Monoclonal Antibodies Specific for Ag c/g and Ag a1/d Polymorphism of Human Low Density Lipoprotein

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INTRODUCTION

Human low density lipoprotein (LDL) is the major cholesterol carrier in the blood stream. It occurs as spherical particles and contains one large protein molecule, apolipoprotein B (apo B).

A genetic polymorphism of this protein was discovered in 1961 by Allison and Blumberg. Subsequently, 5 pairs of Ag epitopes behaving as products of allelic genes were defined using human allotypic antisera: x/y, a1/d, c/g, t/z, and h/i (Bütler and Brunner 1974). The determination of these epitopes has become a usefull tool in paternity investigations, forensic medicine, twin diagnosis, family- and population genetic studies.

The scarcity of human allotypic antisera poses a problem because relatively few polytransfused patients produce anti-Ag antibodies and transfusion of whole blood is replaced more and more by transfusion of blood components lacking allotypic LDL. Therefore, we produced monoclonal antibodies (MAb) against Ag epitopes and obtained anti-Ag c and d in a first attempt.

MATERIALS AND METHODS

LDL was prepared by ultracentrifugation and Ag phenotyping of sera and LDL preparations was performed by passive hemagglutination inhibition using human allotypic antisera (Bütler et al. 1967). Apo B concentrations were determined by radial immunodiffusion.

Female Balb/c mice were immunized with LDL of phenotype y+x-a1-d+c+g-t+z-h-i+ (LDL 39). The spleen cells were fused with X63-Ag8.653 mouse myeloma cells using polyethylenglycol 4,000 d. Pooled supernatants from 2 days of HAT-selected hybridoma cultures were assayed by ELISA for antibodies binding to LDL of different Ag phenotype (LDL 39, 24, T168 and 405; see Table 1). Hybridomas which produced antibodies to LDL 39 but not to at least one of the other LDL were cloned by limiting dilution. MAb were produced in ascites, purified on a Bakerbond Mab 4.6 x 250 mm HPLC column and biotinylated with biotin-X-NHS (biotinyl-ε-aminocaproic acid N-hydroxy succinimide ester).

For ELISA inhibition, flat-bottomed microtitration plates were coated with LDL 39 and remaining free sites blocked with casein hydrolysate. The plates were incubated with serum or LDL in 2-fold dilutions together with a constant amount of biotinylated MAb. The plates were washed and incubated with alkaline phosphatase-conjugated streptavidin and developed with p-nitrophenylphosphate. The absorbance was measured at 405 nm in a

Advances in Forensic Haemogenetics 2
Edited by W. R. Mayr

Titertek Multiscan MS automated reader. To determine the apo B content in serum, the MAb was replaced by a polyclonal rabbit anti-human apo B antiserum. These plates were washed and incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG followed by incubation with substrate.

RESULTS AND DISCUSSION

Hybridoma cultures were screened as described in the methods. Two MAb - D2E1 and H11G3 - were selected and further cloned by limiting dilution. MAb F5D5 recognizes a common epitope on LDL and was used as a control.

Table 1. Ag phenotypes of individual LDL with respect to the 5 epitopes expressed on the immunogen LDL 39 and determination of the specificity of MAb D2E1, H11G3 and F5D5 by ELISA inhibition

LDL	Ag epitopes[a]					µg apo B/ml at 50% inhibition			+/- evaluation[b]		
	y	d	c	t	i	D2E1	H11G3	F5D5	D2E1	H11G3	F5D5
39	+	+	+	+	+	71	172	37	+	+	+
21	+	+	-	+	+	537	62	43	-	+	+
24	+	-	-	-	+	327	407	30	-	-	+
T168	-	-	-	+	+	457	631	29	-	-	+
209	-	+	-	+	+	468	100	25	-	+	+
405	+	+	+	+	-	61	37	25	+	+	+
Recognized epitope:									c	d	common

[a] LDL 39, 21, 24, T168 and 405 are homozygous with respect to all 5 pairs. LDL 209 is heterozygous with respect to the pair a1/d and homozygous with respect to the other 4 pairs

[b] + for strong inhibition (<200 µg apo B/ml at 50% inhibition) and - for weak inhibition (>300 µg apo B/ml)

The specificity of the 2 selected MAb was investigated by ELISA inhibition with 6 different LDL preparations previously phenotyped with human antisera by passive hemagglutination inhibition (Table 1). The values for the apo B concentrations at 50% inhibition were determined graphically from the inhibition curves and submitted to +/- evaluation (Table 1). According to the Ag phenotypes of these 6 LDL, D2E1 recognizes Ag epitope c,

as binding to coated LDL 39 was strongly inhibited by LDL 39 and 405, the only two LDL bearing this epitope. H11G3 recognizes Ag d, since strong inhibition was observed with LDL 39, 21, 209 and 405, the 4 LDL bearing this epitope. F5D5 was inhibitable by all 6 LDL as this MAb recognizes a common epitope on apo B.

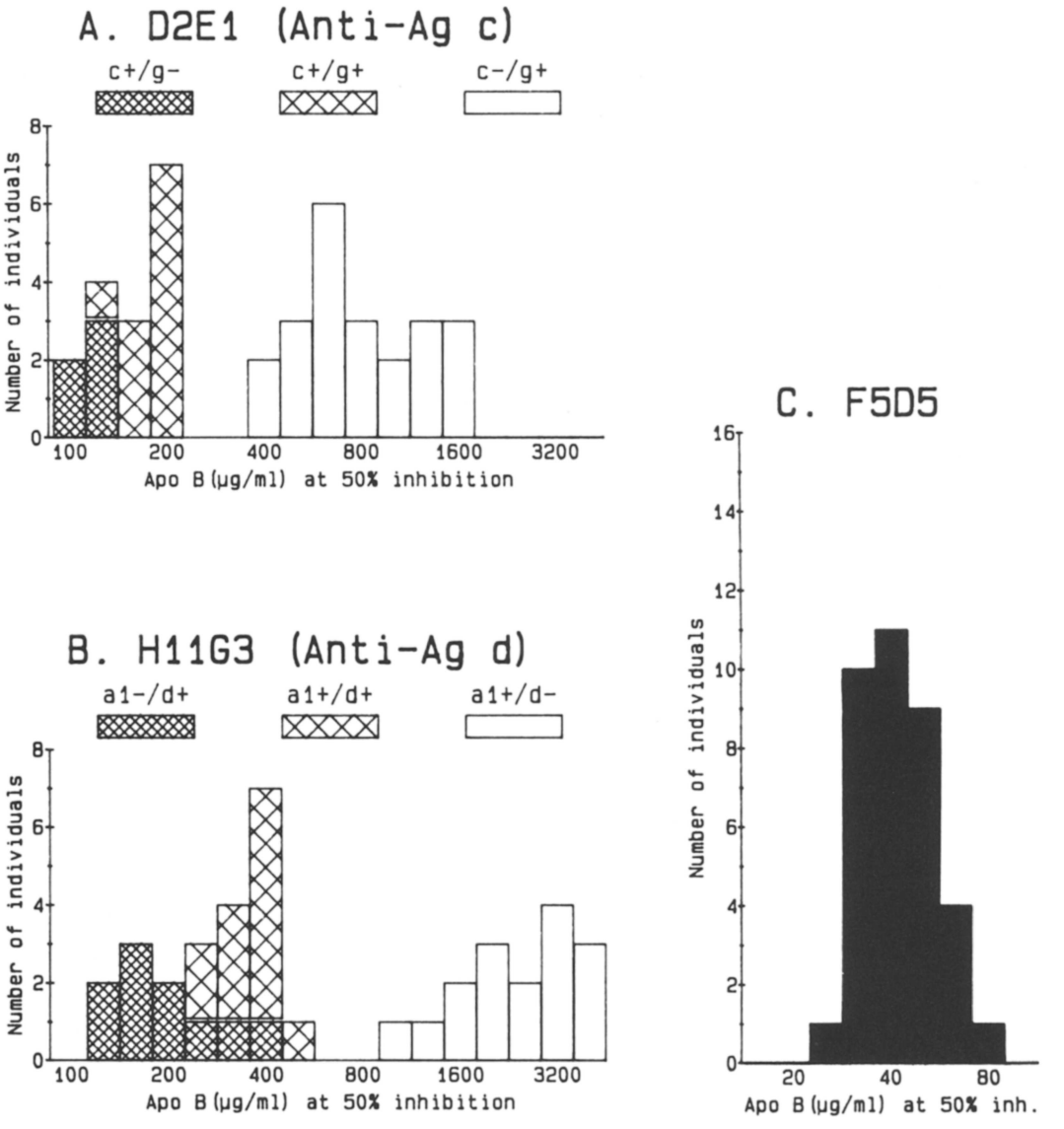

Figure 1. Reactivity of MAb with a panel of 38 sera from unrelated individuals in ELISA inhibition. ELISA inhibition was performed as described in the methods. The results are expressed as apo B concentration at 50% inhibition.

The suitability of the 2 MAb for Ag penotyping in serum was investigated by ELISA inhibition. A panel of 38 sera of unrelated individuals was characterized. The distribution pattern of apo B concentrations required for 50% inhibition is shown in Fig. 1 together with the relevant Ag phenotypes as determined by passive hemagglutination inhibition using human antisera. The ELISA inhibitions show 2 different patterns: on the one hand, a monophasic pattern resembling the standard distribution for MAb F5D5, indicating that this MAb does not recognize a polymorphism on LDL. On the other hand, a biphasic pattern for Mab D2E1 and H11G3 is typical for the recognition of a polymorphism on LDL. D2E1 was strongly inhitited (<240 µg apo B/ml at 50% inhibition) with homo- and heterozygous sera containing Ag epitope c (Ag c+/g- and Ag c+/g+), while inhibition with homozygous sera containing only the allelic epitope g (Ag c+/g+) was weak (>320 µg apo B/ml). H11G3 was strongly inhibited (<480 µg apo B/ml at 50% inhibition) with homo- and heterozygous sera containing Ag d (Ag a1-/d+ and Ag a1+/d+) and weakly (>800 µg apo B/ml) with homozygous sera lacking this epitope (Ag a1+/d-). Bi- or triphasic patterns were also reported with other Ag c specific MAb in various assay systems (Robinson et al. 1986, Tikkanen et al. 1986, Young et al. 1986 and Duriez et al. 1987).

So far, only Ag c specific MAb have been described in the literature. If - in addition to the anti-Ag d specific H11G3 - MAb against further Ag epitopes can be produced, it should be possible to replace the human antisera in Ag phenotyping. Thus, the problems imposed by the shortage of these antisera could be overcome.

REFERENCES

Allison AC and Blumberg BS (1961) An isoprecipitation reaction distinguishing human serum-protein types. Lancet I:634-637

Bütler R, Brunner E, Vierucci A and Morganti G (1967) Comparative studies on anti-Ag sera in immunodiffusion and in passive hemagglutination methods. Vox. Sang. 13:327-345

Bütler R and Brunner E (1974) Ag(i): Detection of an antithetical factor to Ag(h). Vox. Sang. 27:550-555

Duriez P, Bütler R, Tikkanen MJ, Steinmetz J, Vu Dac N, Bütler-Brunner E, Luyeye I, Bard JM, Puchois P and Furchart JC (in press) A monoclonal antibody (BIP45) detects Ag(c,g) polymorphism of human apolipoprotein B. J. Immunol. Methods

Robinson MT, Schumaker VN, Bütler R, Berg K and Curtiss LK (1986) Ag(c): Recognition by a monoclonal antibody. Arteriosclerosis 6:341-344

Tikkanen MJ, Enholm C, Bütler R, Young SG, Curtiss LK and Witztum JL (1986) Monoclonal antibody detects Ag polymorphism of apolipoprotein B. FEBS-Letters 202:54-58

Young SG, Bertics SJ, Curtiss LK, Casal DC and Witztum JL (1986) Monoclonal antibody MB19 detects genetic polymorphism in human apolipoprotein B. Proc. Natl. Acad. Sci. USA 83:1101-1105