Separation of swine plasma LDL from $Lpb^{2/3}$ heterozygotes into two apoB allelic haplotypes, Lpb^{2} and Lpb^{3}, with apoB epitope specific antibodies

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Abstract Studies were performed to investigate the separation of Lpb (lipoprotein B) species present in plasma of heterozygous swine bearing the Lpb^{2} and Lpb^{3} apoB mutant genes. Low density lipoprotein (LDL) fractions from $Lpb^{2/2}$ and $Lpb^{3/3}$ homozygotes were coupled to a matrix and used to isolate affinity-purified antibodies anti-Lpb2 and anti-Lpb3 from swine alloimmune sera, one with specificity for the Lpb2 epitope(s) and the other for Lpb3. These antibodies in turn were used to construct two immunosorbers, anti-Lpb2 and anti-Lpb3 Sepharose columns. To separate the two Lpb haplotype populations present in LDL, a density gradient ultracentrifuge subfraction (d 1.032–1.043 g/ml) obtained from $Lpb^{2/3}$ heterozygous pigs was applied to the specific immunosorbers. The retained fraction from the anti-Lpb2 column reacted in the double immunodiffusion test with anti-Lpb2 and anti-Lpb13 immune sera but not with either anti-Lpb3 or anti-Lpb12, while the unretained fractions reacted with anti-Lpb3 and anti-Lpb12 but not with either anti-Lpb2 or anti-Lpb13. The reaction patterns obtained with the two sets of alloimmune sera indicate the existence of two separate lipoprotein populations in LDL: one lipoprotein carrying the Lpb2 and Lpb13 epitopes corresponding to the Lpb^{2} apoB allele, and the other carrying the Lpb3 and Lpb12 allotypes specified by the Lpb^{2} gene. Immunoblotting with anti-Lpb2 and anti-Lpb3 and silver staining showed that the epitopes of both isolated LDL subpopulations are associated with apoB-100. Neutral lipid analyses showed no differences between the isolated Lpb2 and Lpb3 lipoprotein species from the $Lpb^{2/3}$ heterozygotes. These studies demonstrate that plasma LDL subfractions from Lpb heterozygous swine can be separated into two haplotype populations, each corresponding to the product of one apoB gene, and reveal a new insight into the phenotypic expression of plasma LDL, and the LDL phenotype-genotype relationship. Furthermore, this approach will facilitate studies on metabolic differences of two structurally distinct LDL, unaffected by in vitro manipulation, exposed to the metabolic milieu of one individual.—**Rapacz, J., Jr., J. Hasler-Rapacz, J. Rapacz, and W. J. McConathy.** Separation of swine plasma LDL from $Lpb^{2/3}$ heterozygotes into two apoB allelic haplotypes, Lpb^{2} and Lpb^{3}, with apoB epitope specific antibodies. *J. Lipid Res.* 1989. **30:** 199–206.

Supplementary key words low density lipoproteins • apoB • allotypic epitopes • haplotype population

Observations on lipoproteins of specific flotation rates in sera of humans with a myocardial infarction and in sera of rabbits with dietary-induced serum hypercholesterolemia and atherosclerosis led Gofman and co-investigators (1) to suggest that, in addition to the role of dietary cholesterol in atherosclerosis, defects might exist in lipoproteins that affect the catabolism of these molecules leading to hypercholesterolemia and atherosclerosis. Results of subsequent studies have led to a premise that plasma lipoprotein variants are potentially involved in half of all deaths in industrialized countries (2).

Among plasma lipoproteins, low density lipoproteins (LDL), which transport two-thirds of the plasma cholesterol, have attracted attention after it became evident that the main protein of LDL, apolipoprotein B (apoB), is the essential constituent for the assembly of three lipoprotein classes: chylomicrons, very low density lipoproteins (VLDL) and LDL (3) and that the LDL level is correlated with plasma cholesterol concentration and is a risk factor for coronary artery disease.

A close resemblance of swine lipoproteins to human lipoproteins with regard to composition and density distribution (4) made swine an attractive species for the study of genetic polymorphisms of lipoproteins. Based on the assumption that structural genes of apolipoproteins mutated during speciation, producing a series of alleles specifying alloantigenic epitopes in the corresponding apolipoproteins, investigations in swine led to production of alloantigen specific polyclonal antibodies for probing

Abbreviations: LDL, low density lipoprotein; SRID, single radial immunodiffusion; DID, double immunodiffusion; TBS, Tris-buffered saline.

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the existence and extent of genetically mediated polymorphism in the most complex and large apolipoprotein, apoB (5).

A total of 16 allotypic variants, associated with apolipoprotein B have been identified (5–7) and are designated Lpb 1–8 and Lpb 11–18 (8). Genetic studies indicated that the Lpb epitopes are inherited as co-dominant traits and are specified by eight allelic genes, each determining one individual (mutant) and seven common (shared) epitopes, except for Lpb^8, which carries two mutant and six common epitopes. Any given pair of haplotypes differs from each other by two pairs of mutually exclusive epitopes (one mutant and one common; e.g., Lpb 2,13 versus Lpb 12,3 in Lpb^2 and Lpb^3, respectively).

Serological tests to determine whether LDL contain a single apoB haplotype per particle showed that LDL of $d > 1.01$ g/ml carries two independent populations in plasma of each of the 28 Lpb heterozygotes (7, 9). Subsequent studies showed that one of the Lpb genotypes exhibits inherited hyperlipoproteinemia and hypercholesterolemia associated with accelerated atherosclerosis (10).

The purpose of this study was to confirm unequivocally the original finding on the monoallelic Lpb expression of LDL and extend these findings by devising a technique for isolation of the two gene products from the population of Lpb particles in the plasma of an individual swine. Application of this approach to humans and other species will permit the isolation and metabolic studies of two populations of LDL in an individual heterozygote at the single gene level.

MATERIALS AND METHODS

Origin of plasma

The LDL fractions used for affinity chromatography were obtained from plasma derived from Lpb-pretested and overnight-fasted pigs (Immunogenetic Project Herd (IPH), University of Wisconsin, Madison, WI), representing eight Lpb genotypes: homozygote $Lpb^{2/2}$and $Lpb^{3/3}$ and heterozygotes $Lpb^{1/3}$, $Lpb^{2/3}$, $Lpb^{3/4}$, $Lpb^{3/5}$, $Lpb^{3/7}$, and $Lpb^{3/8}$.

Isolation of LDL and LDL subfractions

The LDL (d 1.006–1.073 g/ml) of the homozygotes used for coupling were isolated by preparative ultracentrifugation as described by Alaupovic, Lee, and McConathy (11) and dialyzed against 0.1 M $NaHCO_3$ (pH 8.5) buffer containing 0.5 M NaCl.

The plasma of heterozygotes $Lpb^{2/3}$ was separated into five layers by a density gradient subfractionation method for LDL (12). Layer 3 (L_3, *d* 1.032–1.043 g/ml) was collected under nitrogen, dialyzed, and used to separate, by affinity, two Lpb haplotype populations of LDL containing the following epitopes: Lpb 11, 2, 13, 14, 15, 16, 17, 18 and Lpb 11, 12, 3, 14, 15, 16, 17, 18 (7).

Origin and specificity of antibodies

Swine allotypic antisera, anti-Lpb2, anti-Lpb3, and anti-Lpb11 through Lpb18 used in these studies have been described previously (5, 6, 13, 14). The anti-Lpb3 reacts with a single antigen, the mutant epitope of an apoB haplotype specified by the Lpb^3 allelic gene, and anti-Lpb2 detects the mutant epitope determined by the Lpb^2 allele. Anti-Lpb12 and anti-Lpb13 recognize corresponding common epitopes each present in seven Lpb haplotypes (7, 8).

Preparation and coupling of LDL and affinity-purified antibodies to the matrix

Cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals AB, Uppsala, Sweden) was used as the matrix to couple LDL fractions and affinity-purified antibodies (15, 16). To protect against decreasing efficiency of the LDL columns and to insure longer antigenic activity of LDL, the LDL-Sepharose 4B was cross-linked with 0.031% glutaraldehyde (glutaraldehyde, Grade I, specially purified, 25% aqueous solution, Sigma Chemical Co., St. Louis, MO) (17).

All affinity columns (2 × 50 cm) were prepared as previously described (18) with Sephadex G-25 (Pharmacia, Uppsala, Sweden) at the lower end (1/2 volume of the column, 15–20 cm) to separate the particles of interest from the dissociating agent, and at the upper end (2–3 cm). The Sepharose-4B with either the bound LDL ligand or affinity-purified antibody was packed between the G-25 layers. After coupling LDL fractions and antibodies, the immunosorbers were washed with 30 ml of 3 M sodium thiocyanate (NaSCN) (J. T. Baker, Phillipsburg, NJ). For equilibration of columns and for elution of unretained fractions, a buffer containing 0.025 M Tris-HCl (Sigma; J. T. Baker), pH 7.5, 0.15 M NaCl (Sigma), 0.05% Na_2EDTA (Aldrich, Milwaukee, WI), and 0.01% NaN_3 (Sigma) was used. Retained fractions were eluted with 20 ml of 3 M sodium thiocyanate followed by the equilibration buffer.

As illustrated in **Fig. 1**, both LDL (d 1.006–1.073 g/ml) fractions from the homozygotes were used, after coupling to CNBr-activated Sepharose 4B, for absorption of the alloimmune sera over the alternate LDL-column (e.g., anti-Lpb3 over LDL-Lpb2/2 Sepharose) to remove nonspecific components and for affinity purification of the corresponding antibodies, anti-Lpb3 and anti-Lpb2. The affinity-purified antibodies (anti-Lpb2 or anti-Lpb3) specific for their corresponding Lp-B were used to construct the anti-Lpb2 and anti-Lpb3 immunosorbers.

For preparation of affinity purified antibodies, 21 ml of anti-Lpb2 and 35 ml of anti-Lpb3 were used. After adsorption of the antibodies from the alloimmune sera (Fig.

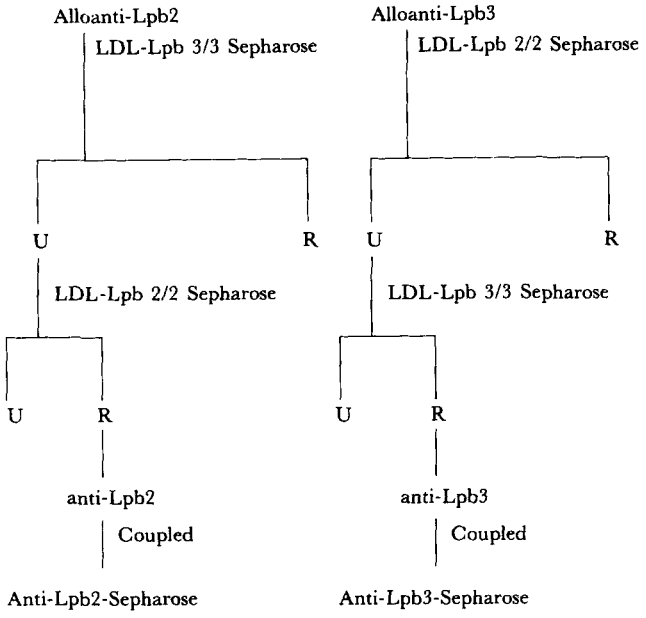

Fig. 1. Scheme for the preparation of the immunosorbers and quantitative data; U, unretained fraction; R, retained.

1), the affinity-purified antibodies (3–6 mg) were coupled to an excess of Sepharose (approximately 25 ml) as outlined. The binding capacity for the Lpb2 immunosorber was 0.54 mg and 0.23 mg for the Lpb3 immunosorber. The low capacity of the immunosorbers was in part due to the small volume of antiserum processed and the allotypic nature of the antibodies.

Isolation of Lpb2 and Lpb3 antigen

One ml of L_3 from heterozygotes $Lpb^{2/3}$ was chromatographed over either anti-Lpb2 or anti-Lpb3 immunosorbers. Due to a limited capacity of immunosorbers, unretained fractions were rerun two or three times to completely separate the two Lpb haplotype populations of LDL. The unretained and retained fractions were collected separately, placed in dialysis tubing, and concentrated with polyethylene glycol flakes (mol wt 18,500; Polysciences, Inc., Warrington, PA) to the original volume for futher analyses. Recoveries of total protein applied to each immunosorber averaged 28 ± 5% (n = 3) for the anti-Lpb2-Sepharose and 35 ± 2% (n = 3) for the anti-Lpb3-Sepharose. Recoveries of the unretained and retained fractions were in the range of 14–18%.

Immunological analyses

The unretained and retained fractions eluted from the anti-Lpb2 and anti-Lpb3 immunosorbers were tested with anti-Lpb2, anti-Lpb3, anti-Lpb12, and anti-Lpb13 antisera in the single radial (SRID) and double immunodiffusion (DID) tests for the detection of Lpb2, Lpb3, Lpb12, and Lpb13 epitopes in the heterozygous $Lpb^{2/3}$ and homozygous $Lpb^{2/2}$ and $Lpb^{3/3}$ sera. In addition, these fractions were also tested with anti-Lpb11 and Lpb14–Lpb18 in the DID test. The DID and SRID tests were performed in 1% Noble agar gel (Difco Lab., Detroit, MI) prepared in 0.86% Bacto hemagglutination buffer (Difco), pH 7.3, with 0.01% NaN_3. For the DID test 6 μl of a normal serum or plasma per well was used with variations from 6 to 16 μl of the alloimmune serum and 6–10 μg of unretained or retained fractions. For the SRID test 6 μl of plasma and layers was used. The tests were incubated at room temperature, read between 18–24 hr, then reread and photographed after 28–36 hr.

Electrophoretic analyses

Gradient polyacrylamide gel (Bio-Rad, Richmond, CA) electrophoresis 2.5–15% (PAGE-SDS) was performed according to an LKB (19) method. High molecular weight standards (BRL, Bethesda, MD) were used and run simultaneously for determination of molecular weights. The proteins were stained with either 0.25% Coomassie Brilliant Blue R (Sigma) and/or silver stain (Bio-Rad).

The electrophoretically separated proteins were transferred to nitrocellulose paper (Fisher, Pittsburgh, PA) by electroblotting (20). Nitrocellulose transblots were fixed with 10% trichloroacetic acid (TCA) (Sigma) for 15 min and washed with distilled water. Sheets were blocked with 3% powdered nonfat dry milk for 90 min at room temperature. Next, nitrocellulose was incubated in TBS-Tween (0.01 M Tris, 0.15 M NaCl, 0.05% Tween-20) buffer (Tween-20, J. T. Baker), pH 7.4, for 90 min at room temperature. The nitrocellulose sheet was placed first with the allotypic antibody (anti-Lpb2 or anti-Lpb3) diluted in Tris-buffered saline (TBS) (0.01 M Tris, 0.25 M NaCl, pH 7.5), for 90 min at room temperature and then overnight at 4°C, followed by washing three times in TBS-Tween at room temperature. The second biotinylated antibody, anti-pig IgG (Sigma) diluted in TBS (1:1000) was incubated for 120 min followed by three washing cycles of TBS-Tween. Bands were visualized using BRL's substrate solution (NBT and BCIP) in 0.1 M Tris, pH 9.5, 0.1 M NaCl, 0.05 M $MgCl_2$ (Sigma).

Analytical methods

Neutral lipid content of whole plasma, ultracentrifugally isolated fractions, unretained and retained fractions from the heterozygotes $Lpb^{1/3}$, $Lpb^{2/3}$, $Lpb^{3/4}$, $Lpb^{3/5}$, $Lpb^{3/7}$, $Lpb^{3/8}$, and homozygotes $Lpb^{2/2}$ and $Lpb^{3/3}$ were determined by gas-liquid chromatography (21).

For protein determination of each ligand, Bio-Rad dye reagent concentrate (Bio-Rad) was used (22).

RESULTS

Swine plasma samples of the $Lpb^{2/3}$ genotype (n = 4) were analyzed by the DID test and showed a nonidentity reaction (**Fig. 2A**) with anti-Lpb2 and anti-Lpb3 alloimmune sera consistent with earlier studies (6, 7). The test indicated that the majority of Lpb2 and Lpb3 epitopes are expressed on two independent molecules. The same pattern was observed when, instead of whole plasma, layer 3 (L_3, d 1.032–1.043 g/ml) was used. This layer was chosen for more detailed studies because it contained the majority of LDL_2 carrying apolipoprotein B as confirmed by SRID test (Fig. 2B, C), using allotypic antisera recognizing the Lpb2 and Lpb3 antigens, respectively. This layer in human plasma contains 41–65% of plasma apolipoprotein B (23). As illustrated, the distribution of Lpb-2 and Lpb-3 epitopes appears to be very similar in each of the tested $Lpb^{2/3}$ heterozygote LDL subfractions.

Layer 3 from the $Lpb^{2/3}$ heterozygous pigs was chromatographed on the anti-Lpb2 and anti-Lpb3 immunosorbers to isolate Lpb2 and Lpb3 epitopes. The retained fraction from the anti-Lpb2 immunosorber formed a precipitation band only with anti-Lpb2 (Fig. 2D, a-2R) but did not react with anti-Lpb3 antibodies (Fig. 2F, a-2R). The unretained fraction reacted only with anti-Lpb3 (Fig. 2F, a-2U) but did not react with anti-Lpb2 antibodies (Fig. 2D, a-2U). As a control, layer 3 (D–G, L_3) was used and reacted with both anti-Lpb2 and anti-Lpb3 antibodies. The retained fraction from the anti-Lpb3 immunosorber reacted with anti-Lpb3 (Fig. 2G, a-3R), but did not react with anti-Lpb2 antibodies (Fig. 2E, a-3R), while the unretained fraction reacted with anti-Lpb2 (Fig. 2E, a-3U), but not with anti-Lpb3 (Fig. 2G, a-3U).

To confirm the earlier hypothesis of different haplotype populations (6) containing a common epitope alternative to the mutant epitope, additional DID tests were per-

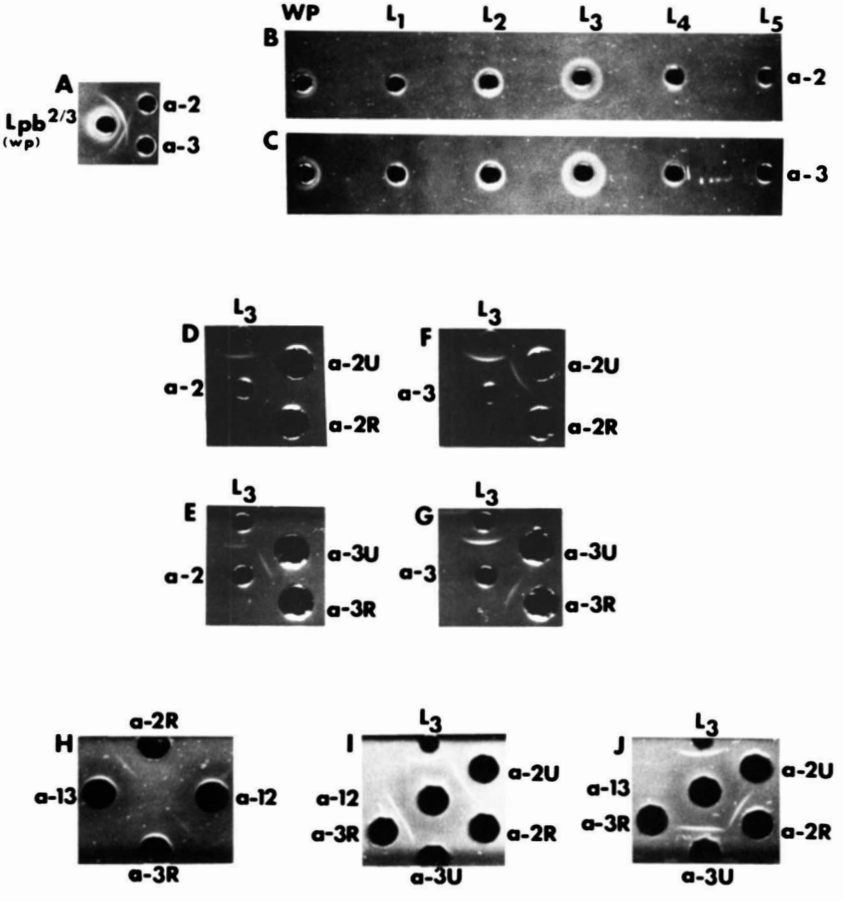

Fig. 2. Immunodiffusion patterns of separated Lpb mutant epitopes. Double immunodiffusion (DID) (A, D–J); single radial immunodiffusion (SRID) analyses (B, C) of whole plasma (WP-A, B, C); different gradient density layers (B, C, L_1–L_5; D–J, L_3); anti-Lpb2 retained (a-2R, D, F, H–J) and unretained (a-2U, D, F, I, J); anti-3 retained (a-3R, E, G–J) and unretained (a-3U, E, G, I, J) eluted from immunosorber, derived from $Lpb^{2/3}$ heterozygous pigs tested with anti-Lpb2 (A, B, D, E, H); anti-Lpb3 (A, C, F, G, H); anti-Lpb12 (I) and anti-Lpb13 (J) epitope specific reagents.

formed (Fig. 2 H,I, J). As shown in Fig. 2-J, epitope Lpb13 was detected only in the retained fraction (Fig. 2, H and J, a-2R) from the anti-Lpb2 and unretained fraction from anti-Lpb3 immunosorber (Fig. 2J, a-3U), whereas Lpb12 is present in the retained fraction from anti-Lpb3 (Fig. 2, H and I, a-3R) and unretained fraction from anti-Lpb2 immunosorber (Fig. 2I, a-2U). Other alloreagents, anti-Lpb11 and anti-Lpb14-Lpb18 reacted with all fractions, retained and unretained, as expected.

The electrophoretic studies on 2.5–15% gradient polyacrylamide gel-SDS treated with silver stain showed the presence of a single protein band for the isolated L_3 from heterozygotes $Lpb^{2/3}$ as well as for retained and unretained Lpb2 and Lpb3 fractions corresponding to apoB-100 (**Fig. 3**). Immunoblotting experiments of L_3 and isolated fractions present further evidence that these proteins are indeed apoB (**Fig. 4**) and contain the mutant epitope of the corresponding apoB haplotypes. These data demonstrate that the LDL of heterozygous sera can be separated into two LDL haplotype populations, corresponding to two mutant epitopes specified by the two apoB allelic genes.

The neutral lipid analyses by gas–liquid chromatography are presented (**Table 1**) for Lpb2, Lpb3, and the parent LDL subclass isolated lipoproteins. No qualitative or quantitative differences were observed between the parent LDL subclass and isolated lipoproteins of each haplotype, suggesting that each haplotype undergoes very similar metabolic processing in the vascular compartment. Similar results were obtained when Lpb3 isolated from the LDL (d 1.006–1.073 g/ml) of a number of different Lpb genotypes (*Lpb 1/3, 2/3, 3/3, 3/4, 3/5, 3/7* and *3/8*) were compared (**Table 2**). As can be seen, no striking differences were noted between the parent LDL fraction and the isolated Lpb3. The only exception was the $Lpb^{3/4}$ heterozygote, where Lpb3 had more TG and less CE than the parent LDL. However, these analyses represent only one animal and require additional studies for confirmation. Comparisons of various heterozygotes with the Lpb^{3} haplotype again demonstrated no striking compositional differences between the LDL or Lpb3 subfractions. The only exception was the $Lpb^{3/5}$ heterozygote with low TG in both the LDL and isolated Lpb3 fraction and high TG observed in the Lpb3 fraction isolated from the LDL of the $Lpb^{3/4}$ heterozygote.

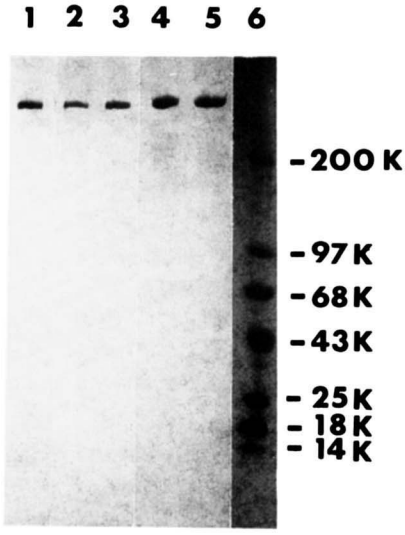

Fig. 3. Polyacrylamide SDS-PAGE patterns of L_3 and its immunoaffinity separated Lpb mutant epitopes. L_3 (1); anti-Lpb2 unretained (2); anti-Lpb3 unretained (3); anti-Lpb2 retained (4); anti-Lpb3 retained (5); and high molecular weight standard (6). Gel stained with silver stain (20 μg protein/well).

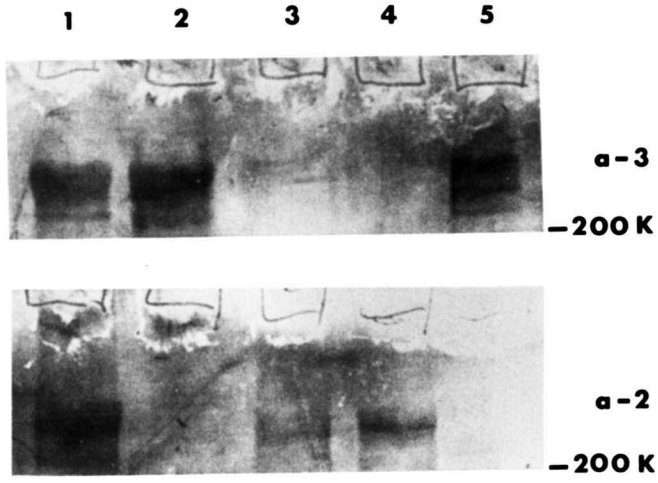

Fig. 4. Immunoblotting pattern of L_3 and immunoaffinity separated epitopes (Lpb2 and Lpb3) using swine apoB epitope specific antibodies. 1, L_3; 2, anti-Lpb2, unretained; 3, anti-Lpb2, retained; 4, anti-Lpb3, unretained; 5, anti-Lpb3, retained; a-3, anti-Lpb3; a-2, anti-Lpb2. 200K, indication of the highest molecular weight standard. The immunoblot is visualized using alkaline phosphotase substrate. Each well contained 20 μg of protein.

DISCUSSION

This study presents an approach for the separation of LDL particles, corresponding to two apoB allelic genes, into two haplotype populations in plasma of heterozygous swine. In addition, partial characterization of the allelic products and comparison of separated allelic products within and between heterozygotes were performed. Double immunodiffusion tests, polyacrylamide gel electrophoresis, and immunoblotting were used to establish this separation for the Lpb heterozygotes previously defined by Rapacz and colleagues (6, 7, 13). No differences between the isolated Lpb2 and Lpb3 lipoprotein species were found by neutral lipid analyses, indicating

TABLE 1. Neutral lipid composition[a] of two Lpb haplotypes isolated from swine heterozygotes of the $Lpb^{2/3}$ genotype (n = 4)

Lipoproteins	Lipid Composition			Lipid:Protein Composition			
	%C	%CE	%TG	%C	%TG	%CE	%PR
L_3	16.70[b] (2.13)	81.66 (3.03)	1.64 (1.23)	9.31 (0.91)	0.90 (0.64)	45.97 (7.02)	43.82 (7.33)
Lpb2 (*anti*-2 L_3-R)[c]	16.70 (1.49)	81.67 (2.26)	1.64 (0.81)	9.54 (1.72)	0.92 (0.48)	46.66 (7.41)	42.73 (8.81)
Lpb2 (anti-3 L_3-U)[d]	16.49 (1.38)	80.85 (2.45)	2.66 (1.57)	9.43 (2.94)	1.34 (0.57)	46.43 (14.92)	42.80 (17.54)
Lpb3 (anti-3 L_3-R)	16.17 (1.53)	82.17 (2.18)	1.67 (0.69)	9.15 (1.63)	1.03 (0.46)	46.57 (7.68)	43.34 (9.04)
Lpb3 (anti-2 L_3-U)	17.01 (1.95)	81.23 (2.58)	1.76 (0.84)	10.87 (2.24)	1.13 (0.62)	51.60 (6.11)	36.40 (8.09)

[a]Determined by gas-liquid chromatography; C, cholesterol; CE, cholesteryl esters; TG, triacylglycerol.
[b]Mean (SD).
[c]L_3-R, retained fraction of layer 3 (d 1.032–1.043 g/ml).
[d]L_3-U, unretained fraction of layer 3 (d 1.032–1.043 g/ml).

that these two Lpb species might undergo similar metabolic processing. These investigations also confirmed the existence of the mutant and shared epitopes as components of a very complex apolipoprotein B phenogroup (7, 8).

The purpose of this study was to confirm these findings and to devise a technique for isolation of the two gene products from the population of Lpb particles in the plasma of an individual swine. Application of this approach to humans and other species would permit the isolation and metabolic studies of two populations of LDL at the single gene level from the same heterozygote individual. This method has special value to search for metabolic defects of lipoproteins associated with structural mutants of apoB genes. Because of its specificity, the method of immunoaffinity chromatography appeared most suitable for separation of two very similar lipoproteins (16). As illustrated, separated fractions from immunosorbers retained from anti-Lpb2 and retained from anti-Lpb3 contained only Lpb2 and Lpb3 mutant epitopes, respectively (Fig. 2). The independence of phenotypic expression of these two apoB genes as two different haplotype populations in LDL was reported earlier (6, 7, 13) and has been confirmed by the present studies.

Earlier studies on swine LDL (6, 13) demonstrated that in each heterozygote pig there are two haplotypic populations of LDL particles corresponding to the products of two Lpb genes but in lipoproteins of d < 1.01 g/ml the heterozygous haplotypes seem to form multimers (9) containing the products of both *Lpb* alleles. Thus, it appears that in analogy to human lipoproteins (24), each swine LDL particle contains one B-100 molecule while swine triglyceride-rich lipoproteins contain two or more B-100 molecules, Lpr apolipoprotein (25), and other apolipoproteins. Similar complex lipoproteins containing several

TABLE 2. Neutral lipid composition of the Lpb^3 haplotype isolated from various Lpb heterozygotes

Lpb genotype	n	Parent LDL			Anti-Lpb3 Retained		
		%C	%CE	%TG	%C	%CE	%TG
LDL $Lpb^{1/3}$	1	9.90	80.82	9.29	9.49	82.34	7.57
LDL $Lpb^{2/3}$	2	10.05	86.49	3.46	8.92	86.59	4.39
LDL $Lpb^{3/3}$	1	9.36	87.32	3.32	7.65	86.80	5.54
LDL $Lpb^{3/4}$	1	11.47	85.32	3.21	8.27	80.10	11.63
LDL $Lpb^{3/5}$	3	12.98[a] (0.80)	85.29 (0.67)	1.73 (0.36)	14.64 (1.67)	84.50 (1.62)	0.85 (0.19)
LDL $Lpb^{3/7}$	1	9.56	83.65	6.79	10.62	85.34	4.04
LDL $Lpb^{3/8}$	1	10.22	86.74	3.04	10.04	86.36	3.60

[a]Mean (SD).

apolipoproteins in the same lipoprotein particle are typical of human triglyceride-rich lipoproteins (26, 27). Such complexes can be degraded to less complex lipoproteins by the action of lipoprotein lipase and can be isolated in the density class corresponding to LDL (27).

Earlier investigations (7) indicated that Lpb lipoproteins of LDL class stem from a very complex genetic locus with allelic genes each expressing the corresponding molecules. It was proposed that the demonstrated genetic polymorphism of the apoB locus evolved through mutations at different parts of the complex primordial structural apoB gene consisting of information for the common epitopes only. Mutations, which appeared during speciation, gave rise to the individual or mutant epitopes eliminating the corresponding common allotype from the structural gene. This process gave rise to new allelic apoB genes and formation of mutually exclusive pairs of epitopes in the apoB haplotype (6). As illustrated (Fig. 2), the Lpb^2 has the epitope Lpb13 while Lpb^3 has epitope Lpb12, confirming the earlier findings. The current concept, based on immunogenetic evidence, assumes that the genetic information determining the main lipoprotein family of the LDL class in the mammals studied (swine, monkeys, rabbits) occupies a very complex locus carrying multiple alleles, or is located at closely linked Ag loci in man (28). Studies in rabbits (29) and monkeys (7, 30) indicate the existence of similar complex phenogroups, as found in pigs, expressed on a single LDL particle. Such studies have not yet been reported for the Ag carrying allotypes in humans.

The characterization of lipoprotein particles of retained fractions by determination of lipid composition indicated no differences in each tested Lpb2 or Lpb3 haplotype with respect to neutral lipids (Table 1). The investigated Lpb3 epitope in other heterozygotes seems to have a lower percentage of cholesterol and higher triglyceride while the heterozygotes with Lpb5 epitope seem to have a higher level of cholesterol and lower triglyceride when the LDL (d 1.006–1.063 g/ml) was used. These differences were demonstrated with the use of the entire LDL density spectrum instead of L_3 (1.032–1.043 g/ml) in Table 1. Such observations suggest that the lipid composition of the Lpb particle is determined by the metabolic milieu.

In conclusion, these studies provide a methodological approach to separate two Lpb populations present in swine Lpb heterozygotes. Though lipid compositional differences were not found in the present study for Lpb2 and Lpb3, studies using other heterozygotes may reveal that particular apoB mutations are associated with structural alterations leading to altered lipid and/or protein:protein interactions.

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