Decreased Binding of Apolipoprotein (a) to Familial Defective Apolipoprotein B-100 (Arg^{3500} → Gln)

A STUDY OF THE ASSEMBLY OF RECOMBINANT APOLIPOPROTEIN (a) WITH MUTANT LOW DENSITY LIPOPROTEINS*

(Received for publication, June 29, 1994, and in revised form, September 12, 1994)

Srdan Duvovich†, Winfried März‡, Sasa Frank‡, Hubert Scharnagl‡, Manfred W. Baumstark**†, Rudolf Zechner†, and Gert M. Kostner†‡‡

From the †Institute of Medical Biochemistry, University of Graz, the ‡Department of Clinical Chemistry, Medical Clinic, Albert Ludwig-University, Freiburg, Germany, the §Gustav Embden-Center of Biological Chemistry, Johann Wolfgang Goethe-University, Frankfurt, Germany, and the **Department of Sports Medicine, Medical Clinic, Albert Ludwig-University, Freiburg, Germany

In familial defective apolipoprotein B-100 (FDB), glutamine is substituted for arginine at position 3500 of the amino acid sequence. This mutation alters the structure of low density lipoproteins (LDL) and reduces their binding to LDL receptors. We studied the assembly in vitro of FDB-LDL with two recombinant apo(a) (r-apo(a)) isoforms containing 17 or 18 kringle IV-type repeats, respectively. R-apo(a) complexed to LDL in a concentration- and time-dependent manner. When we mixed normal LDL at protein concentrations from 1 to 10 mg/liter with 200 µg/liter r-apo(a) and incubated for 20 h, 15-44% of r-apo(a) were bound to LDL, forming an artificial Lp(a)-like particle. With LDL from a homozygous FDB patient, only 2-16% of r-apo(a) were complexed; heterozygous FDB-LDL bound 2-30% of r-apo(a). We also studied the effect of r-apo(a) on the interaction of the monoclonal antibody MB47 with normal and mutant apoB-100. FDB-LDL displayed enhanced binding of MB47. Adducts generated from normal LDL and r-apo(a) had an increased affinity for MB47, when compared to LDL alone. In contrast, r-apo(a) did not change MB47 reactivity when incubated with FDB-LDL. Altogether, our findings suggest that domains in apolipoprotein B which are important for the interaction with the LDL receptor play a role in the assembly of Lp(a) as well. They provide, in addition, an explanation for the observation that in Lp(a) of heterozygous FDB patients, the ratio of defective to normal apoB-100 is significantly smaller than in LDL from the same patients.

Elevated lipoprotein (a) (Lp(a)) is a risk factor for cardiovascular disease (reviewed in Refs. 1–4). It is composed of an LDL particle and a specific antigen, apo(a) (5). Apo(a) exists in more than 30 genetic isoforms which differ by their size and number of kringle IV-like repeats (6); the apo(a) size is negatively correlated with plasma Lp(a) levels (7–10). The mechanism underlying this association is still unknown. We showed in humans that Lp(a) concentrations strongly correlate with the rate of synthesis, but not with the fractional catabolic rate (11). In an extension of this work, Rader et al. (7) reported that the variation of Lp(a) levels among individuals with the same phenotype was also caused by differences in the Lp(a) production rate.

Subsequent studies in homozygotic twins and sib pair analyses claimed that the heritability of apo(a) levels was more than 90% (9, 10). There exists, however, a great number of nongenetic factors affecting plasma Lp(a) concentrations; Lp(a) increases in acute phase reactions (12), during pregnancy (13), in many forms of kidney disease (14, 15), and in endocrine disorders (16, 17). Hormonal contraceptives, testosterone, and smoking appear to lower Lp(a) (18–20). The mechanisms of all these changes are unknown.

The role of the LDL receptor in the clearance of Lp(a) is controversial. Lp(a) was found increased in familial hypercholesterolemia (21, 22), suggesting that LDL receptors were involved in the catabolism of Lp(a). However, up-regulation of LDL receptors by hydroxymethylglutaric-CoA reductase inhibitors (23–25) or cholesterolamine (26) did not reduce Lp(a) and other investigators reached the conclusion that defective LDL receptors had no effect on Lp(a) (27–30). There seems to be agreement now that the LDL receptor binds Lp(a) with a significantly lower affinity as compared to LDL (31) and that the LDL receptor most probably plays a minor role for the catabolism of Lp(a) (32). Because the fractional catabolic rate of Lp(a) is approximately equal to that of LDL, alternative pathways must contribute to the clearance of Lp(a), one of which possibly involving the LDL receptor-related protein/a2-macroglobulin receptor (33).

Familial defective apolipoprotein B-100 (FDB) denotes a genetic disorder of lipoprotein metabolism leading to high plasma cholesterol and LDL concentrations (34–37). FDB is caused by an arginine to glutamine mutation at codon 3500 of the apoB gene (35). This substitution leads to a defective binding of apoB-100 to the LDL receptor. We show here that LDL purified from a homozygous FDB patient exhibits an approximately 50% reduced capacity to assemble with recombinant apo(a) (r-apo(a)) in vitro.

EXPERIMENTAL PROCEDURES

Materials—COS-7 cells were obtained from the American Type Culture Collection (ATCC CRL 1651). Antibodies specific for apo(a) and...
apoB were produced in our laboratory (31, 32). Horseradish peroxidase-linked protein A and an enhanced chemiluminescent detection kit (ECL) were obtained from Amersham. Lp(a) isoform standard and reference sera were purchased from Immuno (Vienna, Austria). Replication-defective biotinylated adenovirus (dl3121, streptavidin-polylysine conjugate, and human transferrin-polylysine conjugate were produced at the Department of Molecular Pathology, Vienna, Austria. The specific antibody MB47 was provided by Dr. Linda K. Curtiss (The Research Institute of Scripps Clinic, La Jolla, CA). Recombinant apo(a) containing 17 KIV-type units was obtained from Dr. Dan Eaton (Genentech Inc., San Francisco, CA). Nitrocellulose was from Hoefer Scientific. All other materials were from Sigma.

**Patients**—We studied LDL from four patients with FDB, one homozygote and three heterozygotes. The homozygous patient (F.B.) was the 55-year-old male who was described previously (37, 38). The three heterozygotes (R.W., R.O., and I.K.) belonged to one family; they were on normal diet.

**Lipids and Lipoproteins**—Lipids were measured with enzymatic reagents from Boehringer Mannheim (Mannheim, Germany) or Merck (Darmstadt, Germany). VLDL, LDL, and HDL were quantified in terms of cholesterol after preparative ultracentrifugation (39, 40). ApoA-I, apoA-II, and apoB were measured by nephelometry (Behring, Marburg) (40). ApoB phenotyping was accomplished by immunoblotting (41), and apo(a) by a commercial immunoradiometric assay (Pharmacia, Uppsala, Sweden) (42). Protein was measured according to Lowry et al. (43).

**Electron Microscopy**—LDL were dialyzed against 0.125 mol/liter ammonium acetate, 2.6 mol/liter ammonium carbonate, and 0.26 mol/liter EDTA-Na2. They were mixed with sodium phosphate buffer (final concentration 10 g/liter), applied to Formvar-carbon coated grids, and examined in a Philips EM 300 electron microscope at an accelerating voltage of 100 kV.

**Construction of an Apo(a) Expression Vector—**cDNA clones reported by McLean et al. (44) were used for the production of the apo(a) expression plasmid (pSG5-18) containing DNA sequences coding for 18 KIV-like domains, for the kringle V (KV)-like and the protease domain, following standard recombinant DNA techniques (45, 46). As described elsewhere, a construct was generated encompassing the apo(a) cDNA 5'-untranslated region, the signal sequence, the first 17 KIV through KIV-5, 294 bp of KIV-6, the last 48 bp of KIV-27, KIV-28 through KIV-37, KV, the protease domain, and 67 bp of the 3'-untranslated region. This fragment was ligated into the EcoRI site of the expression vector pSG5 (46). High level expression of r-apo(a) was obtained in COS-7 cells, cultured in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) lipoprotein-deficient serum, to the manufacturer's instructions. The sensitivity of this method was 5 ng.

**Fast Lipoprotein Chromatography**—1 ml of plasma was loaded onto a 500-mm column of Sepharose (50% packed bed, Pharmacia), and eluted with 300 mmol/liter NaCl, 100 mmol/liter Na3PO4, pH 7.4, at a flow rate of 0.3 ml/min-1 (52). The column eluate was fractionated and analyzed for r-apo(a):LDL complexes with a sandwich enzyme immunoassay obtained from Byk-Sangtec (Dietzenbach, Germany). In this assay, anti-apo(a) and anti-apoB serve as capturing and detecting antibodies, respectively (42).

**Immunostaining of Free r-apo(a) and r-apo(a):LDL Complexes by DELFIA—**Lp(a) was determined with a sandwich DELFIA. 96-well plates (Costar, Cambridge, MA) were coated with polyclonal affinity purified anti-apo(a) from sheep. Nonspecific binding sites were blocked with 250 μl of 0.5% (v/v) bovine serum albumin for 30 min. 200-μl aliquots of the samples were added to the wells and incubated for 2 h at 20°C. After three washing steps with 50 mm Tris-HCl, pH 7.7, a polyclonal detection antibody (either anti-apo(a) or anti-apoB from rabbits), labeled with Eu, was added to the wells and further incubated for 2 h at 20°C. Excess antibody was removed by two washing steps with 50 mm Tris-HCl, pH 7.7. 200 μl of enhancement solution (Pharmacia) was added, and after 15 min, the plates were read with a DELFIA reader. For the determination of total apo(a), Eu-labeled anti-apo(a) was used; for the determination of r-apo(a):LDL complexes, Eu-labeled anti-apoB was used. Standard curves were produced with the Lp(a) Reference Standard from Immuno Diagnostika and included in each experiment. The assay was linear between 1 and 100 ng of Lp(a) per well, the coefficient of variation was 2.6%.

**Binding of ApoB-100-specific Monoclonal Antibody MB47 to LDL and r-apo(a):LDL Complexes**—MB47 reactivity with LDL and r-apo(a):LDL complexes was determined by means of a solid-phase competitive enzyme immunoassay (Nunc Immuno Plates). Lp(a) was coated (150 μl of control LDL/10 μg/liter protein in 0.2 mol/liter carbonate buffer, pH 10.0), blocked with 10 g/liter bovine serum albumin (in carbonate buffer), and washed with 200 μl of phosphate-buffered saline containing 0.05% Tween 20.

LDL was desalted on Pharmacia PD-10 columns equilibrated with phosphate-buffered saline containing EDTA-Na2 (0.3 mol/liter), assayed for protein, and adjusted to 10 g/liter bovine serum albumin and 0.05% Tween 20. LDL (final concentration 70 μg/liter apolipoprotein) were pre-incubated with KIV-17 r-apo(a) (5 mg/liter), control incubations were performed with the same dilutions of LDL, but without r-apo(a). The mixtures were incubated overnight with MB47 (ammonium sulfate precipitate of mouse ascites fluid) diluted 1:4000 in phosphate-buffered saline containing 0.3 mol/liter EDTA, 10 g/liter bovine serum albumin, and 0.5% Tween 20, and 100 μl were loaded into LDL-coated microplate wells. After 2 h, the plates were washed three times and the amount of antibody bound to immobilized LDL was determined with peroxidase-conjugated anti-mouse immunoglobulin G (Boehringer Mannheim, diluted 1:750 in phosphate-buffered saline-Tween-20 bovine serum albumin). Color was developed with o-phenylenediamine.

**RESULTS**

We obtained LDL from one homozygote and three heterozygous patients with FDB (Table I). The morphology of FDB-LDL as studied by negative staining electron microscopy revealed no significant differences from normal (not shown). However, slight differences were observed when the chemical compositions of the patients' LDL were compared to normal LDL. The calculated particle radius of LDL from F.B. (homozygous FDB) was smaller than for normal LDL. Consistently, LDL from F.B. contained less phospholipid molecules per particle than normal. This reflects the preponderance of small dense LDL in the patient, which has been described previously (37). On the other hand, LDL from the three FDB heterozygotes turned out all the bigger, due to the increased cholesterol ester content.

In previous experiments, we studied the assembly of Lp(a) in vitro by incubation of r-apo(a) with LDL from normal healthy donors. This way, we generated Lp(a)-like complexes which were indistinguishable from native Lp(a) with respect to hydrated density, composition, and morphology (48, 53). Com-
Lp(a) Assembly in Familial Defective ApoB-100

Clinical data and lipoproteins in a homozygous (+/+ ) and three heterozygous (+/- ) FDB patients

TABLE I
Lipoprotein concentrations were determined by preparative ultracentrifugation.

| Age (years) | F.D. +/+ | R.W. +/+ | R.O. +/+ | I.K. +/+ |
|-------------|----------|----------|----------|----------|
| 45          | 55       | 37       | 49       | 25       |
| Sex         | m        | m        | f        | f        |
| Height (cm) | 178      | 180      | 158      | 168      |
| Weight (kg) | 72       | 88       | 57       | 55       |
| Cholesterol (g/liter) | 3.06 ± 0.14 | 2.67 ± 2.47 |
| Triglycerides (g/liter) | 1.39 ± 0.45 | 1.02 ± 0.47 |
| HDL-cholesterol (mg/dl) | 0.28 ± 0.14 | 0.21 ± 0.08 |
| LDL-cholesterol (mg/dl) | 0.09 ± 0.01 | 0.07 ± 0.06 |
| LDL-cholesterol (g/liter) | 2.20 ± 0.14 | 1.97 ± 1.93 |
| ApoA-I (g/liter) | 1.65 ± 0.05 | 1.29 ± 1.39 |
| ApoA-II (g/liter) | 0.54 ± 0.03 | 0.62 ± 0.47 |
| ApoB (g/liter) | 1.47 ± 0.10 | 1.48 ± 1.11 |
| Lp(a) (units/liter) | 14 ± 4 | 901 ± 900 |
| ApoE phenotype | 3/3 | 3/3 | 3/3 |

* Four blood samples have been obtained from F.B. during this study; mean (± S.D.) lipid and lipoprotein concentrations of these samples are reported.

** Multiply with 0.54 to convert units/liter into milligrams/liter (35).

plexes were formed whether or not living cells were present; the degree of complex formation was time dependent. Fig. 1 displays a time course experiment in which 200 μg/liter r-apo(a) from COS-7 cells transfected with pSG5–18 were incubated for 0.5–24 h with 5 mg/liter LDL-protein and the degree of Lp(a) assembly was measured. A plateau was reached after approximately 16 h. Thus, all subsequent experiments were performed at 20 h incubation. The efficacy of Lp(a) assembly also depended on the ratio of apoB to r-apo(a) in the medium, increasing with higher ratios. For reasons of practicality we carried out most studies at a fixed concentration of 200 μg/liter r-apo(a) and variable apoB concentrations ranging from 0.5 to 10 mg/liter.

We were interested to see whether FDB-LDL behaved similarly to normal LDL with respect to complex formation with r-apo(a). To study this, LDL from a homozygous FDB patient and from a normal individual were incubated for 20 h with 200 μg/liter r-apo(a). The complex formation was monitored by sub-marin SDS-agarose gel electrophoresis and Western blotting using anti-apo(a) as detecting antibody (Fig. 2). With normal LDL, two major bands were observed, one migrating to the position of S4 and the other slightly faster than the F isoform of the phenotyping standard. In addition to anti-apo(a), the slow migrating band also reacted with anti-apoB (not shown). The fast migrating band reacted with anti-apo(a) only. As shown elsewhere (48, 53), addition of reducing agents, e.g. mercaptoethanol, caused the slow migrating band to disappear, while only one fast migrating apo(a) band was seen. Thus, the slow migrating band consisted of the apo(a)-LDL heterodimer, which is dissociated by mercaptoethanol, but not by SDS alone. When the same experiment was performed with FDB-LDL, only small amounts of heterodimers were found (Fig. 2).

In another approach to examine the assembly of r-apo(a) with LDL, we added r-apo(a) at a final concentration of 5 mg/liter to apo(a)-negative plasma, incubated at room temperature, and separated the mixture by fast flow size exclusion chromatography. We then analyzed the column fractions for r-apo(a)-LDL complexes. As expected, r-apo(a)-LDL complexes were produced, eluting at slightly higher apparent molecular masses than LDL (Fig. 3). In the FDB homozygous plasma, the concentration of LDL (LDL-C 2.52 g/liter) was approximately 1.6 g/liter. In spite of this, only half the amount of r-apo(a)-LDL complexes was formed in the patient's plasma, compared to the normal plasma.

Both SDS-agarose gel electrophoresis and size exclusion chromatography are semi-quantitative means to study Lp(a) assembly. To approach the problem in quantitative terms, similar incubations as described in Fig. 2 were performed with different concentrations of normal and mutant LDL, followed by the measurement of free and LDL-complexed apo(a) using DELFIA (Fig. 4). At all LDL concentrations, the amounts of r-apo(a) found in the artificial Lp(a) complex were significantly lower with mutant LDL than with normal LDL. For FDB-LDL, the amount of r-apo(a) complexed to LDL ranged between 6.0 ± 0.8 and 21.2 ± 3.2% of total r-apo(a), as opposed to normal LDL, where values between 18.5 ± 2.1 and 42.4 ± 5.1% were found.

To confirm these findings, a different batch of r-apo(a) containing 17 kringle IV-like domains (described in Ref. 49) was studied (Fig. 5). In the incubation mixtures containing 10 mg/liter normal LDL-protein and 200 μg/liter r-apo(a), 44% of total r-apo(a) was complexed with LDL and formed an Lp(a)-like particle; heterozygous FDB-LDL bound 20–36% r-apo(a), and homozygous FDB-LDL only 16%. At lower LDL concentrations, the amount of r-apo(a) complexed to FDB-LDL was always significantly smaller than the amount of r-apo(a) complexed to normal LDL.

The monoclonal antibody MB47 recognizes an epitope near the receptor binding domain of apoB-100. We reasoned, if r-apo(a) indeed bound to this region, MB47 reactivity might

FIG. 1. Time course of Lp(a) assembly. 200 μg/liter r-apo(a) (18 KIV) were incubated with 5 mg/liter LDL-protein. At the time intervals indicated and the Lp(a) and r-apo(a)-LDL-complexes were analyzed by DELFIA as described under "Experimental Procedures." Results are expressed in percent of total r-apo(a) complexed to LDL.

FIG. 2. Assembly of normal LDL and FDB-LDL with recombinant r-apo(a). R-apo(a) containing supernatant from COS-7 cells transfected with pSG5–18 (200 μg/liter r-apo(a)) were incubated overnight with normal and FDB-LDL at the apoB concentrations indicated below each lane. Aliquots of the incubation mixtures corresponding to 10 ng of apo(a) were subjected to SDS-agarse gel electrophoresis and transferred to nitrocellulose. Apo(a) was detected with a polyclonal antibody and ECL as described under "Experimental Procedures." Lane 1, apo(a) isoform standard containing the isoforms F, S1, S2, S3, and S4 according to the nomenclature of Utermann (70); lanes 2–5, pooled normal LDL; lanes 6–9, LDL from F.B. (FDB homozygote).
affected by the attachment of apo(a). Therefore, we studied the interaction of normal LDL and FDB-LDL with MB47 before and after adding r-apo(a) (17 KIV). As expected, FDB-LDL exhibited a higher affinity for MB47 than normal LDL. When r-apo(a) was complexed to normal LDL, the resulting complexes displayed a higher affinity for MB47 than the LDL alone. In contrast, no change in MB47 reactivity was observed when FDB-LDL were preincubated with r-apo(a) (Fig. 6).

**DISCUSSION**

In studies which are published elsewhere (48, 53), we investigated the assembly of Lp(a) in eukaryotic cells transfected with expression vectors containing variable lengths of apo(a) cDNA. All data we obtained were compatible with a two-step model of extracellular Lp(a) assembly. This assumption is in line with reports from other groups using systems similar to ours (54, 55), transgenic mice (56), or cultured primary baboon hepatocytes (57). Considering all these earlier findings together, it appears that apo(a) is synthesized in the liver and, in a first step, loosely complexes to LDL outside the hepatocyte. In that complex, the sulfhydryl group of cysteine 4057 comes close to a free sulfhydryl group of apoB-100 and forms a stabilizing interaction of normal LDL and FDB-LDL with recombinant apo(a). 0.5–10 mg/liter LDL-apoB were incubated with 200 mg/liter r-apo(a) (18 KIV for 20 h) and the amount of r-apo(a) complexed to LDL was determined by DELFIA (see "Experimental Procedures"). The values are expressed as the percentage of r-apo(a) complexed to LDL; they are means ± S.D. of four experiments, each carried out in triplicate. *Squares,* normal LDL; *circles,* FDB-LDL.

**FIG. 4.** Assembly of normal and FDB-LDL with recombinant apo(a). 0.5–10 mg/liter LDL-apoB were incubated with 200 mg/liter r-apo(a) (18 KIV for 20 h) and the amount of r-apo(a) complexed to LDL was determined by DELFIA (see "Experimental Procedures"). The values are expressed as the percentage of r-apo(a) complexed to LDL; they are means ± S.D. of four experiments, each carried out in triplicate. *Squares,* normal LDL; *circles,* FDB-LDL.

**TABLE II**

| Particle radius (nm) | r-apo(a) LDL complexes | LDL complexes |
|---------------------|------------------------|--------------|
|                     | FDB                    | normal LDL   |
| 9.7 ± 0.3*          | 10.2 ± 0.1*            | 9.78 ± 0.32  |

*Significantly different from control LDL (p < 0.05).

**FIG. 5.** Assembly of normal and FDB-LDL with recombinant apo(a). 200 mg/liter r-apo(a) (17 KIV) was incubated with increasing amounts of LDL and the percentage of apo(a) complexed to LDL was determined using DELFIA for total r-apo(a) and for r-apo(a):LDL complexes (for details see "Experimental Procedures"). LDL were obtained from pooled plasma of three normal individuals (solid bars), from three heterozygous FDB patients (hatched bars), and one homozygous FDB patient (crosshatched bars). Values are means ± S.D. from three independent experiments, each performed in triplicate.

**FIG. 6.** Effect of r-apo(a) on the binding of the monoclonal antibody MB47 to normal and FDB-LDL. Normal (left) and FDB-LDL (right) (final concentration 70 mg/liter apoB) were incubated with r-apo(a) (5 mg/liter). Control incubations were carried out with exactly the same LDL preparations, but without r-apo(a). The binding of the apoB-100-specific monoclonal antibody MB47 was determined in a solid phase competitive enzyme immunoassay as described under "Experimental Procedures." All competition curves were obtained in a single experiment. **BB** is the ratio of antibody bound to the solid phase in the presence of LDL or r-apo(a):LDL complexes divided by the amount of antibody bound in the absence of competitor. Each data point represents the mean of triplicate determinations. Circles, r-apo(a):LDL complexes; squares, control LDL without r-apo(a). The dotted line in the right panel corresponds to the competitive curve of normal LDL (squares in the left panel).
disulfide bond in the second step. The latter assumption is based on the observation that the substitution of cysteine residue 4057 for serine (55) or for arginine (48) by site-directed mutagenesis led to a significant decrease of stable Lp(a) assembly. We reasoned, if our two-step model for Lp(a) assembly was correct, then not only the substitution of cysteine residue 4057 in apo(a), but also a conformational change in apob-100 which interferes with the noncovalent attachment of apo(a) to the LDL surface should reduce complex formation and Lp(a) synthesis. To evaluate this hypothesis, we examined the binding of r-apo(a) to LDL from patients with familial defective apoB-100 (Arg3600 → Gln). Using different strategies and r-apo(a) from two independent sources containing either 17 or 18 KIV-like repeats, we demonstrated that the association of r-apo(a) with FDB-LDL is greatly reduced.

Circumstantially, these findings would imply that the apoB-100 receptor binding domain is involved in the binding of apo(a) to LDL. When tested in vitro, Lp(a) exhibits reduced binding to the LDL receptor compared to LDL (31, 32, 58). This concurs with the idea that apo(a) partially masks the apoB-100 receptor binding domain. The latter contains two lysine-rich segments extending from residues 3147 to 3157 and from residues 3359 to 3367 (59, 60). Its conformation is profoundly altered in FDB (61-63). We, therefore, speculated that the conformational change in the apoB-100 receptor binding domain was responsible for the reduced ability of FDB-LDL to interact with apo(a).

To throw additional light on this hypothesis we examined whether the binding of r-apo(a) to LDL affected the affinity for the apoB-100 specific monoclonal antibody MB47. The MB47 epitope consists of two non-linear domains including amino acid residues 3429-3453 and 3507-3523; it is located in the vicinity of the apoB-100 receptor binding domain (64, 65). We fully confirmed previous work showing that MB47 binds to FDB-LDL with higher affinity than to normal LDL (37, 66). Interestingly, complexation of r-apo(a) to normal LDL increased their reactivity with MB47, suggesting that the MB47 epitope was involved in the binding of apo(a) and/or underwent a conformational change on apo(a) attachment. In contrast, FDB-LDL already possess a conformation, which is similar to that in vitro binding of apo(a) and or underwent a conformational change in the apoB-100 receptor binding domain was responsible for the reduced ability of FDB-LDL to interact with apo(a).

Alternatively, FDB-LDL may already possess a conformation which is similar to that induced by the binding of apo(a) to normal LDL.

We wish to reconcile our findings with a report by Perombelon et al. (67) who studied Lp(a) in 31 members of two FDB families. They found higher Lp(a) concentrations in FDB heterozygotes, compared to unaffected relatives. They also analyzed Lp(a) levels of family members with identical apo(a) phenotypes, with or without FDB. In five cases, where a direct comparison was possible, FDB patients again exhibited higher Lp(a) levels than the healthy family members. Obviously, this would be in line with the idea that Lp(a) is metabolized by LDL receptors. However, Perombelon et al. (67) also performed a detailed analysis of FDB-Lp(a) in vitro which was in sharp contrast to this assumption. When they removed the apo(a) moiety of Lp(a) from heterozygous FDB patients, the resulting LDL-like particles (i.e. Lp(a)−) contained a smaller proportion of defective particles (50%) than LDL from the same patient (60-75%) (67). Finally, when the same families were re-examined, no differences in Lp(a) concentrations were detected between affected and unaffected siblings with the same apo(a) allele (29, 68), suggesting that LDL receptors do not significantly contribute to Lp(a) catabolism in humans. Further support for this view comes from work showing that defective LDL receptors have almost no effect on Lp(a) concentration (27-30) and that hydroxymethylglutaryl-CoA reductase inhibitors do not lower Lp(a) (23-25).

In heterozygous FDB, the ratio of mutant to normal apoB-100 ranges from 60:40 to 75:25 (34, 67, 69). If Lp(a) assembly was in fact occurring in the plasma compartment, the ratio of Lp(a) particles containing mutant apoB-100 to those with "wild type" apoB-100 should be close to that for LDL, i.e. between 60:40 and 75:25. However, according to Perombelon et al. (67) this ratio is 50:50. Our results may provide a plausible solution to this paradox: although circulating at a higher concentration than normal LDL, the defective LDL complexes less apo(a) in FDB heterozygotes. As a net effect, the concentration of Lp(a) molecules endowed with the defective apoB-100 is shifted toward approximately equal concentration of Lp(a) with normal apoB-100.

Plasma Lp(a) concentrations are highly heritable and the apo(a) locus accounts for more than 90% of the interindividual variation in plasma Lp(a) concentrations (9). It is, therefore, believed that sequence differences at the apo(a) locus determine the rate of Lp(a) biosynthesis. Rader et al. (7), however, reported differences in the Lp(a) production rate among individuals with the same phenotype, and a large number of nongenetic factors is gradually becoming recognized which modify Lp(a) concentrations (12−14, 16−20). Here we demonstrate experimentally that genetic variation altering apoB-100 structure and metabolism may influence Lp(a) biosynthesis.

In summary, we show that FDB-LDL exhibits reduced capacity to assemble with two different recombinant apo(a) molecules containing 17 or 18 kringles IV repeats. These findings not only provide a possible explanation for the observations of Perombelon et al. (67), but in extension of this suggest that the LDL surface, and in particular the epitopes of apoB-100 which are important for LDL receptor binding, are involved in Lp(a) assembly as well. This hypothesis which has a potential bearing on Lp(a) homeostasis in vivo is now pursued further in our laboratories.

Acknowledgments—We thank Dr. Linda K. Curtiss, The Research Institute of Scripps Clinic, La Jolla, CA, for the monoclonal antibody MB47 and Dr. Dan Eaton, Genentech Inc., San Francisco, CA, for providing recombinant apolipoprotein(a). The technical assistance of Bettina Donserhak, Margarete Fruhmann, Ulrike Stein, Margit Stullschnig, Daniela Wittmann, and Harald Grillohofer is appreciated.

REFERENCES
1. Utermann, G. (1989) Science 246, 904-910
2. Stehney, W. M., and Fries, G. (1989) J. Clin. Invest. 85, 1709-1715
3. Kostner, G. M., and Krempler, F. (1992) Circ. Res. Lipidol. 3, 279-284
4. Edelberg, J. M., and Pizzo, V. S. (1991) Fibrosclerosis 5, 135-143
5. Pfister, J. W., Heidemann, C., A., M., Jr., Mortis, J. D., and Dahlen, G. H. (1993) J. Biol. Chem. 258, 4582-4589
6. Koschinsky, M. L., Beisiegel, U., Henoe-Bruns, D., Eaton, D. L., and Lawn, R. M. (1990) Biochemistry 29, 640-644
7. Rader, D. J., Cain, W., Schel, L. A., Usber, D., and Brewer, H. B., Jr. (1993) J. Clin. Invest. 91, 443-447
8. Kauf, H. K., Kochl, M., Menzel, H. J., Sandholzer, C., and Utermann, G. (1992) Hum. Genet. 90, 2153-2163
9. Boerwinkle, E., Leffert, C. C., Lin, J., Lachner, C., Chiesa, G., and Hobbs, H. H. (1992) J. Clin. Invest. 90, 52-60
10. Austin, M. A., Sandholzer, C., Schel, Y. V., Newman, B., Krause, R. M., and Utermann, G. (1992) Eur. J. Hum. Genet. 1, 861-870
11. Kostner, G. M., Roscher, A., Haslauer, J., and Reusser, S. (1983) J. Biol. Chem. 258, 4582-4589
12. Noma, A., and Kawade, M. (1989) Atherosclerosis 78, 145-150
13. Zechner, R., Desny, G., Schudel, J. O., Heith, P. F., and Kostner, G. M. (1986) Metab. Exp. 35, 333-336
14. Irish, A. B., Simons, L. A., Saville, S., Hayes, J. M., and Simons, J. (1992) Aust. J. Exp. Biol. Med. Sci. 22, 239-243
15. Wanner, C., Rader, D. W., Krämer, J., Brewer, H. B., Jr., and Schollmeyer, P. (1985) Ann. Intern. Med. 103, 263-269
16. Buildingblock, M. W., v. Stein, T., Jakob, E., Luley, C., Berg, A., and Keul, J. (1993) in Hormones in Lipoprotein Metabolism (Steinmetz, A., Schneider, J., and Schudel, J. O., Eds.), Chapter 3, pp. 69-73
