# Identification of a mouse monoclonal antibody, LHLP-1, specific for human Lp(a)

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**Abstract** Heretofore, immunologic reagents used to define and quantify human Lp(a) have been polyclonal in origin and therefore heterogeneous in antigenic specificity. We report here the isolation of a mouse monoclonal antibody, LHLP-1, monospecific for Lp(a). The antigen reactive with LHLP-1 was expressed in both lipoprotein Lp(a) as well as apolipoprotein Lp(a) delipidated by SDS treatment; however, disulfide reduction of apolipoprotein Lp(a) inhibited LHLP-1 reactivity. The antigen reactive with LHLP-1 on Lp(a), therefore, appears not to require lipid for expression of its conformationally dependent (disulfide-inhibitable) epitope. Antigen reactivity was virtually absent in the apoB and other proteins contained in very low density, low density, and high density lipoprotein particles. Immunologic quantification of Lp(a) in individual serum samples with a rabbit reference antiserum or LHLP-1 showed good correlation. We conclude that the monoclonal antibody LHLP-1 identifies an antigen unique to Lp(a) and that this antibody may therefore be useful in the further characterization and measurement of human Lp(a). — **Duvic, C. R., G. Smith, W. E. Sledge, L. T. Lee, M. D. Murray, P. S. Roheim, W. R. Gallaher, and J. J. Thompson.** Identification of a mouse monoclonal antibody, LHLP-1, specific for human Lp(a). *J. Lipid Res.* 1985. **26:** 540–548.

**Supplementary key words** hybridoma • immunoblot • electroimmunoblot • inhibition enzyme immunoassay

Lp(a) is one of the least well characterized human serum lipoproteins. While it is known that Lp(a) shares some features with low density lipoproteins (LDL), e.g., both contain apolipoprotein B (1, 2) and have similar lipid compositions (1, 3), Lp(a) and LDL also differ significantly. For example, Lp(a) is found primarily in the density range 1.050–1.080 g/ml, or higher, whereas the majority of LDL is found in the 1.006–1.063 g/ml density range (4, 5). In addition, the pre-beta electrophoretic mobility of Lp(a) on agarose (3, 6) or paper (7) differs from that of LDL, perhaps reflecting the greater amounts of sialic acid in the Lp(a) particle (2, 3). There is an additional antigenic determinant, the Lp(a) antigen, on the Lp(a) particle that is not present on LDL (1, 8). Finally, recent work (9) has revealed considerable heterogeneity in both Lp(a) lipoprotein particle density and size of the apolipoprotein unique to Lp(a).

The significance of Lp(a) in human disease processes is also far from established. It has been shown that Lp(a) levels were elevated in cases of proven coronary heart disease (8, 10–14). Lp(a) concentration was not, however, correlated with age, sex, or cholesterol or triglyceride concentrations (14). Measurement of Lp(a) levels in human plasma may, therefore, be another independent factor relevant to the risk of developing heart disease that is independent of LDL cholesterol, triglyceride, apoB, or other positive risk factors associated with the atherosclerotic process.

Immunological quantification of Lp(a) has previously been performed solely with heterogeneous (polyclonal) antibody populations. Monoclonal antibodies provide an additional means with which to study and quantify lipoproteins (15–18). The goal of the investigation described here was to develop a monoclonal antibody to Lp(a) and to characterize the nature and reactivity of this antibody.

## MATERIALS AND METHODS

### Preparation of lipoproteins

Plasma lipoproteins were prepared from single donor plasma without the addition of proteolytic inhibitors according to the method of Havel, Eder, and Bragdon (19)

Abbreviations: Lp(a), lipoprotein a; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; apoB, apolipoprotein B; apoA-I, apolipoprotein A-I; apoE, apolipoprotein E; DMEM, Dulbecco's modified Eagle's medium; HAT, hypoxanthine, aminopterin, thymidine medium; IEIA, inhibition enzyme immunoassay; PBS-T, phosphate-buffered saline containing Tween-20; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; DTT, dithiothreitol; *r*: correlation coefficient; PAGE, polyacrylamide gel electrophoresis.

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with a Beckman L5-50 centrifuge and a 50.1 rotor. The plasma or lipoprotein preparations were adjusted to the appropriate density by the addition of solid KBr. In this way, lipoproteins of the following density ranges were obtained: $d < 1.006$, $1.006 < d < 1.063$, and $1.063 < d < 1.21$ g/ml. Additional preparations with the following densities were also prepared: $d < 1.019$, $1.019 < d < 1.050$, $1.050 < d < 1.080$, and $1.080 < d < 1.21$ g/ml. All preparations of $d > 1.21$ g/ml were also retained for analysis. The preparations were dialyzed extensively against 10 mM phosphate-buffered saline, pH 7.4, containing 0.01% EDTA and 0.01% sodium azide. Protein determinations were done by the Coomassie blue binding assay of Bradford (20) with bovine gamma globulin as standard.

Lp(a) was purified by gel filtration chromatography by a modification of the method of Albers and Hazzard (21). Briefly, a 9.5-ml portion of a $1.050 < d < 1.080$ g/ml lipoprotein fraction was applied to a 2.5 × 90 cm column of 6% agarose (Bio-Gel A-5m, 100–200 mesh, Bio-Rad Laboratories, Richmond, CA). Eluting buffer was 0.1 M Tris-HCl, 0.15 M NaCl, 1 mM EDTA, pH 8.2; flow rate was about 5 ml/hr and 5-ml fractions were collected after monitoring for absorbance at 280 nm. The eluates were also assayed for Lp(a) reactivity by an inhibition enzyme immunoassay to be described below. Appropriate fractions were pooled and concentrated by negative pressure dialysis (Bio-Molecular Dynamics, Beaverton, OR). A purified Lp(a) standard and reference antibody were the generous gifts of Dr. J. Albers.

### Production of monoclonal antibodies

Balb/c mice were injected intraperitoneally with 500 μg of an $1.063 < d < 1.21$ g/ml density fraction containing Lp(a) in an equal volume of Freund's complete adjuvant. The mice were boosted twice more after 1-wk intervals with 500 μg of protein without adjuvant. Fusion was conducted 3 days after the final injection by a modification of the method of Kennett et al. (22). The mouse myeloma cell line, X63-Ag8.653, of Kearney et al. (23) was used. Myeloma cells ($10^7$) were fused with erythrocyte-free splenocytes ($10^8$) from immunized mice by centrifugation in the presence of 30% polyethylene glycol 1000 (J. T. Baker, Phillipsburg, NJ). Residual polyethylene glycol was washed from the cell pellet and the cells were then suspended in 30 ml of Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY) containing 10% alpha medium (Gibco) and 20% fetal calf serum.

The cells were then added to 96-well microculture plates at one drop per well and incubated in a humidified incubator containing 5% $CO_2$. The following day an additional drop of the same medium containing 0.1 mM thymidine, 0.1 mM hypoxanthine, and 0.8 μM aminopterin (HAT) was added. An additional two drops of medium with HAT was added 1 week later. Macroscopic colonies were visible by 2 weeks after fusion. Supernatant fluid from visible clones was assayed for immunoreactivity to a fraction containing Lp(a) by enzyme immunoassay. Antibody-secreting clones were then expanded into larger tissue culture plates and supernatant fluids from these cultures were used as a source of antibody.

Growth was observed in 20 wells of four microculture plates. Three clones produced antibody with potential Lp(a) specificity and one of these clones, LHLP-1, was investigated in detail. In studies not described here (24), Ouchterlony analysis of supernatant fluids of LHLP-1 showed antibody of only the $IgG_1$ subclass with kappa light chain. In addition, three different techniques were used to demonstrate that the product produced by LHLP-1 was truly monoclonal. First, immunoelectrophoresis of ascites from mice injected intraperitoneally with LHLP-1 cells showed that a product with a narrow mobility range predominated after reaction with rabbit antiserum to mouse IgG. Second, supernatant with LHLP-1 cells grown in cell culture contained a limited number of well-defined mouse immunoglobulin bands after isoelectric focusing. Finally, the LHLP-1 clone has been subcloned at limiting dilution and antibody reactivity as determined by enzyme immunoassay was apparently identical with that of the parent clone.

In some cases, ascitic fluid was used as a source of antibody. To obtain ascites, Balb/c mice were primed by intraperitoneal injection of Pristane (Aldrich Chemical Co., Milwaukee, WI) 1 week prior to injection intraperitoneally with $10^7$ hybridoma cells. When the mice appeared swollen, the ascitic fluid was collected by draining the peritoneal cavity with a large-gauge needle.

### Immunological procedures

Ouchterlony analysis and immunoelectrophoresis were performed as previously described (24). Goat antiserum to human apoA-I and to human apoB were obtained from Dr. P. Roheim and rabbit antiserum to human Lp(a) was obtained from Dr. J. Albers.

To assay for the presence of Lp(a) in a specimen, an inhibition enzyme immunoassay (IEIA) was done. Fifty μl of a solution of antigen ($1.050 < d < 1.080$ g/ml) containing 26 ng of total protein was added to each well of a 96-well polystyrene microtiter plate (Immulon 2, Dynatech, Alexandria, VA) in 0.2 M carbonate buffer, pH 9.5. This amount of antigen was determined from checkerboard titrations of both antibody and antigen to give adequate absorbance for the assays described below. The plate was incubated overnight at 4°C. Immediately prior to use, the plate was washed three times with phosphate-buffered saline containing 0.05% Tween-20 (PBS-T) (25). Equal volumes of a dilution of the test sample and 1:200 dilution of monoclonal antibody to Lp(a) were mixed in test tubes

and incubated for 45 min at 37°C. Fifty μl of this mixture was then added to each well of a PBS-T-washed microtiter plate that had been previously coated with antigen. After incubation for 45 min at 37°C and three washes with PBS-T, 50 μl of a 1:500 dilution of alkaline phosphatase-labeled goat antiserum to mouse IgG (Miles Lab., Kankakee, IL) was added and incubated for 45 min at 37°C. After three washes with PBS-T, 100 μl of substrate (*p*-nitrophenyl phosphate) was then added in 0.92 M triethanolamine buffer, pH 9.8, and absorbance at 405 nm was measured on a Titertek Multiskan automated plate reader (Flow Laboratories, McLean, VA). In studies not reported here (in preparation), within-day and between-day variation were about 8% and 12%, respectively. Placement of samples on the plate does not seem generally critical although isolated instances of aberrant behavior of outside rows and columns have been noted (unpublished observations). An expanded treatment of Lp(a) quantification by enzyme immunoassay will be the topic of a subsequent paper (in preparation). The enzyme immunoassay with the reference rabbit antiserum was identical to the mouse assay just described, except that the rabbit antiserum was used as initial detection reagent and an alkaline-phosphatase-labeled goat anti-rabbit IgG (Miles) was used as developing reagent. Data were analyzed by calculation of a 50% inhibition dose (26, 27) based on an antigen standard kindly supplied by Dr. J. Albers. A typical curve obtained by inhibition of a fixed amount of LHLP-1 with Lp(a) is shown in **Fig. 1**. Similar standards were included on every plate to calibrate dose-response variables. We have no evidence based on comparisons of logit-log slopes for any significant deviation from parallelism with the samples analyzed here.

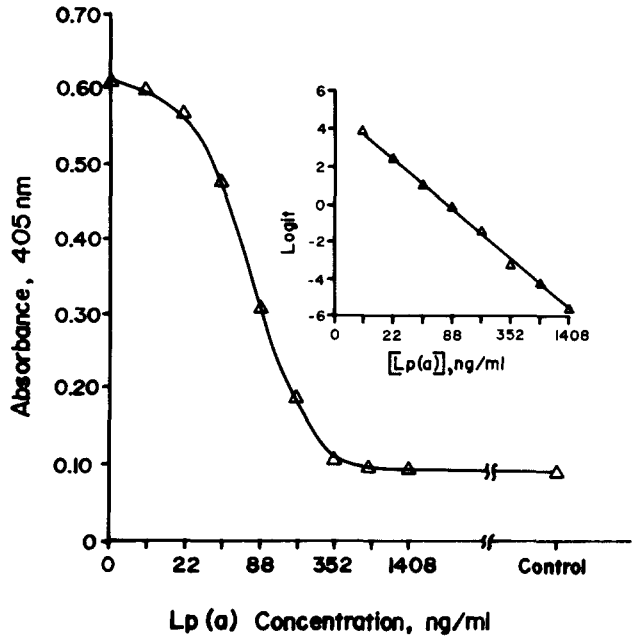


**Fig. 1.** Inhibition enzyme immunoassay. Varying concentrations of standard Lp(a) were used to inhibit an equal volume of a 1:200 LHLP-1 supernatant dilution and residual antibody was measured as described in Materials and Methods. Control indicates absorbance measured in a control well containing no LHLP-1. The inset shows the weighted logit-log transformation of this data (27): slope (± 1 SD) = 1.90 ± 0.07; 50% inhibition point (± 1 SD) = 78.0 ± 2.0 ng; $r$ = 0.996.

### Agarose electrophoresis and immunoblotting

Isoelectric focusing was performed in agarose with a pH gradient of 3.5–10 (Ampholine, LKB, Rockville, MD) (28). Samples of supernatant or ascites were applied and run for 3 hr at 4 watts constant power. Duplicate plates were used either for protein staining or for immunoblotting as described below. Electrophoresis of lipoproteins in 0.5% agarose was performed by a modification of the method of Noble (29) for 2 hr at 200 V. Agarose was prepared in 0.025% Tris-tricine buffer, pH 8.6 (30), containing 0.4% albumin. Plates were run in triplicate with one plate stained for lipid with Sudan Black B (0.2% in 60% ethanol) and the other plates used for immunoblotting. Occasionally, a spot of BSA-Evans Blue was applied between wells to mark electrophoretic movement. After completion of the electrophoretic run, immunoblotting plates were overlaid with nitrocellulose paper (Millipore Corporation, Bedford, MA). The gels were then pressed dry by overlaying the nitrocellulose with filter paper, paper towels, and a 1-kg weight. After most liquid had been extracted from the gels (about 15 min), the nitrocellulose paper was removed and washed in PBS-T (31). Following washing, the nitrocellulose paper was incubated in a 1:10 dilution in PBS-T of a supernatant containing LHLP-1 or a 1:500 dilution of goat anti-apoB antibody for 1 hr. After washing, the nitrocellulose was immersed in a 1:500 dilution of peroxidase-labeled rabbit anti-mouse IgG (Miles) or a 1:500 dilution of peroxidase-labeled rabbit anti-goat IgG (Cappel) for 1 hr. After washing, the paper was reacted with peroxidase substrate (4-chloronaphthol, 0.5 mg/ml, in PBS, pH 7.4, and 0.015% $H_2O_2$). After washing in water, the nitrocellulose was dried.

### Electroimmunoblotting

Polyacrylamide gel electrophoresis in linear 3.5–10% acrylamide gels employed the discontinuous buffer system of Laemmli (32). Samples were mixed with one-half volume of sample buffer (6.9% SDS, 30% glycerol, 188 mM Tris, pH 6.8, and bromphenol blue) and applied to the wells. Only when indicated, some samples were treated by boiling at 100°C for 2 min in the presence or absence of 6.7 mM dithiothreitol (final concentration). After electrophoresis, the gel was either stained with Coomassie blue (0.2% in 50% methanol and 10% acetic acid) or used to localize antigenic reactivity by immuno-

electroblotting. Except for the use of Tris-glycine buffer without methanol, the transfer method used was essentially that of Towbin, Staehelin, and Gordon (33). Electrophoresis was conducted for 4 hr at 60 volts with temperature regulated to between 10–12°C. The nitrocellulose membrane was then removed and blocked by immersion in PBS-T. After three washes in PBS-T, the blot was immersed in a 1:10 dilution of LHLP-1 antibody in PBS-T or a 1:500 dilution of goat anti-apoB antibody and shaken overnight at room temperature. After four washes in PBS-T, the blot was incubated with a 1:500 dilution of peroxidase-labeled rabbit anti-mouse IgG or a 1:500 dilution of peroxidase-labeled rabbit anti-goat IgG for 60 min at room temperature. After four washes in PBS-T, the blot was developed in the chloronaphthol-peroxide substrate until bands were visible. The blot was then washed with water and dried.

## RESULTS

### Localization of antibody reactivity by agarose electrophoresis and immunoblotting

To localize antibody reactivity to a lipoprotein particle, agarose electrophoresis of plasma, VLDL ($d < 1.006$ g/ml), LDL ($1.019 < d < 1.05$ g/ml), the density fraction $1.05 < d < 1.080$ g/ml, purified Lp(a), and HDL ($1.08 < d < 1.21$ g/ml) was done simultaneously on three gels. One of the gels was stained for lipid, another immunoblotted with monospecific rabbit antibody to apoB, and the third gel was immunoblotted with LHLP-1. The lipid stain in **Fig. 2** (top) demonstrated that the purified Lp(a) (lane 5) migrated in a pre-beta position that was slightly slower than VLDL ($d < 1.006$ g/ml, lane 2). Also evident were the pre-beta and beta particles of the $1.05 < d < 1.08$ g/ml fraction (lane 4) and the presence of pre-beta as well as alpha lipoproteins in HDL ($1.09 < d < 1.21$ g/ml, lane 6). Plasma showed lipids of alpha, pre-beta, and beta mobility (lane 1).

When the second gel (Fig. 2, middle) was immunoblotted for apoB, reactivity was confined to pre-beta- and beta-migrating lipoproteins and was absent in lipoproteins with alpha mobility. When an identical gel was immunoblotted versus LHLP-1 (Fig. 2, bottom), bands were observed in the $1.050 < d < 1.080$ g/ml fraction, the purified Lp(a), and in the HDL preparations in the position of a pre-beta protein. VLDL had pre-beta mobility but no immunoreactivity to LHLP-1. Also evident was a pre-beta band in whole serum.

To determine whether the particle to which LHLP-1 reacted contained apoA-I or apoB, the $1.05 < d < 1.08$ g/ml density fraction was first reacted with goat antibody to human apoA-I or apoB, the mixture was electrophoresed in agarose, and then immunoblotted with

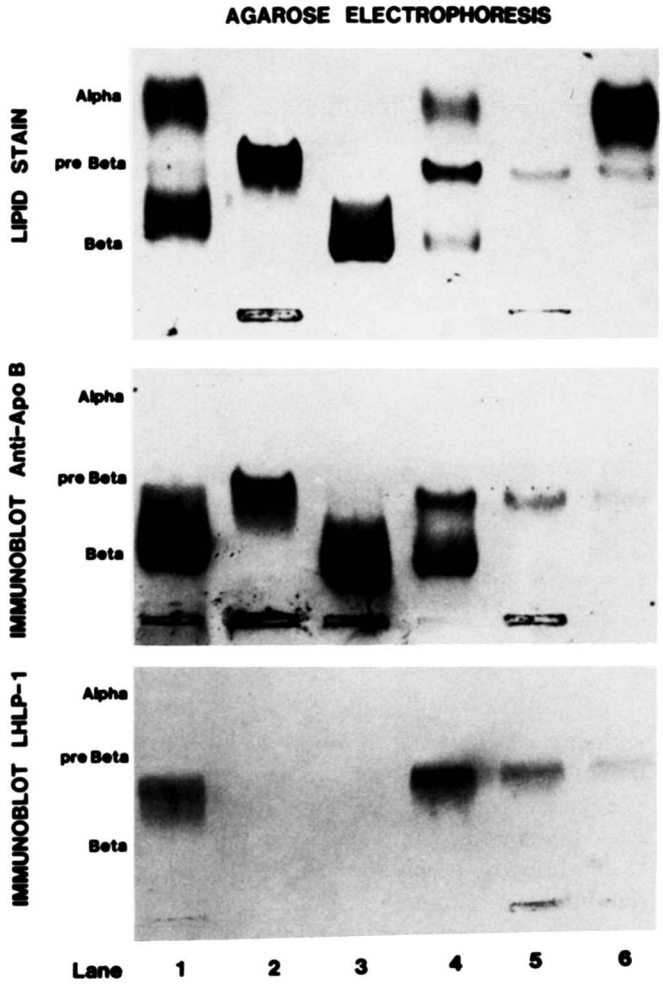


**Fig. 2.** Electrophoresis and immunoblots of lipoprotein preparations in 0.5% agarose containing 0.4% bovine serum albumin. Top: lipid stain (Sudan Black). Middle: immunoblot with rabbit anti-apoB after transfer to nitrocellulose. Bottom: immunoblot with LHLP-1 after transfer to nitrocellulose. Lane 1: unfractionated, intact plasma, 880 μg protein; lane 2: VLDL ($d < 1.006$ g/ml), 12.3 μg protein; lane 3: LDL ($1.019 < d < 1.05$ g/ml), 9.4 μg protein; lane 4: $1.05 < d < 1.08$ g/ml, 20.5 μg protein; lane 5: purified Lp(a), 5.7 μg protein; lane 6: HDL ($1.08 < d < 1.21$ g/ml), 47.2 μg protein.

LHLP-1 antibody. **Fig. 3** shows that pretreatment of this density fraction with anti-apoA-I had no effect on the appearance of the pre-beta particle (lane 6), but that treatment with anti-apoB completely abolished reaction of LHLP-1 with all particles in the $1.05 < d < 1.08$ g/ml density fraction (lane 5). These results suggested that the LHLP-1 immunoreactive particle contained apoB and not apoA-I.

### Correlation between results obtained with monoclonal antibody or rabbit reference antiserum

As an additional measure of the specificity of LHLP-1, independent determinations of the relative amounts of Lp(a) in fresh, normolipemic plasma samples were done

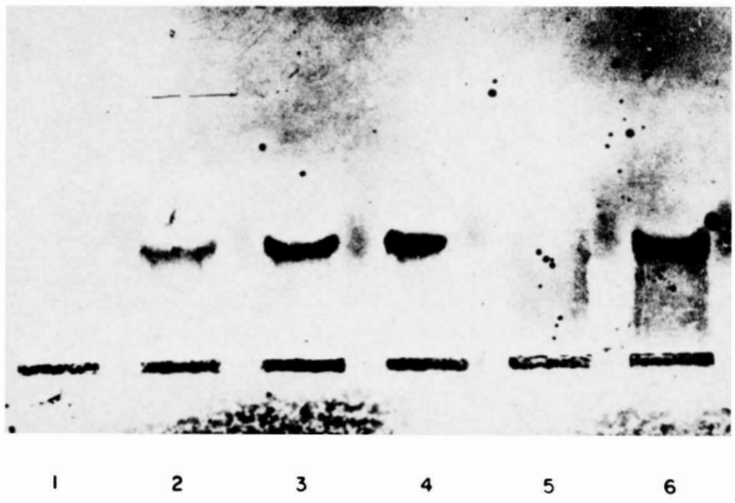


**Fig. 3.** Immunoblot with LHLP-1 of an Lp(a)-containing 1.050 < d < 1.080 g/ml lipoprotein fraction (11.2 μg of protein) treated with goat antiserum to apoA-I or apoB for 1 hr at 37°C. The spots between lanes are dye spots transferred to the nitrocellulose as a mobility marker. Lane 1: buffer control (no serum or lipoprotein); lane 2: normal goat serum alone + lipoprotein fraction; lane 3: goat anti-mouse serum + lipoprotein fraction; lane 4: lipoprotein fraction alone; lane 5: goat anti-apoB serum + lipoprotein fraction; lane 6: goat anti-apoA-I serum + lipoprotein fraction.

with either LHLP-1 or rabbit anti-Lp(a). In these assays, one unit was defined by an antigen standard kindly supplied along with the reference rabbit antiserum by J. Albers. Dilutions of the standard antigen were used versus each antibody preparation in the standard inhibition immunoassay. From the resulting standard curves, apparent concentrations of individual plasma samples were determined. Preliminary studies shown in **Table 1** determined the distribution of LHLP-1 immunoreactivity by percentage in three single donor plasmas. The results were basically consistent with specific activity determinations in that about 50% of total LHLP-1 reactivity was present in the 1.05 < d < 1.08 g/ml density fraction regardless of the individual donor. Variable amounts were present in the 1.08 < d < 1.21 and d > 1.21 fractions. These results were quite similar to the distribution studies described by Gaubetz et al. (1) with Lp(a) prepared in the presence of proteolytic inhibitors. In other work (24), purified Lp(a) was enriched approximately 10,000 times in LHLP-1 reactivity when compared to the original plasma (estimated Lp(a) content by IEIA = 328 μg/ml).

In another test of LHLP-1 antigenic specificity, the results of the independent Lp(a) determinations with the standard rabbit antibody and monoclonal methods were compared in order to assess the correlation between each set of values (**Fig. 4**). In both instances there was a distribution from less than 1 to greater than 20 micrograms of Lp(a) in the assay. The correlation coefficient between each set of values was 0.982 (n = 9, $P < 0.01$, ref. 34).

## Reactivity of LHLP-1 with the apoproteins of Lp(a)

The evidence cited above demonstrated that LHLP-1 reactivity exists to the intact Lp(a) particle. The nature of the antigenic moiety within the particle was further characterized by SDS polyacrylamide gel electrophoresis followed by electroblotting. As shown in the Coomassie blue stain in **Fig. 5**, SDS-treated Lp(a) (lane 1) showed a single band of higher apparent molecular weight than that of apoB (lane 4). After boiling with DTT reduction (lane 3), the high molecular weight band in Lp(a) split into two bands, one of which had a mobility identical to that of apoB and the other which had a mobility between that of intact Lp(a) and apoB. Electroblotting with rabbit anti-apoB (**Fig. 6**, left) showed apoB reactivity to be present in intact Lp(a) (lane 1), in the band with mobility of apoB

TABLE 1. Distribution of recovered immunoreactive Lp(a) among different ultracentrifugation fractions of individual plasma samples

| Fraction | Recovery in Percent | | |
|---|---|---|---|
| | Sample A | Sample B | Sample C |
| Plasma | 100[a] | 100[a] | 100[a] |
| Density (g/ml) | | | |
| d < 1.006 | ND[b] | 1.7 | 0.75 |
| d < 1.03 | 2.04 | ND | ND |
| 1.006–1.050 | ND | 0.60 | 0.74 |
| 1.03–1.040 | 0.10 | ND | ND |
| 1.040–1.063 | 1.93 | ND | ND |
| 1.050–1.080 | ND | 50.0 | 52.9 |
| 1.063–1.21 | 83.1 | ND | ND |
| 1.080–1.21 | ND | 21.0 | 13.68 |
| d > 1.21 | 9.93 | 13.0 | 27.16 |
| Total recovery | 97.1 | 86.3 | 95.21 |

[a]Micrograms of Lp(a) protein/ml of undiluted plasma (estimated by IEIA titration with LHLP-1): A, 294 μg/ml; B, 249 μg/ml; C, 452 μg/ml.
[b]Not determined.

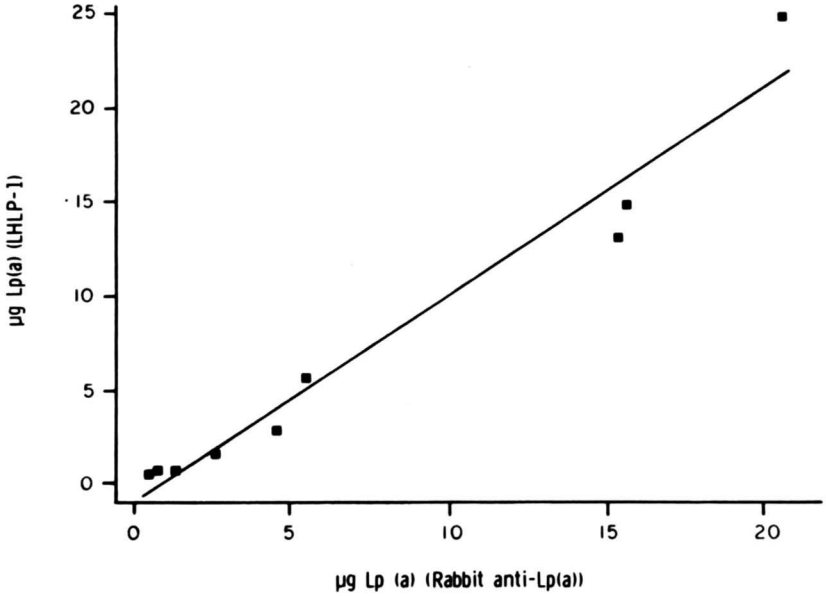


**Fig. 4.** Interassay comparison of immunoreactive Lp(a) in nine serum samples selected to give a wide range of Lp(a) content per sample (six males, three females; ages 22–56). The purified Lp(a) used to standardize both the LHLP-1 and rabbit immunoassays was kindly supplied by Dr. John Albers. (The correlation coefficient for these data is $r = 0.982$.)

after reduction of Lp(a) (lane 3), and in apoB (lane 4). Little apoB reactivity was present in the intermediate band derived from reduced Lp(a) (lane 3). Electroblotting with LHLP-1 detection reagent showed that immunoreactivity was confined to the intact Lp(a) band (Fig. 6, right, lanes 1 and 2) with little residual activity present in the intermediate band formed upon reduction of Lp(a) (lane 3). LDL apoB was nonreactive (lane 4) as was the apoB contained in reduced Lp(a) (lane 3). Boiling did not destroy the antigen detected by LHLP-1 (lane 2) or the anti-apoB (left, lane 2).

## DISCUSSION

Immunochemically defined high serum levels of the Lp(a) lipoprotein may constitute an independent risk factor for coronary heart disease (14). Present methods of preparation of antisera for immunochemical quantification of Lp(a) are cumbersome because they require extensive absorptions to remove apoB cross-reactivity. As has been demonstrated by the work of others with hybridoma antibody to human apoE and apoB (15–18), monoclonal antibodies should provide standards for immunoassays of lipoproteins. Accordingly, we have developed a stable hybridoma cell line, LHLP-1, which produces antibody with apparent specificity for the intact human Lp(a) particle.

The criteria met by LHLP-1 for Lp(a) specificity are several. First, LHLP-1 immunoreactivity is confined to a pre-beta particle significantly enriched in the $1.050 < d < 1.080$ g/ml lipoprotein density fraction. VLDL, another pre-beta particle, has virtually no LHLP-1 reactivity. The particle reactive with LHLP-1 did not contain apoA-I but

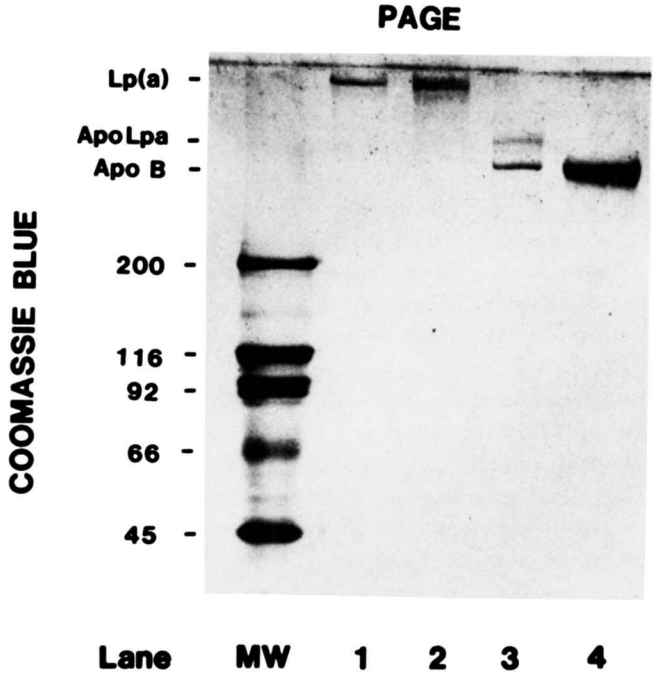


**Fig. 5.** Gradient (3.5 to 10%) polyacrylamide gel electrophoresis (PAGE) of purified Lp(a) (5.3 μg protein) and LDL (8.6 μg of protein). MW, standard molecular weight markers (in kilodaltons); lane 1: untreated Lp(a); lane 2: Lp(a) after boiling at 100°C; lane 3: Lp(a) after boiling at 100°C and reduction with 6.7 mM dithiothreitol; lane 4: untreated LDL.

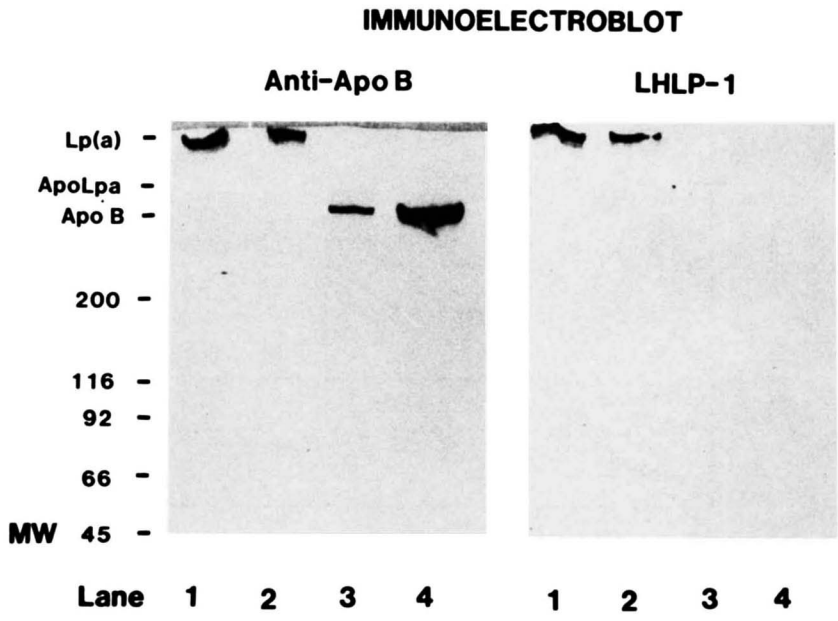


**Fig. 6.** Electroimmunoblot of purified Lp(a) (5.3 μg of protein) and LDL (8.6 μg of protein). Left: electroimmunoblot with rabbit anti-apoB after transfer to nitrocellulose. Right: electroimmunoblot with LHLP-1 after transfer to nitrocellulose. MW, standard molecular weight markers (in kilodaltons); lane 1: untreated Lp(a); lane 2: Lp(a) after boiling at 100°C; lane 3: Lp(a) after boiling at 100°C and reduction with 6.7 mM dithiothreitol; lane 4: untreated LDL.

did contain apoB. Purified Lp(a) enriched LHLP-1 immunoreactivity approximately 10,000-fold over that of serum. Further, the results of immunoassays done with an Lp(a) reference rabbit antiserum or LHLP-1 antibody showed high correlation. This suggested that the two antibody preparations recognized similar species in the plasma samples analyzed. Finally, LHLP-1 antibody recognized the unique apoprotein band contained in Lp(a), but did not react with apoB contained in LDL. Based on these results, it can be concluded that LHLP-1 antibody reacted to the Lp(a) particle.

However, as shown by preliminary distribution studies of LHLP-1 reactivity in various density fractions, immunoreactivity may not *necessarily* be confined to the Lp(a) particle. Significant LHLP-1 reactivity can be found in denser regions, including the $d > 1.21$ g/ml fraction. This suggests several possibilities that deserve further investigation. First, the LHLP-1 antigen may be degraded upon storage and become denser. Gaubetz et al. (1) have discussed in detail the possible role that proteolysis may play in such observations. Second, alternative forms of the antigen may be synthesized or metabolized such that the antigen is not necessarily associated with the Lp(a) lipoprotein particle. Immunoaffinity chromatography should prove useful in the isolation of such non-Lp(a) particles, if they exist. However, immunoblotting of whole serum failed to detect these hypothesized particles as electrophoretically distinct from typical Lp(a) lipoprotein (Fig. 2). Finally, Fless, Rolih, and Scanu (9) have described a number of density variants of Lp(a) characterized by the presence of apoB and unique antigenically specific apoLp(a) proteins of varying sizes. The purified Lp(a) samples characterized here did not display this heterogeneity but were similar to those studied by Gaubetz et al. (1). None of the samples we have analyzed quantitatively for presence of LHLP-1 reactivity had more than about 5% of such reactivity in densities lower than 1.05–1.063 g/ml. It is likely that our samples did not contain the low density Lp(a) characterized by Fless et al. (9), although we cannot yet exclude the possibility that such materials are unreactive with LHLP-1 antibody.

Of particular interest to structural aspects of the Lp(a) particle is our observation that LHLP-1 immunoreactivity existed to the large molecular weight protein found in the non-reduced but detergent-treated Lp(a). While resistant to boiling, the antigenic determinant was sensitive to reduction. This suggests higher order protein structure is necessary for maintenance and/or expression of the LHLP-1 reactive epitope. Finally, it must be noted that conventional LDL ($1.019 < d < 1.05$ g/ml) contained large amounts of apoB that was nonreactive with LHLP-1 antibody. These results are consistent with those of both Gaubetz et al. (1) and Fless et al. (9) who demonstrated Lp(a) reactivity as confined to a large molecular weight band in SDS-treated but non-reduced Lp(a) lipoproteins. However, LHLP-1 immunoreactivity differs significantly from the polyclonal antibody preparations studied by these workers in that reduced forms of Lp(a) are poorly

immunoreactive with LHLP-1.

These results can serve as the basis for further speculation. Lp(a) apoB is associated with a high molecular size protein (1, 9), and unique LHLP-1 antigen reactivity (this paper), while LDL apoB is associated with neither. It is not likely that Lp(a) and LDL apoBs are segregated as the result of different metabolic fates since turnover studies (35) failed to show significant catabolic differences between isolated Lp(a) and LDL. Rather, the results suggest that the apoBs of Lp(a) and LDL might have effective segregation during biosynthesis either as the result of different sites of biosynthesis or as the result of compartmentalization (physical or temporal) during biosynthesis. The heterogeneity of the unique protein moiety associated with the Lp(a) of certain individuals (9) adds additional complexity. Further work is needed, therefore, to clarify the relationship between apoB, various molecular forms of the Lp(a) protein, and LHLP-1 immunoreactivity. Finally, the reactivity of LHLP-1 antibody with Lp(a) described here should provide the basis of assays for immunological quantification of Lp(a).

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