Expression of Human Apolipoprotein B90 in Transgenic Mice

DEMONSTRATION THAT APOLIPOPROTEIN B90 LACKS THE STRUCTURAL REQUIREMENTS TO FORM LIPOPROTEIN(a)*

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Lipoprotein(a) (Lp(a)) is a lipoprotein formed by the disulfide linkage of apolipoprotein(a) (apo(a)) to the apoB100 of a low density lipoprotein particle. Earlier site-directed mutagenesis studies of apo(a) demonstrated that apo(a) cysteine 4057 is required for the disulfide linkage; however, the cysteine residue within apoB100 that is involved in the disulfide bond has not been identified. We previously demonstrated that the apoB100 produced by human apoB transgenic mice binds to apo(a) and forms Lp(a) (Linton, M. E., Farese, R. V., Jr., Chiesa, G., Grass, D. S., Chin, P., Hammer, R. E., Hobbs, H. H., and Young, S. G. (1993) J. Clin. Invest. 92, 3029–3037). To further explore the structural features of human apoB that are required for the formation of Lp(a), we used a transposon-interrupted human apoB gene clone to develop transgenic mice that express high levels of a truncated form of human apoB, apoB90, which contains the amino-terminal 10% of apoB containing amino acid sequences that are essential for the formation of Lp(a).

Lipoprotein(a) (Lp(a)) is a lipoprotein that can be found in the plasma of humans as well as old world monkeys (1–3). It consists of a complex between the apolipoprotein (apo) B100 of a low density lipoprotein (LDL) particle and apolipoprotein(a) (apo(a)), a glycoprotein that is homologous to plasminogen, a serine protease. High concentrations of Lp(a) in the plasma have been shown to be a strong risk factor for premature coronary heart disease in many human populations (4, 5), although it is noteworthy that several recent studies have failed to identify Lp(a) as a significant risk factor for coronary disease (6–8). The reason(s) for the atherogenicity of Lp(a) are not known with certainty, but several possible mechanisms have been suggested (3).

The structural features of apo(a) and apoB that lead to their association and formation of Lp(a) are incompletely understood. Strong evidence exists, however, that the association of apo(a) and apoB involves a disulfide bond between cysteine residues of the two molecules. Although Lp(a) cannot be dissociated into apo(a) and LDL-apoB100 by either chaotropic agents or by heating to 100 °C (9), Lp(a) can be completely dissociated by disulfide reducing agents such as dithiothreitol (10, 11). Studies with reducing agents cannot, however, prove the existence of a sulfhydryl linkage because both apoB100 and apo(a) also have multiple internal disulfide bonds (12, 13), and it could be argued that reducing agents could dissociate apo(a) and apoB100 by breaking internal disulfide bonds, thereby altering the conformation of one or both molecules and interfering with a noncovalent interaction. Stronger evidence in favor of a disulfide linkage has come from recent functional studies of mutant apo(a) molecules in which the best candidate for a free cysteine residue, Cys4057 (13), was changed to other amino acid residues (14, 15). Kochinsky et al. (15) and Brunner et al. (14) mutated Cys4057 to several other amino acid residues and then expressed the mutant apo(a) proteins in cultured cells. Unlike native apo(a), the mutant apo(a) did not bind to human LDL-apoB100 and form Lp(a). Although one still could argue that the substitution of other amino acid residues for Cys4057 might alter the conformation of the apo(a) molecule, these mutagenesis experiments, taken together with the earlier studies, constitute persuasive evidence that apo(a) and apoB100 are disulfide-linked.

The structural features of apoB100 that are essential for its interaction with apo(a) have not yet been identified. Most investigators presume that a specific domain of apoB100 binds to apo(a), thereby bringing Cys4057 of apo(a) into close proximity with a free cysteine of apoB100 to facilitate the formation of a disulfide bond. Trieu and McConathy (16) have reported the existence of hydrophobic interactions between LDL and apo(a) that may be relevant to the formation of Lp(a), but to date neither the specific region of the apoB100 molecule that binds to apo(a) nor the apoB cysteine residue involved in the disulfide linkage has been identified with certainty. Molecular modeling

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§ The abbreviations used are: Lp(a), lipoprotein(a); apo, apolipoprotein; LDL, low density lipoprotein; LB, Luna-Bertani broth; RIA, radioimmunoassay; BSA, bovine serum albumin; VLDL, very low density lipoprotein.

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Human ApoB90 Cannot Form Lp(a)

Human apo(a) transgenic mice are capable of interacting with apo(a) to form bona fide Lp(a) particles (20). Expression of human apo(a) in the mouse is useful for studying apo(a)-human apoB interactions because the endogenous mouse apoB lacks the structural features required for complexing with apo(a) (9). To examine the structural features of apoB required for the formation of Lp(a), we generated transgenic mice expressing a truncated form of human apoB, apoB90, and then tested the capacity of apoB90 to form Lp(a) particles.

MATERIALS AND METHODS

Transgenic Mice—Human apo(a) transgenic mice (9) were obtained from Robert Hammer (University of Texas Southwestern Medical Center, Dallas, TX). We previously generated transgenic mice expressing human apoB100 by microinjecting murine syngeneic metaphase plate clones with a P1 bacteriophage carrying the human apoB gene (20). For the in vitro assays of Lp(a) formation, we used the plasma of the F1 offspring of human apoB transgenic founder 102D; the mean concentration of human apoB100 in the plasma of chow-fed female and male mice was 63 and 39 mg/dl, respectively. For the breeding experiments with apo(a) transgenic mice, we used the F1 offspring of the human apoB transgenic founder 1095. The human apoB100 level in these mice was ~8 mg/dl.

To generate transgenic mice expressing human apoB90, we interrupted p158 (the 5′ and 3′ ends of which are derived from natural human apoB90) with a mini-Tn10 transposon containing a neomycin resistance gene (from the plasmid pT2neo), obtained from Nat Sternberg of the DuPont Merck Pharmaceutical Company, Glenolden, PA, according to techniques outlined in a recent review (21). Briefly, Escherichia coli cells (strain NS3529) harboring the P1 clone, p158, were grown in Luria-Bertani broth (LB) containing 50 μg/ml of kanamycin (the P1 vector contains a kanamycin resistance gene). The cells were made competent and transformed with the transposon-containing plasmid, pZTneo5, which contains an ampicillin resistance gene. The transposable element within pZTneo5 contains a chloramphenicol resistance gene and an RSVneo gene. The transposable element also contains a NotI restriction site, which is useful for localizing the site of transposition. Following the transformation, the cells were plated on LB agar plates containing chloramphenicol, ampicillin, and kanamycin. A single colony was grown in 10 ml of LB containing chloramphenicol (25 μg/ml) and kanamycin (50 μg/ml) until an A600 of 0.05 was reached. Then, isopropyl-1-thio-&-D-galactosidase (Boehringer Mannheim) and purified from an Escherichia coli expression system.

To isolate E. coli colonies containing a transposon-interrupted p158 plasmid, various amounts of the phage-infected cells were spread onto LB agar plates containing chloramphenicol, ampicillin, and kanamycin. A total of 200 colonies were grown in LB containing kanamycin, and P1 plasmid DNA was isolated from the colonies using the QIAGEN-tip 500 columns (Qiagen, Inc., Chatsworth, CA) according to the manufacturer's procedures. To purify the insert of p158/neo8 for microinjection of murine syngeneic metaphase plate clones, the DNA was digested with MluI, a restriction enzyme that cleaves at four sites within the P1 vector sequences but does not cleave within the insert of p158/neo8. A 9-kilobase MluI fragment containing the transposon-interrupted apoB gene (and approximately 8 kilobases of P1 vector sequences) was purified from a 1% pulsed-field agarose gel by digesting the agarose with β-agarase according to the procedures described by Schedl et al. (23) for the purification of yeast artificial chromosome DNA. The β-agarase and all purification enzymes were purchased from New England Biolabs (Beverly, MA). After digestion with agarase, the DNA-containing solution was spun in a microcentrifuge at 14,000 rpm for 10 min to sediment undigested agarose gel fragments; the DNA was dialyzed against a TE (10 mM Tris, 1 mM EDTA) buffer containing 100 mM NaCl, 30 μg spermine, 70 μg spermidine and then adjusted to a concentration of 5 μg/ml. The purity of the insert DNA was determined by gel electrophoresis in the transgenic core facility of the Gladstone Institute of Cardiovascular Disease and by David Grass of DNX Biotherapeutics, Inc., at Princeton, NJ, according to standard techniques (24).

To identify transgenic founder animals, we analyzed mouse plasma for the presence of human apoB production by Western blot and/or by radiomunoassay (RIA), as described below. All mice were fed a mouse chow diet.

Monoclonal Antibodies—To identify human apoB in mouse plasma samples, we used two different apoB106-specific monoclonal antibodies, MB47 and MB43, which we previously had generated and characterized (5, 26). Antibody MB47 binds near amino acid 3500; antibody MB43 binds between amino acids 4027 and 4081 (27). The apoB-specific monoclonal antibody C1.4 (28), which has an epitope near amino acid 500, was provided by Gustav Schonfeld of the Washington University School of Medicine in St. Louis, MO. The apoB-specific monoclonal antibodies MB47, MB43, and C1.4 were obtained from the laboratory of Robert Hammer (University of Texas Southwestern Medical Center, Dallas, TX). The monoclonal antibody C1.4, which has an epitope near amino acid 500, was obtained from Francesco Mancini (University of Texas, Southwestern).

We generated another apo(a)-specific antibody, LPA6, for use in this study. To develop antibody LPA6, we isolated human LDL (d = 1.019–1.063 g/ml) from a human subject with high plasma levels of Lp(a). The LDL was isolated by ultracentrifugation for 24 h; the "dense" LDL that floated to the top of the ultracentrifuge tube was used to immunize mice. Fusions of splenic lymphocytes with P3Ag8.653.1 myeloma cells were performed as described previously (29). Hybridoma colonies were screened initially for binding to the immunogen by solid phase RIA. An apo(a)-specific antibody, LP4, was subsequently identified by its ability to bind to recombinant apo(a) (a gift of Richard Smith of J & J Biotechnology, La Jolla, CA). Antibody LPA6 is an IgG2a κ antibody and does not bind to human plasma in a solid phase RIA.

Radioimmunossays of Human ApoB—To measure the concentration of human apoB in the plasma of transgenic mice, human plasma lipoproteins, or the plasma fractions obtained from Superoxide 6 chromatography, we used the solid phase "sandwich RIA" described in detail by Linton et al. (20). Briefly, this RIA utilized two human apoB-specific monoclonal antibodies, MB47 and C1.4. Ninety-six-well polyvinyl chloride plates were coated with antibody MB47. After blocking the plates with bovine serum albumin (BSA), we added the samples to be tested to the plate and incubated them for 16 h at 4 °C. After the plates were washed, the human apoB that had been captured by the immobilized antibody MB47 was detected with 125I-labeled antibody C1.4. In experiments to assess the human apoB concentration in transgenic mouse plasma, specific apoB-containing mouse plasma fractions containing apo(a) and apoB90, the mouse polyvinyl chloride plates were coated with phosphate-buffered saline containing 2 μg/ml of monoclonal antibody 2D8 rather than antibody MB47. In this RIA, 125I-C1.4 was used to detect the apoB captured by the immobilized antibody 2D8.
sample buffer). The separated proteins were electrophoretically transferred onto a nitrocellulose membrane for Western blot analysis (30). Chromatographic Separation of the Plasma Lipoproteins—To analyze the distribution of cholesterol, triglycerides, and human apoB in the plasma of transgenic mice, plasma samples were fractionated on a Superose 6 10/50 column (Pharmacia Biotech Inc.) (20, 31). The cholesterol and triglyceride concentrations in each fraction was determined with kits from Abbott Diagnostics (North Chicago, IL) and Boehringer Mannheim, respectively. The relative content of human apoB in each fraction was assessed by the solid phase sandwich RIA (20).

Incubation of Transgenic Mouse Plasma with Apo(a)—The formation of Lp(a) in vitro was assessed according to the techniques described by Chiesa et al. (9). In this assay, recombinant human apo(a) from a human apo(a) transgenic mouse plasma (5 μl) was incubated with a sample of human apoB-containing lipoproteins (1 μg) in a 0.15 M NaCl solution (total incubation volume, 40 μl); after a 4-h incubation at 37 °C, the mixture was size-fractionated on a 4% SDS-polyacrylamide gel under nonreducing conditions as well as reducing conditions (incubated with 3% 2-mercaptoethanol for 10 min at 90 °C). The separated proteins were then electrophoretically transferred to a sheet of nitrocellulose membrane for Western blotting with a horseradish peroxidase-labeled anti-human apo(a) monoclonal antibody, IgG-1A2. Because Lp(a) migrates at a much higher molecular weight than the free apo(a), the formation of Lp(a) can be detected on an IgG-1A2 Western blot of the separated proteins at the high molecular weight band (and the disappearance of the lower molecular weight, free apo(a) band). We tested four samples for their capacity to form Lp(a) in this assay system. First, we tested the plasma of a human subject with homozygous hypobetalipoproteinemia (32) who exclusively synthesized apoB45.2 (and absolutely no apoB48 or apoB100). This subject has a small amount of Lp(a) that migrates at a slightly higher molecular weight than the free apo(a) band. We also tested the plasma of human apoB100 transgenic mice and the plasma of human apoB90 transgenic mice. As a positive control for Lp(a) formation, the apo(a) transgenic mouse plasma was incubated with human d < 1.063 g/ml lipoproteins, which were prepared from the plasma of a normolipidemic human subject by ultracentrifugation (5). Finally, as a negative control, the plasma of a nontransgenic mouse plasma. Before setting up the incubations, the concentration of apoB in each sample was determined by both the sandwich RIA and a Western blot analysis using monoclonal antibody 1D1 or MB43. Each of the incubations of apo(a) with the human apoB-containing samples contained equal amounts of apo(a) (5 μl of plasma) and apoB (1 μg). The monoclonal antibodies we used to assess apoB concentration bound apoB90 and apoB100 with equal affinity because no differences in the slopes of the apoB90 and apoB100 binding or displacement curves were noted in three different RIA formats. The first was a competition assay in which we determined the capacity of apoB100 samples to displace apoB90 from antibody 1D1. The second was a sandwich RIA using antibody MB43 as the capture antibody and 125I-C1.4 as the detecting antibody (20), and the third was an otherwise identical sandwich RIA using monoclonal antibody 2D8 as the capture antibody and 125I-C1.4 as the detecting antibody. All apo(a) incubations also were performed in the presence of 100 μM epsilon-aminocaproic acid (Sigma), a lysine analog that interferes with the binding of human LDL to apo(a) (34).

Lipoprotein (a) Sandwich RIA—To assess the formation of Lp(a) during the incubation of apo(a) with human apoB-containing lipoproteins, we used a sandwich RIA, using antibody LPA6 as the "capture" antibody and LPA1.4 to detect the Lp(a) that was captured by LPA6. Each well of the flexible polynyl chloride 96-well plates was incubated with 50 μl of phosphate-buffered saline containing LPA6 (5 μg/ml) for 16 h at 4 °C. The plates were washed four times with phosphate-buffered saline containing 0.1% RIA-grade bovine serum albumin, 0.05% Tween 20, and 0.4% sodium azide (SPRIA) and then incubated with 0.5 μl of 200 μl of SPRIA containing 2.0% BSA (SPRIA-BSA). A total of 5 μl of each of the in vitro Lp(a) incubation mixtures (described in the preceding two paragraphs) were adjusted to a volume of 50 μl with SPRIA-BSA and added to the LPA6-coated plates in triplicate. After a 16-h incubation at 4 °C, the plates were washed six times with SPRIA, and the wells were counted in a γ counter.

RESULTS AND DISCUSSION

In 1993 we generated human apoB100 transgenic mice, using a P1 bacteriophage clone, p158, that spanned the entire human apoB gene; these mice were bred with apo(a) transgenic mice to develop mice expressing high levels of Lp(a) (20). In this study, we interrupted p158 with a transposon to generate a mutant apoB gene clone, p158/neos, encoding a carboxyl-terminal truncated apoB protein, apoB90. Microinjection of the 91-kilobase MuI fragment of p158/neos into fertilized ICR eggs yielded 92 offspring, 12 of which expressed human apoB90 in their plasma. An additional six offspring were obtained from microinjected C57/B16 × SJL F1 hybrids; some of these animals expressed apoB90. The apoB90 concentration in the plasma of founder animals ranged from 5 to 60 mg/dl, similar to the range of apoB100 concentrations in the apoB100 transgenic mice (20). DNA sequencing of p158/neos predicted that the construct should code for a truncated apoB protein containing the amino-terminal 4084 amino acids of apoB100. To confirm that the apoB protein in the plasma of the mice was of the predicted size, we performed Western blots of plasma samples from a human apoB100 transgenic mouse and an apoB90 transgenic mouse, using selected apoB-specific monoclonal antibodies (Fig. 1). A Western blot using antibody 1D1 revealed that the plasma of both the apoB90 and the apoB100 mice contained human apoB48. As expected, apoB90 was bound by antibody MB43 (the epitope for antibody MB43 is located between amino acids 4027 and 4081) but was not bound by antibody Bso1 6 (the epitope for antibody Bso1 6 is located between amino acids 4157 and 4189). Of note, these Western blots demonstrated that the concentration of apoB90 in the plasma of the apoB90 transgenic animal was equal to that observed in the "high expressing" apoB100 animals.

To assess the distribution of cholesterol, triglycerides, and human apoB in the plasma of the transgenic animals, the plasma from one apoB90 transgenic mouse and one apoB100 transgenic mouse was fractionated by Superose 6 chromatography. Compared with the plasma of the male apoB100 transgenic animal, the plasma of the male apoB90 transgenic animal had a slightly lower LDL cholesterol peak (Fig. 2A) but a slightly higher LDL triglycerides peak (Fig. 2B). The amounts of apoB in the plasma of the apoB90 and apoB100 mice were similar (Fig. 2C). Almost all of the human apoB90 and human apoB100 was found in LDL-sized particles. The apoB90 lipoproteins were, as expected (35–38), slightly smaller than the lipoproteins containing apoB100. One could argue that the smaller size distribution of the apoB90 particles might reflect more extensive metabolism of these particles in the plasma. However, we have used p158 and p158/neos to generate rat hepatoma cell lines that secrete human apoB100 and apoB90. When the cell culture medium from these cells was subjected to Superose 6 chromatography, we found that the apoB90-containing lipoproteins were slightly smaller than the apoB100-containing lipoproteins.2 Of note, Chiesa and co-workers (9) have shown that the size of the apoB-containing lipoproteins is

2 S. McCormick, M. Linton, and S. Young, unpublished observations.
relevant for in vitro assays of Lp(a) formation; human LDL readily complexed with apo(a) to form Lp(a), whereas larger apoB-containing lipoproteins (such as VLDL) were less effective in forming Lp(a).

To assess the capacity of apoB90 to form a complex with recombinant human apo(a), the plasma of the apoB90 transgenic mouse was incubated with the plasma of human apo(a) transgenic mice at 37 °C for 4 h. Following the incubation, the mixtures were size-fractionated on SDS-polyacrylamide gels, and Western blotting was performed with the apo(a)-specific antibody, IgG-1A2. In this assay system, Lp(a) was distinguished from apo(a) by its size. No Lp(a) formation was detected after incubation of the apoB90 transgenic mouse plasma with apo(a) (Fig. 3A, lane 4). Similarly, no Lp(a) was formed during the incubation of apo(a) with the plasma of a human subject who synthesized only apoB45.2 (Fig. 3A, lane 5). In contrast, the incubation of apo(a) with human d < 1.063 g/ml lipoproteins or with the plasma of a human apoB100 transgenic mouse (Fig. 3A, lanes 2 and 3, respectively) resulted in abundant Lp(a) formation. In fact, virtually all of the free apo(a) in these incubations was converted to Lp(a). The formation of Lp(a) was also eliminated when a reducing agent, 3% 2-mercaptoethanol, was added to the incubation mixtures before electrophoresis on the SDS-polyacrylamide gels (Fig. 3B). We also found that the formation of Lp(a) could be prevented by including 100 mM e-aminocaproic acid in the in vitro incubations (Fig. 3C). It is important to note that the amount of human apoB in each of the in vitro incubations was the same, as judged by a Western blot using monoclonal antibody MB43 (Fig. 3D) or by a human apoB sandwich RIA (Fig. 4, shaded bars). A monoclonal antibody-based sandwich RIA was also used to assess the formation of Lp(a) in the in vitro incubations (Fig. 4, solid bars). In this assay, antibody LPA6 was used as the "capture antibody," and 125I-C1.4 was used to quantify the bound Lp(a). Substantial 125I-C1.4 binding to the Lp(a) was observed upon incubation of apo(a) with human d < 1.063 g/ml lipoproteins or with the plasma of the apoB100 transgenic mice, whereas little 125I-C1.4 binding was observed upon incubation of mixtures consisting of apo(a) and plasma samples containing either apoB45.2 or apoB90.

We previously demonstrated that mice expressing both human apoB100 and apo(a) have bona fide Lp(a) in their plasma (20). To test the possibility that apoB90 might associate with apo(a) in vivo, we established a mating between a female apoB90 transgenic animal and a male that was hemizygous for the apo(a) transgene. One of the four offspring expressed both transgenes. In that animal, all of the apo(a) circulated free of the lipoproteins, and no Lp(a) was detectable, even though the animal had high plasma levels of apoB90, ~50 mg/dl (Fig. 5A). In parallel, we established a mating between an apo(a) male and an apoB100 female (from human apoB100 transgenic line 1095, which expresses relatively low levels of apoB190). One of the four offspring expressed both transgenes. Even though this animal had an apoB100 level of ~8 mg/dl, virtually all of the apo(a) was in the form of Lp(a) (Fig. 5B).
observations on the Lp(a) of a human subject who is a compound heterozygote for familial hypobetalipoproteinemia, H. J. B. H. J. B. had two mutant apoB alleles, one yielding apoB37 (1728 amino acids) (39) and another yielding apoB86 (3896 amino acids) and apoB100 (40). The d < 1.063 g/ml lipoproteins of H. J. B. contained a small amount of both apoB86 and a larger amount of apoB100; a nonreduced SDS-gel of the high density lipoprotein fraction (d = 1.075-1.21 g/ml) revealed a large amount of Lp(a) but no free apoB86 or apoB100. When the Lp(a) was subjected to a disulfide reducing agent, it dissociated into apo(a) and apoB100, but we were never able to detect any apoB86 resulting from the dissociation of the Lp(a) (41).

The carboxyl-terminal 10% of apoB100 contains two cysteine residues, Cys3190 and Cys3215. A possibility consistent with our results is that one of these carboxyl-terminal cysteines is involved in the disulfide linkage. Of these 2 cysteines, the studies of Coleman et al. (19) would favor Cys3190, because that residue, unlike Cys3215, is accessible to labeling with a fluorescent sulf-hydryl probe. However, our data do not permit us to exclude the possibility that a more remote cysteine is involved in the disulfide linkage. Molecular modeling studies (17) and immunochromatographic studies (18) have suggested the possibility that apoB Cys3215 might be involved in the disulfide linkage. It is conceivable that the carboxyl-terminal 10% of apoB100 contains sequences that are required for the noncovalent association of apo(a) and apoB100 and that this noncovalent association facilitates the formation of a disulfide bond involving a more remote cysteine, such as Cys3190. We believe that site-directed mutagenesis of each of the 4 carboxyl-terminal cysteine residues of apoB100 will ultimately be required to establish the identity of the cysteine residue that is involved in the disulfide linkage between apoB and apo(a). An equally important topic of investigation, however, will be the identification of the structural features within the carboxyl-terminal 10% of apoB100 that facilitate its interaction with apo(a). Advances in molecular techniques (42, 43) will make the generation of subtle mutations within the full-length apoB gene clone practical and will allow us in the future to undertake more detailed studies of the structural requirements for Lp(a) formation.

In 1992, Sternberg (44) pointed out that it should be possible to interrupt Pt1 clones with transposons to generate truncated proteins, thereby facilitating the investigation of protein structure/function relationships. To our knowledge, our study is the first application of Sternberg's suggestion, and it represents the first example of the expression of a mutant form of human apoB in transgenic mice.

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Fig. 4. Relative amounts of Lp(a) formed during the incubation of apo(a) with human d < 1.063 g/ml lipoproteins, human apoB100 transgenic mouse plasma, human apoB90 transgenic mouse plasma, and the plasma of the human apoB45.2 homozygote. The amount of human apoB in each incubation mixture (shaded bars) and the amount of Lp(a) formed during the incubations (solid bars) were assessed by monoclonal antibody-based sandwich RIAs as described under "Materials and Methods." Each bar shows the specific counts bound per well. Identical data were obtained from four independent experiments with different apo(a)-apoB incubations. Equivalent results for Lp(a) formation were obtained using a different sandwich RIA, in which antibody MB47 was used as the capture antibody and 121-LPA6 (iodinated by Bolton-Hunter reagent) was the detecting antibody.

A. Apo(a) + ApoB90

B. Apo(a) + ApoB100

This study, which involved both in vitro incubation studies and in vivo studies with apoB90/apo(a) transgenic mice, demonstrated that human apoB90 cannot form Lp(a). The failure of apoB90 to form Lp(a) very strongly suggests that the carboxy-terminal 10% of apoB100 is required for Lp(a) formation. The results do not, however, completely exclude the possibility that all of the important structural features for Lp(a) formation exist within apoB90 but that these structures are not in the proper conformation because of secondary factors related to the length of apoB90. For example, altered lipid composition of apoB90-containing lipoproteins might alter the conformation of apoB90 on the surface of the lipoprotein, affecting its ability to bind to apo(a). In any case, our data indicating that apoB90 cannot form Lp(a) are consistent with the preliminary clinical
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