Evaluation of an antibody with putative anti-RH1/MNS3 compound specificity

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Background

Although RH and MNS blood group systems are classically considered independent, the underlying membrane proteins GPA, GPB as well as RhAG, RhD, RhCE are associated within the ankyrin-1 complex. Physical proximity in the red-cell membrane may facilitate the emergence of compound antibody specificities. We report a 42-year-old gravida 2, para 1 woman with transfusion-dependent T-LGL leukemia. During her 2nd pregnancy, antibody screening detected an anti-KEL3 and a serologically atypical anti-MNS3, only reacting in the presence of RH1. The aim of this study was to characterize this unusual antibody specificity using detailed serological, genetic and protein structure analyses.

Methods

Investigations comprised serological typing for the ABO, RH, KEL and MNS systems, direct and indirect antiglobulin testing, including papain-treated cells, as well as in-house test cells treated with trypsin/DTT. To exclude anti-LW1, cord blood cells with defined RH:1/RH:-1 and MNS:3/MNS:-3 phenotypes were analyzed. Serial antibody titrations were performed. Adsorption-elution analyses with MNS:3 and RH:1/RH:-1 test cells were conducted to assess the suspected compound specificity of the putative anti-RH1/MNS3 (in-house). The clinical relevance was assessed by monocyte

monolayer assay (MMA). Genetic investigations were performed using targeted long-read sequencing enriching for *MNS*, *RHD/RHCE/RHAG*, as well as for other loci encoding proteins of the ankyrin-1 complex. Structural data of this membrane complex was used to evaluate relative positions of GPB and RhD allowing assessing the plausibility of a compound antigen.

Results

The patient was typed as RH:1,2,-3,4,5; KEL:-1,2,-3 and MNS:1,-2,-3,4; with a negative DAT. The anti-MNS3 antibody demonstrated an atypical serological pattern. Reactivity was observed exclusively with test cells co-expressing MNS3 and RH1 (IAT 2+, papain negative, trypsin 3+, DTT 2+), whereas MNS:3/RH:-1/KEL:-3 cells were consistently non-reactive. Other clinically relevant antibody specificities were excluded. Only cord blood cells expressing both RH1 and MNS3 showed 2+ reactions. During pregnancy, the antibody titer remained stable at 64. Adsorption studies further supported a compound specificity, as the putative anti-RH1/MNS3 could only be adsorbed using RH:1/MNS:3 red cells, while RH:-1/MNS:3 adsorption cells failed to remove reactivity. MMA did not point to clinical relevance of the antibody (<3%). Genetic analyses did not reveal the presence of altering genetic variants in genes involved in ankyrin-1 complex formation (*GYPA*/*GYPB, RHD/RHCE, RHAG, ANK1, AQP1, CD47, EPB42, ICAM4, SLC2A1, SLC4A1, SPTA1, SPTB*). Structural data suggests that GPB is positioned closer to RhAG than to the RhCE subunit with an estimated separation of ~38 Å, which provides limited support for a direct RH1/MNS3 compound antigen.

Conclusion

Serological studies revealed an atypical antibody in a pregnant patient showing reactivity only with RH:1/MNS:3 red blood cells. While adsorption experiments supported a putative compound anti-RH1/MNS3 specificity, genetic analyses did not reveal variants in the associated genes that could point to an altered membrane complex conformation in the patient. Moreover, the spatial organization of GPB and RhD within the erythrocyte membrane macrocomplex is not supportive of such an arising conformational epitope, but unknown scaffold proteins could modify the subunits' conformation. The patient delivered a healthy RH:1/KEL:-3/MNS:-3 neonate.