Com	Metabolism of ^{125}I-LDL at 37°C,‡ % control: Binding	Internalization	Degradation
Parental CHO	Yes	Yes	100	100	100
320-2b-2	—	—	8	15	3
320-5a-2	Yes	No	6	7	4
320-6a-1	Yes	No	3	5	1
320-6b-2	Yes	No	0	2	1
320-7a-1	Yes	No	5	5	1
320-9a-1	Yes	No	3	4	2
320-9a-4	Yes	No	2	4	2
320-9b-1	Yes	No	9	6	1
320-12a-1	Yes	Yes	77	58	59§

Clones were isolated after two rounds of Mev/LDL/Com–amphotericin B selection. After the first round of selection, cells were grown in 3% newborn calf lipoprotein-deficient serum. After the second round of selection, the cells were grown in 10% newborn calf serum. The fourth digit in the clone designation indicates the independent stock of mutagen-treated cells from which the clone was derived.

* Each clone was tested in one of six separate experiments, each of which included parental CHO cells for controls.

† On day 0, clones were seeded into a 96-well microtiter plates at a concentration of 32,000 cells per well in 0.2 ml of 3% newborn calf lipoprotein-deficient serum. On day 1, the monolayers were re-fed with 0.2 ml of 3% newborn calf lipoprotein-deficient serum containing 0.25 mM mevalonate (Mev) in the presence of the following: no further additions, 5 μM compactin, or 5 μM compactin and LDL (30 μg of protein per ml) (Mev/LDL/Com). On day 3, the wells were washed, incubated for 4–7 hr with 0.1 ml of 1% newborn calf lipoprotein-deficient serum containing 300 μg of amphotericin B per ml, rewashed, fixed, and stained. In all cases, the clones were resistant to amphotericin B when they were preincubated with compactin in the absence of LDL (data not shown).

‡ On day 0, clones were seeded into 6-well plates at a concentration of 250,000 cells per well in 3 ml of 3% newborn calf lipoprotein-deficient serum. On day 2, the medium was replaced with 1 ml of medium B containing ^{125}I-LDL (10 μg of protein per ml; 257 cpm/ng of protein) in the presence (single incubations) or absence (duplicate incubations) of unlabeled LDL (400 μg of protein per ml). After incubation for 5 hr at 37°C, the amount of ^{125}I-LDL degradation products excreted into the medium and the amounts of surface-bound and internalized ^{125}I-LDL were determined. Each clone was tested in one of four separate experiments, and the high-affinity values are expressed as the percentage of control values for the parental CHO cells. The mean (SD) of control values for binding, uptake, and degradation were: 87 (14) ng/mg, 378 (80) ng/mg, and 3,378 (377) ng/5 hr per mg, respectively.

§ In another experiment (data not shown), clone 320-12a-1 exhibited saturation kinetics for ^{125}I-LDL degradation that were essentially identical to those of the parental CHO cells.

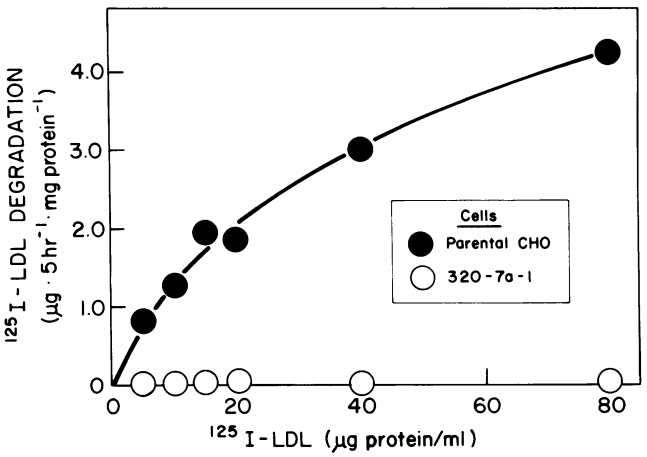

FIG. 2. Saturation kinetics for the degradation of ^{125}I-LDL at 37°C by monolayers of parental CHO cells and clone 320-7a-1. On day 0, parental CHO (●) and clone 320-7a-1 (○) cells were seeded in 3% newborn calf lipoprotein-deficient serum into the wells of 24-well dishes at a concentration of 60,000 cells per well. On day 2, each monolayer received 0.5 ml of medium B containing the indicated amount of ^{125}I-LDL (198 cpm/ng of protein). After incubation for 5 hr at 37°C, the amounts of ^{125}I-LDL degradation products excreted into the medium were determined.

Previously isolated LDL receptor-defective CHO clones fall into two classes (11, 13). The *icc* class of mutants (e.g., clone 11-2) can induce LDL receptor activity in the *cbc* class (e.g., clone 14-2) when the cells are in close proximity during cocultivation (11). Table 3 shows that cocultivation of Mev/LDL/Com–amphotericin B-selected clones with *cbc* clone 14-2 induced LDL receptor activity. Thus, these clones fall into the *icc* class. Unlike parental CHO cells or clone 14-2, clone 11-2 is hypersensitive to the toxic lectin ricin (11). Clones 320-9b-1 and 320-6a-1 (Fig. 3) and the other receptor-defective clones (data not shown) do not share the ricin hypersensitivity of clone 11-2.

Defects that prevent the expression of LDL receptor activity also might inhibit the expression of other endocytic pathways (e.g., a pleiotropic defect in receptor recycling through coated pits and vesicles). CHO cells normally express the mannose 6-phosphate receptor pathway and can be infected by VSV, a lipid envelope virus that can enter some cells through coated pits (15–17). Clone 320-7a-1 expressed normal levels of mannose 6-phosphate (lysosomal enzyme) receptor activity (data not shown). In addition, the parental and receptor-defective clones were efficiently infected by VSV (data not shown), although there were minor quantitative differences (2- to 3-fold) in plaque formation on the different clones. Thus, the defects in endocytosis in these clones are not likely to be a consequence of pleiotropic disruptions in the coated pit–coated vesicle pathway of endocytosis.

Cholesterol auxotrophs should become sterol deficient (amphotericin B resistant) and eventually die if incubated in the absence of an exogenous source of sterol (e.g., in 3% newborn calf lipoprotein-deficient serum). The Mev/LDL/Com–amphotericin B protocol was slightly modified by growing colonies after selection in 10% newborn calf serum to permit the survival of sterol auxotrophs. Mutagen-treated CHO cells survived at a frequency of 1.9×10^{-4} after two rounds of this modified selection. One of 10 clones that were analyzed in detail (clone 320-8a-2) was LDL receptor-defective and fell into the *icc* class of mutants (Table 3). The remaining clones were ex-

Table 3. Complementation of clone 14-2 by cocultivation with receptor-defective clones

Clone	^{125}I-LDL degradation, % control*	
	Pure clone	Mixture with clone 14-2
Parental CHO	100	—
14-2	5†	—
11-2	6	45
320-2b-2	3	49
320-5a-2	6	44
320-6a-1	1	37
320-6b-2	2	48
320-7a-1	0.4	51
320-9a-1	3	43
320-9a-4	5	46
320-9b-1	2	34
320-8a-2‡	3	43

On day 0, pure clones or mixtures of clone 14-2 and the indicated clones (cell ratio of 4:1, respectively) were seeded into 24-well plates at a total concentration of 60,000 cells per well in 1.5 ml of 3% newborn calf lipoprotein-deficient serum. On day 2, the medium was replaced with 0.5 ml of medium B containing ^{125}I-LDL (10 μg of protein per ml; 204 cpm/ng of protein) in the presence (single incubations) or absence (duplicate incubations) of unlabeled LDL (400 μg of protein per ml). After incubation for 5 hr at 37°C, the amounts of ^{125}I-LDL degradation products excreted into the medium were determined.

* The high-affinity values for degradation from two independent experiments are expressed as the percentage of the parental CHO control (2,353 or 1,924 ng/5 hr per mg) measured in the same experiment.

† Average from two experiments.

‡ Clone isolated by the modified Mev/LDL/Com–amphotericin B procedure (see *Results*).

amined for their ability to grow in 10% newborn calf serum or in 3% newborn calf lipoprotein-deficient serum in the presence or absence of either mevalonate or LDL.* Fig. 4 shows that parental CHO cells (left column) and LDL-receptor-defective clone 320-7a-1 (right column) can grow under all of these conditions. In contrast, four representative clones (center columns) isolated by using the modified procedure could not grow without an exogenous source of cholesterol (10% newborn calf serum or LDL), suggesting that these clones are sterol auxotrophs. The inability of mevalonate to serve as a source of sterol suggests that the defects in these cells affect steps distal to the synthesis of mevalonate.

DISCUSSION

This paper reports a two-step procedure for the isolation of mutant CHO cells with LDL receptor-defective phenotypes. In the first step, cells were preincubated overnight in Mev/LDL/Com medium, which contains lipoprotein-deficient serum. Cells with defects in the expression of the LDL pathway of receptor-mediated endocytosis were rendered cholesterol deficient while cells that internalized LDL normally were cholesterol replete (unpublished data). In the second step, the preincubation medium was removed and the cells were incubated with amphotericin B, a polyene antibiotic that forms toxic complexes with sterols (refs. 3, 4, 7, 8; unpublished data). Only those cells with the desired mutant phenotypes were cholesterol deficient and, thus, survived the treatment with amphotericin B.

* In other experiments the addition of 20 mM mevalonate permitted growth of parental CHO cells in 3% newborn calf lipoprotein-deficient serum when cellular mevalonate synthesis was inhibited by compactin (data not shown).

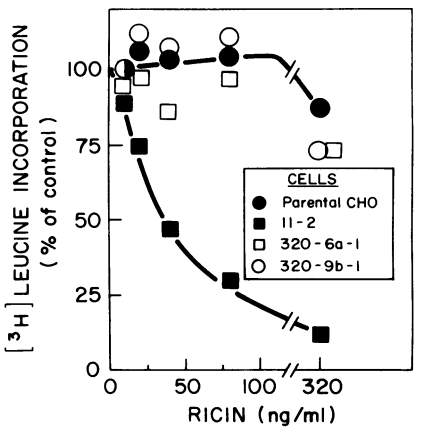

FIG. 3. Effects of ricin on protein synthesis in parental and LDL receptor-defective CHO cells. On day 0, parental CHO (●), clone 11-2 (■), clone 320-6a-1 (□), and clone 320-9b-1 (○) cells were seeded in 3% newborn calf lipoprotein-deficient serum into the wells of 24-well dishes at a concentration of 80,000 cells per well. On day 2, the medium was removed, and each monolayer received 0.5 ml of 3% newborn calf lipoprotein-deficient serum containing the indicated amount of ricin. After a 4-hr incubation at 37°C, the medium was removed, the monolayers were washed, and the cells were re-fed (0.5 ml per well) with 3% newborn calf lipoprotein-deficient serum containing 2.5 μCi of [^{3}H]leucine per ml. After a 2-hr incubation, the amount of radioactivity incorporated into each monolayer was determined. The 100% control values were: parental CHO, 35,492 cpm per well; clone 11-2, 12,301 cpm per well; clone 320-6a-1, 32,748 cpm per well; and clone 320-9b-1, 26,533 cpm per well.

The altered phenotypes of the isolated clones are believed to arise as a result of mutation because the appearance of such cells required mutagenesis with ethyl methanesulfonate, and the phenotypes were stably transmitted when the cells were cloned and grown under nonselective conditions. The estimated frequency of induced mutations in the LDL pathway (2.6×10^{-5} per cell) was similar to the frequency observed for the induction of receptor-defective mutations isolated by using toxic and fluorescent reconstituted LDL (13). The LDL receptor-defective mutants isolated in this study appear to affect only the LDL pathway and are all members of the *icc* class of mutations, one of two previously defined classes of LDL receptor-defective mutations. The *icc* class of mutants can induce LDL receptor activity in the *cbc* class when the cells are in close

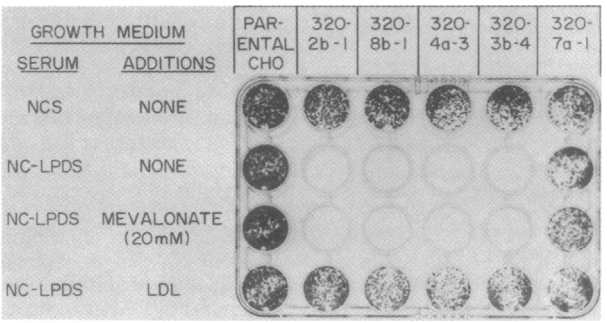

FIG. 4. Growth in lipoprotein-deficient medium of parental CHO cells and mutant clones isolated by the modified Mev/LDL/Com–amphotericin B procedure: Dependence on LDL. On day 0, parental CHO cells (left column), LDL receptor-defective clone 320-7a-1 (right column), and clones isolated by using the modified selection procedure (center columns) were seeded at a concentration of 1,000 cells per well into a 24-well plate in 1.5 ml of either 10% newborn calf serum or 3% newborn calf lipoprotein-deficient serum containing no further additions or mevalonate (20 mM) or LDL (30 μg of protein per ml). On day 6, the cultures were fixed and stained.

proximity during cocultivation (11). Complementation analysis by cell fusion and immunological analysis with anti-LDL receptor antibodies should help to define the genetic and biochemical complexity of this set of mutations.

Amphotericin B and other polyene antibiotics have been used to isolate cells with alterations in cholesterol metabolism (5–8). The Mev/LDL/Com–amphotericin B procedure modified for postselection growth in 10% newborn calf serum was essentially the same as the method of Saito *et al.* (6) and permitted rapid isolation of clones that are probably cholesterol auxotrophs with defects distal to mevalonate synthesis. The auxotrophs presumably could not take up the LDL in Mev/LDL/Com medium because of the deleterious effects of growth in lipoprotein-deficient medium prior to incubation in Mev/LDL/Com. It should be possible to use amphotericin B to isolate auxotrophs in specific steps of the cholesterol biosynthetic pathway. Such auxotrophs could grow in newborn calf lipoprotein-deficient serum only if the appropriate biosynthetic intermediate were added to the culture medium. By temporarily incubating mutagen-treated cells in newborn calf lipoprotein-deficient serum without the added intermediate, specific auxotrophs could be depleted of sterols, rendered amphotericin B resistant, and therefore selected. For example, mutants defective in steps proximal to or including the HMG-CoA reductase reaction might be isolated by using mevalonate as the intermediate.

The Mev/LDL/Com–amphotericin B selection procedure is more rapid (one round of selection takes 30–36 hr) and efficient than our previous method, which used toxic and fluorescent reconstituted LDL (13). It should be possible to use this procedure to generate large numbers of mutants so that the receptor-mediated endocytosis of LDL can be defined and characterized in greater genetic and biochemical detail. Because the cells need only be exposed to selection conditions for a short time (overnight), it may be possible to modify the Mev/LDL/Com–amphotericin B method to permit the isolation of cells with conditional-lethal mutations. By crosslinking other receptor ligands (e.g., lysosomal enzymes, peptide hormones) and antibodies to LDL, it should be possible to adapt the Mev/LDL/Com–amphotericin B selection to isolate mutants with defects in other endocytic pathways. The reversion of such mutants by DNA-mediated gene transfer may prove to be a useful tool for isolating the genes involved in receptor-mediated endocytosis.

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