Case Report: Putative Compound Anti-RH1/MNS3

M. Gueuning[1], C. Azzato[2], S. Koller[1,3], A. Komarek[2], Y-L. Song[2], C. Rütsche[4], A. Floch[5,6], G. A. Thun[1], S. Meyer[1,3], C. Engström[2]

1 Blood Transfusion Service Zurich (SRC), Department of Research and Development, Schlieren, Switzerland

2 Blood Transfusion Service Zurich (SRC), Department of Immunohematology, Schlieren, Switzerland

3 Blood Transfusion Service Zurich (SRC), Department of Molecular Diagnostics and Cytometry, Schlieren, Switzerland

4 Institute for Laboratory Medicine and Blood Donation Service, Spital Thurgau AG, Münsterlingen Cantonal Hospital, Switzerland

5 Univ Paris Est Créteil, INSERM U955 Equipe Transfusion et Maladies du Globule Rouge, IMRB, Créteil, France

6 Laboratoire de Biologie Medicale de Référence en Immuno-Hematologie Moléculaire, Etablissement Français du Sang Ile-de-France, Créteil, France

A. 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 (ab) specificities. We report a 42-year-old gravida 2, para 1 woman with transfusion-dependent T-LGL leukemia. During her 2nd pregnancy, ab screening detected an anti-KEL3 and a serologically atypical anti-MNS3, only manifesting in the presence of RhD.

B. Aims

The aim of this study was to characterize this unusual ab specificity using detailed serological, genetic and protein structure analyses.

C. 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, 8 cord blood cells with defined RH:1/RH:-1 and MNS:3/MNS:-3 phenotypes were analyzed. Serial ab titrations were performed. Absorption/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). Genetic investigations were performed using a custom targeted long-read capturing assay designed to enrich for MNS, RH and RHAG loci. The library was sequenced on an Oxford Nanopore MinION flowcell. Structural data of the ankyrin-1 complex was used to evaluate the relative positions of GPB and RhD within the complex and to assess the plausibility of a compound antigen.

D. 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 ab demonstrated an atypical serological pattern. Reactivity was observed exclusively with test cells co-expressing MNS:3 and RH:1 (IAT 2+, papain negative, trypsin 3+, DTT 2+), whereas MNS:3/RH:-1/KEL:-3 cells were consistently non-reactive. Other clinically relevant ab specificities were excluded. Only cord blood cells expressing both RH:1 and MNS:3 showed 2+ reactions. During pregnancy, the ab titer remained stable (32-64). 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. Genetic analyses did not reveal the presence of altering SVs or SNVs in *GYPA*, *GYPB, RH, RHAG* or *RHCE*. 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. Genetic analyses using adaptive sampling are currently being performed on other genes involved in the complex (*ANK1, AQP1, CD47, EPB42, ICAM4, SLC2A1, SLC4A1, SPTA1, SPTB*).

E. Summary / Conclusions

Serological studies revealed an atypical antibody in a pregnant patient showing reactivity only with RH:1/MNS:3 red cells. While adsorption experiments supported a putative compound anti-RH:1/MNS:3 specificity, genetic analyses did not reveal variants in the currently investigated genes that could explain an altered membrane complex conformation in the patient. Extended investigation of the involved genes may shed light on a possible formation of a conformational epitope arising from the spatial organization of GPB and RhD protein within the erythrocyte membrane macrocomplex. The patient delivered a healthy RH:1/KEL:-3/MNS:-3 neonate. No evidence of HDFN was observed, and the DAT was negative.