

Clinical approach after identification of a rare anti-En^a in a prenatal sample

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The antigens associated with the MNS blood group system (ISBT 002) are located on glycophorin A (GPA) and glycophorin B (GPB). The most frequently encountered antibodies to antigens in this system by a transfusion medicine service are those directed against M, N, S, and s. Individuals lacking GPA typically have red blood cells that lack M, N, and En^a, whereas those lacking both GPA and GPB lack M, N, and En^a as well as S, s, and U. Such individuals may develop a rare antibody, anti-En^a, directed against determinants on GPA. This antibody is capable of causing hemolytic transfusion reactions and hemolytic disease of the fetus and newborn. This case report describes a pregnant woman found to have anti-En^a. Molecular testing supported an M^k phenotype that was found in several members of her immediate family. *Immunohematology* 2019;35:159–161.

Key Words: En^a, antibody, prenatal, MNS

Introduction

The MNS blood group system (ISBT 002) consists of red blood cell (RBC) antigens located on glycophorin A (GPA) and glycophorin B (GPB). The *GYP A* and *GYP B* genes encoding these glycophorins are located on the long arm of chromosome 4.^{1,2} The most frequently encountered antibodies to antigens in this system by a transfusion medicine service are those directed against M, N, S, and s. In rare cases, individuals may lack GPA or both GPA and GPB on their RBCs because of gene deletion, and such individuals may develop a rare antibody, anti-En^a, to determinants located on GPA. Anti-En^a does not target a precise antigen on GPA but rather is an “umbrella” term for antibodies directed at external regions of GPA made by rare people who lack all or part of GPA. Three broad categories of anti-En^a have been defined according to the effect of protease enzymes on the antigenic determinants that each detects, namely anti-En^aTS, -En^aFS, and -En^aFR.³ The results from a previous study by Tanner and Anstee⁴ showed that RBCs lacking GPA, the major sialoglycoprotein on the RBC surface, lack En^a. This deficiency reduces the overall zeta potential of an intact RBC resulting in RBCs with a higher propensity to agglutinate than RBCs expressing GPA.⁴ Antibodies against En^a have been associated with hemolytic transfusion reactions and hemolytic disease of the fetus and newborn (HDFN).⁵

We present a case of the rare M^k phenotype with allo-anti-En^a in a maternal prenatal sample. We discuss the clinical ramifications of this finding.

Case Report

A 28-year-old Caucasian woman, never transfused, primigravida at approximately 23 weeks' gestation, presented for routine prenatal care at her local community hospital. A routine type and antibody detection test was performed and found to be positive, and her sample was flagged for further testing by the Immunohematology Reference Laboratory (IRL) at Miller-Keystone Blood Center (Bethlehem, PA). The IRL identified anti-En^a by indirect antiglobulin testing. The presence of this antibody is worrisome for both mother and fetus because anti-En^a of an IgG isotype has been implicated in both acute and delayed hemolytic transfusion reactions and HDFN.³ The patient was referred to the Maternal Fetal Medicine department at Penn State Hershey Medical Center (Hershey, PA).

The initial antibody detection test performed at Hershey Medical Center demonstrated weak positive reactivity with all three reagent screening RBCs and with 11 of 11 panel RBCs at the antiglobulin phase using a solid-phase red cell adherence assay. The direct antiglobulin test was negative. Further testing by our IRL revealed that the patient's RBCs typed M–N–S–s– with licensed RBC antisera (Immunacor, Norcross, GA). This finding prompted further testing of the patient's plasma against a U– and a U+^{var} reagent RBC, both of which were reactive. This reactivity was not detected when the RBCs were treated with ficin.

These results, together with the patient's non-African ethnicity, allowed the IRL to rule out anti-U and anti-Wr^b. Classically, it was expected that both of these antibodies would remain reactive with ficin-treated reagent RBCs. Furthermore, the testing of the patient's plasma against ficin-treated reagent RBCs in low-ionic-strength solution (tube method) resulted in no agglutination. These findings are consistent with a subtype of En^a that is sensitive to ficin treatment, historically known

as En^aFS. However, because of the lack of M^kM^k or En(a-) reagent RBCs available for testing against the patient's plasma, these findings were not confirmed.

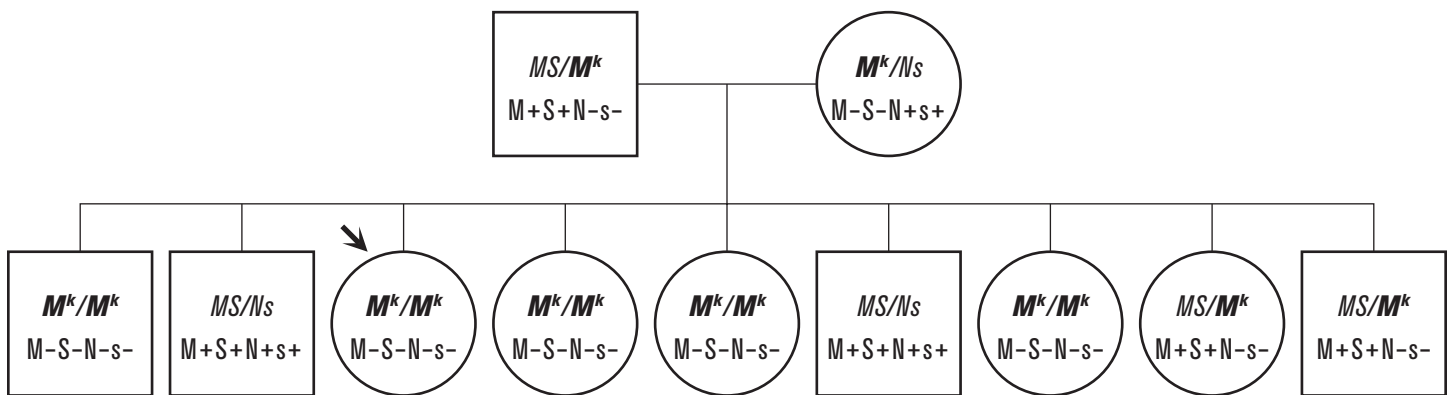
IgG titers were then performed without enhancement media at 37°C for 60 minutes. The initial titer was 1, and 1 month later, the titer was 2; these results are within procedural variation and the titer difference was considered insignificant by the IRL. The sample was referred to the National Molecular Laboratory at the American Red Cross (Philadelphia, PA) for performance of RBC genotyping by PreciseType HEA Molecular BeadChip (Immucor). This molecular platform interrogates targets to predict expression of M, N, S, s, and U. The patient sample failed to amplify for all the assays that interrogate *GYPA* and *GYPB* targets. RBCs from the presumed father of the fetus demonstrated an M+N-S+s+ phenotype by serological and molecular methods from the same laboratories as the patient. The patient's molecular and serologic findings from testing of the patient's sample were consistent with an M^k phenotype, which is a null phenotype for antigens on both GPA and GPB, encoded by homozygosity for *GYP*OIN*. No typing of the patient's RBCs with antisera against other high-prevalence antigens in the MNS system was performed.

Given the exceedingly rare occurrence of En(a-) individuals (<0.1%), clinical encounters with alloantibodies to En^a are limited. If transfusions were required for the mother or fetus, our options were autologous donations, directed donations from family members who match the patient's phenotype, or via rare blood donor registries. Fortunately, this patient came from a large family, most of whom were tested both serologically and molecularly. A blood group genotyping platform (ID CORE XT; Grifols, Barcelona, Spain) that also

interrogates *GYPA* and *GYPB* targets to predict the common antigens in the MNS system was used to analyze samples from the family members at Grifols laboratory. Four siblings were predicted to express the same M^k phenotype as the patient. These results were confirmed, also by Grifols laboratory, by allele-specific polymerase chain reaction (PCR) and agarose gel electrophoresis, which showed an absence of products for *GYPA* exons 2-7; *GYPB* exons 1-2 and 4-6; and *GYPB* pseudo-exon 3 (data not shown). A pedigree demonstrates the likely inheritance pattern of the *GYPA* and *GYPB* genes and the MNS antigens (Fig. 1). Given the rarity in the general population and prevalence within this family, we can postulate that M^k phenotype inheritance is due to consanguinity among the parents in the pedigree, although an inquiry to confirm this was not pursued.

The patient's antibody titers remained unchanged throughout her pregnancy (minimum 1; maximum 2), with low reactivity in a mononuclear monolayer assay (MMA) performed by Grifols laboratory. The MMA is used to predict an antibody's ability to promote macrophagocytic adherence and phagocytosis upon exposure to a cognate antigen with or without a source of complement.

The consensus in this case was to manage this as a high-risk pregnancy with frequent intensive monitoring, including antibody titers. At term, the patient underwent induction of labor and successfully delivered a healthy baby boy by vaginal route. The delivery was without event, no transfusions were necessary antepartum or postpartum, and a neonatal sample revealed no bound RBC antibodies (i.e., negative direct antiglobulin test) or evidence of hemolysis.



Haplotypes: *MS*: *GYPA**M - *GYPB**S *Ns*: *GYPA**N - *GYPB**s *M^k*: *GYPA**deletion - *GYPB**deletion

Fig. 1 This pedigree describes the inheritance of the *GYPA* and *GYPB* alleles and expression of these antigens within the MNS blood group system. Arrow notes the predicted phenotype of the proposita.

Discussion

Because En^a is a high-prevalence antigen, there are few case reports of anti-En^a. This rarity affords the medical community limited data about the potential clinical sequelae of this antibody, which is concerning in a pregnant woman. The identification of the antibody resulted in this case being monitored as a high-risk pregnancy, based on previous case reports.⁶ Providing this patient with rare En(a-) RBCs (or M^kM^k RBCs) via national or international blood donor registries would have been exceedingly difficult. Donors in a registry do not guarantee that RBC units of a desired phenotype will be available when needed. Autologous donation was also considered for the purposes of antepartum or postpartum use as well as future use, if needed, in the form of frozen RBCs. The allogenic donor registry and autologous donation approaches were discussed and understood by the patient. These approaches were unnecessary, however, because the patient had many compatible family members available and willing to donate blood by directed allogenic donations.

The M^k phenotype within this family is due to deletions involving exons 2–7 of the *GYP A* and exons/pseudo-exon 1–6 of the *GYP B* genes rather than a mutation in both the *GYP A* and *GYP B* genes.⁷ This genetic alteration results in the absence of GPA, GPB, and the constitutive antigens of the MNS blood group system. We surmise that our patient was exposed to the En^a present on the GPA of her baby's RBCs *in utero*, or perhaps during an undetected miscarriage, which led to a subsequent alloimmunization. However, given the infrequency in which fetal antigens contact the maternal immune system and the low titer of the maternal antibody, there is a possibility that the anti-En^a may have been non-RBC related (“naturally-occurring”).

In conclusion, this case report describes the identification of a rare anti-En^a in a prenatal patient and the subsequent testing of family members in preparation for the delivery. The clinical finding of a rare antibody for which there are limited data requires leveraging every resource available to predict its behavior and provide safe blood products to patients who may require transfusion.

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