A maternal warm-reactive autoantibody presenting as a positive direct antiglobulin test in a neonate

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Autoimmune hemolytic anemia in pregnancy is a rare cause of hemolytic disease of the newborn. This report describes a neonate with a mild hemolytic process and a positive direct antiglobulin test (DAT) presenting as the first manifestations of a maternal warm-reactive autoantibody. A full-term male neonate, blood group O, had a strongly positive DAT and laboratory evidence suggestive of a mild hemolytic process. The neonate's mother was also group O and had a negative antibody screen. Umbilical cord blood testing revealed a panreactive eluate though the antibody was not detected in cord serum. The neonate's mother was also found to have a positive DAT. A panagglutinin was identified in an eluate of her red cells, although the autoantibody could not be detected in her serum by a variety of sensitive techniques. There was no clinical or laboratory evidence of maternal hemolysis. ***Immunohematology*** 1997;13:6–8.

Red blood cell (RBC) autoantibodies, with or without overt evidence of hemolysis, have an incidence of approximately 1 in 200,000 in 20 to 50-year-olds.[1] However, the incidence rises to 1 in 50,000 in pregnant women.[2] The implicated autoantibody typically is warm-reactive and IgG.[3] Though these antibodies are capable of crossing the placenta and coating fetal RBCs, they rarely cause clinically evident hemolytic anemia in the neonate.[4] We report the case of a neonate presenting with a positive direct antiglobulin test (DAT) and laboratory data suggestive of a mild hemolytic process. An investigation of the neonate's DAT uncovered the first evidence of a maternal warm-reactive autoantibody.

Case Report

A full-term male neonate was born to a healthy, 23-year-old gravida 4, para 2 woman. Her clinical history was only exceptional for an ectopic pregnancy. Her antenatal course was unremarkable, and she had no history or clinical evidence of autoimmune disease. Her hemoglobin concentration at admission was 92 g/L (9.2 g/dL), and her hematocrit was 0.29 (29%). She was blood group O and had a negative prenatal antibody screen. RBCs from cord blood and heel-stick samples tested as blood group O but had a strongly positive DAT. The neonate's total bilirubin level was 121 μmol/L (7.1 mg/dL) 8 hours after delivery. The hematocrit was 0.60 (60%). Twenty-four hours after delivery, the hematocrit was 0.64 (64%), the total bilirubin was 132 μmol/L (7.7 mg/dL), and the reticulocyte count was 0.035 (3.5 %). A peripheral blood smear showed no abnormalities of erythrocyte morphology (including the presence of spherocytes), but four nucleated RBCs/100 white blood cells were identified. No RBC antibodies were detected in the cord blood sample. However, an eluate of the neonate's RBCs revealed a panagglutinin by indirect antiglobulin test (IAT). A maternal sample showed a strongly positive DAT, but no antibodies were detected in the serum by IAT. An eluate of the mother's RBCs also revealed a panagglutinin by IAT. The mother was discharged 24 hours after delivery. The neonate was released from the hospital 48 hours after delivery with a hematocrit of 0.65 (65%) and a total bilirubin of 120 μmol/L (7.0 mg/dL).

Materials and Methods

We performed all serologic testing according to the manufacturers' instructions. The DAT for the mother and the neonate initially was performed in a gel-based microtube system (ID-Micro Typing System™, Ortho Diagnostic Systems, Inc., Raritan, NJ). The DAT was repeated using a standard test tube–based agglutination method as part of an ongoing gel system validation procedure. The anti-human globulin reagent (rabbit anti-human IgG) was obtained from Ortho Diagnostic Systems, Inc. RBC eluates, obtained from cord and maternal blood samples, were prepared by acid-glycine treatment of erythrocytes (Elu-Kit® II, Gamma Biologicals, Inc., Houston, TX). The IAT was performed with cord and maternal serum and with the eluates from

cord and maternal RBCs. A variety of methods were used to provide maximal sensitivity in antibody detection: gel-based microtube agglutination, test tube–based agglutination with polyethylene glycol enhancement (PeG from Gamma Biologicals, Inc.) using untreated RBCs, test tube–based agglutination with ficin-modified RBCs (Gamma Biologicals, Inc.), and a solid-phase assay (Capture-R® Ready-ID® system, Immucor, Inc., Norcross, GA). We initially followed the manufacturers' instructions regarding the length of RBC-serum incubation (gel technique = 40 minutes; PeG = 40 minutes; solid-phase assay = 1 hour) in order to enhance antibody uptake. The strength of test tube–based agglutination reactions were determined using the Marsh scoring system.[5] We followed the manufacturers' instructions in determining the relative strengths of reactions in the gel and solid-phase formats.

Results

RBCs and eluates

Both the mother's and neonate's RBCs were group O. The neonatal DAT was strongly positive (3+) using the gel technique and maintained this strength of reactivity 48 hours after delivery. The initial DAT was microscopically positive by test tube–based agglutination but was negative 48 hours later. The eluate, prepared from umbilical cord RBCs, revealed an IgG panagglutinin with 1–2+ reactivity in gel- and test tube–based assays. The maternal DAT was also strongly reactive (2+) by the gel technique. An IgG panagglutinin was also identified in the maternal eluate using both gel- and test tube–based assays.

Serum samples

A negative IAT was obtained on the maternal serum using the gel technique. A 40-minute incubation (maximum time allowable using this technique) did not alter this result. A negative result was also obtained by standard test tube agglutination. We additionally evaluated the maternal serum by solid-phase assay with a 1-hour incubation. The cord serum also failed to react by the solid-phase technique. There was a weak (+/–) nonspecific pattern of reactivity of maternal serum against ficin-modified RBCs with both a 15-minute and 40-minute incubation. However, reactivity to all of the major clinically significant blood group antigens was ruled out.

Discussion

This case describes a positive neonatal DAT resulting from the passage of maternal autoantibody across the placenta. The autoantibody could not, however, be identified in either cord or maternal serum using a variety of extremely sensitive antibody detection techniques. This implies that the autoantibody was of relatively low titer and was effectively concentrated on antigen-positive neonatal and maternal RBCs. We have seen previous examples of warm-reactive autoantibodies in prenatal patients detectable by solid-phase assay that could not be detected by LISS- or PEG-enhanced test tube methods (unpublished observations).

In our case, the positive neonatal DAT was the first indication of a maternal autoantibody. Autoimmune diseases predominantly affect women in their childbearing years, and pregnancy is known to adversely influence the course of a variety of autoimmune disorders (e.g., systemic lupus erythematosus and myasthenia gravis).[1,2,6] Autoimmune hemolytic anemia and flare-ups of this disease are also associated with pregnancy.[7] As with most other autoimmune diseases, however, neonates are most commonly asymptomatic or develop a mild, self-limiting, and transient disorder.[6,8] This is the general rule with warm-reactive autoantibodies in pregnancy although cases of severe neonatal anemia have been reported.[3,4]

Though the affected neonate in this report had a positive DAT, there was only suggestive evidence of a mild hemolytic process (elevated total bilirubin value at birth and nucleated RBCs on the peripheral blood smear). Despite a positive DAT, there was no obvious clinical or laboratory evidence of hemolysis in the mother. She was taking no medications and had no associated history of an autoimmune disorder. It is interesting to note, however, that the detection of an autoreactive RBC antibody (with or without hemolysis) may be the presenting finding in patients who later develop overt evidence of lymphoproliferative or autoimmune disorders.[9]

The prevalence of positive DATs in pregnancy identified by the gel system is unknown. In our case, the maternal RBC autoantibody was only detected as a consequence of the need to explain a strongly positive neonatal DAT. The neonate's DAT could not be attributed to maternal-fetal ABO incompatibility or a maternal alloantibody, the most common causes of positive neonatal DATs. We were, however, sensitive to the possibility of a maternally acquired autoantibody by a similar case occurring at our institution 3 months before the case under discussion. This suggests that the

phenomenon of warm-reactive autoantibodies in pregnancy, at least in our patient population, may not be particularly uncommon.

The apparent sensitivity of the gel-based DAT may be contributing to our ability to detect low-titer autoantibodies. Although a direct comparison of the sensitivity of different antibody detection methodologies can be misleading, in our case the gel method was clearly superior to a sensitive test tube–based DAT method. In fact, this neonate's DAT was so weak in the test tube that it initially might have been read as a negative if there had not been a previous, strongly reactive gel DAT for comparison. To address the prevalence and clinical relevance of positive DATs in pregnancy, we have begun a prospective study in which DAT testing will be performed on all routine prenatal evaluations. This should help us assess the prognostic significance of a positive DAT during pregnancy in our patient population. This analysis will also benefit the general blood banking community as more institutions adopt gel-based test methodologies.

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