EXPRESSION OF RHD IS LINKED TO RHD/RHCE GENOTYPE

E. Meyer1, Y. Merki1, C. Gassner1, Y.-L. Song2, S. Meyer1, C. Engström2, B. M. Frey2
1Department of Molecular Diagnostics (MOC), 2Immunohematology, Blood Transfusion Service Zurich, SRC, Schlieren, Switzerland

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 the 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, 1995). 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 RhCE phenotypes were included into this study (rr(21), R1r(20), R1R1(23), R0(15), R2(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 RHCEc 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 RhDCE phenotypes. In contrast, RHFe e polymorphic site is not associated with decreased RhD expression. In addition, RHAG protein is equally expressed across all RHCE 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).

Discussion/Conclusions

Sophisticated FACS methods reveal different expression of RhD on RBCs according to RhCE/RHCE phenotype/genotype. RHCEc 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, RHEFe (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 RHCEc may cause variant RhD expression. RHEFe 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, RhC, RHAG), their transcriptional regulations seem to be distinct.