Case report: moderate hemolytic disease of the newborn due to anti-G

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The only previously published case of anti-G in a pregnant woman indicated that anti-G alone caused little, if any, fetal or neonatal hemolysis. This report describes an affected fetus with amnionitic fluid OD 450 absorbance values in the moderate zone of the Liley prediction graph who required prolonged phototherapy after birth until day of life 20. Anti-G was identified and anti-C and -D excluded in the mother's serum. In contrast to the previous report, this report shows anti-G alone can cause moderate HDN and that fetal monitoring and treatment may be necessary. ***Immunohematology* 2006;22:166–170.**

Key Words: hemolytic disease, newborn, anti-G, anti-C, anti-D

Antibodies to the antigens in the Rh system are well-known causes of HDN. The D antigen is a very potent immunogen and anti-D can cause severe HDN. Anti-C has also been shown to cause HDN, although generally less severe. First elucidated by Allen and Tippett in 1958, the G antigen is present on almost all D+ or C+ RBCs and absent from virtually all RBCs that are D– and C–.[1] The apparent codistribution of the G antigen with either the C or D antigen causes anti-G to appear serologically as anti-C plus anti-D activity.[1] Issitt and Tessel were the first to use a serial double-elution procedure using D+C– RBCs followed by D–C+ RBCs to identify the presence of anti-G in approximately 30 percent of sera with anti-C+D activity.[2] Case series have shown that anti-D is absent in 10 of 22 (45%)[3] and 2 of 7 (30%)[4] of alloimmunized pregnant women with apparent anti-D plus anti-C. Although it appears that a significant proportion of apparent anti-D plus anti-C is because of the presence of anti-G, the significance of an apparent titer of anti-D actually due to anti-G in predicting the severity of HDN is not known. The only previously published case of a pregnant woman with anti-G having a titer of 16 against R_2R_2 RBCs suggested that anti-G alone had caused practically no fetal or neonatal hemolysis.[5] We report a case of moderate HDN because of anti-G alone with a titer of 8 against R_2R_2 RBCs where increased amniotic fluid OD 450 nm absorbance values in the moderate zone of the Liley prediction graph and normal middle cerebral artery Doppler studies were followed by postpartum hyperbilirubinemia, anemia, and the requirement for prolonged phototherapy until day of life 20.

Case Report

A 31-year-old group O, rr (dce/dce), gravida 5 pregnant woman was referred to the maternal-fetal medicine department at our facility at 17 weeks, 6 days gestational age because of a history of recurrent spontaneous abortions. Prenatal antibody screening test results from the referring hospital had revealed anti-C and anti-D with titers of 64 and 32, respectively. The patient had received Rh immunoglobulin (RhIG) prophylaxis after all procedures and abortions except for a spontaneous abortion at 6 weeks' gestation before the current pregnancy. She denied ever receiving a blood transfusion. Because of her history of multiple pregnancy losses and a positive antinuclear antibody screen, the patient was placed on prophylactic subcutaneous heparin. Fetal middle cerebral artery peak systolic velocity (MCA PSV), measured by ultrasound Doppler at 21 weeks', 6 days' gestation, was below the median value for gestational age, suggesting no significant fetal anemia. Given these findings, amniocentesis was not performed and the patient was instructed to return in three weeks. The patient returned at 25 weeks, 1 day gestational age, at which time it was noted that fetal growth lagged established dating by two weeks. However, MCA PSV was still without evidence of fetal anemia and there was no

evidence of hydrops by ultrasound. The patient was offered amniocentesis to rule out chromosomal anomaly as the etiology for fetal growth restriction. Amniotic fluid was also analyzed for ΔOD_{450} which was 0.120. This value is in the lower portion of the affected zone of OD_{450} curve by Queenan,[6] which is valid for pregnancies earlier than 27 weeks' gestation. These results suggest that the fetus was affected with HDN but was not severely anemic. Serial amniocentesis at 28, 30, and 32 weeks all returned with ΔOD_{450} values in the low-affected zone of the Queenan chart and low zone 2 of the Liley chart while MCA PSV values also remained within the normal range, suggesting no worsening fetal hemolysis or anemia. The fetus was also followed for asymmetric intrauterine growth restriction by serial ultrasound. The decision was made to deliver the fetus by Caesarean section at 33 weeks' gestational age secondary to oligohydramnios with an amniotic fluid index of 3.4 cm (normal 5–25 cm) and absent end-diastolic flow in the umbilical artery on Doppler ultrasound. A viable male infant weighing 1300 g with APGAR scores of 8 and 9, whose RBCs typed as group O, D+C+E–c+e+, was delivered. The physical exam showed no ascites or peripheral edema. A peripheral serum sample collected on day 1 of life showed a bilirubin of 3.6 mg/dL (1.0–10.5 mg/dL), Hb of 15.7 g/dL (13.5–19.5 g/dL), and Hct of 47.3% (42–60%). Phototherapy was initiated with a preliminary diagnosis of moderate HDN. While on phototherapy, the bilirubin would decrease, maintaining levels below 10 mg/dL. However, when phototherapy was discontinued, the bilirubin would increase, reaching a maximum of 18.1 g/dL on day of life 13. The direct bilirubin level was consistently between 0.0 and 0.1 mg/dL, supporting an absence of obstructive liver disease. On day of life 20, phototherapy was stopped and the bilirubin remained stable at 16.5 to 16.7 mg/dL over the course of six days until it started to decrease. The Hb and Hct continually declined to values of 8.8 gm/dL (10.0–18.0 gm/dL) and 25.7% (31–55%), respectively, on day of life 30. The neonate received no exchange transfusions or RBC transfusions. At a follow-up visit at age 3 months, the infant's Hb and Hct were 11.5 g/dL and 34.6%, respectively and the infant was not jaundiced. The mother received RhIG prophylaxis before discharge.

Materials and Methods

A peripartum maternal serum sample was used to determine antibody specificity and titers. The specificity testing was performed at the American Red Cross Blood Services, Greater Chesapeake and Potomac Region reference laboratory. The serum was first tested to determine initial reactivity to R_1R_1 (DCe/DCe) commercial reagent RBCs and to exclude other clinically significant antibodies by both LISS- and PEG-AHG, according to the manufacturer's protocol (Panocell and Gamma PEG, ImmucorGamma, Houston, TX; ORTHO Antibody Enhancement Solution, Ortho-Clinical Diagnostics, Raritan, NJ). The sample was tested against rare r^Gr RBCs. The patient's serum was then adsorbed onto ficin (Sigma-Aldrich, St. Louis, MO)-treated r′r′ (dCe/dCe) donor RBCs five times to adsorb anti-C, anti-G, or both to exhaustion. The absorbed serum was tested against two R_0r (Dce/dce) reagent RBCs. The eluate prepared from the first adsorbing aliquot of r′r′ (dCe/dCe) RBCs was adsorbed onto ficin-treated R_2R_2 (DcE/DcE) donor RBCs to adsorb out anti-G, if present (Gamma ELU-KIT II, Gamma Biologicals, Inc., Houston, TX). The absorbed eluate was tested against two r′r′ (dCe/dCe) reagent RBCs. Titrations were performed on the anti-C and anti-D using r′r′ (dCe/dCe) and R_2R_2 (DcE/DcE) reagent RBCs (Immucor) and a saline-AHG method with 1-hour incubation at 37°C.

The DAT (Anti-IgG, Murine Monoclonal Gamma-Clone, ImmucorGamma, Norcross, GA) was performed on the infant's cord blood sample that was manually washed 6 times. The eluate was tested against an antibody identification panel (Panocell–16, Immucor) to identify anti-D and anti-C activity and to exclude other clinically significant antibodies.

ABO and Rh typing of RBC samples from the mother and infant were performed with monoclonal reagents according to manufacturers' instructions (Immucor and Gamma Biologicals, Inc.).

Results

Testing of the patient's serum and of the eluate from the infant's cord blood sample with selected panel RBCs revealed reactivity with R_1R_1 (DCe/DCe) RBCs and excluded other clinically significant antibodies. Testing with r^Gr, D–G+ RBCs was strongly reactive. After antibody adsorption from the patient's serum with ficin-treated r′r′ (dCe/dCe) donor RBCs, reactivity against R_0r (Dce/dce) RBCs was not detected, excluding the presence of anti-D. An eluate prepared from the r′r′ (dCe/dCe) adsorbing RBCs showed no reactivity with r′r′ (dCe/dCe) RBCs after adsorption of anti-G with ficin-treated R_2R_2 (DcE/DcE) RBCs, thereby excluding the presence of anti-C. Using this double-

elution procedure and eliminating contributions from anti-C or anti-D through serial adsorptions, it was shown that anti-G alone was responsible for the reactivity with R_1R_1 (DCe/DCe) RBCs and r^Gr RBCs (Table 1). The anti-G titer with r′r′ (dCe/dCe) RBCs was 16; the titer with R_2R_2 (DcE/DcE) RBCs was 8. The DAT of the infant's RBCs was 3+ and the eluate demonstrated 3+ reactivity with R_1R_1 (DCe/DCe), r′r (dCe/dce), and r′r′ (dCe/dCe) RBCs, 2+ reactivity with R_0r (Dce/dce) and R_2R_2 (DcE/DcE) reagent RBCs and no reactivity with seven different r″r (dcE/dce) and rr (dce/dce) reagent RBCs.

Table 1. Adsorption and elution procedure

Sample	Panel RBCs	Results	Conclusion
Peripartum maternal serum sample	R_1R_1 (DCe/DCe)	Strongly reactive	Anti-C, D, or G present
Peripartum maternal serum sample	r^Gr	Strongly reactive	Anti-G present (+/– Anti-C or D)
Absorbed serum, after adsorption onto ficin-treated r′r′ (dCe/dCe) RBCs to adsorb anti-C, anti-G, or both	R_0r (Dce/dce)	Nonreactive	Anti-D excluded
Eluate from r′r′ (dCe/dCe) adsorbing RBCs, after adsorption onto ficin-treated R_2R_2 (DcE/DcE) RBCs to adsorb anti-G	r′r′ (dCe/dCe)	Nonreactive	Anti-C excluded

Discussion

Most cases of HDN associated with anti-G have been in association with anti-D, anti-C or both.[4,5,7–9] Cash and colleagues[5] described the first case where anti-G alone was identified as the cause of a positive DAT in an infant which resulted in the absence of clinical evidence of HDN. In their case report, the newborn did not require transfusion and the bilirubin reached a peak of 11.9 mg/dL at day of life 4. Although the presence of anti-C was not definitively excluded in the mother, the newborn's RBCs were C–. The titer of the antibody was 64 against r′r RBCs and 16 against R_2R_2 RBCs. They concluded that anti-G alone without anti-C, anti-D, or both may not be sufficient to cause severe HDN and suggested the question of whether identification of anti-G and exclusion of anti-C and anti-D could indicate a benign clinical course and alter clinical management such that amniocentesis would not be indicated.

The clinical significance of anti-G remains controversial. Palfi et al.[10] identified anti-G+C in 4 of 27 samples in their study of alloimmunized pregnant women, none of which caused severe HDN. They proposed that low concentration of antibody, the occurrence of IgM antibodies, or both, were possible explanations for this finding. In agreement with the case reported by Cash et al.,[5] they concluded that anti-G+C alloimmunization may have a decreased risk of HDN. This has been disputed by others. Hadley et al.[7] reported a case of severe HDN due to anti-G+C in a D– infant with anemia who required multiple exchange transfusions. The levels and functional activities of both anti-G and anti-C were evaluated with the IAT, the chemiluminescence test (CLT), and flow-cytometric techniques. They found that 6 of 7 anti-G-containing eluates bound higher levels of IgG anti-G to r′r (C+) RBCs than to R_2r (D+) RBCs, which paralleled their results using flow-cytometric analysis. Using the CLT, they found the response of r′r (C+) RBCs sensitized with an eluate containing anti-G (69%) to be consistent with severe HDN. However, r′r (C+) RBCs sensitized with anti-C after adsorption with R_2R_2 RBCs showed a weak CL response that was not consistent with HDN. They also tested 28 serum samples from alloimmunized pregnant women with over 5 IU/mL anti-C+D with 2 of 28 containing levels of anti-G that were consistent with moderate to severe HDN by the CLT. They concluded that anti-G may cause moderate to severe HDN in those women with greater than 5 IU/mL anti-C+D (approximately 7%) and that HDN caused by anti-G is probably not rare. Similarly Lenkiewicz et al.[8] reported a case of moderate HDN due to anti-G+C in a D–C+ newborn with hyperbilirubinemia requiring phototherapy. The levels and functional activities of both anti-G and anti-C were evaluated with the IAT and the CLT. Their results showed both the level and the functional activity of anti-G to be greater than those of anti-C. They concluded that anti-G, and not anti-C, was responsible for the moderate HDN and that anti-G should be regarded as clinically significant in the alloimmunized pregnant woman.

We report a second case of HDN caused solely by anti-G but in an infant who expressed both the C and D antigens on his RBCs. By using a serial double-elution procedure, we identified anti-G alone as the sole antibody present. Differential adsorption techniques were performed, excluding the possibility of a concomitant anti-D or anti-C contributing to the hemolysis. In our case, hyperbilirubinemia requiring a

prolonged stay in the hospital for phototherapy argues that anti-G alone is sufficient to cause at least a moderate HDN in an infant that expresses both the D and C antigens on his RBCs. However, it would seem that differentiation of anti-G from anti-D and anti-C would not be relevant for decisions of patient monitoring.

Our case suggests that differentiating anti-D plus anti-C from anti-G may not be relevant for the purpose of suggesting an indication for amniocentesis given the increasing availability of MCA PSV to predict fetal anemia. Recent articles have suggested that MCA PSV may be superior to amniotic fluid ΔOD_{450} for the diagnosis of fetal anemia in cases of RBC alloimmunization in the hands of an experienced ultrasonographer[11,12] and many centers have now replaced serial amniocentesis using ΔOD_{450} with serial MCA Doppler. In our case, despite the fact that the patient was referred to our institution with an anti-D titer of 32 and anti-C titer of 64, which would be considered critical if performed in our lab, a decision was made to not perform amniocentesis because of the reassuring MCA PSV results. Only later, when the patient had an additional indication for amniocentesis, was the ΔOD_{450} measurement made. The patient was then followed for the remainder of her pregnancy with both MCA PSV and amniocentesis, the results of which indicated a stable level of hemolysis without the development of significant anemia.

Despite the noninvasiveness and increased availability of MCA PSV, current recommendations still do not consider use of this modality until a critical titer is reached. Thus, titration of the antibodies is a critical step, although, in cases of anti-D plus anti-C, the critical titer is not known. Complicating this situation is the fact that, although review articles would make one tend to believe that the situation with anti-D is well established where defined titers (32[12] or 8 to 32[13]) are recommended and standard methodology is recommended (use of R_2R_2 reagent RBCs[13]), laboratories are still reporting widely discrepant results as our case illustrates (referral laboratory titer result of 64 vs. our result of 8). Further complicating the situation with anti-D plus anti-C is the variation in the phenotype of the RBCs used to determine and compare the anti-D and anti-C titers (R_2R_2 and r′r,[5,7,13] R_2R_2 and R_1R_1,[3] R_0r and r′r RBCs[2,4,9,10]). The recommendations of the subcommittee of the Scientific Section Coordinating Committee of the AABB recommended the use of r′r and R_2R_2 RBCs when comparing the titer of anti-C with anti-D,[13] although we used r′r′ RBCs and R_2R_2 RBCs in the interest of comparing RBCs that would have more comparable (homozygous) expression of antigens. In commenting on the comparability of our anti-D titer result to other labs, we think it is relevant that we have been participating in the College of American Pathologists Survey of anti-D titers and our results have been consistent with the largest peer groups. Therefore, we think our titer result would likely be consistent with most labs that participated in this survey. Hopefully, increased participation in titer proficiency surveys will improve the consistency of titer results among laboratories and facilitate comparison of titer results in case studies.

Finally, although we do not feel that identifying anti-G and excluding anti-D and anti-C has relevance in deciding whether or not to nonserologically monitor a pregnancy for HDN, especially with the availability of MCA PSV, we agree with the recommendations of Shirey et al.[4] that identifying or excluding anti-D is relevant. One study has indicated that although anti-G seems to mask the antigenic sites of C and D, it does not prevent the eventual development of anti-D, meaning that RhIG would be indicated in a patient with anti-G but without anti-D.[9] Also, there are medicolegal reasons for the exclusion of anti-D, such as excluding questions of paternity for a D– couple, avoiding inadequate prophylaxis with RhIG, and avoiding confusion regarding previous transfusion history as anti-G and anti-C can be found in recipients of D– products.[9,10]

References

1. Allen FH, Tippett PA. A new Rh blood type which reveals the Rh antigen G. Vox Sang 1958;3:321-30.
2. Issitt PD, Tessel JA. On the incidence of antibodies to the Rh antigens G, rh_i (Ce), C and CG in sera containing anti-CD or anti-C. Transfusion 1981; 21:412-8.
3. Maley M, Babb R, Chapman CE, Fitzgerald J, Cavanagh G. Identification and quantification of anti-D, -C and -G in alloimmunized pregnant women. Transfus Med 2001;11:443-6.
4. Shirey RS, Mirabella DC, Lumadue JA, Ness PM. Differentiation of anti-D, -C, and -G: clinical relevance in alloimmunized pregnancies. Transfusion 1997;37:493-6.
5. Cash K, Brown T, Strupp A, Uehlinger J. Anti-G in a pregnant patient. Transfusion 1999;39:531-3.

6. Queenan JT, Tomai TP, Ural SH, King JC. Deviation in amniotic fluid optical density at a wavelength of 450 nm in Rh-immunized pregnancies from 14 to 40 weeks' gestation: a proposal for clinical management. Am J Obstet Gynecol 1993 May;168(5):1370-6.
7. Hadley AG, Poole GD, Poole J, Anderson NA, Robson M. Haemolytic disease of the newborn due to anti-G. Vox Sang 1996;71:108-12.
8. Lenkiewicz B, Zupanska B. Clinical significance of anti-G. Transfus Med 2002;12:221.
9. Yesus YW, Akther JE. Hemolytic disease of the newborn due to anti-C and anti-G masquerading as anti-D. Am J Clin Pathol 1985;84:769-72.
10. Palfi M, Gunnarsson D. The frequency of anti-C + anti-G in the absence of anti-D in alloimmunized pregnancies. Transfus Med 2001;11:207-10.
11. Bullock R, Martin WL, Coomarasamy A, Kilby MD. Prediction of fetal anemia in pregnancies with red-cell alloimmunization: comparison of middle cerebral artery peak systolic velocity and amniotic fluid OD 450. Ultrasound Obstet Gynecol 2005; 25:331-4.
12. Moise, KJ. Red blood cell alloimmunization in pregnancy. Semin Hematol 2005;45:169-78.
13. Judd WJ. Practice guidelines for prenatal and perinatal immunohematology, revisited. Transfusion 2001;41:1445-52.

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