Evolution of low density lipoprotein structure probed with monoclonal antibodies

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Abstract To assess the relationship of apoB structures in different species of animals, the expressions of apoB epitopes in the sera or plasmas of 23 different mammalian species and one marsupial, and on the low density lipoprotein (LDL) from three species of apes, six species of monkeys, and eight non-primates were measured in competitive radioimmunoassays. The abilities of the sera or LDL to compete with ^{125}I-labeled human LDL for binding to seven monoclonal antihuman LDL antibodies immobilized on microtiter plates were determined. LDL of apes bound to most antibodies, while monkey LDL bound to two or three antibodies. Other mammalian LDL bound only weakly to two of the antibodies or to none. The two monoclonal antibodies binding the LDL of more species were those antibodies which also inhibited the binding to and degradation of LDL by human fibroblasts. The rank order of binding of the LDL of a given species to the antibodies correlated with the rank order inhibition of binding and degradation of ^{125}I-labeled human LDL in the human fibroblast system. This suggests that epitopes spatially located near the recognition site of apoB for cellular receptors have a greater tendency to be conserved.—**Nelson, C. A., M. A. Tasch, M. Tikkanen, R. Dargar, and G. Schonfeld.** Evolution of low density lipoprotein structure probed with monoclonal antibodies. *J. Lipid Res.* 1984. **25:** 821–830.

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The various forms of apolipoprotein B (apoB) (1–3), comprise large proportions of the apoproteins of chylomicrons and VLDL, and the various apoB's are virtually the only apoproteins of human LDL (4). ApoB contains recognition sites that mediate the binding of LDL to the apoB,E (LDL) receptors of a variety of cells (5, 6). LDL internalization is vital for the delivery of cholesterol to cells and for the control of intracellular cholesterol synthesis (7). Studies of apoB primary sequence and conformation have been stymied because of the protein's apparent high molecular weight, lack of obvious repeating units, and its tendency towards aggregation (8–10). However, immunochemical studies conducted by several groups of workers, using monoclonal antibodies produced against apoB-containing lipoproteins, have demonstrated the presence of several epitopes on apoB (11, 12), the sharing of some of the epitopes by the B-100, B-74, B-48, and B-26 subspecies of apoB (11, 12), and the proximity of some epitopes to cellular recognition domains (11, 13).

We have produced a library of seven monoclonal antihuman LDL antibodies. All of the antibodies react with apoB-100; some also react with apoB-48, apoB-74, and apoB-26 (14). Two of the antibodies inhibit the binding of ^{125}I-labeled LDL to fibroblasts, suggesting they are directed against the cellular recognition domains of apoB (13, 15). The availability of these antibodies has made it possible to perform studies of apoB antigenic sites of various animal species in order to identify any epitopes which may be shared. Presumably, any functionally important structures on the molecule would be conserved, whereas other less crucial structures could be lost or gained during evolution (16, 17). Since an important function of apoB is the mediation of binding of some lipoproteins to cellular receptors, we tested the hypothesis that the cell recognition domains of apoB would be conserved whereas other domains may not be conserved. We determined the immunoreactivity of the LDL from various animal species with our seven monoclonal antibodies to human LDL (apoB), including the two antibodies that inhibit the binding of human LDL to fibroblast receptors.

METHODS

Lipoprotein preparation

Sera from two chimpanzees, two gorillas, two orangutans, two lion-tailed macaques, and the non-primates

Abbreviations: LDL, low density lipoproteins; VLDL, very low density lipoproteins; TMU, tetramethylurea; PBS, phosphate-buffered saline; HDL, high density lipoproteins.

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found in **Table 1** were obtained frozen from Dr. Baever of the St. Louis Zoo. Sera from another gorilla and a second sun bear were obtained fresh from the same source. Sera from three chimpanzees and three gorillas were obtained fresh from Dr. W. E. Greer of the Gulf South Research Institute at New Iberia, LA. Plasmas from all the monkeys except the lion-tailed macaque were obtained fresh from Dr. L. L. Rudel of the Department of Comparative Medicine, Bowman Gray School of Medicine. The plasma from Bowman Gray and sera from the Gulf South Research Institute contained 0.01% azide, 10^{-4} M EDTA, and dithiobisnitrobenzoic acid (1 mM), which were added immediately after the bloods were drawn. Human, rat (Sprague-Dawley), and rabbit (White New Zealand) plasmas were collected in the laboratory, and pig plasma was obtained fresh from a slaughter house. These plasmas contained 10^{-4} M EDTA, and the antibiotics as indicated below. To sera from the St. Louis Zoo, penicillin (100 μg/ml), streptomycin (50 μg/ml), azide (0.01%) and EDTA (10^{-4} M) were added as soon

TABLE 1. Cholesterol contents of LDL/HDL ratios in animal sera

Animal Species		Serum Cholesterol	LDL/HDL
		mg/dl	*ratio A_{280nm} areas*
Apes			
Chimpanzee (*Pan troglodytes*)	#1	125	
	#2	192	1.4
	#3	223	
Gorilla (*Gorilla gorilla*)	#1	316	
	#2	274	0.9
	#3	397	
Orangutan (*Pongo pygmalus*)	#1	191	1.60
	#2	251	
Monkeys			
Asian			
Lion-tail macaque (*Macaca silenus*)		134	
Cynomolgus (*M. fascicularis*)		120	0.5
Rhesus (cholesterol-fed) (*M. mulatta*)		513	2.1
Stumptailed macaque (cholesterol-fed) (*M. arctoides*)		637	1.3
African			
African green (*Cercopithecus aethiops*)		191	0.6
Patas (*Erythrocebus patas*)		199	0.3
New World			
Squirrel (*Saimiri sciureus*)		134	0.2
Non-primates			
Carnivora			
Sun bear (*Helarctos malayanus*)		187	0.4
Polar bear (*Thalarctos maritimus*)		290	1.4
Spectacled bear (*Tremarctos ornatus*)		324	4.6
Leopard (*Felis pardus*)		111	0.7
Cheetah (*Acinonyx jubatus*)	#1	181	0.3
	#2	259	
Perissodactyla			
Tapir (*Tapirus indicus*)	#1	45	
	#2	96	
Zebra (*Equus grevyi*)	#1	37	
	#2	74	
Marsupialia			
Kangaroo (*Megaleia rufa*)	#1	47	
	#2	51	
Proboscidea			
Elephant (*Elephas maximus*)		35	1.6

Rabbit (Lagomorpha), rat (Rodentia), and pig (Artiodactyla) plasmas were also studied (see Table 2).

as they were obtained and, if frozen, as thawing took place.

Sera or plasmas were adjusted to density 1.25 g/ml by addition of solid KBr. Solvent of density 1.22 g/ml density containing 5×10^{-4} M EDTA was layered over that solution. Ultracentrifugation was carried out at 45,000 rpm in a Ti 50.3 Beckman angle rotor or at 36,000 rpm in an SW40 swinging bucket rotor for 40 hr at 15°C. The lipoprotein-containing supernatants were withdrawn and concentrated by vacuum dialysis, the density was adjusted to just greater than 1.06 g/ml, and 2.5 ml was transferred to clean 13-ml tubes of the SW40 rotor. Solvent of density 1.06 g/ml (2.5 ml) was overlayered, followed by solvents of the following densities (g/ml), 2.5 ml each: 1.04, 1.03, and 1.019, all formed with KBr and containing 5×10^{-4} EDTA and 0.01% azide. Using KBr, redistribution of the salt occurred within 20 hr in the SW40 rotor at 15°C and 36,000 rpm so that a continuous gradient was obtained (**Fig. 1** and ref. 18). Fractions were obtained by piercing the bottom of the tube and collecting drops. Representative elution patterns are shown in Fig. 1. Some monkeys produced an Lp(a)-like lipoprotein very close to LDL in density. Therefore, to obtain further purification of LDL, a further density gradient ultracentrifugation of the region in question was carried out. NaCl solvents of densities 1.05, 1.04, 1.03, and 1.02 g/ml were used instead of KBr solvents because the NaCl produced a much flatter gradient.

Estimates of the apoB contents of LDL consisted of determining TMU-precipitable protein (4). Some LDL preparations also were examined by SDS-polyacrylamide linear gradient gel (3–12%) electrophoresis (19).

Competitive binding immunoassays

The monoclonal antibody preparations used here were described previously (12, 15). Competitive binding assays were carried out by binding of 150 μl (~5 μg/ml) of monoclonal antibody in PBS (0.15 M NaCl plus 0.01 M potassium phosphate, pH 7.0), to 96-well microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA). Incubation was carried out overnight at 22°C under humidified conditions. The plates were rinsed twice with PBS, and blocked with 3% BSA in PBS for 3–6 hr, humidified at 22°C. After rinsing twice with PBS, serum diluted 1:50 to 1:1600 in PBS-1% BSA or 0.5 to 64 μg of LDL protein/ml (final concentrations) of purified LDL in 1% BSA in PBS were added. Duplicate doses were used and each dose was added in a 120-μl volume. Human plasma and/or human LDL also were included as indicated. Six wells with no competitor were included (Bo), as were wells with no antibody (blanks). ^{125}I-Labeled human LDL (~10 ng/well, same donor used throughout), 110,000 cpm (50 μl), was added to each well, and incubation was

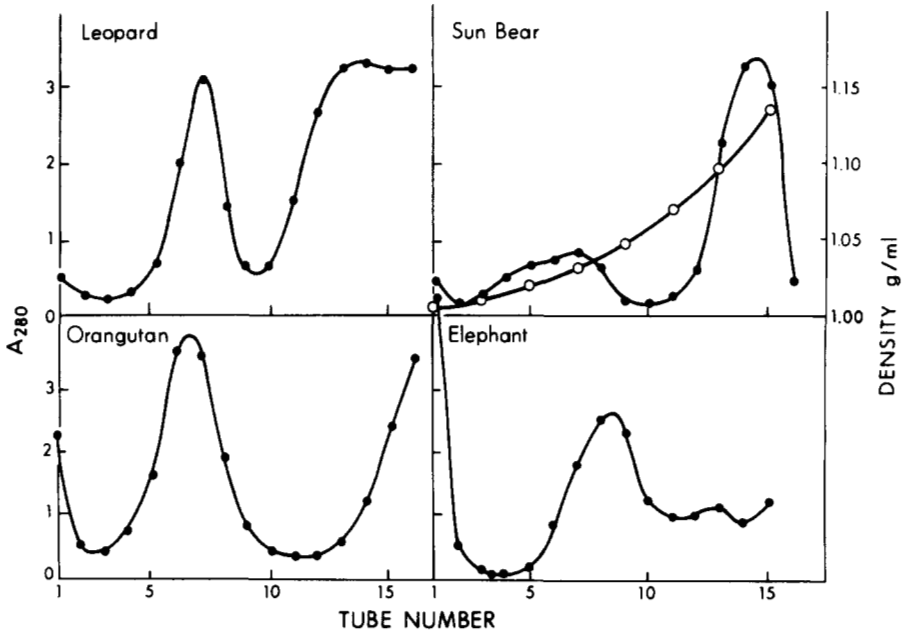

Fig. 1. Ultracentrifugal density gradient elution profiles of the indicated animal lipoproteins (previously isolated in 1.22 g/ml KBr) in KBr gradients. The pycnometrically measured densities are shown for the sun bear. The sun bear sample was freshly obtained sample (not frozen) while the others had been frozen. In tubes 1 and 2 are the VLDL (<1.01 g/ml). Tubes 5 through 9 contained LDL (1.02–1.50 g/ml). Tubes 11 or later contained HDL (>1.07 g/ml).

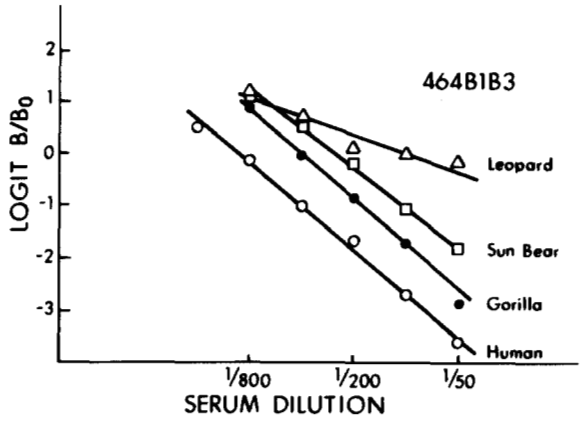

Fig. 2. Competitive displacement curves produced by animal sera or plasmas. For calculating relative quantities of sera, horizontal lines are drawn at logit B/Bo = 0, and the quantities of apoB detected in animal sera or LDL relative to human serum or human LDL are determined. Results are given in Fig. 3.

carried out overnight at 4°C. The wells were rinsed three times, cut, and counted on a Packard gamma spectrophotometer. Logit $B/Bo = \ln\left(\frac{B/Bo}{1 - B/Bo}\right)$ versus ln LDL concentration plots were constructed according to Rodbard and Lewald (20). B equals the ^{125}I-labeled human LDL (net cpm) bound to antibody in the presence of the competing serum or LDL. Bo equals the binding (net cpm) of labeled LDL in the absence of competitors. The relative binding compared to that of a standard human preparation was determined from the ratio of LDL concentrations found at a logit B/Bo of 0. Slopes of regression lines also were compared (20). Bo ranged from 3,000 to 11,000 cpm, blanks were <250 cpm. Coefficients of variation of replicates averaged 5%.

To compare immunoreactivity to biologic activity, competitive assays also were carried out of the uptake and degradation of ^{125}I-labeled human LDL by cultured human fibroblasts, in the presence of competing LDL from various animal species (21, 22).

RESULTS

Initially, 1:40–1:1000 dilutions of sera or plasmas from the animals listed in Table 1 were used in competitive assays with ^{125}I-labeled human LDL as tracer and each of seven different antihuman LDL monoclonal antibodies. A set of representative competitive displacement curves using antibody 464B1B3 is shown in **Fig. 2.** Slopes of some of the regression lines were equal to the slope of the human serum curve, some slopes were less (i.e., flatter). In order to obtain some quantitative estimates of relative competitive potencies, the serum dilutions of the different animal sera needed to yield equivalent degrees of inhibition with human serum were computed from these and similar curves (**Fig. 3**). Thus, the numbers represent differences due both to apparent affinities of an-

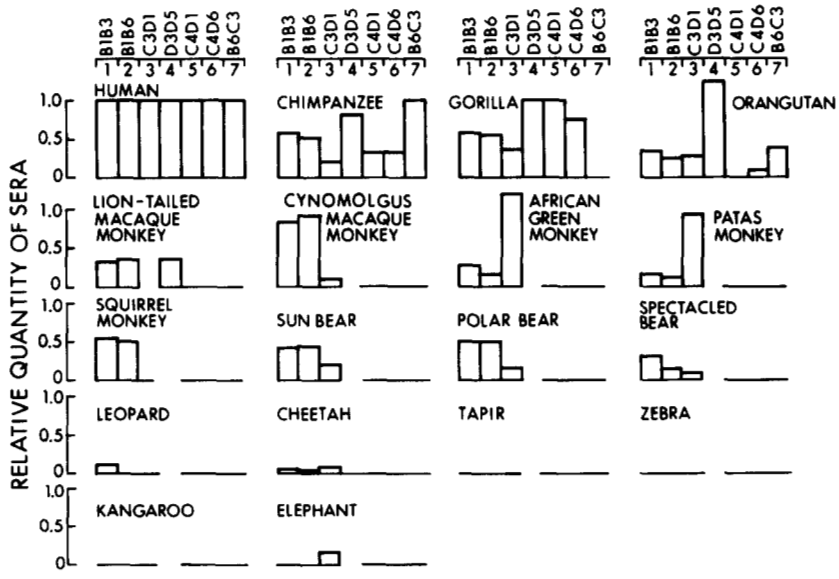

Fig. 3. The quantities of animal serum or plasma relative to human plasma necessary to produce equivalent degrees of inhibition of binding of ^{125}I-labeled human LDL to each of the seven antibodies. When more than one serum from a given species was available, averages are given. The numbers of individual animals tested are given in Table 1. Relative quantity of sera = μl human plasma ÷ μl of animal serum or plasma at logit B/Bo = 0.

tibody-serum interactions (slopes of curves) and to the positions of the curves produced by animal sera or plasmas relative to a curve produced by the human serum (shifts of curves to the right, see Fig. 2). Ape and monkey sera or plasmas bound to more of the seven antibodies than did the sera or plasmas of the non-primates. Sera of more species interacted with antibodies 464B1B3 and 464B1B6 than with the other antibodies. To test whether a single freezing and thawing affected immunoreactivity, paired samples of fresh and frozen–thawed sera of human, sun bear, and chimpanzee were tested with five of the antibodies. Relative quantities of frozen–thawed sera were either the same or at most 30% greater than fresh sera, i.e., immunoreactivity was either unchanged or enhanced by one freeze–thaw cycle. Not only did the patterns of reactivity with the library of antibodies differ between species, but the relative competitive potencies of sera, even for two individuals of the same species, also varied by as much as 2- to 3-fold in assays using any given antibody (data not shown). However, the pattern of reactions was the same for all animals of a given species.

Another way of assessing the immunoreactivity of animal plasmas or sera is to perform radioimmunoassays in which the standard is a human LDL preparation rather than a human plasma sample. This allows for the calculation of apparent concentrations of apoB mass (**Table 2**). In addition to assaying many of the animal species present in Table 1 and Fig. 3, these assays also included plasmas from pigs, rats, and rabbits. Again, more species reacted with antibodies 464B1B3 and 464B1B6 than with other antisera.

Inasmuch as the cholesterol contents (23) and LDL/HDL ratios of different sera differed greatly (Table 1), and apoB contents generally are strongly and positively correlated with cholesterol concentrations (24, 25), the results obtained with sera could have been greatly affected by the variations of apoB contents in the various animal sera. Consequently, isolated LDL was tested directly in order to avoid these confounding effects. LDL species were isolated by density gradient rather than sequential ultracentrifugation to avoid any preconceived assumptions of the density range of LDL. In fact, most LDL did peak at densities close to that of human LDL. The sun bear contained a component lighter than LDL which was not studied, and the orangutan serum contained a very dense HDL (HDL_3) compared to the others. It is not known whether this is a true reflection of HDL heterogeneity or an artifact of freezing and thawing. HDL structure is not stable to freezing and thawing (26). More than 80% of the proteins in all LDL preparations tested were precipitable by TMU (**Table 3**) and on the SDS-gels the vast majority (>85%) of the apoB appeared to be apoB-100, although small amounts of the smaller forms of apoB and other apoproteins were also seen in several samples.

Representative competitive displacement curves produced by isolated LDL of several animal species with antibody 464B1B3 are shown in **Fig. 4.** Slopes of displacement curves (calculated from logit B/Bo vs. ln dose plots) for human LDL ranged from −0.94 to −1.15 in different assays. Slopes for the different animals species fell into two groups, one ranging from −0.72 to −1.42, the other from −0.1 to 0.0. Correlation coefficients

TABLE 2. Apparent contents of apoB epitopes in animal plasmas and sera

Animal Species	B1B3	B1B6	C3D1	C4D1	C4D6	B6C3
			μg/ml			
Apes						
Chimpanzee (223)	457	524	0	318	238	360*
Gorilla (397)	2013*	1475	960*	1307	1163	276*
Monkeys						
Lion-tailed macaque (206)	308	261	0	16	0	15*
Others						
Pig (217)	747*	877*	0	0	0	0
Cheetah (259)	64	36	0*	0	0	0
Rat (52)	0	0	0	0	0	0
Rabbit (28)	120	140		0		
(1800)		1045*	0		0	0
Tapir (96)	135*	44	0	46	0	145
Zebra (73)	0	11*	0	40*	0	0
Kangaroo (51)	54*	0	0	78*	0	340*

Results are means of radioimmunoassays performed in duplicate wells. Six doses of samples were used (dilutions 1:40–1:1000). Unstarred reuslts represent parallelism between standard curves and samples over at least three doses of sample. Starred results represent lack of parallelism. A minus (−) sign designates that the sample was not done, a zero (0) that no significant displacement was produced by the maximum dose of sample. Values in parentheses are cholesterol concentrations for the indicated animal (in mg/dl).

TABLE 3. Percent of apoB in various LDL preparations

Species	% ApoB*
Human	97.8
Chimpanzee	85.1
Gorilla	90.9
Orangutan	85.8
Lion-tailed macaque	87.8
Sun bear	97.9
Polar bear	96.9
Leopard	90.1
Rabbit	84.2
Rat	81.0

Determined as % of LDL protein precipitable by 50% tetramethylurea. On SDS-polyacrylamide gel electrophoresis the LDL of the following species of nonhuman primates contained >85% apoB; cynomolgus, rhesus, African green, Patas, and squirrel monkeys.

ranged from 0.979 to 0.999. Coefficients of variation for replicates averaged 5%. The apparent quantities of apoB in animal LDL relative to human LDL were calculated at B/Bo = 0.5 using curves with slopes of −0.72 to −1.42. Data for individual animals are given in **Table 4.** Curves with slopes −0.1 to 0.0 were deemed to be produced by nonreactive LDL because the relative quantities of LDL would be <0.01. Most LDL species inhibited binding of ^{125}I-labeled human LDL to antibodies 464B1B3 and 464B1B6 and the relative quantity of LDL appeared to be greatest in primates. Antibody 465C3D1 bound all LDL of apes; among monkeys, only African green and patas LDL reacted, but LDL from other species did not bind. The poor binding of non-primate LDL is somewhat in contrast to the results with sera that did inhibit binding of ^{125}I-labeled human LDL to this antibody. This suggests that some other material (perhaps Lp(a) or complement) was present in sera which contributed to the reaction with 465C3D1, or that the epitope in question lost activity during LDL isolation. Chimpanzee and gorilla LDL inhibited binding to antibody 465C4D1 while chimpanzee, gorilla, and orangutan LDL inhibited binding to antibody 465B6C3. Preparations of LDL isolated from individual animals did vary somewhat in their immunologic reactivities (Table 4). Heterogeneities in the expression of these epitopes also were found among individual human LDL preparations (27).

Correlation of antibody binding with cell metabolism

To assess whether the abilities of the LDL of various species to compete with ^{125}I-labeled human LDL for

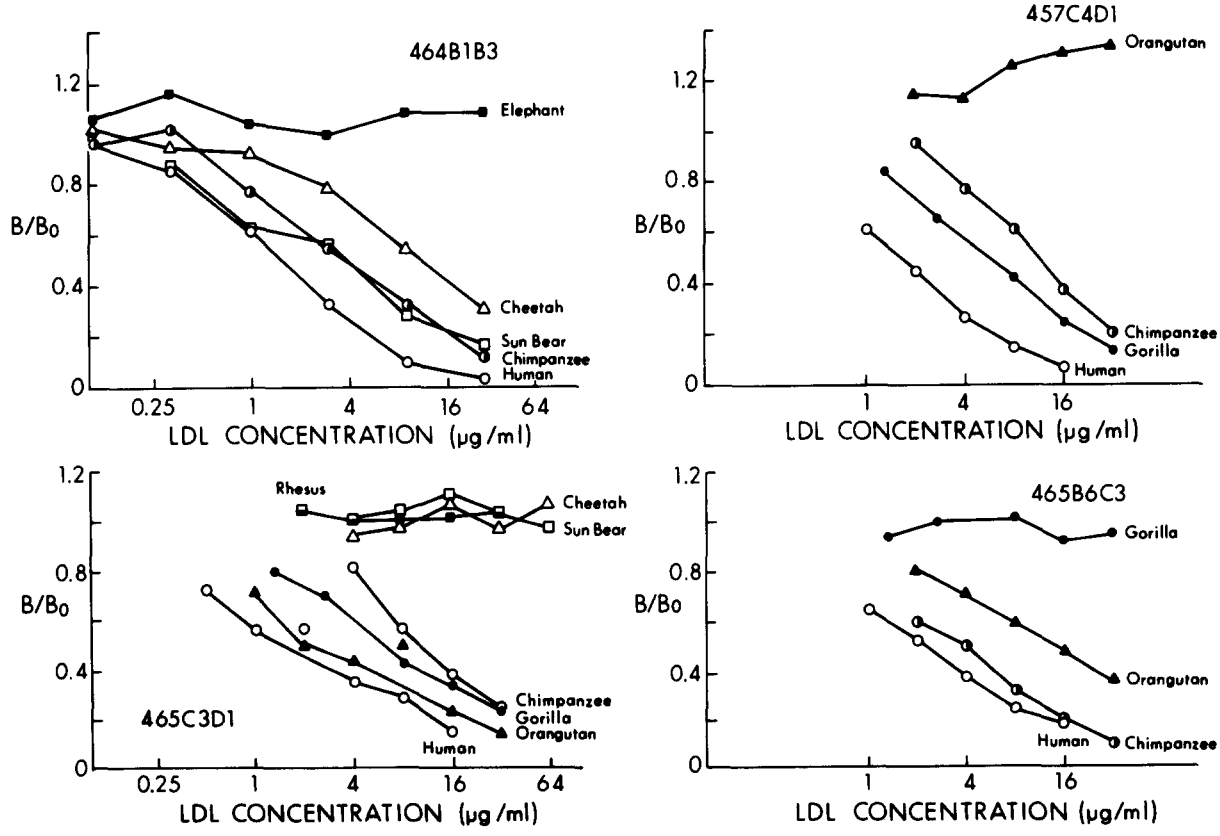

Fig. 4. Representative plots of logit B/Bo versus ln LDL concentration in µg protein/ml for four antibodies. Coefficients of variation of duplicate points were 5%.

TABLE 4. Relative binding of LDL from various animals to anti-human LDL monoclonal antibodies

LDL Donor Species	Monoclonal Antibody						
	464B1B3	464B1B6	465C3D1	475C4D1	457C4D6	465B6C3	465D3D5
Human	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Apes							
Chimpanzee #1	0.26		0.29, 0.31			1.18	
#2	0.36		0.33, 0.37			0.30	
#3	0.30	0.38	0.42, 0.29	0.19	0.17	0.80	0.85
Gorilla #1	0.25		0.41			0.26	
#2	0.37		0.23			0.14	
#3	0.27		0.53				
#4	0.40	0.52	0.31	0.29	0.26	0.15	0.82
Orangutan	0.27	0.35	0.55	0.14	0	0.13	0.43
Monkeys							
Cynomolgus #1	1.7	1.5	0	0	0	0	0
#2	0.26						
#3	0.52						
Rhesus #1	1.0	0.83	0				
#2	0.26	0.35	0	0	0		0
#3 (chol.-fed)	1.16	1.0	0	0		0	
Stumptail #1 (chol.-fed)	0.43	0.42	0	0	0	0	0
#2	0.17						
#3	0.18						
African green #1	0.61	0.74	1.9	0	0	0	0
#2	1.4						
#3	1.3						
Patas #1	0.56	0.54	1.0	0	0	0	0
#2	0.32						
#3	0.30						
Squirrel #1	0.80	0.96	0	0	0	0	0
#2	1.40						
Non-primates							
Sun bear	0.18	0.25	0	0	0	0	0
Polar bear	0.13	0.10	0	0	0	0	0
Cheetah	0.09	0.03	0	0	0	0	0
Leopard	0.14	0.05	0	0	0	0	0
Rabbit	0.10	0.09	0	0	0	0	0

Results are apparent concentrations of apoB per mass LDL protein relative to the apparent apoB contents of human LDL in individual animals.

binding to antibodies ranked with their abilities to compete with ^{125}I-labeled human LDL for binding to cellular LDL receptors, some LDL which bound poorly to antibodies 464B1B3/6, such as leopard and polar bear LDL, and some LDL which bound well to those antibodies, such as rhesus and chimpanzee LDL, were used as competitors against ^{125}I-labeled human LDL for interacting with cultured human fibroblasts (**Fig. 5**). Leopard and polar bear LDL inhibited human LDL uptake and degradation poorly (50% inhibitory concentrations for degradation were too large to determine for the bear, and were 193 μg/ml for the leopard), while chimpanzee and rhesus LDL were more inhibitory relative to human LDL (respective 50% inhibitory concentrations were 122, 67, and 67 mg/ml).

To confirm that antibodies 464B1B3 and 464B1B6 did indeed inhibit the uptake and degradation of ^{125}I-labeled human LDL by human fibroblasts, 5 μg/ml of ^{125}I-labeled human LDL was incubated with human fibroblasts in the presence and absence of 25 μg/ml of the antibodies. Uptake and degradation were inhibited by 69 and 66%, respectively, by antibody 464B1B3 and 79 and 84%, respectively, by antibody 464B1B6. In parallel experiments, rhesus monkey cultured fibroblasts were incubated with ^{125}I-labeled rhesus LDL, with and without antibody 464B1B3. This antibody also inhibited the cell

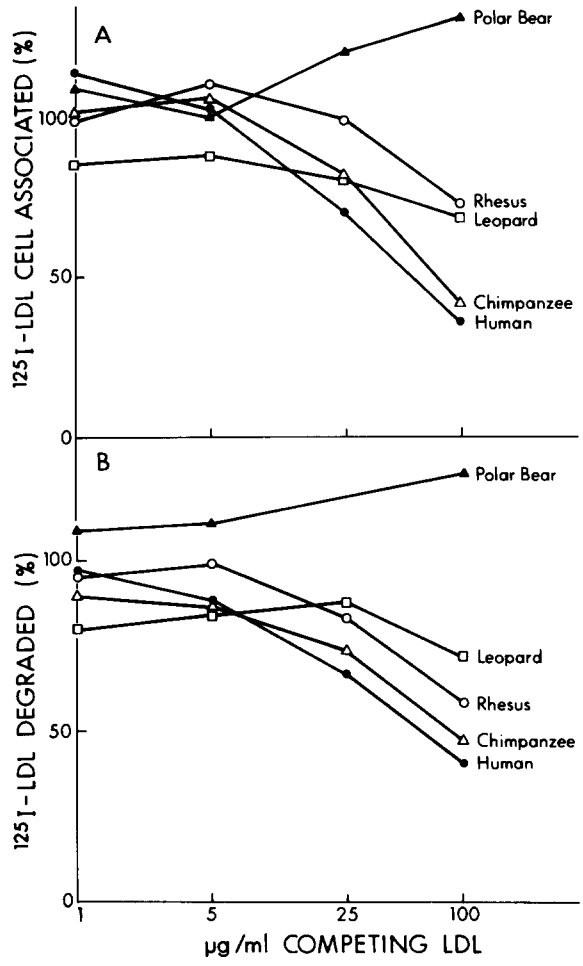

Fig. 5. Competition between animal LDL and human ^{125}I-labeled LDL for uptake (A) and degradation (B) by normal human cultured fibroblasts. Five μg of protein/ml of human ^{125}I-labeled LDL and the indicated amounts in μg of protein/ml of animal LDL were incubated for 4 hr at 37°C in lipid-free media. Uptake represents counts associated with cells after cells are washed several times to remove nonspecifically bound ^{125}I-labeled LDL. Degradation represents 10% trichloroacetic acid soluble non-iodide counts in media. Results are means of two dishes per point. Coefficients of variation were 5%.

association and degradation of ^{125}I-labeled rhesus LDL by 72 and 76%, respectively, whereas several monoclonal antihuman apoA-I antibodies did not.

DISCUSSION

The aim of these experiments was to ascertain whether and to what extent the apoB of various mammalian and a marsupial species shared some structural features with each other and with human apoB. Seven monoclonal antibodies known to be directed against at least five distinct non-overlapping epitopes were used as probes (13). Since two of the antibodies (464B1B3 and 464B1B6) appear to be directed against epitopes which are spatially related to the domain on apoB recognized by cellular LDL receptors (13, 15), it was of particular interest to observe whether these epitopes would be conserved during the evolution of species. Initial experiments were performed using whole plasmas or sera, rather than isolated LDL because it was felt that any cross-reactivity would provide some preliminary information on the presence of apoB-like molecules. It was recognized that for accurate quantitation it would be necessary to use LDL. It turned out that the results obtained with LDL and unfractionated plasmas on sera were qualitatively similar. The experimental data indicated that apes share the most epitopes with human LDL, followed by monkeys and then the non-primate species. Comparable results were obtained by others using polyclonal antihuman LDL antisera (28–30) where individual epitopes could not be evaluated. The patterns of cross reactions between human LDL and the LDL of animals also is similar to results obtained with alkaline phosphatase and HLA antigens in assays using monoclonal antibodies (31, 32).

For the sake of uniformity, it would have been preferable to have used fresh plasmas throughout rather than some samples consisting of frozen sera, fresh plasmas, and fresh sera, but had this restriction been imposed, it would have been impossible to obtain specimens from a large proportion of the animal species. Nevertheless, several lines of evidence indicate that the results may be reliable: *a*) no differences in apoB are noted between fresh and frozen plasma when paired samples are analyzed by radioimmunoassays for apoB performed with polyclonal rabbit antihuman LDL antisera (23) or mouse antihuman LDL monoclonal antibody 464B1B3 (33); *b*) conditions much more disruptive of apoB structure, i.e., chemical modification of arginine or lysine residues (13) or limited proteolysis of LDL (15), do not inactivate the immunoreactivities of the 464B1B3 or 464B1B6 epitopes; *c*) 457C4D1, 475C4D6, 465B6C3, 465C3D1, and 465D3D5 epitopes are detectable on both frozen and thawed human and many non-human primate LDL, but not on many non-primate LDL, suggesting that the low levels or absence of reaction in non-primates is not due to artefactual inactivation of epitopes; *d*) paired unfrozen fresh and frozen and thawed sera of several species gave relative quantities which were within 30% of each other. In sum, these data suggest that LDL structure remains reasonably intact after a single freezing and thawing, and that the immunoreactivities of the most important epitopes (464B1B3 and 464B1B6) under study survive particularly well.

Another potentially confounding factor could have been differences in the apoprotein compositions of LDL. Since 85–98% of LDL proteins were TMU-precipitable

(Table 3) and apoB was by far the predominant apoprotein, it is unlikely that any non-apoB protein components of the different LDL could explain the species differences. The chemical compositions of LDL, i.e., the LDL-lipids, could also have affected the absolute values of the apparent apoB contents of the various LDL (27), but it is unlikely that the patterns of reactivity with the different antibodies would have been affected.

Of great interest was the selective conservation across species of epitopes related to the cellular binding of LDL, epitopes defined by antibodies 464B1B3 and 464B1B6. The results imply that the structures of those epitopes and perhaps of the cellular recognition sites on apoB are similar to the analogous human structures. It may not be too far fetched to extrapolate similarities in the structures of LDL receptors as well (34). On the other hand, the animals that cross-react poorly with the above antibodies may possess either no apoB cellular recognition sites and LDL receptors, or the complementary structures of the cellular receptor-apoB recognition site pairs differ materially from those of the cross-reacting species.

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