Membrane Receptors for Very Low Density Lipoprotein (VLDL) Inhibitor of Lymphocyte Proliferation

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Physiologic concentrations of human plasma very low density lipoproteins inhibit the DNA synthesis of lymphocytes stimulated by allogeneic cells or lectins. In this report we have compared the effects of isolated lipoproteins [very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL)] and lipoprotein-depleted plasma (LDP) on DNA synthesis by phytohemagglutinin-stimulated human lymphocytes. The relative potency for the inhibition of lymphocyte proliferation was VLDL > LDL > HDL > LDP. Fifty percent inhibition of DNA synthesis was observed at a VLDL protein concentration of 1.5-2.0 mg/ml. We have further demonstrated the presence of specific receptors for VLDL on human lymphocytes. Native VLDL was more effective than LDL in competing for 

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ecent studies indicate that plasma lipoproteins can regulate several aspects of lymphocyte function. Studies by Waddell et al., Chisari, and Morse et al. demonstrate that plasma VLDL completely inhibited DNA synthesis by mitogen-stimulated human lymphocytes. In a series of elegant studies, Curtiss and Edginton have shifted the emphasis of lipoprotein inhibitors to a subclass of LDL termed LDL-inhibitor (LDL-In), and the existence of saturable cell surface receptors for human lymphocytes for LDL-In. In this report we have returned to study in greater detail the lymphocyte growth inhibitory and binding properties of human VLDL and to compare VLDL and LDL for inhibitory effects on lymphocyte proliferation.

MATERIALS AND METHODS

Cells

Peripheral blood mononuclear cells (PBM) from normal donors were isolated from heparinized venous blood by a modification of the method of Boyum. Blood from fasting volunteers was collected in 0.1% EDTA, and the cell-free plasma was separated by centrifugation. Pooled plasma was used for lipoprotein isolations within 24 hr of collection. VLDL (d < 1.006 g/ml), LDL (d = 1.006-1.063), HDL (d = 1.063-1.213) and LDP (d > 1.213) were isolated by sequential ultracentrifugation flotation according to standard techniques in a IEC B-60 ultracentrifuge (International Equipment Co., Needham Heights, Mass.) using solid KBr for density adjustments. Lipoprotein fractions were washed twice by flotation through a KBr solution of appropriate density. All lipoprotein fractions were dialyzed for 48 hr with four changes of a buffer containing 20 mM Tris-I-Cl, 0.14 M NaCl, and 0.01% EDTA. Purity of lipoprotein preparations was assessed by polyacrylamide gel electrophoresis. Total proteins were measured by the method of Lowry et al., modified as specified for lipoproteins by Markwell et al. Lipoproteins were assayed for their concentrations of cholesterol and triglycerides with a Technicon Auto Analyzer (Technicon Instrument Co., Tarrytown, N.Y.). Lipoproteins were characterized for apoprotein content using tetramethylurea and SDS gel electrophoretic techniques. Rabbit antiserum against human apo-LDL(B) (Behring Diagnostics, Somerville, N.J.) was used for further identification of the apo-B moiety of

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LDL. The relative proportions of cholesterol, triglycerides, apo-B, apo-C1, II, and III and apo-AI and AI in VLDL, LDL, and HDL corresponded to published reports. Lipoprotein-depleted fetal calf serum (d > 1.215) was prepared by the layering of KBr solution (d = 1.215) over fetal calf serum, followed by centrifugation at 260,000 gaze 2 hr. The top layer that contained lipoproteins was discarded, and the LDFCS was dialyzed as described above.

Iodination of Lipoproteins

The iodination technique used was that of MacFarlane as modified by Shepard, Bedford, and Morgan. In brief, 50 μl of 0.003 M iodine monochloride in 2 M NaCl solution was added to approximately 2 mCi 125I (125I sodium, pH 8–10, carrier free, New England Nuclear, Boston, Mass.) LDL (1 mg protein/ml), which were previously dialyzed against a 1 M glycine-NaOH buffer (pH 10) were diluted to 2 ml with the glycine-NaOH buffer and then were added to the iodine monochloride-125I mixture. The solution was then passed through a Sephadex QAE-25 ion exchange column (Pharmacia, Piscataway, N.J.). One-millilitre aliquots were collected and the radioactivity was measured in a gamma counter (Nuclear-Chicago, Des Plaines, Ill.). Approximately 4% of the 125I was incorporated into VLDL. Radiolabeled lipoproteins recovered in the void volume were dialyzed exhaustively against Tris-NaCl-EDTA buffer. After dialysis the 125I-VLDL contained less than 1 atom of iodine for each VLDL molecule and had a final specific activity of approximately 500 cpm/μg of protein. The 125I-VLDL recovered from the void volume did not differ from untreated VLDL when subjected to lipoprotein polyacrylamide gel electrophoresis. Greater than 97% of the radioactivity was precipitated by incubation with 10% trichloroacetic acid. LDL were iodinated similarly. After separation of the insoluble and soluble apoproteins with 6% TCA using an automated cell harvester (Otto Hiller Co., Madison, Wisc.). The filters were air dried overnight and then counted in a gamma counter (Nuclear of Chicago, Des Plaines, Ill.). All binding assays were conducted in duplicate.

Ligand-Receptor Reversibility

For 48-hr of incubation in 10% LDFCS, lymphocytes (4 x 10⁶ cells) were placed in buffer A with added 125I-VLDL for 60 min at 4°C. Lymphocytes were washed as described above and then treated by a single agitation with buffer B (50 mM NaCl and 10 mM Hepes, pH 7.4) containing either no heparin or 10 mg/ml of heparin (Grade II, 151 USP U/mg anhydrous, Sigma, St. Louis, Mo.) as described by Goldstein et al. The lymphocytes were incubated at 4°C for fixed intervals, after which the cells were pelleted by centrifugation at 15,000 gaze 2 min, the supernatant was discarded, and the amount of 125I-VLDL bound to the cells was determined without washing further.

Degradation of 125I-VLDL by Lymphocytes

The noniodine 125I-labeled lipoprotein degradation products soluble in 10% TCA were assayed in the initial cell supernatant. One millilitre of the supernatant was added to 0.25 ml of 50% TCA. After 30 min at 4°C the tubes were centrifuged at 1000 g for 10 min, and 1.0 ml of the supernate was removed and treated with 1 μl 10% H₂O₂ for 5 min at room temperature. Two milliliters of CHCl₃ were added and the tubes were thoroughly mixed, after which 0.5 ml of the aqueous phase was removed. The effectiveness of this procedure to remove free iodide was evaluated. In a pilot experiment this method was able to remove more than 99% of added free 125I. A blank value due to the presence of small amounts of noniodine acid-soluble material (0.1% of total added radioactivity) in the 125I-VLDL preparations was determined by incubation of 125I-VLDL in culture medium without cells. This blank value was subtracted from the experimental value.

Effect of Colchicine on Degradation of VLDL

Lymphocytes that were incubated in 10% LDFCS for 48 hr at 37°C were removed from the stock flask, and the cell concentration was adjusted to 4 x 10⁶ cells/ml. 125I-VLDL were added to control lymphocytes and to lymphocytes that had been preincubated with 10 μM colchicine for 2 hr. The uptake and degradation of 125I-VLDL were then investigated.

Statistics and Miscellaneous Calculations

The molar content of VLDL was calculated from an estimated molecular weight of 10⁷ daltons with a composition of 7% protein and 93% lipid. For LDL, an estimated molecular weight of 3 x 10⁶ daltons and a composition of 20% protein and 80% lipid were
LYMPHOCYTE RECEPTORS FOR VLDL INHIBITOR

Used, Scatchard slopes were calculated by least square regression analysis. Limits of error of the Scatchard analysis was determined from the error variance. As described by Curtiss and Edgington, the mean binding affinity (Ka) of the ligand-receptor interaction at half saturation of receptor sites was calculated from the equation, \[ Ka = \frac{(L \cdot R_b)}{(L)(R_f)}, \] where \( (L \cdot R_b) \) is the molar concentration of ligand-receptor complex, \( (L) \) is the molar concentration of free ligand, and \( (R_f) \) is the molar concentration of unoccupied receptor.

RESULTS

Effect of Human Lipoproteins on PHA-Stimulated Lymphocyte DNA Synthesis

Figure 1 summarizes the effects of isolated lipoproteins and lipoprotein-depleted plasma on DNA synthesis by PHA-stimulated human lymphocytes. VLDL inhibited PHA-stimulated lymphocyte DNA synthesis in a dose-dependent fashion. Fifty percent inhibition of \(^3\)H-thymidine uptake by lymphocytes was observed at VLDL protein concentrations of 1.5–2.0 \(\mu\)g/ml and at LDL protein concentrations of 70–80 \(\mu\)g/ml. VLDL were nearly 40 times more potent than LDL based on protein content. Twelve-hundred micrograms of HDL protein was needed to achieve 50% inhibition of DNA synthesis. HDL were inactive at concentrations up to 400 \(\mu\)g/ml. The effect of lipoprotein-depleted plasma was minimal.

Biologic Activity of \(^{125}\)I-VLDL and Native VLDL

To confirm the biologic significance of \(^{125}\)I-VLDL as a probe to characterize lymphocyte receptors for VLDL, it was necessary to demonstrate that \(^{125}\)I-VLDL possessed biologic inhibitory activity on PHA-stimulated lymphocytes. At a VLDL protein concentration of 4–5 \(\mu\)g/ml, the lymphocyte DNA synthesis inhibitory effect of native and trace-iodinated VLDL (using approximately 0.01% of \(^{125}\)I routinely used), were 75% ± 8% and 63% ± 5%, respectively. At a VLDL protein concentration of 43–46 \(\mu\)g/ml, the DNA synthesis inhibitory effects of native and trace-iodinated VLDL were 84% ± 4% and 76% ± 6%, respectively.

In a similar study, the biologic activity of trace-iodinated LDL was compared to native LDL. At a LDL protein concentration of 76–80 \(\mu\)g/ml, the DNA synthesis inhibitory effect of native and trace-iodinated LDL were 58% ± 4% and 49% ± 6%, respectively.

Characterization of VLDL Binding to Lymphocytes

When human lymphocytes were incubated for 48 hr in the absence of lipoproteins (LDFCS) and then exposed to \(^{125}\)I-VLDL at either 37°C or 4°C, an initial rapid phase of binding of \(^{125}\)I-VLDL lasted 30–60 min and was followed by a slower binding phase (Fig. 2A). When \(^{125}\)I-LDL were added to lymphocytes, a similar binding response was observed (Fig. 2B). Lymphocytes accumulated approximately two times more VLDL or LDL at 37°C than at 4°C, which suggests that at 37°C \(^{125}\)I-lipoproteins were internalized after binding. In the presence of a sixfold excess of unlabeled VLDL, the binding of \(^{125}\)I-VLDL was reduced by more than 80%, indicating that unlabeled VLDL...
Fig. 2. (A,B) The Effect of time and temperature on the simultaneous binding of $^{125}$I-VLDL and $^{125}$I-LDL to lymphocytes. After 24-hr incubation in 10% LDFCS, the lymphocytes were placed in buffer A and maintained at either 37°C or 4°C. $^{125}$I-VLDL (26.16 µg/ml; 171 cpm/ng protein) or $^{125}$I-LDL (305.0 µg/ml; 70 cpm/ng protein) were added, and duplicate cell incubations were terminated at fixed intervals over a 3.5 hr period. The total amount of $^{125}$I-lipoproteins bound to lymphocytes was determined as described in Materials and Methods using specific activity measurements (cpm/ng protein) for VLDL and LDL, radioactivity present in cell pellets, and lymphocyte numbers per pellet. Results are expressed as nanograms of $^{125}$I-VLDL or $^{125}$I-LDL protein bound per $2 \times 10^6$ cells.

Fig. 3. Effects of unlabeled plasma lipoprotein fractions on $^{125}$I-VLDL binding to lymphocytes at 4°C. The lymphocytes ($4 \times 10^6$ cells/ml) were preincubated for 48 hr in 10% LDFCS. The cells were then washed once and suspended in buffer A at a concentration of $4 \times 10^6$ cells/ml. Unlabeled lipoprotein fractions were added at varying concentrations along with 12.9 µg/ml $^{125}$I-VLDL. The cells were harvested 2 hr later as described in Materials and Methods.
were competing with \(^{125}\)I-VLDL for a limited number of binding sites (Fig. 3). At protein concentrations between 0 and 200 \(\mu\)g/ml, unlabeled VLDL were five times more effective than LDL in competing for \(^{125}\)I-VLDL binding sites. The competing effect of HDL was minimal.

The binding of \(^{125}\)I-VLDL to lymphocytes at 4°C and 37°C was found to be dependent on the presence of calcium in the incubation media. Binding of \(^{125}\)I-VLDL increased 7–8-fold in the presence of 100 \(\mu\)M Ca, as compared to an absence of added CaCl\(_2\) in buffer A (Table 1).

Reversibility of the Ligand-Receptor Interaction

In order to ascertain that lymphocyte uptake of VLDL was due to cell surface receptor binding, it was necessary to demonstrate that the binding process was not only specific, but also reversible. Goldstein et al.\(^{23}\) reported that heparin was able to release \(^{125}\)I-LDL from its receptor sites on the surface of normal human fibroblasts. In the present study, we have investigated the effect of heparin on cell-surface-bound \(^{125}\)I-VLDL at 4°C. Lymphocytes were first incubated with \(^{125}\)I-VLDL for 60 min at 4°C, then washed extensively before treatment with heparin. The amount of \(^{125}\)I-VLDL bound after the heparin treatment was determined at fixed intervals. As shown in Fig. 4, approximately 60% of the \(^{125}\)I-VLDL originally bound to lymphocyte were removed by 2-min incubation with 10 mg/ml heparin as compared to 20% loss of \(^{125}\)I-VLDL in the presence of buffer without heparin.

Effect of 25-Hydroxycholesterol and Cholesterol on \(^{125}\)I-VLDL Uptake by Lymphocytes

Inclusion into a lipoprotein-depleted medium of a mixture of 25-hydroxycholesterol and cholesterol during the incubation period was shown by Ho et al.\(^{19}\) to prevent the induction of enhanced degradation of \(^{125}\)I-LDL by human lymphocytes. Curtiss and Edgington showed that while \(^{125}\)I-LDL receptor binding to lymphocytes was decreased following incubation with sterols, \(^{125}\)I-LDL-inhibitor receptor binding was not affected.\(^{5}\) Hence, we were interested in determining whether VLDL binding could also be down regulated. In our study, lymphocytes were cultured in the presence of a mixture of 25-hydroxycholesterol and cholesterol in 10% LDFCS for 48 hr. The binding assay was then performed at 37°C with \(^{125}\)I-VLDL. Incubation of human lymphocytes for 48 hr in the presence of 25-hydroxycholesterol and cholesterol had no significant effect on lymphocyte uptake of \(^{125}\)I-VLDL (Table 2).

Scatchard Analysis

The number of VLDL receptors per human lymphocyte was estimated by a modified Scatchard plot. The binding of \(^{125}\)I-VLDL by lymphocytes was performed at 4°C in a series of \(^{125}\)I-VLDL concentrations between 8 and 0.5 \(\mu\)g/ml (Fig. 5). Extrapolation to saturation gave an estimate of 65 ± 3 ng of VLDL protein bound per 2 \(\times\) 10⁶ cells. Assuming that the receptor is a single molecular complex capable of interacting with VLDL in an equimolar fashion, it is calculated that each human lymphocyte possesses approximately 28,000 ± 1300 VLDL receptors. Half saturation of this calculated number of receptors occurred at a free VLDL concentration of 11.4 nM. From these values, the mean binding affinity (K_a) was estimated to be approximately 8.77 ± 10⁻¹⁰ liter/mole.

Uptake and Degradation of \(^{125}\)I-VLDL

In the next series of experiments we studied the uptake and degradation of \(^{125}\)I-VLDL by human lymphocytes at 37°C and 4°C as described by Goldstein and Brown.\(^{17,18}\) As shown in Fig. 6, VLDL were not degraded at 4°C, even though VLDL binding to the cell surface was observed (see previous experiments). Although the uptake of VLDL reached a plateau within 2 hr of incubation (Fig. 2A), the
Fig. 4. Release of surface-bound $^{125 \text{I}}$-VLDL from normal lymphocytes by heparin. After 48 hr of incubation in 10% LDFCS, lymphocytes ($4 \times 10^6$ cells) were placed in buffer A with added $^{125 \text{I}}$-VLDL (53 $\mu$g/ml; 79 cpm/ng) for 60 min at 4°C. Lymphocytes were washed as described in Materials and Methods and then treated with buffer B containing either no heparin or 10 mg/ml of heparin. The cells were incubated at 4°C for fixed intervals, after which the medium was removed, and the amount of $^{125 \text{I}}$-VLDL bound to the cells was determined.

Fig. 5. Estimation of the number of $^{125 \text{I}}$-VLDL molecules bound by lymphocytes by the method of Scatchard. Cells were preincubated in 10% LDFCS for 48 hr. Between 0.5 and 8.0 $\mu$g of $^{125 \text{I}}$-VLDL (321 cpm/ng protein) were then added to $2 \times 10^6$ lymphocytes in buffer A, allowed to incubate for 2 hr at 4°C, and the cells harvested as described. Extrapolation of the linear regression line to bound/free of zero results in 66.3 ng $^{125 \text{I}}$-VLDL protein bound to $2 \times 10^6$ lymphocytes that represents approximately 28,000 ± 1300 receptors per cell.

Fig. 6. Effect of time and temperature on $^{125 \text{I}}$-VLDL degradation by lymphocytes. Lymphocytes were cultured for 48 hr in 10% LDFCS before assay. All points represent the mean of duplicate assays containing $4 \times 10^6$ cells in buffer A. 6.9 $\mu$g/ml of $^{125 \text{I}}$-VLDL (29 cpm/ng protein) were added, and the cells were incubated at either 4°C or 37°C for the times indicated after which the total amount of degraded $^{125 \text{I}}$-VLDL by lymphocytes was determined as described in Materials and Methods.
Table 3. Effect of Colchicine on $^{125}$I-VLDL Degradation by Lymphocytes

<table>
<thead>
<tr>
<th>Incubation Period</th>
<th>Degradation of $^{125}$I-VLDL (ng Protein/4 x $10^5$ Cells)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>5 min</td>
<td>0 ± 4</td>
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<tr>
<td>90 min</td>
<td>7 ± 1</td>
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<td>180 min</td>
<td>116 ± 15</td>
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<tr>
<td>24 hr</td>
<td>1,280 ± 16</td>
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*Duplicate sets of lymphocytes were incubated with 10% LDFCS for 48 hr at 37°C. Some tubes were pretreated for 2 hr with colchicine sufficient to achieve a final concentration of 10 $\mu$M. Control cultures were preincubated with an equivalent volume of saline. At zero time, $^{125}$I-VLDL (29 cpm/ng protein) were added to achieve a final concentration of 6.9 µg protein/ml. The lymphocytes were incubated for fixed incubation periods and degradation of $^{125}$I-VLDL was determined as described in Materials and Methods.

†$p < 0.0125$ difference (Student’s $t$ test) between $^{125}$I-VLDL degradation in the presence of colchicine as compared to control cultures.

Continuous accumulation of degraded material in the extracellular fluid was observed for 24 hr at 37°C (Table 3). In the next experiment the effect of the microtubule inhibitor, colchicine, on lymphocyte metabolism of $^{125}$I-VLDL was studied. $^{125}$I-VLDL were added to control lymphocytes and to lymphocytes that had been preincubated with 10 $\mu$M colchicine for 2 hr. The uptake and degradation of $^{125}$I-VLDL were then investigated. Cell uptake of $^{125}$I-VLDL was not significantly affected by pretreatment of lymphocytes with colchicine (data not shown). In comparison, 40% inhibition of $^{125}$I-VLDL degradation at 24 hr was noted with colchicine (Table 3).

To determine whether the mechanism for VLDL degradation by lymphocytes is related to specific recognition of VLDL as compared to other lipoproteins, we have studied the degradation of $^{125}$I-VLDL in the presence of unlabeled lipoproteins. A quantity of 95 µg/ml of unlabeled VLDL reduced receptor-mediated degraded of $^{125}$I-VLDL by 60%. In comparison to VLDL, a six-fold excess of unlabeled...

Fig. 7. Inhibition of lymphocyte degradation of $^{125}$I-VLDL at 37°C by unlabeled VLDL and LDL. Lymphocytes were preincubated for 48 hr in 10% LDFCS. The cells were then washed and suspended in buffer A at a concentration of 4 x $10^6$ cells/ml. Nonradioactive VLDL and LDL were added at varying concentrations along with 44 µg/ml of $^{125}$I-VLDL (12.8 cpm/ng protein). The lymphocytes were then incubated for 24 hr at 37°C, and the degradation of $^{125}$I-VLDL was determined. Control values (no cells) were subtracted from the experimental values.
LDL (575 μg/ml) reduced the degradation of 125I-VLDL by only 30% (Fig. 7).

**DISCUSSION**

Physiologic concentrations of human plasma VLDL and LDL inhibit DNA synthesis by lymphocytes stimulated by allogeneic cells or lectins.1-3 In the present study we have confirmed that all of the classes of normal human plasma lipoproteins inhibited, in a dose-dependent fashion, PHA-induced 3H-thymidine incorporation into DNA by human lymphocytes. Based on protein content, VLDL were the most potent inhibitors, followed by LDL, and then HDL. Fifty percent inhibition of 3H-thymidine uptake by PHA-stimulated lymphocytes was observed at VLDL protein concentrations of less than 2.0 μg/ml. Chisari2 reported that all subclasses of VLDL classified according to flotation rates were equally inhibitory to lymphocyte DNA synthesis, indicating the heterogeneous distribution of inhibitory activity throughout VLDL subclasses. Morse et al. found that the intermediate density lipoproteins (d = 1.006–1.010) were the most potent inhibitors of lymphocyte proliferation, followed by VLDL and then LDL.3

To understand the events leading to the suppressive effects on lymphocyte proliferation, we have studied the binding and degradation of 125I-VLDL by human lymphocytes. Our findings indicate that after the binding of VLDL to specific lymphocyte receptors, VLDL proteins were degraded and excreted from the cell as acid-soluble products. The binding of 125I-VLDL at 4°C was characterized by an initial rapid binding phase followed by a plateau phase. In competition binding experiments, which were performed at 4°C, 125I-VLDL binding by human lymphocytes showed a specificity for VLDL as compared to LDL or HDL. Reversibility of lipoprotein (125I-LDL) binding to fibroblast membrane has previously been demonstrated using heparin.23 In our experiments, heparin induced a rapid release of 60% of 125I-VLDL bound to human lymphocytes, suggesting reversibility of the ligand-receptor interaction. As previously shown with 125I-LDL,5 125I-VLDL binding to lymphocyte receptors required Ca2+. However, in contrast to LDL, the presence of hydroxycholesterol and cholesterol during the 48-hr incubation did not increase the uptake of 125I-VLDL by normal lymphocytes. This result suggests that the metabolic regulation of VLDL receptors differs from that of LDL receptors. Based on a Scatchard plot analysis of 125I-VLDL binding, we estimated that there were 28,000 ± 1300 receptors per lymphocyte after 2 days of incubation in 10% LDFCS. After 36 hr of lymphocyte incubation with 125I-LDL, Ho et al. reported 1800–3600 particles of LDL bound per lymphocyte at saturation.24 We have found that after 48 hr of incubation, there were approximately 5500 ± 200 particles of 125I-LDL bound per lymphocyte at saturation (unpublished data).

The present study also showed that colchicine partially inhibited 125I-VLDL degradation by human lymphocytes. This observation indicates that the degradation of VLDL requires microtubule-dependent cellular activities such as endocytosis, digestive vacuole formation, and secretory degranulation.25–27 Colchicine, however, had no effect on receptor-binding of VLDL. These results with VLDL are similar to previous studies of LDL binding and degradation, and indicate that the high affinity uptake and degradation of VLDL by lymphocytes is dependent on the prior binding of VLDL to specific cell surface receptor sites.24 In contrast to LDL, which possess only one type of apoprotein (apo-B), apo-B and apo-C (I, II, and III) constitute 40% and 50%, respectively, of the total protein moiety of VLDL.14 Since the VLDL iodination technique that we employed was not specific for a single type of apoprotein,15 the specific cell binding by each apoprotein species in VLDL could not be individually assessed. Hence, it is possible that the differences that we reported between VLDL and LDL binding to lymphocytes might be due to differences in apoprotein content. Alternatively, the differences in VLDL and LDL binding may be related to differences in lipid composition.

Even though many previous studies5,11,28 have shown that plasma VLDL and LDL exert growth inhibitory activity on many types of cells, the mechanism by which lipoproteins inhibit cell proliferation is obscure. Chisari2 suggested that VLDL may inhibit PHA-stimulated DNA synthesis by either suppression of prereplicative protein synthesis or sterol metabolism. He further suggested that inhibition depended on the biologic expression of the apolipoprotein B moiety that VLDL shares with LDL, and that VLDL may represent the physiologic progenitor of these molecules. Hui et al.29 reported that LDL inhibition of PHA-stimulated DNA synthesis was due to prevention of Ca2+ accumulation, which was the first biochemical event occurring in PHA-stimulated lymphocytes. Recently, it has been reported that mitogen stimulation of lymphocytes can be inhibited by human LDL that have undergone autoxidation by extensive oxygenation at 37°C in the absence of EDTA,30 while no significant inhibitory effect was noted with nonoxidized lipoproteins. The presence of EDTA during our lipoprotein dialysis and the slower rate of oxidation of VLDL as compared to LDL would tend to lessen the extent of VLDL oxidation occurring in our study.30
LYMPHOCYTE RECEPTORS FOR VLDL INHIBITOR

In recent in vivo experiments in mice, Chisari has proposed that the immunoregulatory effect of human plasma VLDL are caused by inhibition of early inductive events and quantitative restriction in the clonal expansion and entry of primed lymphocytes into the memory cell pool. Experiments dealing with the relative sensitivity of different lymphocyte subpopulations to suppression by VLDL, the distribution of VLDL receptors on such subpopulations, and the composite immunologic effect of interacting subpopulations will be essential to a better understanding of the physiologic role of lipoprotein growth regulators. In studies of the LDL-inhibitor effect on lymphocytes, Curtiss and Edgington have shown that T-suppressor, T-helper, and B lymphocytes are all sensitive to and can be regulated by LDL-In, with T-suppressor cell populations being the most sensitive.

Current information is insufficient to explain the biologic role of plasma lipoprotein cell growth inhibitors. Several studies, including the current one, have demonstrated that plasma VLDL and LDL have inhibitory effects on PHA-stimulated lymphocyte proliferation at considerably lower concentrations than occur in normal plasma. Curtiss and Edgington, in their studies of LDL-inhibitor, suggested that the binding properties of LDL-In to lymphocytes could theoretically account for the observed suppression of lymphocyte proliferation. We would, likewise, propose that the biologic properties of plasma VLDL can account for the observed VLDL suppression of lymphocyte proliferation. Using our estimate of a mean binding affinity for the VLDL receptor complex of $8.77 \times 10^7$ liter/mole and an approximate value of 80 µg of VLDL protein per milliliter normal male adult serum, we estimate that in the physiologic state in blood 91% of lymphocyte VLDL receptors would be occupied with VLDL molecules. At a hypothetical plasma VLDL protein concentration of 20 µg/ml, which produced 90% inhibition of DNA synthesis by PHA-treated lymphocytes in our in vitro study, 72% of VLDL receptors would theoretically be occupied with VLDL molecules in vivo. It seems reasonable to suggest therefore, that under physiologic conditions in circulating blood and considering the high affinity of VLDL for its receptor, normal plasma concentrations of VLDL are capable of profoundly suppressing lymphocyte DNA synthesis. It should be stated, however, that to date receptor binding studies have been performed under nonphysiologic conditions; hence, caution should be used in directly applying such in vitro data to the physiologic state in vivo. To overcome this limitation, future lymphocyte receptor binding experiments should be performed using assay conditions that more closely mimic the microenvironment in circulating blood. The reader should also recognize that much lower concentrations of lipoproteins are found in prenodal lymph fluid and presumable in interstitial fluid as compared to blood, which theoretically should result in a lesser VLDL growth inhibitory effect in lymph nodes and other major sites of lymphocyte proliferation.

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