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Clearance of red cells by monoclonal IgG3 anti-D *in vivo* is affected by the VF polymorphism of FcγRIIIa (CD16)

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SUMMARY

Human red cells (RBC) coated with IgG anti-D are cleared from the circulation to the spleen by macrophages which express IgG receptors (FcγR). Polymorphisms of FcγRIIa and FcγRIIIa affect IgG binding *in vitro*, and may alter the efficiency of clearance of immune complexes *in vivo*. In a RBC clearance study, 22 Rh D-negative subjects were given 100–400 μg human monoclonal or polyclonal IgG anti-D i.m. followed 48 h later by ^{51}Cr-labelled D$^+$ RBC. The half lives of the infused D$^+$ RBC were determined, together with the coating levels of anti-D on the D$^+$ RBC. *FcγRIIA* and *FcγRIIIA* genotyping was performed. Large ranges of phagocytosis and extracellular lysis of RBC *in vitro*, and of half lives of RBC *in vivo,* were observed. Clearance of RBC coated with monoclonal IgG3 anti-D (BRAD-3) was more rapid in five subjects homozygous for FcγRIIIa-F/F158 than in three subjects expressing the FcγRIIIa-V158 allele ($P = 0{\cdot}024$). This effect was not observed, however, for those individuals given polyclonal anti-D. There was also no significant difference in the efficiency of RBC destruction *in vitro* or of RBC clearance *in vivo* between the subjects analysed for individual genotypes or alleles or combinations of alleles. In conclusion, the presence of the FcγRIIIa-V158 allele compromised the efficiency of removal of RBC coated with IgG3 anti-D.

Keywords IgG Fc receptor (FcγR) FcγR polymorphisms anti-D red blood cells (RBC) clearance of RBC

INTRODUCTION

Human red blood cells (RBC) coated with IgG anti-D (EA-IgG) are removed from the circulation by the spleen [1] following their interaction with mononuclear phagocytic cells. Macrophages express three classes of IgG receptors, FcγRI, FcγRIIa and FcγRIIIa [2]. Any or all of these FcγR could, in theory, participate in the interaction with the opsonized RBC. Infusion of the anti-FcγRIIIa antibody, 3G8, to a patient with severe refractory immune thrombocytopenic purpura caused impaired clearance of IgG-sensitized RBC, a doubling of the half life, as well as a dramatic though transient increase in platelet counts [3]. These workers also found clearance of IgG-coated RBC in chimpanzees (using chimpanzee alloantibodies) to be greatly reduced by prior administration of 3G8 [4]. Human spleen cryostat sections bound EA-IgG solely by FcγRIIIa, again being inhibited by 3G8 [5]. Although FcγRIIa may also be blocked by 3G8, by the Fc after the Fab has bound to FcγRIIIa, it is likely that macrophage FcγRIIIa is the main, or primary, receptor for IgG anti-D opsonized RBC in the spleen, resulting in their removal from the circulation [6].

FcγRI (CD64) is a nonpolymorphic high affinity receptor which is normally occupied by cytophilic IgG, preventing interaction with immune complexes unless it is displaced, when efficient binding, phagocytosis and extracellular lysis of opsonized cells occurs.

FcγRIIa (CD32) represents a low affinity receptor, binding immune complexes or cell-bound IgG. FcγRIIa contains a polymorphism and the allotype FcγRIIa-H131 was found to bind human IgG2, unlike FcγRIIa-R131 [7]. FcγRIIa has a higher affinity for IgG3 than IgG1 [8]. Cells expressing FcγRIIa-H131 were shown to mediate greater EA-rosette formation with RBC coated with high levels (100 000 IgG/RBC) of human monoclonal IgG1 or IgG3 anti-D than FcγRIIa-R131 transfectants. Moreover, cells expressing any of the FcγRIIa allotypes bound more EA-IgG3 than EA-IgG1 [9]. Similar data was obtained with neutrophils, although blocking experiments indicated FcγRIIIb

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contributed to binding of EA-IgG3 [10]. Phagocytosis of target RBC was mediated solely by FcγRIIa-H131 transfectants towards EA-IgG3, and only to a modest degree even at high levels of sensitization [11]. At physiologic levels of opsonization, EA-IgG3 but not EA-IgG1 (at 20 000 IgG/RBC) bound to K562 cells (expressing FcγRIIa) [12], whereas EA-IgG1 (at 13 000 IgG/RBC) were lysed by monocytes (FcγRI$^+$, FcγRIIa$^+$) solely through FcγRI [9]. Thus it is probable that only EA-IgG3 at high (nonphysiological) sensitization levels of anti-D may interact with FcγRIIa.

FcγRIIIa (CD16) is also polymorphic at residue 158 in the membrane-proximal domain [13]. This was later found to influence binding of IgG. FcγRIIIa effectively binds complexed IgG and has some affinity for monomeric IgG. NK cells (which express FcγRIIIa) from FcγRIIIa-V/V158 individuals had more cytophilic IgG than those from FcγRIIIa-F/F158 subjects, and bound more IgG1, IgG3 and IgG4 [14]. Similarly, monocytes from FcγRIIIa-V/V158 homozygotes bound more IgG1 than monocytes from FcγRIIIa-F/F158 individuals [15]. It is possible that the presence of cytophilic IgG could obstruct interaction of the receptor with immune complexes or opsonized cells. Alternatively, the FcγRIIIa-V158 allotype with higher affinity for IgG may more efficiently clear immune complexes. FcγRIIIa expressed on NK cells and monocytes are differently glycosylated, with only the former having high mannose-type oligosaccharides, which appears to confer a lower affinity for IgG on monocyte (macrophage) FcγRIIIa. Whereas NK cell FcγRIIIa was blocked by 2 mg/ml IgG, monocyte FcγRIIIa was only partially (30%) blocked at this IgG concentration, which is below normal serum levels [16]. This may enable it to efficiently interact with immune complexes. Thus clearance of EA-IgG (anti-D) *in vivo*, which is most likely to occur predominantly through monocyte/macrophage FcγRIIIa, may be influenced by the polymorphism of this receptor.

The ability of human monoclonal and polyclonal anti-D to mediate clearance of D-positive (D$^+$) RBC transfused into D-negative subjects has been compared [17], as part of a study of the ability of passive monoclonal anti-D to prevent immunization to D$^+$ RBC. Anti-D was injected i.m. two days before infusion of ^{51}Cr-labelled D$^+$ RBC; in this situation the rate of removal of the cells is slower than when RBC are opsonized *in vitro* before infusion [18]. The number of molecules of anti-D on the RBC was determined by flow cytometry [19], and the ability of the subjects' monocytes and NK cells to phagocytose and lyse EA-IgG was assessed in *in vitro* phagocytosis assays and antibody dependent cell-mediated cytotoxicity (ADCC) assays, respectively. A wide range of clearance rates and functional activities were observed in the subjects. Therefore the role of the polymorphisms of FcγRIIa and FcγRIIIa on the clearance of EA-IgG was analysed.

MATERIALS AND METHODS

Red cell clearance study

Twenty-two D-negative male volunteers were injected i.m. with 100–400 μg of anti-D (human monoclonal anti-D produced by EBV-transformed B cell lines, BRAD-3 (IgG3) and BRAD-5 (IgG1), or polyclonal IgG anti-D immunoglobulin). Two days later, 3 ml ^{51}Cr-labelled D$^+$ RBC were injected i.v. and samples taken at 3 min and 1, 3, 5, 24, 48 and 72 h for gamma counting and estimation of RBC half life ($t_{50\%}$) and clearance rate ($0{\cdot}693/t_{50\%}$) [17]. The number of molecules of anti-D on the D$^+$ RBC in samples taken 3 h after injection was determined by flow cytometry for 14 of these subjects [19]. The study was approved by the local Research and Ethics committees and the volunteers gave written informed consent.

Monocyte EA-rosette/phagocytosis assay

Peripheral blood mononuclear cells were isolated from blood taken from the volunteers just prior to the injection of RBC and 24, 48, 72 h and 8 months later, incubated for 1 h at 37°C in wells of a 24-well plate and nonadherent cells removed by washing. D$^+$ RBC were sensitized with serial dilutions of anti-D, washed and adjusted to 1% and 50 μl EA-IgG added to the wells. Unsensitized D$^+$ and D-negative RBC were also used. To standardize the assay, the target D$^+$ red cells were from a single donor (group O R_1R_2) and the anti-D serum (single donation) was stored frozen in aliquots. After incubation for 2 h at 37°C, cells were fixed in 1% glutaraldehyde, rinsed and stained with 0·4% Trypan Blue. The percentage of monocytes with adherent or phagocytosed RBC was determined microscopically.

NK cell ADCC

Peripheral blood mononuclear cells were isolated from the same samples of blood that were taken for the monocyte assay, and depleted of adherent cells by incubation for 1 h at 37°C in plastic flasks. The nonadherent cells (6×10^5/well in medium with 10% human AB serum) were incubated in triplicate in U-well microplates with ^{51}Cr-labelled papainized D$^+$ RBC (4×10^4/well) for 16 h at 37°C in the presence or absence of anti-D [20]. The RBC and anti-D were those used for the monocyte assay above. The anti-D was used neat and at four 10-fold dilutions. Maximum lysis was achieved by addition of 1% Triton X-100. Radioactivity was determined in aliquots of supernatants, and the percentage haemolysis calculated:

$$\%\ \text{haemolysis} = \frac{\text{cpm (test)} - \text{cpm (control)}}{\text{cpm (max)} - \text{cpm (control)}} \times 100$$

FcγRIIA and FcγRIIIA genotyping

This was performed by allele-specific polymerase chain reaction genotyping methods [14,21] using DNA prepared from EBV-transformed B cell lines from the subjects. The specificities of the genotyping assays were confirmed by direct sequencing.

RESULTS

Data on the subjects studied, the dose and type of anti-D injected, *FcγRIIA* and *FcγRIIIA* genotypes, *in vitro* monocyte and NK cell function at the time of RBC infusion, RBC half lives *in vivo*, coating levels of IgG anti-D on the D$^+$ RBC *ex vivo*, and the RBC clearance rate with respect to bound IgG are given in Table 1.

FcγRIIa and FcγRIIIa genotype and allele frequencies

More subjects were homozygous for the *FcγRIIA-R/R131* genotype (41%) than the *H/H131* genotype (14%) and the allele frequencies were 0·63 (R131) and 0·37 (H131). For the FcγRIIIA polymorphism, there were 9% and 77% of subjects homozygous for the *V/V158* and *F/F158* genotypes, respectively, and the allele frequencies were 0·16 (V158) and 0·84 (F158).

No effect of FcγRIIa and FcγRIIIa genotypes on monocyte and NK cell function

The results for monocyte and NK cell function and RBC half lives are summarized in Table 2. In general, monocyte and NK

Table 1. Experimental and clinical data

Subject number	Anti-D, dose (μg)	FcγRIIA-131	FcγRIIIA-158	% monocyte phagocytosis	% NK haemolysis	RBC half life, $t_{50\%}$ (hours)	Molecules IgG/RBC	Clearance rate/molecules bound (×10^{-5})
1	BRAD-3 (100)	RH	FF	4	92	41·0	nd	nd
2	BRAD-3 (200)	RR	FF	16	82	14·6	nd	nd
4	BRAD-3 (300)	RH	FF	28	104	9·9	3 600	1·94
6*	BRAD-3 (300)	RR	FF	7	100	28·1	1 500	1·67
8*	BRAD-3 (300)	RR	FF	5	81	8·7	8 000	1·00
9	BRAD-3 (300)	RH	FF	39	93	2·5	10 000	2·77
10	BRAD-3 (300)	HH	FF	41	100	10·9	3 800	1·68
11*	BRAD-3 (300)	RR	FF	27	nd	4·4	4 300	3·67
17	BRAD-5 (300)	RR	FF	36	95	8·7	2 700	2·96
21*	Polyclonal (100)	RH	FF	35	80	6·9	nd	nd
23	Polyclonal (100)	RH	FF	14	76	6·6	nd	nd
24	Polyclonal (100)	RR	FF	21	62	1·2	nd	nd
25	Polyclonal (100)	RR	FF	nd	nd	3·8	4 000	4·55
26	Polyclonal (100)	RR	FF	64	77	2·8	5 600	4·43
27	Polyclonal (100)	RH	FF	21	88	9·7	3 000	2·37
28	BRAD-3 + 5 (400)	HH	FF	20	107	2·7	9 300	2·76
29	BRAD-3 + 5 (400)	RH	FF	13	82	32·1	5 000	0·43
3	BRAD-3 (200)	RH	VV	41	87	26·7	nd	nd
20	Polyclonal (100)	RR	VV	34	72	2·3	nd	nd
5	BRAD-3 (300)	RH	VF	36	97	24·2	3 400	0·85
7	BRAD-3 (300)	HH	VF	16	67	12·9	3 000	1·80
22	Polyclonal (100)	RH	VF	48	92	6·7	nd	nd

*HLA B8 DR3 (DR17). nd, not determined. Subject numbers taken from [17]. The figures for percentage phagocytosis and haemolysis are the results obtained using anti-D at 1/2 and 1/10 dilutions in the assays, respectively.

Table 2. Analysis of experimental and clinical data with respect to FcγRIIA and FcγRIIIA genotypes and alleles of the subjects

	FcγRIIA genotype or allele					FcγRIIIA genotype or allele					Combinations of FcγRIIA and FcγRIIIA alleles			
	R/R 131	R/H 131	H/H 131	R 131	H 131	V/V 158	V/F 158	F/F 158	V 158	F 158	R 131 V 158	R 131 F 158	H 131 V 158	H 131 F 158
Number of subjects	9	10	3	19	13	2	3	17	5	20	4	17	4	12
(% of total)	(41)	(45)	(14)	(86)	(59)	(9)	(14)	(77)	(23)	(91)	(18)	(77)	(18)	(55)
% phagocytosis by monocytes														
Mean	26·3	27·9	25·7	27·2	27·4	37·5	33·3	24·4	35·0	25·8	39·8	25·9	35·3	26·3
SEM	6·7	4·5	7·8	3·8	3·8	3·5	9·3	4·0	5·3	3·6	3·1	6·9	6·9	3·9
% haemolysis by NK cells														
Mean	81·3	89·1	91·3	85·9	89·6	79·5	85·3	87·9	83·0	87·5	87·0	86·7	85·8	89·8
SEM	4·9	2·6	12·3	2·6	3·2	7·5	9·3	3·2	5·8	3·0	5·4	2·8	6·6	3·4
Half life, $t_{50\%}$														
Mean	8·3	16·6	8·8	12·7	14·8	14·5	14·6	10·2	14·6	10·9	15·0	12·5	17·6	13·8
SEM	2·9	4·2	3·1	2·7	3·4	12·2	5·2	2·6	4·8	2·3	6·1	2·8	4·7	3·5

cell function of the subjects remained relatively constant both during the clearance study and several months later. Data from the subject groups were analysed by the unpaired *t*-test. There were no significant differences in mean values of *in vitro* EA-IgG phagocytosis by monocytes or lysis by NK cells or *in vivo* RBC clearance between any of the groups of subjects, either homozygous or heterozygous for the FcγRIIa or FcγRIIIa polymorphisms. Although the mean percentage phagocytosis by monocytes appeared to vary with respect to the FcγRIIIa-V/F158 genotype, this was not significant and was consistent with the involvement of only FcγRI in this assay. Although EA-IgG were lysed by NK cells through interactions with FcγRIIIa, there was little variation in the percentage haemolysis between subjects. The numbers of subjects with FcγRIIa-H/H131, FcγRIIIa-V/V158 and FcγRIIIa-V/F158 unfortunately were too small for meaningful statistical analysis. Due to the unequal distribution of genotypes, multiple comparisons were not performed. In addition, there was no difference in *in vitro* functional activity or the

Fig. 1. Half lives of RBC after injection of BRAD-3 in eight subjects, stratified according to *FcγRIIIA* genotypes. Subject 3 (■, -V/V158), subjects 5 and 7 (▲, -V/F158) and subjects 2, 4, 9, 10 and 11 (●, -F/F158).

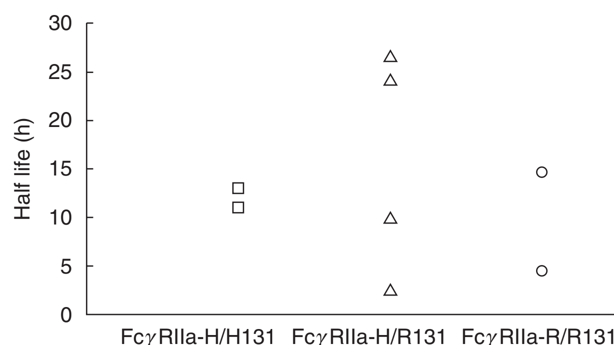

Fig. 2. Half lives of RBC after injection of BRAD-3 in eight subjects, stratified according to *FcγRIIA* genotypes. Subjects 7 and 10 (□, -H/H131), subjects 3, 4, 5 and 9 (△, -H/R131) and subjects 2 and 11 (○, -R/R131).

rate of red cell clearance *in vivo* between the subjects grouped according to their FcγRIIa allotype, or combinations of FcγRIIa and FcγRIIIa haplotypes.

Association between expression of the FcγRIIIa-V158 allele and reduced in vivo *RBC clearance of EA-IgG3*

FcγRIIIa has been proposed the primary receptor utilized in clearance or haemolysis of EA-IgG, and polymorphisms may influence either of these functions. When comparing all subjects for FcγRIIIa genotype or allele using the unpaired *t*-test, there were no significant differences in RBC half lives (Table 2). In contrast, it was observed that in subjects given BRAD-3 (monoclonal IgG3 anti-D) the RBC half lives were significantly different when categorizing the subjects into two groups, those who were homozygous for FcγRIIIa-F/F158 (five individuals) and those who expressed the FcγRIIIa-V158 allele (three individuals). Clearance of RBC was observed to be more rapid in the FcγRIIIa-F/F158 homozygotes (Fig. 1). Three subjects (numbers 1, 6 and 8) given BRAD-3 were excluded from this analysis; their monocytes exhibited very low phagocytic ability (4%, 7% and 5% of their monocytes phagocytosed EA-IgG *in vitro*) compared to the eight included subjects (mean 30·5%). Subjects 6 and 8 with low phagocytosis expressed the HLA haplotype B8 DR3 (DR17) (Table 1) [17], and it is known that some individuals with this haplotype exhibit reduced monocyte phagocytosis [22] and slower clearance [23] of anti-D-coated RBC. Although subject 11 was also HLA B8 DR3 (DR17), phagocytosis by his monocytes was within the range of the other included subjects. In addition, subject 1 was given a low dose of anti-D and had a markedly low plasma concentration of anti-D (1·8 ng/ml) [17] compared to a mean of 10·65 ng/ml (range 4·9–16·4 ng/ml) [17] for the eight included subjects, and this would have severely restricted the amount of anti-D bound to the infused D^+ RBC. The mean RBC half life was observed to be 8·5 ± 2·2 h for the five included FcγRIIIa-F/F158 subjects, and 21·3 ± 4·2 h for the three possessing the FcγRIIIa-V158 allele ($P = 0{\cdot}024$). This difference was not attributable to variation in coating levels of BRAD-3 on the RBC *in vivo*, as the mean clearance rate/molecules of anti-D bound was not different between the two groups (2·5 ± 0·5, and 1·3 ± 0·5, respectively, $P = 0{\cdot}181$). The *in vitro* functional activities of the subjects were also similar.

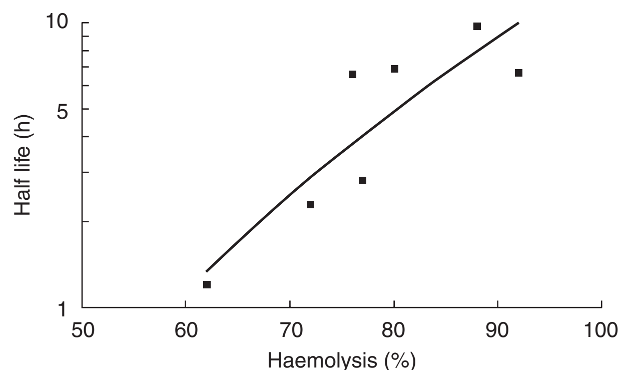

Fig. 3. Relation between haemolysis of EA-IgG by NK cells *in vitro* and clearance of EA-IgG *in vivo* by seven subjects (20, 21, 22, 23, 24, 26, 27) injected with polyclonal anti-D. Slope $R^2 = 0{\cdot}7631$.

Polymorphisms of FcγRIIa do not affect RBC clearance rates

When the subjects shown in Fig. 1 were grouped according to their *FcγRIIA* genotypes, no difference was apparent in the half lives of RBC *in vivo* (Fig. 2).

Comparison of RBC destruction in vitro *and* in vivo

Large differences in RBC half life were obtained between subjects with the same *FcγRIIIA* genotype, but there was less variation with haemolysis *in vitro*. It was observed, however, that when comparing one group of subjects, those given polyclonal anti-D (subjects 20–27, Table 1), there was a correlation between the half life of EA-IgG *in vivo* and the NK cell-mediated haemolysis of EA-IgG *in vitro* elicited by polyclonal anti-D (Fig. 3).

DISCUSSION

The genotypes of both *FcγRIIA* and *FcγRIIIA* in this small sample of Caucasians from the south-west of England differed slightly in frequency from most published studies. There was an increased representation of the *FcγRIIa-R/R131* and *FcγRIIIa-F/F158* genotypes, although when larger numbers of subjects from this region were genotyped, results were similar to previous reports. The *FcγRIIA* genotypes (33% R/R131 and 16% H/H131) and allele frequencies (0·59 R131, 0·41 H131) of 78 subjects were slightly

outside the range of genotypes (16–32% R/R131 and 18–29% H/H131) and allele frequencies (0·42–0·59 R131 and 0·45–0·58 H131) found in 10 other studies [24–33]. The *FcγRIIIA* genotypes (9% V/V158 and 59% F/F158) and allele frequencies (0·25 V158, 0·75 F158) of 32 subjects were similar to published data (7–13% V/V158, 26–66% F/F158, 0·33–0·44 V158, 0·56–0·62 F158) [14,15,33,34].

The situation whereby allogeneic D^+ RBC are cleared from the circulation after binding passive anti-D *in vivo* is more complex than binding of IgG to isolated leucocytes under experimental conditions. Many factors may affect the overall clearance observed, including the amount of IgG on the RBC [35,36], the expression of FcγRIIa and FcγRIIIa [37] and regulation of adhesion molecules by cytokines [38]. Furthermore, the nature of the anti-D may influence interaction with FcγR as, for example, monoclonal IgG1 anti-D's differ in their capacity to promote clearance [39]. The type of clearance study may determine the outcome; antibodies that promote clearance when bound to RBC *in vitro* before injection may not do so when injected i.m., which was the procedure used here. For instance, BRAD-3 cleared D^+ RBC more rapidly than polyclonal anti-D when precoated autologous RBC were infused into D^+ subjects [36] but more slowly when injected i.m. into D-negative subjects two days before D^+ RBC [17]. In these reports, removal of RBC from the circulation started within minutes or hours, respectively. The clearance study analysed here (RBC and anti-D injected separately) is not strictly comparable to most previous ones when RBC precoated with polyclonal anti-D were infused [23,33,37]. However, it more closely represents the clinical situation whereby passive anti-D is given to D-negative women to prevent immunization to fetal D^+ RBC [39].

The lack of association of *FcγRIIA* genotypes or alleles with clearance of RBC coated with anti-D found in our study may be because there is little interaction of these cells with $FcγRIIa^+$ effector cells [11]. At physiological levels of opsonization (10 000–20 000 IgG/cell) only about 5% of monocyte phagocytosis of EA-IgG3 was shown to be a result of activation of FcγRIIa, while this receptor was not utilized for interaction with EA-IgG1 anti-D [40]. In contrast to our data obtained with normal subjects (Fig. 2), a small association between polymorphisms of FcγRIIa and clearance of cells precoated with anti-D has been reported in patients with SLE [33].

The use of monoclonal IgG3 anti-D (BRAD-3) in some subjects may have presented a more sensitive test of the roles of FcγR polymorphisms than that of polyclonal anti-D, which comprises both IgG1 and IgG3, but predominantly IgG1. This is because IgG1 and IgG3 anti-D display a dichotomy of functional activity *in vitro*. In rosette assays, BRAD-3 was found more efficient than IgG1 or polyclonal anti-D at mediating binding of RBC to to FcγRI on monocytes [12], to FcγRIIa on K562 cells or transfectants [9] and to FcγRIIIa on splenic macrophages [41] or NK cells [42]. However, BRAD-3 mediated little haemolysis by $FcγRIIIa^+$ NK cells in ADCC assays, in contrast to polyclonal anti-D or some IgG1 monoclonal anti-D [17,41,43]. The initial step in splenic clearance of RBC may be adherence of EA-IgG to FcγRIIIa on macrophages, because the high affinity FcγRI would be blocked *in vivo* by cytophilic IgG [6]. After binding of EA-IgG to macrophages cytophilic IgG could be displaced from FcγRI allowing signalling through this receptor. If binding of EA-IgG to FcγRIIIa is compromised, and because EA-IgG3 signal poorly through FcγRIIIa, the net result may be slow clearance of EA-IgG3 from the circulation. This may be the explanation why polymorphisms of FcγRIIIa were seen to influence clearance of RBC by BRAD-3 but not by polyclonal anti-D.

The observed association of reduced clearance of RBC mediated by IgG3 anti-D with expression of the FcγRIIIa-V158 allele may well have clinical relevance. It has been documented that donors whose NK cells readily bind IgG express this allele, whereas donors with a low binding phenotype are F/F homozygotes [14,15]. Furthermore, patients with systemic lupus erythematosus (SLE) and haematologic cytopenias were more likely to have the FcγRIIIa-F/F158 genotype, i.e. expression of the V158 allele appeared to confer protection from autoantibody mediated cell clearance and destruction [33]. In contrast, fewer patients with idiopathic thrombocytopenic purpura (ITP) had the FcγRIIIa-F/F158 genotype [34]. Even if FcγRIIIa is not the sole FcγR recruited for EA–IgG interactions, any reduction in the ability of FcγRIIIa to bind EA-IgG may affect the total response achieved. The evidence so far suggests that NK cells, monocytes and macrophages expressing FcγRIIIa-V158 may bind more monomeric cytophilic IgG, blocking the receptor, and partially preventing interactions with IgG-coated cells. In contrast, macrophages expressing FcγRIIIa-F/F158 may have unoccupied receptors and may therefore be able to bind immune complexes more readily than those expressing FcγRIIIa-V/V158, leading to an increased ability to bind and clear these immune complexes and opsonized particles. This hypothesis is supported by results of the clearance study reported here showing that subjects expressing the FcγRIIIa-V158 allele removed RBC coated with IgG3 anti-D from the circulation more slowly than individuals homozygous for FcγRIIIa-F/F158.

These findings are, however, unlikely to affect the design of monoclonal anti-D for Rh D prophylaxis to prevent Rh D immunization of Rh D-negative women and subsequent haemolytic disease of the newborn. A 3 : 1 blend of BRAD-5:BRAD-3 has an IgG anti-D subclass profile of 75% IgG1 and 25% IgG3, approximating that of polyclonal anti-D [43] where IgG3 is only a minor component. This mixture of monoclonal antibodies has shown efficacy at preventing Rh D immunization in a large multicentre study [44,45] where the FcγRIIIa-V/F158 genotypes of the recipients were unknown.

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