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The First Reported Case of Anti-Do^b Causing an Acute Hemolytic Transfusion Reaction

G. Halverson[a]
E. Shanahan[a]
I. Santiago[a]
R. Mabile[a]
T. Thurrell[a]
A. M. Strupp[a]
C. F. W. Wolf[a]
P. Spruell[b]
M. K. Moulds[b]

[a] New York Hospital-Cornell Medical Center, New York, N.Y., USA;
[b] Gamma Biologicals, Houston, Tex., USA

Abstract

The antibodies of the Dombrock blood group system have only rarely been encountered in transfusion practice, and anti-Do^b has not previously been implicated in an acute hemolytic transfusion reaction. We have encountered the first such case involving a chronically transfused black female with hemoglobin SS disease and multiple antibodies in her serum. During a previous admission for sickle cell crisis, the patient received 3 units of compatible blood with no untoward effects. Serum obtained 21 days later contained, in addition to the known antibodies, anti-S plus an unidentified antibody showing characteristics of HTLA. Blood lacking the E, K1, Fy(a), Jk(b) and S antigens was obtained, and 2 least incompatible units were transfused. While administering the second unit, the patient complained of fever and low back pain, and hemoglobinemia was detected. Anti-Do^b was identified in the post-reaction samples by absorption-elution tests, and the patient was confirmed to be Do(a+b–). The first unit transfused during this hemolytic episode tested Do (b+). This case, and a similar case involving anti-Do^a reported in 1986, strengthens the belief that Dombrock antibodies are clinically significant and illustrates the need for their differentiation, prior to transfusion from less clinically significant HTLA antibodies.

Introduction

Acute hemolytic transfusion reactions due to an incompatibility other than ABO are rare, and it has never been reported in a patient with anti-Do^b [1–3]. In this case, the occurrence of several additional alloantibodies made the identification of anti-Do^b much more difficult. This case study describes several techniques that were useful in identifying the cause of the hemolytic reaction and underscores the need for caution when transfusing to a multiply immunized recipient with weakly incompatible blood.

Case Report

A 40-year-old black female with sickle cell disease had a long history of multiple hospital admissions and red cell transfusions for treatment of sickle cell crisis. Anti-E, -K, -Fy^a and -Jk^b had previously been identified in her serum. The patient's direct antiglobulin test (DAT) was negative when last tested with polyspecific antihuman globulin serum.

During hospital admission on April 9, 1989, she was transfused 3 units of antigen-negative, crossmatch-compatible blood with no reported untoward reactions. The patient's hemoglobin rose from 7.0 g/dl before transfusion to 10.1 g/dl on April 11, 1989, when she was discharged. She was readmitted on April 21, 1989, with a hemoglobin

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G. Halverson, BS, MT(ASCP)SBB, DLM
Blood Bank and Transfusion Service, F-540
New York Hospital-Cornell Medical Center
525 E. 68th Street

of 8.8 g/dl and was experiencing symptoms of fever, productive cough and pain consistent with sickle cell crisis. The patient's hemoglobin continued to decline, and by April 24, 1989, it had returned to the pretransfusion value of 7.0 g/dl. A graph of her hemoglobin values over the course of time is shown in figure 1.

Blood samples were sent to the blood bank for compatibility testing. A new antibody, anti-S, was identified in her serum. One of the 3 units transfused on April 9, 1989, tested S+. Another antibody showing characteristics of HTLA was suspected due to a high serum *titer* of 256. The DAT was now weakly positive with both IgG and C3d coating the RBCs. Although no longer in sickle cell crisis, the patient's hemoglobin continued to drop to 6.1 g/dl, and transfusions became necessary. Because it was felt that the additional serum reactivity was probably due to high-titered HTLA antibody(ies) alone, units of E-, K1-, Fy(a)-, Jk(b)- and S-antigen-negative blood were crossmatched, and the 2 least incompatible units were transfused.

Transfusions were started on April 28, 1989. The first unit was transfused over a 4-hour time period with no reported signs or symptoms of a transfusion reaction. Six hours after the first transfusion had been begun, the second transfusion was started. During this infusion the patient experienced vomiting, fever to 38 °C, chills and lower back pain. The posttransfusion serum sample was hemolyzed, and dark urine was noted. The patient's hemoglobin rose initially to 8.0 g/dl but rapidly declined over the next 36 h to the pretransfusion level of 6.1 g/dl. The patient did not receive any additional units of blood and was discharged several days later.

She was readmitted 2 years later in sickle cell crisis with a hemoglobin level of 6.4 g/dl. Although transfusions were considered, the patient did not receive any blood. She received erythropoietin and recovered sufficiently to be discharged without any further treatment.

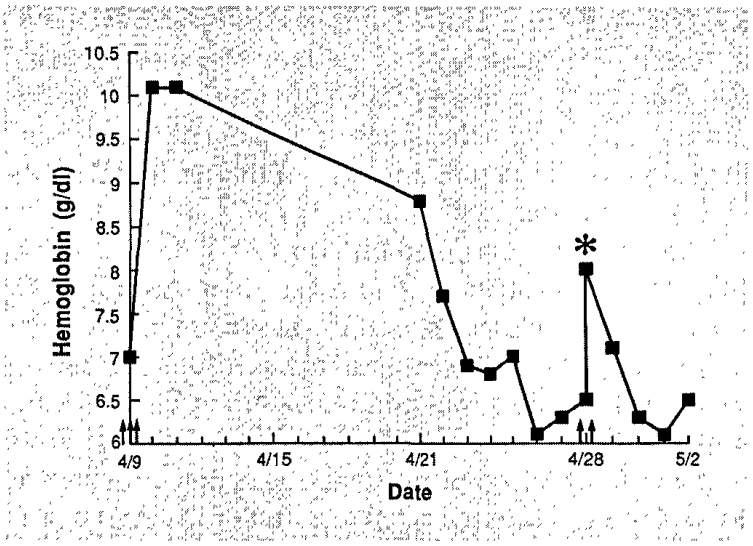

Fig. 1. Hemoglobin values over time during hospital admission. Transfusions are indicated with arrows. The asterisk indicates the acute hemolytic transfusion reaction.

Methods and Materials

All initial testing was done using standard methods. Additional studies (rapid acid eluate, Gamma Biologicals, Houston, Tex., USA; antibody absorption-elution studies, dithiothreitol, DTT, denaturation and monocyte monolayer assays) were performed using standard procedures [4].

Preparation of Autologous Cells by Hypotonic Saline Wash

EDTA-anticoagulated red cells were washed 4–6 times with 0.3% (hypotonic) NaCl solution. Samples were centrifuged at 1,000 *g* for 1 min. The hypotonic washes were followed by 2 consecutive washes with 0.9% NaCl with centrifugation after each wash at 200 *g* for 2 min [5]. This method has been shown to hemolyze red cells containing normal chains of hemoglobin A (AA or AS RBCs). Red cells that are homozygous (SS) are resistant to hemolysis.

Results

The patient's prereaction sample from April 27, 1989, and immediate postreaction blood samples were evaluated. The postreaction sample was markedly hemolyzed, the DAT was positive, showing mixed field appearance with anti-C3d, and was negative for monospecific IgG. Elution studies were nonreactive on both samples before and after reaction.

Since she was a sickle cell patient, the prereaction EDTA sample drawn on April 27, 1989, was used in a hypotonic wash procedure that selectively hemolyzes red cells with normal hemoglobin A to obtain autologous RBCs (SS hemoglobin) free of the previously transfused donor RBCs [5]. The patient's phenotype is group 0, Rh: 1, 2, –3, 4, 5, K: –1, Js(b+) Fy(a–b+), Jk(a+b–), M+s+, Do(a+b–), Le(a–b+), Cr(a+), Hy(a+), At(a+), Lu (b+), Sl(a–), JMH+, Jo(a+). The harvested autologous RBCs typed Do(b–) as did the sample when left unseparated, thus indicating that the previously transfused Do (b+) cells from 3 units given on April 9, 1989, were no longer present. Due to the severity of the transfusion reaction, a rapid acid eluate was prepared from the available (pretransfusion only) EDTA sample. Only one reagent RBC was microscopically reactive, and ficin-pretreated reagent RBCs did not help to reveal any antibody specificity. Since this patient's serum was reactive with eight E–, K–, Jk(b–), S–, Fy(a–), Sl(a–) reagent RBCs, and the patient's own RBCs typed Do(b–), absorption and elution studies were performed on the prereaction serum sample drawn on April 27, 1989. A single absorption was performed for 60 min at 37 °C using equal amounts of patient's serum untreated RBCs. The phenotype of the absorbing cell was rr, K–, Jk(a+b–), MNss, Fy(a–b–), Do(a–b+) and Sl(a–). A rapid acid eluate prepared from this absorbing RBC demonstrated a anti-Do^b. The anti-Do^b reacted equally with untreated and ficin-pretreated cells. No E–,

K–, Jk(b–), Fy (a–), Do(b–), Sl(a–) RBCs, were available to enable direct testing of the patients's neat serum sample. One Do(b–), Sl(a+) RBC tested was found to be weakly reactive with the patient's serum. Polyspecific antihuman globulin serum was used in this testing. Results are shown in table 1.

The first unit transfused on April 28, 1989, tested Do (b+) as did all 3 units she had received on April 9, 1989. The second unit administered during the reported reaction on April 28, 1989, was found to be Do(b–). A titration study on both units was completed using the pre- and postreaction serum samples for comparison. The first unit reacted with the pre- and postreaction samples to a titer of 1,024. The second unit reacted to a titer of 32 with the pre- and 16 with the postreaction samples.

Two Do(b+) RBCs were tested against the patient's serum sample. Both cells were negative for E, K, Fy^a, Jk^b and S antigens. These RBCs reacted 2+ and 3+, respectively, using a saline antiglobulin technique. These same cells, when DTT treated, reacted 1+ only. It is possible that this residual reactivity was due to anti-Sl^a, which is not denatured by DTT treatment. A previous report describing the survival of Sl^a-positive RBCs in a patient with anti-Sl^a showed no decrease in survival [6].

Reagent RBCs were sensitized with the patient's serum in the presence and absence of fresh normal serum as a source of complement. These sensitized RBCs were washed and tested in the monocyte monolayer assay. Results, shown in table 2, are expressed as percent reactivity. The Do(b+) sensitized RBCs were positive at 53% alone and 61% with fresh normal serum. The Do(b–) sensitized RBCs were positive for Jk^b and S and gave a 52% positive monocyte monolayer assay. A negative control red cell lacking Jk^b, S and Sl^a was not available for testing. These results do not permit an assessment of the effectiveness of destruction by anti-Do^b alone.

Table 1. Anti-Do^b identified in absorption-elution studies (sample date April 27, 1989)

	RBCs tested							Neat serum[1] (LISS AGT)	Eluate[1] (polyspecific AGT)
	E	K	Fy^a	Jk^b	S	Do^b	Sl^a		
1	0	0	0	0	0	+	+	1+	n.t.
2	0	0	0	0	0	+	0	1+ s	2+
3	0	0	0	0	0	+	w+	1+ s	2+
4	0	0	0	0	0	+	0	1+ s	2+
5	0	0	0	0	0	+	0	1+ s	2+
6	0	0	0	0	0	0	+	w+	negative

LISS AGT = Antiglobulin test; n.t. = not tested; w = weak, s = strong.
[1] Scale from 1 to 4.

Table 2. Monocyte monolayer assay (date of specimen May 4, 1989)

RBC phenotype	AGT	MMA
Do(a–b+), E–, K–, S–, Fy(a–), Jk(b–)	3+	53%
Do(a–b+), E–, K–, S–, Fy(a–), Jk(b–) + FNS	3+	61%
Do(a+b–), E–, K–, S+s+, Fy(a–), Jk(a+b+)	2+	52%

AGT = Antiglobulin test; MMA = monocyte monolayer assay; FNS = fresh normal serum.

Discussion

Antibodies of the Dombrock system are rarely reported, and are more often found in patients with multiple antibody specificities [1, 2, 7]. Anti-Do^a was first described by Swanson et al. in 1965, and antithetical anti-Do^b was first described by Molthan et al. [1] in 1973. Approximately 66% of Northern Europeans are Do (a+), and 82% are Do(b+) [8]. The Do(a+) phenotype is reported to be lower in blacks while the Do(b+) phenotype is higher [1], although no calculated phenotype frequencies for blacks could be found in the literature. The reactivity of Dombrock antibodies was reported to be eliminated by the use of 0.2 *M* DTT by Greene et al. [7] in 1989.

The first case of anti-Do^b causing a delayed reaction and significant RBC destruction was reported by Moheng et al. [2] in 1985. The first report of an acute transfusion reaction due to a Dombrock system antibody (Do^a) was reported by Kruskall et al. [3] in 1986. In that report the authors had suspected that a nonneutralizable HTLA antibody (titer 64) was present.

We believed at first that only an insignificant, high-titer antibody was present in this patient's sample, but it is now known that anti-Do^b was also present and was probably responsible for the acute hemolytic event observed.

Together, these reports confirm that Dombrock system antibodies can cause significant RBC destruction and underscores the need to differentiate these antibodies from the clinically insignificant HTLA antibodies prior to transfusion.

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