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Studies of Lp-Lipoprotein as a Quantitative Genetic Trait*

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Abstract. Sera from 11 individuals which were classified as Lp(a−) by direct gel diffusion and by absorption tests showed the presence of Lp(a) lipoprotein when the sera were concentrated 120-fold. This Lp(a) antigen was demonstrated by gel diffusion to be indistinguishable from Lp-lipoprotein isolated from known Lp(a+) sera. The Lp(a) preparations from sera classified Lp(a−) also showed electrophoretic mobility in immunoelectrophoresis and schlieren diagrams in ultracentrifugal analyses similar to Lp-lipoprotein from Lp(a+) sera. The proposal is made that observed individual variations in tests for the Lp-lipoprotein reflect a quantitative genetic trait and that it is likely that different individuals produce Lp-lipoprotein in widely varying amounts. The consistency of this proposal with certain previous observations on the Lp system is discussed.

Introduction. The Lp(a) antigenic factor was first described by Berg[1] as a polymorphic genetic variant of human serum β-lipoprotein detected by immunodiffusion with specific antiserum and inherited as a simple autosomal dominant trait. The Lp-lipoprotein differs from other such polymorphic proteins in that it has been detected only by heteroimmune antisera. Although anti-Ag antibodies, which detect another genetically independent polymorphism in β-lipoprotein, are usually found in multiply transfused individuals,[2] isoimmune anti-Lp(a) has not been reported. There have been reports of quantitative differences in the level of the Lp factor in sera from Lp(a+) individuals.[3, 4] Also, several instances have been reported[5, 6] in which Lp(a+) children, not apparently illegitimate, have resulted from matings where parents were both classified as Lp(a−). For these reasons it seemed possible that the Lp-lipoprotein may be present in all individuals but in widely varying amounts, with individuals classified as Lp(a−) having a serum level of the factor below the threshold for detection. If this were the case, the Lp variation among individuals would have to be viewed as a quantitative genetic trait. In order to evaluate this hypothesis, several whole sera which had been classified by routine immunodiffusion methods as Lp(a−) were reexamined for the presence of Lp-lipoprotein in serum subfractions in which Lp-lipoprotein, if present, would be highly concentrated. This report describes the study of some of the Lp(a−) sera that did show the presence of the Lp-lipoprotein in highly concentrated serum subfractions. In addition, a method for preparation of Lp-lipoprotein using dextran sulfate precipitation is described.

Materials and Methods. Specific Lp(a) antisera were prepared and absorbed and gel diffusion analyses were performed as previously described.[7]

Immunoelectrophoresis was performed by a modification of the method of Sheidegger[8] using Gelman immunoelectrophoresis equipment (Ann Arbor, Mich.). Six slides were run at one time at approximately 10 ma (400 V) for 1 hr according to the Gelman handbook instructions. Slides were washed, dried, and stained with amido-schwartz 10B reagent.[9]

Preparation of lipoproteins: Fresh human serum was obtained from adult male donors and kept at 2 to 10°C throughout the isolation procedure. Disodium ethylene diaminetetraacetate (Na_2EDTA) adjusted to pH 7.0 was added to 0.01 to 0.03%. Salt solutions were prepared as previously described.[7] Beta (LDL) and Lp-lipoproteins were precipitated from 200 to 250 ml of whole serum by addition of 40 mg of dextran sulfate (mol wt 2×10^6, Pharmacia, Uppsala, Sweden) per deciliter of serum. This mixture was allowed to interact for 3 hr with occasional gentle mixing, and then centrifuged at 2500 *g* for 20 min. The precipitate was separated and redissolved in an approximately equal volume of NaBr solution (15.6 gm/dl, $\rho = 1.12$) and then dialyzed overnight against NaBr of this same concentration.

Isopycnic density-gradient ultracentrifugation was used for isolation of Lp-lipoprotein and for its separation from as much of the non-Lp beta (LDL) as possible. The gradients were made in no. 40 Spinco centrifuge tubes by adding 4.2 ml of lipoprotein solubilized in NaBr density 1.12, and then successively layering 4.2 ml of NaBr, density 1.085, and NaBr, density 1.04. These gradients were centrifuged in a Spinco model L ultracentrifuge (Spinco Division, Beckman Instruments, Palo Alto, Calif.) for 24 hr at 100,000 *g*. The linearity of the gradients was independently demonstrated by refractive index measurements on NaBr gradients in the absence of protein, using a Bausch and Lomb (Rochester, N.Y.) dipping refractometer.

After centrifugation, 13 fractions of 1 ml each were isolated by cutting the tubes with a specially designed tube cutter.[10] The Lp-lipoprotein is found in fractions 6 through 9, numbering from the top of the tube. When sera which were classified as Lp(a−) were being fractionated, fractions 6 through 9 were concentrated by pervaporation to a final volume of 1 to 2 ml or at least a 120-fold concentration of the material in these fractions relative to the original serum. Whole sera classified as Lp(a+) were fractionated by the same method without pervaporation, resulting in a 20-fold increase in concentration as compared with whole serum. The concentrated fractions were dialyzed against NaBr, density 1.004, in preparation for analytical ultracentrifugation in the Spinco model E ultracentrifuge at 60 K, 20°C.

Results. Gel immunodiffusion, immunoelectrophoresis, and analytical ultracentrifugation were employed to test for the presence of Lp-lipoprotein in the preparations from sera which typed Lp(a−) by usual test procedures, as well as those which typed Lp(a+).

Figure 1*a* shows gel diffusion tests of whole sera from three individuals classified as Lp(a−) and one classified as Lp(a+). Figure 1*b* shows the gel diffusion pattern of 20-fold concentrated, partially purified preparations of the same three Lp(a−) sera and the Lp(a+) serum. Figure 1*c* shows the reactions of the same Lp(a−) sera after approximately 120-fold concentration. (The Lp(a+) preparation in Fig. 1*c* is the same as that used for tests shown in Fig. 1*b*, i.e., a 20-fold concentrated preparation.) Only the preparations from Lp(a−) sera which had been concentrated 120-fold showed precipitin reactions with anti-Lp(a) antiserum (Fig. 1*c*). All three such concentrated preparations gave precipitin lines and showed reactions of identity with the Lp(a+) preparation.

Figures 2*a* and *b* show immunoelectrophoretic patterns of two 120-fold concentrated Lp(a−) preparations. There is a faint but definite reaction of these preparations with anti-Lp(a) antiserum. The electrophoretic mobility of the

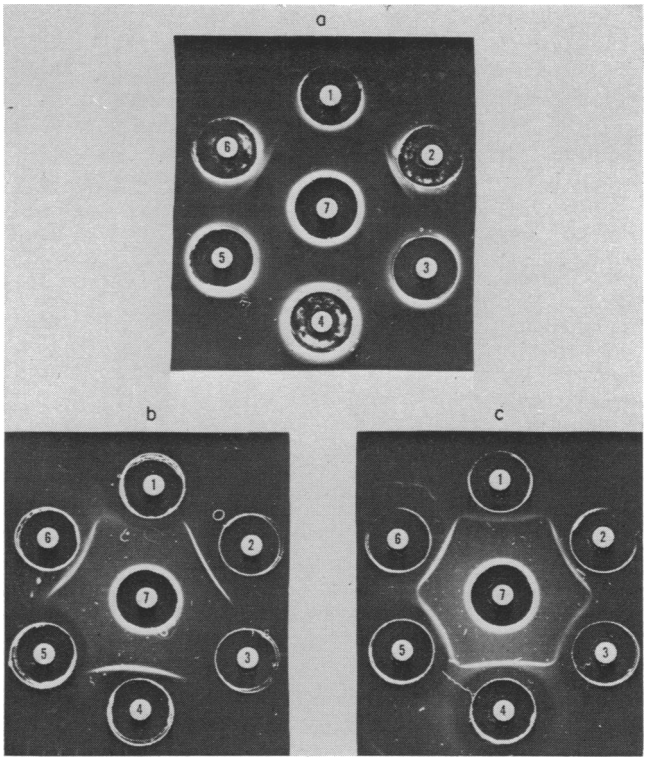

FIG. 1.—Agarose gel diffusion tests.

(*a*) *Wells 2, 4, 6*—Lp(a+) serum; *Wells 1, 3, 5*—three sera classified Lp(a-); *Well 7*—anti-Lp(a+) serum.

(*b*) *Wells 2, 4, 6*—Lp(a+) serum concentrated 20-fold during isolation procedure; *Wells 1, 3, 5*—same sera as shown in Fig. 1*a*, but concentrated 20-fold during isolation procedure; *Well 7*—anti-Lp(a+) serum.

(*c*) *Wells 2, 4, 6*—Lp(a+) serum concentrated 20-fold during isolation procedure; *Wells 1, 3, 5*—same sera shown in Figs. 1 and 2, except concentrated 120-fold during isolation procedure; *Well 7*—anti Lp(a+) serum.

antigen from sera classified Lp(a−) appeared to be the same as that of the antigen from the Lp(a+) serum.

Figure 3 shows analytical ultracentrifuge diagrams of three Lp preparations. Figures 3*a* and *b* show preparations concentrated 20-fold (*a*) and 120-fold (*b*) from one Lp(a−) serum. Figures 3*c* and *d* show preparations concentrated 20-fold (*c*) and 120-fold (*d*) from another Lp(a−) serum. Figure 3*e* shows a 20-fold concentration of a preparation from the Lp(a+) serum.

Small peaks which travel at approximately the same rate as the faster peak in the Lp(a+) preparation (Fig. 3*e*) can be seen in one 20-fold and both 120-fold concentrated preparations from sera originally typed as Lp(a−). This peak is absent in preparations from sera typed Lp(a−) but not concentrated.

Eleven sera that were typed Lp(a−) and subjected to the same concentration and test procedures described above showed the presence of the Lp-lipoprotein in preparations concentrated approximately 120-fold.

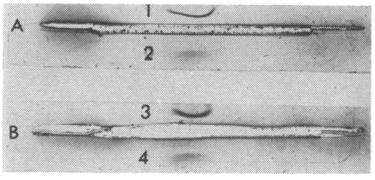

FIG. 2.

FIG. 2.—Immunoelectrophoresis of Lp-antigen preparations. *Troughs A* and *B* contain anti-Lp(a+) serum. *Wells 1* and *3* contain an Lp(a+) antigen preparation. *Wells 2* and *4* contain two different 120-fold concentrated Lp antigens prepared from sera which, unconcentrated, classified Lp(a−).

FIG. 3.—Analytical ultracentrifuge diagrams of Lp preparations. (*a*) and (*c*) 20-fold concentrated preparations from two sera classified Lp(a−). (*b*) and (*d*) 120-fold concentrated preparations from the same two sera seen in Figs. 3*a* and *b*. 3*e* 20-fold concentrated preparation from serum classified Lp(a+).

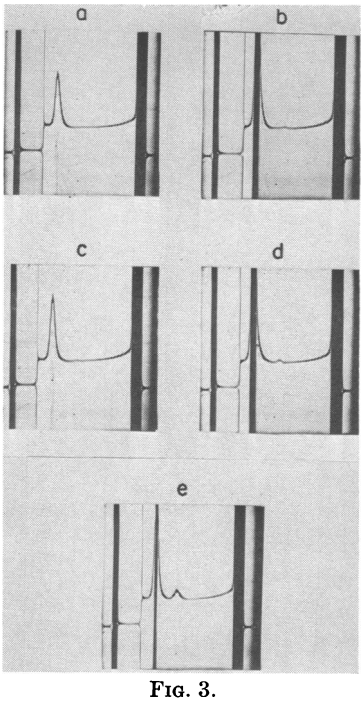

FIG. 3.

Discussion. The presence of a lipoprotein indistinguishable from Lp-lipoprotein in nominally Lp(a−) sera has been demonstrated by three independent tests: (1) the presence of a lipoprotein giving a reaction of identity in gel diffusion tests with antigen from an Lp(a+) serum; (2) the presence of a lipoprotein possessing electrophoretic mobility which appears to be identical with that of the Lp(a+) antigen on immunoelectrophoretic test; and (3) the presence of a small peak traveling at a similar rate as Lp-lipoprotein peaks in analytical ultracentrifuge studies.

These data support our suggestion that the individual differences being detected in tests for the Lp-lipoprotein are the result of quantitative variations in serum level of the lipoprotein. It seems probable that all individuals produce some Lp-lipoprotein which, in sera classified Lp(a−), can be detected only after partial purification and, most important, 20- to 120-fold concentration of the preparation. If this proposition is valid it could explain several interesting observations made previously with regard to the Lp system. Namely:

(1) All anti-Lp sera reported to date are heteroimmune. No isoimmune anti-Lp antisera have been found, even in multiply transfused individuals who often make antibodies to the Ag lipoprotein antigen.[11]

(2) There is a wide variation in reported frequencies of Lp(a+) individuals in comparable populations. Our studies show an Lp(a+) frequency of approximately 50% in Caucasian populations, while other investigators[12] report frequencies of about 35%. These variations could be due to differences in the strength of antisera which detect different minimum levels of Lp(a) factor.

(3) In our earlier work with this system,[7] a serum that was classified as Lp(a−) was found to induce Lp(a) antibodies when injected into a rabbit over a prolonged immunization schedule.

(4) Attempts to find antibody antithetical to anti-Lp(a) by immunization with Lp(a−) sera have failed in our laboratory and others.[13]

(5) Observations of wide quantitative variation among sera classified as Lp(a+) have previously been reported.[3]

Although we have tested only a limited number of "Lp(a−)" sera by our concentration procedures, it is significant that Lp(a) lipoprotein was found in all of the 11 cases examined. The relatively large amount of serum needed for the concentration procedure precludes routine examination of large numbers of Lp(a−) sera. However it appears reasonable at this point to conclude that most, if not all, sera classified by the usual immunodiffusion methods as Lp(a−) do, in fact, contain very low levels of a lipoprotein indistinguishable from the Lp-lipoprotein found in Lp(a+) sera. Qualitative differences cannot, of course, be entirely ruled out with present data. However, it seems clear that the genetic control of this trait must not involve merely determination of simple presence or absence of the factor as previously suggested,[1] but determination of Lp-lipoprotein serum levels.

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