Apolipoprotein B: its role in the control of fibroblast cholesterol biosynthesis and in the regulation of its own binding to cellular receptors

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Abstract Apolipoprotein B transports cholesterol in plasma as low density lipoprotein (LDL) and targets its delivery to cells by binding to a specific plasma membrane receptor. The cellular consequences of apoB binding to its receptor were investigated to determine whether it suppresses cholesterol biosynthesis and reduces the number of cellular receptors for the apoprotein. Upon preincubation of fibroblasts with lipoprotein-deficient medium alone or supplemented with either LDL or apoB complexed to BSA (apoB–BSA), LDL suppressed cholesterol biosynthesis, but apoB enhanced it. Similarly, fibroblasts preincubated in medium supplemented with LDL bound decreased amounts of either ^{125}I-labeled LDL or ^{125}I-labeled apoB–BSA to their receptors, while preincubation with apoB–BSA increased the binding relative to the controls. These latter results occurred in association with a decrease in cellular cholesterol content, indicating that apoB in the medium bound cholesterol and removed it from the cells, thus stimulating both cholesterol synthesis and cellular binding of apoB. Accordingly, fibroblast cholesterol synthesis and the number of functional LDL receptors are not suppressed by the binding of the apoprotein to the receptor, and the known role of apoB remains that of transporting cholesterol in plasma and delivering it to the cell. A possible physiologic role for apoB in depleting cells of cholesterol is presently unknown since apoB is not known to exist free in plasma; however, these findings demonstrate such a functional capability for this apoprotein.—**Shireman, R. B., and W. R. Fisher.** Apolipoprotein B: its role in the control of fibroblast cholesterol biosynthesis and in the regulation of its own binding to cellular receptors. *J. Lipid Res.* 1979. **20:** 594–598.

Supplementary key words low density lipoprotein

The importance of apolipoprotein B in the transport and delivery of cholesterol to the cells of the body has been clarified through the work of Goldstein and Brown (1); they have elucidated the biochemical steps in this metabolic sequence and termed them "the LDL pathway". Thus, LDL in plasma binds to a specific plasma membrane receptor, undergoes endocytosis and degradation in the lysosome, and the cholesteryl esters are hydrolyzed to liberate free cholesterol within the cell. As a consequence of this sequence of reactions, the cellular synthesis of cholesterol is suppressed through a reduction in the activity of HMG-CoA reductase, the activity of acyl-CoA: cholesterol acyl transferase is stimulated, and the number of LDL receptors present in the plasma membrane is suppressed (1). It has also been shown that cholesterol and its oxygenated derivatives, which traverse the plasma membrane in the absence of the carrier lipoprotein, also have the capability of modulating each of these metabolic reactions (2, 3). The question may then be posed as to whether the regulation of these metabolic reactions is solely under the control of the cellular content of cholesterol, which is delivered by LDL, or whether the metabolic regulation results in part as a direct consequence of the binding of LDL with its plasma membrane receptor. We have recently shown that the binding of LDL to its plasma membrane receptor results from the direct interaction of apolipoprotein B with the receptor, and that once bound, apoB is internalized and undergoes proteolytic hydrolysis in a manner analagous to the internalization and hydrolysis of native apoB when it exists within the LDL macromolecule (4).[2] This finding has made it feasible to address the question of whether the binding of apoB to the cellular plasma membrane receptor on fibroblasts *1*) results in the suppression of cholesterol synthesis, and *2*) results in a reduction in the number of available LDL receptors in the cellular plasma membrane. Both of these reactions

Abbreviations: LDL, plasma low density lipoprotein; apoB, apolipoprotein B, the major apoprotein of LDL; BSA, bovine serum albumin; apoB–BSA, the water-soluble complex formed between apoB and BSA.

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[2] Although apoB is added to the tissue culture medium in a soluble complex with BSA, when one measures the cellular binding and uptake of ^{125}I-labeled BSA in the presence or the absence of apoB, there is no significant difference, and the amount of ^{125}I-labeled BSA taken up by the cells is small (less than 10%) compared to the uptake of apoB. It would thus appear that when apoB–BSA binds to the cell receptor, the complex dissociates and BSA is released while apoB is internalized (5).

have been shown normally to be suppressed by the presence of LDL.

EXPERIMENTAL PROCEDURES

LDL was isolated from human plasma in the presence of 0.02% sodium azide, 0.01% merthiolate, and 0.01% EDTA by differential density preparative ultracentrifugation (6). Lipoprotein-deficient serum was prepared by adjusting the density of human serum to 1.20 g/ml with solid KBr and centrifuging for 20 hr at 40,000 rpm. The lipoproteins were removed at the meniscus, and the infranatant was dialyzed against an isotonic Tris buffer at pH 7.4 (7) prior to filtration sterilization. Apolipoprotein B was prepared by ether–ethanol extraction of LDL solubilized in 6 M guanidine, as previously described, and the apoB was mixed with bovine serum albumin in a ratio of 1:5 by weight prior to dialysis against phosphate-buffered saline and filtration sterilization in preparation for tissue culture studies (4).

Normal human fibroblasts, purchased from the American Type Tissue Culture Collection, Rockville, MD, were maintained in culture and used in the 13–16th passages. The conditions for culture were as described by Brown and Goldstein (8). Twenty hr prior to the addition of radioisotopes to the cells, the medium in the tissue culture dishes was replaced with a basic tissue culture medium containing 5% lipoprotein-deficient serum to which were added saline, LDL, and BSA or apoB complexed with BSA. In the studies of cholesterol biosynthesis, after the 20-hr incubation, 5 μCi of sodium[^{14}C]acetate (Amersham Searle) was added to each tissue culture dish, and the incubation was continued for an additional 6 hr. Thereafter, the medium was removed from the plates and saved, and the cells were washed three times with cold phosphate-buffered saline. The cells were dissolved in 0.1 N sodium hydroxide and, after removal of an aliquot for measurement of cellular protein content by the method of Lowry et al. (9), the solution was made 70% in ethanol and 1 N in sodium hydroxide prior to saponification for 6 hr at 60–65°C. Total steroids were then extracted with hexane which was backwashed with 1 ml of 0.1 M sodium acetate. A portion of the hexane extract was counted for ^{14}C in a Packard Tricarb scintillation counter using a Permablend #3 (Packard Co.) scintillation solution, while the remainder of the hexane extract was set aside for the measurement of cellular cholesterol. The ^{14}C-labeled sterol content of the tissue culture medium removed after 6 hr of incubation was determined following saponification and extraction with hexane in the same manner as described for the tissue culture cells. The cellular cholesterol content of the fibroblasts was measured by gas–liquid chromatography using a Hewlett Packard Model 402 instrument by a method developed by Dr. Gerhard Freund of the University of Florida. The separation was accomplished on a 6-ft glass column containing 3% QF-1 on 80/100 mesh Gas-chrome Q (Applied Science Co.). Cholesterol dissolved in hexane was applied to the column at 240°C and eluted as a symmetrical peak whose area was linearly related to the cholesterol content over the range of 0.5–2.5 μg of cholesterol. Cholesterol was the only steroid recovered in measurable quantity from the fibroblasts.

To measure the effect of preincubation of fibroblasts with apoB on the functional expression of the cellular LDL receptor, fibroblasts were preincubated for 20 hr in tissue culture medium containing 5% lipoprotein-deficient serum only or supplemented with LDL or apoB complexed with BSA. The medium was then replaced with fresh medium containing either ^{125}I-labeled LDL or ^{125}I-labeled apoB–BSA and incubated with the cells for 2 hr at 37°C. Specific binding of the radioiodinated LDL or apoB was measured precisely as previously reported (4).

RESULTS

Role of apoB in the control of cellular cholesterol synthesis

Fig. 1 presents the data from one of a series of experiments in which fibroblasts were preincubated for 20 hr in lipoprotein-deficient medium alone or in lipoprotein-deficient medium supplemented with LDL, BSA, or apoB complexed with BSA; the concentrations of these proteins in the medium are indicated in the table. The incorporation of ^{14}C from [^{14}C]acetate into the total sterol fraction of the cells is recorded in the top panel of the figure. The middle panel shows the incorporation of radioactivity into the sterol fraction recovered from the medium; the bottom panel records the total cholesterol content of the fibroblasts. By dividing the values in the top panel by those in the bottom panel, the changes in cellular cholesterol specific activity can be appreciated.

The data demonstrate that, as expected, cells grown in the presence of LDL incorporate very little [^{14}C]-acetate into cholesterol but have a large amount of intracellular cholesterol. By contrast, cells grown in lipoprotein-deficient medium alone or supplemented with BSA have enhanced cholesterol synthesis from acetate and a decreased cellular cholesterol content. When fibroblasts are incubated in the presence of

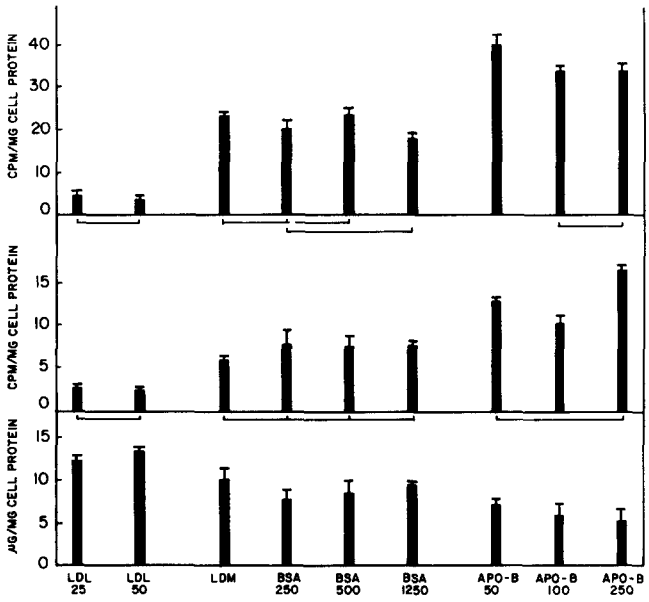

Fig. 1. Cholesterol synthesis and cholesterol content of fibroblasts preincubated for 20 hr in medium as specified along the abscissa where the concentrations of added proteins are in μg/ml. For cells incubated in BSA or apoB, these were added to the lipoprotein-deficient medium (LDM). Solubilized apoB was prepared in a 5-fold weight excess of BSA, hence the medium containing 50 μg/ml apoB also contained 250 μg/ml BSA, etc. After preincubation, 5 μCi of sodium[^{14}C]acetate was added to each flask and the incubation was continued for 6 hr, after which the medium and cells were harvested. Top panel: Total [^{14}C]cholesterol (nonsaponifiable sterols) from cells. Middle panel: Total [^{14}C]-cholesterol from medium. Bottom panel: Total cholesterol content of cells in μg cholesterol/mg cell protein. The horizontal lines at the bottom of the top and middle panels connect samples which do not differ from each other statistically, as analyzed by Duncan's New Multiple Range Test (16). In the bottom panel, statistically significant differences occurred only between the LDL and the apoB samples.

apoB–BSA, there is a greater increase in cellular synthesis of cholesterol, and the sterols recovered from the medium are also more highly labeled. The cholesterol content of the cells appears to be lower than that of control cells, although these differences do not reach statistical significance.

These data clearly show that, unlike LDL, apoB does not suppress cellular cholesterol synthesis, but rather appears to stimulate cholesterol synthesis within the cell. The fall in cellular cholesterol content associated with an increase in the specific activity of the cholesterol in the medium of cells incubated with apoB suggests a net transfer of cholesterol from the cell and a derepression of cellular cholesterol synthesis.

Role of apoB in the control of its own cellular binding

Fig. 2 presents the results of a representative experiment comparing the binding and uptake of ^{125}I-labeled apoB by fibroblasts which had been preincubated in lipoprotein-deficient medium alone or supplemented with LDL or apoB–BSA. Prior incubation with LDL clearly suppresses the binding of ^{125}I-labeled apoB in a manner analogous to that previously reported for ^{125}I-labeled LDL (10). By contrast, preincubation for 20 hr with apoB–BSA results in an enhancement of cellular binding of ^{125}I-labeled apoB. In a parallel set of experiments in which the cellular binding of ^{125}I-labeled LDL was measured after preincubation with apoB–BSA, similar results were observed.

DISCUSSION

The binding of LDL to fibroblasts results in a reduction in endogenous cellular cholesterol synthesis as a consequence of the suppression of HMG-CoA reductase (11). When free cholesterol is presented to the cell, the endogenous synthesis of cholesterol by the cell is also suppressed (2, 3, 11); however, when cells are incubated with HDL, cellular cholesterol synthesis is not suppressed, and there is no evidence of the net transfer of cholesterol into the cell (11). The question of whether delipidated apoB itself exerts a direct influence on cellular cholesterol biosynthesis, however, remained to be answered.

From the data shown in Fig. 1, it is clear that whereas cells incubated in the presence of LDL have suppressed cholesterolgenesis, apoB does not suppress cholesterol biosynthesis. This finding agrees with the

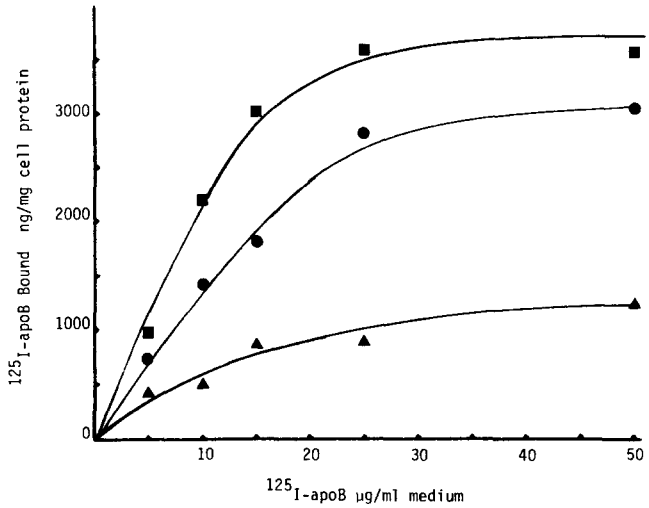

Fig. 2. ^{125}I-Labeled apoB specific binding and uptake by fibroblasts after 20 hr of preincubation with (●) lipoprotein-deficient medium alone, medium supplemented with (■) 50 μg/ml of apoB complexed with BSA, or medium with (▲) 50 μg/ml of LDL. Fibroblasts were then incubated for 2 hr at 37°C in fresh lipoprotein-deficient medium containing ^{125}I-labeled apoB–BSA in the amounts indicated.

conclusion of others that exogenous cholesterol is the major determinant of cellular cholesterolgenesis (11), and that partially delipidated LDL, from which cholesterol has been extracted, fails to suppress cholesterolgenesis after binding to fibroblasts (12).

The addition of delipidated apoB to the tissue culture medium actually appears to enhance cholesterol synthesis. Previously, Brown, Faust, and Goldstein (13) demonstrated that an increased cellular cholesterol content is associated with a decrease in endogenous cholesterol synthesis in cultured fibroblasts, and the data shown in Fig. 1 suggest a decreased cellular cholesterol content in fibroblasts grown in the presence of apoB. It would appear that apoB in the medium is binding cholesterol, resulting in a net loss of cholesterol from the cells. It has previously been reported by Stein and Stein (14) and Bates and Rothblatt (15) that a net efflux of cholesterol occurs from cells grown in the presence of HDL; this also occurs with cells grown in the presence of apoB. The metabolic consequence of such a cellular depletion of cholesterol is then an enhancement of HMG-CoA reductase activity, shown here by increased acetate incorporation into cholesterol.

The number of functional LDL receptors in fibroblasts is controlled by the availability of LDL to the cell; Brown and Goldstein (10) have also determined that in chloroquine-treated cells, LDL does not suppress the availability of the plasma membrane receptor, thus implying that not only binding but also endocytosis and lysosomal hydrolysis of LDL are required to suppress the number of its cellular receptors. It has also been shown that oxygenated derivatives of cholesterol, which will enter the cell independently of LDL binding and uptake, can suppress the number of plasma membrane receptors for the lipoprotein (10). Thus, there is strong evidence indicating that the cholesterol that is liberated after the cellular disassembly of LDL is an active agent in suppressing the number of receptor sites for LDL on the plasma membrane; yet the question remained whether the apoprotein that actually binds to the receptor plays a role in controlling the number of receptors which in fact recognize the protein itself. The data in Fig. 2 show that prior incubation of cells with apoB, in contrast to LDL, not only fails to suppress the cellular mechanism for binding and endocytosis of apoB, but actually augments this cellular activity. Thus, binding of apoB to its receptor does not signal the cell to suppress receptor synthesis.

The enhancement of apoB receptor binding by preincubation with apoB is not a surprising observation in view of the finding of enhanced cholesterol synthesis of fibroblasts preincubated with apoB. As a consequence of the depletion of the cellular cholesterol content by incubation with apoB, the cell responds both by enhanced cholesterol biosynthesis and by increasing the number of its functional apoB receptors.

Since apoB apparently does not circulate freely in plasma, it presumably does not play a physiologic role in removing cholesterol from cells. The observations reported here do help in clarifying the role of apoB: to transport cholesterol in plasma and to target its delivery to cells. ApoB appears to have no direct role in the control of cellular cholesterol synthesis nor in regulating the number of cellular sites for its own plasma membrane receptor. Although not tested in these experiments, it also seems reasonable to assume that apoB does not control the activity of cellular acyl CoA:cholesterol acyl transferase.

The authors wish to acknowledge the research contribution of Lois Broer, a medical student at the University of Florida, who was supported by a summer research fellowship from the Florida Heart Association. We also appreciate the use of Dr. Gerhard Freund's method for measuring cholesterol in cells, and the help of Dr. Ronald Marks in the statistical analyses. This work was supported by NIH grant HL10316-13.

Manuscript received 22 September 1978; accepted 1 December 1978.

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