Characterization of a Human Hepatic Receptor for High Density Lipoproteins


Characterization of the membrane receptor for the low density lipoproteins (LDL) has led to insights into cellular receptor physiology as well as mammalian lipid transport. Results with LDL have stimulated the search for specific receptors for other plasma lipoproteins. Receptors for high density lipoproteins (HDL) have been identified in human fibroblasts and smooth muscle cells. Specificity for this receptor has been difficult to define since normal HDL contains several apolipoproteins, and particles containing apolipoproteins B and E have been shown to compete for HDL binding. In the present study, we demonstrate that HDL isolated from a patient devoid of apolipoprotein E was bound specifically by human hepatic membranes. This binding reached saturation within 2 hours and was EDTA-resistant. Assuming a single receptor model, we found that 2.9 x 10^15 receptors/mg membrane protein bound with an affinity $K_D = 3.5 \times 10^{-7}$ M at 0 to 4°C and $K_D = 1.9 \times 10^{-7}$ M at 37°C. The binding was effectively competed with intact HDL, with HDL that had undergone selective arginine and lysine residue modification, and with antibodies to apolipoproteins A-I and A-II. However, LDL, asialofetuin, and HDL which had undergone tyrosine modification by nitration, and anti-apolipoprotein B did not compete with apo A-I HDL binding. In contrast to LDL binding, the human hepatoma cell line, HEPG2, increased HDL binding with cholesterol loading that was specific for HDL. Thus, hepatic tissue can modulate its recognition of HDL. Finally, hepatic membranes from a patient lacking normal hepatic LDL receptors bound apo A-I HDL normally. These data indicate that a saturable, specific regulatable receptor for apo E-free HDL is present in human liver.

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arterial smooth muscle cells. Therefore, unique, saturable HDL receptors that may have a role in reverse cholesterol transport have been identified in peripheral cells.10-12

Since the liver plays a central role in lipid and lipoprotein metabolism, receptors identified in peripheral cells have been sought in the liver. Specific membrane-bound hepatic receptors for apo B and apo E have been identified in a number of mammals including man.13-20 Specific binding for HDL, independent of apo E, has also been evaluated. If HDL were to facilitate cholesterol removal from the body, hepatic delivery of cholesterol from peripheral cells to the liver might be receptor-mediated. In animal models, radiolabeled HDL is cleared by the liver21-23 and metabolized by both parenchymal and nonparenchymal liver cells.24-28 However, the HDL metabolism in these animal models is not specific for HDL. Lipoprotein particles containing apo B or apo E competed for HDL binding in studies conducted in rats29,30 swine,31 and rabbits.28 Therefore, the significance of HDL binding to liver has been clouded by the apparent lack of binding site specificity.

We evaluated the characteristics of HDL binding to human hepatic membranes by using HDL isolated from a patient who had no circulating apo E.32 These studies indicate that human liver contains a specific, saturable, calcium-independent receptor for HDL that is unique and distinct from the hepatic LDL receptor.

Methods

Liver Membrane Preparation

Biopsy specimens of human liver from normal and familial hypercholesterolemic subjects were obtained intraoperatively after informed consent had been given. Use of intraoperative hepatic biopsies for research purposes was approved by the Human Experiments Committee at the University of Pittsburgh. Tissue was placed immediately in a beaker and all processing occurred at 4°C in a manner similar to that described previously.30 After the tissue was minced with a razor blade, it was washed with an ice-cold buffer containing 150 mM NaCl, 1 mM EDTA, and 10 mM Tris-HCl (pH 8.0). Homogenization was performed by six strokes of a motor-driven Teflon pestle in a buffer containing 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl (pH 8.0). The homogenized preparations (10 mg/ml) were centrifuged for 10 minutes at 1000 g. The supernatant solution was recentrifuged for 25 minutes at 10,000 g, followed by ultracentrifugation at 100,000 g for 60 minutes. The pellet from this ultracentrifugation was resuspended in a buffer containing 150 mM NaCl and 10 mM Tris-HCl (pH 8.0) and flushed 10 times through a 22-gauge needle. These membranes were recentrifuged 15 minutes at 100,000 g, and the membrane pellets were then frozen in dry ice and stored in liquid nitrogen until they were used in the binding assays.

Lipoprotein Preparation

HDL₃ (d = 1.125-1.210 g/ml) and LDL (d = 1.030-1.050 g/ml) were prepared from 500 ml of plasma collected in 0.1% EDTA by plasmapheresis after a 12- to 14-hour fast. HDL₃ was isolated from a subject lacking plasma apolipoprotein E in the plasma.29 Lipoproteins were separated by preparative ultracentrifugation at 4°C (24 hours for LDL and 48 hours for HDL₃) by using KBr for density gradient adjustment.31 After centrifugation, subfractions were dialyzed at 4°C against 150 to 250 volumes of phosphate-buffered saline (pH 7.0, Gibco, Grand Island, New York). Each isolated lipoprotein fraction was sterilized by 0.45 μm filtration (Millipore Corporation, Bedford, Massachusetts) and used within 2 weeks of preparation. Apo A-I was isolated from HDL (d = 1.063-1.21 g/ml) from normal volunteers. After chloroform/methanol (2:1, vol/vol) delipidation, apo A-I was purified by gel filtration on Sephadex G-200 in 8 M urea as reported previously.32 Each preparation was a single band on 7.5% NaDodSO₄ polyacrylamide gel electrophoresis. Apo A-I was iodinated by the iodine monochloride method and a modification of the technique described for lipoproteins.34 A 60% to 80% efficiency of iodination was achieved and ~0.5 mol of iodine was incorporated per mole of apo A-I. The iodinated apo A-I preparation used for binding assays was free of incompetent (nonself-associating) monomer as evaluated by high-performance liquid chromatography.35 The ¹²⁵I-apo A-I was incubated with apo E-free HDL₃ for 30 minutes in a 37°C shaker bath. ¹²⁵I-apo A-I associated with HDL₃ (¹²⁵I-apo A-I HDL) was separated from free ¹²⁵I-apo A-I by a 36-hour, 4°C, 140,000 g ultracentrifugation at a 1.21 g/ml density in a 40.3 Beckman rotor (Beckman, Fullerton, California). From 86% to 94% of the radiolabeled apo A-I remained associated with the HDL. After a 4°C, 16- to 25-hour dialysis against 300 to 500 volumes of phosphate-buffered saline to remove KBr and after Millipore filtration, the protein concentration was determined by the method of Lowry with use of ovalbumin as standard. Specific activities for the ¹²⁵I-apo A-I HDL were 2.7 to 3.6 × 10⁶ Bequerels/mg HDL protein.

Aliquots of HDL underwent arginine modification as described for lipoproteins.37 Acetylation was performed utilizing the diketene reagent as reported by Mahley and co-workers.38 Charge modification of the HDL was confirmed by agarose gel electrophoresis. Modification of tyrosine residues in HDL was performed by nitration of either radiolabeled or unlabeled HDL by a method developed by Eliot Brinton and co-workers based on previously described methods.39 Tetraniotromethane (TNM) (Aldrich Chemical Company, Incorporated, Milwaukee, Wisconsin) was dissolved in 95% ethanol and added to HDL₃ (1 mg protein/ml) in a 0.15 M NaCl, 5 mM EDTA, pH 7.4 buffer at a final TNM concentration of 0.3 mM. Samples were incubated at 25°C for 5 minutes, and the HDL was extensively dialyzed against a 0.15 M NaCl, 5 mM EDTA at 4°C overnight.
Binding of $^{125}$I-Apo A-I HDL to Liver Membranes

Frozen liver membrane preparations were thawed and resuspended through a 22-gauge needle in a 50 mM NaCl, 30 mM Tris-HCl (pH 7.5) buffer and adjusted to a membrane protein concentration of 10–12 mg/ml. Membranes were then sonicated for five 4-second pulses at 55 W-setting with an ultrasonics microtip (Heat Systems Ultrasonics, Incorporated, Plainview, New York). Preliminary experiments demonstrated that $^{125}$I-apo A-I HDL binding to hepatic membranes was linear from 50 μg to 200 μg hepatic membrane protein per assay. Membrane pellets were suspended in a buffer containing 25 mM NaCl and 50 mM Tris-HCl (pH 7.5), for a final membrane protein concentration of 5 to 10 mg/ml. From 10 to 25 μg of this membrane suspension was then added to the assay, which resulted in a final assay buffer containing 50 mM NaCl, 20 mM Tris-HCl (pH 7.5), and either 1 mM CaCl$_2$ or 30 mM EDTA. $^{125}$I-apo A-I HDL was added at the concentrations indicated in the respective tables and figure legends with or without unlabeled HDL to a total assay mixture volume of 0.1 ml. Incubations were carried out at 0° C or 37° C for the indicated time period. Bound $^{125}$I-apo A-I HDL was separated from free ligand by a 30-minute, 100,000 gcentrifugation of 50 μl of the assay mixture through 125 μl phosphate-buffered saline (PBS) in a 30° angle rotor by an air-driven ultracentrifuge (Beckman, Palo Alto, California). The supernatant was removed from the pellets by vacuum aspiration, and the pellet was washed once with 125 μl of PBS. The cellulose nitrate tube tips containing the membrane pellet were sliced and the radioactivity in the pellet was quantitated and the cellular protein content was determined by the method of Lowry.

Data Analysis of Membrane Binding Assays

Binding data were analyzed by using MLAB, an on-line computer modeling program developed at the National Institutes of Health. This system permits the fitting of experimental data to theoretical functions by the Marquardt-Levenberg curve-fitting method. Variable parameters in the theoretical function are adjusted by iteration so as to minimize the sum of the squares of the differences between experimental and theoretical data. Primary data were analyzed directly, rather than as linear transformations as described by Scatchard, in order to simplify error analysis. Final parameter values are given ± standard error. The functions used were:

Single Class of Sites:

$$SBL = \frac{(FL \cdot R)/(KD + FL)}{(XS + L) + 1} - C + B$$

where SBL corresponds to specifically bound ligand, FL to free ligand, R to the number of receptor sites per assay, KD to the equilibrium dissociation constant, and the subscripts 1 and 2 correspond to the first and second sites respectively.

Cellular Metabolism Studies

The regulation of hepatic HDL$_g$ metabolism was assessed by using the well-differentiated human hepatoma cell line, HEPG2. From 2 to 4 × 10$^5$ cells were plated in 2 cm$^2$ wells (Costar, Cambridge, Massachusetts) on Day 1 in RPM1 1640 medium containing 10% fetal calf serum, penicillin, and streptomycin. On Day 3, the medium was replaced by a totally defined serum-free medium, which did or did not contain 50 μg cholesterol/ml. The cholesterol was added to the culture medium after it had been dissolved in ethanol (5 mg/ml) as previously outlined. After a 24-hour incubation in either the cholesterol-free, serum-free medium or in medium containing 50 μg cholesterol/ml, the medium was changed to include the concentrations of $^{125}$I-apo A-I HDL indicated in the figure legend in either the absence or the presence of 10-fold excess unlabeled HDL. After a 2-hour, 37° C incubation with the radiolabeled HDL, the medium was removed and the cells were washed six times with ice-cold phosphate-buffered saline (pH 7.0). The cells were solubilized with 0.5 ml of 1 N NaOH. The cell-associated radioactivity was quantitated and the cellular protein content was determined by the method of Lowry.

Results

The time course of $^{125}$I-apo A-I HDL binding to hepatic membranes was assessed at 0° C and 37° C (Figure 1). The total and nonspecific binding, defined as the binding of the $^{125}$I-apo A-I HDL occurring in the presence of a constant 10-fold excess of unlabeled HDL, was dependent on both incubation time and temperature. In contrast to hepatic membrane binding of LDL, the binding of the radiolabeled HDL in the presence of 30 mM EDTA was not significantly lower.
The effect of temperature and EDTA on the time course of $^{125}\text{I}$-apo A-I HDL binding to human hepatic membranes. From 80 to 110 $\mu$g of hepatic membrane protein was incubated with 25 $\mu$g/ml of $^{125}\text{I}$-apo A-I HDL for the indicated length of time at either $0^\circ$ or $37^\circ$ C. Total binding (●), binding in the presence of 30 mM EDTA (○), and binding in the presence of 500 $\mu$g/ml unlabeled HDL (▲) were determined. Values represent the mean ± SE of triplicate samples.

than the binding observed in the presence of 1 mM calcium and more than 90% of the specific binding at saturation was EDTA-resistant. However, as observed previously with LDL binding, the incubation at $37^\circ$ C resulted in a nearly twofold increase in the binding of radiolabeled HDL. Therefore, the binding of $^{125}\text{I}$-apo A-I HDL to human hepatic membranes was sensitive to the incubation temperature, reached equilibrium by 2 hours, and was EDTA-resistant.

The effect of HDL concentration on specific HDL-hepatic membrane binding was also evaluated at both $0^\circ$ and $37^\circ$ C (Figure 2). Increasing ligand concentration resulted in increased HDL binding at both incubation temperatures. In addition, the binding reached saturation at both temperatures. The primary data were analyzed directly by using the equations given in the Methods section, and receptor number and affinity are given in Table 1. If we assume a single receptor model, the affinity of the HDL-membrane interaction was increased twofold by changing the incubation temperature from $0^\circ$ to $37^\circ$ C. Fitting these data to a two-receptor model provided no additional information. Therefore, $^{125}\text{I}$-apo A-I HDL binding to hepatic membranes is saturable and can be accounted for by a single receptor site in which affin-

Table 1. Human Hepatic Membrane HDL Receptor Number and Affinity at $0^\circ$ C and $37^\circ$ C

<table>
<thead>
<tr>
<th>Model</th>
<th>Receptor number</th>
<th>Dissociation constant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/ mg membrane</td>
<td>Number $\times 10^{15}$/ mg membrane</td>
</tr>
<tr>
<td>Single-receptor site $0^\circ$</td>
<td>0.80 ± 0.05</td>
<td>2.85 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>0.81 ± 0.03</td>
<td>2.88 ± 0.11</td>
</tr>
<tr>
<td>Two-receptor site $0^\circ$</td>
<td>0.80 ± 0.20</td>
<td>2.85 ± 0.71</td>
</tr>
<tr>
<td>Site A</td>
<td>23.4 ± 3.8 $\times 10^5$</td>
<td>833 ± 13.5 $\times 10^5$</td>
</tr>
<tr>
<td>Site B</td>
<td>0.61 ± 0.05</td>
<td>2.17 ± 0.18</td>
</tr>
<tr>
<td>37$^\circ$</td>
<td>124 ± 1819</td>
<td>441 ± 6476</td>
</tr>
</tbody>
</table>

The receptor number and affinity were calculated by using the specific binding data as described in Methods and by assuming either a single- or a two-receptor site model. Values represent means ± SE.
ity, but not receptor number, are affected by the incubation temperature.

The specificity of the HDL-hepatic membrane interaction was assessed with competition binding assays. Increasing concentrations of HDL, asialofetuin, and LDL, ligands known to interact with specific hepatic receptors, were incubated with the hepatic membranes in the presence of 50 μg/ml 125I-apo A-I HDL at both 0° and 37° C (Figure 3). Unlabeled HDL competed effectively with the radiolabeled ligand; however, LDL and asialofetuin were unable to decrease the binding of 125I-apo A-I HDL binding.

Chemical modification of lysine and arginine residues prevents LDL from interacting with its receptor.37,38 The lysine and arginine residues in HDL were modified and the ability of these preparations to inhibit 125I-apo A-I HDL membrane binding was assessed (Figure 4). Despite the charge modification of these amino acids, the chemically altered HDL preparations were as effective as the native HDL in inhibiting 125I-apo A-I HDL binding. Modification of HDL by nitration affects tyrosine residues. In contrast to acetylated and acetoacetylated HDL, nitrated HDL did not effectively compete for the 125I-apo A-I HDL binding. Thus, LDL-, asialofetuin-, and tyrosine-modified

Figure 2. The effect of increased ligand concentrations on the specific binding of 125I-apo A-I HDL to human hepatic membranes. From 92 to 106 μg of hepatic membrane protein was incubated for 2 hours at the indicated temperatures and with the indicated lipoprotein concentrations. The specific binding was determined as the difference in observed binding in the absence and presence of tenfold excess unlabeled HDL for each 125I-apo A-I HDL concentration. Values represent the mean of duplicate samples for a given ligand concentration.

Figure 3. The competition of 125I-apo A-I HDL with unlabeled HDL, LDL and asialofetuin. Hepatic membranes (98 to 102 μg protein per assay) were incubated for 2 hours at either 0° or 37° C with 50 μg/ml 125I-apo A-I HDL and unlabeled HDL (△), asialofetuin (●), or LDL (○). The initial binding of 125I-apo A-I HDL bound per mg membrane protein at 37° C and 0° C, which represents 100%, was 462 and 356 ng, respectively.
HDL did not compete for $^{125}$I-apo A-I HDL binding, while arginine and lysine modification of HDL did not alter binding to its receptor. The inability of nitrated HDL to compete with radiolabeled HDL was paralleled by the reduced level of direct binding observed with radiolabeled HDL that had undergone nitration (Table 2). However, nitration did not alter the specific activity of the radiolabeled HDL. In addition, no significant apoprotein aggregation was generated as determined by autoradiography of sodium dodecyl sulfate gel electrophoresis of the nitrated $^{125}$I-HDL. Total and competitively bound nitrated $^{125}$I-HDL were only 40% and 32% that of unmodified HDL. Therefore nitration, which modifies tyrosine residues, alters HDL binding to hepatic membranes.

Finally, the apolipoprotein specificity for the HDL-membrane interaction was assessed immunologically (Table 3). Both LDL and anti-apo B antibodies were ineffective in reducing the HDL-membrane binding. In contrast, anti-apo A-I, anti-apo A-II, and unlabeled HDL reduced binding by 60% or more. Therefore, the binding of $^{125}$I-apo A-I HDL to human hepatic membranes demonstrated a unique specificity.

Individuals homozygous for familial hypercholesterolemia (FH) lack a normally functioning LDL receptor in both nonhepatic and hepatic tissues. The ability of $^{125}$I-apo A-I HDL to bind to FH hepatic membranes was evaluated (Table 4). At saturation, these hepatic membranes demonstrated a significant reduction in the number of expressed LDL receptors.

| Table 2. Effect of Nitration on Direct $^{125}$I-HDL Binding |  |
|---|---|---|
| Bound $^{125}$I-HDL (ng/mg protein/2 hr) | Total | Non-specific | Specific |
| Unmodified $^{125}$I-HDL | 661 ± 23 | 236 ± 16 | 425 ± 28 |
| Nitrated $^{125}$I-HDL | 269 ± 10 | 137 ± 8 | 132 ± 13 |

Hepatic membranes (128 μg protein) were incubated for 2 hours at 37° C with 100 μg of unmodified and nitrated $^{125}$I-HDL in the absence (total) and presence (non-specific) of tenfold excess unlabeled HDL3. Values represent the means ± SE of triplicate samples.

| Table 3. Inhibition of $^{125}$I-HDL Binding by LDL, HDL3, Anti-Apo A-I, Anti-Apo A-II, and Anti-Apo B |  |
|---|---|---|
| Percent of total $^{125}$I-HDL binding | Excess LDL | 79.5 ± 0.8 |
| Anti-apo B | 23.8 ± 0.7 |
| Anti-apo A-I | 39.0 ± 0.3 |
| Anti-apo A-II | 41.2 ± 2.8 |

Human hepatic membranes (108 μg/assay) were incubated with 1 μg $^{125}$I-HDL/ml in the presence of 100-fold excess LDL and HDL and with antibodies titrated to bind 3 μg of apolipoprotein. Total binding (100%) was 94 ± 9 ng $^{125}$I-HDL bound/mg membrane protein. Values represent the means ± SE of triplicate assays.

| Table 4. $^{125}$I-Apo A-I HDL Binding to Normal and Homozygous Familial Hypercholesterolemic Hepatic Membranes |  |
|---|---|---|
| $^{125}$I-Apo A-I HDL binding (ng/mg membrane protein) | Total | Non-specific | Specific |
| Normal (n = 3) | 1107 ± 141 | 390 ± 171 | 717 ± 26 |
| Familial hypercholesterolemia | 1542 | 714 | 828 |

From 86 to 122 μg of hepatic membrane protein from normal and familial hypercholesterolemia individuals were incubated for 2 hours at 37° C with 350 μg $^{125}$I-apo A-I HDL/ml in the absence (total binding) or presence (non-specific binding) of tenfold excess unlabeled HDL. Specific binding is the difference between total and nonspecific binding. Values for normal membranes represent the means ± SE.
ceptors. However, both the total and specific binding of $^{125}$I-apo A-I HDL to these membranes were normal or slightly increased. This indicates that the hepatic HDL receptor is genetically distinct from the LDL receptor in humans.

Finally, we assessed the regulation of hepatocyte HDL binding in vitro using the well differentiated human hepatoma cell line, HEPG2. HEPG2 saturably bound $^{125}$I-apo A-I HDL after cholesterol preincubation (Figure 5). The enhanced HDL binding with cholesterol preincubation contrasted with the 26% decline in $^{125}$I-LDL binding and internalization observed in these cells under identical conditions. Furthermore, this upregulation of HDL binding is also present in normal human hepatocytes after cholesterol exposure for 24 hours (data not shown). Therefore the HEPG2 reflect normal human hepatocyte regulation of HDL binding.

As was noted with the hepatic membranes, the upregulated binding of HDL by HEPG2 was specific (Figure 6). Neither LDL nor acetate-acetylated LDL competed for HDL binding. In contrast, at 5 $\mu$g unlabeled HDL protein/ml, only 50% of the $^{125}$I-HDL binding was present. Thus the specificity of the regulated $^{125}$I-HDL binding in HEPG2 paralleled the specificity demonstrated in the isolated hepatic membranes.

![Figure 5](http://atvb.ahajournals.org/) The effect of cholesterol loading on $^{125}$I-apo A-I HDL binding by the human hepatoma cell line, HEPG2. From 3 to 5 x $10^5$ HEPG2 were plated in 2 cm² wells on Day 1 in medium containing 10% fetal calf serum. After 48 hours, the medium was changed. The preconfluent HEPG2 were incubated with either serum-free medium (○) or a serum-free medium containing 50 $\mu$g cholesterol/ml (●) for 24 hours. The specific binding is defined as the difference in binding observed in the absence and presence of tenfold excess unlabeled HDL after a 2-hour, 37°C incubation. Values represent the mean ± SE of triplicate samples.

![Figure 6](http://atvb.ahajournals.org/) Competition of $^{125}$I-HDL binding by LDL, acetate-acetylated LDL, and HDL in HEPG2 cells. After cholesterol loading HEPG2 cells as described in Figure 5, cells were incubated with 5 $\mu$g $^{125}$I-HDL for 2 hours at 37°C in the absence (total binding) or presence of the indicated concentrations of unlabeled lipoprotein. Values represent the mean of duplicate wells.

**Discussion**

The apolipoproteins serve numerous physiologic functions in lipoprotein metabolism. In addition to the physical solubilization of lipid into aqueous media, apolipoproteins serve as enzyme cofactors and as recognition ligands for specific cellular receptors. Apo B-containing lipoproteins have received the most attention as the ligand in cellular receptor-lipoprotein interactions. The specific receptor for apo B-containing lipoproteins is calcium-dependent, protease-sensitive, saturable, upregulated in cholesterol-depleted cells in vitro, and sensitive to arginine and lysine residues. The putative role of LDL and its receptor pathway is to deliver cholesterol to tissues.

Physiologic roles independent of a solubilization function have been sought for apo A-I and apo A-II, the principle apolipoprotein of HDL. The initial in vitro tissue culture studies did not suggest a receptor-recognition role for apo A-I or apo A-II. However, recent studies indicate that HDL binding and internalization by nonhepatic cell lines may, in fact, be regulated. In addition to providing cholesterol for steroidogenic tissues, HDL binding and internalization increased when fibroblasts, smooth muscle cells, or endothelial cells were loaded with cholesterol. The enhanced binding was correlated with enhanced cholesterol efflux mediated by HDL. Therefore, binding of HDL by nonhepatic, nonsteroidogenic tissues, which appears to be mediated by apolipoproteins, has supported the concept.
of the delivery of cholesterol from peripheral cells to the liver.\(^5\)

The reverse cholesterol transport concept assumes that HDL cholesterol can be delivered to the liver. Partial hepatectomy\(^49\) and metabolic turnover studies\(^50,51\) in the rat indicate that HDL cholesterol is efficiently delivered to the liver without concomitant degradation through lysosomal hydrolysis of the entire HDL particle. Utilizing metabolic turnover techniques and multicompartmental analysis, Schwartz and co-workers have demonstrated that both cholesteryl ester\(^52\) and free cholesterol\(^53\) in HDL are preferential sources of biliary cholesterol in man. Therefore, a receptor for HDL in liver membranes could facilitate the delivery of HDL cholesterol through a pathway independent of the LDL lysosomal delivery pathway.

We have demonstrated in the present study that the binding of HDL to human hepatic membranes is saturable and specific and does not depend upon the presence of apo E. Furthermore, binding is distinct from that reported previously for plasma lipoproteins in other mammalian species. Unlike LDL binding to human liver, HDL binding is EDTA-resistant\(^18\) and the affinity of HDL binding is affected by temperature. The calcium-independent nature of HDL binding to human liver has also been reported previously in swine\(^37\) and rabbits.\(^28\) However, unlike previous evaluations of HDL binding to liver, the present study indicates a unique specificity of HDL binding to human membranes. Studies using either hepatocyte cultures or hepatic membranes have previously demonstrated some degree of competition of HDL, binding by other density classes of lipoproteins.\(^27,28,44,56,57\) Whether expressed as \(\mu\)g/ml or normalized to a molar concentration, asialofetuin and anti-apo B did not decrease LDL binding of \(^{125}\)-apo A-I HDL to hepatic membranes (Figure 4, Table 3). In addition, the HDL binding to human hepatic membranes was not affected by arginine and lysine residue modification, which impair apo B and apo E binding.\(^37,38\) However, nitration, which reportedly modifies tyrosine residues and decreases HDL metabolism by nonhepatic tissues,\(^58,59\) and anti-apo A-I and anti-apo A-II attenuated HDL binding. These studies were performed by using HDL isolated from a patient with no circulating apo E.\(^39\) As a result, the HDL binding observed in these studies can not be mediated by an apo E interaction with the hepatic apo E or apo B, E receptor. Although the chemical characteristics of the HDL membrane binding site are yet to be defined, the saturability and specificity demonstrated in the present studies are characteristic of receptors. Most well-characterized receptors are protein, but recently glycolipids have not only elicited transmembrane signals\(^54\) but have also induced adsorptive endocytosis.\(^55\) Further evaluation of the chemical nature of the hepatic HDL receptor is warranted. Therefore, binding of HDL to human hepatic membranes characterized by these membrane studies indicates that a receptor for HDL apolipoproteins that is distinct from any previously described hepatic lipoprotein receptor is present in human liver.

The study of isolated cellular membrane fractions allows for the rapid separation of bound and free ligand which is necessary for accurate determination of receptor number and affinity. Intact cell studies require sequential washes that disrupt the equilibrium between bound and free ligand.\(^60\) This loss of equilibrium results in an overestimation in the affinity of binding. Although cell membrane binding studies lead to useful estimates of binding kinetics, intact cells are necessary for the study of receptor regulation. The current investigation indicated that HDL binding can be regulated. HEPG2, a well-differentiated human hepatoma cell line, modulated the binding of apo E-free HDL. As was observed in nonhepatic cells in vitro,\(^11\) preincubation of both HEPG2 and normal hepatocytes with cholesterol-enriched medium led to enhanced HDL binding by these cells. Although upregulation of HDL binding by hepatocytes with cholesterol depletion (rather than cholesterol loading) would be more attractive for a reverse cholesterol transport role for this HDL receptor, such a scheme may be too simplistic. In contrast to nonhepatic tissues, which have only a few pathways by which cholesterol is metabolized, the hepatocyte can secrete cholesterol directly into bile, can convert cholesterol to bile acids, and can package cholesterol into lipoproteins during lipoprotein synthesis. Therefore, understanding the effect of cholesterol enrichment of the entire cell as well as the subcellular cholesterol compartments and understanding the metabolic fate of subcellular cholesterol pools are necessary to assess the role a receptor for HDL in liver would have in reverse cholesterol transport.

Finally, this hepatic HDL receptor appears to be genetically distinct from the hepatic LDL (apo B, E) receptor. The hepatic membranes from an LDL receptor-negative familial hypercholesterolemic homozygote also demonstrated defective hepatic LDL receptors.\(^19\) However, these membranes bound apo E-free HDL normally (Table 4). Therefore, in addition to the unique specificity and amino acid requirements, the binding of HDL appears to be genetically distinct from the hepatic LDL receptor system. These combined data indicate that human hepatic membranes manifest a unique and genetically distinct receptor for HDL that can be regulated in vitro.

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