β-VLDL in Hepatic Lipase Deficiency Induces ApoE-Mediated Cholesterol Ester Accumulation in Macrophages

Murray W. Huff, Cynthia G. Sawyez, Philip W. Connelly, Graham F. Maguire, J. Alick Little, Robert A. Hegele

Hepatic lipase-deficient subjects in the Ontario kindred are compound heterozygotes for hepatic lipase mutations (Ser277→Phe and Thr399→Met). Cholesteryl ester-rich β-very-low-density lipoprotein (β-VLDL) accumulates in plasma and such subjects have premature atherosclerosis. To determine a possible mechanism, we hypothesized that hepatic lipase-deficient β-VLDL, homozygous for apolipoprotein (apo) E3, would cause cholesteryl ester accumulation and foam cell formation in macrophages. β-VLDL and pre-β-VLDL were isolated by Pevikon electrophoresis and incubated with J774 macrophages, cells that do not secrete apoE. β-VLDL increased cellular cholesteryl ester content 13-fold, whereas pre-β-VLDL increased cholesteryl ester sevenfold. β-VLDL increased acyl CoA:cholesterol acyltransferase activity fourfold (measured as [14C]oleate incorporation into cholesteryl ester). Preincubation of hepatic lipase-deficient β-VLDL with the anti-apoE monoclonal antibody 1D7, which inhibits binding of apoE to low-density lipoprotein receptors, inhibited cellular cholesteryl ester accumulation by 75%, whereas the anti-apoB blocking monoclonal antibody 5E11 failed to inhibit cellular cholesteryl ester accumulation. In contrast to hepatic lipase deficiency, β-VLDL from type III subjects (E2/E2) failed to increase cellular cholesteryl ester or acyl CoA:cholesterol acyltransferase more than 1.5-fold. Thus, hepatic lipase-deficient β-VLDL readily induces cholesteryl ester accumulation in J774 macrophages, a process mediated by functional apoE3. This may explain the premature atherosclerosis observed in this kindred.

KEY WORDS • β-VLDL • hepatic lipase deficiency • apolipoprotein E • macrophages • foam cells

Hepatic lipase (HL) is an important enzyme in the metabolism of very-low-density lipoprotein (VLDL) remnants and high-density lipoproteins (HDL). This enzyme has both triglyceride lipase and phospholipase activities and is located at the surface of hepatic nonparenchymal cells. Three kindreds have been reported in whom the probands are completely deficient in HL activity. Subjects in the Ontario kindred, who exhibit complete hepatic lipase deficiency (HLD), have premature atherosclerosis and elevated total cholesterol, total triglycerides, and VLDL concentrations. In addition, the low-density lipoproteins (LDL) and HDL in these subjects are triglyceride rich and phospholipid rich. The VLDL elevation is characterized by the presence of cholesteryl-rich β-VLDL, an abnormal lipoprotein that is enriched in apolipoprotein (apo) E. It has been suggested that the modification of the VLDL remnant by HL is required for apoE transfer to HDL. The proband in this family is homozygous for normal apoE3.

The Ontario subjects with complete HLD have been shown by Hegele et al.9 to be compound heterozygotes for two missense mutations. One mutation occurs in exon 6 of the HL gene, which results in a Ser277→Phe substitution of the mature protein. The other involves a mutation in exon 8, which results in a Thr399→Met substitution. While subjects heterozygous for each mutation have variably depressed plasma HL activity, compound heterozygotes for both mutations demonstrate complete phenotypic expression of HLD. Site-directed mutagenesis and in vitro expression of these mutant forms of HL confirm the decreased specific activity observed in vivo. The premature atherosclerosis present in the Ontario family is thought to be due in part to the accumulation of β-VLDL secondary to HLD. Cholesteryl ester–rich β-VLDL accumulates in the plasma of cholesterol-fed animals, and it is thought that this lipoprotein causes the atherosclerosis observed in these animal models. Canine and rabbit β-VLDL are rich in apoE that readily binds to the B/E receptor. This β-VLDL is avidly taken up by macrophages in culture via the B/E receptor, causing cholesteryl ester accumulation and foam cell formation. Connelly et al.5 demonstrated that β-VLDL from the brother of the proband in the Ontario kindred (B2, who is completely...
TABLE 1. Plasma and Lipoprotein Lipid Concentrations of VLDL Donors

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma</th>
<th>LDL</th>
<th>HDL</th>
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<tbody>
<tr>
<td></td>
<td>C</td>
<td>C/TG</td>
<td>C</td>
</tr>
<tr>
<td>Heptic lipase deficient*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2A</td>
<td>8.25</td>
<td>1.66</td>
<td>2.02</td>
</tr>
<tr>
<td>B2B</td>
<td>8.04</td>
<td>9.59</td>
<td>3.88</td>
</tr>
<tr>
<td>Type III hyperlipoproteinemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.05</td>
<td>6.65</td>
<td>2.87</td>
</tr>
<tr>
<td>2</td>
<td>6.78</td>
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<td>3.10</td>
</tr>
<tr>
<td>3</td>
<td>5.97</td>
<td>2.53</td>
<td>1.66</td>
</tr>
</tbody>
</table>

VLDL indicates very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; C, cholesterol; TG, triglycerides.

*B2A and B2B are samples from the same hepatic lipase-deficient patient (B2) obtained 2 years apart.

B2 was homozygous for apolipoprotein E3 and the type III patients were all homozygous for apolipoprotein E2. Values are expressed as mean±SEM in millimoles per liter.

Hepatic lipase (HL) carries out hydrolysis of triglycerides to free fatty acid and glycerol, which is subsequently converted to diacylglycerol by diacylglycerol O-acyltransferase (DGAT) 1 and 2 and reesterified to triglycerides. HL deficient) binds readily to and stimulates cholesterol esterification in human skin fibroblasts. The only other naturally occurring example of a HL deficient individual is type III HL deficient hyperlipoproteinemia. Most patients with type III hyperlipoproteinemia are homozygous for a mutant form of apolipoprotein E, apoE2, that differs from normal apoE (apoE3) by a single amino acid substitution (Cys158→Arg) in the apoE molecule. The β-VLDL is rich in apoE2, which is dysfunctional with respect to binding to hepatic receptors, resulting in the accumulation of β-VLDL in plasma. Rare cases of hyperlipidemia with β-VLDL accumulation have been reported in which patients are homozygous or heterozygous for other mutations in apoE. In contrast to HL, type III subjects have normal postheparin lipolytic activities and relatively low levels of LDL and HDL. Type III β-VLDL is thought to contribute to atherosclerosis; however, β-VLDL from type III patients with the common Cys158 variant is taken up poorly by macrophages in culture due to the receptor-binding impairment. This study was conducted to determine a potential mechanism for the premature atherosclerosis observed in HL. We assessed whether β-VLDL from subjects with HL could induce cholesterol ester accumulation and foam cell formation in cultured macrophages.

Methods

Patients

The Ontario kindred with HL has subjects with significant premature coronary artery disease. The three brothers, homozygous for HL, all had clinically defined coronary disease before age 50. The clinical characteristics of the HL subject (B2) used in the present studies has been described previously. He developed angina at the age of 50 years, had coronary bypass surgery at age 53, and had a myocardial infarction at age 58. At the time of the present study, B2 was 57 years old and was taking l-thyroxine, diltiazem, hydrochloride, and hydrochloethazide. Lovastatin (60 mg/d) had been withdrawn 4 weeks before blood sampling. He was determined to be apoE3/E3 by restriction isotyping and isoelectric focusing gel electrophoresis. The type III dysbetalipoproteinemic subjects were recruited from the Lipid Clinics at University Hospital, London, Ontario, and St Michael’s Hospital, Toronto. They were classified as type III according to the criteria of the Lipid Research Clinics protocol. None of these patients displayed fasting chylomicronemia or had a metabolic disorder such as obesity, diabetes, renal dysfunction, or hypothyroidism. In addition, none of these subjects were being treated with drugs known to influence lipid metabolism. All of the type III subjects were homozygous for apoE2 as determined by isoelectric focusing. The lipid and lipoprotein profiles of the subjects studied in these experiments were determined following the Lipid Research Clinics protocol and are summarized in Table 1. These studies were approved by the University of Western Ontario Health Sciences Standing Committee on Human Research and the Human Ethics Committee at the University of Toronto. All subjects gave informed consent.

Lipoprotein Isolation

Approximately 50 mL blood was collected from subjects who had fasted for at least 12 hours and placed in tubes containing Na2EDTA at a final concentration of 0.15%. Plasma was obtained by centrifugation at 1000g for 25 minutes at 4°C. Approximately 25 mL plasma was immediately layered under buffer A (1.006 g/mL density solution containing 0.195 mol/L NaCl, 1 mmol/L tris(hydroxymethyl)aminomethane [Tris] [pH 7.4], 1 mmol/L Na2EDTA, 10 μmol/L phenylmethylsulfonyl fluoride, 3 mmol/L Na3citrate, and 0.10 mmol/L merthiolate) in a 37.5-mL Beckman Quickseal tube (Beckman Instruments, Mississauga, Ontario). VLDL (S 60 to 400) was isolated by ultracentrifugation in a Beckman 60 Ti rotor for 18 hours at 40 000 rpm at 12°C using a Beckman L8 ultracentrifuge. VLDL samples were washed through an equal volume of buffer A in a Beckman 70.1 Ti rotor at 40 000 rpm at 12°C for 18 hours. Lipoprotein-deficient serum (LPDS) was isolated from the plasma of fasting, healthy laboratory personnel as described previously. The LPDS was free of apoE as assessed by enzyme-linked immunosorbent assay (ELISA).

Isolation of β-VLDL

Whole VLDL was separated into β- and pre-β-migrating VLDL by Pevikon block electrophoresis as described previously. The lipoproteins were eluted from Pevikon C-870 using 0.15 mol/L NaCl containing 0.01% Na2EDTA, pH 7.5. Aliquots of each fraction were analyzed by analytical agarose gel electrophoresis, and fractions having β- or pre-β-mobility were...
**Lipid Analyses**

Total plasma and lipoprotein cholesterol and triglycerides were analyzed using Technicon enzymatic reagents (cholesterol, T1684-01; triglyceride, T1868-01; Technicon Instruments Inc, Tarrytown, NY), and assays were standardized by the Lipid Standardization Laboratory, Centers for Disease Control and Prevention, Atlanta, Ga. The cholesterol, phospholipids, cholesteryl esters, and triglycerides in the Pevikon fractions were analyzed as described by Kuksis et al. Lipoprotein samples were stored at 4°C and used for tissue culture experiments within 1 week. ApoE phenotypes and genotypes were determined for the VLDL donors used in these studies.

**Determination of Cellular Lipid Content**

Cellular lipid analyses were carried out by using modifications to methods described previously. Cells were washed twice with buffer B (0.15 mol/L NaCl, 50 mmol/L Tris, and 0.2% bovine serum albumin [BSA], pH 7.4) and twice with buffer B without BSA. Lipids were extracted in situ using two 30-minute incubations with 1.0 mL hexane/isopropanol (3:2, vol/vol). The remaining cells were digested for 16 hours at room temperature in 1.0 mL of 0.1 N NaOH, and cell protein was determined. The lipid extracts were pooled and dried under nitrogen, resuspended in chloroform/methanol (2:1, vol/vol), and separated by thin-layer chromatography (TLC) with petroleum ether/diethyl ether/acetic acid (84:15:1, vol/vol). Free cholesterol, esterified cholesterol, and triglyceride spots were identified (after exposure to iodine) and eluted from the TLC gel with isopropanol. Recovery was assessed by using [1α,2α(3H)]cholesteryl oleate and glycerol tri[1,2,3]oleate (Amersham, Oakville, Ontario). To each cholesteryl ester and free cholesterol sample, 5α-cholestane was added as an internal standard, the samples were saponified, and the mass of cholesterol was determined by gas-liquid chromatography. The triglyceride sample was adjusted to 1.0 mL with isopropanol, and the mass was determined by using the method of Neri and Frings.
TABLE 2. Plasma Concentrations of VLDL Pevikon Fractions

<table>
<thead>
<tr>
<th>Fraction</th>
<th>TG</th>
<th>Chol</th>
<th>Protein</th>
<th>FC</th>
<th>CE</th>
<th>PL</th>
<th>TG</th>
<th>B</th>
<th>E</th>
<th>C</th>
<th>CE</th>
<th>TG</th>
<th>B</th>
<th>ApoE</th>
<th>ApoE</th>
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<tbody>
<tr>
<td>HLD Pre-β-VLDL</td>
<td>63</td>
<td>12</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>22</td>
<td>60</td>
<td>3.3</td>
<td>0.5</td>
<td>1.4</td>
<td>0.12</td>
<td>2.3</td>
<td>18.1</td>
<td>0.17</td>
<td>0.38</td>
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<tr>
<td>β-VLDL</td>
<td>61</td>
<td>36</td>
<td>15</td>
<td>9</td>
<td>18</td>
<td>23</td>
<td>39</td>
<td>7.2</td>
<td>0.8</td>
<td>2.5</td>
<td>0.45</td>
<td>2.5</td>
<td>5.4</td>
<td>0.12</td>
<td>0.34</td>
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<tr>
<td>Type III Pre-β-VLDL</td>
<td>71</td>
<td>15</td>
<td>8</td>
<td>6</td>
<td>14</td>
<td>19</td>
<td>50</td>
<td>3.2</td>
<td>1.3</td>
<td>4.0</td>
<td>0.28</td>
<td>4.4</td>
<td>15.6</td>
<td>0.41</td>
<td>0.33</td>
</tr>
<tr>
<td>β-VLDL</td>
<td>55</td>
<td>33</td>
<td>22</td>
<td>9</td>
<td>32</td>
<td>20</td>
<td>26</td>
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<td>1.25</td>
<td>3.9</td>
<td>3.2</td>
<td>0.29</td>
<td>0.50</td>
</tr>
</tbody>
</table>

VLDL indicates very low-density lipoprotein; TG, triglycerides; Chol, cholesterol; FC, free cholesterol; CE, cholesteryl ester; PL, phospholipid; B, E, and C, apolipoproteins B, E, and C; apo, apolipoprotein; HLD, hepatic lipase deficient.

Pre-β-VLDL and β-VLDL fractions of total VLDL (Sf 20 to 400) were separated by Pevikon electrophoresis. Values for hepatic lipase deficiency are the mean of two determinations and the values for type III are the mean of three determinations. Lipids were analyzed by gas-liquid chromatography and the apolipoproteins were analyzed by enzyme-linked immunosorbent assay.

Cholesterol Esterification Assay

The incorporation of [1-14C]oleic acid into cellular cholesteryl esters was determined as described previously. Lipoproteins were added to duplicate dishes of J774 cells and incubated for 5 hours in DMEM containing 5% LPDS. Each dish received 0.04 µCi [1-14C]oleic acid (Amersham) complexed with fatty acid-free BSA (Sigma) in a molar ratio of 5:1. The cells were washed three times with buffer B without BSA, lipids were extracted as described above, and the cell protein was determined. The lipids were separated by TLC as described above, using [1a,2a(n)-1H]cholesteryl oleate to assess recovery. The cholesteryl ester bands were scraped from the plates and counted in Aquasol-2 (Dupont Canada, Mississauga, Ontario) using a Beckman LS 3801 counter.

Results

The VLDL from HLD patient B2 was separated into subfractions using Pevikon electrophoresis. Fractions containing β-migrating (P1 and P2) and pre-β-migrating (P5 and P6) VLDL, as shown in Fig 1, were analyzed for lipids and apolipoprotein content; the results are shown in Table 2. The β-VLDL fraction contained more cholesteryl ester, apoB, apoE, and apoC and fewer triglycerides relative to the pre-β-VLDL fraction. The weight ratios of cholesteryl ester to triglycerides were higher, whereas the ratios of triglycerides to apoB and of apoE to apoB were lower in the β-VLDL fraction compared with the pre-β-VLDL fraction. The VLDL from three type III patients, all having the apoE2/E2 phenotype, was also separated into β-VLDL and pre-β-VLDL by using Pevikon electrophoresis. The β-VLDL fraction contained more cholesteryl ester, apoB, apoE, and apoC and fewer triglycerides than the pre-β-VLDL fraction. In the β-fraction, the ratios of cholesteryl ester to triglycerides and of apoE to apoC were higher, whereas the ratios of triglycerides to apoB and of apoE to apoB were lower than in the pre-β-VLDL fraction. With respect to composition, values for the Pevikon fractions from the HLD patient were similar to those of the type III patients. However, the type III β-VLDL had a higher cholesteryl ester/triglyceride ratio and had a threefold greater apoE content than the corresponding fraction in the HLD patient.

Fig 2 demonstrates that both the pre-β-VLDL and β-VLDL subfractions from the HLD subject are capable of inducing increases in the cholesteryl ester (Fig 2A) and triglyceride (Fig 2B) content of J774 cells after 16 hours of incubation. The cellular free cholesterol concentration did not change with any of the additions (data not shown). Fig 2 also shows that the β-VLDL fraction caused greater increases in cellular esterified cholesterol content when compared with the pre-β-fraction, even though both fractions were added at equal amounts of lipoprotein cholesterol. The β-VLDL fraction induced the same degree of cholesteryl ester accumulation as equal amounts of rabbit β-VLDL and normal human LDL, although the latter was added at a threefold higher cholesterol concentration. The HLD pre-β-VLDL caused a twofold greater increment in cellular triglycerides than the β-fraction, reflecting the greater triglyceride/cholesterol ratio of the pre-β-fraction.

ApoE was the ligand responsible for the majority of the cholesteryl ester accumulation caused by both the β-VLDL and pre-β-VLDL fractions from the HLD patient (Fig 3). Fab fragments of monoclonal antibody 1D7, which specifically inhibits the interaction of apoE with the LDL receptor, inhibited cellular cholesteryl ester accumulation induced by pre-β-VLDL and β-VLDL by 75% and 80%, respectively (Fig 3A). Inhibition of both fractions with the control apoE monoclonal antibody 6C5 had no effect on the increase in cellular cholesteryl ester. Antibody 6C5 is known to bind to apoE close to the TV-terminal, an epitope not involved in receptor binding. ApoE was not involved in cellular triglyceride accumulation, since neither 1D7 nor 6C5 antibody had any effect on the increase in cellular triglyceride levels (Fig 3B).

ApoB was not involved in the cellular accumulation of either cholesteryl ester or triglycerides (Fig 4). Fab fragments of monoclonal antibody 5E11, which specifically blocks the binding of apoB to the LDL receptor, and monoclonal antibody 1D1, which binds to an epitope of the apoB molecule not involved in receptor recognition, had no influence on the increments of cellular lipids induced by the two VLDL fractions from the HLD patient.

It was determined that the increase in cellular cholesteryl ester induced by β-VLDL was associated with an increase in acyl CoA:cholesterol acyltransferase (ACAT) activity (Fig 5). The incorporation of [14C]oleate into cholesteryl ester was stimulated 2.5-fold. Preincubation of β-VLDL with the specific antibody 1D7 inhibited the increase in ACAT activity by 85%, confirming the role of apoE as the primary ligand responsible for uptake.
The VLDL from three type III (E2/E2) patients was separated into β-VLDL and pre-β-VLDL fractions (Fig 1) and compared directly with the corresponding fractions from the HLD patient; the results are shown in Fig 2. Neither type III VLDL fraction caused any marked increase in cellular cholesteryl ester content. The type III β-fraction caused only a 2.5-fold increment in cellular cholesteryl ester. In striking contrast, a 12-fold increase was observed for the same fraction from the HLD patient even though equal amounts of lipoprotein cholesteryl were added to cells. Similarly, the pre-β-VLDL from type III subjects increased cellular cholesteryl ester content 1.8-fold, whereas the increase was 4.5-fold for the corresponding fraction from the HLD patient. In contrast, both VLDL fractions from the type III subjects caused increases in cellular triglyceride levels similar to the two fractions from the HLD patient when the results were adjusted for differences in particle triglyceride composition. The type III pre-β-VLDL and β-VLDL increased ACAT activity by 1.2-fold and 1.4-fold, respectively (data not shown).

**Discussion**

The uptake of remnants of triglyceride-rich lipoproteins by macrophages and the resulting accumulation of cholesteryl esters may contribute to the presence of lipid-filled foam cells and the atherosclerosis associated with some forms of dyslipidemia. The present studies clearly show that the cholesterol-enriched β-VLDL fraction from the HLD patient markedly stimulated cholesteryl ester and triglyceride accumulation in the mouse J774 macrophage cell line. Pre-β-VLDL from this patient also enhanced cellular cholesteryl ester accumulation, but to a lesser extent. This finding, together with the finding that the concentration of β-VLDL is approximately threefold to fourfold higher than that of pre-β-VLDL in this patient, suggests that in vivo the β-fraction may be a primary contributor to foam cell formation.

The association of HLD with an increase in the plasma concentration of β-VLDL indicates that HL plays an important role in the hepatic clearance of VLDL remnants, their conversion to LDL, and the transfer of apoE to HDL. The extent of cholesteryl ester accumulation induced by HLD pre-β-VLDL is similar to that induced by type IV hypertriglyceridemic VLDL and fivefold higher than that observed for normal VLDL. The amount of apoE in HLD β-VLDL is increased relative to normal VLDL but similar to that reported for hypertriglyceridemic VLDL in type IV and...
type V patients.\textsuperscript{16} The experiments with the specific anti-apoE and anti-apoB antibodies strongly suggest that apoE is the primary ligand mediating uptake and that apoB is probably not involved. J774 macrophages do not secrete apoE, suggesting that the increased concentration of lipoprotein-bound apoE was capable of mediating the cellular accumulation of cholesteryl ester. In previous studies, Connelly et al\textsuperscript{16} demonstrated that the \( \beta \)-VLDL fraction from this patient could readily compete with radiolabeled LDL for binding to fibroblasts. This effect was abolished by trypsin treatment, and since trypsin is thought to inactivate apoE but not apoB, these results suggest that the \( \beta \)-VLDL bound to the LDL receptor of fibroblasts via apoE. Pre-\( \beta \)-VLDL–induced cholesteryl ester accumulation in J774 cells was also mediated by apoE. This finding is consistent with our previous results showing that hypertriglyceridemic VLDL (predominantly pre-\( \beta \)-VLDL) is capable of inducing cholesteryl ester accumulation in J774 macrophages, a process largely mediated by apoE.\textsuperscript{16}

Although apoE mediates the majority of uptake of both HLD \( \beta \)-VLDL and pre-\( \beta \)-VLDL by the J774 macrophage, it is possible that other characteristics of this particle contribute to its enhanced uptake. A high ratio of apoE to apoC has been shown to increase hepatic uptake of VLDL.\textsuperscript{35,36} The ratio of apoE to apoC was similar in both the \( \beta \)-VLDL and pre-\( \beta \)-VLDL fractions from HLD and type III patients (0.34 and 0.38, respectively), which is higher than that reported previously by us for apoE-rich VLDL from normal subjects (0.12 to 0.15).\textsuperscript{27} Thus, the increased apoE relative to apoC may contribute to the enhanced uptake of HLD \( \beta \)-VLDL.

Although we did not directly study the receptor involved in mediating the \( \beta \)-VLDL uptake by J774 cells, it is likely that LDL receptor–mediated uptake was the major route. Ellsworth et al\textsuperscript{13} demonstrated that \( \beta \)-VLDL from cholesterol-fed animals is taken up by J774 macrophages by the LDL receptor. Recently, a VLDL receptor very similar to the LDL receptor has been described that binds apoE-containing lipoproteins with high affinity. Although this receptor has not been demonstrated in macrophages, it is possible that it could mediate the uptake of HLD VLDL.\textsuperscript{38} Also, macrophages express the LDL receptor–related protein,
which has been shown to bind β-VLDL from cholesterol-fed rabbits, presumably mediated by apoE. However, binding has been shown only if this lipoprotein is enriched with a substantial excess of added apoE, fourfold over that present on rabbit β-VLDL, a lipoprotein containing much more apoE per particle (apoE/apoB, 1.2) than the HLD β-VLDL as isolated for the present experiments (apoE/apoB, 0.12). It is possible that the conformation of apoE present in the HLD β-VLDL preparation is such that it is a preferred ligand for the LDL receptor-related protein. The small amount of cholesteryl ester accumulation caused by HLD β-VLDL in the presence of the anti-apoE 1D7 antibody may have been mediated by the macrophage membrane-bound protein MBP 190 described by Gian turco et al. This protein, which is distinct from the LDL receptor, does not require apoE as a ligand and mediates the binding of triglyceride-rich lipoproteins to P388D macrophages.

The present studies compared the ability of HLD VLDL to induce cholesteryl ester accumulation in macrophages with that of β-VLDL from patients with type III hyperlipoproteinemia who are homozygous for apoE2. Type III hyperlipoproteinemia is the only other condition with naturally occurring β-VLDL in humans. The β-VLDL from both types of patients was similar in composition, although the core lipids of the β-VLDL from the type III subjects were enriched in cholesteryl esters at the expense of triglycerides. Type III β-VLDL also had a greater apoE/apoB ratio, indicating a higher apoE content per lipoprotein particle. In contrast to β-VLDL from the HLD patient, type III β-VLDL failed to induce a marked increase in cellular cholesteryl ester content, despite being added to cells at equal cholesterol concentrations. Also, type III pre-β-VLDL did not induce any significant cholesteryl ester accumulation compared with the HLD pre-β-VLDL fraction. These results indicate that the interaction of the two forms of β-VLDL with macrophages differs markedly and that normal apoE3 plays a major role. The presence of sufficient apoE3 on the HLD β-VLDL allows these lipoproteins to bind readily to the macrophage LDL receptor, mediating uptake, whereas the defective apoE2 isoform present in type III β-VLDL does not. Thus, in the HLD subjects in vivo, arterial wall macrophages may readily take up β-VLDL mediated by apoE. However, in type III subjects, if the β-VLDL contrib-
FIG 5. Bar graph showing cholesterol esterification in J774 macrophages incubated with the Pevikon β-migrating very-low-density lipoprotein (VLDL) (S 20 to 400) fraction (50 μg lipoprotein cholesterol per milliliter medium) from the hepatic lipase-deficient (HLD) patient B2. The β-VLDL was also incubated with cells after preincubation with Fab fragments of the anti-apolipoprotein E monoclonal antibody 1D7 (100 μg Fab fragments/50 μg lipoprotein cholesterol for 30 minutes at 37°C). Incubations were carried out in the presence of [14C]oleic acid complexed to albumin for 5 hours. Incorporation of [14C]oleate into cellular cholesteryl esters was determined after separation of cellular cholesteryl esters by thin-layer chromatography. The values are the results of duplicate determinations for two experiments expressed as mean±SEM. Chol indicates cholesterol; Pn, protein.

utes to atherosclerosis, foam cell formation must occur by an as-yet-unknown alternate mechanism. A mechanism of foam cell development by HLD VLDL based on the downregulation of the LDL receptor may be less likely than uptake by a receptor that is refractory to exogenous cholesterol concentrations, such as the LDL receptor-related protein. However, arterial wall macrophages may resemble J774 cells in that their LDL receptors are poorly regulated.16 Hypertriglyceridemic VLDL can induce the same degree of cholesteryl ester accumulation whether the cells are preincubated in LPDS or in the presence of serum, suggesting poor regulation of the LDL receptor.16 Tabas et al.41 have shown that J774 macrophages have a high rate of ACAT activity and are unable to efficiently downregulate their LDL receptors. Although we have provided indirect evidence that both the HLD pre-β-VLDL and β-VLDL are taken up by the macrophage LDL receptor, this raises the question as to why the HLD β-VLDL accumulates in plasma and is not cleared by the hepatic LDL receptor. It is possible that for HLD β-VLDL to be a ligand for the LDL receptor it must be completely hydrolyzed by lipoprotein lipase (LPL) and/or HL to expose apoE epitopes. In previous experiments, Evans et al.42 demonstrated that type IV hypertriglyceridemic VLDL (E3/E3), as isolated from plasma, can be taken up by HepG2 cells only after lipolysis by LPL. In similar experiments with HLD β-VLDL, lipolysis by LPL was also required for uptake by HepG2 cells, even though these cells express active HL (A.J. Evans, PhD, and M.W. Huff, PhD, unpublished data, May 1991). This suggests that, in vivo, lipoproteins that accumulate in the d < 1.006 g/mL fraction of plasma in HLD are incompletely hydrolyzed by peripheral endothelial cell-bound LPL. We have shown that LPL is secreted from J774 cells, and lipolysis of hypertriglyceridemic VLDL is required for uptake by these cells.43 It is also possible that lipolysis of HLD β-VLDL and pre-β-VLDL is required for uptake by J774 cells. Thus, in vivo, for HLD β-VLDL and pre-β-VLDL to become ligands for the hepatic LDL receptor, complete lipolysis by LPL followed by interaction with HL at the hepatocyte cell surface may be required. In the microenvironment of the macrophage cell surface, LPL secreted by the macrophage is sufficient to render these lipoproteins ligands for the LDL receptor.

The results of this study with type III β-VLDL are consistent with our previous findings16 and those of Ishibashi et al.44 We demonstrated that this lipoprotein increased macrophage total cellular cholesterol content only modestly. Ishibashi et al found that radiolabeled type III VLDL (E2/E2) was degraded to a lesser extent than normal VLDL in autologous monocyte-derived macrophages. The marked accumulation of cholesteryl esters in macrophages induced by HLD β-VLDL is somewhat analogous to the results of Innerarity et al,17 who demonstrated that cholesterol ester−enriched β-VLDL isolated from the plasma of cholesterol-fed dogs binds to macrophages via interaction with normal apoE3 and is inhibited when this apoE is exchanged for dysfunctional apoE2.

Several aspects of these studies support the concept that lipoprotein cholesteryl ester and triglycerides accumulate in macrophages by different mechanisms.43 The anti-apoE antibody 1D7 blocks HLD β-VLDL− and pre−β-VLDL−induced cholesteryl ester accumulation but not triglyceride accumulation. Type III VLDL fractions induce similar increments in macrophage triglycerides as their HLD β-VLDL counterparts. This is consistent with the idea that macrophage LPL hydrolyzes lipoprotein triglycerides in the media, and the resulting free fatty acids are taken up and re-esterified into triglycerides. The cholesteryl ester−enriched remnant is then taken up by a receptor-mediated mechanism mediated by apoE.

In summary, the increased plasma concentration of β-VLDL and its avid uptake by cultured macrophages suggest that this lipoprotein promotes the formation of cholesterol-rich foam cells and may explain the premature atherosclerosis that has been documented in this family with HLD.

Acknowledgments

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