

Monoclonal anti-D: Reactivity with various D phenotypes.

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At the MRC Blood Group Unit we have, over the years, tested blood samples from numerous individuals having unusual expression of the Rh antigen D. Therefore, when monoclonal anti-D antibodies became available, we were very interested to see how these reagents would react when tested against the different forms of the D antigen. Reported here are some of the results of testing 21 human monoclonal anti-D against cells of various D phenotypes, including D<sup>u</sup>, Rh:33 and D categories.

19 of the antibodies discussed here were made by Drs. Hughes-Jones and Thompson and are listed in Table 2 and 3. The antibodies were produced by EBV transforming B cells from the peripheral blood of recently boosted donors and then fusing them with mouse myeloma cells to form heterohybridomas (Thompson et al 1986). The various cell lines originated from 5 donors. Where more than one monoclonal antibody has been obtained from a single donor, there is evidence that they are different (class, subclass, light chain type, migration in PAGE). The exceptions to this are FOG 3, FOG A and FOG C, which are probably the same antibody.

Two other monoclonal antibodies, UCH D4 (Crawford et al 1983) and HD7 (Lowe et al 1986), were tested in parallel. UCH D4 is an anti-D produced by an EBV transformed human lymphoblastoid cell line; the first stable EBV transformed anti-D producing cell line to be reported. HD7, a human-human monoclonal anti-D, was obtained by fusing a human lymphoblastoid line (W1-L2-729-HF2) to lymphocytes from an immunised donor.

Before giving details of the investigation a reminder of the complexities of the D antigen seems appropriate. The expression, on red cells, of the Rh antigen D can vary both quantitatively and qualitatively. People whose cells lack part of the D antigen may when immunised, make an alloanti-D. Tippett and Sanger (1962,1977) analysed results of cross-testing cells and sera of D positive individuals who had made anti-D and classified these D antigens into 6 categories. A seventh category was added more recently (Lomas et al 1986), whereas category I has been discarded. The interaction between cells and serum of people with D on their cells and anti-D in their serum is shown in Table 1. Categories III, IV and V are now sub-divided. Cells of two of

the sub-divisions, IV<sub>a</sub> and V<sub>a</sub>, express the low frequency marker antigens Go<sup>a</sup> and D<sup>w</sup> respectively. Anti-Tar identifies another low frequency antigen expressed by cells with a category VII D antigen.

Category VI cells have the least D as determined by anti-D made by D- and D+ people. A different, characteristic form of weak D is associated with the infrequent Rh antigen Rh33. (For review of Rh see Issitt 1985).

The most difficult D phenotype to define is D<sup>u</sup>. This is a term for a quantitative and not qualitative variation, a weakening of D expression. It's definition is dependent on the reagents and techniques used in a particular laboratory. Tippett and Sanger made no attempt to fit D<sup>u</sup> samples into categories.

The monoclonal antibodies were mainly studied by agglutination tests, read microscopically. Saline suspensions (3%) of untreated and papain treated cells were used against the IgM antibodies and papain treated cells only against the IgG antibodies. Titrations (Table 2,3) of the antibodies when tested against CDe/cde cells showed the activity of all antibodies to be greatest with enzyme treated cells; the indirect anti-globulin test (IAT) did not enhance the reaction. Even for the IgG anti-D IAT gave lower titres than when papain treated cells were tested (Table 2).

Category III<sub>a</sub> and III<sub>c</sub> samples were strongly agglutinated by all antibodies. Unfortunately no category II sample was available for testing. Category VII cells when enzyme treated could also be detected by all reagents. However, HAM B did not agglutinate untreated DVII cells and 3 other antibodies reacted weakly (Table 3).

Most of the IgM antibodies agglutinated IV<sub>a</sub> and IV<sub>b</sub> cells with the exception of HAM 1 and HD7. These two reagents were not able to detect the DIV antigen even when papain treated cells were used (Table 3). Category V<sub>a</sub> was surprisingly difficult to detect especially on untreated cells. Three reagents, FOM 1, HAM A and HAM B did not detect V<sub>a</sub>; the others did so to varying degrees. The strength of reactions of some antibodies varied, from positive or weakly positive to negative, for cells from members of the same category or subdivision of a category. The order of strength of reactions was the same as that found with polyclonal anti-D.

Three antibodies, FOM A, HAM A and HAM 2, reacted with the characteristic weak D antigen of c(D)(e)/cde the complex associated with the infrequent antigen Rh33. FOM A could detect the antigen on untreated cells, the other two antibodies required papain treated cells. MAD reacted very weakly with papain treated Rh:33 cells.

DVI is usually easier to detect with polyclonal anti-D

antibodies than is the D of c(D)(e)/cde Rh:33. However, of the monoclonal antibodies tested here only one, HD7, was able to detect DVI consistently. Of the IgG anti-D antibodies (Table 2) only one, UCH D4, reacts with some category VI samples. Most IgG antibodies detect category IV but REG A, and 8D6 usually, miss category IVa and IVb, and GAD 2 reacts very weakly. UCH D4 is able to make the distinction between IVa and IVb samples. Category Va cells give the same sort of variable pattern as that found with the IgM anti-D. Four antibodies, REG A, 8D6, GAD and GAD 2 miss category Va completely.

D<sup>u</sup> cells were very difficult to detect with both the IgG and the IgM antibodies. Several D<sup>u</sup> samples which also lack G antigen were almost impossible to detect and some samples classified as D<sup>u</sup> by polyclonal reagents did not react with any of the monoclonal anti-D by the methods used. It is known that the strength of the D antigen of cells classified as D<sup>u</sup> is very variable. Some D<sup>u</sup> samples react with most anti-D by the anti-globulin test or when enzyme treated cells are used, whereas others react with only a few anti-D. Because the MRC Blood Group Unit is sent 'problem' blood samples it is likely that most of the samples tested will be on the lower end of the D<sup>u</sup> scale, that is, those with very weak D antigens. This may explain the difficulty experienced when trying to detect these antigens.

In contrast to polyclonal anti-D, the IgG monoclonal anti-D did not agglutinate saline suspensions of homozygous -D- and .D. cells. Nor did they agglutinate D+ cells of samples with reduced sialic acid levels such as En(a-) cells.

Blocking of the D antigen was attempted by incubating CDe/cde cells with the IgG anti-D antibodies. The 'blocked' cells were then tested with the monoclonal IgM anti-D and one polyclonal anti-D agglutinin. One antibody, FOG B, was able to block the action of all the IgM anti-D but the other 10 IgG monoclonal anti-D had little or no blocking effect. This may suggest that FOG B reacts with a common epitope or, perhaps, binding to it's epitope interferes with the ability of the other anti-D to bind to a different epitope. Blocking tests with DIII, DIV, DV and DVII were attempted: the pattern of reactions was not easy to interpret. So far use of monoclonal anti-D has not revealed the number of epitopes which make up the complete D antigen.

Tests, not only with these 21 antibodies but also with many others, have revealed only one monoclonal anti-D that behaves in the same way as an anti-D made by a category D person. HD7 gives the same pattern of reactions as an anti-D made by some category DIVa individuals.

Although the work reported here involved mainly the use of agglutination tests with untreated and papain treated cells, the antibodies were used by a number of other methods. No single method was able to detect the D antigen of all known D phenotypes. However, these monoclonal anti-D can be used to

distinguish the different D phenotypes and, when correctly standardised, will lessen the dependence on the small quantities of the special polyclonal reagents available.

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Table 1. Classification of people with D on their cells who have made anti-D

cells	anti-D from D+ people						Anti-		
	II	IIIa	IVa	Va	VI	VII	Go <sup>a</sup>	D <sup>w</sup>	Tar
D II	-	+	+	+	+	nt	-	-	-
D III	+	-	+	+	+	w	-	-	-
D IV	-	w	-	+	+	+	+	-	-
D V	+	-	+	-	±	-	-	+	-
D VI	-	-	+	-	-	-	-	-	-
D VII	+	-	+	±	+	-	-	-	+

nt not tested

Table 2. IgG monoclonal anti-D: reactivity with various D phenotypes

Antibody	Ig		D category					Titre against CDe/cde cells	
	sc	lc	IVa	IVb	Va	VI	Rh:33	p	IAT
FOG A	G3	κ	+	+	+/-	-	-	32	8
FOG B	G1	λ	+	+	+	-	-	256	256
FOG C	G3	κ	+	+	+/-	-	-	64	32
FOG 1	G1	κ	+	+	+	-	w	64	32
FOG 3	G3	κ	+	+	+/-	-	-	128	32
PAG 1	G1	λ	+	+	+/-	-	w	64	16
REG A	G1	κ	-	-	-	-	-	256	32
8D6	G1		w/-	w/-	-	-	-	32	2
GAD	G3		+	+	-	-	-	64	32
GAD 2	G3	λ	w	w	-	-	-	64	32
UCH D4	G1	κ	+	-	+	w/-	-	>512	16

Category IIIa, IIIc and VII gave strong positive results against all antibodies.

Papain treated cells (3% saline suspension) used throughout except for IAT

IAT = Indirect antiglobulin test using untreated cells and anti-human IgG

Ig sc = Immunoglobulin subclass

Ig lc = Immunoglobulin light chain type

For other symbols see legend of Table 3.

Table 3. IgM monoclonal anti-D: reactivity with various D phenotypes

Antibody	Ic	IVaIVb			D category		VI		VII		Rh:33		Titre against CDe/cde cells IAT		
		S	S	S	S	P	S	P	S	P	S	P	S	P	
FOM A	λ	+	+	+	+	+	+	+	+	+	+	+	4	4	1
FOM 1	λ	+	+	-	-	-	-	-	-	-	-	-	16	16	4
HAM A	k	+	+	-	-	-	-	+	+	+	+	+	16	64	4
HAM B	λ	+	+	-	-	-	-	-	-	-	-	-	64	128	8
HAM 1		-	-	-	-	+	+	+	+	+	+	+	8	8	8
HAM 2	λ	+	+	+/-	-	+	+	+	+	+	+	+	64	128	8
MAD		+	+	-	-	+	w/-	+	+	+	-	w	>512	>512	64
MAD 1	λ	+	+	-	-	+	+/-	+	+	+	-	-	a	a	a
MAD 2	λ	+	+	-	-	+	w	+	+	+	-	-	128	128	8
HD7	k	-	-	+	+	+	+	+	+	+	-	-	2	4	2

Category IIIa, IIIC gave strong positive results by all methods against all antibodies.

Ic = Immunoglobulin light chain type.

S = results of agglutination tests using 3% saline suspension of untreated cells.

P = results of agglutination tests using 3% saline suspension of papain treated cells.

IAT = Indirect antiglobulin test using polyspecific anti-human globulin and untreated cells

a = insufficient reagent available for titration.

Strength of reaction order +, +<sup>w</sup>, W, -

+/-, w/- = positive or weakly positive with some samples, negative with others.