Recognition of vesicular lipoproteins by the apolipoprotein B,E receptor of cultured fibroblasts[1]

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Abstract Vesicular lipoproteins (e.g., lipoprotein-X) are found in plasma in cholestasis or following infusion of Intralipid or phospholipid. To investigate the metabolism of vesicular lipoproteins, we isolated them from the plasma of subjects with cholestasis or following chronic or single Intralipid infusion. Cholestasis and chronic Intralipid therapy were found to be associated with elevated plasma concentrations of apoE, as determined by radioimmunoassay. Vesicular lipoproteins purified from each of the three types of plasma contained apoE, as well as other proteins. In cholestasis, in which levels of apoE were up to five times normal, a major portion of the plasma apoE was on vesicular lipoproteins. Normalized for apoE content, all preparations of vesicular lipoproteins displaced ^{125}I-labeled LDL from apoB,E receptors of cultured fibroblasts identically. This displacement was inhibited by monoclonal antibodies that block receptor binding of apoE. Vesicular lipoproteins containing ^{125}I-labeled apoE were internalized and degraded by fibroblasts. Different preparations caused small losses or gains of cellular cholesterol, with appropriate stimulation or suppression of apoB,E receptors. Thus, vesicular lipoproteins contain apoE, and apoE mediates their interaction with the apoB,E receptor. Our results suggest that the catabolism of cholesterol-rich vesicular lipoproteins, formed during cholestasis or following infusions of Intralipid or phospholipid, may be receptor-mediated. —**Williams, K. J., A. R. Tall, I. Tabas, and C. Blum.** Recognition of vesicular lipoproteins by the apolipoprotein B,E receptor of cultured fibroblasts. *J. Lipid Res.* 1986. **27:** 892–900.

Supplementary key words lipoprotein-x • cholestasis • parenteral nutrition • intravenous fat emulsion • liposome • phospholipid • cholesterol • atherosclerosis

A vesicular lipoprotein is a phospholipid-cholesterol bilayer that surrounds an aqueous core and contains adsorbed apoproteins. Vesicular lipoproteins occur in cholestasis, in which they have been called "lipoprotein-X" (2–5). Vesicular particles also occur following infusion of dispersed phospholipid (6), Intralipid (an emulsion of phospholipid and triglyceride) (7–11), or chylomicrons (12).

In a recent study, artificial vesicular particles prepared in vitro by adding biliary lipids to normal plasma were used as a model to explore the metabolism of lipoprotein-X (13). The artificial particles were shown to contain no apoE, and they did not interact with the fibroblast apoB,E receptor (13). However, in earlier studies, apoE had been detected on the vesicular lipoproteins that occur in vivo in cholestasis (3, 4, 14) or during Intralipid therapy (8). Based on these earlier studies and the known receptor binding of apoE (15–18), it was suggested that apoprotein receptors that recognize apoE may be involved in the catabolism of vesicular lipoproteins (19). It was also hypothesized (19) that receptor-mediated catabolism of vesicular lipoproteins may account for the observed delivery of drugs entrapped in liposomes to the liver (20) and may play a role in the observed anti-atherogenic effects of phospholipid infusions (21–23).

To determine whether human vesicular lipoproteins do contain apoE, we isolated these particles from the plasma of patients with cholestasis or subjects who received Intralipid Infusions. Apolipoprotein-E content was then determined by use of a well-characterized radioimmunoassay (24). Because vesicular lipoproteins have been described to contain apoC-III (4, 5, 8, 14), which can interfere with receptor recognition of apoE (16, 25), we also characterized the interaction of vesicular lipoproteins with the fibroblast apoB,E receptor.

MATERIALS AND METHODS

Human subjects

Patients with cholestasis and plasma cholesterol concentrations above 350 mg/dl were referred to us by the inpatient gastroenterology consultation service or were

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; HDL$_c$, high density lipoprotein that contains apoE; Lp-X, lipoprotein-X; RIA, radioimmunoassay; TCA, trichloroacetic acid.

[1] A preliminary report of this work was presented at the 58th Scientific Sessions of the American Heart Association (1).

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identified through inspection of the records of clinical ultrasound examinations at Presbyterian Hospital in New York City (see Table 1 for specific diagnoses). Patients receiving long-term therapy with Intralipid were referred to us by the parenteral nutrition consultation service. Normal volunteers were solicited by advertisement and were admitted to the Clinical Research Center overnight, where they each received 1000 ml of 10% Intralipid over 8 hr. The experimental protocols involving human subjects were approved by the Institutional Review Board at Columbia-Presbyterian Medical Center, and informed consent was obtained from each subject.

Samples of blood were obtained after an overnight fast and were anticoagulated with EDTA or citrate. Samples of blood from subjects receiving Intralipid were obtained at least 12 hr after the end of the preceeding day's infusion. Red cells were sedimented, and plasma was stored at 4°C for no more than 24 hr before use.

Materials

LDL was prepared ultracentrifugally (26). Radioiodinated LDL was prepared by the iodine monochloride method (27). Monoclonal antibodies that block apoE binding to the LDL receptor were the generous gift of Dr. Karl Weisgrabber of the Gladstone Foundation Laboratories. Non-immune mouse myeloma IgG (Litton Bionetics Inc., Kensington, MD) was used as control antibody.

Isolation of vesicular lipoproteins

To verify the purity of isolated vesicular lipoproteins, we relied on the absence of apoB, as previously described (3, 4, 8, 28-30), and on the low ratio of cholesteryl ester to unesterified cholesterol. Vesicular lipoproteins contain abundant cholesterol, but only 1-2% cholesteryl ester, whereas potentially contaminating lipoproteins are cholesteryl ester-rich. Using these two criteria, we found that previously published techniques (4, 30) for isolating lipoprotein-X gave preparations contaminated with particles that contained no apoB but were rich in cholesteryl ester. Therefore, we added a gel filtration step to remove these particles, which were smaller than lipoprotein-X. This modified, three-step procedure was as follows. First, plasma was subjected either to heparin-polyanion precipitation (31, 32) or to ultracentrifugation at a density of 1.063 g/ml (26). Second, the resuspended pellet from precipitation or the supernatant from ultracentrifugation was chromatographed on a 2.5 × 95 cm bed of cross-linked 6% agarose, previously equilibrated with 0.05 M sodium phosphate buffer, pH 7.4, with 0.02% sodium azide. Finally, the void fractions from the 6% agarose column were pooled and applied to a 2.5 × 35 cm bed of hydroxyapatite, previously equilibrated with 0.05 M sodium phosphate buffer. Unbound material was eluted with this buffer. Bound lipoproteins were eluted with a linear gradient of sodium phosphate concentrations, from 0.05 M to 0.65 M (30).

Individual fractions from the final step of hydroxyapatite chromatography were assayed for their content of free and esterified cholesterol by an enzymatic method (33). A typical elution profile is shown in **Fig. 1.** Fractions eluting early in the gradient contained no apoB detectable by electrophoresis of protein in SDS polyacrylamide gels (34) or by Ouchterlony immunodiffusion (35) against rabbit antiserum to human apoB (Calbiochem-Behring Corp., La Jolla, CA). These fractions also contained virtually no cholesteryl ester. Fractions eluting late in the gradient (after fraction #72) contained cholesteryl ester and apoB, and were presumably mainly VLDL. Fractions that eluted early in the phosphate gradient and contained less than 2.0% cholesteryl ester were pooled, concentrated by vacuum dialysis, dialyzed against 0.9% saline/0.3 mM EDTA, pH 7.4, sterilized by filtration through a Millex-GS 0.45 μm filter (Millipore, Bedford, MA), and stored under argon at 4°C.

Analysis of the vesicular lipoproteins

Preparations of vesicular lipoporteins were assayed for contents of free and esterified cholesterol (33), protein (36), phospholipid (37, 38), and triglyceride (37, and Worthington Triglyceride Reagent Set, Cooper Biomedical Inc., Malvern, PA), and for protein species by SDS-

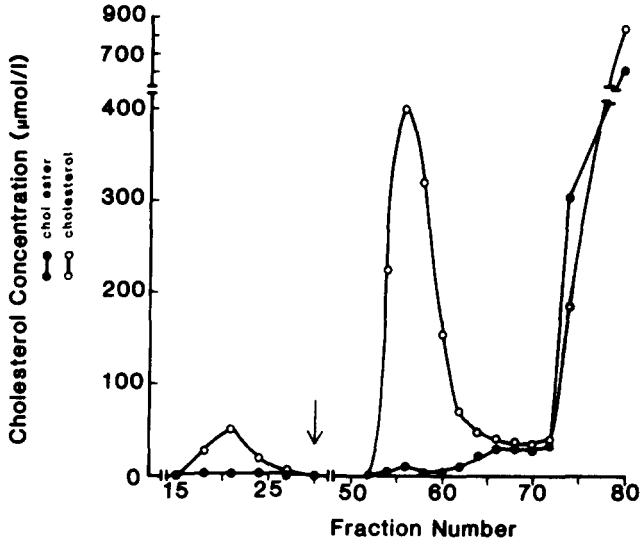

Fig. 1. Purification of vesicular lipoproteins by hydroxyapatite chromatography. Plasma from a subject who received a single infusion of Intralipid was subjected to precipitation with heparin and polyanions, then the precipitated lipoproteins were gel filtered. The void fractions from gel filtration were pooled, applied to a hydroxyapatite column, and eluted with 0.05 M sodium phosphate buffer. Bound material was eluted with a phosphate gradient of 0.05 M to 0.65 M; the arrow marks the beginning of this gradient. Concentrations of cholesterol (O) and cholesteryl ester (●) in the fractions from hydroxyapatite chromatography are shown.

polyacrylamide gel electrophoresis using mercaptoethanol as a reducing agent (34). ApoB and apoE contents were also qualitatively estimated by Ouchterlony diffusion analysis against monospecific, polyclonal anti-apoB or anti-apoE antibodies (35). ApoE content was quantitated by RIA, as described in detail previously (24). The apoE RIA had a working range of 0.8 to 12 ng, a within-assay coefficient of variation of 9%, and a between-assay coefficient of variation of 3% (24).

ApoE concentrations and distributions in human plasma

Total apoE concentration in plasma from subjects either with cholestasis and hypercholesterolemia or following Intralipid infusion was assayed by RIA (24). To determine which lipoproteins carried the apoE, 3.0 ml of plasma from a cholestatic subject was chromatographed on a 6% agarose column (1 × 115 cm) and eluted with 0.06 M phosphate buffer (17). Even-numbered fractions from gel filtration were assayed for cholesterol (39) and for apoE (24). The remaining void fractions were pooled and applied to a hydroxyapatite column (1.5 × 10.5 cm). The column was washed with 0.05 M phosphate buffer, to elute unbound material. Bound material was then eluted with a phosphate gradient (0.05 M to 0.65 M), to separate apoB-containing lipoproteins from the vesicular lipoproteins (30). Hydroxyapatite column fractions were assayed for apoE.

Binding and internalization studies

Human foreskin fibroblasts, frozen in the 6th–8th passage, were thawed, plated at 10^5 cells per 35 mm dish, and grown to subconfluency in Dulbecco's Modified Eagle's Medium (DME) supplemented with 100 units of penicillin/ml, 100 units of streptomycin/ml, 292 μg of glutamine/ml, and 10% (v/v) fetal calf serum. Incubation was at 37°C in an atmosphere of 8% CO_2 and 92% air. The cells were then grown to near confluency in 5% (v/v) lipoprotein-deficient fetal calf serum (LpDS), to stimulate the activity of apoB,E receptors (27).

Competitive displacement studies between ^{125}I-labeled LDL and unlabeled vesicular lipoproteins were conducted at 4°C (27). These studies were also performed in the presence of anti-apoE monoclonal antibodies that block apoE binding to the apoB,E receptor (18). The ability of cholesterol on the vesicular lipoproteins to suppress fibroblast apoB,E receptors during a 24-hr incubation at 37°C was assessed by subsequently measuring dextran-releasable binding of ^{125}I-labeled LDL to the cell surface (27); also measured were total cholesterol contents of lipid-extracted fibroblasts from selected wells (40). All results of these assays on fibroblasts were normalized for cellular protein content.

To measure directly the degradation of vesicular lipoproteins by fibroblasts, radioiodinated particles were prepared. Because vesicular lipoproteins have a low ratio of protein to lipid, radioiodination was accomplished indirectly. ApoE, purified by sequential heparin-Sepharose and DEAE-cellulose chromatography, was radioiodinated by the iodine monochloride procedure (24, 27). This isolated ^{125}I-labeled apoE (9.3 μg of apoE mass, 1.9×10^8 cpm) was then incubated with a preparation of vesicular lipoproteins (1.2 mg of total protein, 62 μg of apoE) for 1 hr at 25°C, then overnight at 4°C. The incubation mixture (2 ml total volume) was then passed over a 1 × 30 cm bed of 6% agarose and eluted with 0.9% saline/0.3 mM EDTA, pH 7.4, to separate vesicular particles from free protein and possible discoidal recombinants. One-ml fractions were collected and monitored for radioactivity. The peak eluting at the void volume spanned approximately six fractions; the three fractions with the highest radioactivity were pooled for use as radiolabeled vesicular lipoproteins. This preparation contained 51.8% of initial protein mass and 20.8% of initial radioactivity and had a specific activity of 58.7 cpm/ng of total protein. Degradation of the radiolabeled vesicular lipoproteins by fibroblasts was assayed by measuring release of TCA-soluble, chloroform-insoluble ^{125}I radioactivity into the culture media (27).

RESULTS

Characterization of donor plasma and vesicular lipoproteins

The total apoE concentrations in the plasmas of patients with cholestasis or from subjects receiving Intralipid are shown in **Table 1.** Cholestasis with hypercholesterolemia was associated with markedly elevated apoE levels that correlated with the degree of hypercholesterolemia ($r = 0.93$, $0.02 < P < 0.05$, $n = 5$) (41). Chronic infusions of Intralipid were associated with moderately elevated plasma apoE levels. Acute infusions produced only slight elevations in plasma apoE concentration.

To determine how much of the apoE in cholestatic plasma was transported on vesicular lipoproteins, plasma was fractionated by gel filtration and the distributions of apoE and cholesterol were determined (**Fig. 2**). Approximately 57% of the total recovered apoE was in the fractions that contained Lp-X, VLDL, and LDL (35–57 ml elution volume). These fractions were pooled, then subjected to hydroxyapatite chromatography, to separate Lp-X from the apoB-containing particles. A profile similar to that in Fig. 1 was obtained; 37% of the total recovered apoE was in the Lp-X peak. Thus, 21% (37% of 57%) of plasma apoE was associated with Lp-X. Thirty-three percent was on VLDL/LDL (the second bound hydroxyapatite peak), 42% was on HDL_c (the second apoE peak from gel filtration of whole plasma; see Fig. 2), and 4% was unidentified.

TABLE 1. ApoE concentrations in plasma samples that contain vesicular lipoproteins

Origin of Lp-X	Underlying Diagnosis	Concentration of Plasma Components		
		ApoE	Cholesterol	Triglycerides
		μg/ml	*mg/dl*	
Cholestasis and hypercholesterolemia	Adenocarcinoma of unknown primary	269.6	593	254
	Adenocarcinoma of unknown primary	209.8	618	
	Acute choledocholithiasis	180.7	465	
	Primary biliary cirrhosis	87.7	388	93
	Alcoholic hepatitis and cirrhosis	62.3	354	210
Chronic Intralipid therapy	Chronic depression and weight loss[a]	50.2	168	
	Prolonged postoperative ileus[a]	60.0	132	
Single Intralipid infusion	Volunteer #1, before	32.7	169	60
	Volunteer #1, after	38.3	169	79
	Volunteer #2, before	28.9	179	110
	Volunteer #2, after	50.2	203	410

ApoE concentrations were measured by RIA. The concentrations of apoE in normal plasma are 36 ± 11 μg/ml (mean ± SD) (17).
[a]Same patient, 2 weeks apart; surgery was for a perforated ulcer.

To obtain vesicular lipoproteins of sufficient purity for use in cell culture studies, it was necessary to use the three-step isolation procedure outlined in the Methods section (see Fig. 1). The vesicular lipoproteins isolated by this method from cholestatic patients, from patients receiving Intralipid chronically, and from subjects receiving single infusions of Intralipid showed similar lipid compositions. Cholesteryl esters comprised < 0.2% and triglycerides comprised < 3.0% of total lipid mass. The molar ratio of free cholesterol to phospholipid was consistently between 0.6 and 0.7. Although the protein content of these particles was consistently 1.5–3.6% of total lipid mass, the protein species in the different preparations varied considerably (**Fig. 3**). Albumin was the major protein, presumably trapped in the aqueous cores of the vesicular lipoproteins (4). ApoA-I and C-apoproteins were variably present. No apoB was detected by protein electrophoresis or by Ouchterlony analysis. The content of apoE was 2–5% of total protein in preparations from cholestatic plasma or from plasma following chronic Intralipid therapy. In contrast, following single infusions of Intralipid, apoE was only 0.4% of total protein, as assessed by RIA, and was undetected by protein electrophoresis.

Interaction of vesicular lipoproteins with cells

Each preparation of vesicular lipoproteins (i.e., from cholestatic plasma, from plasma taken during chronic Intralipid therapy, and from plasma following single infusions of Intralipid) was able to inhibit binding of ^{125}I-labeled LDL to fibroblasts. When the results were expressed in terms of lipid or total protein content, the preparations showed markedly different potencies (not shown). However, when expressed in terms of apoE content, all preparations displaced ^{125}I-labeled LDL identically (**Fig. 4**), suggesting that apoE was the ligand mediating the competitive binding. The vesicular lipoproteins (per mg of apoE) were 15–20 times as potent as unlabeled LDL (per mg of apoB) in displacing ^{125}I-labeled LDL, which suggests roughly equivalent potency per mole of apoprotein. This result agrees with previous measurement of the relative affinities of apoE and apoB for the apoB,E receptor (17). At sufficient excess of vesicular lipoprotein apoE, the binding of ^{125}I-labeled LDL to the fibroblasts was essentially completely eliminated.

Because some preparations of vesicular lipoproteins contained several proteins in addition to apoE, we sought additional evidence that displacement of ^{125}I-labeled LDL was caused specifically by the apoE on the vesicular lipoproteins. The ability of vesicular lipoproteins to

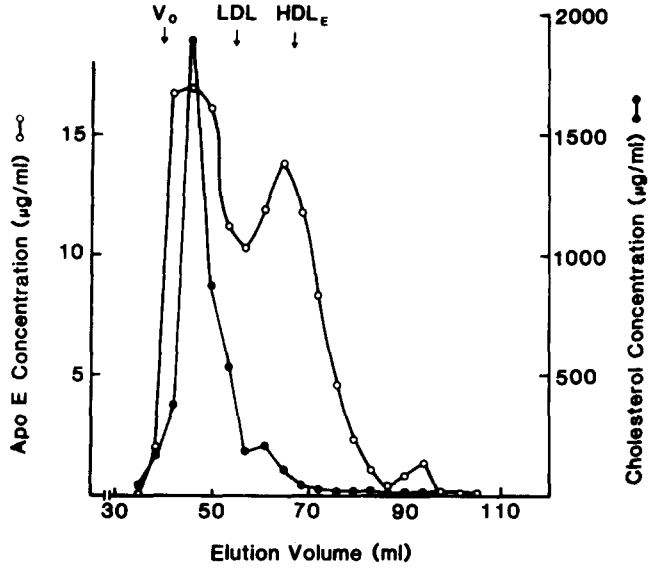

Fig. 2. Distribution of apoE (○) and total cholesterol (●) in gel-filtered plasma from a patient with cholestasis and hypercholesterolemia. Three ml of plasma was gel-filtered through a 1 × 115 cm bed of 6% agarose. The elution volumes of the void (V_o), normal LDL, and HDL_e are indicated.

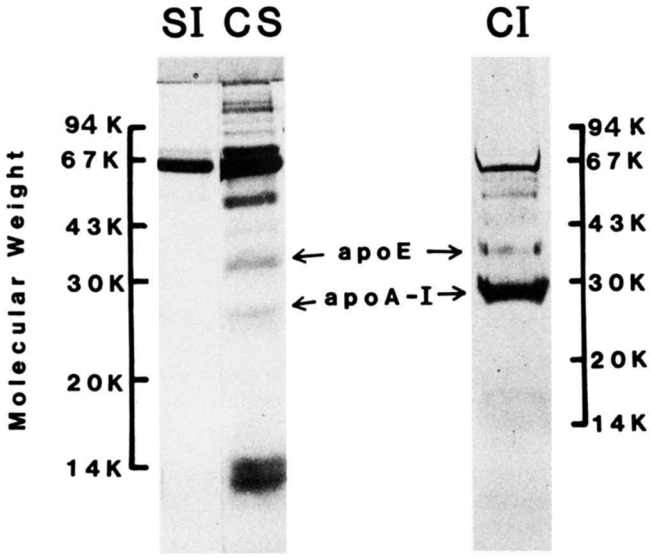

Fig. 3. SDS-polyacrylamide gel electrophoresis of the proteins of vesicular lipoproteins. Molecular weight standards (STD) were phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and alpha-lactalbumin (14,400). The vesicular lipoproteins were isolated from cholestatic plasma (CS), and from plasma following single infusions (SI) or chronic infusions (CI) of Intralipid. The two lanes on the left (SI and CS) had stacking gels of 4% acrylamide and running gels of 12% acrylamide. The lane on the right (CI) had a polyacrylamide gradient of 6–16%. Bands that comigrated with the apoE and apoA-I bands of human VLDL are indicated.

displace ^{125}I-labeled LDL from the apoB,E receptor of fibroblasts was inhibited by monoclonal antibodies that block apoE binding to apoB,E receptors (**Fig. 5**). A 40:1 mass ratio of antibody to apoE reduced the inhibition of dextran-releasable ^{125}I-labeled LDL binding from 94% inhibition (2.6 mg of ^{125}I-labeled LDL protein bound/mg cellular protein) to 41% inhibition (26.1 mg of ^{125}I-labeled LDL protein bound/mg cellular protein). Thus, the majority of the competitive displacement by vesicular lipoproteins was attributable to apoE.

To document intracellular catabolism of vesicular lipoproteins, particles containing ^{125}I-labeled apoE were incubated with fibroblasts. These radiolabeled vesicular lipoproteins were internalized and degraded, as demonstrated by the release of TCA-soluble, chloroform-insoluble ^{125}I radioactivity into the media (**Fig. 6**), consistent with receptor-mediated uptake of the particles.

The ability of vesicular lipoproteins to change the cholesterol content of fibroblasts and to alter receptor status is displayed in **Fig. 7A and B.** Vesicular lipoproteins poor in apoE (from an Intralipid-infused subject) and vesicular lipoproteins rich in apoE (from a cholestatic subject) were relatively ineffective at producing either net delivery of cholesterol mass to the cells or suppression of cellular apoB,E receptors. At the highest concentration tested, vesicular lipoproteins poor in apoE produced a 25% loss of cellular cholesterol, which was associated with further stimulation of apoB,E receptor activity. At maximal concentration, apoE-rich vesicular lipoproteins produced a moderate net gain (20%) in cellular cholesterol and an 18% suppression of apoB,E receptor activity. A comparable amount of total LDL cholesterol produced a more pronounced (143%) gain of cellular cholesterol and a more pronounced (80%) suppression of apoB,E receptors. For all lipoprotein preparations, the effect on receptor status was linearly related to the effect on cellular cholesterol content (Fig. 7B).

DISCUSSION

The present study shows that human vesicular lipoproteins contain apoE and interact with the apoB,E receptor of fibroblasts, as previously hypothesized (19). Our results suggest that vesicular particles prepared in vitro by mixing plasma with biliary lipids, as described in a prior study (13), are not adequate models for the vesicular lipoproteins that develop in vivo. The previously reported absence of receptor binding by artificial vesicular particles isolated from plasma–bile mixtures is consistent with our finding that binding depends on apoE, which the artificial particles lacked (13).

In our study, vesicular lipoproteins were internalized and degraded, and the ligand for their binding was apoE. The evidence that apoE mediates the cellular interactions was, first, that all preparations of vesicular lipoproteins

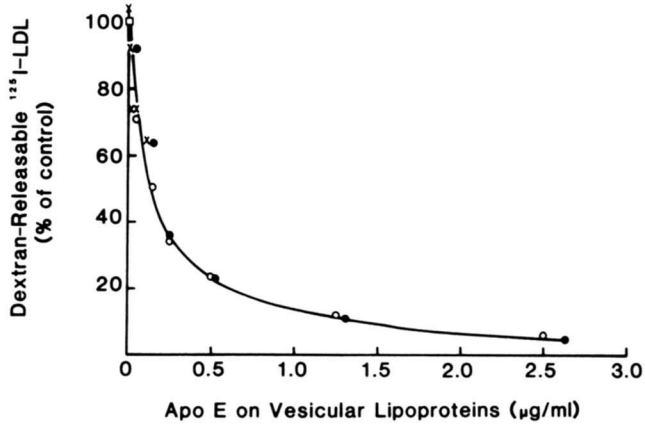

Fig. 4. Competitive displacement of ^{125}I-labeled LDL by vesicular lipoproteins. Human fibroblasts grown to near confluency in DME/LpDS were incubated for 2 hr at 4°C with media containing 1.0 μg of ^{125}I-labeled LDL protein/ml and the indicated concentrations of vesicular lipoprotein apoE. The cells were then washed, and dextran-releasable radioactivity was measured, from which the mass of dextran-releasable ^{125}I-labeled LDL protein was calculated. Binding of ^{125}I-labeled LDL in the absence of vesicular lipoproteins (100% on the y-axis) (□) was 52.7 ng of ^{125}I-labeled LDL protein/mg of cellular protein. The experiment was performed with vesicular lipoproteins isolated from three situations: (○) from cholestatic plasma; (●) from plasma following chronic Intralipid infusion; (X) from plasma following a single infusion of Intralipid. Each point represents the mean of two determinations.

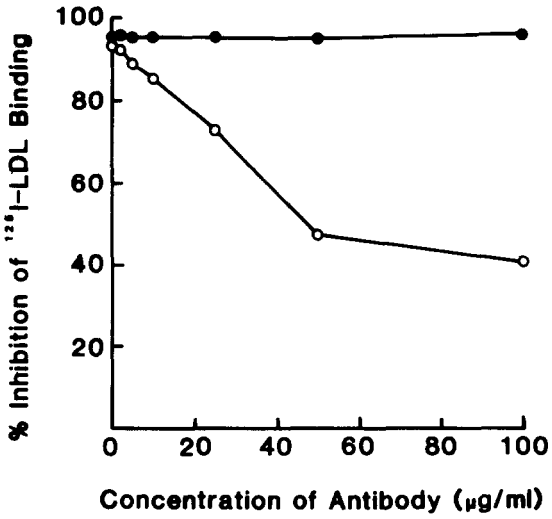

Fig. 5. Effect of monoclonal blocking antibodies to apoE on the ability of vesicular lipoproteins to inhibit dextran-releasable binding of ^{125}I-labeled LDL to fibroblasts. Media containing 2.51 μg of cholestatic vesicular lipoprotein apoE/ml, 1.0 μg of ^{125}I-labeled LDL protein/ml, and the indicated concentrations of antibody were incubated for 90 min at 25°C, to allow antibody binding, then chilled to 4°C. Fibroblasts were incubated for 2 hr at 4°C with these media, and dextran-releasable radioactivity was measured. Binding of ^{125}I-labeled LDL without vesicular lipoproteins and without antibodies (0.0% inhibition on the y-axis) was 43.9 ng of ^{125}I-labeled LDL protein/mg of cellular protein; (○) with monoclonal anti-apoE antibodies; (●) with nonimmune mouse myeloma IgG.

fell on the same displacement curve when normalized for apoE content and, second, that the majority of this displacement was blocked by monoclonal antibodies to apoE. Similar approaches have been used previously to establish apoE as the ligand mediating the receptor binding of β-VLDL isolated from subjects with primary dysbetalipoproteinemia (18) and of HDL_2 isolated from subjects with abetalipoproteinemia (17). Our finding that apoE on vesicular lipoproteins has the same potency in displacing ^{125}I-labeled LDL as has been reported for the apoE on HDL_2 (17) suggests that little, if any, apoE was sequestered within the vesicular particles.

The appearance of lipoprotein-X in cholestatic plasma has been attributed to regurgitation of bile into the blood stream (2–5). Evidence to support this hypothesis includes the closely matching lipid compositions between lipoprotein-X and bile (5), the appearance of lipoprotein-X in the plasma of dogs following implantation of the bile duct into the inferior vena cava (5), and the appearance of vesicular particles in the bile canaliculi of isolated livers from cholestatic, but not control, rats (3).

There is additional evidence to suggest that the accumulation of vesicular lipoproteins in cholestatic plasma may be the result of impaired clearance as well. First, with normal hepatic function, the liver is the major site of clearance of infused vesicles (19, 20). Second, the presence of apoB,E receptors in the liver (16, 42) and our demonstration that lipoprotein-X binds to apoB,E receptors support the suggestion that hepatic clearance of vesicular particles could be receptor-mediated (19). Finally, high serum concentration of bile salts, such as are seen in cholestasis, have been shown to suppress hepatic apoB,E receptors in vivo (42). Therefore, we conclude that the accumulation of lipoprotein-X and apoE in plasma in cholestasis may be partly a consequence of impaired clearance, caused by suppression of hepatic apoB,E receptors by high serum concentrations of bile salts. In our subjects with normal hepatic function, rapid hepatic clearance of apoE-rich vesicular lipoproteins may have been responsible for the inability of Intralipid infusions to elevate plasma apoE levels beyond about 60 μg/ml. It has been shown that administration of cholestyramine, a potent inducer of hepatic apoB,E receptors (43), causes a marked reduction in the concentration of unesterified cholesterol in plasma in cholestasis (44–46). Our data on the receptor-binding of vesicular lipoproteins suggest that the efficacy of cholestyramine in lowering plasma cholesterol in cholestasis may partly result from enhanced hepatic removal of lipoprotein-X particles.

Although we demonstrated uptake and degradation of vesicular lipoproteins by fibroblasts, the vesicular lipoproteins were relatively inefficient at causing either accumulation of cellular cholesterol or suppression of

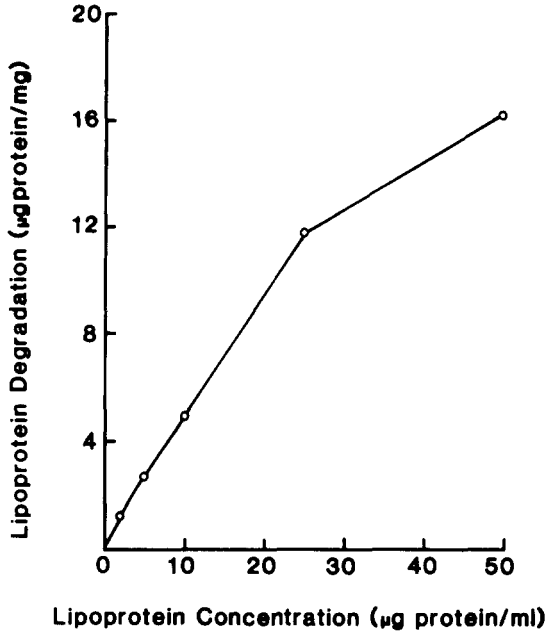

Fig. 6. Degradation of radiolabeled vesicular lipoproteins by fibroblasts. Fibroblasts and empty tissue culture wells were incubated for 4 hr at 37°C with media that contained vesicular lipoproteins labeled with ^{125}I-labeled apoE. Degradation was determined by measuring TCA-soluble, chloroform-insoluble ^{125}I radioactivity in the media. Degradation by fibroblasts was calculated by subtracting the spontaneous degradation in cell-free wells from the degradation in fibroblast-containing wells. Spontaneous degradation was 3.9–5.4% of total degradation.

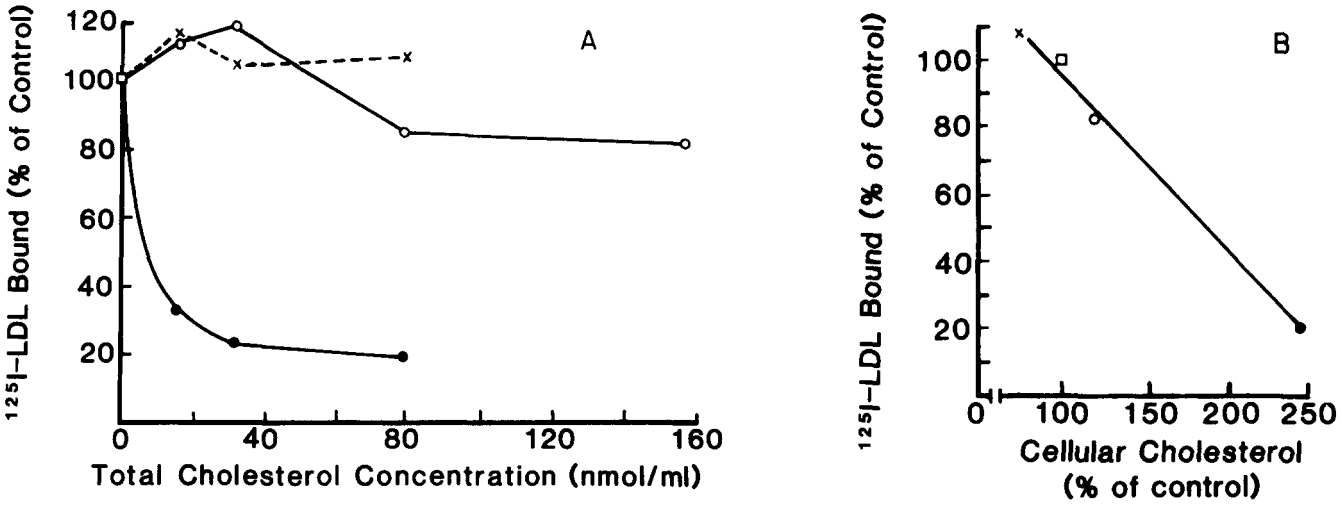

Fig. 7. Panel A: Effect of cholesterol-containing lipoproteins on cellular apoB,E receptor status. Fibroblasts grown to subconfluency in DME/LpDS were incubated for 24 hr at 37°C in DME/LpDS plus the indicated concentrations of total cholesterol. The cholesterol was on the following types of particles: (○), cholestatic vesicular lipoproteins; (X), vesicular lipoproteins isolated from human plasma following a single infusion of Intralipid; or (●), human LDL. The control incubation (□) was 24 hr in DME/LpDS with no added lipoproteins. Following the 24-hr incubation, the cells were washed, then incubated for 2 hr at 4°C in media containing 10 μg of ^{125}I-labeled LDL protein/ml with or without a 50-fold excess of unlabeled LDL. Dextran-releasable radioactivity was measured. As an indication of apoB,E receptor status, specific binding of ^{125}I-labeled LDL to the cells was calculated as the difference between the amounts bound in the presence and absence of excess unlabeled LDL. Panel B: ApoB,E receptor status plotted against cellular cholesterol content. The cholesterol contents of the fibroblasts corresponding to the extreme right-hand points of each curve in the top panel were assayed by gas–liquid chromatography. The symbols for the cholesterol-containing lipoproteins in the 24-hr incubation are the same as in panel A. The control value for specific binding of ^{125}I-labeled LDL was 54.8 ng of ^{125}I-labeled LDL protein/mg of cellular protein and the control value for cellular cholesterol content was 17.9 nmol/mg of cellular protein.

cellular apoB,E receptors. Previous studies have shown that phospholipid vesicles are capable of removing cholesterol from cells (6, 47). Thus, our results could reflect simultaneous cholesterol delivery by receptor-mediated endocytosis and cholesterol removal by nonspecific means.

Based on previously published values for the diameters of vesicular lipoproteins (4, 5, 9, 14) and for the surface areas occupied by phospholipid and cholesterol molecules at lipid–water interfaces (48), we conclude that the preparations of vesicular lipoproteins isolated following single infusions of Intralipid contained significantly fewer than one apoE molecule per vesicular particle, and, therefore, contained particles with no apoE. The preparations isolated from cholestatic plasma or during chronic Intralipid therapy contained on average about one apoE molecule per particle, which does not eliminate the possibility that apoE-free particles were present in these preparations as well. A subpopulation of lipoprotein-X that contains no apoE has been previously reported (14). However, the absence of apoE was demonstrated for material that had been isolated by more than 48 hr of ultracentrifugation, during which the apoE might have been stripped from the particle surface. Also, the absence of apoE was demonstrated by protein electrophoresis, not by more sensitive, immunologic techniques (14). In our competitive binding studies, displacement of ^{125}I-labeled LDL depended solely on the apoE contents of the different preparations of vesicular lipoproteins, implying that apoE-free particles were not significantly involved. During co-incubation with cultured fibroblasts, apoE-free subpopulations of vesicular particles might have promoted cellular loss of cholesterol, without participating in receptor-mediated re-uptake.

It has been suggested that the ability of phospholipid infusions to produce regression of experimental atherosclerosis can be explained by several mechanisms, including the creating of vesicular lipoproteins, which would act as additional cholesterol acceptors (19), and the phospholipid enrichment of pre-existing lipoproteins, especially HDL (6, 19, 49). Our data provide support for the hypothesis that vesicular lipoproteins are at least partly responsible for phospholipid-induced regression (19). Vesicular lipoproteins have been shown to arise in plasma and acquire endogenous cholesterol following infusion of dispersed phospholipid (6), as was used in the original regression studies (21–23). We have shown that vesicular lipoproteins occurring in vivo do contain apoE, and might therefore be able to deliver their cholesterol to the liver for excretion, as hypothesized (19). If there is a competition between peripheral and hepatic apoprotein receptors for uptake of these particles, the administration of cholestyramine might favor hepatic clearance, and thereby accelerate phospholipid-induced regression of atherosclerosis. Pharmacologic manipulation of hepatic apoprotein receptors may also prove useful in directing

liposomally encapsulated drugs toward or away from the liver, as desired.

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