Monoclonal Antibodies to Human Plasma Low-Density Lipoproteins. I. Enhanced Binding of $^{125}$I-Labeled Low-Density Lipoproteins by Combined Use of Two Monoclonal Antibodies

Simon J. T. Mao, James G. Patton, Juan-José Badimon, Bruce A. Knutte, Michael C. Alley, and Alan D. Cardin

Four monoclonal antibodies (IgG2b) to human plasma low-density lipoproteins (LDL) have been characterized. The binding affinities of each monoclonal antibody to $^{125}$I-labeled LDL were moderately high, ranging from $10^5$ to $10^{10}$ L/mol at 4°C, but were reduced by at least 50–70% at 37°C. The maximum binding of each monoclonal antibody was unique, ranging from 20 to 95% of total $^{125}$I-labeled LDL, suggesting that LDL particles were immunologically heterogeneous. One antibody, LP-34, had both high and low binding affinities to LDL. Another, LP-47, exhibited high affinity for isolated LDL, yet reacted poorly with native LDL in plasma, indicating that the conformation of isolated LDL differs from that of native LDL in plasma. Unlike polyclonal serum antibodies, a mixture of four monoclonal antibodies failed to precipitate LDL, but did show a drastic increase in binding to LDL. We found that only two of our monoclonal antibodies were necessary for such synergistic enhancement. We propose that one of the monoclonal antibodies may serve as a catalytic reagent, and discuss the clinical significance of this finding.

Additional Keyphrases: apolipoprotein · radioimmunoassay · antibody binding affinity · antigen–antibody interaction

A major advance in molecular immunology and biochemistry has been the production of homogeneous monoclonal antibodies (1, 2). Fusion techniques (3) and potential uses of monoclonal antibodies (4, 5) have recently been reviewed. Human plasma low-density lipoproteins (LDL) are the vehicles for the transport and uptake of cholesterol. Brown and Goldstein have shown that LDL are bound by high-affinity receptors on cell membranes, internalized via endocytosis, and degraded in lysosomes (6, 7).

LDL are heterogeneous in size and chemical composition (8–13). Recently, Kane et al. (11) reported the presence of at least four major molecular forms of apolipoprotein (apo) B in circulating plasma and noted that LDL contain three of these forms: B-100, B-74, and B-26. Another study (14) also suggests that another apo B (B-48), a major component of chylomicrons, is not a precursor of apo B in LDL. Because the difference in the amino acid composition of each apo B species is slight, monoclonal antibodies will probably be necessary to distinguish such subtle changes in an antigenic determinant (4).

The purpose of the present study is to explore the interaction between LDL and their monoclonal antibodies. We have found that the stability of the antigenic structure of LDL differs under various temperature conditions (15). Understanding the binding properties of LDL to antibodies should allow us to map the antigenic sites, develop immunoassays for apo B, and probe the structure and metabolism of LDL. Recent studies have implicated overproduction of LDL as one of the factors causing hypercholesterolemia (16, 17), and a technique to decrease the concentrations of plasma LDL by using anti-LDL antibody affinity columns has been reported (18). Our finding of the enhancement of the binding of LDL by the combined use of two monoclonal antibodies could provide a clinical aspect to the selection of monoclonal antibodies for affinity column preparations.

Materials and Methods

Preparative Procedures

*Production of monoclonal antibodies.* The preparation, IgG subclass, and affinity of the L1,G2,F2 monoclonal antibody have been described previously (15, 19). Production of three new hybridoma lines against LDL (LP-22, LP-34, and LP-47) have followed similar techniques. In general, all hybridomas were prepared by fusing spleen cells of immunized female Balb/c mice with a nonsecreting myeloma cell line (FO) (3). To screen for antibody production, we incubated 50–100 μL of the culture media from each well with 20 000 dpm of $^{125}$I-labeled LDL ($^{125}$I-LDL, specific activity ~2 Ci/g) and equilibrated this mixture in a radioimmunoassay buffer containing, per liter, 10 g of bovine serum albumin, 0.1 mol of sodium borate, 1 mmol of Na2EDTA, and 0.1 g of Na2SO4 (pH 8.5). After incubation at 4°C for 16 h, we added 100 μL of staphylococcus containing protein A (IgG SORB; The Enzyme Center, Inc., Boston, MA 02111) to separate unbound $^{125}$I-LDL. The mixture was incubated at 4°C for 10–30 min, washed with 2 mL of radioimmunoassay buffer, centrifuged, and the radioactivity counted. Wells containing antibody activity were then cloned and subcloned by limiting dilution (2, 15, 19) and further expanded in tissue culture flasks.

*Preparation and characterization of monoclonal antibodies.* We fractionated 100 mL of the media containing monoclonal antibodies by precipitation with 100 mL of saturated ammonium sulfate, then dissolved the precipitate in 100 mL of 0.15 mol/L NaCl solution; this procedure was repeated five times. Finally, we reconstituted the precipitate in 20 mL of 0.15 mol/L NaCl solution and dialyzed this against 16 L of a radioimmunoassay buffer but without the albumin. The immunoglobulin class of each antibody was determined and confirmed by immuno-electrophoretic and immunodiffusion techniques as IgG2b subclass by using rabbit antibodies specific to mouse IgG1, IgG2a, IgG2b, and IgG3 (15). Because the LP-34 monoclonal antibody gave a nonlinear Scatchard plot analysis, we further purified the antibody by diethylaminoethyl-cellulose column chromatography (20).
and by LDL-Sepharose affinity column chromatography (21). The purified LP-34 monoclonal antibody showed a single band on polyacrylamide gel electrophoresis in sodium dodecyl sulfate (22), with a relative molecular mass of ~120,000–150,000. It also showed one major band on iso-electric focusing gel electrophoresis. The purified LP-34 monoclonal antibody gave a Scatchard plot almost identical to that obtained with ammonium sulfate-fractionated antibody.

Isolation and iodination of LDL. Plasma LDL (d 1.019–1.063) were obtained by sequential ultracentrifugation (23) in a Beckman 60 Ti rotor (Beckman Instruments, Inc., Palo Alto, CA 94304) as previously described (15). We added EDTA, 1 mmol/L, to all solutions to prevent hydroperoxidative degradation of lipoproteins. The isolated LDL were refloated at d 1.063 to remove excess plasma albumin and immediately dialyzed against 8 L of a standard buffer containing, per liter, 10 mmol of Tris HCl, 0.1 mol of NaCl, 1 mmol of EDTA, and 0.1 g of NaN₃ (pH 7.4). The protein content of the LDL, determined by a modification of Lowry's method in the presence of sodium dodecyl sulfate (24) with bovine serum albumin as the standard, was in close agreement with the value obtained by amino acid analysis. LDL were radioiodinated with Na¹²⁵I according to a modification of McFarlane's procedure (25) as previously described (15). In brief, we iodinated 250 μg of LDL (in 10 μL of 1 mL/L glycine buffer containing 1 mmol of EDTA per liter, pH 10.0) with 1 mCi of Na¹²⁵I (Amersham Corp., Arlington Heights, IL 60005) for 30 s. Unbound Na¹²⁵I was fractionated on a Bio-Gel P-2 column and the ¹²⁵I-LDL were then exhaustively dialyzed against 8 L of a standard buffer. The final specific activity of ¹²⁵I-LDL used for the direct binding assays and radioimmunoassays was ~2.2 Ci/g. Approximately 90 ± 2% of the dialyzed ¹²⁵I-LDL was precipitable by trichloroacetic acid (200 g/L), whereas only 6% of the radioactivity remained in the liquid moiety, as judged by ethanol/ether extraction (1/3 by vol). More than 95% of the ¹²⁵I-LDL was contained within a single peak when co-eluted with unlabeled LDL on a Bio-Gel A-15 M column (26). At least 95% of the ¹²⁵I-LDL was bound by conventional mouse serum anti-LDL (present in excess). We also iodinated LDL with unlabeled NaI under the same conditions described above. The unlabeled iodinated LDL competed equally with isolated LDL in standard displacement curves of the LDL radioimmunoassay. In addition, the ¹²⁵I-LDL and isolated LDL competed equally for binding to the antibody as assessed by the maximal binding at various dilutions. Thus, iodination did not alter the antigenic structure of LDL.

Preparation of conventional serum antibodies. Anti-LDL antiserum was obtained from adult female Balb/c mice (Jackson Laboratories, Bar Harbor, ME 04609) as previously described (15).

Analytical Procedures

Direct binding assay of ¹²⁵I-LDL to monoclonal antibodies and Scatchard plot analysis. For binding assays, we incubated 100 μL of ¹²⁵I-LDL (~20,000 dpm, containing about 10 ng of protein) with 100 μL of monoclonal or polyclonal antibodies at different dilutions in radioimmunoassay buffer and at different temperatures (4, 24, and 37 °C) for 18–20 h. Maximal binding was achieved usually with less than 8 h of incubation, whereas polyclonal serum antibodies required 14 h. To stop the reaction, we added 100 μL of IgG SORB (27) that had been preincubated at 4, 24, and 37 °C, respectively. After 10–30 min of incubation, the assay tubes were washed with 2 mL of standard radioimmunoassay buffer at 4 °C, and bound ¹²⁵I-LDL were pelleted by centrifugation at 2000 × g for 30 min at 4 °C.

Similar techniques were used for Scatchard plot analyses (28). Bound LDL were determined by incubation of increasing doses of unlabeled LDL in the presence of ¹²⁵I-LDL (15). The reaction mixture contained 100 μL of ¹²⁵I-LDL, 100 μL of unlabeled LDL (ranging from 7 to 7500 ng), and 100 μL of antibody, and was incubated for 20–24 h (15).

To study the binding response of monoclonal antibodies to ¹²⁵I-LDL prepared from normal men, we isolated LDL from fresh plasma collected from five healthy male volunteers into EDTA, 1 g/L. Lipid profiles and ages of each individual are given in Table 1. The efficiency of radio labeling for the LDL from the five normal subjects was 50–60% and the final specific activity was 2.2 (SD 0.25) Ci/g. No significant differences among the final specific activities of each individual's LDL were noted.

For calculations, the percent bound of total ¹²⁵I-LDL is expressed by B/B₀, where B₀ is the total ¹²⁵I-LDL bound to antibodies minus nonspecific binding, and T is the total ¹²⁵I-LDL used in each assay. All the assays were performed in triplicate.

Radioimmunoassay. We used 12 × 75 mm polystyrene disposable culture tubes (Lancer; American Scientific Products, McGaw Park, IL 60086). Conventional antibodies to LDL were titered (1:2000–1:4000) so as to bind ~70% of the total ¹²⁵I-LDL. Because the monoclonal antibodies (LP-22 and LP-47) did not bind 100% of the total ¹²⁵I-LDL, they were titered so as to bind ~50% of the maximal binding. Monoclonal antibodies LP-22, LP-34, and LP-47 were diluted 640-, 80-, and 640-fold, respectively. The assay included two steps (15, 27). First, we added 100 μL of antibodies to 100 μL of ¹²⁵I-LDL containing ~10 ng of LDL (20,000–22,000 dpm) and 100 μL of unlabeled LDL or diluted plasma and allowed the reaction to proceed at 4 °C for 15–24 h. Second, we added 100 μL of IgG SORB to all tubes to separate unbound ¹²⁵I-LDL as described above in the direct binding assay. To evaluate nonspecific binding, we used either normal hybridoma media or nonimmune mouse serum; typical nonspecific binding was 2–3% of the total ¹²⁵I-LDL added.

For calculations, the percent bound = (B/B₀)/100, where B is the total bound ¹²⁵I-LDL in the presence of unlabeled LDL standards or plasma minus nonspecific binding, and B₀ is the total bound ¹²⁵I-LDL in the absence of unlabeled LDL or plasma minus the nonspecific binding.

Lipopysis of very-low-density lipoproteins and its effect on their immunoreactivity as determined by radioimmunoassay. In a lipolysis experiment, performed as previously described (29), VLDL obtained from normal subjects were adjusted to

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Table 1. Lipid and Protein Composition of Plasma and LDL Prepared from Five Normal Men

<table>
<thead>
<tr>
<th>Age, yrs</th>
<th>Cholesterol, g/L</th>
<th>HDL-chol, mg/dL</th>
<th>Triglycerides, mg/dL</th>
<th>Phospholipids, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>1.47</td>
<td>0.40</td>
<td>0.57</td>
<td>1.46</td>
</tr>
<tr>
<td>24</td>
<td>1.24</td>
<td>0.36</td>
<td>0.52</td>
<td>1.48</td>
</tr>
<tr>
<td>30</td>
<td>1.21</td>
<td>0.43</td>
<td>0.42</td>
<td>1.37</td>
</tr>
<tr>
<td>34</td>
<td>1.39</td>
<td>0.52</td>
<td>0.58</td>
<td>1.51</td>
</tr>
<tr>
<td>35</td>
<td>1.71</td>
<td>0.44</td>
<td>0.79</td>
<td>1.64</td>
</tr>
</tbody>
</table>

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a triglyceride concentration of 1 g/L (apolipoprotein concentration of 0.136 g/L). To 100 μL of VLDL substrate (containing 50 g of bovine serum albumin per liter), we added various amounts (10–100 μL) of bovine milk lipoprotein lipase (EC 3.1.1.3), 10 mg/L, and incubated the mixture, in a final volume of 300 μL, at 37 °C for 60 min. VLDL samples without lipase treatment were also incubated under the same conditions. We used [14C]triolein to monitor the degree of triglyceride hydrolysis. To detect apo B immunoreactivity, we included VLDL containing 200 ng of apolipoproteins so that the VLDL dose would displace ~50% of [125I]-LDL from monoclonal antibodies; the rest of the radioimmunoassay was as described above. Lipoprotein lipase did not interfere with the binding of [125I]-LDL to monoclonal antibodies nor was the standard displacement curve affected. These findings were confirmed by independent observations in the absence of VLDL as a comparator in the assays.

For calculations, the percent immunoreactivity or the percent inhibition = 100 – [(B/B0) × 100], where B is the total bound [125I]-LDL in the presence of unlabeled VLDL (in the lipase-treated or untreated sample), and B0 is the total bound [125I]-LDL in the absence of VLDL. Nonspecific binding was subtracted before all calculations.

Lipid analyses. Triglyceride content of VLDL and plasma was measured by an enzymic kit method (Dow Chemical Co., Indianapolis, IN 46268) as previously described (30). Cholesterol and phospholipid contents were determined by a Beckman enzymic kit (Beckman Instruments, Inc., Carlsbad, CA 92008) procedure and a commercial enzymic kit (Nippon Shoji Kaisha, Ltd., Osaka, Japan) procedure (31), respectively.

Results

Binding Properties of [125I]-LDL to Monoclonal Antibodies

Previously we have reported that the binding of L1C6F2 monoclonal antibody to LDL is thermal dependent (15). We have now investigated three more monoclonal antibodies, obtained independently. For all the monoclonal antibodies the binding with [125I]-LDL was temperature dependent, being optimal at 4 °C (Figure 1). Only monoclonal antibody LP-47 bound [125I]-LDL to the same extent at both 4 °C and 24 °C.

The degree of maximal binding (B0/T) of each monoclonal antibody to [125I]-LDL was different (Figure 1, Table 2), which suggests that LDL are immunochemically heterogeneous and that the antigenic structures present in each LDL particle are not identical. Polyclonal serum antibodies, on the other hand, bound 95% of the [125I]-LDL (Figure 1) and this binding was not temperature dependent. Thus, the heterogeneous nature of LDL is not readily apparent unless highly specific monoclonal antibodies are used for binding experiments.

Scatchard Plot Analyses

In theory, for a given polypeptide antigen, each monoclonal antibody can recognize only one unique antigenic determinant (1). Scatchard plots (28) permit verification of the concept of single epitope–single affinity (32). As Figure 2 shows, a single-affinity linear plot was obtained with monoclonal antibodies LP-22 and LP-47 but not LP-34. This suggests that LDL possess both high- and low-binding-
affinity sites to monoclonal antibody LP-34, and that the antigen determinants (for LP-34 monoclonal antibody) are probably not uniformly exposed on LDL particles. The binding characteristics and affinities between 125I-LDL and monoclonal antibodies are given in Table 2; because the Scatchard plot (Figure 2) was not linear, we calculated the binding affinity by extrapolating from the average value of the curve. The monoclonal antibody with the highest affinity (7.5 × 10^9 L/mol) at 4°C was LP-34; as the temperature increased, the binding affinity decreased by at least 70%. The polyclonal antibodies, on the other hand, bound LDL with an affinity (4.8 × 10^10 L/mol) one to two orders of magnitude greater than monoclonal antibodies and were not significantly altered by temperatures from 4°C to 37°C.

Binding Response of Monoclonal Antibodies to 125I-LDL Prepared from Normal Men

The maximum binding of 125I-LDL to each monoclonal antibody being different, we decided to study whether LDL isolated from different normal individuals would yield different binding properties to each clone. We isolated LDL from five normal men, radiiodinated the samples, and used them in binding assays. The plasma and LDL lipid and protein composition for each subject are listed (Table 1). For the labeled LDL from each normal individual the binding pattern to a given monoclonal antibody was similar at 4°C (data not shown).

Immunoreactivity of Apo B in VLDL after Lipolysis

After hydrolysis of VLDL triglycerides with bovine milk lipase, we determined the immunoreactivity of apo B by inhibition radioimmunoassay. Increasing the degree of lipolysis decreased the immunoreactivity of apo B (Table 3) for all of the monoclonal antibodies tested. Lipid extracts from VLDL, LDL, or a sonicated triglyceride emulsion did not compete with 125I-LDL for binding to the monoclonal antibodies.

Enhancement of 125I-LDL Binding to Monoclonal Antibodies by Another Monoclonal Antibody

The antigen determinant of a protein antigen is relatively small (33, 34), and monoclonal antibodies, unlike serum antibodies, recognize only a single antigenic site. Immunoprecipitation by a monoclonal antibody is usually not possible (15). Thus, we attempted to mix our four monoclonal antibodies to create artificial "polyclonal" serum antibodies to see if they could be used as a precipitating reagent. The mixture failed to precipitate LDL, but the binding of 125I-LDL to the mixed monoclonal antibodies increased greatly (Table 4).

In theory, the mixture of 25 μL of each monoclonal antibody (final vol, 100 μL) should yield maximally about 800 cpm of bound 125I-LDL. However, the total binding (6378 cpm) was much greater (Table 4), indicating that the artificial polyclonal antibodies enhance the antigen–antibody interaction and may stabilize the antigen–antibody complexes. We also observed this enhancement when we used incubation at 4°C.

To delineate which specific monoclonal antibody might play the crucial role in such enhancement, we tested combinations of two or three selected monoclonal antibodies for their binding affinity to 125I-LDL. Monoclonal antibodies were diluted to bind a small but detectable amount of labeled antigen (~5%). The L.CαF2 monoclonal antibody, although possessing the lowest binding affinity to LDL (5 × 10^9 L/mol), bound 10-fold more LDL in the presence of LP-34 monoclonal antibody than in its absence (Table 4).

We also determined the binding affinities of the various

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**Table 2. Summary of the Characteristics of LDL Monoclonal Antibodies (all IgG2b)**

<table>
<thead>
<tr>
<th>Clone</th>
<th>Scatchard plot</th>
<th>% of maximal binding at 4°C</th>
<th>% of maximal binding at 37°C</th>
<th>% of maximal binding at 10°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.CαF2a</td>
<td>Linear</td>
<td>0.5</td>
<td>0.25</td>
<td>20–30</td>
</tr>
<tr>
<td>LP-22</td>
<td>Linear</td>
<td>4.5</td>
<td>1.2</td>
<td>70</td>
</tr>
<tr>
<td>LP-34</td>
<td>Curvilinear</td>
<td>7.5</td>
<td>1.9</td>
<td>95</td>
</tr>
<tr>
<td>LP-47</td>
<td>Linear</td>
<td>1.3</td>
<td>0.29</td>
<td>55</td>
</tr>
<tr>
<td>Conventional</td>
<td>Curvilineara</td>
<td>48.0</td>
<td>48.0</td>
<td>95</td>
</tr>
</tbody>
</table>

a Data derived from a previous study (19).

b Binding to total 125I-LDL.

c Polyclonal antibodies.

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**Table 3. Effect of Triglyceride Hydrolysis on VLDL Immunoreactivity**

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>% Immunoreactivity* at triglyceride hydrolysis* of 125I-LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.CαF2</td>
<td>52.8</td>
</tr>
<tr>
<td>LP-22</td>
<td>42.5</td>
</tr>
<tr>
<td>LP-34</td>
<td>69.2</td>
</tr>
<tr>
<td>LP-47</td>
<td>47.2</td>
</tr>
</tbody>
</table>

* % inhibition of 125I-LDL (from monoclonal antibodies) by VLDL = 100 – [(B/B0) × 100] (see Materials and Methods).

* The % of hydrolysis listed resulted from the addition of 0, 10, 20, 50, or 100 μL, respectively, of lipoprotein lipase (10 mg/L).
combinations of monoclonal antibodies. The combination of L1C6F2 and LP-34 gave affinities of 4.8 × 10⁹ L/mol at 4°C and 2.0 × 10⁹ L/mol at 37°C. The combination of four monoclonal antibodies (L1C6F2 + LP-22 + LP-34 + LP-47) gave affinities of 6.4 × 10⁹ L/mol and 1.5 × 10⁹ L/mol at 4°C and 37°C, respectively. Although the total binding of the mixtures to 125I-LDL increased significantly at 37°C, the binding affinity still did not reach that attainable by conventional antisemur (4.8 × 10¹⁰ L/mol). It will be interesting to discover whether adding more lines of monoclonal antibodies to the mixtures will produce a combined affinity equal to or greater than that obtained with conventional antisera, or whether it will be possible to generate one LDL monoclonal antibody having an affinity as high as that of polyclonal antibodies without the cooperative binding effects of other monoclonal antibodies.

Decrease of Monoclonal Antibody Binding to 125I-LDL Previously Incubated at 37°C

Possible explanations for the low-binding affinity at 37°C are that increasing the temperature induces (a) a conformational change in the structure of the monoclonal antibodies, (b) a change in the orientation of apo B in LDL (35), (c) a dissociation of the 125I from the LDL particles, or (d) some combination of these possibilities.

Accordingly, we devised the following strategies: (a) preincubating monoclonal antibodies at 37°C followed by reaction with LDL; and (b) preincubating 125I-LDL at 37°C followed by reaction with monoclonal antibodies (combinations of L1C6F2 and LP-34). After each preincubation, we reacted antigen with antibody at 37°C, rather than at 4°C, to avoid any reversible conformational changes of either (18). As shown in Figure 3, preincubation of monoclonal antibodies (combinations of L1C6F2 and LP-34) at 37°C for up to 20 h did not affect the binding. However, preincubating the 125I-LDL significantly decreased the binding. Precipitation of preincubated 125I-LDL by trichloroacetic acid showed no dissociation of 125I molecules from LDL, suggesting that only the antigenic structure of LDL is affected by temperature.

Standard Displacement Curves

Standard displacement curves for unlabeled LDL in radioimmunoassays are shown in Figure 4. The assay is specific for both LDL (Figure 4) and VLDL (Table 3) because apolipoproteins A-I, A-II, C-II, and C-III and high-density lipoproteins (HDL), at doses up to 2000 ng, did not displace 125I-LDL from antibodies. The range of sensitivity was from 50 to 800 ng of added unlabeled LDL but varied with each monoclonal antibody. The immunoreactivity of apo B in plasma essentially paralleled that of the LDL standard. However, plasma (up to 330 nL) failed to displace 125I-LDL from monoclonal antibody LP-47 (Figure 4C), suggesting that one cannot assume that use of a monoclonal antibody as a reagent will replace conventional antibodies in radioimmunoassays to determine concentrations of apo B in plasma. Moreover, the antigenic structure of native LDL in plasma may be different from that of isolated LDL. Some part of native LDL appears to become antigenic either in the process of antibody production in vivo or during the isolation procedure after ultracentrifugation.

Discussion

The monoclonal antibodies to LDL we have produced are fundamentally different from conventional polyclonal antibodies; for example, all four of our lines exhibit thermal-dependent binding to LDL. This thermal dependence is not, however, the result of temperature affecting the binding between monoclonal antibodies (IgG2b) and protein A because the interaction of protein A and IgG is not temperature dependent at 4–37°C (15, 27). Use of a second antibody (rabbit anti-mouse IgG) technique instead of protein A yielded a similar observation (15).

Although the specific antigenic site for each monoclonal antibody has not been investigated, it is unlikely that all the monoclonal antibodies tested recognize the same determinant. Several lines of evidence suggest that each monoclonal antibody is unique: (a) monoclonal antibody LP-47 bound to 125I-LDL equally at 4°C and 24°C; (b) monoclonal antibody LP-34 bound almost 100% of the LDL population with a curvilinear Scatchard plot; (c) monoclonal antibody LP-47 bound 125I-LDL but the binding was only slightly displaced by native LDL in plasma; and (d) monoclonal antibody L1C6F2, the low-affinity antibody that bound only 20–30% of 125I-LDL, drastically enhanced the binding to LDL in the presence of LP-34 monoclonal antibody.

Monoclonal antibody LP-34 recognized all populations of
LDL and produced a curvilinear Scatchard plot (Figure 2), indicating that LDL contain both low- and high-affinity antigenic site(s) for the monoclonal antibodies. The possible mechanism for the high- and low-affinity site(s) may be the heterogeneity of LDL structure and chemical composition (11–16). The molecular orientation of apo B may vary according to its lipid and protein composition (36, 37). Thus, we conclude that Scatchard analyses can also be used to study the homogeneity of the antigenic structure.

Schonfeld et al. (38), using conventional rabbit antibodies to LDL, demonstrated that apo B immunoreactivity to VLDL depended on the extent of lipolysis of VLDL triglycerides, increasing by 10–15% after complete lipolysis. Their observation is not consistent with our present finding that apo B loses substantial immunoreactivity after lipolysis. Because the conformation of apoproteins is altered upon the dissociation of lipids (39–42), the removal of triglycerides from VLDL might lead to a conformational change in apo B and, therefore, decrease the immunoreactivity in our system. Recent studies (43, 44) have suggested that monoclonal antibodies to protein antigens are extremely conformation specific and recognize only the topographic antigenic sites of the protein. Other reports (45–47) have shown that monoclonal antibodies are powerful reagents in detecting a single amino acid substitution for a given protein. The experiments with LP-47 monoclonal antibody, which recognized only isolated LDL (used for immunization) and not plasma LDL, further strengthen the conclusion that in-vitro reactivity of antibodies is dependent on the form of the antigen used for immunization.

Unlike the reports of monoclonal antibodies to human IgG (48, 49), our mixture of four monoclonal antibodies did not mimic the reactivity of serum polyclonal antibodies in terms of ability to precipitate LDL, binding affinity, and thermal-dependent binding properties to $^{125}$I-LDL. To our knowledge, high-affinity monoclonal antibodies capable of precipitating LDL have not been produced.

The finding that the combination of $L_1C_6F_2$ and LP-34 monoclonal antibodies enhanced the binding is fascinating. The binding of one monoclonal antibody may decrease the binding of another antibody (50) because of steric hindrances, in that one molecule of antigen containing multiple antigenic sites cannot be bound by all antibody populations (51). We suggest that association of monoclonal antibody to one domain of LDL modulates the environment of another domain. We have not examined in detail the structural basis and mechanisms for such enhancement; however, one possibility would be that the binding of one monoclonal antibody to $^{125}$I-LDL enhanced another antibody to bind to $^{125}$I-LDL, eventually allowing both antibody molecules to bind. In this case, however, the counts of bound $^{125}$I-LDL would not be increased after separation by protein A because our antibodies were not radiolabeled. An attractive hypothesis, as illustrated in Figure 5, is that the first monoclonal antibody serves as a catalytic-like reagent. The maximal binding of the second monoclonal antibody to LDL is "catalyzed" by the first monoclonal antibody. Once the first monoclonal antibody has bound to LDL and has established the binding of the second antibody to LDL, the first antibody may then dissociate from LDL (under equilibrium conditions) and

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**Fig. 4.** Standard displacement curves and specificity of LDL radioimmunoassays at 4 °C

Plasma was pooled from 10 normal men. Monoclonal antibodies LP-22 (A), LP-34 (B), LP-47 (C), and conventional serum antibodies (D) were diluted 840-, 80-, 640-, and 2000-fold, respectively. Human apolipoproteins A-I, A-II, C-II, C-III, and E, up to 10 μg, did not displace $^{125}$I-LDL from antibodies.
To test this hypothesis, we studied the binding of 125I-LDL to monoclonal antibody LP-34 in the presence and absence of monoclonal antibody L1C6F2 at 4 °C for 18–20 h. A small dose of L1C6F2 (the antibody known to maximally bind only 20–30% of total 125I-LDL, Table 2) sharply increased the binding and reduced by five- to 10-fold the dose of LP-34 (the antibody known to bind 95% of 125I-LDL, Figure 1) necessary for maximal binding to LDL. The dose of L1C6F2 used in this experiment bound less than 10% of 125I-LDL by itself.

The fact that most complex antigens contain multiple epitopes, each potentially recognizable by one or more individual monoclonal antibodies, has important immunologic and clinical implications. For example, in-vivo recognition of a foreign protein by the immune system may require concerted binding by many antibodies. In the present experimental situation, combined use of individual monoclonal antibodies to a given complex antigen may provide a basis for selectivity similar to that which is operative in vivo. Because heterogeneous antibodies are capable of increasing the binding to antigens, this may also explain why a given protein contains multiple antigenic sites (21, 33, 34, 40, 51). Clinically, the significance is that one may be able to combine two monoclonal antibodies and prepare LDL immunoabsorbent columns to remove excess apo B-containing lipoproteins in patients with hypercholesterolemia. This technique, performed with conventional serum antibodies, has been shown to be beneficial (18).

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References


