Interaction of Swine Lipoproteins with the Low Density Lipoprotein Receptor in Human Fibroblasts*

(Received for publication, October 23, 1975)

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HDL₃, a cholesterol-rich lipoprotein that accumulates in the plasma of cholesterol-fed swine, was shown to resemble functionally human and swine low density lipoprotein in its ability to bind to the low density lipoprotein receptor in monolayers of cultured human fibroblasts. This binding occurred even though HDL₃ lacked detectable apoprotein B, which is the major protein of low density lipoprotein. After it was bound to the low density lipoprotein receptor, HDL₃, like human and swine low density lipoprotein, delivered its cholesterol to the cells, and this, in turn, caused a suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, an activation of the cholesterol-estersifying system, and a net accumulation of free and esterified cholesterol within the cells. Swine HDL₃, like human high density lipoprotein, did not bind to the low density lipoprotein receptor nor did it elicit any of the subsequent metabolic events. HDL₃, like human low density lipoprotein, was incapable of producing a metabolic effect in fibroblasts derived from a subject with the homozygous form of familial hypercholesterolemia, which lack low density lipoprotein receptors. These results indicate that two lipoproteins that have been associated with atherosclerosis—low density lipoprotein in humans and HDL₃ in cholesterol-fed swine—both can cause the accumulation of cholesterol and cholesteryl esters within cells through an interaction with the low density lipoprotein receptor.

A distinctive cholesterol-carrying lipoprotein accumulates in the plasma of swine that have been fed cholesterol (1). This lipoprotein, designated HDL₃, differs from low density lipoprotein (LDL), the major cholesterol-carrying lipoprotein of normal swine plasma, in its protein components (1). Whereas LDL contains exclusively apoprotein B, HDL₃ contains a protein termed the “arginine-rich apoprotein,” variable amounts of apoprotein A-I and the fast-migrating C apoproteins, and no detectable apoprotein B (1).

Previous studies by Assmann and co-workers have indicated that despite the structural differences between HDL₃ and LDL, the two lipoproteins are functionally similar in that both suppress the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), the rate-controlling enzyme of cholesterol synthesis, in cultured swine smooth muscle cells (2). Suppression of HMG CoA reductase activity by LDL has been shown in human fibroblasts to require the binding of the lipoprotein to a cell surface receptor (3, 4). This receptor heretofore has been shown to interact only with human lipoproteins containing apoprotein B (3). The finding that swine HDL₃ suppressed HMG CoA reductase activity in swine cells raised the possibility that this lipoprotein, although lacking apoprotein B, might be capable of interacting with the LDL receptor.

The current studies were, therefore, designed to determine whether HDL₃ can bind to the LDL receptor in human fibroblasts, and whether this binding is followed by the same sequence of metabolic events that follows the binding of human LDL—namely, internalization of the surface-bound lipoprotein by endocytosis (4, 5), hydrolysis of its protein and cholesteryl ester components in lysosomes (5–7), and transfer of the liberated free cholesterol to the cellular compartment (8), where it suppresses HMG CoA reductase activity (9) and activates an acyl CoA:cholesteryl acyltransferase facilitating its own re-esterification (10, 11). Human fibroblasts were used...
for these studies because of the availability of naturally occurring mutant cells that lack the LDL receptor (3, 4). Derived from subjects with the homozygous form of familial hypercholesterolemia, these mutant cells do not bind, internalize, or degrade LDL with high affinity, and are, thus, unable to suppress HMG CoA reductase activity and stimulate cholesterol ester formation in the presence of LDL (12).

**EXPERIMENTAL PROCEDURE**

Materials—DL-3-Hydroxy-3-methyl[1-14C]glutaric acid A (11.7 mCi/mmol) and [1-14C]oleic acid (51.8 mCi/mmol) were purchased from New England Nuclear Corp. 14C-Sodium (carrier-free in 0.05 N NaOH) was obtained from Schwarz/Mann. Tissue culture supplies, thin layer chromatographic materials, and reagents for assays were purchased from sources as previously reported (9).

**Cells**—The normal human fibroblast strain and the homozygous familial hypercholesterolemia fibroblast strain used in these studies were derived from subjects D. S. and M. C., respectively, as previously reported (12). The regulation of lipoprotein and cholesterol metabolism in these cells is typical of that of the indicated genotype. All cells were grown in monolayers and were used between the 5th and 20th passage. Cell lines were maintained in a humidified incubator (5% CO2) at 37° in 75-cm2 stainless steel flasks containing 10 ml of growth medium consisting of Eagle's Minimum Essential Medium supplemented with penicillin (100 units/ml); streptomycin (100 μg/ml); 20 mM Tricine-chloride, pH 7.4; 24 mM NaHCO3; 1% (v/v) nonessential amino acids; and 10% (v/v) fetal calf serum. All experiments were carried out using a standard format. Confluent monolayers of cells from stock flasks were dissociated with 0.05% trypsin/0.02% EDTA solution and were seeded (Day 0) at a concentration of 1 × 106 cells per dish into 60 × 15-mm Petri dishes containing 3 ml of growth medium with 10% fetal calf serum. On Day 3, the medium was replaced with 3 ml of fresh medium containing 10% fetal calf serum. On Day 6, when the cells were in late logarithmic growth, each monolayer was washed with 2 ml of Dulbecco's phosphate-buffered saline, after which 2 ml of fresh medium containing 5% (v/v) human lipoprotein-deficient serum (LPDS) were added (final protein concentration, 2.5 mg/ml). Experiments were carried out on Day 7 after the cells had been incubated for 24 hours in the presence of LPDS. All incubations were carried out at 37°.

**Swine Lipoproteins**—LDL, HDL<sub>s</sub>, and HDL<sub>c</sub> were obtained from miniature swine fed a basic hog chow containing 1% cholesterol and 15% lard. LDL and HDL<sub>c</sub>, obtained by differential ultracentrifugation (13), LDL and HDL<sub>s</sub> were then separated by Geon-Pevikon block electrophoresis (1, 13). The apoprotein content of each lipoprotein was monitored by polyacrylamide gel electrophoresis using 11 g of acrylamide and 0.46 g of bisacrylamide per 100 ml of 50 mM Tris buffer, pH 8.2, containing 0.1 g of sodium dodecyl sulfate per 100 ml (14). HDL<sub>c</sub> was shown to be devoid of detectable apoprotein B by two criteria: (a) no stainable protein remained at the origin during sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1); and (b) no precipitation was observed on an Ouchterlony immunodiffusion plate using an antibody to swine LDL. It was felt that these techniques would have been able to detect apoprotein B if it had accounted for more than 5% of the HDL<sub>c</sub> protein. Prior to incubation with the cultured cells, the lipoprotein fractions were dialyzed against Buffer A (0.15 M NaCl and 0.3 mM EDTA, pH 7.4).

**Human Lipoproteins**—Human LDL (density 1.019 to 1.063 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 as previously described (9). 14C-labeled LDL was prepared as previously described (3, 4). Prior to incubation with the cultured cells, the lipoprotein fractions were dialyzed against Buffer A.

**Assays**—The activity of HMG CoA reductase in cell-free extracts (9), the specific high affinity binding and uptake and proteolytic degradation of 125I-LDL by cell monolayers (3, 4), and the rate of 14C-cholesterol incorporation into cholesteryl esters by cell monolayers (10) were measured exactly as described in the cited references. For the experiments in Figs. 4 and 5, the sodium-[14C]leucine-albumin solution was prepared by a modification (10) of the method described by Van Harken et al. (15). The cellular content of free and esterified cholesterol was measured by a previously described gas-liquid chromatographic method (8). The protein content of the lipoprotein fractions and the cell extracts was determined by a modification of the method of Lowry et al. (16) using bovine serum albumin as a standard.

**RESULTS**

When incubated with human 125I-LDL at 37°, normal human fibroblasts bind the lipoprotein at the receptor site, internalize it, and degrade it. When the system reaches a steady state, the total cellular content of 125I-LDL is directly proportional to the number of receptors occupied with LDL, and hence, measurement of the total cell-bound 125I-LDL serves as an index of 125I-LDL binding to the receptor (3-5). The ability of various lipoproteins to compete with human 125I-LDL for binding to the LDL receptor is shown in Fig. 1A. Unlabeled human LDL competed with the 125I-LDL at the receptor site and, hence, reduced the total cellular uptake. Fifteen percent more 125I-LDL was displaced by unlabeled swine LDL compared to unlabeled human LDL. On the other hand, as previously described for human HDL (3), swine HDL<sub>c</sub> was much less effective than LDL in competing with 125I-LDL for its binding site. The binding behavior of unlabeled swine HDL<sub>c</sub> was nearly identical with that of swine LDL and human HDL<sub>c</sub>, all three lipoprotein fractions displacing 50% of the bound radioactivity in the range of 15 to 20 μg of protein per ml (Fig. 1A).

Consistent with these findings was the observation that the proteolytic degradation of human 125I-LDL, which depends on LDL binding to its receptor (4), was competitively inhibited by unlabeled human LDL, swine LDL, and swine HDL<sub>c</sub>, but not by swine HDL<sub>s</sub> (Fig. 1B).

The ability of the various lipoprotein fractions to suppress HMG CoA reductase activity was correlated with their ability to bind to the LDL receptor. Thus, human LDL, swine LDL, and swine HDL<sub>c</sub>, but not swine HDL<sub>s</sub>, suppressed the enzyme activity of normal human fibroblasts (Fig. 2). That suppression by swine LDL and swine HDL<sub>c</sub> did, in fact, involve binding to the LDL receptor was confirmed by the observation that neither of these lipoprotein fractions suppressed HMG CoA reductase activity in cells from a subject with the receptor-negative form of homozygous familial hypercholesterolemia, which lack functional LDL receptors (Fig. 3).

Another metabolic consequence of LDL binding to its receptor in normal human fibroblasts is enhancement of the
The present studies demonstrate that HDL₄, derived from the plasma of cholesterol-fed swine, is capable of binding to the LDL receptor in human fibroblasts and of eliciting all of the known cellular functions of LDL, i.e. suppressing HMG CoA reductase activity, activating the cholesterol-esterifying system, and causing a net accumulation of free and esterified cholesterol within the cell. The parameters of lipoprotein composition important in the interaction of LDL and HDL, with the cell surface receptor remain to be defined. The binding of human LDL to its receptor in human fibroblasts is believed to involve the protein LDL receptor in human fibroblasts. HMG CoA reductase activity (9). (b) Selective modification of cholesterol within the cell. On the other hand, swine HDL₄, as reported previously for human HDL, did not interact with the LDL receptor in human fibroblasts.

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Interaction of Swine Lipoproteins with the LDL Receptor

TABLE I
Content of free and esterified cholesterol in normal human fibroblasts incubated in the presence of human LDL and swine HDL.

Cell monolayers were grown under standard conditions, and on Day 6, the medium was replaced with 2 ml of growth medium containing 10% LPDS. After 48 hours (Day 8), fresh growth medium containing 10% LPDS and 20 μg protein per ml of the indicated lipoprotein fraction was added to each dish. After incubation at 37° for 24 hours, cells from two dishes were pooled and harvested for measurement of their cholesterol content as described under “Experimental Procedure.” Each value represents the average of duplicate determinations.

<table>
<thead>
<tr>
<th>Lipoprotein fraction</th>
<th>Cellular cholesterol content</th>
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<tbody>
<tr>
<td></td>
<td>Free</td>
</tr>
<tr>
<td>None</td>
<td>34.3</td>
</tr>
<tr>
<td>Human LDL</td>
<td>44.9</td>
</tr>
<tr>
<td>Swine HDL&lt;sub&gt;α&lt;/sub&gt;</td>
<td>47.3</td>
</tr>
</tbody>
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human LDL protein with maleic anhydride destroys its ability to bind to the LDL receptor.\(^1\) (c) Cell surface \(^{125}\)I-LDL binding can be prevented, and bound \(^{125}\)I-LDL can be dissociated from its receptor by exposure of the LDL to heparin and other sulfated glycosaminoglycans that are known to form soluble complexes with LDL through ionic interactions with its protein component.\(^3\) (17)

If the protein component of LDL<sub>α</sub> is involved in the binding reaction, then the current observation that swine HDL<sub>α</sub>, which is lacking apoprotein B, can also bind to the LDL receptor suggests that the protein of HDL<sub>α</sub> may contain a region homologous with apoprotein B. It is likely that this region resides in the arginine-rich apoprotein of HDL<sub>α</sub>, since HDL<sub>α</sub> contains the A and C apoproteins but lacks the arginine-rich apoprotein, does not bind to the LDL receptor. Lipoprotein size and lipid composition may also be important in the binding of lipoproteins to the LDL receptor, since LDL<sub>α</sub> and HDL<sub>α</sub> are similar in size and in cholesterol content, whereas HDL<sub>α</sub> is smaller in size and has a lower ratio of cholesterol to protein.\(^1\)

Regardless of the mechanism by which HDL<sub>α</sub> binds to the LDL receptor, the binding itself may play an important role in the production of the atherosclerosis associated with cholesterol feeding in swine. Thus, two lipoproteins implicated in atherosclerosis, namely, LDL in humans and HDL<sub>α</sub> in swine, both can cause the accumulation of free and esterified cholesterol in peripheral cells through their interaction with the LDL receptor.

REFERENCES

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