Anti-Holley detected in a primary immune response

V.J. BARRETT, M.M. O'BRIEN, J.J. MOULDS, P. SPRUELL, V. JACKSON, AND J.R. STUBBS

Anti-Holley (Hy) has been reported as an IgG antibody occurring in previously transfused or multiparous black patients. In this case anti-Hy was identified in a 16-year-old black, primigravida female admitted at 32 weeks gestation because of premature rupture of the membranes. On admission, her blood type was determined to be A_2B, D-positive and an antibody screen was negative. A second antibody screen, performed 4 days later, was positive in all three cells. Anti-Hy was subsequently identified. The antibody was reactive at room temperature, 37°C, and in the antiglobulin phase. IgG and IgM components of anti-Hy were demonstrated in the maternal serum, documenting a primary immune response. This resulted in serologic findings not previously described for anti-Hy. A direct antiglobulin test on the newborn red cells was negative and there was no clinical evidence of hemolytic disease of the newborn (HDN). A monocyte monolayer assay performed with maternal serum yielded negative results. Recent scientific information has resulted in the placement of Hy in the Dombrock blood group system. Alloantibodies to Dombrock system antigens have not been associated with severe HDN. ***Immunohematology*** 1996;12:62–65.

Holley (Hy) is a high-incidence antigen present on the red blood cells (RBCs) of >99.9 percent of the Caucasian population.[1] Anti-Hy was first reported in 1967 in a nontransfused, black female during her third pregnancy.[2] The newborn infant had a positive direct antiglobulin test (DAT). There was no evidence of hemolytic disease of the newborn (HDN) in her two older children. Beattie and Castillo[3] reported a hemolytic transfusion reaction caused by anti-Hy in a 46-year-old black male. Hsu et al.[4] documented decreased survival of ^{51}Cr-labeled Hy-positive RBCs in a patient with anti-Hy. A case of mild HDN due to anti-Hy was reported by Lacey, Moulds, and Sobonya.[5] The antibody has been described exclusively in black individuals who have had alloimmune exposure (i.e., pregnancy or blood transfusion). Previously, anti-Hy has been described as an IgG antibody that causes weakly positive reactions in the antiglobulin phase of serologic testing. Described here is an anti-Hy with IgG and IgM components produced as a primary immune response in a primigravida nontransfused black female. The mixture of IgG and IgM resulted in serologic findings never before described in association with anti-Hy.

Case Report

A 16-year-old, primigravida black female was admitted at 32 weeks gestation for management of premature rupture of the membranes, mild preeclampsia, and onset of premature labor. She had no prior history of transfusions and no significant previous medical history. On the day of admission she was found to be A_2B, D-positive with a negative antibody screen. Her hemoglobin was 12.7 g/dL. Keflex was administered as a prophylactic measure. On the 4th day of hospitalization, a second type and screen was ordered and results revealed an alloantibody reacting weakly with all reagent RBCs tested. Subsequently, the alloantibody was shown to be anti-Hy existing in both IgG and IgM forms. The antibody was reactive at room temperature, 37°C, and antiglobulin phases. A crossmatch of the patient's serum and the RBCs of one of her siblings was performed and found to be incompatible. The American Red Cross was contacted to supply Hy-negative units, if needed. The infant was delivered by cesarean section on the 8th hospital day and found to be A, D-positive with a negative DAT and no clinical evidence of HDN. The hemoglobin was 18.5 g/dL and the hematocrit was 55.9%. The infant was treated for suspected but unconfirmed congenital sepsis, respiratory distress, and pseudomonas conjunctivitis. Transient hypermagnesemia, hypoglycemia, and neonatal hyperbilirubinemia (15.5 mg/dL) were also present. All values returned to normal and the infant was discharged 8 days after birth. Hy typing was not done on the cord sample, and attempts to get mother and child back for further testing were unsuccessful.

Materials and Methods

Standard serologic techniques were employed for all tests performed.[6] Initial studies with a commercial three-cell screen (Gamma Biologicals, Inc., Houston, TX) and an eight-cell panel (Immucor, Inc., Norcross, GA) were performed using a low-ionic-strength additive (LO-ION, Gamma Biologicals) and monoclonal anti-IgG (Gamma Biologicals). Additional antibody screens were performed using 0.2M dithiothreitol (DTT)-treated cells and chloroquine diphosphate-treated cells. Additional anti-

body identification studies were performed using polyethylene glycol (PEG) (Gamma Biologicals), bovine albumin, papain, and ficin (American Red Cross). Overall, serologic studies included tests conducted and read at room temperature, 37°C, and by the anti-human globulin test. Antibody titration studies and an antigen profile on the patient's RBCs were performed. At Gamma Biologicals, Inc., Consultation Service, an aliquot of serum was treated with 2-mercaptoethanel (2-ME) to inactivate IgM. A monocyte monolayer assay (MMA) was performed on the patient's serum by the American Red Cross National Reference Laboratory (Rockville, MD).

Results

The patient was found to be A_2B, C+D+E–c+e+, M+N–S+s+, P_1+P+, Le(a–b+), K–k+, Kp(a–b+), Js(a–b+), Fy(a+b–), Jk(a+b–), Ge:2, Lu(b+), Vel+, Gy(a+^{w}), Hy–, Do(a–b+). The initial antibody screen performed on admission was negative. An antibody screen ordered 4 days later was found to contain anti-Hy, reactive by LO-ION, bovine albumin, PEG, ficin, and papain techniques, and with chloroquine-treated cells. The antibody was nonreactive with 0.2M DTT-treated cells. The reactivity of the antibody was weak; however, the titer was found to be 256 at a saline antiglobulin phase. The DAT on the maternal RBCs was negative. 2-ME-treated serum was reactive only at the antiglobulin phase; however, a saline diluent control was reactive from room temperature through the antiglobulin phase, demonstrating that a portion of the reactivity was due to IgM. The MMA, performed to predict clinical significance of the antibody, was found to be within the normal range of less than 3 percent using two different Hy-positive RBC samples and two different monocyte sources. Actual results ranged from 2.0 to 2.8 percent. The newborn infant's RBCs were found to be A, D-positive with a negative DAT.

Discussion

Antibodies to the Hy and Gy^a antigens have been categorized in the past as high-titer, low-avidity (HTLA). Some examples of anti-Hy (including the one described here) and anti-Gy^a have shown serologic characteristics consistent with those described for HTLA antibodies. However, anti-Hy and anti-Gy^a have also shown in vivo behavior uncharacteristic of HTLA antibodies. As previously described, anti-Hy has been shown to cause accelerated destruction of Hy-positive RBCs.[3,4] Moulds et al.[7] reported fever and chill reactions following the transfusion of incompatible RBCs to two Gy(a–) women with anti-Gy^a stimulated by pregnancy. It appears, therefore, that anti-Hy and anti-Gy^a can be clinically significant in some settings, and this potential clinical significance distinguishes these antibodies from others in the HTLA group.

Moulds et al.[7,8] provided evidence of a phenotypic link between the Hy and Gy^a antigens. They reported that the Gy(a–) phenotype observed in a white individual also typed as Hy-negative, while the Hy-negative phenotype seen in black individuals typed as Gy(a+^{w}). Spring and Reid[9] confirmed the association with immunochemical studies that showed Gy^a and Hy are carried on the same novel glycoprotein. Their results also suggested that glycosylphosphatidylinositol (GPI) provides the majority of Gy^a and Hy attachment to the RBC membrane. The findings of Telen et al.[10] further support GPI attachment of Hy and Gy^a. The high incidence Jo^a antigen was demonstrated to be phenotypically linked to Gy^a and Hy by Weaver et al.,[11] and evidence for the location of Jo^a on the Gy^a/Hy glycoprotein has been provided by Spring et al.[12] Finally, Banks, Hemming, and Poole[13] have concluded that the Dombrock antigens (Do^a and Do^b) also reside on the Gy^a/Hy glycoprotein. These authors also state that the Gy(a–), Hy-negative, Jo(a–) phenotype represents the null phenotype for the Dombrock blood group system, and they propose that the Gy^a/Hy glycoprotein be called the "Dombrock-active glycoprotein." Gy^a, Hy, and Jo^a have been formally assigned to the Dombrock blood group system by the ISBT Working Party on Terminology.[14]

This is the first reported example of anti-Hy identified during primary immunization and consisting of a mixture of IgM and IgG components. The antibody developed during the third trimester in a primigravida with no transfusion history. The patient's RBC phenotype for Dombrock system antigens was consistent with that of other reported Hy-negative individuals. The anti-Hy showed relatively weak agglutination reactions at the antiglobulin phase while displaying a titer of 256. The antibody demonstrated similar reactivity with untreated and enzyme-treated RBCs, was reactive with chloroquine-treated RBCs, and was not reactive with DTT-treated RBCs. MMA results suggested minimal risk for HDN with this first pregnancy. The above in vitro findings are consistent with characteristics of previously described examples of anti-Hy. The presence of an IgM compo-

nent in this case, however, resulted in agglutination reactions at room temperature and at 37°C. Such findings have not been previously described. Treatment of the patient's serum with 2-ME confirmed the role of the IgM component. With 2-ME treatment, serum reactivity at room temperature and 37°C was abolished while reactivity in the antiglobulin phase remained.

Anti-Hy appears to pose little risk of HDN. In this case, a negative DAT on the infant's RBCs and the negative MMA results provide serologic support for the lack of HDN. In the one documented case of mild HDN due to anti-Hy, the authors reported minimal adverse clinical effects.[5] It has been postulated that poor expression of the Hy and Gy^a antigens on the RBCs of newborns provides protection from HDN.[1] Interestingly, anti-Do^a and anti-Do^b have not been documented as causes of HDN despite full expression of the corresponding antigens on newborn RBCs.[15] Anti-Jo^a has never been reported to cause HDN. It appears, therefore, that antibodies directed toward Dombrock system antigens are of minimal risk for HDN, even in the setting of full antigen expression at birth.

In conclusion, we have detected anti-Hy in a nontransfused, primiparous black female. Because the antibody was detected during the primary immune response, there was an IgM component. Due to the presence of the IgM component, anti-Hy reactivity was noted at room temperature and at 37°C in addition to the antiglobulin phase. These findings, therefore, are not consistent with previous characterization of anti-Hy as an IgG antibody reacting only at the antiglobulin phase.[1] It is quite possible that antibodies directed against Dombrock blood group antigens are of minimal risk for HDN. However, there appears to be a risk of transfusion reaction when blood bearing Dombrock blood group antigens is transfused to patients with corresponding antibodies.[3,16,17]

Acknowledgments

The authors are grateful to the staff and supervisor of the Transfusion Service at the University of South Alabama Medical Center; to the American Red Cross Blood Services Gulf Coast Region in Mobile, AL; to the American Red Cross National Reference Laboratory in Rockville, MD; and to Gamma Biologicals, Inc., Consultation Service in Houston, TX, for technical assistance.

The authors express their appreciation to Martha Hunn and Cheryl Moyles for their clerical assistance in the preparation of this document.

References

1. Issitt PD. Applied blood group serology. 3rd ed. Miami, FL: Montgomery Scientific Publications, 1985.
2. Schmidt RP, Frank S, Baugh M. Antibodies to high incidence antigenic determinants (anti-So, anti-El, anti-Hy, and anti-DP) (abstract). Transfusion 1967;7:386.
3. Beattie KM, Castillo S. A case report of a hemolytic transfusion reaction caused by anti-Holley. Transfusion 1976;15:476–80.
4. Hsu TCS, Jagathambal K, Sabo BH, Sawitsky A. Anti-Holley (Hy): characterization of another example. Transfusion 1975;15:604–7.
5. Lacey P, Moulds M, Sobonya J. Clinical and serological observations in hemolytic disease of the newborn caused by anti-Holley (abstract). Transfusion 1978;18:379.
6. Walker RH, ed. Technical manual. 11th ed. Bethesda, MD: American Association of Blood Banks, 1993.
7. Moulds JJ, Polesky HF, Reid M, Ellisor SS. Observations on the Gy^a and Hy antigens and the antibodies that define them. Transfusion 1975;15:270–4.
8. Moulds JJ, Ellisor SS, Reid ME, Harrison M. Association of Holley-Gregory blood group systems (abstract). Transfusion 1973;13:363.
9. Spring FA, Reid ME. Evidence that the human blood group antigens Gy^a and Hy are carried on a novel glycosylphosphatidylinositol-linked erythrocyte membrane glycoprotein. Vox Sang 1991;60:53–9.
10. Telen MJ, Rosse WF, Parker CJ, Moulds MK, Moulds JJ. Evidence that several high-frequency human blood group antigens reside on phosphatidylinositol-linked erythrocyte membrane proteins. Blood 1990;75:1404–7.
11. Weaver T, Kavitsky D, Carty L, et al. An association between the Jo^a and Hy phenotypes (abstract). Transfusion 1984;24:426.
12. Spring FA, Reid ME, Nicholson G. Evidence for expression of the Jo^a blood group antigen on the Gy^a/Hy-active glycoprotein. Vox Sang 1994;66:72–7.
13. Banks JA, Hemming N, Poole J. Evidence that the Gy^a, Hy and Jo^a antigens belong to the Dombrock blood group system. Vox Sang 1995;68:177–82.
14. Daniels GL, Moulds JJ, Anstee DJ, et al. ISBT working party on terminology for red cell surface antigens. Vox Sang 1993;65:77–80.
15. Salmon C, Cartron J, Rouger P. The human blood groups. New York: Masson Publishing USA, Inc., 1984.
16. Halverson G, Shanahan E, Santiago I, et al. The first reported case of anti-Do^b causing an acute hemolytic transfusion reaction. Vox Sang 1994;66:206–9.
17. Banks JA, Parker N, Poole J. Evidence to show that Dombrock (Do) antigens reside on the Gy^a/Hy glycoprotein. Transfus Med 1992;2:68.

Vicki J. Barrett, MS, MT(ASCP)SBB, Assistant Professor and Clinical Director, University of South Alabama, Department of Medical Technology, 1504 Springhill Avenue, Room 2309, Mobile, AL 36604-3273; M. Margaret O'Brien, MD, Department of Pathology, University of South Alabama Medical Center, Mobile, AL; John J Moulds, MT(ASCP)SBB, President and Chief Operations Officer, Gamma Biologicals, Inc., Houston, TX; Peggy Spruell,

MT(ASCP)SBB, Consultation and Education, Gamma Biologicals, Inc.; Valerie Jackson, MT(ASCP)SBB, Assistant Director, Reference and Consultation, American Red Cross Blood Services, Gulf Coast Region, Mobile, AL; James R. Stubbs, MD, Director, Transfusion Service, Department of Pathology, University of South Alabama Medical Center, Mobile, AL.

If you have any questions concerning the *Immunohematology Journal of Blood Group Serology and Education,* the *Immunohematology Methods and Procedures* manual, or the National Reference Laboratory for Blood Group Serology, including the Rare Donor Registry, please contact Delores Mallory by phone at (215) 451-4905 or by fax at (215) 451-2538 Monday and Tuesday, or by phone at (301) 738-0706 or by fax at (301) 738-0704 Wednesday through Friday. To contact Ms. Mallory on the Internet, address your correspondence to the following: mallory@usa.redcross.org

Attention SBB and BB Students: You are eligible for a free one-year subscription to *Immunohematology*. Ask your education supervisor to submit the name and complete address for each student, and the inclusive dates of the training period to *Immunohematology*, Musser Blood Center, 700 Spring Garden Street, Philadelphia, PA 19123-3594.