Apolipoprotein E Is the Determinant that Mediates the Receptor Uptake of β-Very Low Density Lipoproteins by Mouse Macrophages

Thomas L. Innerarity, Kay S. Arnold, Karl H. Weisgraber, and Robert W. Mahley

Beta very low density lipoproteins (B-VLDL) from cholesterol-fed animals and from patients with Type III hyperlipoproteinemia are internalized by a receptor-mediated process in mouse macrophages. Once internalized, the cholesteryl esters of B-VLDL are hydrolyzed in lysosomes, and the released cholesterol is re-esterified, resulting in a massive accumulation of cholesteryl esters. In the present study, competitive binding experiments demonstrated that canine apo E HDL (lipoproteins that contain almost exclusively apolipoprotein E) inhibited the receptor-mediated uptake of 125I-B-VLDL. The incorporation of human apo E into B-VLDL was also shown to modulate binding. Reductively methylated B-VLDL (methyl B-VLDL) were not taken up by macrophages and did not stimulate cholesteryl ester synthesis. When unmodified human apo E-3 was incorporated into the lipoprotein in place of the canine methyl apo E, these hybrid B-VLDL (methyl B-VLDL [E-3]) were internalized and degraded and were as effective as native B-VLDL in stimulating cholesteryl ester synthesis in macrophages. In the reverse experiment, the incorporation of methyl apo E-3 into native canine B-VLDL (B-VLDL [methyl E-3]) reduced the binding activity of the B-VLDL and abolished their ability to stimulate cellular cholesteryl ester synthesis. Canine B-VLDL into which apo E-2(Arg158→Cys) had been incorporated had less ability to stimulate cholesteryl ester synthesis (20%) than did native B-VLDL, but they were more active than B-VLDL [methyl E-2] or B-VLDL [methyl E-3], which had virtually no activity. These results demonstrate that apo E is the determinant mediating the receptor binding and uptake of B-VLDL by mouse macrophages. (Arteriosclerosis 6:114–122, January/February 1986)

One of the consequences of diet-induced hypercholesterolemia in various experimental animal models is the induction of fatty streaks and more advanced atherosclerotic lesions. Recently, it has been shown that foam cells accumulate in the intima of vessels of nonhuman primates in less than 1 month after the onset of cholesterol feeding. The majority of the foam cells are macrophages that have accumulated lipids, primarily in the form of cholesteryl ester lipid droplets stored in the cytoplasm.

From the Gladstone Foundation Laboratories for Cardiovascular Disease, Cardiovascular Research Institute, Departments of Pathology and Medicine, University of California, San Francisco, California.

Address for reprints: Thomas L. Innerarity, Ph.D., The Gladstone Foundation Laboratories, P.O. Box 40608, San Francisco, California 94140.

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The in vivo mechanism whereby blood monocytes migrate into the arterial wall and acquire lipid is unknown. However, in vitro studies have established that specific types of lipoproteins can be taken up by cultured mouse peritoneal macrophages and can convert these cells to cholesteryl ester-loaded cells resembling foam cells (for review, see reference 5). Abnormal lipoproteins that are taken up by macrophages include low density lipoproteins (LDL) that have been acetylated, acetocacetylated, malondialdehyde-treated, oxidized, isolated from the artery wall, and complexed with dextran sulfate. These chemically modified lipoproteins are recognized by distinct receptors on the macrophage cell surface. Naturally occurring lipoproteins capable of causing cholesteryl ester accumulation in macrophages are the B-VLDL. The B-VLDL are induced by diets high in fat and cholesterol and also accumulate in the plasma of patients with the genetic lipid abnormality,
Type III hyperlipoproteinemia. The primary genetic defect in Type III hyperlipoproteinemia is a gene that codes for abnormal forms of apo E that do not bind normally to either apo B, E(LDL) or apo E receptors. The presence of the receptor-defective apo E accounts for the accumulation of B-VLDL and cholesterol feeding, presumably secondary to the down-regulation of the expression of the apo B,E(LDL) receptor. In either situation, the B-VLDL are heterogeneous and represent two distinct lipoprotein classes.

Both subclasses of β-VLDL bind to lipoprotein receptors on macrophages. These receptors mediate the internalization of the β-VLDL and their subsequent degradation within the lysosomes. The cholesteryl esters contained within the β-VLDL are hydrolyzed in the lysosomes, and the liberated cholesterol enters the cytoplasm where it is re-esterified. The mouse macrophage receptor for B-VLDL is subject to feedback down-regulation by an accumulation of cholesteryl esters, although in comparison to the efficient down-regulation of the apo B,E(LDL) receptor, the B-VLDL are heterogeneous and represent two distinct lipoprotein classes.

In the current study, we conclude that apo E in B-VLDL is the predominant apolipoprotein recognized by the macrophage receptor that is responsible for B-VLDL binding.

Methods

Lipoprotein Isolation and Characterization

Canine β-VLDL (d < 1.006 g/ml) and apo E HDL (d = 1.006–1.02 g/ml) were isolated from the plasma of dogs fed a semisynthetic diet containing hydrogenated coconut oil and cholesterol. Human β-VLDL (d < 1.006) that contained either the apo E-2(Arg142→Cys) or the apo E-3(Cys 112→Arg, Arg142→Cys) variant were isolated from the plasma of Type III hyperlipoproteinemic patients. The canine and human d < 1.006 lipoproteins were isolated by ultracentrifugation in a 60 Ti rotor (Beckman) at 50,000 rpm for 16 hours at 4°C and then were washed by recentrifugation under the same conditions. The B-VLDL were separated from the pre-B-VLDL in the d < 1.006 fraction by Pevikon block electrophoresis. The apo E HDL were isolated by centrifugation at d = 1.006 to 1.02 in a 60 Ti rotor at 50,000 rpm for 16 hours at 4°C and were separated from the LDL by Pevikon block electrophoresis. Human LDL (d = 1.02–1.05) were isolated as described previously.

Triglyceride and total cholesterol concentrations were determined enzymatically as described by the manufacturer (Bio-Dynamics, Boehringer Mannheim Corporation, Indianapolis, Indiana). The lipoprotein phospholipid concentration was determined by phosphorus analysis, and the protein concentration was determined by the method of Lowry et al. The β-VLDL were iodinated by the iodine monochloride procedure described by Bilheimer et al. and had a specific activity of 200 to 800 cpm/mg of protein. The apo E HDL were iodinated by the Bolton-Hunter procedure and had a specific activity of 200 to 400 cpm/mg of protein.
Human apo E-2(Arg158→Cys) and apo E-3 were isolated from the d < 1.02 ultracentrifugal fraction of plasma by Sephadex G-200 column chromatography of the delipidated d < 1.02 lipoproteins as described. The apolipoprotein content of the lipoproteins and the purity of the apo E were determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

Apolipoproteins and lipoproteins were reductively methylated as described previously. The apolipoproteins, 2.5 to 3 mg in saline-EDTA (0.15 M NaCl and 0.01% EDTA, pH 7.4), were diluted to 1.5 times original volume with 0.3 M sodium borate buffer, pH 9. The modifications were performed at 4°C by adding a sodium borohydride solution (2 mg/ml in 0.1 M sodium borate buffer, pH 9) at a ratio of 0.35 mg of borohydride/mg of protein to apolipoprotein, followed by 1-μl additions of 37% Formalin at 6-minute intervals with gentle mixing after each addition. After 30 minutes (six additions of Formalin), an additional one-half the original amount of sodium borohydride was added and six 1-μl additions of Formalin were continued at 6-minute intervals. After the last addition (total of 60 minutes), the modified protein was dialyzed extensively against saline-EDTA. The B-VLDL were reductively methylated by the same procedure, but sodium borohydride was added at a ratio of 0.4 mg/mg of lipoprotein protein, and 1 μl of a 1:5 dilution of 37% Formalin was added at each time point.

The apo E was reduced with 0.5% (vol/vol) β-mercaptoethanol (final concentration, 0.5%, vol/vol) for 1 hour at 37°C. The β-mercaptoethanol was removed by dialysis against an oxygen-free, 0.1 M ammonium bicarbonate buffer that had been prepared by degassing under vacuum for 2 hours while bubbling nitrogen through the buffer.

The reduced apo E was then incorporated into radiolabeled β-VLDL or methyl β-VLDL at a 4:1 to 20:1 protein/protein ratio and was incubated at 37°C for 2 hours. The mixture was layered on a d = 1.006 to 1.063 KBr gradient with a cushion of 0.5 ml of d = 1.21 solution in 1/2 in. × 2 in. polyallomer tubes (Beckman). The tubes had been pretreated with polyvinyl alcohol to improve wettability. After spinning at 55,000 rpm for 16 hours at 4°C in a SW55 rotor, 10 fractions of 0.6 ml each were obtained. The lipoproteins with the incorporated apolipoproteins floated at d < 1.006, while the free apolipoproteins sedimented to the bottom of the tube. The β-VLDL-containing fractions, determined by radioactivity and absorbance at 280 nm, were pooled and dialyzed against saline-EDTA. The hybrid lipoproteins were characterized by lipid analysis and SDS-polyacrylamide slab gel electrophoresis. Autoradiograms were prepared with XS-5 Kodak film.

Unstimulated peritoneal macrophages were obtained from Swiss-Webster mice by peritoneal lavage, as previously described. Macrophages were identified by size distribution analysis on the Channelizer (Coulter Electronics, Hialeah, Florida), and the cell suspension in Dulbecco’s modified Eagle's medium (DMEM) was plated at 5 × 10² macrophages per 16-mm petri dish well. After a 1.5- to 2-hour incubation at 37°C, the cells were washed three times with DMEM and then incubated at 37°C for 18 to 20 hours in DMEM containing 20% fetal calf serum to duplicate the conditions that were originally used to describe the uptake of β-VLDL by mouse macrophages. Fibroblasts were maintained in culture, and binding studies at 4°C were conducted as described.

The cellular uptake and proteolytic degradation of the radiolabeled lipoproteins by fibroblasts or macrophages were determined as described. Non-specific uptake and degradation were determined by adding unlabeled canine hypercholesterolemic d < 1.006 lipoproteins at a final concentration of 500 μg protein/ml along with the radiolabeled lipoproteins. Specific binding values were obtained by subtracting non-specific values from the total cellular uptake and degradation. Cholesterol esterification by macrophages during incubation with ¹⁴C-oleate-albumin complex and lipoproteins was determined by a 16-hour incubation at 37°C. Each 16-mm plate of cells received 300 μl of DMEM containing no serum, various concentrations of lipoprotein cholesterol, and 0.2 mM ¹⁴C-oleate-albumin complex. After the incubation period, the cells were placed on ice, then rapidly washed three times with 1 ml of cold phosphate-buffered saline containing 2 mg/ml of bovine serum albumin followed by two 10-minute incubations with the same buffer and, finally, a rapid wash with cold phosphate-buffered saline. The lipids in the cells were extracted in situ with 3:2 hexane/isopropanol containing 15,000 dpm ¹⁴C-cholesterol oleate as an internal standard. The ¹⁴C-cholesterol esters were determined by liquid scintillation counting after thin-layer chromatography on Whatman LKSD prechannneled plates with 90:10:1 hexane/diethyl ether/ammonia used as the solvent system. Cellular protein was determined by dissolving the lipid-solvent-extracted cells with 0.1 M NaOH.

Canine β-VLDL and apo E HDL₉ were used to demonstrate the specificity of the uptake of β-VLDL by mouse macrophages in competitive binding experiments. While β-VLDL contain apo B-100 and/or apo B-48, apo E, C apolipoproteins, and minor amounts of other apolipoproteins, canine apo E HDL₉ possess apo E as the predominant, if not exclusive, apolipoprotein. As shown in Figure 1, unlabeled β-VLDL and apo E HDL₉ were equally effective.
Figure 1. Ability of canine β-VLDL (■) and canine apo E HDLc (●) to compete with 125I-β-VLDL (A) and 125I-apo E HDLc (B) for cellular binding and uptake by mouse peritoneal macrophages. Each 16-mm dish of macrophages received 0.35 ml of Dulbecco’s modified Eagle’s medium (DMEM) containing 10% lipoprotein-deficient serum, 1 μg/ml of either 125I-β-VLDL or 125I-apo E HDLc, and the indicated concentration of unlabeled lipoproteins. The cells were incubated for 5 hours at 37° C.

competitors for uptake by macrophages. In other experiments (not shown), apo E HDLc did not always compete as effectively as β-VLDL; however, in all experiments, the apo E HDLc were very efficient competitors for 125I-β-VLDL binding and degradation. By comparison (data not shown), human LDL at concentrations up to 200 μg of protein/ml were not effective in competing with β-VLDL for binding to the macrophages (as described previously in references 10 and 11).

Earlier studies demonstrated that incubation of mouse peritoneal macrophages with apo E HDLc, in contrast to β-VLDL, does not lead to a massive accumulation of cholesteryl esters.10 However, as shown in Figure 2, incubation of the cultured macrophages with apo E HDLc stimulated the formation of cholesteryl 14C-oleate but were not as effective as β-VLDL. Incubation of the macrophages with human LDL did not result in appreciable stimulation of cholesteryl ester formation (addition of 50 and 100 μg of LDL protein/ml resulted in the formation of 3.6 and 4.2 nmol of cholesteryl ester per milligram of cellular protein, respectively). The ability of apo E HDLc to compete with β-VLDL for cellular uptake and to stimulate receptor-mediated cholesterol esterification strongly implicates apo E as playing an important role in the binding of both β-VLDL and apo E HDLc to mouse macrophage receptors.

To verify these results, we took advantage of two observations: 1) reductive methylation of the lysine residues of the apolipoproteins inhibits the receptor-mediated binding and cellular uptake of β-VLDL by macrophages;21 and 2) as shown below, exogenously introduced apo E can be incorporated into a β-VLDL particle by displacing the methylated endogenous apo E. Use of a combination of these two procedures is outlined in Figure 3. In one case, canine 125I-β-VLDL were incubated with unmodified human apo E-3 or reductively methylated apo E-3 to create hybrid β-VLDL particles possessing receptor-active apo E-3 or receptor-inactive methyl apo E-3. Alternatively, 125I-methyl β-VLDL were incubated with unmodified human apo E-3 to restore receptor-active apo E to these lipoprotein particles.

Figure 2. Cholesteryl ester formation in macrophages after incubation with canine β-VLDL and canine apo E HDLc. Each 16 mm dish of macrophages received 0.45 ml of DMEM containing 0.2 mM 14C-oleate-albumin complex (27.6 dpm/pmol) and the indicated concentration of apo E HDLc or β-VLDL. The cells were incubated for 16 hours at 37° C, and the cellular content of cholesteryl 14C-oleate was determined. The cholesteryl-to-protein ratio was 3.5 and 7.7 for apo E HDLc and β-VLDL, respectively.
Figure 3. Procedure for the preparation of β-VLDL, β-VLDL [E-3], methyl β-VLDL, methyl β-VLDL [E-3], and methyl β-VLDL [methyl E-3].

Figure 4 shows the apo E region of SDS-polyacrylamide gels of native and methylated canine 125I-β-VLDL after the incorporation of unmodified or methylated human apo E-3. Shown is the apo E region of the gels stained with Coomassie blue and the corresponding autoradiograms of the same gels (the canine β-VLDL, including the apo E in these particles, were radioiodinated with 125I). The canine apo E and human apo E migrate with different apparent molecular weights; therefore, it is possible to estimate the relative concentration of each. The introduction of unmodified human apo E-3 or human methyl apo E-3 displaced most of the canine 125I-apo E from the native β-VLDL or from the canine methyl β-VLDL.

Cellular uptake and degradation of canine 125I-methyl β-VLDL and hybrids (derivatives) of these particles (shown in Figure 4) by fibroblasts and macrophages were examined (Figure 5). The 125I-methyl β-VLDL and the 125I-methyl β-VLDL incubated with methyl apo E were not taken up or degraded by either fibroblasts or macrophages. However, the 125I-methyl β-VLDL into which the unmodified human apo E was incorporated were taken up and degraded by both cell types (Figure 5). This demonstrates that the introduction of receptor-active apo E-3 is sufficient to restore the receptor-binding activity to the β-
VLDL. Conversely, when methyl apo E-3 was incorporated into native 125I-B-VLDL, cellular uptake by macrophages was decreased by 97%, as compared to 125I-B-VLDL or 125I-B-VLDL with unmodified apo E-3.

The ability of β-VLDL to stimulate cholesterol esterification in macrophages is a characteristic of the receptor-mediated uptake process of β-VLDL. A clear illustration of the role of apo E in the binding and uptake process was apparent when β-VLDL-induced cholesterol esterification was examined (Figure 6). The native canine β-VLDL, native β-VLDL with apo E-3 incorporated, and native β-VLDL that had been subjected to the incubation and reisolation procedures had similar abilities to stimulate cholesteryl ester synthesis. The introduction of methyl apo E-3 into the canine β-VLDL abolished the ability of the lipoprotein particles to stimulate cholesterol esterification (Figure 6, top). Methyl β-VLDL or reisolated methyl β-VLDL produced little cholesterol esterification in macrophages. However, with the introduction of unmodified apo E-3 into the methyl β-VLDL (methyl β-VLDL [E-3]), the lipoproteins were taken up and stimulated the esterification of cholesterol to levels equivalent to incubation of the cells with native β-VLDL (Figure 6, bottom). As expected, incorporation of methyl apo E-3 into methyl β-VLDL had no effect. These results demonstrate that apo E is the predominant apolipoprotein determinant responsible for β-VLDL binding and uptake by fibroblasts and macrophages.

Previous studies have demonstrated that apo E-2 isolated from patients with Type III hyperlipoproteinemia bind poorly to both the apo B,E(LDL) and the apo E receptors. The apo E variant apo E-2(Arg158→Cys), when combined with the phospholipid dimyristoylphosphatidylcholine (DMPC), possessed only 0.5% to 1% of the normal apo E-3 DMPC binding activity to the apo B,E(LDL) receptor. In the present study, as summarized in Table 1, the apo E-2 isoform incorporated into canine β-VLDL was less active in mediating uptake, as determined by oleate incorporation into cholesteryl esters. Cholesteryl ester formation induced by β-VLDL with apo E-2 was much less (~20% of the value obtained for β-VLDL) than that observed with the native canine β-VLDL or β-VLDL with apo E-3.

As expected, the β-VLDL with methyl apo E-2 had no biological activity. However, incorporation of unmodified apo E-2 into methyl β-VLDL increased the ability of the β-VLDL to stimulate cholesteryl ester synthesis to levels equivalent to those of unmodified β-VLDL with apo E-2 (Table 1).

In addition to apo E-2(Arg158→Cys), other variants of apo E that have defective apo B,E(LDL) receptor binding have been identified. It has been demonstrated that the substitution of cysteine for arginine at residue 142 in the variant apo E-3*(Cys112→Arg, Arg142→Cys) has a deleterious effect on its ability to bind to the apo B,E(LDL) receptor. This variant was recombined with DMPC, which it expressed about 20% of the normal apo E-3 binding activity to the apo B,E(LDL) receptor. In previous studies it was determined that β-VLDL containing apo E-3*(Cys112→Arg, Arg142→Cys) were taken up by macrophages and caused cholesteryl ester accumulation. As shown in Figure 7, the human Type III β-VLDL containing apo E-3*(E-3* β-VLDL) were more active in stimulating cholesteryl ester synthesis in macrophages than were the Type III β-VLDL containing the apo E-2 (E-2 β-VLDL). The E-2 β-VLDL that had normal apo E-3 incorporated were most active. Thus, the same hierarchy of apo E binding activity previously noted for the apo B,E(LDL) receptor (E-3>E-3*>E-2) was also observed for apo E binding and uptake by mouse peritoneal macrophages.

Table 1. Incorporation of Human Apolipoprotein E-2 Into Canine β-VLDL Inhibits the Ability of β-VLDL to Stimulate Cholesteryl Ester Formation in Mouse Macrophages

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>14C-oleate → cholesteryl 14C-oleate (nmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp 1</td>
</tr>
<tr>
<td>No lipoprotein</td>
<td>0.2</td>
</tr>
<tr>
<td>β-VLDL</td>
<td>13.3</td>
</tr>
<tr>
<td>β-VLDL [E-3]</td>
<td>18.4</td>
</tr>
<tr>
<td>β-VLDL [E-2]</td>
<td>1.5</td>
</tr>
<tr>
<td>β-VLDL [methyl E-2]</td>
<td>ND</td>
</tr>
<tr>
<td>Methyl β-VLDL</td>
<td>0.3</td>
</tr>
<tr>
<td>Methyl β-VLDL [E-2]</td>
<td>2.0</td>
</tr>
</tbody>
</table>

The β-VLDL were prepared as outlined in Figure 3. The β-VLDL samples were added at a total final cholesterol concentration of 50 µg/ml. The other conditions are identical to those described in the legend to Figure 7. ND = not determined.
by guest on January 22, 2018

The ability of human apo E-2 B-VLDL, apo E-2 B-VLDL [E-2], apo E-3 B-VLDL, and apo E-2 B-VLDL [E-3] to stimulate cholesteryl \(^{14}\)C-oleate formation in mouse peritoneal macrophages. The ratio of apo E to B-VLDL was 15:1 (wt/wt). The cells were prepared as described in the legend to Figure 6.

Discussion

Previous studies have determined the apolipoprotein specificity of the apo B,E(LDL) and apo E receptors. The apo B,E(LDL) receptor (also referred to as the LDL receptor) recognizes both apo B-100 and apo E. The binding of both of these apolipoproteins is calcium-dependent and is mediated by the availability of positively charged lysine and arginine residues within the apolipoprotein structure. If a limited number of either of these amino acid residues are chemically modified, the apolipoprotein binding to the receptor is abolished. The lysine and arginine residues in apo E that are crucial for binding activity have been localized to the receptor-binding domain, a region in the vicinity of residues 140 to 150 of the apo E molecule. This receptor-binding domain is very rich in basic amino acids (7 of 11 residues), as shown by the sequence His-Leu-Arg-Lys-Leu-Arg-Lys-Arg-Leu-Arg. Recently, the complete amino acid sequence of the human apo B,E(LDL) receptor has been determined from cDNA analysis of a receptor mRNA, and a region of the receptor protein postulated to interact with apo B and apo E has been tentatively identified. The putative ligand binding domain consists of seven or eight repeat sequences of about 40 amino acid residues. Within these repeated sequences, a region enriched in negatively charged amino acids may represent the ligand binding sites. This region possesses a consensus sequence of Asp-Cys-X-Asp-Gly-Ser-Asp-Glu. The importance of these regions as the ligand binding sites is based on the identification of the receptor-binding domain of apo E responsible for mediating ligand binding, a region rich in positively charged amino acids, as described above. The existence of multiple binding sites on the apo B,E(LDL) receptor was predicted previously from binding studies demonstrating that: 1) fewer apo E-containing particles (apo E HDL) are required to saturate all available receptors as compared to the number of apo B-containing LDL; and 2) apo E-containing particles bound with a much higher affinity than LDL. Based on these and other data, it was suggested that the apo B,E(LDL) receptor possessed multiple binding sites (up to eight individual sites), a postulate strengthened by the sequence analysis of the receptor.

The second well-characterized lipoprotein receptor is the apo E receptor. In contrast to the apo B,E(LDL) receptor, which has been found in almost all cells and tissues, the apo E receptor has been localized to the liver. The ligand specificity of this receptor differs from the apo B,E(LDL) receptor in that it binds only apo E-containing lipoproteins and not apo B-containing LDL. The apo E and apo B,E(LDL) receptors have a number of properties in common. The binding interaction requires Ca\(^{2+}\), and the binding can be abolished by selective chemical modification of arginine and lysine residues of the apo E. The binding domain for apo E appears to be similar for both receptors, since mutant forms of apo E with single amino acid substitutions in the region of residues 140 to 160 disrupt the binding to both receptors. Furthermore, a polyclonal antibody prepared against the apo B,E(LDL) receptor cross-reacts with the apo E receptor and the binding of apo E-containing lipoproteins. Recently, the apo E receptor has been isolated and purified from canine liver and has been shown to represent a receptor distinct from the apo B,E(LDL) receptor.

A number of lipoproteins that contain apo E and apo B-100, such as triglyceride-rich VLDL and cholesterol-rich hepatic \(\beta\)-VLDL, can bind to both the apo B,E(LDL) and apo E receptors. Monoclonal antibodies against apo E or apo B, which block receptor binding, have been used to demonstrate that these lipoprotein particles bind primarily via apo E, and not apo B-100, to the apo B,E(LDL) and apo E receptors. Intestinally derived \(\beta\)-VLDL and chylomicron remnants contain both apo E and apo B-48. Evidence derived from monoclonal antibody studies, as well as direct binding experiments, indicates that apo B-48 does not bind to either the apo B,E(LDL) or the apo E receptor. Binding of these particles is exclusively mediated by apo E.

The present studies establish that apo E is the major determinant responsible for \(\beta\)-VLDL binding to receptors on mouse peritoneal macrophages. Gianturco et al. have presented indirect evidence suggesting that the role of apo E is to modulate the binding of triglyceride-rich VLDL to macrophage receptors and that apo B-48 is involved in the binding of chylomicrons or triglyceride-rich VLDL. While the present studies do not totally exclude the possibility that apo B-48 could be involved in modulating binding, it is clear that apo B-48 is not a major determinant. Furthermore, the hepatic \(\beta\)-VLDL, which con-
tain apo B-100 and apo E but not apo B-48, are taken up by receptors on mouse macrophages. The importance of apo E in mediating β-VLDL binding to the macrophage receptor was demonstrated by two different experiments. The first experiment demonstrated that the canine lipoprotein apo E HDL, which contains only apo E, bound to receptors on macrophages and inhibited receptor-mediated uptake of canine apo B-VLDL. In the second series of experiments, the apo E content of canine B-VLDL was manipulated by incubating the B-VLDL with chemically modified apo E (reductive methylation that abolished receptor binding activity). The B-VLDL possessing the methylated apo E lost their ability to bind to the receptor. Conversely, when unmodified apo E was incorporated into methylated B-VLDL, receptor binding activity was restored. Our conclusion is that apo E is the major protein determinant responsible for β-VLDL binding to mouse macrophage receptors. Consistent with these results are the recent findings of Wang-Iverson et al. Apolipoprotein E was identified as the ligand mediating the recognition of normal human VLDL by receptors on human monocyte macrophages.

The nature of the receptor (or receptors) on mouse peritoneal macrophages that mediates the uptake of β-VLDL is unclear. As first reported by Goldstein et al., the properties of the lipoprotein receptor(s) on mouse peritoneal macrophages differ markedly from those of the classic LDL receptor. Human and canine LDL bound poorly to mouse macrophages and were weak competitors for β-VLDL binding. Moreover, incubation of mouse macrophages with LDL stimulated more cholesteryl ester formation compared to the marked stimulation of cholesteryl ester formation induced by β-VLDL. Another difference between the putative β-VLDL receptor and the typical apo B,E(LDL) receptor was their sensitivity to regulation by intracellular cholesterol. The putative β-VLDL receptor on mouse peritoneal macrophages was resistant to regulation by intracellular cholesterol while the expression of the classic LDL receptor was very sensitive to the concentration of intracellular cholesterol.

Recent studies from our laboratory (C. Koo, M. Wemetette-Hammond, R.W. Mahley, and T.L. Innerarity, unpublished data) suggest that mouse peritoneal macrophages possess apo B,E(LDL) receptors that exhibit unusual properties. These properties are similar to those ascribed to the β-VLDL receptor. Many properties of the macrophage receptor for β-VLDL are identical to those of the classic apo B,E(LDL) receptor: 1) they bind apo E-containing lipoproteins with high affinity; 2) they require divalent cations; 3) selective chemical modification of lysine residues abolishes binding; and 4) the hierarchy of binding of the apo E variants is similar (E-3>E-2>E-3>E-2). However, other evidence from studies of macrophages from Watanabe heritable hyperlipoproteinemic rabbits and from humans with the homozygous form of familial hypercholesterolemia indicates that the β-VLDL receptor is distinct from the apo B,E(LDL) receptor. These macrophages, which lack functional LDL receptors, degraded β-VLDL presumably via the β-VLDL receptor. Regardless of whether the uptake of β-VLDL by mouse peritoneal macrophages is by two distinct lipoprotein receptors or by a unique apo B,E(LDL) receptor, the present study defines the predominant role of apo E as the receptor-binding determinant for β-VLDL.

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T L Innerarity, K S Arnold, K H Weisgraber and R W Mahley

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