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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 and RhCE resp., which compose together with Rhesus associated glycoprotein (RhAG), Band 3 and ankyrin the ankyrin complex (AC) binding the red blood cell (RBC) membrane to α-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 an adapted flowcytometry approach.

Aim

In this study, we describe a flowcytometric method for measurement of the RhD antigen density of various RhCE phenotypes.

Methods

Analysis was performed on a flowcytometer (FACSCanto II, Becton Dickinson (BD)) using BD FACSDiva software and identical instrument settings for all samples examined. RBCs were incubated with saturating concentration of PE-conjugated anti-RhD antibodies BRAD-3/BRAD-5/FOG-1 (IBGRL, Bristol, UK). Debris was excluded by gating the RBCs 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, the samples from common phenotypes were assessed for expression of RhAG using BRIC-69PE (IBGRL). Genotyping for *RHD/RHCE* and *RHD* zygosity were assessed by RBC-Ready Gene ZygoFast (inno-train, Kronberg, Germany). Unfortunately no genomic DNA was available form --D.-- samples for genotyping.

Results

A total of 151 samples from healthy blood donors with defined RhCDE phenotypes were included into this study (rr(21), R1r'(2), R1r(20), R1R1(23), R0r(15), R2r(18), R1R2(27), R2R2(22), --D.--(3)). Variant expression of RhD by different RhCE phenotypes using BRAD-3-PE is shown in Figure 1. RhD is weakly expressed in the presence of RhC antigen (Cepellini effect). *RHD* gene dose dependent variant expression of RhD is mitigated by *RHC/c* genotype. --D.-- phenotypes express excessive amount of RhD epitopes on the RBC membrane as compared to phenotypes co-expressing RhCE protein. When only samples with confirmed phenotypes by genotyping 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, repealing serologic RhCDE phenotype. 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).

Figure 1: Variant expression of RhD by different RhCE phenotypes using BRAD-3-PE

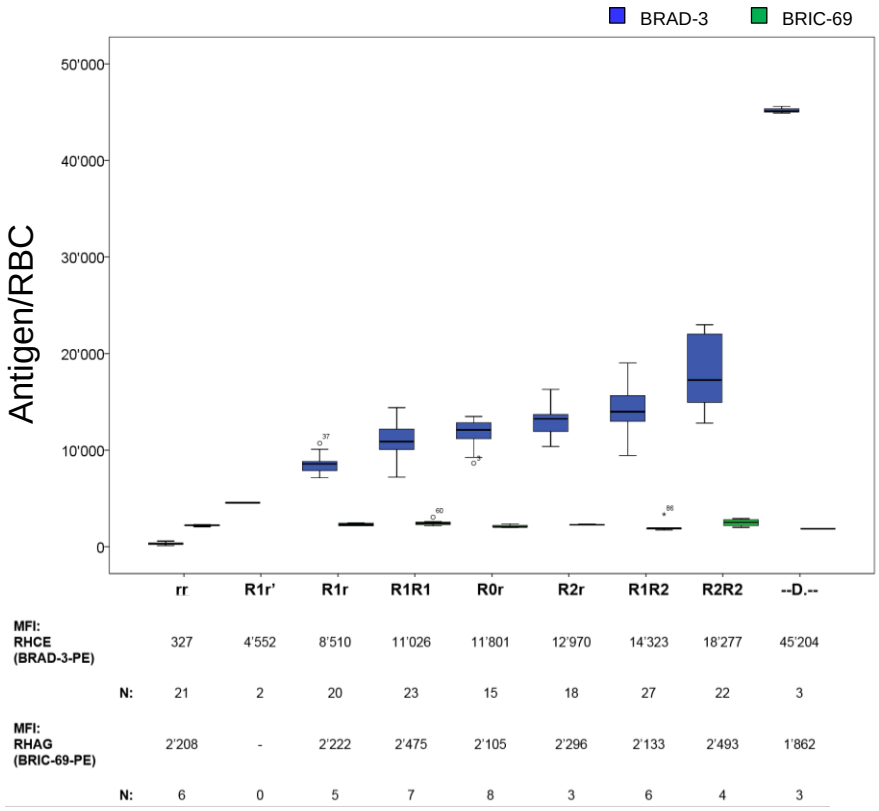
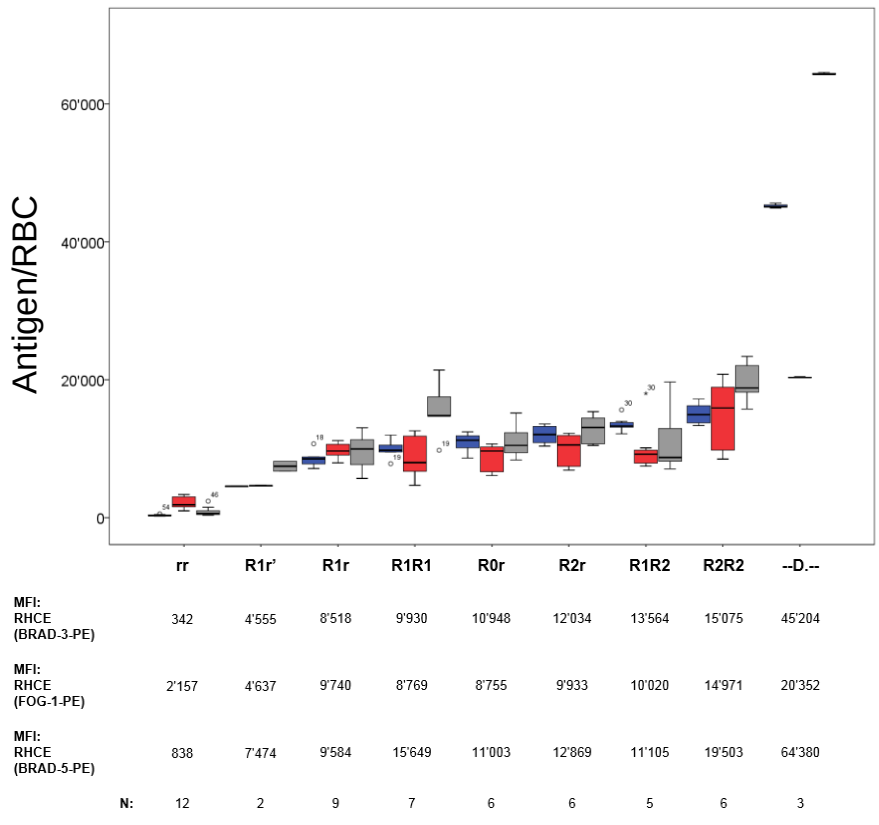


Figure 2: Expression of RhD epitopes across RhCDE phenotypes using anti-RhD antibodies BRAD-3/BRAD-5/FOG-1



Conclusions

Sophisticated FACS methods reveal variant expression pattern 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* single nucleotide variant (SNV) may cause variant RhD expression. *RHE/e* SNV mirroring the homologous sequence of *RHD* in exon 5 is not recognized 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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