EXPRESSION OF RHD IS LINKED TO RHD/RHCE GENOTYPE

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Background

RHD and *RHCE* represent homologous genes in head-to-head position on chromosome 1 (chr1, p36.11). They encode for the proteins RhD resp. RhCE which compose together with Rhesus associated glycoprotein (RhAG), Band 3 and ankyrin the ankyrin complex (AC) linking the red blood cell (RBC) membrane to a-spectrin of RBC cytoskeleton (S.E. Lux, BLOOD, 2016). Cooperatively, the proteins of AC are important for maturation and physiologic properties of RBCs. Many proteins of the RBC membrane express blood group antigens on their extracellular surface and are therefore of concern in transfusion medicine. Cepellini et al. described weakened hemagglutination reactions of RHD+ RBCs in the presence of an RhC+ antigen (Cepellini et al, PNAS, 1955). We attempted to further elucidate the expression of RhD/RhAG proteins in various RhCE/*RHCE* pheno-/genotypes using a sophisticated flow cytometry approach.

Aim

In this study, we investigated a flow cytometric method for measurement of the antigen-density of various RHCE-phenotypes.

Methods

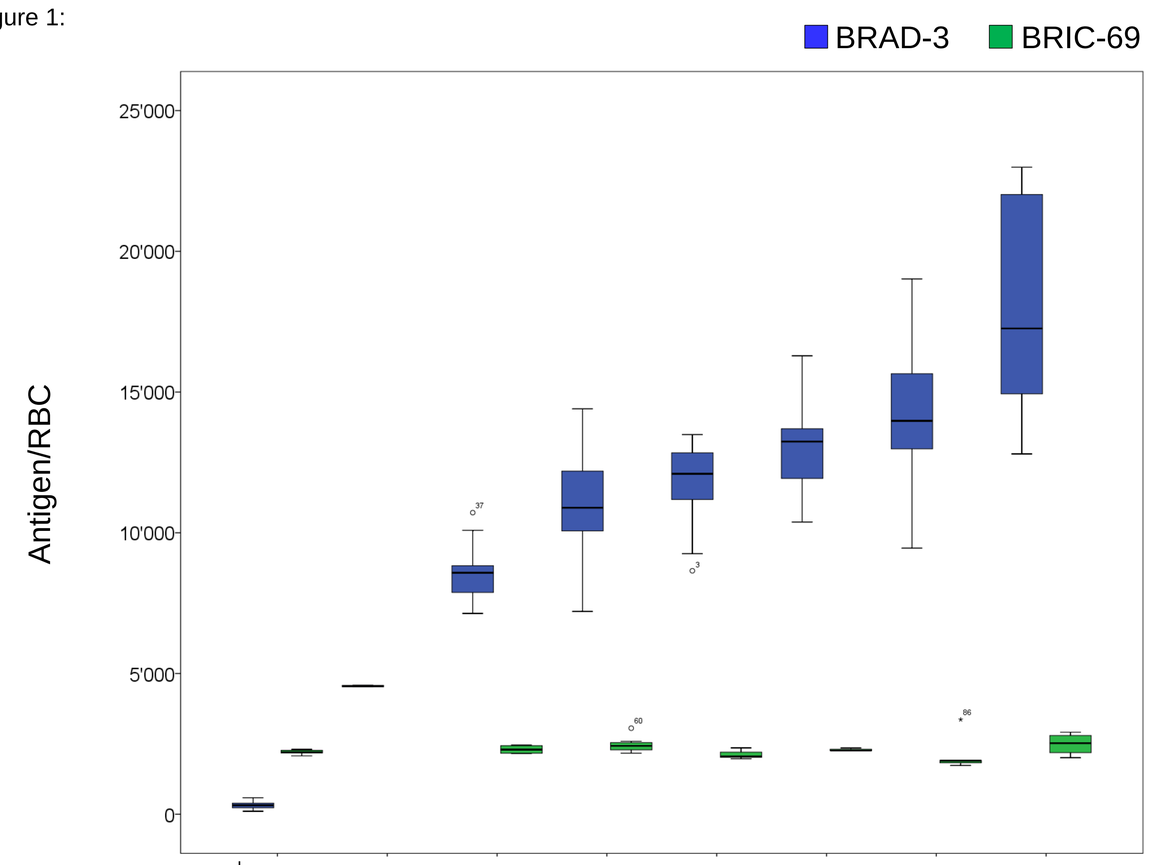
Analysis was performed on a flow cytometer (FACSCanto II, Becton Dickinson (BD)) using BD FACSDiva software and identical instrument settings for all samples. Optimized number of RBCs was incubated with saturating concentration of PE-conjugated anti-RhD antibodies BRAD-3/BRAD-5/FOG-1 (IBGRL, Bristol, UK). Debris was excluded by RBC gating in FSC/SSC plot. QuantiBRITE-PE beats (BD) were applied according to manufacturer's instruction to quantify the relative expression of RhD epitopes. In addition a representative number of samples from common phenotypes were assessed for expression of RhAG using BRIC-69PE (IBGRL).

Results

A total of 146 samples from healthy blood donors with serologically defined RhCDE phenotypes were included into this study (rr(21), R1r(20), R1R1(23), R0r(15), R2r(18), R1R2(27), R2R2(22)). Variant expression of RhD by different RhCE phenotypes using BRAD-3-PE is shown (Figure 1). RhD is weakly expressed in the presence of RhC antigen (Cepellini effect). Effect of *RHD* gene dose on RhD protein expression is mitigated by *RHC/c* genotypes. When only samples with molecularly confirmed phenotypes were assessed, the *RHDCE* genotype predicts consistently the strength of RhD protein expression. Outlier samples (3) were retrospectively genotyped and revealed *RHDCE* genotypes as expected from the strength of RhD expression falsifying RhCDE phenotypes. In contrast, *RHE/e* polymorphic site is not associated with decreased RhD expression. In addition, RhAG protein is equally expressed across all RhCDE phenotypes (Figure 1). Similar results were obtained with alternative anti-D antibodies such as BRAD-5-PE and FOG-1-PE, although different antibody's avidity precludes quantitative comparison of antigen expression on RBCs (Figure 2).

Variant expression of RhD by different RhCE phenotypes using BRAD-3-PE

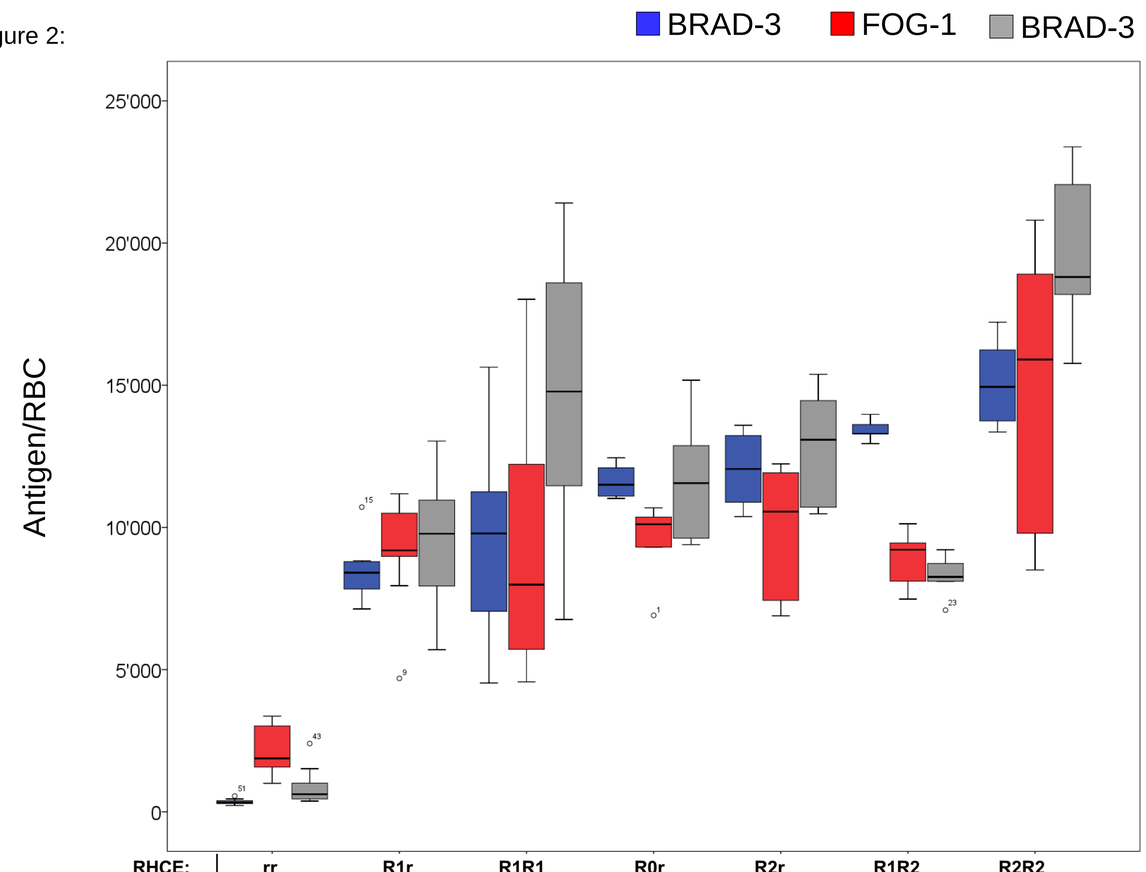
Figure 1:



	rr	R1r'	R1r	R1R1	R0r	R2r	R1R2	R2R2
MFI: RHCE (BRAD-3-PE)	327	4'552	8'510	11'026	11'801	12'970	14'323	18'277
N:	11	2	20	23	15	18	27	22
MFI: RHAG (BRIC-69-PE)	2'208	-	2'222	2'475	2'105	2'296	2'133	2'493
N:	6	0	5	7	8	3	6	4

Uniformly expression of RhD epitopes across all RhCDE phenotypes using anti-RhD antibodies BRAD-3/BRAD-5/FOG-1

Figure 2:



RHCE:	rr	R1r	R1R1	R0r	R2r	R1R2	R2R2
MFI, BRAD-3-PE	342	8'442	9'509	11'612	12'034	13'428	15'075
MFI, FOG-1-PE	2'158	9'179	9'493	9'582	9'933	8'879	14'479
MFI, BRAD-5-PE	839	9'606	14'727	11'699	12'869	8'283	19'502
N=	12	9	7	6	6	5	6

Discussion/Conclusions

Sophisticated FACS methods reveal different expression of RhD on RBCs according to RhCE/*RHCE* phenotype/genotype. *RHC/c* polymorphic sites (c.48G>C, c.201A>G, c.203A>G of exon 1, exon 2 resp. and intron 2) are in linkage with RhD expression, confirming the observation by Cepellini et al. In contrast, *RHE/e* (c.676C>G, exon 5) is not in linkage with RhD expression. Based on epigenomic signature it is conceivable that altered transcription factor binding sites (TBS) of *RHD* mirrored by homologous *RHC/c* may cause variant RhD expression. *RHE/e* SNP mirroring the homologous sequence of *RHD* in exon 5 is not recognised as TBS. In addition, although AC comprises all three Rh proteins (RhD, RhCE, RhAG), their transcriptional regulations seem to be distinct.

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