Interaction of Plasma Lipoproteins With Human Platelets

By Linda K. Curtiss and Edward F. Plow

Human plasma low density lipoproteins (LDL) and high density lipoproteins (HDL) were radiiodinated and their interaction with washed human platelets was assessed. Both ligands were bound by the platelet at 37 °C, and an apparent equilibrium was attained within two hours. Minimal binding was observed at 22 °C or 4 °C. The specificity of these interactions was indicated by the observations that: (a) labeled and nonlabeled lipoproteins interacted with the platelet with the same apparent affinity; (b) nonlabeled lipoproteins inhibited binding, whereas unrelated plasma proteins did not; and (c) the platelet-bound ligands exhibited the appropriate apoprotein chain compositions when analyzed by polyacrylamide gel electrophoresis. Binding of HDL and LDL was found to be independent of the state of platelet activation and did not require divalent ions. Binding of HDL to the platelet was saturable, and a class of sites that maximally bound 1.585 ± 390 HDL particles, with a dissociation constant of 3.1 × 10⁻⁸ mol/L, was identified. Binding of LDL to the platelet was more complex, but evidence for a class of sites that bound 7.075 ± 4.800 LDL particles, with a dissociation constant of 4 × 10⁻⁸ mol/L, was found. LDL was a poor inhibitor of ¹²⁵I-HDL binding to the platelet, whereas HDL was an effective inhibitor of ¹²⁵I-LDL binding. The capacity of HDL to bind or inhibit LDL binding was not dependent on its apoprotein E content. These results are most readily interpreted in terms of two types of lipoprotein interaction sites on platelets: (1) an HDL binding site that does not bind or interacts poorly with LDL, and (2) an LDL binding site that recognizes or is otherwise altered by HDL. The HDL site may be similar to the LDL receptor expressed by steroidogenic tissues in terms of apoprotein specificity. The LDL site is not the same as the LDL receptor of most extrahepatic cells.

Platelets and lipoproteins are intimately involved in the pathogenesis of a wide variety of diseases, including atherosclerosis, thrombosis, and coronary heart disease. Evidence accumulating over the past two decades suggests the possibility of a direct relationship between plasma lipoproteins and the hemostatic function of platelets. Particularly pertinent in this regard are the reports of abnormal platelet function observed in the disease. 

Recent studies have demonstrated that lipoproteins can alter the aggregation response of platelets. Aviram and Brooks found that low density lipoproteins (LDL) enhanced, whereas high density lipoproteins (HDL) reduced thrombin-induced platelet aggregation. Hassal et al showed that plasma LDL levels were correlated with enhanced platelet sensitivity to aggregation by epinephrine. These observations imply an interaction of these lipoproteins with the platelet surface as the initial mechanism for altering platelet function. Specific receptors for lipoproteins have been identified on a variety of circulating and tissue-fixed cells. LDL receptors, as expressed by most extrahepatic cells, possess a dual specificity that permits lipoproteins containing either apoproteins (apo) B or E to interact. This relaxed specificity allows either four apo B-containing LDL particles or one apo E-containing HDL particle to bind to a single LDL receptor. In hepatic as well as steroidogenic tissues, such as testes, ovaries, placenta, and adrenals, independent binding sites for HDL have been reported. Though not definitively established, a role for apo A1 in determining the specificity of these HDL receptors has been suggested. Several laboratories have now demonstrated specific binding of certain lipoproteins to washed human platelets. Aviram and Brook have reported that gel-filtered platelets from normal subjects bind both LDL and HDL and have found minimal cross-reaction between the binding sites for these ligands. In contrast, Koller et al found that HDL inhibited LDL binding and that LDL inhibited HDL binding. In this study, we have
systematically examined the binding, specificity, and interrelationships between the interactions of HDL and LDL with human platelets.

MATERIALS AND METHODS

Lipoprotein Isolation and Characterization

Blood was obtained from humans following approved institutional protocols in accordance with assurance GO 108 XB on file with Health and Human Services (HHS). Lipoproteins were isolated from pooled fresh fasting plasma that was obtained from normal healthy donors by plasmapheresis. During the course of these studies, the lipoproteins were isolated from eight different plasma pools by sequential ultracentrifugation using KBr for density adjustment, as previously described, and in the presence of 0.1% (wt/vol) EDTA, 1 mg/mL gentamycin sulfate, 0.2% sodium azide, 5 mmol/L benzamidine, 10 mmol/L diisopropylfluorophosphate, and 10 μg/mL soybean trypsin inhibitor. The lipoprotein fractions used were LDL (density = 1.019 to 1.063 g/mL) and HDL (density = 1.063 to 1.25 g/mL). Lipoproteins were dialyzed thoroughly against 0.15 mol/L NaCl, 0.3 mmol/L EDTA, 5 mmol/L benzamidine, and 0.0005% α-tocopherol, pH 7.4, filter sterilized and stored at 4°C. Half of the nonprecipitable radioactivity was free iodide, as determined by chloroform extraction, and less than 7% of the acid-precipitable radioactivity was extractable into organic solvents containing 0.1% Coomassie Brilliant Blue R250 in 50% trichloroacetic acid, destained, photographed, and dried. Dried gels containing the radioiodinated lipoproteins were analyzed by autoradiography (X-Omat; Eastman Kodak, Rochester, NY) and the autoradiographs were quantitated by soft laser densitometric scanning.

Platelets

Platelets were isolated from fresh human blood drawn into acid citrate dextrose by differential centrifugation and gel filtration, as previously described. The gel filtration was performed on a 2.5 × 40 cm column of Sepharose 2B equilibrated with divalent ion-free Tyrode's buffer, pH 7.2, containing 2% bovine serum albumin. Freedom from divalent ions was assured by passage of the equilibration buffer over a 1 × 5 cm precolumn of Chelex 100 (BioRad). The effectiveness of the washing procedure in removing autologous plasma lipoproteins was assessed by adding 125I-HDL or 125I-LDL to the platelet-rich plasma. Based on the recovery of radioactivity in the isolated platelet suspension, 7.0 ng of HDL and 12.8 ng of LDL were present per 10^8 platelets. As isolated, the platelet exhibited typical aggregation and secretory responses to a variety of stimuli. Specifically, the platelets exhibited full-scale aggregation responses measured in a Sienco dual channel aggregometer to 5 μM of thrombin and 4 μg/mL of collagen. Adenosine diphosphate (ADP) (5 μmol/L) induced aggregation-required addition of exogenous fibrinogen. Thrombin-induced secretion of 3H-serotonin was complete at 5 μL/mL of thrombin. Platelet counts were determined electronically. For selected experiments, platelet lysis induced by LDL or HDL was assessed using 51Cr-labeled platelets.

Binding of the Lipoproteins to Platelets

All labeled and nonlabeled lipoprotein preparations were dialyzed into Tyrode's buffer immediately before use, and the labeled ligands were precentrifuged for five minutes at 11,000 rpm in a Beckman microfuge. In a typical experiment, platelets at a final concentration of 10^9/mL and 125I-HDL or 125I-LDL at 5 to 20 μg/mL were mixed and incubated at the desired temperature. At selected time points, triplicate 0.05-mL aliquots were removed and layered onto 0.3 mL of 20% sucrose in Tyrode's-albumin buffer. The tubes were centrifuged at 22°C for 2.5 minutes at 11,000 rpm in a Beckman microfuge, and the tips of the tubes containing the platelets were amputated and counted for radioactivity. Platelets spun at 37°C ± 2°C gave comparable binding. Calculation of the bound lipoproteins was based on the specific activities of the radiolabeled ligands, and results are expressed as nanograms of lipoprotein protein bound per 10^8 platelets. Binding isotherms were subjected to nonlinear curve-fitting analysis, utilizing the "Ligand Program" of Munson and Rodbard on the Hewlett-Packard HP 9836 computer.

Polyacrylamide Gel Electrophoresis

The apoproteins were analyzed by polyacrylamide slab gel electrophoresis of the lipoproteins in the presence of 1% SDS. The gels were prepared according to Lautemili, using a 25 mmol/L Tris-glycine buffer at pH 8.6, as described previously. The upper stacking gel contained 1% SDS and 3% acrylamide, and the lower running gel was either a 3% to 6% or a 7.5% to 20% acrylamide gradient that also contained 1% SDS. Lipoproteins were delipidated by boiling for three minutes in electrophoresis buffer containing 1% SDS and electrophoresed for 18 hours at 35 mA for the 7.5% to 20% gel or 8 mA for the 3% to 6% gel. The gels were stained for protein with 0.1% Coomassie Brilliant Blue R250 in 50% trichloroacetic acid, destained, photographed, and dried. Dried gels containing radioiodinated lipoproteins were analyzed by autoradiography (X-Omat; Eastman Kodak, Rochester, NY) and the autoradiographs quantitated by soft laser densitometric scanning.
Radioimmunoassays

The apoprotein AI, B, CI, and E content of each of the plasma lipoprotein classes was quantitated immunochemically in a solid-phase competitive radioimmunoassay. Assays were performed, as described,22 in flexible round-bottom polyvinyl chloride microtiter plates (Dynatech, Alexandria, Va). The wells were coated, as described,22 so as to achieve a final bound lipoprotein concentration of 50 ng/well. To accomplish this, very low density lipoprotein (VLDL) was used at 50 µg/mL, LDL at 4.4 µg/mL, and HDL at 24 µg/mL. After postcoating, 0.025 mL of lipoprotein (0.1 to 1,000 µg/mL) was added and allowed to compete with the bound antigen for binding to limiting amounts of ascites fluid containing human apoprotein-specific monoclonal antibodies. The antibodies specific for apo AI and apo CI were IgGₐ antibodies secreted by cloned and stable hybridomas generated from the fusion of P3 X 63.653 mouse myeloma cells with spleen cells of mice immunized with the isolated apoproteins. They were used at dilutions of 1 :90,000 and 1 :300,000, respectively. The apo B-specific antibody (B24) has been extensively described,22 and was used at a dilution of 1 :1,000,000. The apo E-specific antibody (Emab-3) is a mouse IgGₐ monoclonal antibody that binds all apoprotein E isoforms and was a generous gift of Dr L. C. Smith, Baylor.24 It was used at a dilution of 1 :5,000. After incubation for 18 hours at 4 °C, the wells were washed free of unbound material. The amount of mouse monoclonal antibody bound was quantitated by a second four-hour incubation at 4 °C with 10 ng/well of an immunochemically purified 125I-goat anti-mouse Ig, which has been previously described.22 Non-specific binding was determined by replacing the specific ascites fluids with similar dilutions of their respective control mouse myeloma ascites fluids. Apo AI, apo CI, and apo E were quantitated by reference to standard curves generated with the purified apoproteins. The apoproteins were used at 0.1 to 20 µg/mL, were a gift of Drs L. C. Smith and J.T. Sparrow, Baylor, and were isolated as described.35 Apo B was quantitated by reference to a standard curve generated with LDL of density 1.032 to 1.035 g/mL as competitor and was used at 0.1 to 10 µg/mL. This LDL standard did not contain immunochemically detectable apo AI, CI, E, or human serum albumin.

RESULTS

Human plasma HDL (density, 1.063 to 1.25 g/mL) and LDL (density, 1.019 to 1.063 g/mL) were isolated by density gradient ultracentrifugation in KBr and radioiodinated to specific activities of 1 to 3 µCi/µg. Interaction of these radiolabeled ligands with washed human platelets was assessed by centrifuging the platelets through 20% sucrose to separate bound from free ligand. As previously described, this centrifugation system provides greater than 90% platelet recovery without cell lysis or induction of platelet secretion.29,36 In the absence of platelets, the application of 5.5 to 6.0 × 10² cpm of ¹²⁵I-HDL or ¹²⁵I-LDL onto the sucrose solution resulted in the recovery of less than 100 cpm in the tip of the tube after centrifugation.

In initial experiments, the interaction of the two lipoproteins with washed human platelets was assessed as a function of time and temperature. As shown in Fig 1, each lipoprotein bound to the washed platelets at 37 °C, and the time course of the binding reaction was similar for ¹²⁵I-HDL and ¹²⁵I-LDL. In each case, binding increased linearly for approximately 90 minutes and reached an apparent equilibrium by 120 minutes. At equilibrium, approximately sixfold more ¹²⁵I-LDL was bound to the cells than ¹²⁵I-HDL on a protein basis. With both ligands, the interactions were markedly temperature dependent. At 22 °C and 4 °C, binding of each lipoprotein at 180 minutes was less than 5% of that observed at 37 °C. In all subsequent studies, lipoprotein binding was measured at 37 °C.

Four approaches were utilized to establish that the binding of the lipoproteins to the platelet was specific. First, it was determined that the observed interaction was not due to radioiodination of the lipoproteins. Each radiolabeled lipoprotein and homologous nonlabeled lipoprotein was added to the platelets in varying proportions, while a constant total lipoprotein concentration was maintained. At 120 minutes, plots of the cpm bound v percent of the radiolabeled lipoprotein present were found to be linear for both HDL and LDL (Fig 2). The linear correlation coefficients of r ≥ .991.
indicated that each labeled and nonlabeled pair of lipoproteins interacted with the platelet with the same apparent affinity. In separate experiments, $^{125}$I-HDL labeled with the Bolton-Hunter reagent or by the lactoperoxidase procedure were found to be equivalent in their reactivity with the platelet.

Because less than 2% of the added $^{125}$I-HDL and $^{125}$I-LDL was bound by the platelets, we attempted to verify that the observed binding was not limited to a small population of each of the added $^{125}$I-ligands. A constant amount of $^{125}$I-HDL (7.5 $\mu$g/mL) or $^{125}$I-LDL (15 $\mu$g/mL) was added to platelets in the absence or presence of a 20-fold excess of its homologous nonlabeled ligand. After one hour at 37°C, the unbound fraction of each $^{125}$I-ligand was transferred to a fresh platelet suspension for a second one-hour incubation at 37°C. This same second unbound fraction was then transferred successively to two additional platelet suspensions for identical incubations. Binding of the radiiodinated lipoprotein in the presence of excess nonlabeled lipoprotein in each instance was less than 10% of the total binding observed in the absence of nonlabeled lipoprotein. No decrease in the percent ligand bound to platelets was observed from the first through the fourth incubation. Therefore, the observed specific binding of $^{125}$I-HDL and $^{125}$I-LDL to platelets was not limited to a small subpopulation of the HDL and LDL ligands.

As a third criterion of specificity, the radiolabeled material binding to the platelets was characterized. $^{125}$I-HDL and $^{125}$I-LDL were incubated with platelets for 120 minutes. After phase separation, the unbound ligands and the ligands extracted from the platelet pellets with SDS were analyzed by polyacrylamide gel electrophoresis. The autoradiograms shown in Fig 3 indicate a qualitative identity in apoprotein composition of the starting, bound, and unbound ligands for either HDL or LDL. From densitometric scans, >95% of the radioactivity of the $^{125}$I-LDL preparation was associated with large molecular weight apo B, and this percentage remained the same in the bound and unbound ligand. No apo B was detected in the bound, unbound, or starting preparation of $^{125}$I-HDL. In the starting $^{125}$I-HDL, apo A1 and apo AII constituted 54% and 33% of the radioactivity, respectively, and this proportion was maintained in the unbound $^{125}$I-HDL (57% and 34%, respectively). In the platelet-bound $^{125}$I-HDL, apo A1 and apo AII also accounted for >90% of the radioactivity, but their relative proportion was altered to 75% apo A1 and 15% apo AII. In addition, the low molecular weight peptides present in the starting and unbound $^{125}$I-HDL
(probably apo AI monomers or apo C) were not observed in the platelet-bound 125I-HDL. The increase in the apo AI content of the platelet-bound 125I-HDL ligand was observed in two separate analyses with different preparations of HDL. Thus, the platelet-bound lipoproteins were authentic HDL and LDL, and a selection of HDL containing only apo AI may occur in binding to the platelet. Because all HDL particles contain apo AI and all LDL particles contain apo B, these observations are consistent with the studies above indicating that platelet binding was a function of a major rather than a minor component of the labeled ligands.

In separate experiments, the acid precipitability of the extracted radioactivity in 10% phosphotungstic acid was assessed. For 125I-HDL, the platelet-bound radioactivity was 85.3% precipitable compared to 93.0% precipitation of 125I-HDL incubated at 37 °C for two hours in the absence of platelets. For 125I-LDL, 88.2% of the ligand and 90.3% of the platelet-bound radioactivity were precipitable. Thus, protein-associated radioactivity accounted for at least 85% of the bound radioactivity, and no apparent degradation of the apoprotein chains of the platelet-associated lipoproteins was detected during the 120-minute incubation at 37 °C.

As a fourth approach to validate specificity, the capacity of the nonlabeled lipoproteins and unrelated proteins to inhibit the platelet interaction of 125I-HDL and 125I-LDL was assessed. As shown in Table 1, nonlabeled HDL and LDL inhibited the binding of the homologous radiolabeled ligands by greater than 90%, which confirmed previous estimates of a nonspecific binding of ≤10%. In six experiments using at least two different preparations of each labeled ligand, the nonspecific binding (residual binding in the presence of excess nonlabeled lipoproteins) was 6.4% ± 1.5% and 5.7% ± 3.4% for 125I-HDL and 125I-LDL, respectively. Nonlabeled LDL and HDL also inhibited the binding of 125I-HDL and 125I-LDL, respectively, and this cross-competition will be considered in detail later. However, a tenfold molar excess of unrelated proteins produced no inhibition of the binding of either ligand (Table 1). It was noted that 125I-LDL binding was inhibited 33% by a 50-fold molar excess of ovalbumin, but not the other proteins.

To establish optimal conditions for interaction of the lipoproteins with the platelets, the effects of divalent ions and platelet stimulation on ligand binding were evaluated. Previous interactions had been measured in the absence of added divalent cations. The observation that 3 mmol/L EDTA did not affect the binding of either 125I-HDL or 125I-LDL verified divalent ion independence of the interactions (Table 2). Moreover, calcium and magnesium inhibited the binding of both HDL and LDL in a dose-dependent fashion. At a 1 mmol/L concentration of calcium, specific 125I-LDL binding was inhibited 100%. The inhibition of specific 125I-HDL binding was less extensive and did not exceed 59%, even at concentrations of 2 mmol/L calcium. The residual binding of 125I-HDL in the presence of calcium or magnesium was specific, as inclusion of a 100-fold excess of nonlabeled HDL in the reaction mixtures inhibited the total ligand binding by ≥95% in the presence or absence of the divalent ions.

### Table 1. Specificity of HDL and LDL Binding to Human Platelets

<table>
<thead>
<tr>
<th>Competitor</th>
<th>125I-HDL</th>
<th>125I-LDL</th>
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<tbody>
<tr>
<td>None</td>
<td>471 ± 65</td>
<td>3,587 ± 140</td>
</tr>
<tr>
<td>LDL</td>
<td>101 ± 25</td>
<td>175 ± 26</td>
</tr>
<tr>
<td>HDL</td>
<td>48 ± 6</td>
<td>191 ± 13</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>543 ± 85</td>
<td>3,616 ± 250</td>
</tr>
<tr>
<td>Transferrin</td>
<td>487 ± 15</td>
<td>3,596 ± 114</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>432 ± 50</td>
<td>3,630 ± 341</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>ND</td>
<td>3,575 ± 226</td>
</tr>
<tr>
<td>Soybean trypsin</td>
<td>ND</td>
<td>3,608 ± 157</td>
</tr>
<tr>
<td>RNAase</td>
<td>ND</td>
<td>3,625 ± 121</td>
</tr>
</tbody>
</table>

125I-HDL or 125I-LDL, at concentrations of 7.5 or 15.0 μg/mL, respectively, were added to a platelet suspension (10⁶ cells/mL) containing the indicated competing proteins. Each competitor was added at a 10 molar excess, relative to the final concentration of the radiolabeled ligand. Binding was measured after a two-hour incubation at 37 °C. Results are the mean of triplicate determinations. ND, not determined.

### Table 2. Effect of Divalent Ions on LDL and HDL Binding to Human Platelets

<table>
<thead>
<tr>
<th>Addition</th>
<th>125I-HDL</th>
<th>125I-LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>590 ± 12</td>
<td>2,400 ± 40</td>
</tr>
<tr>
<td>EDTA (3 mmol/L)</td>
<td>550 ± 30</td>
<td>2,670 ± 280</td>
</tr>
<tr>
<td>Calcium (2 mmol/L)</td>
<td>259 ± 26</td>
<td>ND</td>
</tr>
<tr>
<td>Calcium (1 mmol/L)</td>
<td>290 ± 90</td>
<td>130 ± 3</td>
</tr>
<tr>
<td>Calcium (0.01 mmol/L)</td>
<td>500 ± 10</td>
<td>1,320 ± 78</td>
</tr>
<tr>
<td>Magnesium (1 mmol/L)</td>
<td>280 ± 15</td>
<td>380 ± 10</td>
</tr>
<tr>
<td>Magnesium (0.01 mmol/L)</td>
<td>416 ± 25</td>
<td>2,170 ± 85</td>
</tr>
</tbody>
</table>

125I-HDL or 125I-LDL, at final concentrations of 7.5 or 15.0 μg/mL, respectively, were added to a platelet suspension (10⁶ cells/mL) in Tyrode's albumin buffer, pH 7.2, containing the indicated divalent ion (chloride salts) or chelating agent. Binding was measured after two hours at 37 °C, and results are the mean of triplicate determinations. Nonspecific binding in the presence of a 50-fold excess of cold ligand was 28 ± 3 and 133 ± 7 ng/10⁶ platelets for HDL and LDL, respectively. Specific binding, as used in the text, was defined as the total binding minus the nonspecific binding. ND, not determined.
inhibitory patterns for both $^{125}$I-LDL and $^{125}$I-HDL binding also were obtained when sulfates rather than the chloride salts of the divalent ions were utilized. Evidence presented in Table 3 indicated that interaction of the lipoproteins with the platelet was also independent of platelet stimulation. ADP and thrombin were utilized as representative platelet stimuli, and, to insure the absence of stimulation during the binding reaction, a combination of prostaglandin E₁ (PGE₁) and theophylline was utilized. These stimuli and inhibitors had little effect on the quantitative binding of either $^{125}$I-HDL or $^{125}$I-LDL. The greatest change was noted with LDL binding in the presence of thrombin, and this represented only a 14% increase in the total nanograms bound compared to the nonstimulated cells. In separate experiments, we verified that the kinetics of ligand binding were not altered by these stimuli and inhibitors.

Utilizing nonstimulated platelets in the absence of divalent ions and a two-hour incubation at 37 °C, the capacity to saturate the platelet binding sites for the lipoproteins was assessed. In these experiments, increasing concentrations of radiolabeled $^{125}$I-HDL or $^{125}$I-LDL were added to the platelets in the absence or presence of a 50-fold excess of the homologous nonlabeled lipoprotein. Residual binding of the radiolabeled ligands in the presence of excess nonlabeled lipoprotein was defined as being “nonspecific” and was subtracted from the binding in the absence of nonlabeled ligand to yield the “specific” binding. The specific binding isotherm for $^{125}$I-HDL is shown in Fig 4A. When the concentrations of $^{125}$I-HDL added exceeded 15 μg/mL, saturation was attained. Scatchard analysis of the data (Fig 4B) could be interpreted in terms of a capacity to saturate the platelet binding sites for the divalent ions and a two-hour incubation at 37 °C, the straight line with a correlation coefficient of $r = .93$.

Assuming a mean particle protein molecular weight of $2.5 \times 10^7$ for HDL, duplicate experiments with two preparations of $^{125}$I-HDL yielded a $K_d$ of $3.1 \pm 2.8 \times 10^{-4}$ mol/L with 1,585 ± 390 HDL particles bound per platelet. In addition, the data from a single experiment were subjected to nonlinear curve-fitting computer analysis. The data were accommodated reasonably by a one-site, but not a two-site, model, and similar values for $K_d$ and receptor number were derived. The results of the analysis with $^{125}$I-LDL are shown in Fig 5. The specific binding isotherm (Fig 5A) suggested saturable binding, although it was clearly less definitive than with $^{125}$I-HDL. Nevertheless, the data shown could be reasonably accommodated by a linear Scatchard plot (Fig 5B), with a linear correlation coefficient of $r = .85$. With a particle protein molecular weight of 5 x 10⁸, the $K_d$ derived from two separate determinations was $4.0 \pm 1.8 \times 10^{-8}$ mol/L with 7,075 ± 4,800 particles bound per platelet. Curve-fitting analysis of these data yielded similar binding parameters in a one-site model. Experimentally, we attempted to extend the binding isotherm in the range of 50 to 200 μg/mL of added $^{125}$I-LDL. At 100 μg/mL $^{125}$I-LDL, the total binding increased 3.5-fold compared to 50 μg/mL, and the “nonspecific” binding in the presence of 2 mg/mL nonlabeled LDL increased 15-fold. These observations suggest the presence of either a very high-capacity, low-affinity LDL binding site or ligand self-association. Attempts to analyze these data in either a one-site or two-site model were unsuccessful, probably because the nonspecific binding was not a constant percent of ligand input.

As previously noted in Table 1, the binding of each

Table 3. Effect of Platelet Stimulation on HDL and LDL Binding to Human Platelets

<table>
<thead>
<tr>
<th>Addition</th>
<th>Total ng Bound/10⁹ Platelets</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>$^{125}$I-HDL</td>
</tr>
<tr>
<td>None</td>
<td>504 ± 15</td>
</tr>
<tr>
<td>ADP (10 μmol/L)</td>
<td>510 ± 5</td>
</tr>
<tr>
<td>Thrombin (2 U/mL)</td>
<td>499 ± 22</td>
</tr>
<tr>
<td>PGE₁ (1 μg/mL)</td>
<td>518 ± 14</td>
</tr>
</tbody>
</table>

+ theophylline (1 μmol/L) | 518 ± 14 | 1,950 ± 35 |

Stimuli and inhibitors were added to platelets (10⁹/mL) in divalent ion-free Tyrode’s albumin buffer, pH 7.2, followed immediately by addition of either $^{125}$I-HDL (7.5 μg/mL) or $^{125}$I-LDL (15.0 μg/mL). Binding was measured in triplicate after two hours at 37 °C. Nonspecific binding in the presence of a 20-fold molar excess of cold ligand was 38 ± 12 and 162 ± 14 ng/10⁹ platelets for HDL and LDL, respectively.

![Fig 4. Concentration-dependent binding of $^{125}$I-HDL to platelets. (A) Platelets at a final concentration of 5 x 10⁷/mL were incubated at 37 °C for 120 minutes with increasing concentrations of $^{125}$I-HDL. Data have been corrected for nonspecific binding. (B) Scatchard plot of the data shown in A. “Bound/Free” is the $^{125}$I-HDL bound (ng/10⁹ platelets)/ng $^{125}$I-HDL added.](image-url)
radiolabeled lipoprotein was inhibited by both nonlabeled lipoproteins. This phenomenon was further investigated by examining the effects of increasing concentrations of the nonlabeled lipoproteins on the binding of each ligand. In preliminary experiments, it was established that concentrations of nonlabeled LDL and HDL as high as 1 mg/mL did not cause platelet lysis during a two-hour incubation at 37 °C, as assessed by the recovery of 51Cr-labeled platelets. The effects of varying concentrations of HDL and LDL on 125I-HDL binding are shown in Fig 6A. As anticipated, nonlabeled HDL effectively inhibited the binding of radiolabeled HDL. The concentration of nonlabeled HDL required for 50% competition was 5.7 μg/mL (2.3 x 10^{-8} mol/L) and was in reasonable agreement with the K_d for the binding of 125I-HDL. Nonlabeled LDL was only inhibitory at very high concentrations. An LDL concentration of 200 μg/mL (4 x 10^{-7} mol/L) was required for 50% inhibition of 125I-HDL binding; no competition was observed with LDL at 50 μg/mL, a concentration that approached apparent saturation of the measurable LDL binding sites on the platelet (see Fig 5A). The effect of the higher LDL doses on 125I-HDL binding could reflect occupancy of a second low-affinity LDL binding system or nonspecific effects. With 125I-LDL as the ligand (Fig 6B), nonlabeled LDL produced 50% inhibition at 16 μg/mL (3 x 10^{-8} mol/L), and this also was consistent with the K_d of the interaction derived from Scatchard analyses. However, HDL not only produced complete inhibition, but also was more effective than LDL in inhibiting the binding of 125I-LDL. A concentration of 2 μg/mL (0.8 x 10^{-8} mol/L) HDL was required for 50% inhibition, and this was fourfold less than required with nonlabeled LDL.

On cells such as fibroblasts, the binding of HDL to the LDL receptor is dependent on its apo E content. To determine if the capacity of HDL to bind to platelets or to compete with 125I-LDL binding was dependent on its apo E component, apo E-free HDL was prepared by heparin-agarose absorption. A control sample was generated in parallel by treating the same HDL preparation with unsubstituted agarose beads. The apoprotein A1, B, C1, and E content of sample was quantitated by solid-phase radioimmunoassays with monoclonal antibodies to each apoprotein (Table 4). No apo E was detected in the heparin-agarose-adsorbed HDL, representing a reduction of at least 97% in apo E. The low content of apo B present in the HDL preparation was also reduced by the heparin-agarose absorption, whereas the apo A1 and C1 content were unaltered. Each of the HDL preparations was then tested for its capacity to inhibit the binding of 125I-HDL or 125I-LDL to the platelet. The concentrations required for 50% inhibition are summarized in Table 5. With 125I-HDL (untreated) as the ligand, similar concentrations of HDL absorbed with heparin-agarose or agarose were required for 50% inhibition. These preparations also inhibited 125I-LDL binding at
similar concentrations. Thus, HDL binding or its capacity to inhibit LDL binding was independent of its apo E content.

**DISCUSSION**

The abnormalities in platelet composition and function in patients with hyperlipoproteinemia suggest that the circulating levels of lipoproteins influence the properties of the platelet. On this basis, we have sought to evaluate the direct interaction of plasma lipoproteins with the platelet utilizing LDL and HDL as prototype lipoprotein particles. When radiiodinated, each of the lipoprotein ligands bound to the platelets in a time-dependent and temperature-dependent interaction. The specificity of the interaction for lipoproteins was established by the observations that: (a) labeled and nonlabeled lipoproteins interacted with the platelet with the same apparent affinities; (b) a constant proportion of the labeled ligands was bound in sequential binding assays; (c) the platelet-bound HDL and LDL resembled the added lipoprotein in apoprotein composition; and (d) the interaction was inhibited by nonlabeled lipoproteins, but not by unrelated proteins or glycoproteins. Characteristics of the interaction included: (a) a marked temperature dependence; (b) a lack of divalent ion dependence; (c) independence of the state of platelet stimulation; and (d) absence of extensive apoprotein degradation during a two-hour incubation. At 37°C in the absence of divalent ions and platelet stimulation, a saturable component of both HDL and LDL could be demonstrated, indicating the presence of a discrete number of binding sites for these lipoproteins on the platelet. Scatchard analyses of the specific binding isotherms for either HDL or LDL could be interpreted in terms of a single class of binding site with respect to affinity. The dissociation constants for the binding of HDL and LDL to the platelets were similar (3 to 4 x 10⁻⁸ mol/L), but the capacity for LDL was 4.5-fold higher. The existence of an additional high-capacity, low-affinity LDL binding site could not be experimentally excluded.

To consider the relationship between HDL and LDL binding, the capacity of nonlabeled lipoproteins to inhibit binding of each radiolabeled ligand was assessed. With ¹²⁵I-HDL, inhibition was observed with both HDL and LDL; however, LDL was a much poorer inhibitor (40-fold) and at least 100 μg/mL LDL was required for significant inhibition of ¹²⁵I-HDL binding. From the specific binding isotherms of ¹²⁵I-LDL, concentrations of 20 to 40 μg/mL LDL saturated the high-affinity platelet binding sites for this lipoprotein. This implied that the inhibition of ¹²⁵I-HDL binding by LDL was not due to the interaction of LDL with this site on the platelet. Inhibition by LDL must, therefore, arise from nonspecific or indirect effects or from a lower affinity interaction with the platelet. On this basis, it is proposed that the platelet possesses a high-affinity binding site for HDL that does not bind LDL or binds LDL very poorly. HDL receptors have been identified on steroidogenic tissues, such as testes, ovaries, and adrenals. These receptors bind apo E-free HDL with high affinity in the absence of divalent ions, do not bind LDL, do not necessarily facilitate the degradation of the apoprotein chains of HDL, and have a suggested specificity for apo A-containing HDL particles. These characteristics, including the capacity to bind apo E-free HDL and a selective specificity for apo AI-containing HDL, suggest that the platelet binding sites for HDL share many of the characteristics of the HDL receptors expressed by steroidogenic cells. However, only direct binding studies utilizing isolated apo AI will confirm this hypothesis.

Platelet binding of ¹²⁵I-LDL was effectively inhibited by both HDL and LDL. Such cross-inhibition is characteristic of the LDL receptor of fibroblasts, which binds apo E-containing HDL and apo B-containing LDL.¹⁵ The platelet binding site for LDL did not, however, appear to recognize the apo E components of HDL. The temperature and divalent ion sensitivity of LDL binding further distinguished...
the platelet binding site from the LDL receptor on extrahepatic cells. Based on these properties, LDL binding to platelets may be dependent on either the lipid or the apoprotein constituents of the lipoprotein. Inhibition of LDL binding by HDL also may reflect recognition of common nonapoprotein components of the particles, as lipoprotein receptors with specificities for both LDL and apo E free-HDL have been reported, and these receptors appear to interact with phospholipids as well as apoproteins. In addition, the data of Koller et al suggest that such inhibition may be of a more complex variety than simple competition for the same binding site.

In sum, our data suggest the existence of two binding sites for lipoproteins on platelets: an HDL binding site that interacts poorly with LDL, and an LDL binding site that either reacts well with HDL or is altered by HDL binding. The postulation of distinct HDL and LDL binding sites is consistent with the conclusions of Koller et al. In fact, the affinity and number of HDL and LDL particles bound per platelet measured by these investigators and in our study are quite similar (LDL, $K_a = 6.2 \times 10^7$ L/mol and 1,470 molecules/platelet compared to our data of $K_a = 2.5 \times 10^7$ L/mol and 7,075; HDL, $K_a = 9 \times 10^7$ L/mol and 3,200 molecules/platelet compared to our data of $K_a = 3.2 \times 10^7$ L/mol and 1,585 molecules/platelet). Nevertheless, certain characteristics of the interactions are quite distinct in the two studies. Specifically, we found that LDL was a poor inhibitor of HDL binding and that the binding of both ligands reached a rapid equilibrium (15 minutes), was temperature independent, and occurred in the presence of divalent ions. Our data, as well as that of Koller et al, contrast considerably with the observations of Aviram and Brooks, who found that $^{125}$I-LDL binding was not affected by HDL as well as in a number of other major parameters. Further studies are required to resolve the basis for these differences.

The role of these platelet binding sites in the maintenance or alteration of platelet function by lipoproteins is unknown. Presumably, such interactions initiate the exchange or transfer of lipids between the lipoproteins and the platelet, and this could result in either the loss of platelet lipid or the utilization and processing by the cells of lipid constituents of the lipoproteins, such as those recently discussed by Glass et al. In the system utilized in this study, physiologic concentrations of divalent ions inhibited high-affinity LDL binding to the platelet. However, this does not exclude an influence of LDL on platelet cholesterol content through a collisional exchange mechanism. In addition, a significant component (~50%) of HDL binding was not inhibited by calcium or magnesium. Therefore, from a physiologic perspective, there is good reason to examine in detail the high-affinity, EDTA-sensitive interaction of HDL with platelets and to assess the consequences of this interaction on platelet lipid composition and function.

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LK Curtiss and EF Plow