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Monoclonal antibody detects Ag polymorphism of apolipoprotein B

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A monoclonal antibody (MB-19) was used to investigate the polymorphism of apolipoprotein B in a large family and in unrelated subjects. Apolipoprotein B was shown to exhibit high-, intermediate- or low-affinity binding to this antibody. Thus, MB-19 bound strongly to the Ag(c) epitope, an Ag antigenic domain previously characterized by human antisera, while it bound only weakly to the allelic epitope Ag(g). It proved therefore useful for the detection of the two corresponding allelic apoB species designated $apoB_c$ (high-affinity binding) and $apoB_g$ (low-affinity binding), and for confirming their co-dominant transmission. Intermediate binding resulted from the presence of a mixture of both apoB populations in heterozygous subjects.

Ag polymorphism *Apolipoprotein B* *LDL* *Monoclonal anti-apoB antibody* *ApoB polymorphism*

1. INTRODUCTION

The genetic variation in human low-density lipoprotein (Ag polymorphism) was discovered using antisera obtained from multiply transfused patients [1]. Subsequently, 5 pairs of Ag antigens behaving as products of allelic genes were defined [2,3] and located in apolipoprotein B (apoB) [4]. The existence of polymorphic species of apoB is of interest because this apoprotein is recognized by the low-density lipoprotein (LDL) receptor.

Using previously characterized monoclonal antibodies [5,6] Schumaker et al. [7] described a common apoB polymorphism. This initial report was based on a semiquantitative method, which may have resulted in misclassification of some LDL samples, as pointed out by Young et al. [8], and therefore to the conclusion that the polymorphism was not related to the Ag system [7]. Using one of the antibodies (MB-19) we explored the matter further. We now report that the Ag antigenic determinants Ag(c) and Ag(g) can be distinguished using this antibody. Moreover, the co-dominant inheritance of two allelomorphic apoB species, designated $apoB_c$ and $apoB_g$, was confirmed.

2. MATERIALS AND METHODS

2.1. *Subjects*

The Ag phenotypes as well as the reactivity of apoB with antibody MB-19 were studied in an East Finnish family with hypercholesterolemia, and in a group of healthy unrelated individuals.

2.2. *LDL*

LDL (density 1.019–1.050 g/ml) was isolated by sequential ultracentrifugation [9] and its apoB content (LDL-apoB) determined using a conventional anti-apoB antiserum (Orion Diagnostica, Helsinki).

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2.3. *Ag phenotyping*

The determination of Ag antigenic determinants was carried out by a passive hemagglutination inhibition method [10] using human antisera against the Ag factors x, y, a_1, d, c, g, t, z and h.

2.4. *The enzyme-linked immunosorbent displacement assay (ELISA)*

The reactivity of apoB-containing LDL with antibody MB-19 was assessed by ELISA as follows (fig.1). Flat-bottomed microtitration plates (Flow Laboratories, CT) were coated with 200 μl standard LDL (1 μg/ml in PBS) by incubation for 4 h at 23°C. After saturating the extra binding sites with 3% bovine serum albumin (BSA) for 2 h at 23°C the wells received 50 μl of a 1:15000 dilution of ascites fluid containing antibody MB-19 [5,8] and increasing amounts of LDL-apoB BSA-PBS (150 μl) as indicated on the abscissa in fig.1. After incubation (4 h, 23°C), wells were rinsed 3 times with 1% BSA-PBS, followed by addition of a 1:500 dilution in 1% BSA-PBS of alkaline phosphatase-conjugated anti-mouse IgG (Orion Diagnostica, Finland). Following incubation with this second antibody (3 h, 23°C), and subsequent rinsing with 1% BSA-PBS, 0.2% disodium *p*-nitrophenyl phosphate was added to each well. The reaction was stopped after 30 min by 1 N NaOH. The microtitration plates were read at 405 nm using a Titertek Multiskan^R eight-channel photometer (Eflab Oy, Helsinki).

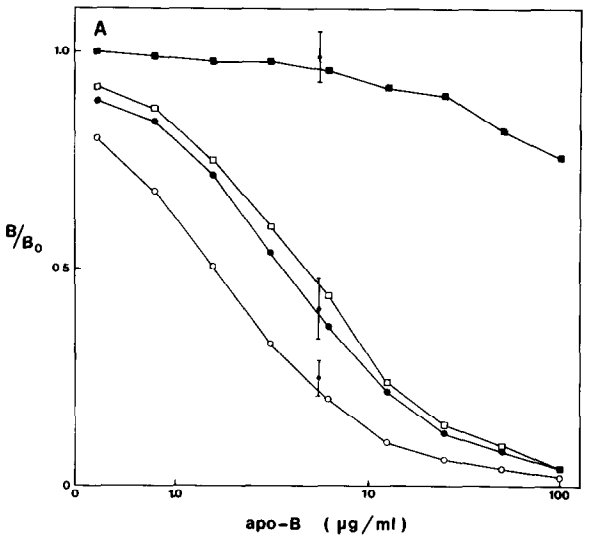

Fig.1. The reactivity of apoB with antibody MB-19 in the family of the proposita (no.12 in family E shown in fig.2). Displacement curves were produced by LDL-apoB obtained from the father (■), mother = proposita (○), daughter (□) and son (●). The results of competition ELISA are expressed as B/B_0, where B denotes absorbance in the presence and B_0 in the absence of competing apoB (contained in LDL). For comparison, the displacement abilities of apoB from a group of unrelated subjects are shown: the vertical bars indicate mean ± SD for different LDL or plasma samples obtained from subjects with weak (n = 10), intermediate (n = 10) and strong (n = 10) binding activities determined using 6.25 μg/ml of apoB.

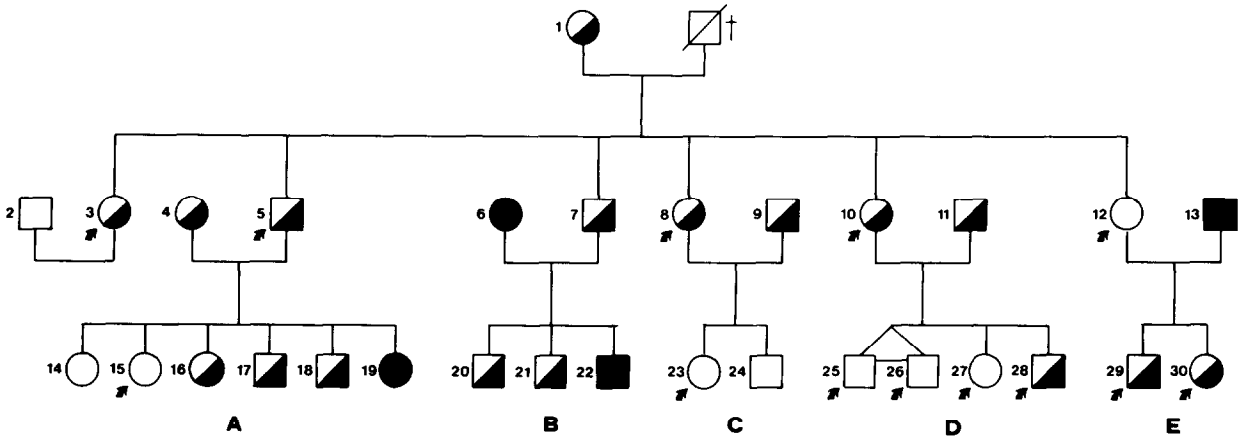

Fig.2. Transmission of apoB immunophenotypes in an East Finnish family. (○, □) High-affinity binding, (◐, ◪) intermediate-affinity binding, (●, ■) low-affinity binding. Arrows indicate subjects with elevated serum cholesterol levels. Lipid analytic data will be reported in detail elsewhere.

3. RESULTS

The competitive displacement assays with antibody MB-19 demonstrated that LDL-apoB exhibited high-, intermediate- or low-affinity binding to this antibody (fig.1). The inheritance of apoB reactivity was investigated in a large family (fig.2). The 9 high-affinity reactors displayed an average B/B_0 of 0.30 ± 0.02 (mean ± SD), the 17 intermediate reactors had an average of 0.45 ± 0.04, and the corresponding value for the 4 low-affinity reactors was 0.91 ± 0.06. Comparison with the corresponding Ag antigen phenotypes revealed that the 9 high-affinity binders were all Ag(c+g−), whereas the 4 low-affinity binders were Ag(c−g+) (table 1). Moreover, all the 17 intermediate-affinity binders were Ag(c+g+).

Consistent results were obtained when the Ag phenotypes and antibody MB-19 binding data from 20 unrelated individuals were compared (table 1). This striking relationship suggests that antibody MB-19 is an anti-Ag(c) reacting strongly with the apoB carrying the Ag(c) domain ($apoB_c$) but weakly with the apoB species containing the Ag(g) domain ($apoB_g$).

Table 1

Expression of Ag phenotypes and apoB reactivity with antibody MB-19 in families and unrelated individuals

	No.[a]	Ag phenotypes					ApoB binding to MB-19
		x y	a_1d	t z	h i	c g	
Family A Mo	4	− +	− +	+ −	− +	+ +	I
Fa	5	− +	+ +	+ −	− +	+ +	I
	14	− +	− +	+ −	− +	+ −	H
	15	− +	− +	+ −	− +	+ −	H
	16	− +	+ +	+ −	− +	+ +	I
	17	− +	+ +	+ −	− +	+ +	I
	18	− +	+ +	+ −	− +	+ +	I
	19	− +	+ +	+ −	− +	− +	L
Family B Mo	6	+ +	+ +	+ −	− +	− +	L
Fa	7	− +	+ +	+ −	− +	+ +	I
	20	− +	− +	+ −	− +	+ +	I
	21	− +	− +	+ −	− +	+ +	I
	22	− +	+ +	+ −	− +	− +	L
Family C Mo	8	− +	+ +	+ −	− +	+ +	I
Fa	9	− +	+ +	+ −	− +	+ +	I
	23	− +	− +	+ −	− +	+ −	H
	24	− +	− +	+ −	− +	+ −	H
Family D Mo	10	− +	+ +	+ −	− +	+ +	I
Fa	11	− +	+ +	+ +	− +	+ +	I
	25	− +	− +	+ −	− +	+ −	H
	26	− +	− +	+ −	− +	+ −	H
	27	− +	− +	+ −	− +	+ −	H
	28	− +	+ +	+ −	− +	+ +	I
Family E Mo	12	− +	− +	+ −	− +	+ −	H
Fa	13	− +	− +	+ −	− +	− +	L
	29	− +	− +	+ −	− +	+ +	I
	30	− +	− +	+ −	− +	+ +	I

4. DISCUSSION

The present results indicate that antibody MB-19 detects the Ag(c/g) polymorphism of apoB previously characterized by human antisera [2]. Without a single exception, individuals homozygous for the Ag(c) allele expressed high-affinity binding apoB, and those homozygous for the Ag(g) allele expressed low-affinity binding apoB. Moreover, apoB from individuals heterozygous for the Ag alleles c and g always exhibited intermediate binding (table 1). Thus, high-affinity binding to MB-19 corresponds to the presence of $apoB_c$, low-affinity binding to the presence of $apoB_g$, whereas intermediate binding reflects the presence of both $apoB_c$ and $apoB_g$. This notion is supported by the data of Young et al. [8] who by Scatchard analyses demonstrated that the intermediate binding pattern results from a mixture of high- and low-affinity binding apoB. There is no evidence that any of the other Ag antigens would modify apoB binding to antibody MB-19. For example, in family E (table 1) the Ag antigens x, y, a_1, d, t, z, h and i were identical in all 4 family members while only c and g varied, demonstrating that only the c/g antigenic pair could contribute to binding differences in this instance.

The apoB species with high- and low-affinity binding properties behave as products of two allelic genes which are expressed in an autosomal co-dominant fashion. For example, in family A

Table 1 (continued)

Other family members	1	− +	+ +	+ −	− +	+ +	I
	2	− +	+ +	+ +	− +	+ −	H
	3	− +	+ +	+ −	− +	+ +	I
Unrelated individuals:							
Strong binders	1	− +	+ −	+ −	− +	+ −	H
	2	− +	− +	+ −	− +	+ −	H
Intermediate binders	3	− +	+ +	+ +	− +	+ +	I
	4	− +	− +	+ −	− +	+ +	I
	5	− +	− +	+ −	− +	+ +	I
	6	+ +	+ +	+ −	+?	+ +	I
	7	+ +	+ +	+ −	− +	+ +	I
	8	− +	+ −	+ +	− +	+ +	I
	9	− +	− +	+ −	− +	+ +	I
	10	+ +	+ +	+ −	− +	+ +	I
Weak binders	11	+ +	+ −	+ −	− +	− +	L
	12	− +	− +	+ −	− +	− +	L
	13	+ +	+ −	+ +	− +	− +	L
	14	+ +	+ −	+ −	− +	− +	L
	15	− +	− +	+ −	− +	− +	L
	16	+ +	+ +	+ −	− +	− +	L
	17	+ −	+ −	+ −	− +	− +	L
	18	+ +	+ −	+ −	− +	− +	L
	19	+ +	+ −	+ +	− +	− +	L
	20	+ +	+ −	+ +	− +	− +	L

[a] Numbers refer to the pedigree shown in fig.2

Mo, mother; Fa, father; H, high-; I, intermediate-; L, low-affinity binding of apoB to antibody MB-19

(table 1, fig.2) father and mother exhibit intermediate binding, two of the children express strong, three intermediate, and one weak binding. This approaches the Mendelian ratio of 1:2:1, and the findings in other members of the pedigree shown in fig.2 also are compatible with this mode of inheritance.

The data in table 1 and from other analyses (not shown) demonstrate that about 50% of unrelated subjects studied show the low-affinity binding pattern, 40% exhibit intermediate binding, and 10% high-affinity binding. This indicates a gene frequency of about 0.70 for the more common allele (coding for $apoB_g$, or low-affinity binding apoB) in unrelated individuals. This is the same as the frequency previously reported for the Ag(g) gene in the Swiss population [2]. Previously, Young et al. [8] reported that in California subjects the low-affinity binding pattern with MB-19 was the prevalent one (40%). These findings, both with regard to distribution of immunophenotypes and association with Ag system, differ from a previous report [7] presumably because of different methodology.

On the basis that MB-19 does not bind to the cellular binding site on apoB [11] it can be assumed that the Ag(c/g) allelic variation does not directly affect the LDL receptor recognition domain. This is supported by the finding that monoclonal antibodies directed against this domain [11–13] do not detect any polymorphisms of apoB [11,14].

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