Biochem. J. (1988) 252, 237–245 (Printed in Great Britain)

Rat monoclonal antibodies to rabbit and human serum low-density lipoprotein

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A total of 16 hybrid myeloma clones secreting monoclonal antibodies (McAb) to rabbit or human serum low-density lipoprotein (LDL) were derived from the fusion of spleen cells from LOU or DA rats immunized with rabbit or human LDL and the rat myeloma lines Y3 Ag1.2.3 or YB2/0. Anti-(rabbit LDL) McAb showed limited reactivity with LDL from human, rhesus-monkey, rat and mouse serum. Six out of seven anti-(human LDL) McAb reacted with rhesus-monkey LDL, and only one showed partial cross-reaction with rabbit LDL. Binding-competition experiments indicated that the epitopes recognized by the anti-(rabbit LDL) IgG could be grouped into two major clusters: McAb in the first cluster reacted either with apo-(lioprotein B-100) (apoB-100) and apo-(lipoprotein B-74) (apoB-74) or with apoB-100 but not with apo-(lipoprotein B-48) (apoB-48), the lower-M_r form of apoB of intestinal origin; the McAb in the second cluster all reacted with apoB-48 in addition to apoB-100 or apoB-100 and apoB-74. The six anti-(human LDL) IgG bound to separate epitopes on LDL. Further data on the epitope specificity of these McAb were obtained by antibody blotting after partial proteolysis of apoB-100 with trypsin or staphylococcal V8 proteinase, and the data confirmed the results obtained with the binding-competition experiments. One McAb to rabbit LDL inhibited the binding of LDL to the fibroblast LDL receptor (50% inhibition at a McAb/LDL molar ratio of 10). A similar result was produced by two other McAb at higher concentrations of antibody.

INTRODUCTION

In man, 60–65% of serum cholesterol is transported in low-density lipoprotein (LDL; ρ 1.019–1.063 g/ml), a lipoprotein rich in cholesteryl esters and cholesterol which contains a single protein (apoB) as apolipoprotein. ApoB is responsible for the binding of LDL to its receptor, a membrane protein which promotes LDL uptake and degradation in several tissues (Goldstein & Brown, 1977; Brown *et al.*, 1981).

A lipoprotein homologous with human serum LDL is present in a number of animal species, although in several species high-density lipoprotein (HDL; ρ 1.063–1.21 g/ml) rather than LDL is the major cholesterol carrier in serum (Chapman, 1980). In the rabbit, a species which is widely used for studies on lipoproteins and atherosclerosis, 45–50% of serum cholesterol is transported in LDL.

In the last few years it has been discovered that the apoB associated with serum VLDL, IDL and LDL is not identical with the apoB associated with chylomicron and chylomicron remnants. Chylomicron and LDL apoB(s) (apoB-48 and apoB-100 respectively) have different M_r values, slightly different amino acid compositions and are synthesized in different tissues [apoB-48 is synthesized in the intestine and apoB-100 is synthesized in the liver (Kane *et al.*, 1980; Kane, 1983)]. Furthermore, whereas apoB-100 binds with high affinity to the LDL receptor, apoB-48 does not (Hui *et al.*, 1984*b*). Nevertheless, the two proteins are immunochemically related, since antisera produced against LDL react with apoB-100 and with apoB-48 (Kane, 1983). More recently, apolipoproteins homologous with human apoB-100 and apoB-48 have been identified in several animal species, including rabbit (Kroon *et al.*, 1985).

The availability of antibodies specific for apoB-100 and apoB-48 may be of considerable interest for animal studies on lipoprotein synthesis, turnover and catabolism, as separate receptors control the catabolism of lipoproteins containing apoB-48 or apoB-100 in liver, and the two receptors appear to be under separate genetic (Kita *et al.*, 1982; Bilheimer *et al.*, 1982) and metabolic (Angelin *et al.*, 1983) control. We report here the derivation and characterization of two sets of rat McAb to rabbit and human LDL. Several McAb react with both apoB-100 and apo-48; others are specific for apoB-100, and do not cross-react with apoB-48.

MATERIALS AND METHODS

Isolation of LDL

LDL was isolated and purified from rabbit, human, rhesus monkey (*Macaca mulatta*), rat and mouse sera by preparative ultracentrifugation in a 50 Ti rotor at 49000 rev./min for 16 h at 4 °C. The density of the serum was

Abbreviations used: VLDL, very-low-density lipoproteins (ρ < 1.006 g/ml); IDL, intermediate-density lipoprotein (ρ 1.006–1.019 g/ml); LDL, low-density lipoprotein (ρ 1.019–1.063 g/ml); HDL, high-density lipoprotein (ρ 1.063–1.21 g/ml); apoB-100, apo-(lipoprotein B-100); apoB-48, apo-(lipoprotein B-48); BSA, bovine serum albumin; TBS, Tris-buffered saline (0.025 M-Tris/HCl/0.15 M-NaCl, pH 7.4); PBS, phosphate-buffered saline (0.025 M-monosodium phosphate/disodium phosphate/0.15 M-NaCl, pH 7.4); DMEM, Dulbecco's modified Eagle's medium; McAb, monoclonal antibody(ies).

raised with solid KBr. Lipoproteins were purified by re-centrifugation, dialysed against 0.01 M-Tris/HCl/0.15 M-NaCl/0.001 M-EDTA, pH 7.4 at 4 °C, and stored at 4 °C after filtration on 0.22 μm-pore-size filters. Purity of LDL was established by agarose-gel and SDS/polyacrylamide-gel electrophoresis. More than 95% of the human and rhesus-monkey LDL protein was apoB-100. Rabbit, rat and mouse LDL contained apoE (3–11%) and traces of apoC species (1–3%) in addition to apoB-100. LDL protein was measured as described by Markwell *et al.* (1978) and used to express LDL concentrations. Rabbit and human LDL were labelled with ^{125}I by using a modification of the lactoperoxidase procedure of Marchalonis (1969).

Immunization with LDL

Groups of four male adult rats (DA and LOU) and mice (C57BL10 and BALB/c) were immunized with rabbit and human LDL in Freund's complete adjuvant (Difco 0638-60-7). Each animal received either 125 μg (rats) or 25 μg (mice) of LDL as multi-site intramuscular injections. Antibody titre in serum (defined as the highest dilution of serum giving an absorbance reading of twice the background in the enzyme-linked solid-phase assay) was measured before, and 3 weeks after, the primary immunization. As the best antibody response was obtained in rats, rat spleen cells were used to derive the antibody-secreting cell lines. The animal used for the fusion received a second injection of LDL (50 μg intravenously in 0.1 ml of 0.01 M-Tris/HCl/0.15 M-NaCl, pH 7.4) 3 days before isolation of spleen cells for fusion.

Derivation of hybrid myelomas

Derivation of hybrid myelomas was carried out essentially as described by Galfre' & Milstein (1981). For generation of anti-(rabbit LDL) McAb, 2.1×10^8 spleen cells from a LOU rat were used for fusion. Spleen cells (1.05×10^8) were fused with 4.2×10^7 cells of the rat myeloma line Y3 Ag1.2.3 (Galfre' *et al.*, 1979) and 1.05×10^8 cells were fused with 4.2×10^7 cells of the rat myeloma line YB2/0 (Kilmartin *et al.*, 1982). For generation of anti-(human LDL) McAb, 1.3×10^8 spleen cells from a DA rat were fused with 5×10^7 cells of the rat myeloma line Y3 Ag1.2.3. At 10–15 days after fusion, culture supernatants were tested for anti-LDL antibodies. Selected positive cultures were cloned in soft agar, and individual clones were picked when at the stage of a few hundred cells and transferred on to 24-well plates. Cells [$(0.5–1) \times 10^6$] were then transferred into 25 cm^2 flasks in 5 ml of 20% (v/v) fetal-bovine serum in DMEM. After 3–4 days, $(2.5–5) \times 10^6$ cells were transferred into 75 cm^2 flasks in 15 ml of 20% fetal-bovine serum in DMEM. At this stage the spent medium was collected and the cells used for the generation of ascites tumours.

Solid-phase enzyme-linked assay for anti-LDL antibodies

96-well plates (Dynatech, M129A) were coated overnight at 4 °C with LDL (250 ng/well) in 0.05 ml of 0.05 M-Na_2CO_3 and blocked with BSA (2.5 mg/well) in 0.05 M-Na_2CO_3. First antibodies were incubated for 1 h at room temperature, and plates were washed three times with Tween 20 (Sigma, P-1379; 1 g/litre in TBS) before incubation for 1 hour with horseradish-peroxidase-conjugated anti-rat IgG [Miles; 61.206; diluted 1:1000 with BSA solution (25 g/litre) in TBS]. Plates were washed as described above and peroxidase detected by addition of 0.005 M-*o*-phenylenediamine (Sigma, P-1526; 0.15 ml/well) and 0.005 M-H_2O_2 (BDH, 28519) in 0.1 M-phosphate/citrate buffer, pH 5.0. The reaction was stopped by addition of 2 M-H_2SO_4 and the A_{490} was measured in a Dynatech MicroReader II microphotometer. Preliminary experiments were performed to optimize the assay, which included coating the wells with different amounts of LDL (25 ng–2.5 μg/well) and washing the plates with various concentrations of Tween 20 or BSA in TBS. The procedure finally adopted, as described above, was sensitive enough to detect anti-LDL antibodies in hybridoma supernatants diluted 5–10 times with minimal background. The coefficient of variation for the assay was 5.8% ($n = 5$).

Binding-competition assay

Binding-competition assays were performed as described by Fisher & Brown (1980) using a modification of the solid-phase enzyme-linked assay. Plates (96-well) were coated with LDL (25 ng/well). McAb were concentrated 10-fold from the spent culture medium by centrifugation at 10000 *g* for 10 min at 4 °C after incubation for 1 h in ice with $(NH_4)_2SO_4$ (353.5 g/litre). Each McAb was incubated with LDL individually and in combination with each of the other McAb in a 1:1 (v/v) ratio. The final amount of McAb bound to LDL was then measured with horseradish-peroxidase-conjugated anti-rat IgG. The binding of a mixture of two McAb to LDL was expressed in the following way:

$$B = A_{(a+b)}/(A_a + A_b) \times 2$$

where $A_{(a+b)}$ is the absorbance measured for the binding of the mixture of McAb a and McAb b and A_a and A_b are the absorbances measured for the binding of McAb a and McAb b separately. For pairs of McAb which bind to LDL with comparable binding affinities, $B = 2$ if no competition exists between McAb a and McAb b: $B = 1$ (or close to 1) if a strong competition exists between the two McAb. Intermediate values are obtained if there is a certain amount of competition between the two antibodies. IgM McAb were not included in the assay because the assay is not suitable for comparison of McAb of different classes.

Assuming the amount of competition between different McAb to be inversely related to the distance between the epitopes which they recognize, a coefficient related to the distance between the different epitopes was calculated and used to derive an epitope map using a clustering technique based on the procedures described by Ward (1963) and Everitt (1981) as implemented in the computer program CLUSTAN (Wishart, 1978).

Antibody blotting of apoB and apoB fragments

Antibody blotting of apoB was carried out after SDS/polyacrylamide-gel electrophoresis of VLDL or LDL protein(s) essentially as described by Burnette (1981). After electrophoresis, gels were equilibrated for 15 min in 0.025 M-Tris/0.2 M-glycine in water/methanol (4:1, v/v) and proteins electrophoretically blotted for 1 h at 0.5 A on to 0.45 μm-pore-size nitrocellulose paper (Schleicher und Schuell, 401196). Strips (0.5 cm) of nitrocellulose paper were cut, blocked for 30 min with BSA (25 g/l in PBS), incubated for 1 h with the different

Table 1. Designation, isotype and apoB specificity of the rat McAb to rabbit and human LDL

The isotype of the McAb was determined by double immunodiffusion using isotype-specific anti-(rat IgG) antisera. The apoB specificity of the McAb was established by antibody blotting of the apoB(s) associated with rabbit and human VLDL ($\rho < 1.006$ g/ml).

Immunogen	Spleen cell	Myeloma	McAb	Isotype	apoB specificity
Rabbit LDL	LOU rat	Y3 Ag1.2.3	MAC22	γ2a	B-100, B-74
			MAC23	γ2a	B-100, B-74, B-48
			MAC24	μ	Unknown
			MAC25	μ	Unknown
		YB2/0	MAC26	γ2b	B-100, B-48
			MAC27	γ2a	B-100
			MAC28	γ2b	B-100
			MAC29	γ2a	B-100
			MAC31	γ2a	B-100, B-74, B-48
Human	DA rat	Y3 Ag1.2.3	MAC127	γ2b	B-100, B-74
			MAC128	μ	Unknown
			MAC129	γ1	B-100, B-74, B-48
			MAC130	γ2a	Unknown
			MAC131	γ2a	B-100, B-48
			MAC132	γ2b	B-100
			MAC133	γ2b	Unknown

antibodies (spent hybridoma culture medium) and washed three times with Tween 20 (1 g/l) in PBS. Strips were then incubated with peroxidase-conjugated anti-rat IgG [diluted 1:1000 with BSA (25 g/l) in PBS], washed three times for 15 min, and incubated in 0.0035 M-4-chloro-1-naphthol (Sigma, C-8890) and 0.005 M-H_2O_2 in PBS/methanol (4:1, v/v). All incubations were performed at room temperature with constant rocking.

Inhibition of binding of rabbit ^{125}I-LDL to fibroblast LDL receptor by anti-LDL McAb

Cultures of rabbit fibroblasts were established from skin biopsies of New Zealand White rabbits and cultured in 10% (v/v) fetal-calf serum in DMEM in the presence of 100 i.u. of sodium penicillin/ml and 100 μg of streptomycin sulphate/ml. Cultures of human skin fibroblasts were generously given by Dr. J. Owen (Royal Free Hospital, Hampstead, London NW3, U.K.). Binding of ^{125}I-LDL to fibroblasts was carried out at 4 °C. Briefly, 25000 cells were plated in 35 mm-diameter dishes, cultured for 4 days in 10% (v/v) fetal-bovine serum in DMEM and for 1 day in 10% (v/v) lipoprotein-deficient fetal-bovine serum in DMEM before the cells were incubated either with ^{125}I-LDL or mixtures of purified McAb and ^{125}I-LDL as detailed in the legend to Fig. 6 (below).

Purification of McAb from ascitic fluid

McAb MAC27, MAC28 and MAC29 were purified from the ascitic fluid of LOU rats injected intraperitoneally with 10×10^6 hybrid cells. Rats were injected intraperitoneally with 2 ml of pristane (Sigma T7640) 2 weeks before injection of the cells. Ascitic fluid was collected from the peritoneal cavity 1–2 weeks after injection of the hybrid cells. McAb were partially purified by $(NH_4)_2SO_4$ precipitation (353.5 g/litre) in ice for 1 h, followed by centrifugation at 10000 *g* for 10 min at 4 °C, dialysed against 0.02 M-triethanolamine buffer, pH 7.7, and finally purified by fast-protein-liquid (ion-exchange) chromatography on a MonoQ column (Pharmacia) using a gradient of NaCl (0–0.35 M) in 0.02 M-triethanolamine, pH 7.7. Purity of McAb was confirmed by agarose-gel electrophoresis and SDS/polyacrylamide (125 g/l)-gel electrophoresis under reducing conditions.

RESULTS

Derivation of rat hybrid myelomas secreting McAb to rabbit and human LDL

Nine cultures secreting antibodies to rabbit LDL and seven cultures secreting antibodies to human LDL were cloned and selected for further study. Table 1 shows the designation of these clones, their parent myeloma and the isotype of the McAb they secrete. The specificity of the McAb for the different forms of apoB was defined by antibody blots, and the data are summarized in Table 1. Although most of the IgG species bound to one or more forms of detergent-solubilized apoB, two anti-(human LDL) monoclonal IgGs (MAC130 and MAC133) did not. These two McAb may react against a conformation-dependent epitope of apoB or to a lipid determinant. None of the IgM species bound to detergent-solubilized apoB (Table 1).

Species specificity of rat McAb to rabbit and human LDL

Rat McAb to rabbit LDL showed considerable species specificity (Fig. 1). Only three anti-(rabbit LDL) monoclonal IgG species (MAC27, MAC28 and MAC31) showed some reactivity (8–19%) with human LDL and none reacted appreciably with rhesus-monkey and rat LDL (Fig. 1). However, all rat anti-rabbit LDL McAb showed partial cross-reactivity (11–34%) with mouse LDL (Fig. 1).

All the anti-(human LDL) McAb except MAC130 reacted equally well with human and rhesus-monkey LDL (Fig. 2). McAb MAC130 showed negligible binding to rhesus-monkey LDL, indicating that the epitope recognized by this McAb was specific for human LDL.

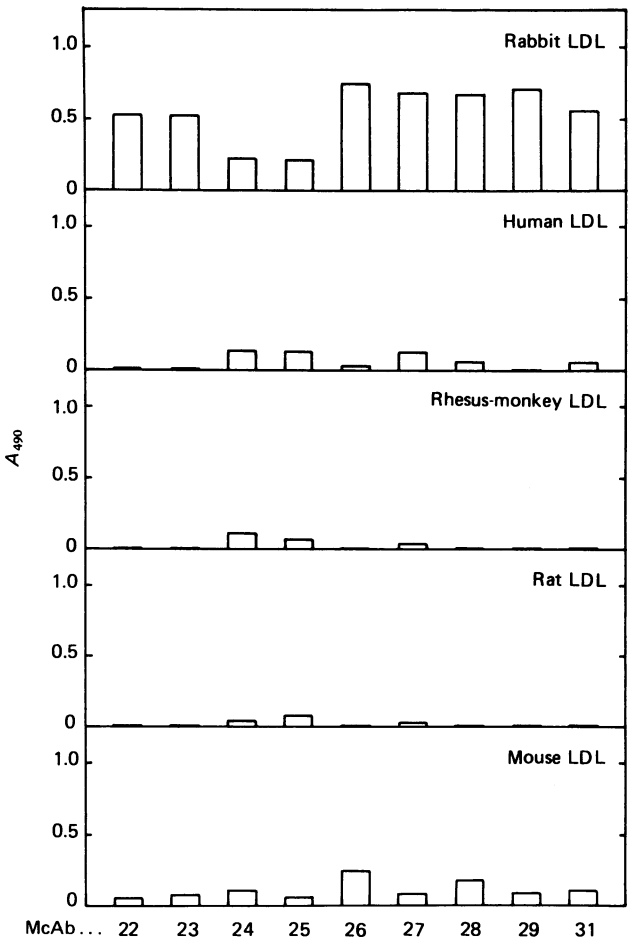

Fig. 1. Reactivity of rat McAb to rabbit LDL with LDL isolated from different species

LDL (ρ 1.019–1.063 g/ml) was isolated and purified from serum, and the binding of McAb to 250 ng of LDL from the species shown in the Figure was determined by using a solid-phase enzyme-linked assay. The assay was carried out on triplicate wells. Numbers at the bottom indicate different McAb.

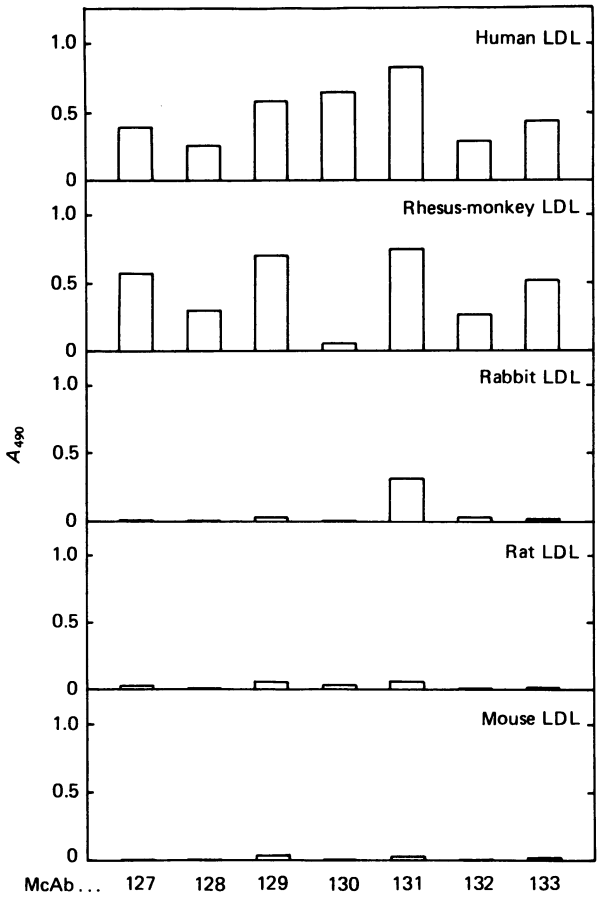

Fig. 2. Reactivity of rat McAb to human LDL with LDL isolated from different species

This was carried out as described in Material and methods and in the legend to Fig. 1 by using a solid-phase enzyme-linked assay. The assay was carried out on triplicate wells. Numbers at the bottom indicate different McAb.

Only one McAb (MAC131) showed partial cross-reactivity (38%) with rabbit LDL, and none reacted significantly with rat or mouse LDL (Fig. 2). Albeit that only three non-primate species were studied, the data in Fig. 2 suggest that most of the antigenic determinants of human LDL are specific for primate lipoproteins.

Epitope specificity of rat McAb to rabbit and human LDL

When excess amounts of individual antibodies (i.e. amounts of McAb which gave a plateau absorbance reading in the solid-phase enzyme-lined assay over a severalfold dilution) or pairs of two McAb were incubated with rabbit LDL and the total amount of antibody bound was measured, the results shown in Table 2 were obtained. Some pairs of McAb (e.g. MAC26 with MAC31) showed strong competition for binding to LDL, whereas the combination of other McAb (e.g. MAC23 with MAC28) showed very little competition. Analysis of the competition data by a clustering technique showed that the epitopes recognized by the different McAb could be grouped in two major clusters (Fig. 3). One included the epitopes recognized by MAC22, MAC29 and MAC27; the other included the epitopes recognized by MAC23, MAC26 and MAC31. The three McAb grouped in the second cluster appeared to be the one recognizing apoB-48, in addition to apoB-100 and/or apoB-74 (Table 1).

Binding-competition experiments with anti-(human LDL) McAb showed that, under the conditions used, none of the McAb was able to compete totally with the binding of another McAb to LDL (Table 3). Analysis of the results by a clustering technique confirmed that the epitopes recognized by the different McAb were distinct (results not shown).

A further characterization of the epitope specificity of the anti-LDL McAb was carried out after partial proteolysis of rabbit and human LDL apoB-100 by trypsin or staphylococcal V8 proteinase. For these experiments LDL was exposed to either trypsin or staphylococcal V8 proteinase, and the peptides produced were separated by SDS/polyacrylamide-gel electrophoresis, transferred to nitrocellulose paper and probed with the different McAb. Fig. 4 shows the results of one of these experiments with rabbit apoB-100. The two IgM (MAC24 and MAC25), which did not bind any

Table 2. Binding-competition assay of McAb to rabbit LDL

The ability of each McAb to compete with the binding of other McAb to rabbit LDL was studied by using a modification of the solid-phase enzyme-linked assay. Briefly, individual McAb and pairs of McAb were incubated with rabbit LDL and the total amount of antibody bound was detected with enzyme-conjugated second antibody. The binding of each pair of McAb was expressed relative to the binding of individual McAb, which was taken as 1 (see the text for details). Data from one experiment are shown and are means (±S.E.M.) for triplicate wells.

		Relative binding						
McAb	McAb...	MAC22	MAC23	MAC26	MAC27	MAC28	MAC29	MAC31
MAC22		–	1.43±0.09	1.43±0.11	1.19±0.07	1.61±0.12	1.02±0.03	1.20±0.12
MAC23				1.25±0.08	1.52±0.09	1.72±0.11	1.38±0.09	1.17±0.02
MAC26					1.15±0.07	1.61±0.10	1.04±0.05	0.99±0.07
MAC27						1.45±0.10	1.19±0.04	1.25±0.11
MAC28							1.40±0.11	1.67±0.09
MAC29								1.19±0.04
MAC31								–

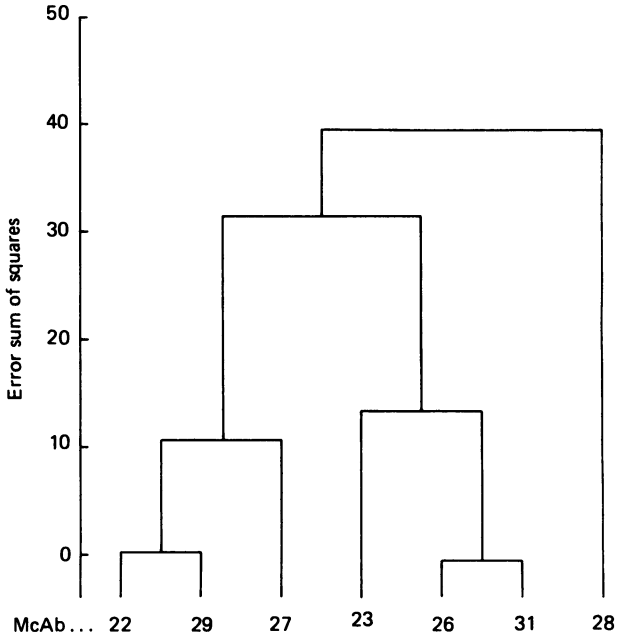

Fig. 3. Epitope map of rat McAb to rabbit LDL as shown by cluster analysis of the binding-competition assay

The dendrogram shows the data presented in Table 2 analysed by a clustering technique based on the procedure of Ward (1963) as implemented in the computer program CLUSTAN (Wishart, 1978). This procedure combines those two clusters, P and Q, whose fusion yields the least increase in the error sum of squares. The coefficient shown in the ordinate scale is twice the increase in the error sum of squares caused by the cluster's fusion.

form of detergent-solubilized apoB (Table 1), did not bind any fragment of apoB-100 (Fig. 4*a* and 4*b*). The IgG bound various tryptic fragments of apoB-100 (Fig. 4*a*) according to four patterns: the first was recognized by MAC22, MAC27 and MAC28, the second was recognized by MAC23 and MAC31, the third was unique for MAC26 and the fourth was unique for MAC29 (Fig. 4*a*). That the epitope recognized by MAC22 and MAC27 is related to, but not identical with, that recognized by MAC28 was shown by the antibody blots of the peptides generated by digestion of apoB-100 with staphylococcal V8 proteinase (Fig. 4*b*). Whereas the V8-proteinase peptide maps for MAC22 and MAC27 appeared only slightly different, that of MAC28 was clearly different (Fig. 4*b*). The tryptic peptide maps for these McAb were identical (Fig. 4*a*). Only minor differences were detected in the V8-proteinase peptide maps for MAC23 and MAC31 (Fig. 4*b*), confirming the proximity of the epitopes recognized by these McAb as indicated by their specificity for the different forms of apoB (Table 1) and the results of the binding competition experiments (Table 2 and Fig. 3).

The four rat McAb to human LDL able to bind detergent-solubilized apoB (MAC127, MAC129, MAC131 and MAC132) were also studied for their ability to bind to specific apoB fragments generated by partial proteolysis (Fig. 5). Each McAb recognized a specific set of peptides generated either by trypsin (Fig. 5*a*) or staphylococcal V8 proteinase (Fig. 5*b*), thus confirming the unique epitope specificity of these McAb.

Inhibition of binding of ^{125}I-LDL to the LDL receptor by anti-LDL McAb

Since it is known that apoB-100, but not apoB-48, is capable of binding to the LDL receptor, three anti-(rabbit LDL) McAb (MAC27, MAC28 and MAC29), which reacted only with apoB-100 (Table 1) were selected for studies on their ability to interfere with the binding of ^{125}I-LDL to its receptor. These McAb were purified from ascitic fluid and incubated at several concentrations with a fixed concentration of ^{125}I-LDL. These mixtures were then incubated with rabbit fibroblasts at 4 °C and the amount of ^{125}I-LDL bound to the fibroblast LDL receptor in the presence or absence of McAb was determined (Fig. 6). MAC28 was able to reduce the binding of ^{125}I-LDL to the fibroblast receptor (50% inhibition at a 10-fold molar excess of McAb). Concentrations 10–20 times higher of MAC27 and MAC29 were required in order to produce the same inhibition of binding (Fig. 6).

The two McAb to human LDL which only reacted with apoB-100 and apoB-74 (MAC127) or with apoB-

Table 3. Binding-competition assay of McAb to human LDL

The procedure outlined in the Materials and methods section and in the legend to Table 2 was followed. The results shown are from one experiment and are means (±S.E.M.) for triplicate wells.

		Relative binding					
McAb	McAb...	MAC127	MAC129	MAC130	MAC131	MAC132	MAC133
MAC127		–	1.36±0.04	2.47±0.11	2.53±0.09	2.32±0.15	1.56±0.11
MAC129				1.41±0.05	1.56±0.04	1.52±0.08	1.85±0.09
MAC130					2.79±0.15	1.70±0.13	1.65±0.07
MAC131						1.97±0.13	1.49±0.09
MAC132							1.34±0.08
MAC133							–

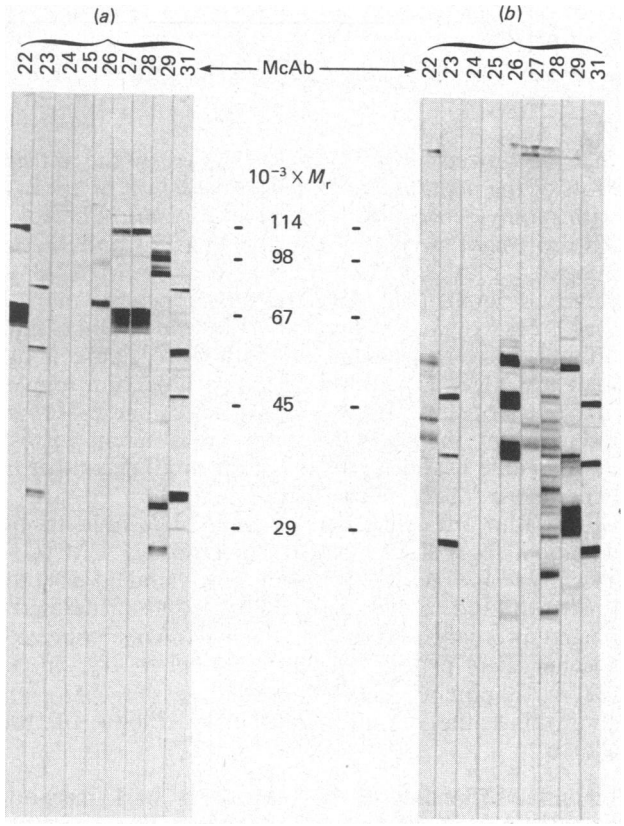

Fig. 4. Reactivity of rat McAb to rabbit LDL with fragments of ApoB-100 generated by trypsin or staphylococcal V8 proteinase

Rabbit LDL (ρ 1.019–1.063 g/ml) was incubated for 2 h at room temperature with trypsin (Sigma T8253) (*a*) or staphylococcal V8 proteinase (Miles, 39-900) (*b*) in 0.05 M-Na_2HPO_4/NaH_2PO_4, pH 7.8, at an enzyme/substrate ratio of 1:100 (w/w) (Drapeau, 1977). The reaction was stopped by addition of SDS and 2-mercaptoethanol, followed by electrophoresis. ApoB-100 fragments were separated on a polyacrylamide (125–200 g/l) gel (Laemmli, 1970), transferred to nitrocellulose paper and probed with the different McAb. Numbers in the centre of the Figure show the position of M_r markers.

100 (MAC132) and the two IgG McAb that did not bind to any form of detergent-solubilized human apoB (MAC130 and MAC133) were used in similar experiments with human fibroblasts. None of them inhibited the binding of human ^{125}I-LDL to the LDL receptor at McAb/^{125}I-LDL molar ratios lower than 100, suggesting that none of these McAb was directed to the binding site for the LDL receptor (results not shown).

DISCUSSION

In the last 4 years, several laboratories have produced mouse McAb to human LDL (Curtiss & Edgington, 1982; Mao *et al.*, 1982, 1983; Marcel *et al.*, 1982; Patton *et al.*, 1982; Tikkanen *et al.*, 1982; Tsao *et al.*, 1982; Milne *et al.*, 1983; Watt & Watt, 1983; Hui *et al.*, 1984*a*) and rat LDL (Sparks *et al.*, 1986).

We have reported here the derivation of two sets of rat McAb to rabbit and human LDL and their characterization in terms of species specificity, apoB specificity and epitope specificity.

Whereas it is generally assumed that LDL apoB (apoB-100) from different species shows considerable immunological cross-reactivity (Goldstein & Chapman, 1976; Goldstein *et al.*, 1977; Chapman 1980), the data presented here indicate that such cross-reactivity is related to (i) the species being considered, (ii) the antibody class and (iii) the epitope specificity of the antibody (Figs. 1 and 2). This indicates that certain epitopes of apoB-100 are more conserved than others among the different species. Recent data from other laboratories indicate that the LDL-receptor apoB-100 recognition site is very conserved in different species (Nelson *et al.*, 1984; Young *et al.*, 1986). Since it is known, however, that the interaction of LDL with the LDL receptor is considerably species-specific (Innerarity *et al.*, 1980; Drevon *et al.*, 1981), it could be of interest to know if the cross-reactivity of animal LDL with antibodies directed to the receptor site of human LDL correlates with the ability of animal lipoproteins to interact with the human LDL receptor.

The availability of McAb specific for apoB-100, and possibly apoB-48, is of potential interest for the quantification of these apolipoproteins in serum, as triacylglycerol-rich lipoproteins containing either apoB-48 or apoB-100 cannot be completely separated by physical methods (Lindgren & Jensen, 1972). IDL and LDL containing apoB-100 are cleared from the blood largely through the hepatic LDL receptor (reviewed by Goldstein *et al.*, 1983). Chylomicron remnants contain apoE in addition to apoB-48, and thus could bind both to the remnant (apoE) receptor and the LDL receptor. However, studies in the WHHL rabbit, which has a

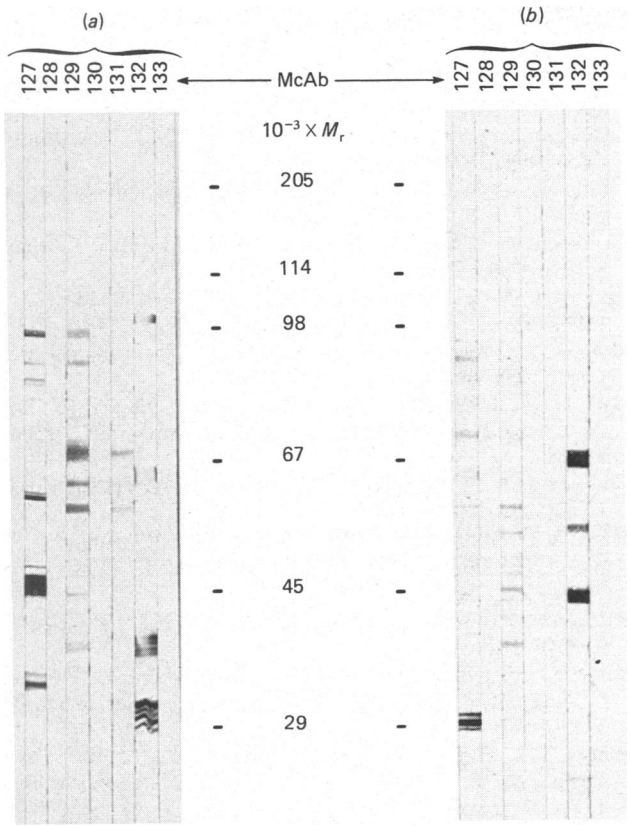

Fig. 5. Reactivity of rat McAb to human LDL with fragments of ApoB-100 generated by (*a*) trypsin or (*b*) staphylococcal V8 proteinase

Human LDL (ρ 1.019–1.063 g/ml) was incubated for 2 h with trypsin or staphylococcal V8 proteinase as indicated in the legend to Fig. 4. The reaction was stopped by addition of SDS and 2-mercaptoethanol, followed by electrophoresis, and apoB fragments were separated by SDS/polyacrylamide-gel electrophoresis, transferred to nitrocellulose and probed with the different McAb. Numbers in the centre of the Figure show the position of M_r markers.

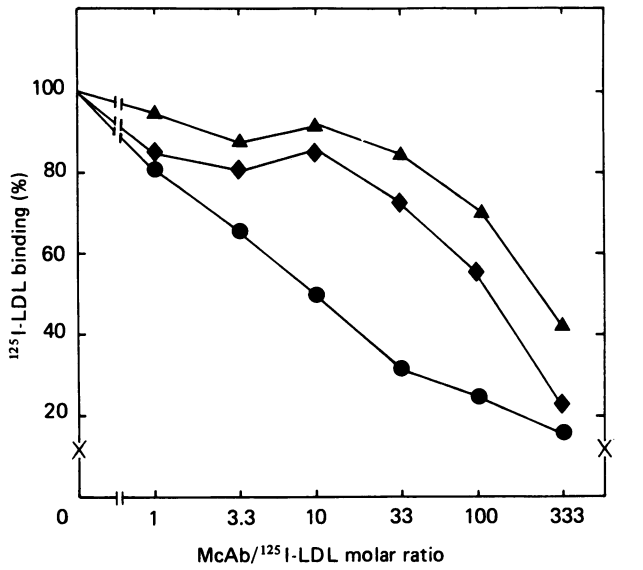

Fig. 6. Binding of rabbit ^{125}I-LDL to fibroblast LDL receptor in the presence of anti-LDL McAb

Skin rabbit fibroblasts were plated at 25000 cells/dish in 35 mm-diameter dishes and cultured for 4 days in 10% (v/v) fetal-calf serum in DMEM. At day 4 the medium was replaced with lipoprotein-deficient 10% (v/v) fetal-calf serum in DMEM. At day 5, cells were washed with PBS and incubated at 4 °C in lipoprotein-deficient 10% (v/v) fetal-calf serum in DMEM/Hepes for 1 h. Either ^{125}I-LDL (5 μg/ml) or mixtures of McAb and ^{125}I-LDL were then added. In the latter case the concentration of ^{125}I-LDL was 5 μg/ml, whereas the concentration of McAb varied from 1.5 to 500 μg/ml. Assuming an M_r of 500000 for apoB and of 150000 for IgG McAb, this corresponded to molar ratios of McAb to apoB from 1:1 to 330:1. The mixtures of ^{125}I-LDL and McAb were preincubated at 4 °C for 6 h before incubation with fibroblasts. Cells were incubated for 45 min at 4 °C in the presence of ^{125}I-LDL or ^{125}I-LDL/McAb mixtures and then washed (three times for 5 min) with BSA (2.5 g/l) in PBS, followed by a 5 min wash with PBS. ^{125}I-LDL bound to the cells was measured after solubilization of cell protein with SDS (10 g/l). The binding of ^{125}I-LDL after preincubation with the different McAb is shown as a percentage of the total binding of ^{125}I-LDL in the absence of McAb. This was 33.6 ng/mg of protein. Low-affinity binding (the binding of ^{125}I-LDL in the presence of 500 μg of unlabelled rabbit LDL/ml) was 3.9 ng/mg of protein and is shown by an '×' on the ordinate scale. McAbs: ▲, MAC27; ●, MAC28; ◆, MAC29.

genetic deficiency of LDL receptor, have shown that hepatic LDL receptor is essential for the clearance of IDL and LDL from serum, but not for the clearance of chylomicron remnants (Kita *et al.*, 1982). Whether the remnant receptor is essential for the clearance of chylomicron remnants in the presence of an active hepatic LDL receptor is not yet known.

The ability to measure separately apoB-48 and apoB-100 in serum would certainly help to define the factors which control the activity of these two receptors, as indicated by the serum levels of their ligand lipoproteins. McAb such as MAC22, MAC27, MAC28, MAC29, MAC127 and MAC132 could be used to quantify apoB-100. McAb specific for human or animal apoB-48 have not yet been produced, although the availability of an antiserum to apoB-48, which does not cross-react with apoB-100 (Kane, 1983), seems to indicate the possibility of generating antibodies specific for apoB-48.

Preliminary studies with murine McAb to human LDL have revealed, however, major limitations in the use of McAb for the assay of apoB in serum. Different binding of several McAb has been shown to VLDL, IDL and LDL (Tsao *et al.*, 1982), VLDL subfractions (Tikkanen *et al.*, 1984) and LDL subfractions (Tsao *et al.*, 1982; Mao *et al.*, 1982). Two approaches, however, may overcome this limitation. The first is based on the use of mixtures of two or more McAb (Mao *et al.*, 1983; Marcovina *et al.*, 1985); the second involves the use of a detergent-based immunoassay. Work on these lines is in progress in a number of laboratories. Furthermore, McAb to human LDL have recently been used for the identification of genetic polymorphism associated with LDL (Schumaker *et al.*, 1984) and the isolation of cDNA species complementary to apoB mRNA (Huang *et al.*, 1985; Knott *et al.*, 1985; Law *et al.*, 1985; Shoulders *et al.*, 1985; Wei *et al.*, 1985).

One McAb to rabbit LDL namely MAC28, was able to inhibit 50% of the binding of ^{125}I-LDL to the LDL receptor at a McAb/^{125}I-LD lipoprotein molar ratio of 10 (Fig. 6). Concentrations 10 times higher of MAC27 and MAC29 were required to produce the same degree of inhibition (Fig. 5). None of the McAb to human LDL which we have produced was able to interfere with the binding of human ^{125}I-LDL to the receptor. McAb able to inhibit the binding of human ^{125}I-LDL to the LDL receptor have been prepared by Tikkanen *et al.* (1982), Milne *et al.* (1983) and Hui *et al.* (1984*a*). These antibodies may prove useful in identifying the LDL-receptor binding site on apoB and define the minimal sequence of apoB required for binding to the receptor. Furthermore anti-LDL antibodies specific for the receptor binding site may permit the production of anti-idiotypic anti-receptor antibodies. Anti-idiotypic antibodies to the insulin receptor (Shechter *et al.*, 1982), to the acetylcholine receptor (Cleveland *et al.*, 1983) and to a receptor for the T-cell I-J determinant (Zupko *et al.*, 1985) have been prepared, and it is conceivable that anti-(LDL receptor) antibodies could be derived using this approach.

E.G. was supported by a long-term fellowship from the European Molecular Biology Organization (EMBO) and later by a Research Scholarship of Corpus Christi College, University of Cambridge. We thank Mr. D. Brown (Department of Applied Biology, University of Cambridge) for assistance in the analysis of binding-competition results, to Mrs. A. Larkins for the isotyping of the McAb, to Mr. M. W. Smith (Central Animal Services, Cambridge) for supplying the rhesus-monkey serum, and to Miss L. Walker for technical assistance. Dr. R. Drew and Dr. A. R. Bradwell of the Department of Immunology, University of Birmingham, Birmingham, U.K., kindly supplied the isotype-specific antisera. Dr. A. Munro and Dr. G. Butcher are gratefully acknowledged for discussions and critical reading of the manuscript before its submission. Part of this work was supported by a grant from the British Heart Foundation.

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Received 3 November 1987; accepted 22 January 1988