Swine Aortic Smooth Muscle in Tissue Culture

Some Effects of Purified Swine Lipoproteins on Cell Growth and Morphology.

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SUMMARY  Smooth muscle cells (SMC) were grown from inner media explants of swine aorta and used as a model for studying the role of lipoproteins in atherogenesis. These cultured cells retain the characteristics of SMC through multiple passages. Cell growth curves, in time, were obtained by using standard counting techniques. SMC grew slowly (0.019 cycle/day) in modified Dulbecco-Vogt medium supplemented with 1.5% swine serum. Purified lipoproteins were prepared from three normolipidemic and two hyperlipidemic (cholesterol-fed) swine. When the medium of 84 growth experiments were supplemented with these lipoproteins, SMC growth rate increased linearly with lipoprotein cholesterol concentration up to 10 mg/dl. At 10 mg/dl of lipoprotein cholesterol, very low density lipoproteins (VLDL) increased growth rate 7.2-fold \(P < 0.01\);

low density lipoproteins (LDL) 5.7-fold \(P < 0.01\); high density lipoproteins (HDL\(_1\) 3.4-fold \(P < 0.02\); and HDL\(_2\), a lipoprotein appearing in the hyperlipidemic swine, 3.0-fold \(P < 0.01\). Addition of 10% lipoprotein-free serum stimulated growth rate 6.0-fold \(P < 0.01\). There was no difference between normo- and hyperlipidemic lipoproteins with respect to cell growth rate. Factors present in the ultracentrifugal bottom, and factors appearing during the platelet release reaction, were shown to contribute to the SMC growth response. Morphological alterations characteristic of intimal foam cells occurred in SMC grown in VLDL at triglyceride levels in excess of 15 mg per 100 ml. Thus there are distinct parallels between SMC response in this model in vitro and atherogenesis in vivo.

THERE IS convincing evidence that the vascular smooth muscle cell is the proliferative cell of atherogenesis. 1-4 Vascular smooth muscle cells (SMC) can synthesize cholesterol 2 as well as collagen, elastin, and mucopolysaccharides. 4 Each of the latter is a connective tissue component of the atherosclerotic plaque and is alleged to complex with lipids in the arterial wall. 5-11 Studies of the arterial intima and media in vivo are limited by our inability to do serial examination at any given point in the arterial tree. Cultured vascular smooth muscle cells therefore appear to provide a more readily controlled model for evaluation of the cellular biology of SMC proliferation. Ross and co-workers 12, 13 have demonstrated that various serum constituents stimulate growth of SMC in tissue culture. In particular, low density lipoproteins (LDL) 13 and a "platelet-dependent factor" 13 stimulate SMC proliferation. The latter is a nondialyzable substance which appears in serum during the platelet release phenomenon. Dzoga and co-workers 14 observed that LDL from experimentally hyperlipemic monkeys and rabbits were more growth-promoting for SMC than LDL from normolipidemic species, even at the same cholesterol level. To date, the effect on SMC growth of the full range of homologous lipoprotein classes from normo- and hyperlipidemic serum has not been reported. In view of the recognized clinical and experimental association between certain hyperlipoproteinemic states and atherosclerosis, we have studied the growth of cultured swine aortic smooth muscle cells in the presence of various purified homologous lipoproteins. Lipoproteins were obtained from both normolipidemic and hyperlipidemic swine. Particular attention was paid to those lipoproteins which appear or which increase during experimental hyperlipidemia.

Methods

CELL CULTURE  The technique was similar to that described by Ross. 14 A small piece of descending thoracic aorta was obtained under sterile conditions from four miniature swine in the age range 1.5–4 months. By careful dissection, the intima and outer two-thirds of the media were removed. The remaining inner media was minced into pieces no greater than 0.5 mm at greatest dimension. Five to ten of these were explanted under sterile cover slip in a 35-mm Falcon plastic Petri dish. These explants were incubated at 37°C under 5% CO\(_2\)-95% air (pH 7.4) in 3 ml of modified Dulbecco-Vogt (D-V) medium 14 supplemented with 10% fetal calf serum and penicillin (50 μg/ml). Cells grew out of the explants within 3–5 days and reached sufficient numbers for passage within 15–20 days. For passage, the cells were suspended in medium after incubation for 6–8 minutes in 0.05 trypsin-0.02% ethylenediaminetetraacetic acid (EDTA) solution. This suspension was passed to a 250-ml plastic flask (Falcon) with approximately 60% plating efficiency. Cells grew to confluency within 5–10 days, depending on the initial plating density. As described below, SMC thus obtained had distinct morphological and electron microscopic characteristics when compared with skin fibroblasts and with cells derived from aortic adventitial explants. SMC could be trypsinized and passed at least 10 times (approximately 40 cell generations) without loss of morphological characteristics. All studies were performed on primary cell lines following their third, fourth, or fifth trypsinization and
passage. Such cells were used to determine the effect of isolated serum lipoproteins on cell growth rate.

Cell growth curves were obtained by inoculating 6-8 x 10^4 cells into each of a series of seven 35-mm Falcon plastic Petri dishes. One dish from each of two duplicate series was counted on days 1, 2, 4, 7, 9, 12, and 16 after inoculation. Cell number per Petri dish was determined by counting a sample of its trypsinized and uniformly suspended cells in a Fuchs-Rosenthal hemocytometer. For each plate, four hemocytometer chambers were counted (25-150 cells each); thus a point on a growth curve represents the average of eight individual determinations. Preliminary evaluation of the plating and counting techniques showed acceptable precision; cell counts on six plates, inoculated at the same time and grown in 1.5% serum for 4 days, showed a coefficient of variation of 6.5%. All cells were begun in D-V medium supplemented with 1.5% pooled swine serum. On the 4th day, isolated lipoproteins, as described below, were added to the medium in varying concentrations.

LIPOPROTEINS

Lipoproteins were obtained from three normolipidemic and two hyperlipidemic swine. The hyperlipidemia of the latter swine resulted from the daily addition to their diet of 15% lard and 1.5% cholesterol. This caused a marked increase in plasma cholesterol from a normal of less than 90 mg/dl to 200-600 mg/dl. Associated with the hypercholesterolemia were striking changes in the lipoprotein type and distribution. These included the appearance of a β-migrating lipoprotein (B-VLDL) in the ultracentrifugal fraction of density less than 1.006 g/ml, the appearance of an α-migrating lipoprotein (cholesterol-induced lipoprotein called HDL2) in the fraction of density 1.02-1.08 g/ml, and an increase in low density lipoproteins (LDL)14. Normolipidemic serum lipoproteins were isolated and purified by sequential density (given throughout in grams per milliliter) ultracentrifugation, isolating and washing in the density ranges less than 1.006 (very low density lipoproteins, VLDL), 1.006-1.063 (LDL), and 1.10-1.21 (HDL). The lipoprotein fractions from the hypercholesterolemic swine included the density class less than 1.006 (VLDL and B-VLDL which were not further separated), the density class 1.02-1.063 (containing LDL and HDL2), 1.10-1.21 (containing LDL2, HDL3, and HDL4), which were separated by Geon-Pevikon electrophoresis, and the density class 1.10-1.21 (containing HDL2). HDL2 thus obtained are devoid of LDL by immunochemical and paper electrophoretic analysis, and have no particles above 130 Å in diameter (i.e., no LDL or HDL2) by negative-staining electron microscopy.15

The isolated fractions were dialyzed at least 48 hours at 4°C against several changes of at least 100 vol of buffer containing 0.15 M NaCl and 0.1 mM EDTA, pH 7.4, and 214,000 g Preference for lipid content (total cholesterol,19 cholesterol ester,21 triglyceride,22 and phospholipid22) and protein22 following the final Millipore filtration. Table 1 summarizes these chemical determinations. With the above technique for lipoprotein preservation, lipid losses during filtration averaged less than 10%.

ELECTRON MICROSCOPY

Electron microscopy was performed on smooth muscle cells from selected growth curves, with the technique described by Ross.19 Cells were fixed in Karnovsky's solution, postfixed with s-collidine-buffered osmium tetroxide, stained in block with uranyl acetate, and dehydrated with progressively concentrated ethanol. Cells were covered with a thin layer of Epon. When this became tacky, an Epon-filled capsule (BEEM) was inverted over selected areas on the Petri dish. After polymerization for 48 hours at 60°C, the BEEM capsules were removed with the SMC embedded in the Epon face. Tangential cuts gave en face sections through the cells. Thin sections were stained with uranyl acetate and examined by electron microscopy.

Results

CELL MORPHOLOGY

Cultured swine smooth muscle cells were distinct from cultured skin fibroblasts and from cells grown from swine aortic adventitial explants. Individually, as seen under the light microscope the SMC were ribbon-like, bipolar cells with a centrally located single nucleus, prominent nucleoli, and longitudinal orientation of the cytoplasmic contents. By contrast, skin fibroblasts and adventitial cells had several cytoplasmic projections, had less dense and less well oriented cytoplasmic constituents, and were spread out in a thinner monolayer, thus appearing to be somewhat larger. In the logarithmic phase of growth, SMC grew at a slower rate than skin fibroblasts and adventitial cells. For example, in D-V medium plus 10% fetal calf serum, typical skin fibroblasts have a logarithmic phase doubling time of 36 hours, whereas the doubling time for swine aortic adventitial cells was 45 hours and for swine SMC, 60 hours. Skin and adventitial cells thus achieve confluence earlier than SMC, and after confluence have a relatively constant population density in culture. By contrast, aortic SMC continue to grow after confluence, usually at a rate approximately equal to their initial logarithmic phase rate. This is accomplished by the piling up of the cells into many cell layers in a characteristic multicentric proliferative pattern. Figure 1 provides examples of the SMC growth patterns.
Electron microscopy of smooth muscle cells in a confluent stationary phase (Fig. 2) demonstrated dense cytoplasmic myofilaments and peripheral dense bodies. In the perinuclear spaces, mitochondria, rough endoplasmic reticulum, and Golgi apparatus were present, but not abundant. Adventitial cells (Fig. 3) grown under similar conditions had less densely arrayed myofilaments and a greater proportion of the other cytoplasmic organelles.

**EFFECTS OF LIPOPROTEIN ON GROWTH RATE**

We observed a lipoprotein-cholesterol concentration-dependent stimulation of SMC growth rate by the various...
lipoproteins. VLDL and LDL in cholesterol-equivalent concentrations were equally stimulatory when added to cells growing in limiting medium (D-V medium + 1.5% swine serum). HDL₃ and HDL₂ were one-half to one-third as stimulatory, at the same cholesterol levels, as LDL and VLDL. The response was the same for lipoproteins from normal and hypercholesterolemic swine. There was no apparent relationship between cell growth rate and lipoprotein phospholipid or triglyceride.

Figure 4 shows typical SMC growth curves. At the point of lipoprotein supplement (day 4), cells growing in limiting medium have achieved essentially stationary growth phase.
Of the isolated lipoproteins, LDL from normolipemic serum and VLDL (isolated at density less than 1.006) and LDL from hyperlipemic serum were significantly more growth-promoting ($P < 0.02$) than HDL$_2$ and HDL$_c$ and only slightly more stimulatory than 10% lipoprotein-free serum ($P > 0.10$). However, LDL and VLDL were only one-half to one-third as growth-promoting as 10% swine whole blood serum ($P < 0.01$).

In the early phase of growth in culture, cell number increases approximately exponentially in time according to the expression

$$\frac{N}{N_0} = e^{kt}$$

where $N$ = number of cells at time $t$, $N_0$ = number of cells at $t_0$, and $k$ = growth constant, the fractional rate of increase in cell number per unit of time. Our data were seen to follow this relationship. Therefore, the cell growth constant was computed as the slope of a linear regression analysis between the natural logarithm of the cell number and time for the period (5–6 days) following addition of the lipoproteins or other serum mixtures. Figure 5 summarizes the results from 84 paired growth curves for cells from three different miniature swine and serum from three normolipemic and two hyperlipemic swine. From the bar graph at the right of the figure, it can be seen that the SMC growth constant averaged 0.019/day in limiting medium (labeled 1.5% PPS, i.e., pooled pig serum). It was 0.110/day in limiting medium plus 10% lipoprotein-free serum from both normal (N) and hyperlipemic (H) swine (referred to as “1.21 bottom” at the center of the bar graph). In limiting
FIGURE 4 A typical growth experiment conducted as described in the text. Cells were inoculated in Petri dishes (10 x 35 mm), and grown initially in modified Dulbecco-Vogt medium supplemented with 1.5% pooled swine serum. At the time (day 4) of addition of isolated serum components to the 1.5% serum base medium, the cells had achieved virtually stationary growth. Dishes were refed every 2 days (arrows). The numbers in parentheses at the right are the lipoprotein-cholesterol concentration in mg/dl. Full details of the lipid and protein composition of the various isolated lipoproteins are given in Table 1. The upper curves are for the normolipidemic serum N-3; the lower curves for the hyperlipidemic serum H-2. The curves labeled 10% and 15% pig serum demonstrate the typical growth response to whole blood serum pooled from five normolipidemic swine. LDL and VLDL = low density and very low density lipoprotein, respectively; HDL-2 and HDL-C = a-migrating lipoproteins.

For example, the largest growth constant measured in 22 additional "recombination" studies was 0.200/day (compared to a growth constant of 0.330/day for 10% whole blood serum). The value 0.200/day resulted when LDL at a final concentration of 6.6 mg/dl cholesterol plus 10% 1.21 bottom were added to limiting medium on the 4th day of the growth curve, and is to be compared to the value of 0.067/day obtained when LDL, alone, was added at 6.6 mg/dl cholesterol. Furthermore, this initial improvement in growth with added 1.21 bottom (seen to a similar extent in all such recombination studies) diminished over 5-7 days, so that the final portion of the growth curve approximated that seen with LDL addition alone. This apparent discrepancy between whole blood serum and recombined serum is best explained by the recent observation of Ross et al. that serum clotted in the absence of platelets produces a slower initial SMC growth response and a much lower stationary phase population density than serum clotted in the presence of platelets. All sera used to obtain lipoproteins and 1.21 bottom for these studies were clotted in the absence of

medium plus 10% whole blood serum (PPS: containing 7.9 mg/dl cholesterol), growth constant averaged 0.330/day. These rates correspond to approximate doubling times of 37, 6.3, and 2.1 days, respectively.

Since initial studies of this type with combined lipoprotein solutions showed little additional growth stimulation with lipoprotein-cholesterol concentrations above 10-15 mg/dl, growth rates were evaluated in detail for all lipoproteins only in the range of 0-10 mg/dl of lipoprotein-cholesterol. Over this range, there was a linear lipoprotein-cholesterol concentration-dependent stimulation of cell growth. Based on a regression analysis of the individual lipoprotein growth data of Figure 5, at the level of 10 mg/dl added cholesterol, cell growth rate with added lipoprotein exceeded that in the limiting medium by factors of 7.2 in VLDL (P < 0.01), 5.7 in LDL (P < 0.01), 3.4 in HDL-4 (P < 0.01), and 3.0 in HDL-C (P < 0.02).

The mitogenic effect of 10% swine whole blood serum could not be recovered by recombining 10% (by volume) 1.21 ultracentrifugal bottom with individual lipoproteins.
LIPOPROTEIN EFFECTS ON AORTIC SMOOTH MUSCLE CELLS/Brown et al.

Platelets. We have found a platelet-dependent factor, comparable to the primate factor of Ross et al.\textsuperscript{16} in the swine serum-SMC system. This factor appears in the 1.21 ultracentrifugal bottom, as determined in 18 additional growth experiments. Blood was obtained from a single normolipidemic swine (N-2); a sample was allowed to clot at room temperature for 2 hours before addition of EDTA, KBr, and ultracentrifugation to obtain 1.21 bottom. The remaining blood was processed by adding EDTA immediately, removing cellular elements with successive 20-minute, 20°C centrifuge runs at 3,000, 3,000 and 20,000 g, followed by preparative ultracentrifugation for lipoproteins and 1.21 bottom. The first sample thus clotted in the presence of a normal complement of platelets, whereas the latter clotted in their virtual absence (<100/mm\(^3\)). The 1.21 bottom samples thus obtained were concentrated 2x with Diaflo filtration (Amicon) and added in varying concentration to the limiting medium of SMC growth curves on day 4. Results are summarized in Table 2.

These studies demonstrate that factors present in lipoproteins, in the 1.21 bottom, and in platelets are capable of contributing to the SMC proliferative response. The mechanisms whereby they interact to produce the whole serum mitogenic activity remain to be determined.

**Table 2** Platelet-Dependent Growth Effects in 1.21 Bottom

<table>
<thead>
<tr>
<th>Added serum % (ref. original concentration)</th>
<th>Whole blood serum</th>
<th>1.21 ultracentrifugal bottom of whole blood serum</th>
<th>1.21 ultracentrifugal bottom of platelet-free plasma serum</th>
<th>Significance of difference between last two columns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1% WBS</td>
<td>17.8</td>
<td>4.6</td>
<td>8.0</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>5%</td>
<td>1.9</td>
<td>3.5</td>
<td>6.0</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>10%</td>
<td>2.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20%</td>
<td>3.2</td>
<td>4.1</td>
<td>-</td>
<td>( P &lt; 0.03 )</td>
</tr>
<tr>
<td>40%</td>
<td></td>
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SMC = smooth muscle cells.

Comparison of the initial SMC growth rate, expressed as the log phase doubling time, in 1% and 10% swine whole blood serum (WBS), and in 1% WBS plus varying concentrations of 1.21 ultracentrifugal bottom obtained from whole blood serum or from platelet-free plasma serum. The doubling time \( T_d \) is computed from the initial growth constant \( k \) by the formula. \( T_d = 0.693/k \).
progressively acquired perinuclear refractile droplets which ranged in diameter from 0.25 μm to 2.5 μm, most commonly between 0.7 and 1.5 μm. As seen with the electron microscope, these homogenous droplets were bounded by a shell of dense material which was much thinner than the unit membrane and lacked the bilamellar structure. Figure 6 illustrates the appearance of cells fed limiting medium plus 20% (vol) N-3 VLDL. They bear a striking resemblance to human atherosclerotic foam cells (see Geer et al., Figs. 10 and 11). These changes correlated with VLDL triglyceride concentration and not with VLDL cholesterol. That is, the changes were very striking in medium supplemented with 20% N-3 VLDL, moderate with 10% N-3 VLDL, and absent with 10% H-2 VLDL (Table 1).

Discussion

Swine smooth muscle cells derived from the inner media of the aorta have morphological features and growth characteristics which distinguish them from other mesenchymal cell lines. Our experience with explantation and repeated passage of swine aortic medial cells is comparable to that of Ross14 for primate aortic cells. The process by which cells grow out of the swine aortic medial explant is documented by Jarmolych et al. with electron microscopic studies.

The studies reported here provide quantitative information on the concentration-dependent influence of certain isolated serum fractions and some of their combinations on the regulation of SMC growth in vitro and cholesterol synthesis. The SMC metabolism in vivo probably also depends on the concentration of various serum proteins in the cellular milieu of the arterial wall. Of particular importance are the lipoproteins, peptide hormones, and released platelet products which diffuse into the arterial intima in normal states, and may increase greatly in response to hypertension, hyperlipidemia, or endothelial injury. The serum-limited medium (D-V plus 1.5% pooled pig serum) was selected because SMC grow slowly in it. Comparison of this in vitro model with the slowly growing intimal smooth muscle cells in vivo is prevented by a lack of available data on the concentration of the various serum components in the intimal cellular milieu. It is clear that the endothelial lining forms an effective diffusion barrier protecting the underlying cells from the full plasma concentrations of proteins at least as small as albumin; however, quantitative data on intimal interstitial protein concentration is lacking. Thus while these in vitro data are generally consistent with present insights into the metabolism of accelerated intimal proliferation, their biological significance and their potential relevance to the atherogenic processes depend on a precise knowledge of the determinants of the concentration of these serum fractions in the arterial wall, which is a matter for continuing investigation.

Stimulation of smooth muscle cell growth by isolated LDL and HDL from homologous normolipidemic primate serum has been reported by Ross and Glomset.18 Their finding that LDL stimulate growth in limiting medium more than HDL is confirmed in this report. Our observation that LDL and VLDL are significant stimulators of SMC growth is in accord with the clinical association between increased LDL and VLDL and premature atherosclerosis. This report presents the first study of the effect on cell growth of the full range of homologous lipoprotein classes from both normo- and hyperlipidemic serum. In this setting, it is shown that normo- and hyperlipidemic lipoproteins do not differ in their capacity to stimulate cell growth when lipoprotein cholesterol is comparable. This observation is at odds with that of Dzoga et al.,19 who found significantly increased growth with hyperlipidemic LDL compared with a cholesterol-equivalent normolipidemic LDL solution. This discrepancy is unexplained, but it may be due to differences in the growth assay technique. Dzoga et al. measured the extent of cell outgrowth from rabbit aortic medial explants as an indication of proliferation. In fact, this technique may measure differences in cell size and mobility as well as replication rate.

These observations with cell growth and morphology add another dimension to the understanding of cell-lipoprotein interactions. The regulation of sterol synthesis by these same lipoproteins has been studied in our laboratories and is reported elsewhere.20 Our findings regarding the regulation by LDL and VLDL of β-hydroxy-β-methylglutaryl (HMG)-CoA reductase activity in these cells were virtually identical with those of Brown et al.77 for normal human skin fibroblasts. Their investigations have implicated a high affinity cell membrane receptor, specific for the B-apolipoprotein common to LDL and VLDL, as the mediator of the cholesterol-dependent suppression of HMG-CoA reductase activity. Thus our observation that HDLc suppresses HMG-CoA reductase was unexpected, since HDLc are immunochemically devoid of B-apolipoprotein.19 HDLc are unique cholesterol-rich α-migrating lipoproteins which are induced by cholesterol feeding in dogs27 and swine.16,19 The apo-protein content of HDLc includes the arginine-rich protein, apo-A-1, and the C apoproteins.18,19 The mechanism by which HDLc interact with the cell to regulate HMG-CoA reductase remains to be determined.

These data, as it stands, permit certain limited speculation. A necessary condition for suppression by lipoproteins of HMG-CoA reductase activity appears to be the delivery of cholesterol to the cell in the form accessible to the site of enzyme synthesis, although not necessarily bound to LDL.18,20 HDLc appear to fulfill this criterion. Since they are comparable to LDL in size and cholesterol content, they may achieve this through the "high affinity receptor" proposed by Goldstein and Brown28 or they may produce allosteric changes in the "low affinity receptor" facilitating increased delivery of cholesterol. A recent report by Steinberg and Nestel91 suggests that the LDL particle must be "internalized" by the cell before it can participate in HMG-CoA reductase regulation. The studies of Bierman et al.99 and of Stein and Stein18 indicate that rat aortic SMC bind lipoproteins by a mechanism not susceptible to trypsin digestion and thorough washing. Such avid binding is presumed to be endocytosis. A recent report by Mahley et al.44 suggests that LDL and HDL are equivalent in terms of binding kinetics and suppression of human fibroblast HMG-CoA reductase, but that they differ in the rate of onset of stimulation of cellular esterification of cholesterol.

The observation that HDLc act like LDL in the regulation
FIGURE 6 Light (a) and electron (b) micrographs of smooth muscle cells (SMC) grown in limiting medium plus 20% normalipidemic (N-3) very low density lipoprotein (VLDL) (30 mg/dl triglyceride, 5.4 mg/dl cholesterol, 2.8 mg/dl protein). After several days of incubation, the cells progressively acquired perinuclear coalescent refractile droplets. The propensity to acquire these droplets correlated with the VLDL triglyceride, occurring whenever they exceeded 15 mg/dl. There was no obvious relationship between these droplets and VLDL cholesterol. N = nucleus; D = droplets; Mv = myofilaments.
of cholesterol synthesis, but like HDL, in promoting cell growth demonstrates further that separate chemical or structural properties of these lipoproteins determine their differing effects on cellular metabolism. There are several hypothetical explanations for the differential effect of LDL, VLDL, and HDL on cell growth: (1) Other growth-promoting factors may be bound selectively to LDL and VLDL; (2) the A-1 peptide, common to HDL and HDL, may itself partially inhibit cell growth, or contain a spectrum of cholesterol esters or other substances which are relatively inhibitory (a hypothesis supported by recent clinical observations); or (3) to achieve optimal growth-promoting effects it may be necessary for cholesterol to remain bound to the B-apolipoprotein after it enters the cell, apparently not necessary condition for suppression of reductase activity.

Whatever the mechanism of their effect, HDL may represent a biologically adaptive response, since increased dietary cholesterol is stored, in part, in a form which can suppress the endogenous production of cholesterol while minimizing the potential vascular proliferative response.

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