Delayed Hemolytic Transfusion Reaction Due to Anti-Js^a in an Alloimmunized Patient With a Sickle Cell Syndrome

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Delayed hemolytic transfusion reactions occur via an anamnestic immune response in patients previously alloimmunized by certain RBC antigens. Conventional pretransfusion antibody screening tests and crossmatches are unable to detect certain antibodies that potentially can cause these reactions because they may be present in low concentrations or have low affinity for their respective antigen or their indicator antigen may be absent from test RBCs. We report the second case of a delayed hemolytic transfusion reaction caused by an undetectable (by routine methods) anti-Js^a in a patient with a sickle cell syndrome (hemoglobin SC disease) and multiple alloantibodies, in whom retrospective indirect antiglobulin tests enhanced by polyethylene glycol revealed the presence of weakly reactive anti-Js^a. (Key words: Anti-Js^a; Transfusion reaction; Sickle cell; Alloimmunization) Am J Clin Pathol 1997;108:658–661.

Delayed hemolytic transfusion reactions (DHTRs) are an uncommon sporadic complication of transfusion. Such reactions result from a secondary immune response in a previously transfused or pregnant patient in whom an alloantibody has developed, which may be below detectable levels at the time of pretransfusion testing. Further difficulties in antibody detection may be encountered when the antibody is directed against a low-frequency RBC antigen, absent from screening RBCs and present in only a small percentage of random blood donors. The most common antibodies implicated in DHTRs are those directed against antigens in the Rh, Kell, Kidd, and Duffy systems.[1] The Kell system is complex, containing more than 20 different antigens.[2,3]Antibodies to some of these antigens have been implicated in severe hemolytic reactions and hemolytic disease of the newborn.[4,5] Anti-Js^a (K6), an antibody to a low-frequency antigen (< 1% of whites, 19.5% of blacks), was first described by Giblett in 1958.[6] That case report involved a previously transfused white patient with metastatic carcinoma. Subsequently, review of the scientific literature has revealed seven additional cases of anti-Js^a, including three cases of alloimmunization after transfusion, two cases of hemolytic disease of the newborn, and one case each of naturally occurring anti-Js^a and anti-Js^a DHTR.[7–13] To our knowledge, we report the second case of DHTR due to anti-Js^a, occurring in a patient with a sickle cell syndrome and multiple RBC alloantibodies.

CASE REPORT

The patient was a 62-year-old black woman (gravida 4, para 3) with hemoglobin SC disease and a history of previous RBC transfusions. In December 1995, the patient underwent elective parathyroidectomy for primary hyperparathyroidism due to parathyroid hyperplasia. In addition, a small focus of papillary carcinoma of the thyroid was found for which she underwent total elective thyroidectomy in June 1996.

For the latter procedure, a preoperative immunohematologic evaluation revealed the patient's RBCs to be A, Rh-positive (R_1r), a negative direct antiglobulin test (DAT), and a positive antibody screening test with anti-E, anti-M, anti-Fy^a and anti-Jk^b identified in the patient's serum. The CBC showed a total WBC count of 5,800/μL (5.8 × 10^9/L); hemoglobin, 9.8 g/dL (98 g/L); hematocrit, 29.6% (0.30); mean corpuscular volume, 75 cu μm (75 fL); and platelets, 157 × 10^3/μL (157 × 10^9/L). Hemoglobin electrophoresis revealed 51% hemoglobin S and 49% hemoglobin C.

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Serum chemistry tests revealed a mild indirect hyperbilirubinemia (total bilirubin, 1.4 mg/dL [24 μmol/L]; direct bilirubin, 0.2 mg/dL [4 μmol/L]) and an elevated lactate dehydrogenase (LDH) 212 U/L. To prepare this patient for the thyroidectomy, it was recommended, based on current literature,[14] that she undergo a simple partial manual exchange transfusion to remove one unit and replace it with two units of RBCs. This exchange was performed without incident using two units of crossmatch-compatible, group O RBCs that were negative for E, M, Fy^a, and Jk^b antigens. Values for the hemoglobin, hematocrit, and hemoglobin electrophoresis following the exchange are given in the Table.

While the patient was at home after discharge from the outpatient transfusion center, approximately 5 hours after transfusion, she experienced fever up to 101°F (38.6°C). She did not seek medical attention for this fever, and this episode was not reported to her physicians. The thyroidectomy was performed without complication 4 days after the RBC exchange transfusion. The patient did not require transfusion during this period. The day after the thyroidectomy (5 days after the exchange), fevers up to 103.5°F (40.0°C) developed that were associated with back and left upper quadrant abdominal pain. A urinalysis revealed dark brown urine (3+ dipstick for "blood") with no RBCs seen on microscopic examination; urobilinogen was elevated, at 2 U. Peripheral blood smears showed marked target cells, occasional sickle cells, and moderate anisocytosis. Supporting laboratory data for hemolysis included a marked indirect hyperbilirubinemia (total bilirubin 4.5 mg/dL [76 μmol/L]; direct bilirubin 0.5 mg/dL [8 μmol/L]), LDH, 965 U/L; and a reticulocyte count of 2.2% (.022). An immunohematologic workup revealed a positive DAT (weak) due to IgG. One of the units transfused to the patient at the time of partial exchange was found to be strongly incompatible with this posttransfusion specimen at the antiglobulin test phase. Antibody identification revealed all previous antibodies and a newly detected anti-Js^a. An eluate prepared from the patient's postexchange (day 9) RBCs also showed anti-Js^a. A polyethylene glycol (PEG)-enhanced crossmatch performed retrospectively on the pretransfusion specimen revealed weak incompatibility at the antiglobulin test phase with RBCs from the incompatible donor unit used for exchange transfusion, further confirming the presence of anti-Js^a. Since discharge on postoperative day 3 (postexchange transfusion day 9), the patient's clinical course has been unremarkable.

MATERIALS AND METHODS

Serologic tests were performed in accordance with standard methods.[15] Antibody screening tests, crossmatches, and antibody identification used low-ionic-strength solution (LISS; Gamma N-Hance, Gamma Biologicals, Houston, Tex) incubated for 10 minutes followed by an indirect antiglobulin test using anti-IgG (Gamma-Clone, Gamma Biologicals). Polyethylene glycol

COMPARISON OF LABORATORY RESULTS BEFORE AND AFTER THE EXCHANGE TRANSFUSION

Variable	*Before*	*Immediately After*	*5–9 Days*
Temperature, °F (°C)	98.2 (37.1)	101 (38.6), at 5 hours	103.5 (40.0), at 5 days
Urinalysis			
Hemoglobin	Negative	Not done	3+
Urobilinogen, U	0.1	Not done	2
Hemoglobin, g/dL (g/L)	9.8 (98)	11.9 (119)	10.5 (105)
Hematocrit, %	29.6 (0.30)	36.6 (0.37)	31 (0.31)
Hemoglobin electrophoresis, %			
S	51	37	Not done
C	49	33	Not done
A	Not done	29	Not done
Bilirubin, mg/dL (μmol/L)			
Total	1.4 (24)	Not done	4.5 (76), day 5
Direct	0.2 (4)	Not done	0.5 (8), day 5
Serum lactate dehydrogenase, U/L	212	Not done	965, day 5
Antibody Screen	Anti-E, anti-M, anti-Fy^a, anti-Jk^b	Not done	Anti-E, -M, -Fy^a, -Jk^b, -Js^a, day 9
Direct antiglobulin test	Negative	Not done	Positive (weak), day 9
Postexchange eluate of patient's RBCs			Anti-Js^a, day 9

(Gamma Peg, Gamma Biologicals) was used as a supplementary, more sensitive technique. Eluates were prepared using a LISS-wash-acid procedure (Elu-Kit II, Gamma Biologicals). For antibody identification, serum and eluates were evaluated using a panel of reagent RBCs at the antiglobulin phase using polyspecific anti-IgG , -C3d.

DISCUSSION

Delayed hemolytic transfusion reactions involve an anamnestic antibody response and commonly occur 5 to 7 days after a transfusion.[1] Because clinical symptoms may be absent or unnoticed, diagnosis often rests on laboratory determination of antibody-mediated hemolysis, which classically includes a positive DAT and antibody screen associated with an elevated LDH and reticulocyte count and the appearance of indirect hyperbilirubinemia and hemoglobinuria. Furthermore, the patient described in this report was clinically symptomatic with fever and severe back and left upper quadrant abdominal pain. Ness et al[16] have differentiated DHTRs from delayed serologic reactions. The positive DAT, eluate results, and supporting laboratory values are the key serologic tests that implicate a serologic reaction, while correlated clinical symptoms in the patient support a hemolytic reaction. Laboratory data obtained from this patient 5 days after the exchange supported a diagnosis of DHTR. Interestingly, this patient experienced a fever of 101°F (38.6°C) within 5 hours of exchange. Although we suspect that this febrile episode was due to transfusion of a unit of Js(a+) RBCs, laboratory data implicating an immediate hemolytic event were not obtained, therefore, this fever could not be distinguished from one associated with a febrile nonhemolytic transfusion reaction.

The transfusion history of the patient described was thoroughly evaluated. During the period from 1956 to 1971, she received a total of seven units of crossmatch-compatible RBCs. These transfusion episodes included two units of RBCs in 1956 and 1961 following vaginal delivery of viable infants, one unit of RBCs in 1961 following tubal ligation, and two units of RBCs in 1971 following a therapeutic abortion and total abdominal hysterectomy. The patient recalls no symptoms associated with these transfusions. Before 1995, this patient's alloimmunization history is undocumented. She did not receive transfusions again until December 1995, when the four alloantibodies were first detected, and she received two units of E-negative, Fy^a-negative, Jk^b-negative, crossmatch-compatible RBCs before a parathyroidectomy. The patient's posttransfusion course was unremarkable.

Patients with sickle cell disease have high rates of alloimmunization to RBC antigens. However, the best method to select blood for their support remains controversial. A common approach is to transfuse blood from the random donor population until alloimmunization occurs and then provide RBCs that are negative for antigens for which alloantibodies have developed.[17] Another approach is to provide extended phenotypically matched RBCs prospectively to all patients with sickle cell disease.[18,19] Still another alternative is to provide blood from randomly selected black blood donors to all nonalloimmunized patients with sickle cell disease without regard to prospective phenotype matching.[20] By using the latter two approaches, alloimmunization to antigens such as C, E, Fy^a, and Jk^b likely will not occur, or they will occur less frequently; this is not the case with Js^a, an antigen found predominantly in the black population (19.5% of blacks and < 1% of whites).[1,21] These relative frequencies are significant because our donor population is made up of more than 90% whites and less than 10% blacks. Thus, the Js^a antigen is essentially absent from RBCs in our blood supply, as well as from virtually all reagent screening cells. This latter point is particularly important, because many transfusion service standard operating procedures call for an immediate-spin crossmatch when a negative antiglobulin antibody screen result is obtained and there is no history of alloimmunization. Therefore, low-frequency antibodies, such as anti-Js^a, would be missed.

The presence of certain antigens on RBCs relative to donor race is notable in this case. Our patient required RBCs that were negative for E, M, Fy^a, and Jk^b. Such an RBC phenotype has a likelihood of being present in 12.1% of black donors but only 1.5% of white donors.[1,20] Thus, by selecting such a phenotype, there is more than an eightfold likelihood that blood would come from a black donor. Consequently, by purposely selecting multiply antigen-negative RBCs for our patient, it is likely that we matched our patient with a black donor. This donor selection bias may be important because of the increased prevalence of certain relatively low-frequency antigens in the black population, such as V (30%) and Js^a (19.5%), which are rare (< 1%) in whites and could lead to alloimmunization.[20] According to Sosler et al,[20] this increased prevalence of V and Js^a does not negate the benefit gained from intraracial transfusion because these antigens are of lower immunogenicity.

In this case, anti-Js^a was not detected using a LISS antibody screen, but was retrospectively detected using the PEG-enhanced technique. Lin et al[22] reviewed two cases of severe hemolytic reactions in two persons due

to anti-E and anti-C, respectively. As in the present case, the units of RBCs given to these patients were compatible on conventional crossmatch but were shown retrospectively to be incompatible by using PEG-enhanced techniques. While enhanced antibody detection methods, such as LISS, are commonly used in transfusion services, PEG and proteolytic enzymes are sometimes used as supplementary techniques. Unfortunately, as evidenced in a review of 10,000 patients by Issitt et al[23] enzyme-enhanced antibody screening often results in the detection of clinically insignificant (cold and warm autoimmune) antibodies, which unnecessarily increases the cost of transfusion. Shirey et al[24] have shown that PEG has a false-positive rate of 1.3% compared with 0.1% for LISS, but that this disadvantage is balanced by the ability of PEG to be more sensitive in the detection of clinically significant antibodies.[24] Considering the relative ease and low cost of PEG-enhanced compatibility tests (antibody screens and crossmatches) and the possibility of preventing hemolytic reactions in patients such as the patient described, laboratorians might consider this type of enhanced testing for the pretransfusion specimens of patients who are already multiply alloimmunized (ie, are "immune responders") and who require repeated RBC support.

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