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

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Introduction

Although RH and MNS blood group systems are considered independent, underlying glycophorin and Rh proteins are associated within the ankyrin-1 membrane complex. Their physical proximity may enable the emergence of compound antibody specificities. We report a gravida 2, para 1 woman with T-LGL leukemia in whom — beside an anti-KEL3 — an atypical anti-MNS3 was detected, only manifesting in the presence of RH1. We aimed to characterize this unusual antibody specificity with serological, genetic and protein structure analyses.

Methods

Investigations comprised serological typing for ABO, RH, KEL and MNS systems, DAT/IAT, including papain-treated cells, and 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 used. Adsorption-elution with MNS:3 and RH:1/RH:-1 test cells were conducted to assess compound specificity of the putative anti-RH1/MNS3 antibody. Clinical relevance was assessed by monocyte monolayer assay (MMA). Genetic investigations were performed using targeted long-read sequencing enriched for loci encoding proteins of the ankyrin-1 complex. Structural data of this

membrane complex was used to evaluate relative positions of GPB and RhD for assessing the plausibility of a compound antigen.

Results

The patient was typed RH:1,2,-3,4,5; KEL:-1,2,-3 and MNS:1,-2,-3,4; with a negative DAT. Reactivity of the detected anti-MNS3 antibody was observed 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 relevant antibody specificities were excluded. Only cord blood cells expressing both RH:1 and MNS:3 showed 2+ reactions. Adsorption studies supported a compound specificity, as the putative anti-RH1/MNS3 antibody could only be adsorbed using RH:1/MNS:3 red cells. MMA did not point to clinical relevance of the antibody. Genetic analyses revealed no altering genetic variants in genes involved in ankyrin-1 complex formation. Structural data suggest that GPB is separated from the RhD subunit by ~38 Å, providing limited support for a direct RH1/MNS3 compound antigen.

Conclusion

While serological experiments suggested an antibody with compound anti-RH1/MNS3 specificity, neither genetic nor protein structure analyses pointed to a putative arising conformational epitope involving GPB and RhD proteins. An unknown scaffold protein modifying the subunits' formation could explain our serological observation.