Isolation of allele-specific, receptor-binding-defective low density lipoproteins from familial defective apolipoprotein B-100 subjects

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Abstract Familial defective apolipoprotein B-100 (FDB) is a genetic disorder apparently caused by a single amino acid substitution (Arg_{3500}→Gln) that disrupts the binding of low density lipoproteins (LDL) to the LDL receptor. The plasma of FDB heterozygotes contains a mixture of normal LDL and LDL that is defective in binding to the LDL receptor. In this study, the monoclonal antibody MB19 (which recognizes an immunogenetic polymorphism in apolipoprotein B-100) was used to determine the percentage of defective LDL in the plasma of FDB heterozygotes and to isolate allele-specific receptor-binding-defective LDL. Several FDB heterozygotes were identified who were heterozygous for the MB19 polymorphism: one apolipoprotein B allotype in each of these individuals bound with low affinity to MB19 and possessed the Arg_{3500}→ Gln mutation, whereas the other apolipoprotein B allotype bound with high affinity to MB19 and normally to the LDL receptor. Using MB19 radioimmunoassay, we determined that an average of 73% (range 65–87) of the total LDL from FDB heterozygotes contained the Arg_{3500}→Gln mutation. Antibody MB19–Sepharose immunoaffinity chromatography was used to separate the receptor-binding-defective LDL from the normal LDL. The isolated LDL contained primarily the Arg_{3500}→Gln mutation and had only about 9% of normal LDL receptor-binding ability. Finally, the MB19–Sepharose chromatography procedure may be useful for isolating other allele-specific LDL that have functionally significant mutations.—**Arnold, K. S., M. E. Balestra, R. M. Krauss, L. K. Curtiss, S. G. Young, and T. L. Innerarity.** Isolation of allele-specific, receptor-binding-defective low density lipoproteins from familial defective apolipoprotein B-100 subjects. *J. Lipid Res.* 1994. **35:** 1469–1476.

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Apolipoprotein (apo) B-100 is the predominant protein component of low density lipoproteins (LDL), which transport about two-thirds of plasma cholesterol in humans (1). Each spherical human LDL particle contains a single molecule of apoB-100 (2). ApoB-100, a protein 4536 amino acids in length, is synthesized in the liver and is essential for the assembly and secretion of triglyceride-rich very low density lipoproteins, the metabolic precursors of LDL (1, 3, 4). A second major role of apoB-100 in lipoprotein metabolism stems from its function as a ligand for the LDL receptor, which mediates effective clearance of LDL from the circulation. Mutations resulting in structural changes in either the LDL receptor or apoB-100 disrupt the efficient receptor-mediated clearance of LDL. While mutations in the LDL receptor gene have long been known as a cause of familial hypercholesterolemia, the existence of a mutation in apoB-100 that disrupts the receptor binding and plasma clearance of LDL has been appreciated only more recently (4–6).

Familial defective apoB-100 (FDB) is characterized by moderate to severe hypercholesterolemia and LDL that bind defectively to the LDL receptor and therefore accumulate in the plasma (4, 6). The receptor-binding defect is strongly associated with a mutation (CGG → CAG) in the codon for residue 3500 of mature apoB-100; the mutation results in the replacement of an arginine by a glutamine (Arg_{3500}→Gln) (7). Several investigations have collectively identified more than 100 heterozygotes with this disorder in North America and Europe (6). The Arg_{3500}→Gln mutation has been estimated to occur at a frequency of 1/500 to 1/700 in these populations (4, 8–10). Additionally, several studies have shown an association

Abbreviations: apo, apolipoprotein; BSA, bovine serum albumin; DMEM, Dulbecco's Modified Eagle's Medium; ED_{50}, effective dose required for 50% competition; FDB, familial defective apolipoprotein B-100; FH, familial hypercholesterolemia; LDL, low density lipoproteins; PBS, phosphate-buffered saline; RIA, radioimmunoassay.

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between FDB and a predisposition to premature atherosclerosis, probably owing to the elevated levels of LDL in these subjects (9, 10).

LDL isolated from FDB heterozygotes have only about one-third the ability of normal LDL to compete with normal ^{125}I-labeled LDL for binding to the LDL receptor in cultured normal human fibroblasts (5). Because each LDL particle has only one copy of apoB-100, FDB heterozygotes have two populations of LDL: one containing normal apoB-100 and the other containing apoB-100 that is defective in binding to the LDL receptor (designated defective LDL). As a result of the inefficient catabolism of the defective LDL, it was considered probable that the defective allotype was present in excess of normal LDL. Because total LDL from FDB heterozygotes have ~33% of normal receptor-binding activity, much of the binding activity would then seem to be due to the normal LDL, with a lesser, but unknown, contribution by the defective LDL.

The aims of this study were to determine the percentage of allele-specific, defective LDL in FDB heterozygotes and to isolate and characterize the receptor-binding activity of the defective LDL. The observation that several FDB heterozygotes were also heterozygous for an MB19 polymorphism was central to our accomplishing these aims. Monoclonal antibody MB19 detects a functionally neutral apoB polymorphism, binding to one apoB allotype ($MB19_1$) with a much higher affinity than to the other ($MB19_2$) (11). In FDB heterozygotes who are also heterozygous for MB19, haplotype analysis has proven that one apoB allotype produces LDL expressing weak binding to both MB19 and the LDL receptor; the other allotype produces LDL with normal affinity for the LDL receptor and high affinity to MB19 (12). Using MB19 in a solid-phase radioimmunoassay (RIA), we were able to determine the percentage of the defective LDL in the plasma of FDB heterozygotes. Moreover, using MB19–Sepharose immunoaffinity chromatography, it was possible to isolate an LDL fraction consisting mostly of the population that is defective in binding to the LDL receptor.

MATERIALS AND METHODS

Subjects

The subjects from whom plasma was obtained for this study were all FDB heterozygotes who also were MB19 heterozygotes. All had total plasma cholesterol levels of 220–260 mg/dl, although the LDL cholesterol levels ranged from 130 to 200 mg/dl. Two of the subjects studied were being treated with lovastatin at the time that blood for the study was drawn. A more comprehensive evaluation of the effect of lipid lowering drugs on amounts of allele-specific LDL in FDB heterozygotes is in progress. Two of the subjects, on whom LDL allotype quantitation was performed, were brothers. All other subjects were unrelated.

Lipoprotein preparation

Plasma from fasting subjects was obtained from whole blood, using 1 mg of EDTA/ml as an anticoagulant. The LDL (d 1.02–1.05 g/ml) were isolated from plasma by sequential centrifugation in an L8-70 ultracentrifuge (Beckman Instruments, Palo Alto, CA) at 59,000 rpm (13) and washed by recentrifugation at d 1.05 g/ml. The isolated LDL were dialyzed against 0.15 M NaCl, 0.01% EDTA (NaCl-EDTA) before use and radiolabeled with ^{125}I or ^{131}I by the iodine monochloride method (14).

Receptor-binding studies

Cultures of normal human fibroblasts were maintained as described (15). Seven days prior to an experiment, cells were plated into 12 × 22-mm multiwell tissue culture dishes at 1.4×10^4 cells/dish. Forty-eight hours before the experiment, the wells were washed once with Dulbecco's Modified Eagle's Medium (DMEM) and then incubated in DMEM containing 10% human lipoprotein-deficient serum. The assays for receptor binding (4°C) were performed as described (16).

Solid-phase competitive radioimmunoassay

The solid-phase interaction of monoclonal antibodies with lipoproteins used a modification of the procedure of Young et al. (11) and Friedel et al. (12). The LDL from a subject homozygous for the high-affinity $MB19_1$ allele were absorbed to Removawells (Dynatech, Chantilly, VA). The wells were washed in phosphate-buffered saline (PBS) containing 0.1% RIA grade bovine serum albumin (BSA) and 0.05% Tween 20; then nonspecific binding sites were blocked with 4% RIA-grade BSA in PBS. After washing as above, dilutions of test LDL in PBS containing 3% RIA-grade BSA were added to the wells as competitor, along with a constant concentration of MB19 ascites fluid. The wells were washed again, and ^{125}I-labeled sheep anti-mouse IgG (Amersham, Arlington Heights, IL) was added as a second antibody. After final washes, the ^{125}I in each well was quantitated by gamma counting. The results were plotted as B/B_{max} versus the log of the concentration of the test LDL, where B and B_{max} represent counts per min in the presence or absence of test LDL competitor, respectively. The competition curves were drawn by computer-generated sigmoidal fit of the points. The concentration of test LDL required to inhibit 50% of the binding of antibody MB19 to the immobilized $MB19_1$ LDL (ED_{50}) was determined from the competition curves. There was a linear relationship between the reciprocal of the ED_{50} and the percentage of $MB19_2$ allotype in the test LDL. Therefore, the percentage of $MB19_2$ allotype in an LDL from an FDB/MB19 heterozygote could be determined by linear regression of points obtained using known mixtures of $MB19_2$ and $MB19_1$ LDL. All results were normalized to the concentration of apoB in each test LDL, determined by performing a simultaneous plate as-

say with antibody MB3, a monoclonal antibody shown to provide an accurate quantitation of the total apoB present in LDL (17).

Haplotype analysis of subjects

The MB19 phenotype of the individuals used in this study was determined by use of the MB19 solid-phase RIA (18). The phenotypes were confirmed by genotyping using ApaLI digestion of a DNA fragment amplified by polymerase chain reaction of genomic DNA (19). The Arg_{3500}→Gln genotype of the subjects was determined by polymerase chain reaction amplification and reaction with allele-specific oligonucleotides (7).

MB19 isolation and affinity chromatography

MB19 IgG was isolated from ascites fluid on a Protein A-Sepharose column (Pharmacia, Uppsala, Sweden) (20). Isolated MB19 IgG was coupled to CNBr-Sepharose 4B (Pharmacia, Uppsala, Sweden) at 17.5 mg IgG/gm dry gel (5 mg/ml swelled gel) at pH 8.6, according to the manufacturer's instructions (21). The MB19-Sepharose was washed in buffer B (0.1 M citric acid, 2 M NaCl, 0.01% EDTA, pH ~2) and then equilibrated in buffer A (0.1 M trisodium citrate, 0.05 M Tris, 0.15 M NaCl, 0.01% EDTA, pH 8.4). Each LDL sample was diluted 1:1 with buffer A or dialyzed against this buffer before it was loaded onto the MB19-Sepharose column (5 mg LDL protein per 4 ml [~2.5 gm dry gel] slurry). The LDL was recirculated over the column for 2–4 h, then eluted with a gradient of increasing concentrations of buffer B, as described in the figure legends. Fractions were pooled, dialyzed against NaCl-EDTA, and concentrated in CentriCell centrifugal concentrators (30,000 molecular weight cutoff) (Polysciences, Warrington, PA). Each MB19-Sepharose column was used only once because much of the LDL could not be eluted from the gel.

RESULTS

Identification of MB19/FDB heterozygotes and determination of allele-specific concentrations of LDL from these subjects

Haplotype analysis of the apoB gene of FDB probands and their kindreds identified several subjects who were heterozygous for FDB and the MB19 polymorphism (double heterozygotes). The pedigree analysis of one family is shown in **Fig. 1.** A comparison of haplotypes from all pedigrees analyzed revealed that the FDB mutation always occurred along with an $MB19_2$ allele. Thus, the defective LDL from FDB heterozygotes would also display low affinity for antibody MB19. Therefore, in each of the double heterozygotes identified, one apoB allele coded for a protein containing the Arg_{3500}→Gln mutation and the $MB19_2$ low-affinity binding site, and the other apoB allele coded for normal apoB and the $MB19_1$ high-affinity binding site. The LDL from the double heterozygote identified as G1737 from the family shown in Fig. 1 were used for many of the studies described below.

Previously we and others reported (11, 12, 22) that the LDL from an $MB19_2$ homozygote, an $MB19_1$ homozygote, and an MB19 heterozygote yield distinctly different displacement curves in competitive solid-phase RIA using antibody MB19. The differences in the displacement curves are the result of different affinities of antibody MB19 for allotypes $MB19_1$ and $MB19_2$ (11) and provide a basis for estimating the percentage of $MB19_2$ (and thus defective-binding) LDL in FDB heterozygotes by solid-phase RIA (12). We assessed the ability of the following LDL to compete with immobilized $MB19_1$ LDL for binding to antibody MB19. Competition curves were generated for LDL from an $MB19_1$ homozygote, an $MB19_2$ homozygote, a normal MB19 heterozygote, an FDB/MB19 heterozygote, and three mixtures of $MB19_1$

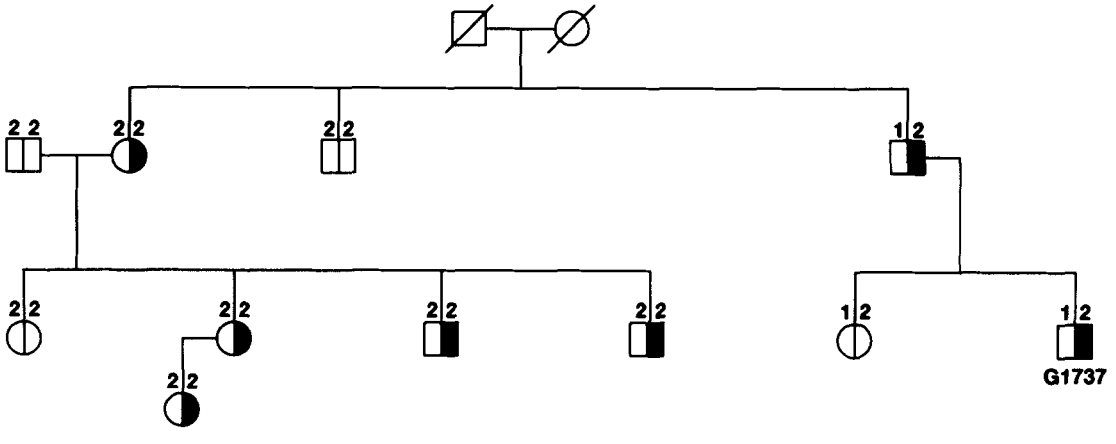

Fig. 1. Pedigree pattern of a kindred with familial defective apoB-100 showing the MB19 apoB allotypes. Half-black symbols represent heterozygotes for FDB (Arg_{3500}→Gln). MB19 allotypes $MB19_1$ and $MB19_2$ are designated 1 and 2. Note the segregation of FDB with the $MB19_2$ allele in the double heterozygotes, such as subject G1737, who was used in this study.

with $MB19_2$ LDL: 20:80, 25:75, and 30:70 (**Fig. 2A**). The displacement curves were generated through computer-assisted sigmoidal fit of the points. From these curves, the ng of apoB necessary to inhibit 50% of the antibody MB19 binding to immobilized $MB19_1$ LDL (ED_{50}) could be calculated (legend, Fig. 2). As would be predicted, LDL mixtures containing a higher percentage of low-affinity $MB19_2$ LDL were less effective competitors (i.e., their displacement curves shifted right) (Fig. 2A). We noted a linear relationship between the reciprocal of ED_{50} and the percentage of $MB19_2$ LDL in the test LDL (Fig. 2B). As shown in Fig. 2, LDL from the FDB heterozygote G1737 had an affinity for MB19 between that of the 80:20 and the 75:25 ratio of $MB19_2$:$MB19_1$ LDL. In the experiment shown in Fig. 2A and 2B, we calculated that 78% of the LDL in subject G1737 were the $MB19_2$ allotype and were defective in binding to the LDL receptor. Using these procedures, the percentage of defective LDL ($MB19_2$ LDL) in the LDL from subject G1737 was determined 12 times from five samples over a 57-month period. An average of $76 \pm 6\%$ of the LDL was found to be $MB19_2$ (defective). Another FDB/MB19 heterozygote, who was tested five times, had an average of 67% $MB19_2$ LDL. Two other FDB/MB19 heterozygotes had 80% and 77% $MB19_2$ LDL, respectively. Thus, we can conclude that approximately three-fourths of the LDL in FDB heterozygotes are receptor-binding-defective but that the amount of defective LDL varies among FDB heterozygotes. Previously, we reported the discovery of a FDB heterozygote (subject JH), whose LDL we estimated contained 83% of the FDB allotype (12). When we reanalyzed this data using the procedures described in this study, we obtained an average defective allotype concentration of $85 \pm 3\%$.

Isolation of the $MB19_2$ LDL particles by antibody MB19 immunoaffinity chromatography

The RIA studies suggested that it might be possible to use MB19-Sepharose immunoaffinity columns to separate the two different LDL allotypes from the total LDL of an FDB/MB19 heterozygote. To establish optimal conditions for the immunoaffinity separation of $MB19_2$ from $MB19_1$ LDL, we isolated $MB19_1$ and $MB19_2$ LDL from the plasma of homozygotes. The $MB19_1$ LDL were radiolabeled with ^{131}I. To minimize the possibility of damaging the $MB19_2$ LDL that were to be characterized, we radiolabeled them with lower-energy ^{125}I. When equal amounts of the radiolabeled LDL were mixed and applied to an MB19-Sepharose column, most of the LDL from both allotypes bound to the column. As shown in **Fig. 3,** when a gradient of pH (8 → 2) and NaCl (0.15 → 2 M) was initiated, the low-affinity ^{125}I-labeled $MB19_2$ LDL eluted quickly and contained little ^{131}I-labeled $MB19_1$ LDL. From this and five other MB19-Sepharose control experiments, we determined that fractions 5–24 provided the best recovery of the most pure ^{125}I-labeled $MB19_2$ LDL; an average of 92% of the LDL from pooled fractions 5–24 were ^{125}I-labeled LDL (allotype $MB19_2$) (**Table 1**). Typically, the recovery of the ^{125}I-labeled LDL from the immunoaffinity column was 10–20%.

The $MB19_2$ LDL from fractions 5–24, eluted from the column under mild conditions, were very similar to the

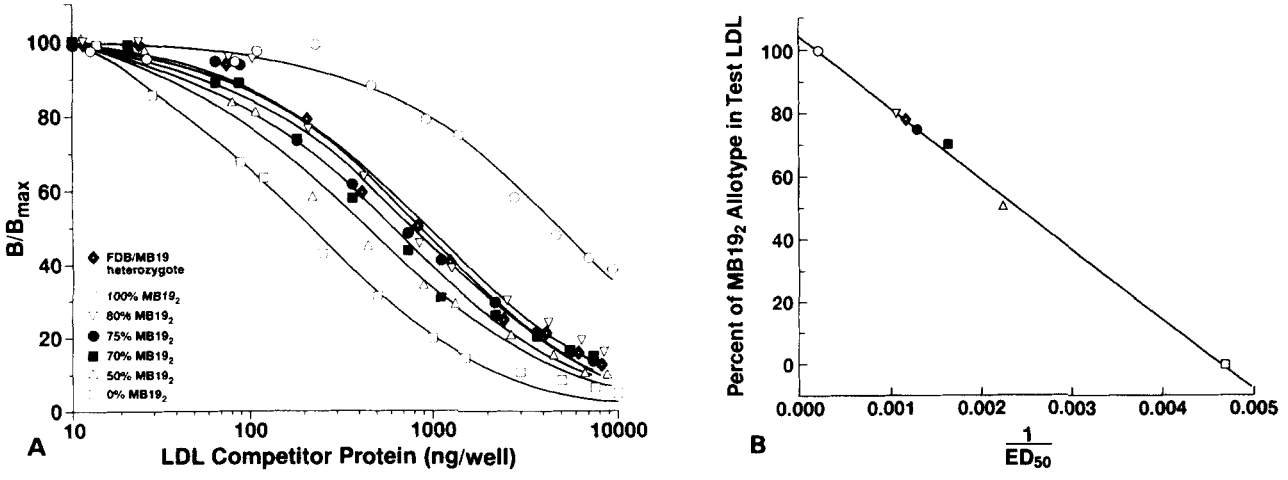

Fig. 2. Panel A: Solid-phase competitive RIA demonstrates the ability of various LDL to compete with immobilized $MB19_1$ LDL for binding (B/B_{max}) to a fixed concentration of antibody MB19. Competitor LDL included: □, an $MB19_1$ homozygote (0% $MB19_2$, ED_{50} = 214 ng); △, an MB19 heterozygote (50% $MB19_2$, ED_{50} = 445 ng); ■, a 70% $MB19_2$: 30% $MB19_1$ mixture (ED_{50} = 603 ng); ●, a 75% $MB19_2$:25% $MB19_1$ mixture (ED_{50} = 761 ng); ▽, an 80% $MB19_2$:20% $MB19_1$ mixture (ED_{50} = 928 ng); ○, an $MB19_2$ homozygote ($MB19_2$ = 100%, ED_{50} = 4597 ng); and ◆, an FDB and MB19 heterozygote (ED_{50} = 851 ng). Panel B: Linear relationship between the reciprocal of the ED_{50} and the percentage of $MB19_2$ allotype in the competitor LDL.

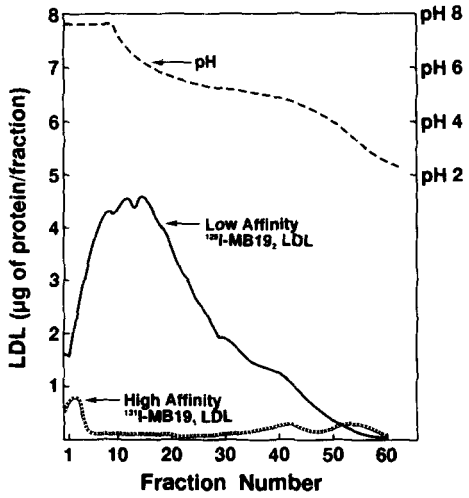

Fig. 3. Antibody MB19 immunoaffinity chromatography of a 50:50 mixture of MB19$_2$ ^{125}I-labeled LDL and MB19$_1$ ^{131}I-labeled LDL on an MB19-Sepharose column. The LDL mixture (1 mg total) was applied to the column (1 × 5 cm) pre-equilibrated in buffer A (see Methods). The column was washed with 15 ml of buffer A (fractions 1-9), followed by a 70-ml linear gradient using buffer B (NaCl: 0.15 → 2 M, pH 8 → 2). The flow rate was 0.4 ml/min, and 2-ml fractions were collected. The protein content of each fraction was estimated using the specific activities of the original LDL.

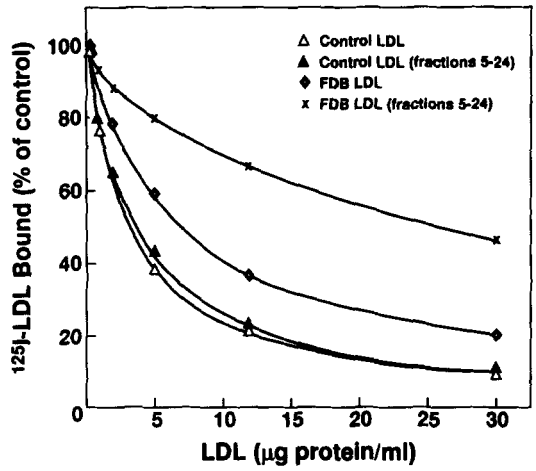

Fig. 4. Ability of various LDL to compete with a fixed concentration of normal ^{125}I-labeled LDL for binding to LDL receptors on normal human fibroblasts. The various LDL were: control MB19$_1$/MB19$_2$ LDL (△), control MB19$_2$ LDL obtained from an MB19-Sepharose column (fractions 5-24) (▲), LDL isolated from a familial defective apoB-100 subject (♦), and MB19$_2$ LDL (fractions 5-24) isolated from an MB19-Sepharose column to which FDB LDL had been applied (×). DMEM containing 25 mM HEPES and 10% human lipoprotein-deficient serum, 2 µg/ml ^{125}I-labeled LDL, and the indicated concentrations of the various unlabeled LDL were added to 22-mm culture wells (0.45 ml/well) containing normal human fibroblasts. After a 2-h incubation at 4°C, the amount of ^{125}I-labeled LDL bound to the fibroblasts was determined. In the absence of competitor, 10.3 ng of ^{125}I-labeled LDL bound per well.

MB19$_2$ LDL applied to the column by the following criteria: lipid composition (data not shown), size, and morphology as determined by electron microscopy of negatively stained LDL particles (mean diameter of native was 17.8 nm versus 18.5 nm for fractions 5-24), and binding to the LDL receptor (**Fig. 4**).

Isolation of allele-specific FDB LDL

To isolate a fraction of LDL greatly enriched in defective apoB-100 from FDB heterozygotes, we used conditions identical to those determined to be optimal for the isolation of MB19$_2$ LDL from a mixture of radiolabeled MB19$_1$ and MB19$_2$ LDL. LDL from subjects heterozygous for FDB and the MB19 polymorphism were applied to an MB19-Sepharose column and eluted. Fractions 5-24 were pooled, and the purity of these allele-specific LDL was assessed by a solid-phase competitive RIA. The ability of LDL contained in fractions 5-24 to compete with antibody MB19 for binding to the immobilized MB19$_1$ LDL was compared to that of LDL from an MB19$_1$ homozygote, an MB19$_2$ homozygote, and an MB19/FDB heterozygote (**Fig. 5**). The results indicated that the FDB allele-specific MB19$_2$ LDL (fractions 5-24) had a displacement virtually identical to that of LDL from the MB19$_2$ homozygote, suggesting that FDB fractions 5-24 consist predominantly of MB19$_2$ LDL.

TABLE 1. Isolation of ^{125}I-labeled MB19$_2$ LDL by MB19-Sepharose chromatography

Experiment	Total LDL	MB19 Sepharose Gel	^{125}I-labeled MB19$_2$ LDL in Fractions 5-24
	mg	*ml*	*%*
1	5.5	2.75	87
2	12	6	89
3	4.8	6	92
4	4.0	5	93
5	1	5	97
Average			92

The LDL-receptor-binding characteristics of the purified fractions 5-24 from FDB LDL were examined. In a competitive binding assay against normal ^{125}I-labeled LDL, FDB LDL (fractions 5-24) displayed only 9.5% of the receptor-binding activity of control LDL, while fractions 5-24 isolated from normal LDL heterozygous for MB19 showed receptor-binding activity virtually identical to that of control LDL (Fig. 4). Seven different preparations of FDB fractions 5-24 LDL had an average of 9% of the receptor-binding activity of normal LDL (**Table 2**). The average concentration of defective LDL protein required to displace 50% of the normal ^{125}I-labeled LDL from the LDL receptors was 26.4 µg/ml versus 2.4 µg/ml for control LDL (Table 2).

We also examined the direct binding of radiolabeled control LDL and FDB LDL isolated by MB19 im-

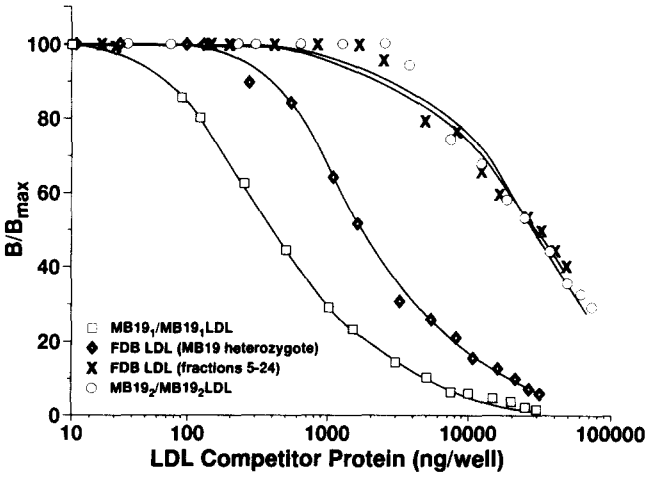

Fig. 5. Ability of various LDL to compete with immobilized $MB19_1$ LDL for binding (B/B_{max}) to antibody MB19 in a solid-phase RIA. LDL were from: an $MB19_1$ homozygote (□), a familial defective apoB-100 subject who was an MB19 heterozygote (♦), fractions 5-24 isolated from an MB19-Sepharose column to which the FDB LDL had been applied (×), and an $MB19_2$ homozygote (○).

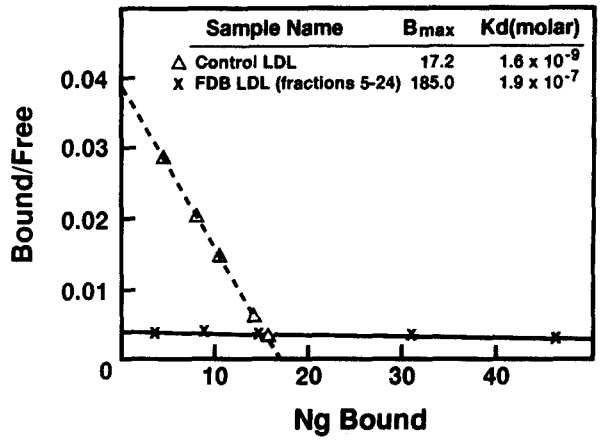

Fig. 6. Scatchard plot for the binding of ^{125}I-labeled control LDL (△) and ^{125}I-labeled defective LDL, FDB fractions 5-24 (×) to normal human fibroblasts. "Bound/free" is the ratio of the lipoprotein bound (ng protein/dish) to the lipoprotein free in the media (ng protein added/0.45 ml). The data have been corrected for nonspecific binding by the addition of 100× unlabeled LDL at each concentration.

munoaffinity chromatography to the LDL receptors on normal human fibroblasts (**Fig. 6**). The FDB LDL (fractions 5-24) had approximately 100-fold lower affinity for the LDL receptor (K_d = 1.9 × 10^{-7} M) than that of control LDL (K_d = 1.6 × 10^{-9} M). These results demonstrated that the substitution of Gln for Arg at apoB-100 residue 3500 greatly reduced the ability of LDL to bind to the LDL receptor.

TABLE 2. Receptor binding activity of FDB LDL from unrelated subjects isolated by MB19-Sepharose immunoaffinity compared with control LDL

	ED_{50}[a]		
FDB Subject	Control	FDB $MB19_2$ LDL (fractions 5-24)	Percent of Normal Binding Activity
	μg/ml		
G1737	2.7	28.2	9.5
	2.8	32.1	8.8
	2.2	25.8	8.5
G14	2.4	32.6	7.4
	1.1	25.7	4.2
G1708	2.4	17.0	14.2
	2.9	23.4	12.5
Average	2.4 (± 0.6)	26.4 (± 5.4)	9.3 (± 3.3)

[a] Concentration of LDL required to displace 50% of ^{125}I-labeled control LDL from LDL receptors on normal human fibroblasts at 4°C.

DISCUSSION

This study describes the quantitative determination of the percentage of defective LDL in FDB heterozygotes and the development of a procedure to isolate allele-specific LDL. Both procedures exploit the observation that the monoclonal antibody MB19 binds to apoB-100 allotype $MB19_2$ with a much lower affinity than to allotype $MB19_1$ (11). Competitive solid-phase RIA allowed us to determine that an average of 75% of the LDL of FDB heterozygotes is the defective $MB19_2$ allotype. Using an immunoaffinity column of MB19-Sepharose, it was possible to isolate a population of $MB19_2$ LDL. More important, the methodology was used to purify the defective allotype from the LDL of FDB heterozygotes. These individuals have one normal apoB-100 allele and one that expresses apoB-100 with a mutation at residue 3500. Because there is only one apoB-100 molecule per LDL particle, the total LDL from FDB heterozygotes are a mixture of normal and defective apoB-100. While total LDL from FDB heterozygotes have about 33% of normal receptor-binding activity in a competitive binding assay, the abnormal LDL isolated from the MB19 immunoaffinity column had only about 9% of normal LDL receptor-binding activity. Part of this binding activity may be due to small amounts of contaminating normal receptor-binding LDL, so the actual binding activity of the FDB LDL may be somewhat less than 9%.

The results from this study help to quantitate the impact of the 3500 mutation on the catabolism of LDL. Defective LDL accumulates in FDB heterozygotes because it is not efficiently cleared via the LDL-receptor-mediated pathway, as is normal LDL. Until this study, the

relative amounts of each LDL allotype present in FDB heterozygotes had not been determined. Previously we demonstrated that total LDL from FDB heterozygotes have an average of 35% of the binding activity of normal LDL in a competitive receptor-binding assay (12). If the mutant LDL had little or no receptor binding activity, then all of the receptor binding activity would be due to the normal LDL and the distribution of allele-specific amounts of LDL would be about 35% normal LDL and 65% FDB LDL. However, now that the binding activity of the FDB LDL is known to be 9%, the distribution of LDL allotypes can be reevaluated from this receptor binding data. The distribution of LDL allotypes in the plasma based on this data is ~72% FDB LDL and ~28% normal LDL. Of the total LDL receptor binding activity from a FDB heterozygote, normal LDL contributes 28%, while the remainder ($0.72 \times 0.09 = 0.065$, or 6.5%) is attributable to the defective LDL. This distribution is similar to the results we obtained in this study using the competitive antibody MB19 solid-phase RIA. With this assay we found that approximately three-fourths of the total LDL from FDB heterozygotes are defective LDL.

If the plasma LDL cholesterol levels from 41 previously identified FDB heterozygotes (4) are analyzed assuming a 25:75 percent distribution of apoB allotypes in plasma, one can draw several intriguing conclusions. The average plasma LDL cholesterol level in these FDB subject heterozygotes, 199 mg/dl (4), would be composed of about 50 mg cholesterol/dl of normal LDL and about 150 mg/dl of defective LDL. It should be noted that the predicted concentration of normal-LDL cholesterol for these patients (50 mg/ml) is about one-half the LDL concentration (120 mg/dl) in age- and sex-matched normal controls (4), suggesting that the 3500 mutation may not have a major impact on the plasma concentration of the normal LDL allotype. If FDB has a gene dosage effect similar to that of familial hypercholesterolemia (FH), then one might expect that FDB homozygotes would have LDL cholesterol levels of ~ 300 mg/dl (150 mg/dl × 2), which would be more than double the plasma LDL cholesterol concentration of normal individuals but less than one-half the concentration of LDL in FH homozygotes (23). In fact, the first report of an FDB homozygote, by März et al. (24), described a patient on a normal German diet without lipid-lowering medication who initially presented with an LDL cholesterol level of 265 mg/dl and, in a second measurement 4 weeks later, 292 mg/dl. Thus, the LDL plasma cholesterol from this initially reported FDB homozygote is remarkably similar to what is predicted by analysis of FDB heterozygotes.

The MB19-Sepharose chromatography procedure and the quantitative solid-phase RIA also may be useful for the analysis of allele-specific LDL in other situations where immunological assays, functional assays, or sequence analysis indicate a genetic variation in apoB-100. For example, Gavish et al. (22) have described an inherited condition of allele-specific differences in amounts of full-length apoB-100 and LDL in plasma. Whether this difference is due to unequal synthesis or to differential catabolism is not known; however, the quantitative MB19 assay should be useful to answer this and similar questions. We have used this assay to demonstrate that the unequal distribution of the allele-specific LDL in one subject is not due to biosynthesis of unequal amounts of apoB allotype. In this FDB heterozygote, about 83% of the LDL were FDB LDL (12). The subject's VLDL and IDL, however, had equal concentrations of $MB19_1$ and $MB19_2$ allotypes (unpublished data). Thus, the dramatic unequal distribution of LDL allotypes was not due to an apoB mutation that reduced the allele-specific synthesis or assembly of apoB-100 from one apoB allele into VLDL but, rather, to the unequal catabolism of the LDL allotypes.

The MB19-Sepharose chromatography procedure has already proven useful as a method for isolating sufficient FDB LDL for physical biochemical studies. Using ^{13}C nuclear magnetic resonance, Lund-Katz et al. (25) found that when [^{13}C]dimethyl derivatives were added to the lysine residues of apoB-100, the LDL had a distinct ^{13}C nuclear magnetic resonance spectrum. In normal LDL, these modified lysines exist in two microenvironments: ~ 50 lysines with a pK of 8.9 and 170 lysines with a pK of 10.5 (25). In defective LDL purified from FDB heterozygotes by MB19 immunoaffinity chromatography, there was a decrease equivalent to seven lysine residues in the number of pK 8.9 lysines. The circular dichroism spectra of the mutant and normal LDL were identical, indicating that the mutation causes no major secondary-structure perturbation of the whole protein (26). From these studies Lund-Katz and coworkers (26) concluded that the single amino acid substitution $Arg_{3500} \rightarrow Gln$ induces a local conformational change in the region of the receptor-binding site of apoB-100.

In summary, the procedures described in this study should facilitate the quantitative determination of allele-specific concentrations of VLDL, IDL, and LDL. Moreover, the MB19 affinity chromatography will make possible the isolation of LDL containing unique apoB mutations from heterozygotes.

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