Overexpression of Low Density Lipoprotein Receptor on Chinese Hamster Ovary Cells Generates Foam Cells

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The atherosclerotic lesion is characterized by the presence of cholesterol-loaded foam cells. Chinese hamster ovary (CHO) cells do not normally store cholesteryl esters because low density lipoprotein (LDL) receptors are suppressed by exposure of these cells to LDL cholesterol. We transfected LDL receptor cDNA linked to the simian virus 40 early promoter into CHO cells (CHO 29) and found that LDL receptor binding in these cells was not suppressed by an excess amount of LDL cholesterol, indicating no regulation of the LDL receptor in CHO 29 cells. Furthermore, CHO 29 cells showed a high activity of LDL uptake and intracellular accumulation of cholesteryl esters. Light-microscopic examination demonstrated the resulting formation of foam cells in CHO 29 cells in the presence of 5 μg LDL/ml. These results demonstrated that foam cell changes in atherosclerotic lesions can be reproduced in CHO cells, whose LDL receptor activity is overexpressed, through the mechanism of LDL receptor-mediated endocytosis of native LDL. (Arteriosclerosis and Thrombosis 1991;11:1310–1314)

Cellular cholesterol balance is regulated by sterol-mediated feedback repression of genes whose products mediate the uptake of exogenous cholesterol and synthesis of cholesterol within the cells. When cells are exposed to an excess of low density lipoprotein (LDL), cholesterol uptake is suppressed through repression of the gene for the LDL receptor. The cultured macrophages and smooth muscle cells take up and degrade native LDL. However, these cells do not accumulate cholesteryl ester, even during prolonged incubation with an excess of LDL because of the suppression of LDL receptor activity. On the other hand, in early atherosclerotic lesions both monocyte-derived macrophages and smooth muscle cells store a large amount of cholesteryl ester, resulting in the generation of foam cells. Researchers have proposed that generation of modified LDL such as oxidized LDL and the subsequent uptake of modified LDL through the scavenger receptor play important roles in foam cell formation in the arterial wall. Recent study has suggested that native LDL can be stored in fibroblast cell lines transfected with simian virus (SV) 40 due to an altered regulation of LDL receptor activity. In the present study, we investigated whether native LDL could be stored in CHO cells whose expression of the LDL receptor was driven by the transfected SV40 promoter and was not suppressed by exogenous LDL.

Methods

Materials

Chinese hamster ovary (CHO-K) cells were purchased from the American Type Culture Collection. Iodine-125-labeled NaI and 125I-protein A were purchased from ICN Biochemicals, Inc., Irvine, Calif. Ham’s F-12 medium (HAM), phosphate-buffered saline (PBS), and newborn calf serum (NCS) were obtained from GIBCO, Grand Island, N.Y. Geneticin (G418) and bovine serum albumin (BSA) were from Sigma Chemical Co., St. Louis, Mo. Other reagents used were all of the highest grade available.

Cell Cultures

Wild-type CHO-K cells were maintained in HAM containing 10% NCS in humidified 5% CO2 incubators at 37°C.
Transfection and Selection of CHO Cells Expressing Human Low Density Lipoprotein Receptor

pLDLR-2 containing full-length human LDL receptor cDNA linked to an early SV40 promoter was kindly provided by T. Yamamoto. pLDLR-2 and pSV2Neo containing a neomycin-resistant gene were cotransfected into CHO-K cells by the CaCl₂ coprecipitation technique. CHO-K cells were seeded at 0.8x10⁶ cells/10-cm dish and cultured in HAM with 10% NCS for 24 hours. Four hours before transfection, the medium was changed to 5 ml Dulbecco's modified Eagle's medium with 10% fetal calf serum (FCS), DNA-CaCl₂ coprecipitates were prepared as follows. Two hundred microliters of the solution containing 10 μg DNA of pLDLR-2, 2 μg DNA of pSV2Neo, and 250 mM CaCl₂ was added dropwise to the same volume of 2x Hank's balanced salt solution buffer (280 mM NaCl, 50 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 1.5 mM Na₂HPO₄ [pH 7.0–7.1]), and the mixture was kept still at room temperature for 30 minutes. Then the mixture was added to each medium and incubated for 6 hours. The cells were washed once with PBS without CaCl₂ and shocked for 90 seconds with 15% glycerol in 1x Hank's balanced salt solution. The cells were washed with PBS and allowed to grow for an additional 48 hours in HAM with 10% NCS. At this time the cells were subcultured and diluted 1:5 in the complete medium supplemented with 600 μg/ml G418. After 3 weeks of G418 selection, each resistant colony was isolated and cultured individually in a 24-well plate. Forty-eight clones were selected for resistance to G418 (600 μg/ml). To screen the clones, specific binding and degradation of ¹²⁵I-LDL in each clone were determined. The positive clones that fully expressed the human LDL receptor were selected and maintained in the complete medium with 300 μg/ml G418. One of the highly expressive transfectants was subjected to limiting dilution in a 96-well plate to confirm a single clone. A strong positive clone was obtained from 48 clones, which had the highest low density lipoprotein receptor activity. When the cells were approximately 80% confluent (~3 days), cells were solubilized in 50 mM tris(hydroxymethyl)aminomethane maleate (pH 6.0) containing 3% sodium dodecyl sulfate, 1% Triton X-100, and 2 mM phenylmethylsulfonyl fluoride. Cell lysates (left, CHO 29 cells; right, control CHO-K cells) were applied to sodium dodecyl sulfate-10% polyacrylamide gels and electrophoresed. Proteins were then transferred to nitrocellulose paper. After the nitrocellulose sheet was incubated with antiserum (IgG-C7) and then with iodine-125-labeled protein A, autoradiography was performed. Molecular size markers are at far left.

FIGURE 1. Immunoblot analysis of Chinese hamster ovary cells overexpressing low density lipoprotein receptor (CHO 29) maintained in Ham's F-12 medium supplemented with 10% newborn calf serum at 37°C in 5% CO₂. CHO 29 cells were selected from 48 clones, which had the highest low density lipoprotein receptor activity. When the cells were approximately 80% confluent (~3 days), cells were solubilized in 50 mM tris(hydroxymethyl)aminomethane maleate (pH 6.0) containing 3% sodium dodecyl sulfate, 1% Triton X-100, and 2 mM phenylmethylsulfonyl fluoride. Cell lysates (left, CHO 29 cells; right, control CHO-K cells) were applied to sodium dodecyl sulfate-10% polyacrylamide gels and electrophoresed. Proteins were then transferred to nitrocellulose paper. After the nitrocellulose sheet was incubated with antiserum (IgG-C7) and then with iodine-125-labeled protein A, autoradiography was performed. Molecular size markers are at far left.

Lipoproteins and Binding Assay for the Low Density Lipoprotein Receptor

Lipoproteins were prepared from human volunteer plasma by sequential ultracentrifugation. Each fraction was floated again at the same density. Lipoproteins were labeled with [¹²⁵I]NaI by a modification of the iodine monochloride method of McFarlane. CHO 29 and control cells were seeded at 5.0x10⁶ cells/3.5-cm well, cultured in HAM with 10% NCS, and used for experiments within 72 hours (approximately 60–80% confluence). After incubation for 48 hours in 5 mg/ml lipoprotein-deficient serum (LPDS), cells were cultured with the indicated amounts of LDL for 24 hours at 37°C. Then 20 μg/ml ¹²⁵I-LDL (98 cpm/ng) was added to the cells at 37°C for 5 hours. LDL receptor binding activity was measured according to the method of Goldstein and Brown. The surface-bound and internalized ¹²⁵I-lipoproteins were exhibited together as LDL receptor binding activity. The contents of cell proteins dissolved in 0.1N NaOH were determined by the method of Lowry et al. Lipid Accumulation in Cells

After incubation with 5 mg/ml LPDS for 48 hours, the cells were cultured with 5 μg/ml LDL or β-very low density lipoprotein (β-VLDL) at 37°C. β-VLDL was isolated from the plasma of 1% cholesterol-fed rabbits by ultracentrifugation. After the indicated time, the cells were harvested and extracted with chloroform/methanol (2:1 vol/vol), and the mass of esterified cholesterol in the extract was enzymatically measured by fluorescence scanning.

Western Blotting

Cells were solubilized in 50 mM tris(hydroxymethyl)aminomethane maleate (pH 6.0) containing 3% sodium dodecyl sulfate, 1% Triton X-100, and 2 mM phenylmethylsulfonyl fluoride. Cell lysates were applied to sodium dodecyl sulfate–(10%) polyacrylamide gels and electrophoresed. Proteins were then transferred to nitrocellulose paper by the method of Beisiegel et al. After the nitrocellulose sheet was incubated with the antiserum (IgG-C7) and then with
FIGURE 2. Line plots showing regulation of low density lipoprotein (LDL) receptor activity by addition of LDL (μg/ml) to Chinese hamster ovary (CHO-K) cells. Control CHO cells (●) and CHO 29 cells (○) were cultured as described in "Methods." After incubation for 48 hours in 5 mg/ml lipoprotein-deficient serum, cells were cultured with LDL for 24 hours at 37°C. Then 20 μg/ml iodine-125-labeled LDL (98 cpml ng) was added to cells at 37°C for 5 hours. Assay was performed in triplicate. 100% indicates 125I-LDL binding activity of cells in the absence of LDL during 24-hour incubation, and 100% binding corresponds to 523 ng LDL/mg cell protein for CHO 29 cells and 70 ng LDL/mg cell protein for control CHO-K cells, respectively.

125I-protein A (2,000 cpm/ng, 0.5 μg/ml), autoradiography was performed.

Results

Forty-eight clones were selected for resistance to G418 (600 μg/ml) after 3 weeks of transfection. The specific binding and degradation of 125I-LDL in each clone were determined to screen the clones. Of 48 clones, CHO 29 cells demonstrated the highest expression of LDL receptor, whose binding activity was approximately 10-fold greater than that of control CHO-K cells in the screening assay. Western blotting with a monoclonal antibody against the human LDL receptor (IgG-C7) detected the 130-kd LDL receptor in CHO 29 cells, whereas no human LDL receptor was detected in control CHO-K cells because IgG-C7 does not recognize the LDL receptor of the Chinese hamster

We also studied the regulation of LDL receptor activity. After the cells were cultured in medium containing no lipoproteins, CHO 29 cells were exposed to various amounts of LDL. As shown in Figure 2, LDL receptor activity was not influenced by the presence of as much as 50 μg/ml LDL in CHO 29 cells, whereas LDL receptor activity was suppressed in control CHO-K cells. The cellular contents of esterified cholesterol were identical when cells were cultured without lipoproteins. With increasing amounts of native LDL in the medium, the cellular contents of esterified cholesterol increased markedly in CHO 29 cells (data not shown), whereas no increase in the contents of esterified cholesterol was observed in control CHO-K cells. When cells were cultured with 5 μg protein/ml LDL, CHO 29 cells gradually stored esterified cholesterol inside cells, whereas no accumulation of esterified cholesterol was observed in the control CHO-K cells (Figure 3). Five micrograms protein/ml β-VLDL can be stored in both cell lines, but β-VLDL is stored less efficiently in control CHO-K cells (Figure 3).

Massive accumulation of esterified cholesterol through LDL receptor-mediated endocytosis of LDL in CHO 29 cells was visualized by light-microscopic examination (Figure 4). In control CHO-K cells incubated with LDL, only occasional small lipid droplets were observed after staining with oil red O. In contrast, CHO 29 cells incubated with the same amounts of LDL showed remarkable staining with oil red O. During the first 2 days lipid droplets accumulated around the nucleus, and then
cytoplasm became filled with lipids and the cells became foam cells.

Discussion

In the present study, nonsuppressed uptake of LDL in CHO cells was achieved by overexpression of the LDL receptor. We visually demonstrated that overexpression of the LDL receptor induces foam cell changes in transfected cells, whose expression of the LDL receptor is driven by the SV40 promoter. It is easy to suppose that overexpression of the cell-surface LDL receptor increases an influx of LDL and that the resulting excessive cholesterol beyond the cellular demand is stored as lipid droplets. Very interestingly, β-VLDL was stored in both CHO 29 and control cells (Figure 3), and large amounts of β-VLDL compared with LDL were endocytosed into control cells. Although the greater accumulation of cholesteryl ester in CHO 29 cells than in control cells is explained by the endocytosis of β-VLDL through overexpressed LDL receptors, we speculate that β-VLDL can be endocytosed through another receptor recognizing apolipoprotein E on lipoproteins, an LDL receptor-related protein (LRP) whose activity is not suppressed by sterol. LRP has been proposed to have a high affinity for lipoproteins containing several molecules of apolipoprotein E, such as β-VLDL. Other examples of the upregulation of LDL receptor activity in vitro have been reported for cells stimulated by growth factors such as platelet-derived growth factor and for transformed cells. Because these examples are thought to be related to foam cell formation, overexpression of the LDL receptor under certain pathological conditions may play a role in atherogenesis. Many investigations have paid attention to modified lipoproteins, including acetylated LDL, oxidized LDL, malondialdehyde LDL, N,N-dimethyl-1,3-propanediamine LDL, and aggregated LDL as a source of cholesteryl ester stored in foam cells. Although the scavenger pathway has been proposed to play an important role in foam cell formation, unregulation of the LDL receptor pathway is another possible mechanism for foam cell formation because we demonstrated apparent lipid accumulation in transformants with high LDL receptor activities in the presence of very small amounts of LDL (5 μg/ml).

We believe that under the condition that enables cells to upregulate LDL receptor activities against the suppressing force, foam cells can be generated by endocytosis of native LDL via the LDL receptor in the process of atherosclerosis, which is slowly progressive throughout life. Our results emphasize the critical role of LDL receptor regulation in protecting cells from the cellular accumulation of cholesteryl
ester when they are exposed to excessive amounts of native LDL and the possible role of unregulated LDL receptor expression in foam cell generation.

References

KEY WORDS: • low density lipoprotein receptor • atherosclerosis • foam cells • overexpression
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