A case of hydrops fetalis, probably due to antibodies directed against antigenic determinants of GP.Mur (Miltenberger class III) cells

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The GP.Mur (Miltenberger class III) phenotype was found to occur in about 6.3 percent of Hong Kong (HK) Chinese blood donors. The incidence of antibodies directed against antigenic determinants of GP.Mur cells (anti-Mi) among patients was 0.34 percent, similar to that in Taiwan Chinese. A case of hydrops fetalis probably attributable to maternal anti-Mi was encountered in an HK Chinese woman during her sixth pregnancy. The anti-Mi was potent (titer 512, score 99). It fixed complement and was a mixture of IgG1 and IgG3. Two biological assays, the monocyte monolayer assay and the chemiluminescence test, were strongly positive. The father was found to be heterozygous for the *GP.Mur* gene. ***Immunohematology*** 1996;12:115–118.

The biochemical and molecular bases of the Miltenberger (Mi) subclasses have been elucidated and reviewed.[1,3] The abnormal glycoprotein in GP.Mur red blood cells (RBCs) is a hybrid glycophorin (GP) of the type (B-A-B).[1,2] The GP.Mur phenotype is rarely seen among Caucasians (1 in 10,000).[4] Certain Asian populations have a higher incidence of the phenotype: 9.7 percent in Thai blood donors,[5] 7.3 percent in Chinese blood donors in Taiwan,[6] and 6.3 percent in Hong Kong (HK) Chinese donors.[7] Anti-Mi usually occurs as a mixture, with or without separable specificities,[8] and has been reported to cause mild to moderate hemolytic transfusion reactions (HTRs) or hemolytic disease of the newborn (HDN).[6,9–12] This article reports on a case of fatal HDN and hydrops fetalis, most probably attributable to anti-Mi. In order to characterize fully the hemolytic potential of the antibody, a monocyte monolayer assay (MMA), a chemiluminescence test (CLT), and IgG sub-class typings were performed. The impact of this case on the selection of antibody screening and panel RBCs for type and screen (T & S), and investigation of HDN among HK Chinese is also discussed.

Case Report

A 40-year-old Chinese woman (LCK) was admitted to the hospital with a diagnosis of impending fetal hydrops at the 18th week of her sixth pregnancy. Her first four pregnancies were uncomplicated. The fifth pregnancy resulted in intrauterine death at the 27th week. The cause of death was not ascertained. The glucose-6-phosphate-dehydrogenase (G6PD) and syphilis serology (VDRL) screens were normal and nonreactive, respectively. Ultrasound studies did not reveal fetal cardiac or renal abnormalities. At the 19th week of gestation, there were severe fetal ascities and hepatosplenomegaly. Intrauterine fetal death was diagnosed and a therapeutic abortion was performed. The fetal corpse was pale and extremely edematous. Blood samples from the father (NML) and the mother were obtained for serologic investigations and RBC phenotyping.

Materials and Methods

Standard serologic techniques were used.[13] RBC panels, polyspecific anti-human globulin (AHG), anti-IgG, and anti-C3b, -C3d were purchased from Ortho Diagnostic Systems, Inc., Raritan, NJ. Alloantibodiy screening was conducted using a saline antiglobulin test (AGT), a polyethylene glycol antiglobulin test (PEG-AGT), and a two-stage enzyme AGT. In-house GP.Mur RBCs were used to screen for the presence of anti-Mi. Confirmation of the specificity of anti-Mi was accomplished by testing the serum with two more examples of GP.Mur cells. Differentiation between IgG and IgM antibodies was carried out by dithiothreitol (DTT) treatment of serum.[14] Titration results were scored according to the method of Marsh.[15]

Western blotting of the father's erythrocyte membranes was performed as previously described.[16] After separation of the membrane components by SDS-polyacrylamide gel electrophoresis, glycophorins A, B, and

GP.Mur-related hybrids were detected using monoclonal antibodies R1.3 and R18. The membrane components of GP.Mur, GP.Hop (MiIV), GP.Hil (MiV), or GP.Bun (MiVI) RBCs all gave positive results with R1.3, but with R18 only those from GP.Mur RBCs gave abnormal bands. The GP.Mur phenotype could, therefore, be readily ascertained. Homozygosity or heterozygosity of GP.Mur status could be identified from the immunostaining pattern, as the band corresponding to the heterodimer of GP.Mur with the α band was missing in homozygotes.[7]

Sera for IgG sub-class typing were obtained from the Central Laboratory, Netherlands, and the typing was performed in accordance with the manufacturer's instruction. GP.Mur RBCs, sensitized with maternal serum, were added to various IgG sub-class antisera at various dilutions in a V-bottom microplate, which was incubated overnight. The microplates were positioned at an angle of 60°. Sera of known IgG sub-classes from previous referrals (anti-D, -E, -JMH, and Miltenberger antibodies) were used as positive controls. RBCs remained as a discrete button if the reaction was positive, while streaming indicated a negative result.

MMA was carried out according to the methods of Nance et al.[17] and Schanfield et al.[18] The assay was performed in duplicate. The assay result was expressed as a percentage of reactive monocytes with adherent and/or phagocytosed RBCs (percentage of reactivity). The CLT was performed by Hadley.[19] The chemiluminescence response was expressed as a percentage of a positive control (PPC) comprising R_1r RBCs sensitized with 10,000 IgG1 monoclonal anti-D molecules/cell. A PPC of > 20 percent differentiated all babies with moderate or severe HDN from normal babies.

DTT treatment of serum was carried out by combining equal volumes of 0.01M (0.01 mol/L) DTT in phosphate-buffered saline (PBS) and test serum, and incubating the mixtures at 37°C for 30 minutes. PBS was used in place of DTT as the negative control.

Results

The mother's RBCs were group A, C+c+D+E+e+; Fy(a+b–); Jk(a+b+); M+N+S–s+; K–k+; Le(a+b+); and P_1+. No alloantibodies other than anti-Mi were detected in the serum. The father's RBCs were group O, C+c+D+E+e+; Fy(a+b–); Jk(a–b+); K–k+; Le(a–b–); P_1+; and of the GP.Mur phenotype. Immunoblotting with R1.3 and R18 revealed that, in addition to the normal GPA and GPB, bands both at the M_r of 38 Kd and 58 Kd, representing a GP.Mur hybrid, were detected (see Fig. 1). The father was therefore heterozygous for the *GP.Mur* gene. It was impossible to perform fetal RBC phenotyping due to the poor quality of the sample.

In tests against GP.Mur cells and father's RBCs, the maternal serum gave a 2+ reaction after incubation at room temperature for 15 minutes, and 4+ agglutination in the AGT and PEG-AGT. The presence of anti-Mi was confirmed by testing with two additional examples of GP.Mur cells. Ms. J. Poole from the International Blood Group Reference Laboratory (IBGRL), Bristol, England, also confirmed the anti-Mi specificity. DTT treatment of the maternal serum showed that the anti-Mi was predominantly IgG. The anti-Mi was found to fix complement and to have a titer of 512 with a score of 99 by AGT. The IgG sub-class typing showed that the anti-Mi was a mixture of IgG1 and IgG3.

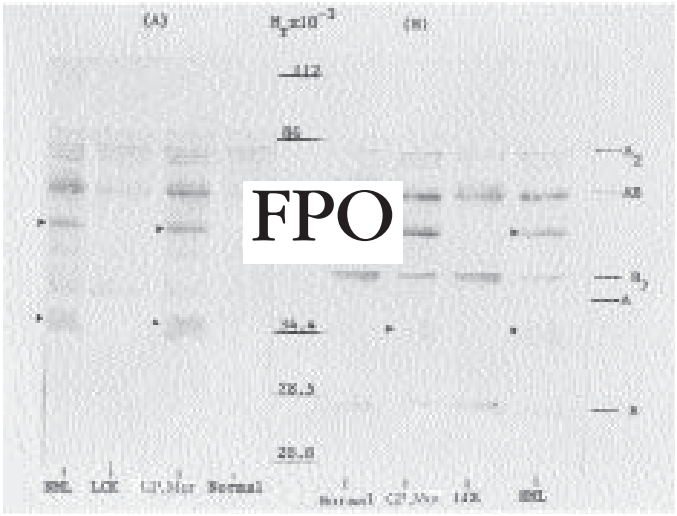

Fig. 1. Immunoblotting of red cell membranes using monoclonal antibodies R18 (Panel A) and R1.3 (Panel B).
→ GP.Mur-related glycoproteins.

The MMA reactivities of maternal serum against RBCs from the father and from a random GP.Mur donor were 87 percent and 84 percent, respectively. In the CLT the maternal serum against GP.Mur RBCs gave a response of 198 percent. Both assays thus forecast that the antibody would cause severe HDN in a child with antigen-positive RBCs.

Discussion

Although the poor quality of the fetal sample in this case rendered it impossible to perform blood group phenotyping, the serologic investigations and functional assays on the maternal serum provided supportive evi-

dence of the hemolytic potential of the anti-Mi. Other causes for fetal hydrops, such as congenital cardiac or renal abnormalities, hemoglobinopathy, chromosome abnormality, or intrauterine infections, were excluded by clinical, hematological, and virological examinations, and chromosome studies.

The maternal serum contained a potent IgG3 and IgG1 anti-Mi that fixed complement. The hemolytic potential of the anti-Mi was further borne out by the strongly positive results obtained in the functional assays in this study (MMA and CLT). The reactivity against the GP.Mur RBCs in an MMA was 87 percent. The result was significant when compared to the 21.5 percent reactivity in a case of mild HDN we had previously encountered.[20] Our findings, therefore, showed that the maternal anti-Mi was the most probable cause of the fatal fetal hydrops. The potency of the antibody in this case was undoubtedly a result of repeated immunization of the mother during some of her previous pregnancies.

Despite the rapid advances in molecular genetics that contribute to the understanding of the Miltenberger subsystem, the question of the clinical significance of anti-Mi remains unresolved. Recently, several cases of HTRs and HDN due to anti-Mi were reported by Lin and Broadberry.[6,11] There is no consensus as to whether it is necessary to include GP.Mur cells for antibody screening, since anti-Mi only occasionally causes an HTR or HDN, and most reported cases are mild or moderate.[6,11,21]

Our report documents an example of anti-Mi with strong hemolytic potential to the extent of probably causing fatal hydrops fetalis. None of the commercially available antibody screening panels currently used include GP.Mur RBCs. In addition, anti-Mi might not be detected in the immediate spin procedure of T & S and would be missed if the electronic crossmatch procedure was in use. Further evidence to support the inclusion of GP.Mur RBCs in screening panels for T & S in countries where the GP.Mur phenotype is prevalent is thereby documented. Further, anti-Mi should be considered in the investigation of HDN in these countries. A pilot antenatal screening program with GP.Mur cells is being conducted in Hong Kong to study the incidence of anti-Mi in pregnancy and its relationship to HDN.

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