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To the editor:

RhIg-prophylaxis is not influenced by FCGR2/3 polymorphisms involved in red blood cell clearance

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Red blood cell (RBC) alloimmunization against the RhD antigen may occur in RhD-negative women during pregnancy or at delivery of a RhD-positive child.[1] The administration of Rh-immunoglobulin (Rh-Ig) prevents anti-D alloimmunization and subsequent hemolytic disease of the fetus and newborn (HDFN) with very high efficiency. Postpartum administration of Rh-Ig decreases the prevalence of new anti-D alloimmunization from 3.5% to 0.5%, and the risk of becoming immunized is halved if Rh-Ig also has been administered in the last trimester of pregnancy.[2] Rh-Ig is produced from pooled plasma from hyperimmunized donors. It is generally accepted that Rh-Ig, which does not fix complement,[3] induces a rapid clearance of circulating fetal D-positive RBC by binding to activating IgG-Fc receptors (FcγRs) on macrophages, preventing recognition of these RBCs by the maternal adaptive immune system.[4,5] To replace polyclonal Rh-Ig in the future, recombinant RhD antibodies are being selected for their ability to induce fast removal of RhD-positive RBCs from the circulation.[6-9] Until now, the in vivo success of these antibodies has been limited.[10] Efforts to replace Rh-Ig by recombinant antibodies are hampered by lack of knowledge about the working mechanism of Rh-Ig.[4]

We hypothesize that women in whom prophylaxis has failed carry genetic risk factors that interfere with the Rh-Ig-mediated prevention of an immune response. The *FCGR2/3* locus encoding FcγR involved in Rh-Ig-mediated RBC destruction is highly polymorphic. Multiple single-nucleotide polymorphisms and copy number variations (CNV) of various *FCGR* genes exist that influence the FcγR expression level and/or function.[11] The *FCGR3A*-158V/F (rs396991) and the *FCGR2A*-131H/R (rs1801274) polymorphisms have profound effects on the affinity for IgG. Increased clearance of opsonized RBC in individuals with the high-affinity *FCGR3A*-158V allele has been described.[12] *FCGR2C* is a pseudogene in most Caucasians, but can contain a single-nucleotide polymorphism creating an open reading frame (*FCGR2C*-ORF allele), leading to functional expression of the activating FcγRIIc.[13,14] Finally, the expression of the inhibitory FcγRIIb on

Table 1. Fcγ*R*-profiles of alloimmunized women compared with healthy controls

		Alloimmunized		
	Controls (N = 199) (%)	All (N = 305) (%)	Adequate Rh-Ig (N = 219) (%)	No Rh-Ig (N = 86) (%)
FCGR2A				
Allele frequency				
131H*	214 (53.5)	316 (53.7)	228 (52.1)	88 (58.7)
131R	186 (46.5)	272 (46.3)	210 (47.9)	62 (41.3)
Phenotype frequency				
At least 1 131H	155 (77.9)	236 (77.4)	172 (78.5)	64 (74.4)
No H	44 (22.1)	69 (22.6)	47 (21.5)	22 (25.6)
FCGR2B				
Allele frequency				
232I†	352 (88.4)	541 (88.7)	388 (88.6)	153 (89.0)
232T	46 (11.6)	69 (11.3)	50 (11.4)	19 (11.0)
2B.1 GT	727 (88.9)	1058 (84.7)	771 (85.6)	287 (82.5)
2B.4 CA‡	42 (5.1)	93 (7.4)§	65 (7.2)	28 (8.0)
Phenotype frequency				
2B.4	38 (19.4)	87 (28.5)§	63 (28.8)§	24 (27.9)
No 2B.4	161 (80.9)	218 (71.5)	156 (71.2)	62 (72.1)
FCGR2C				
Haplotype frequency				
STOP	363 (86.8)	516 (80.9)	375 (81.0)	141 (80.6)
Classical-ORF¶	44 (10.5)	100 (15.7)§	67 (14.5)	33 (18.9)§,‖
Nonclassical-ORF	11 (2.6)	22 (3.4)	21 (4.5)	1 (0.6)§,‖
Phenotype frequency				
At least 1 Classical-ORF	39 (19.6)	91 (29.8)§	63 (28.8)§	28 (32.6)§
No Classical-ORF	160 (80.4)	214 (70.2)	156 (71.2)	58 (67.4)
FCGR3A				
Allele frequency				
158V#	135 (32.8)	229 (36.5)	168 (37.3)	61 (34.7)
158F	276 (67.2)	398 (63.5)	283 (62.7)	115 (65.3)
Phenotype frequency				
At least 1 158V	109 (54.8)	182 (59.7)	132 (60.3)	50 (58.1)
No 158V	90 (45.2)	123 (40.3)	87 (39.7)	36 (41.9)
FCGR3B				
Haplotype frequency				
FCGR3B*01**	164 (39.9)	236 (37.6)	172 (37.8)	64 (37.2)
FCGR3B*02	237 (57.7)	378 (60.3)	272 (59.8)	106 (61.6)
FCGR3B*03**	10 (2.4)	13 (2.1)	11 (2.4)	2 (1.2)
Phenotype frequency				
At least 1 FCGR3B*01	127 (63.8)	188 (61.6)	136 (62.1)	52 (60.5)
No FCGR3B*01	72 (36.2)	117 (38.4)	83 (37.9)	34 (39.5)

Significance levels are indicated by symbols.
*Increased affinity IgG1.
†Increased inhibition of FcγRI signals.
‡Increased FcγRIIb expression.
§Significant ($P < .05$) compared with controls.
‖Significant ($P < .05$) when the adequate prophylaxis group was compared with the group that had not received anti-D.
¶FcγRIIc expression.
#Increased affinity to all IgG.
**Increased affinity IgG3.

myeloid cells is elevated in individuals carrying the promotor haplotype 2B.4 compared with the wild-type promotor 2B.1.[15]

Given the influence of *FCGR2/3* polymorphisms on outcome of immunoglobulin therapies, we set out to determine whether failure of Rh-Ig is related to genetic variations within the *FCGR2/3* locus. For this, we identified a group of women who became RhD-immunized despite adequate prophylaxis and compared their *FCGR* profiles with those of healthy volunteers and of women immunized because of a lack of Rh-Ig prophylaxis.

DNA was obtained from 132 volunteer anti-D plasma donors (all women older than 45 years who were immunized during previous pregnancies), 66 RhD-immunized women identified during routine antibody screening in pregnancy, and 107 women who gave birth to children who had to be treated with intrauterine transfusions due to severe RhD-mediated HDFN (IUT; LOTUS study).[16] Seventy-two DNA samples were obtained from children of the LOTUS study. From all women, information about Rh-Ig prophylaxis was available through a structured questionnaire or a telephone interview with the obstetric caregiver. DNA from 199 volunteers was available; their ethnicity was determined by short tandem repeat marker analysis of 15 autosomal short tandem repeat loci, using the Powerplex 16 System (Promega, Madison, WI), according to the manufacturer's instructions. Samples were obtained with informed consent in accordance with the Declaration of Helsinki.

Genomic DNA was isolated from blood or saliva samples with a DNA extraction kit (QIAamp, DNA blood mini kit; Qiagen Benelux,

Table 2. Fcγ*R*-profiles of children treated with IUT because of severe HDFN compared with healthy controls

	Controls (N = 199) (%)	IUT Children (N = 72) (%)
FCGR2A		
Allele frequency		
131H*	214 (53.5)	77 (53.5)
131R	186 (46.5)	67 (46.5)
Phenotype frequency		
At least 1 131H	155 (77.9)	56 (77.8)
No 131H	44 (22.1)	16 (22.2)
FCGR2B		
Allele frequency		
232I†	352 (88.4)	129 (89.6)
232T	46 (11.6)	15 (10.4)
2B.1 GT	727 (88.9)	258 (86.6)
2B.4 CA‡	49 (6.0)	23 (7.7)
Phenotype frequency		
2B.4	39 (19.6)	17 (23.6)
No 2B.4	160 (80.4)	55 (76.4)
FCGR2C		
Haplotype frequency		
STOP	363 (86.8)	118 (76.6)
Classical-ORF§	44 (10.5)	23 (14.9)
Nonclassical-ORF	11 (2.6)	13 (8.4)
Phenotype frequency		
Classical ORF	39 (19.6)	23 (31.9)‖
No Classical ORF	160 (80.4)	49 (68.1)
FCGR3A		
Haplotype frequency		
158V	135 (32.8)	60 (41.1)
158F	276 (67.2)	86 (58.9)
Phenotype frequency		
At least 1 158V	109 (54.8)	51 (70.8)‖
No 158V	90 (45.2)	21 (29.2)
FCGR3B		
Allele frequency		
FCGR3B*01¶	164 (39.9)	58 (38.2)
FCGR3B*02	237 (57.7)	87 (57.2)
FCGR3B*03¶	10 (2.4)	7 (4.6)
Phenotype frequency		
FCGR3B*01	127 (63.8)	47 (66.2)
No FCGR3B*01	72 (36.2)	24 (33.8)

Significance levels are indicated by symbols.
*Increased affinity IgG1.
†Increased inhibition of FcγRI signals.
‡Increased FcγRIIb expression.
§FcγRIIc expression.
‖Significant ($P < .05$) compared with controls.
¶Increased affinity IgG3.

Venlo, The Netherlands). An *FCGR*-MLPA (MRC Holland, Amsterdam, The Netherlands) was performed as described before.[13,15]

Statistics were performed by χ-square test or by Fisher's exact test. P values <.05 were considered to be statistically significant.

All known FcγR polymorphisms were analyzed for 219 women who were identified as RhD-alloimmunized during first trimester antibody screening despite receiving adequate postnatal Rh-Ig prophylaxis in all previous pregnancies with an RhD-positive child. These were compared with 199 ethnically matched control patients and 86 women who did not receive prophylaxis and were RhD-immunized during pregnancy (Table 1).

We hypothesized that if the protective effect of Rh-Ig is achieved by RBC clearance, a decreased frequency of the high-affinity FcγR alleles (*FCGR3A-158V*, *FCGR2A-131H*) and/or a skewing in CNV of the activating FcγR would be found in RhD-alloimmunized women. We did not detect a difference in the distribution of *FCCR3A* and *FCGR2A* alleles or CNV changes between the RhD-immunized women (despite prophylaxis) and both control groups (Table 1; supplemental Tables 1 and 2, available on the *Blood* Web site). Almost half (n = 107) of the RhD-immunized women received IUT during pregnancy. To ensure this group did not introduce a bias, the group was compared with women without IUT treatment (n = 112). No significant differences were found in Fcγ*R*-polymorphisms (supplemental Tables 3-5).

We determined the *FCGR2/3* profile of 72 children treated with an IUT for severe HDFN. An increased frequency of the *FCGR3A-158V* allele was found (Table 2), and no significant differences in CNV or genotype (supplemental Tables 6 and 7). This indicated that HDFN severity is negatively affected by this high-affinity allele, in agreement with a previously identified association with increased IgG-mediated RBC clearance.[12,17]

B-cell inhibition has also been suggested as a possible mechanism of action of Rh-Ig.[10] The simultaneous cross-linking of the inhibitory FcγRIIb and the cognate B-cell receptor through IgG-opsonized RBC[5] induces down-modulation of the B-cell receptor-signaling, thereby preventing immune activation. This inhibiting effect can be potentially overruled by the expression of the activating FcγRIIc in donors that carry the *FCGR2C-ORF* allele,[18] although the expression of FcγRIIc on B cells has been contested.[19] The contribution of additional antigen-presenting cells cannot be excluded to date. The *FCGR2B* promoter haplotype 2B.4, which is associated with increased FcγRIIb expression, was significantly overrepresented, and an increase in the frequency of the *FCGR2C-ORF* ($P = .012$) allele was detected in RhD-immunized women, irrespective of whether there was adequate prophylaxis or not ($P = .022$; Table 1). The presence of *FCGR2C-ORF* and the *FCGR2B* promoter haplotype 2B.4 is associated with various immunological diseases and may reflect a higher susceptibility to trigger an antibody response by as-yet-unidentified mechanisms.[13,20] In the 86 RhD-immunized women who did not receive Rh-Ig, frequencies of the *FCGR2C-ORF* allele and 2B.4 haplotypes were essentially similar to those found in the alloimmunized women who did receive Rh-Ig (Table 1). Therefore, we conclude that skewing of *FCGR2C-ORF* allele and 2B.4 promoter haplotype is associated with increased immunization risk and independent of the mechanism of action of Rh-Ig. In a previous study on genetic risk factors for anti-D respondership, the *FCGR2C-ORF* allele and 2B.4 promoter haplotype were not investigated.[21]

In conclusion, whereas high-affinity alleles encoding FcγRs, which are known to influence the clearance of anti-D-sensitized red cells[12] are indeed associated with a severe course of HDFN, they do not seem to influence the preventive effect of Rh-Ig. This observation is in agreement with results of recent studies in mouse models on antibody-mediated suppression of RBC alloimmunization, in which prevention of response occurred independent of RBC clearance rate[22,23] A recent murine study suggested that antigen modulation might play a role in immunoprophylaxis[24]; however, FcγR polymorphisms influencing antigen modulation have not been identified to date.[25] Altogether, these observations indicate that the mechanism of action of RhIG differs from the mechanism of action of maternal anti-D. At this time, recombinant antibodies aimed to replace polyclonal plasma-derived Rh-Ig are selected on their ability to clear RBC.[6-9] This, however, may not be the main prerequisite for in vivo success of recombinant anti-D antibodies and has to be reconsidered as the main screening strategy.

The online version of this article contains a data supplement.

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To the editor:

KIT blockade is sufficient for donor hematopoietic stem cell engraftment in Fanconi anemia mice

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Fanconi anemia (FA) results from defects in genes involved in the DNA repair pathway and is characterized by progressive bone marrow failure (BMF) and a high incidence of cancer.[1] Currently, hematopoietic stem cell transplant (HSCT) is the only curative option for the BMF. Due to the underlying DNA repair defect, FA patients have inherent hypersensitivity to agents like cyclophosphamide, busulfan, and ionizing radiation.[2] Recently, fludarabine, reduced-intensity cyclophosphamide, total body irradiation (TBI)–based conditioning followed by a CD34-selected graft has improved the 5-year survival to >80%.[3,4] Despite reduced graft rejection and graft-versus-host disease, conditioning-related toxicity and long-term risk of malignancy are still high.[3-6] Pretransplant conditioning devoid of alkylating agents/TBI would decrease the conditioning-related toxicity and additionally lower the risks of long-term malignancy in FA.