Radioimmunoassay of human plasma Lp(a) lipoprotein

John J. Albers,[1] Janet L. Adolphson, and William R. Hazzard

Department of Medicine and Northwest Lipid Research Clinic, School of Medicine,
University of Washington, Seattle, WA 98195

Abstract A quantitative immunodiffusion assay demonstrated Lp(a) lipoprotein in 91% (911 of 1000) of subjects. In order to quantitate Lp(a) in all plasma, a sensitive and specific double antibody radioimmunoassay was developed. The between-assay coefficient of variation was 8%. Lp(a) levels by radioimmunoassay were highly correlated with those obtained by the less sensitive radial immunodiffusion method ($r = 0.98$, n = 51). All but one of the 89 Lp(a) "negative" subjects by immunodiffusion had detectable levels of Lp(a) by radioimmunoassay. The one subject without detectable Lp(a) had abetalipoproteinemia (without detectable apolipoprotein B by radioimmunoassay). Furthermore, Lp(a) was detected in all three non-human primates examined: patas monkey, baboon, and pig-tail monkey. Quantitation of Lp(a) levels in 90 male myocardial infarction (MI) survivors and their spouses showed that the distribution of Lp(a) levels of MI survivors was significantly higher above the 50th percentile cut-point ($P < 0.02$) and exceeded that of the spouses. Furthermore, the Lp(a) distribution at and above the 50th percentile for the MI survivors who had an MI at age <50 (n = 36) was shifted to values higher than those having an MI at age >50. Thus, high levels of Lp(a) may be associated with premature coronary disease. We conclude that Lp(a) is present in all individuals with apolipoprotein B and that apolipoprotein B appears necessary for the plasma transport of the Lp(a) lipoprotein. Consistent with this hypothesis, quantitative immunochemical precipitation of ^{125}I-Lp(a) indicated that essentially all individual molecules of six purified Lp(a) preparations contain both the Lp(a) antigen and apolipoprotein B.

Supplementary key words myocardial infarction · primate

Based on qualitative gel diffusion studies, the Lp(a) lipoprotein [Lp(a)] was originally considered a genetic variant of low density lipoprotein (LDL) determined by a single autosomal dominant mechanism and confined to 30–40% of the Caucasian population (1, 2). Other studies suggested that the Lp(a) lipoprotein is immunochemically, chemically, and physiochemically distinct from the bulk of the d 1.019–1.063 (LDL) lipoproteins, even though its hydrated density (principally d 1.050–1.10) overlaps that of LDL and that its major protein constituent, apoprotein B, appears

identical to the principal protein moiety of LDL (3, 4). More recent studies (5–7), using a sensitive quantitative radial immunodiffusion (RID) assay for the Lp(a) lipoprotein, have suggested that: *1*) Lp(a) is present in all or nearly all individuals; *2*) Lp(a) is a quantitative genetic trait determined by a multifactoral mechanism; and *3*) Lp(a) and the bulk of the LDL molecules are under separate metabolic and genetic control.

Even the above RID method is less than ideal, however, because of the common circumstance in which the concentration of Lp(a) in whole human plasma lies below its lower limit of sensitivity (10–20% of plasma samples tested). Therefore, the purpose of the present studies was to develop a more sensitive quantitative radioimmunoassay (RIA) in order to: *1*) assess Lp(a) levels in Lp(a) "negative" plasmas; *2*) compare immuno-cross-reactivity of the Lp(a) of three primate species with human Lp(a); and *3*) compare Lp(a) levels in plasma from myocardial infarction survivors and their spouses to assess a possible association between these levels and premature atherosclerosis.

METHODS

Preparation of antigen and antibody

Plasma was obtained from donors shown by the RID assay (5) to contain high levels of Lp(a) antigen (>30 mg/dl). Lipoprotein containing the Lp(a) antigen was isolated by ultracentrifugation and chromatography as previously described (5). The isolated lipoprotein was characterized by agarose electro-

---

phoresis using the Bio-Gram A kit (Bio-Rad Laboratories, Richmond, CA), by Ouchterlony gel diffusion studies, and by quantitative immunoprecipitation (8, 9). Antibody to the Lp(a) antigen was prepared as previously described (5). Immunization of rabbits with purified Lp(a) lipoprotein, intramuscularly, subcutaneously, and intradermally, led to the production of precipitating antisera. This antisera was absorbed with LDL (d 1.030–1.040) until a precipitate no longer formed upon addition of LDL. This absorbed antiserum was designated anti-Lp(a).

Lipoprotein-deficient plasma was isolated by first adjusting whole plasma density to 1.25 g/ml with KBr. Ultracentrifugation was then performed in a 60 Ti rotor at 4°C for 28 hr at 50,000 rpm. The top 10 ml were removed with the use of a tube slicer and the infranatant lipoprotein-deficient plasma was dialyzed against 20 mM borate, 150 mM NaCl, 1 mM EDTA, and 0.05% NaN_3 pH 8.0.

Iodination of the Lp(a) lipoprotein

Radiolabeling of the Lp(a) lipoprotein with ^{125}I was performed by the McFarlane ICl procedure as modified by Bilheimer, Eisenberg, and Levy (10). The reactants were added in amounts to give a specific activity of 200 μCi/mg protein and 20 atoms iodine/mol of Lp(a) lipoprotein, assuming a molecular weight of 5.4×10^6, 27% protein content, 5% labeling efficiency, and 80% counting efficiency. The labeled lipoprotein was chromatographed on a 0.9×60 cm column of Bio Gel A5m, 200–400 mesh (Bio-Rad Laboratories), using 50 mM sodium barbital, 1 mM EDTA and 70 mM KI, pH 8.2, as elution buffer, and the eluted fractions were assayed for radioactivity in an autogamma counter. The first peak of radioactive ^{125}I-Lp(a) was pooled and diluted with 20 mM borate, 150 mM NaCl, 1 mM EDTA, 0.05% NaN_3, and 1% bovine serum albumin (BSA) pH 8.0, henceforth designated RIA buffer. The preparation was then dialyzed exhaustively against RIA buffer plus 20 mM KI but without BSA.

Lp(a) standards

The Lp(a) lipoprotein to be used as standard was dialyzed against RIA buffer without BSA. The concentration of lipoprotein protein was determined by the method of Lowry et al. (11) using BSA (Metrix, Armour Pharmaceutical Company, Chicago, IL) as standard. The protein value was multiplied by 0.7 to convert to absolute protein weight and this "corrected" protein value was multiplied by 3.7 to convert total Lp(a) lipoprotein protein to Lp(a) lipoprotein (5). Results were expressed in terms of total Lp(a)

lipoprotein unless specified. The preparation was diluted with 150 mM NaCl, 0.1% sodium dodecyl sulfate for protein determination or, alternatively, the Lp(a) reaction products were extracted with ether. The Lp(a) lipoprotein was diluted with RIA buffer to make standards ranging from 0.8 to 7.0 μg/ml protein.

Radioimmunoassay

A double antibody radioimmunoassay (RIA) for assessment of the Lp(a) antigen was developed. The ^{125}I-Lp(a) was filtered through a Swinnex-13 0.45 μm Millipore filter (Millipore Corp., Bedford, MA) and diluted to 2 μg/ml total Lp(a) protein with lipoprotein-deficient plasma (d > 1.25 plasma fraction). To each assay tube was added 100 μl of unknown sample or standard, followed by 100 μl of a solution of ^{125}I-Lp(a) (2 μg/ml protein) and then 100 μl of anti-Lp(a) antibody (diluted approximately 1:700 with the d > 1.25 plasma fraction). Lp(a) standards and anti-Lp(a) sera were diluted with d > 1.25 fraction in order to reduce nonspecific precipitation and to minimize the possibility of reactivity of antibodies not specific for the Lp(a) antigen. After incubation at 4°C for 24 hr, 100 μl of nonimmune rabbit serum diluted 1:100 with RIA buffer was added and then 300 μl of sheep anti-rabbit IgG (representing an excess of antibody) was added. After a second incubation at 4°C for 12–16 hr, radioactivity was measured; 1.0 ml of RIA buffer was added, the tubes were centrifuged at 2500 rpm for 20 min in a Sorval RC-3 centrifuge (Ivan Sorval, Norwalk, CT), and the supernatant fluid was decanted. The precipitate was subsequently washed three times with RIA buffer and then measured for radioactivity.

Every assay contained control tubes in which: (1) conditions were designed for maximal immunoprecipitation (1:20 dilution of anti-Lp(a) sera); (2) the specific anti-Lp(a) was omitted from the assay mixture (nonspecific control); and (3) a large excess (50 μg) of unlabeled Lp(a) lipoprotein was added to the usual assay mixture. The standards and nonspecific controls were included at the beginning, the end, and sometimes (depending on the assay size) in the middle of the assay. All samples were assayed in triplicate. A plasma quality control pool, stored at 4°C with 0.05% sodium azide in a sealed Wheaton vial, was assayed several times in each assay.

To obtain the optimal dilution of plasma prior to Lp(a) quantitation by RIA procedure, the concentration of Lp(a) in all samples was first measured by the RID procedure. All samples were diluted in order to give approximately 50% displacement of the ^{125}I-Lp(a).

Characterization of the Lp(a) lipoprotein by double antibody precipitation

The Lp(a) lipoprotein was characterized by quantitative precipitation (8, 9). One hundred μl of anti-Lp(a) or anti-LDL sera was added to 100 μl of ^{125}I-Lp(a) (2 μg/ml). After incubation at 4°C for 24 hr, 50 μl was removed and measured for radioactivity. Then 300 μl of sheep anti-rabbit IgG was added, and the tubes were incubated at 4°C for 12 hr. After centrifugation the precipitates were washed as in the Lp(a) radioimmunoassay.

Subjects and metabolic studies

Plasma samples were obtained after an overnight (12–14 hr) fast from adults participating in a population study of the prevalence of hyperlipoproteinemia being conducted at the Northwest Lipid Research Clinic and from hyperlipidemic subjects referred to the clinic. The prevalence study population has been described previously (12). For estimation of time-to-time variation of Lp(a) levels, fasting plasma was obtained from seven healthy hospital employees under standardized conditions (13). In addition, two healthy males, aged 29 and 58, underwent an assessment of Lp(a) and apolipoprotein B levels before, during, and after a 28-day period of controlled cholesterol feeding. During the cholesterol feeding period, each subject ingested an isocaloric liquid formula diet of normal composition (40% fat [polyunsaturated/saturated, P/S, = 0.5], 45% carbohydrate, 15% protein) except for the presence of 5,000 mg cholesterol/day derived from 20 egg yolks. Myocardial infarction subjects were men who had been hospitalized with a definite myocardial infarction and sampled at least 3 months later.[2]

Plasma from the 13 baboons and 6 pigtail monkeys was obtained from Dr. Ram Pratrap Kushwaha, University of Washington, Seattle, while the patas monkey plasma was received from Dr. Robert Mahley, Nation Heart, Lung, and Blood Institute, Bethesda, Md. All samples were stored at 4°C with 0.05% sodium azide in a sealed Wheaton vial.

Other methods

Apolipoprotein A-I and A-II were quantitated by radial immunodiffusion (12, 14). Both assays have an interassay coefficient of variation of approximately 6% Apolipoprotein B was measured by a sensitive double antibody radioimmunoassay as previoulsy described (15, 16).

---

[2] In collaboration with Dr. Arno Motulsky, Division of Medical Genetics, University of Washington.

RESULTS

Antigen characterization

Agarose electrophoresis of the purified Lp(a) lipoprotein gave a single band with pre-beta mobility. The undelipidated Lp(a) gave no reaction in gel diffusion to anti-human serum albumin, anti-apolipoprotein D, anti-apo-C-II, anti-apo-A-I, anti-apo-A-II, or anti-"arginine rich" apoprotein sera but it reacted with anti-apo-B and anti-Lp(a) sera. The Lp(a) lipoprotein was delipidated with ether–ethanol and partially solubilized in 8 M urea or 6 M guanidine-HCl. The soluble non-apoprotein B proteins reacted only with anti-Lp(a) sera. Lp(a) or delipidated Lp(a) preparations were not evaluated for the presence of apoproteins C-I or C-III.

Antibody characterization

The anti-Lp(a) sera did not react in gel diffusion studies with human serum albumin, C apoproteins derived from VLDL, or the apoproteins A-I, A-II, D, "arginine-rich" peptide, or the d > 1.25 plasma fraction, but reacted with purified Lp(a). Anti-Lp(a) sera precipitated less than 3% of the radioactivity from ^{125}I-labeled LDL or d 1.019–1.050 plasma fraction.

Immunoprecipitation

Double antibody precipitation of six Lp(a) preparations showed that 94 ± 2% (mean ± SD) of the ^{125}I-labeled Lp(a) lipoprotein reacted with anti-Lp(a) sera while 92 ± 1% reacted with anti-LDL sera (specific for apolipoprotein B), whereas 94% reacted with both antisera. These results indicate that the individual molecules of the Lp(a) lipoprotein contain both apolipoprotein B and the Lp(a) antigen. Precipitation of a Lp(a) preparation after 3 weeks of storage at 4°C showed that 92% of the radioactivity was precipitable with anti-Lp(a) sera while 72% was precipitable with anti-LDL sera. This suggests that some Lp(a) antigen was dissociated from the Lp(a) lipoprotein during this 3-week period.

Validation of the assay

Under the conditions described for maximum precipitation, >95% of the radioactivity of the ^{125}I-Lp(a) was precipitable with anti-Lp(a) sera. Furthermore, 100 μg of unlabeled Lp(a) displaced >95% of the ^{125}I-Lp(a). Approximately 3% (3 ± 1) of the radioactivity was precipitated in the absence of specific anti-Lp(a) sera. There was no significant displacement (<3%) of radioactivity by the addition of physiological concentrations of human serum albumin, LDL (d 1.019–1.050), HDL (d 1.125–1.21),
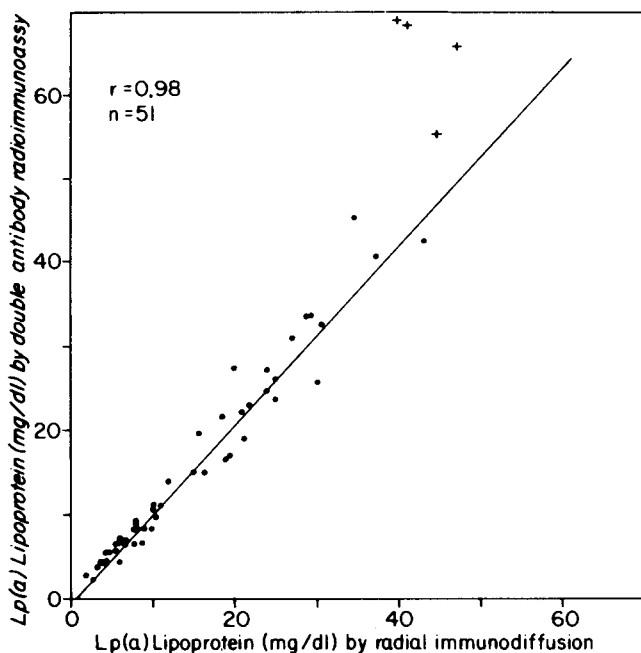
Fig. 1. Comparison of Lp(a) lipoprotein values obtained by radial immunodiffusion with those obtained by double antibody radioimmunoassay. The four samples indicated with a cross (+) were excluded from the calculation of the correlation coefficient.

or apoproteins A-I, A-II, C-II, C-III, or the d > 1.21 plasma fraction. All six VLDL preparations (d < 1.006 plasma fractions) gave significant displacement with Lp(a) concentrations of 0.5 ± 0.1 mg/dl (mean ± SD).

The sigmoidal curve formed by plotting percent bound (B/Bo) vs. log antigen concentration was linearized by applying the logit transformation to B/Bo. The average correlation coefficient (r) of logit B/Bo versus antigen concentration (using least squares regression) was 0.996 for six assays. The percentage of antibody-bound ^{125}I-Lp(a) in the absence of added unlabeled Lp(a) was 58 ± 10 for six assays.

The Lp(a) content of a plasma sample appeared to be independent of dilution of antigen in the assay. Human plasma was diluted 1:5, 1:10, 1:20, 1:30, 1:40, 1:60, 1:80, 1:90, 1:100, and 1:200 in six assays. The average slope of the line formed by plotting logit B/Bo versus log plasma concentration was 0.77, nearly identical with that of the standards in the same assay (0.76).

Interassay variation was calculated from the values of a plasma sample that was included in five assays. The value of the sample showed a coefficient of variation of 7.3% with no systematic changes over a 4 month period. Within-assay variation, determined by analyzing a sample 12 times in each of five assays and calculating the coefficient of variation in the sample values, was 6 ± 3%.

Comparison of RID with RIA

Plasma samples from 55 fasting subjects, found to be positive for Lp(a) by gel diffusion, were assayed for Lp(a) lipoprotein by both the RID and the double antibody RIA procedures. As indicated in **Fig. 1**, Lp(a) lipoprotein as determined by the RIA procedure was highly correlated with the level of plasma Lp(a) determined by the RID method (r = 0.98, n = 51). The slope of the regression line was 1.08, indicating the absolute identity of the results obtained by the two methods. Each of the four plasmas excluded from the regression had Lp(a) levels greater than 39 mg/dl by RID.

RID "negative" samples

Of the 1000 samples assayed for Lp(a) by radial immunodiffusion, 89 did not show a detectable precipitin ring. These 89 samples were assayed by the more sensitive double antibody RIA at a 1:4 dilution. Eighty-eight samples had measurable amounts of Lp(a) (>0.5 mg/dl). The distribution of Lp(a) levels for these samples was slightly skewed with a mode between 2 and 2.5 mg/dl (**Fig. 2**). The single subject without detectable levels of Lp(a) was a subject with abetalipoproteinemia demonstrated to have undetectable apoprotein B by a sensitive double antibody radioimmunoassay (15, 16).

Relationship of Lp(a) to apolipoprotein A

Lp(a) levels have been shown previously (7) not to be significantly correlated with apolipoprotein B, triglyceride, or net cholesterol (total cholesterol minus Lp(a) cholesterol) (7). In this study Lp(a) was not
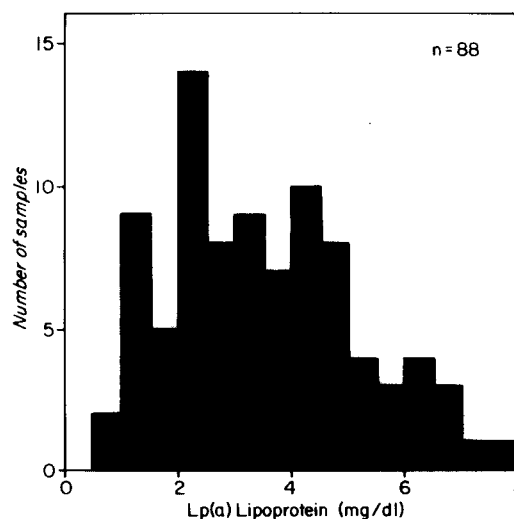

Fig. 2. The distribution of Lp(a) lipoprotein assessed by radioimmunoassay in 88 samples in which Lp(a) could not be detected in whole plasma by immunodiffusion.

correlated with apolipoprotein A-I in either the MI survivors or spouses ($r = -0.16$ and -0.01, respectively). Furthermore, Lp(a) levels were not correlated with A-II in either group (MI survivors, $r = -0.15$; spouses, $r = -0.19$).

Myocardial infarction group

The plasmas of a group of 90 myocardial infarction survivors and their spouses were assayed for Lp(a) lipoprotein by the double antibody radioimmunoassay and their levels compared with those of a group of 340 "healthy" adults who had previously been assayed by RID (5). The Lp(a) lipoprotein levels of the spouses were not significantly different from
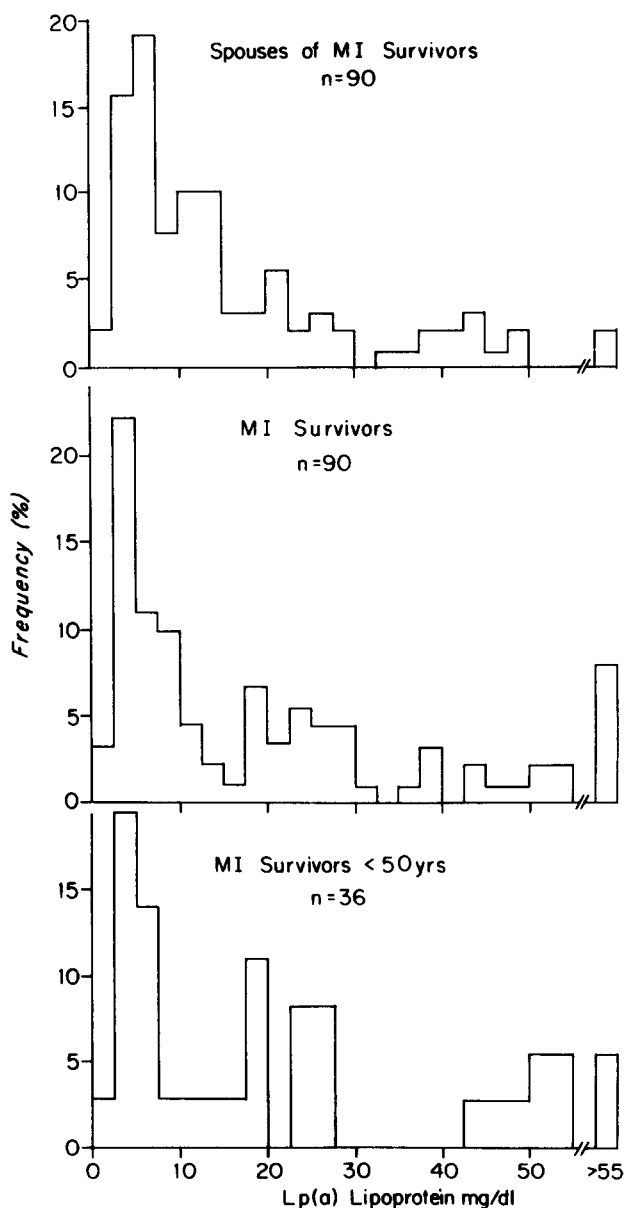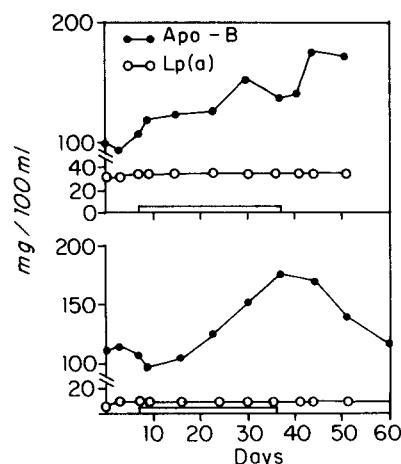

Fig. 4. Relationship of Lp(a) lipoprotein to apoprotein B. Closed circles ● —— ●, apoprotein B; open circles O —— O, Lp(a) lipoprotein; open bar ⬜, dietary period supplemented with 36 g cholesterol/day.

those of the "healthy" adults at any of the percentile cutoffs examined using the Wilcoxon signed rank test for nonparametric distributions (17). However, for the 50th percentile and above, the Lp(a) distribution of the myocardial infarction survivors was significantly shifted to higher levels than the spouse controls, **Fig. 3** ($P < 0.02$). Among the 90 MI survivors, 36 suffered their MI when they were less than 50 years old. These subjects had significantly higher levels ($P < 0.02$) than those subjects who had their MI in the 6th and 7th decades.

Physiological variation of Lp(a) levels

The week-to-week variation of Lp(a) levels was determined by RIA in seven healthy hospital employees for 8–10 weeks. The immunoassay was performed every third week. The levels in these subjects had a mean coefficient of variation of 9.7% (range 12–6%) with no systematic changes in Lp(a) levels. Repeated analysis of Lp(a) levels on the plasma samples or the quality control pool over the 10-week period confirmed the previous conclusion (5) that Lp(a) levels of plasma samples do not change significantly if stored at 4°C in sealed Wheaton vials with 0.05% sodium azide. Assuming an interassay variation of approximately 7%, as determined from the quality control plasma samples, these subjects exhibited little or no physiological variation in fasting Lp(a) levels over the period of study. Furthermore, Lp(a) levels were determined in two subjects who underwent 28 days of high egg yolk cholesterol feeding. Biweekly Lp(a) analysis during an 8-week period showed that their Lp(a) levels remained essentially constant whereas apoprotein B levels increased dramatically in response to cholesterol feeding (**Fig. 4**).

Fig. 3. The distribution of Lp(a) lipoprotein in myocardial infarction survivors and their spouses.

Albers, Adolphson, and Hazzard **Human plasma Lp(a) lipoprotein** 335

Lp(a) in nonhuman primates

Plasma samples from a patas monkey (*Erythrocebus*), 13 baboons (*Papio papio*) and 7 pigtail monkeys (*Macaca nemestrina*) were assayed for Lp(a) by the radial immunodiffusion method using human Lp(a) as standard. All primate sera examined gave precipitin bands with rabbit anti-human Lp(a) sera, which formed lines of identity with human Lp(a). The patas monkey plasma, 4 of 13 baboon plasmas, and 6 of 7 pigtail monkey samples gave precipitin rings by the standard RID method with mean concentrations equivalent to 21, 23 ± 12, and 28 ± 6 mg/dl for the patas monkey, baboon and pigtail monkey, respectively. The concentration of monkey Lp(a) by the RID procedure appeared independent of plasma dilution. In order to compare the cross-reactivity of the Lp(a) of these primates with that of human Lp(a), dilutions of the plasmas were tested for their ability to compete with human ^{125}I-Lp(a) for anti-human Lp(a). The displacement curves (**Fig. 5**) of the nonhuman primate plasmas were not parallel to that of human plasma, indicating the immunological dissimilarity of human Lp(a) from nonhuman primate Lp(a). The affinity of human Lp(a) for anti-Lp(a) was greater than that of all three nonhuman primate species. Assuming that the RID method using heterologous antisera is a valid estimate of Lp(a) levels, approximately 9 times more pigtail monkey Lp(a) and 170 times more baboon Lp(a) than human Lp(a) were needed to displace 50% of the human ^{125}I-Lp(a) from its homologous antibody. The patas monkey plasma did not compete with human Lp(a) in the dose range utilized.
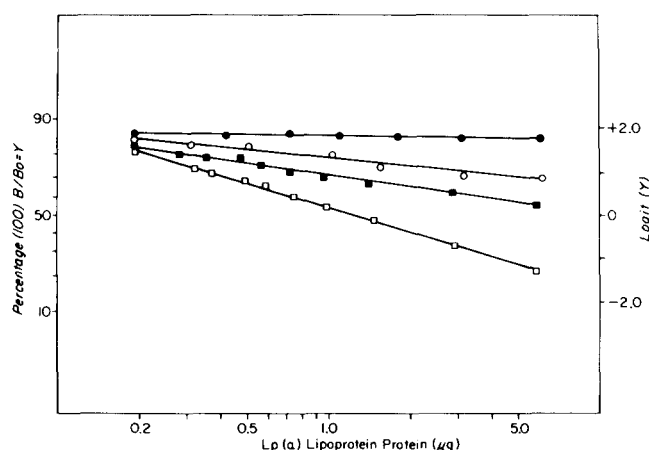

Fig. 5. Competitive displacement of human ^{125}I-Lp(a) from anti-human Lp(a) by human plasma (open squares), pigtail monkey plasma (closed squares), baboon plasma (open circles), and patas monkey plasma (closed circles). Right ordinate, logit Y, and left ordinate Y or 100 (B/Bo) on logit scale.

DISCUSSION

We previously described a simple radial immunodiffusion assay (RID) for the Lp(a) lipoprotein (5). However, the RID assay was concentration-dependent, assay variation increased sharply for concentrations below 8 mg/dl (6), and the precipitate rings were barely discernable for Lp(a) levels <4 mg/dl. Therefore, we have developed a more sensitive double antibody radioimmunoassay.

The described radioimmunoassay, though technically more difficult and time-consuming, appears to meet exacting requirements of specificity and precision. First, Lp(a) could be quantitated in all plasma samples (excepting that from a single subject with abetalipoproteinemia). Second, less than 3% of the total radioactivity was displaced by other apoproteins, including apoprotein B, or the d > 1.25 plasma fraction. Third, the inter-assay coefficient of variation was less than 8%, provided that the plasma dilutions were optimized by first estimating the Lp(a) level by the RID procedure. Since Lp(a) values vary over a wide range and RIA assays exhibit heteroscedasticity (error variance that is nonhomogeneous and dependent on antigen dose), we recommend that the Lp(a) concentration of each plasma sample first be estimated by RID prior to RIA analysis. When so performed, the RID and RIA methods gave comparable plasma Lp(a) values on nearly all samples, even though they are based upon different principles. The RID procedure depends upon diffusion and precipitation, and thus antigen concentration is inversely related to antigen size, whereas the RIA procedure depends upon inhibition of antigen binding and depends upon antigen affinity and antibody accessibility to the antigenic site rather than antigen size (14, 18). The good agreement between the two methods suggests that: *1*) the accessible Lp(a) antigen is a reasonably constant average fraction of the Lp(a) lipoprotein molecules and *2*) the Lp(a) lipoproteins from different donors usually have comparable size distributions and/or Lp(a) antigen accessibility is inversely related to Lp(a) lipoprotein size. It is possible that the RID procedure underestimated the four samples with RID values >39 mg/dl because these samples had larger than average size distributions.

Early studies (1, 2) were interpreted to indicate that the Lp(a) lipoprotein is a qualitative genetic trait, present in some individuals but absent in others. These conclusions were based upon relatively insensitive qualitative double gel diffusion studies. In our studies, by optimizing the gel diffusion methods, we detected Lp(a) in 91% (911 of 1000) of the plasma

samples tested. Of the remaining 89 samples that were Lp(a) negative by the RID procedure, 88 were shown to contain detectable levels by the more sensitive radioimmunoassay technique. Thus, the present study directly confirms our previous suggestions (5, 6) that: *1*) Lp(a) lipoprotein is present in the plasma of all or nearly all individuals; and *2*) Lp(a) concentrations exhibit continuous variation and should therefore be considered a quantitative or metric character. The only individual who lacked the Lp(a) lipoprotein was a subject with abetalipoproteinemia, a rare genetic disorder in which apolipoprotein B is absent. This intriguing observation suggests that apolipoprotein B is necessary for the synthesis and/or secretion (i.e., plasma transport) of the Lp(a) lipoprotein. Consistent with this hypothesis was our finding that essentially all Lp(a) molecules from the six Lp(a) preparations contain both the Lp(a) antigen and apolipoprotein B. The observation that Lp(a) levels remain essentially constant during a 28 day period of controlled cholesterol feeding whereas the apoprotein B levels increased dramatically support the previous suggestion (7) that the Lp(a) lipoprotein and low density lipoprotein are metabolically independent even though they both contain the same structural protein, apoprotein B.

Some sera, from 22 to 67% of those examined, from four nonhuman primate species, (chimpanzee, orangutan, baboon and rhesus) have been reported to react with anti-Lp(a) sera in gel diffusion studies (2). Also, a recent report has demonstrated reactivity with patas monkey plasma (19). It was therefore of interest to learn that all plasmas from all the nonhuman primates examined (patas monkey, baboon, and pigtail monkey) contained material that could be precipitated with anti-human Lp(a) sera. This supports the likelihood that all primates, like man, have the Lp(a) lipoprotein. However, plasma from each of the primates with nearly identical apparent Lp(a) levels by RID were quite different in their ability to displace human ^{125}I-Lp(a) from anti-Lp(a). This observation, though superficially puzzling, is quite plausible if one considers that the two immunochemical methods depend on different principles (see above). Furthermore, the results point out the potential pitfall in quantitating heterologous antigens (i.e., antigens from a species different from that from which the standard was prepared) by radioimmunoassay. For example, if the patas monkey had been tested only by the RIA procedure, no Lp(a) antigen would have been detected. The RID method may also not be appropriate for quantitating heterologous antigens.

From studies in which an insensitive qualitative gel diffusion method was used, it has been reported that only 30–40% of the Caucasian population have detectable Lp(a) lipoprotein or, by past convention, are considered Lp(a+) (1, 2). In a more recent study using comparable gel diffusion methods, 31% (19 of 61) of a group of healthy Finns were considered Lp(a+) while 53% (53 of 100) of subjects with proven coronary heart disease were Lp(a+) (20). Presumably those plasmas not considered Lp(a+) had Lp(a), but at levels below the threshold of detection by the gel diffusion method employed. The threshold of detection for the method approximates the 69th percentile cut-point for the healthy population since 31% had detectable levels. If we assume that Lp(a) levels in the healthy Finnish population are comparable to those previously reported in a population of 340 unrelated fasting adults from the U. S. (5) then the 69th percentile would be 19 mg/dl. Consistent with these data, we found that 31% (28 of 90) of the spouse controls had Lp(a) levels >19 mg/dl while 42% (38 of 90) of the MI survivors and 47% (17 of 36) of the MI survivors less than 50 years old had levels above this arbitrary threshold level (spouse vs. all MI, $x^2 = 5.24$, $0.025 < P < 0.010$). The 95th percentile cutoff is often considered the threshold value between normal and abnormal. We found 4% (4 of 90) of the spouses and 14% (13 of 90) of the MI subjects and 19% (7 of 36) of the MI subjects under 50 years old with Lp(a) levels above this threshold level of 47 mg/dl, referred to as hyper Lp(a) lipoproteinemia. Thus, the MI group had significantly more subjects with hyper Lp(a) lipoproteinemia then the spouses (spouses vs. all MI, $X^2 = 25.5$, $P < 0.001$; spouses vs. MI < 50, $x^2 = 20.9$, $P < 0.001$). Our quantitative Lp(a) analysis showed that the MI survivors differed from the control group at a 50th percentile cutoff and above, while the group who had an MI at age <50 had even higher levels. The above results suggest that high Lp(a) levels may be positively associated with coronary disease and, most importantly, demonstrate that this association is even stronger for subjects with premature coronary heart disease. However, it is possible that the onset of coronary heart disease or an acute myocardial infarction may cause an increase in Lp(a) levels. Thus, the predictive value of Lp(a) levels for premature heart disease needs to be evaluated.■

REFERENCES

1. Berg, K. 1963. A new serum type system in man—The Lp system. *Acta Pathol. Microbiol. Scand.* **59:** 369–382.
2. Berg, K. 1968. The Lp system. *Ser. Haematol.* **1:** 111–136.
3. Albers, J. J., C. H. Chen, and F. Aladjem. 1972. Human serum lipoproteins. Evidence for three classes of lipoproteins in S$_f$ 0–2. *Biochemistry.* **11:** 57–63.
4. Ehnholm, C., H. Garoff, O. Renkonen, and K. Simons. 1972. Protein and carbohydrate composition of Lp(a) lipoprotein from human plasma. *Biochemistry.* **11:** 3229–3232.
5. Albers, J. J., and W. R. Hazzard. 1974. Immunochemical quantification of human plasma Lp(a) lipoprotein. *Lipids.* **9:** 15–26.
6. Albers, J. J., P. Wahl, and W. R. Hazzard. 1974. Quantitative genetic studies of the human plasma Lp(a) lipoprotein. *Biochem. Genet.* **11:** 475–486.
7. Albers, J. J., V. G. Cabana, G. R. Warnick, and W. R. Hazzard. 1975. Lp(a) lipoprotein: relationship to sinking pre-β lipoprotein, hyperlipoproteinemia and apolipoprotein B. *Metabolism.* **24:** 1047–1054.
8. Albers, J. J., and F. Aladjem. 1971. Precipitation of I-125 labeled lipoproteins with specific polypeptide antisera. Evidence for two populations with differing polypeptide compositions in human high density lipoproteins. *Biochemistry.* **10:** 3436–3442.
9. Albers, J. J., and S. Dray. 1969. Allelic exclusion and phenogroup expression in individual molecules of rabbit low-density lipoprotein allotypes. *J. Immunol.* **103:** 163–169.
10. Bilheimer, D. W., S. Eisenberg, R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
11. Lowry, V. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
12. Albers, J. J., P. W. Wahl, V. G. Cabana, W. R. Hazzard, and J. J. Hoover. 1976. Quantitation of apolipoprotein A-I of human plasma high density lipoprotein. *Metabolism.* **25:** 633–644.
13. Lipid Research Clinics Program Manual Laboratory Operations. 1974. DHEW Publication No. (NIH) 75-628, Vol. 1.
14. Albers, J. J., and M. C. Cheung. 1976. Quantitation of apolipoprotein A-II of human plasma high density lipoprotein. *Circulation.* Suppl. 2, **54:** 367.
15. Albers, J. J., V. G. Cabana, and W. R. Hazzard. 1975. Immunoassay of human plasma apolipoprotein B. *Metabolism.* **24:** 1339–1351.
16. Rachmilewitz, D., J. J. Albers, and D. R. Saunders. 1976. Apoprotein B in fasting and postprandial human jejunal mucosa. *J. Clin. Invest.* **57:** 530–533.
17. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. The Iowa State University Press, Ames, Iowa. 3–593.
18. Schonfeld, G., and B. Pfleger. 1974. The structure of human high density lipoprotein and the levels of apolipoprotein A-I in plasma by radioimmunoassay. *J. Clin. Invest.* **54:** 236–246.
19. Mahley, R. W., K. H. Weisgraber, T. Innerarity, and H. B. Brewer, Jr. 1976. Characterization of the plasma lipoproteins and apoproteins of the *Erythrocebus patas* monkey. *Biochemistry.* **15:** 1928–1933.
20. Dahlen, G., M. H. Frick, K. Berg, M. Valle, and M. Wiljasalo. 1975. Further studies of Lp(a) lipoprotein/pre-β lipoprotein in patients with coronary heart disease. *Clin. Genet.* **8:** 183–189.