Acute hemolytic transfusion reaction caused by anti-Co^a

R.B. Covin, K.S. Evans, R. Olshock, and H.W. Thompson

Co^a is a high-frequency blood group antigen in the Colton blood group system expressed on red blood cells (RBCs) of approximately 99.8 percent of random persons. Anti-Co^a has been reported to cause delayed hemolytic transfusion reactions, hemolytic disease of the newborn, and accelerated clearance of RBCs in vivo. Acute hemolytic transfusion reactions (AHTRs) have not previously been reported. A 58-year-old man was hospitalized for vascular surgery. Initial blood bank evaluation revealed anti-Fy^a. The patient received six units of RBCs during his initial hospitalization and developed anti-E. A subsequent sample was sent to the reference laboratory when all units of RBCs appeared incompatible. Additional studies, including alloadsorptions, revealed the presence of anti-E, anti-Fy^a, and an apparent warm autoantibody. One unit of least-incompatible RBCs was transfused during surgery. The patient had an increase in temperature. Hemoglobinuria and a decrease in hematocrit were also noted. Due to the clinical impression of an AHTR, the pre- and postreaction samples were re-evaluated in the reference laboratory and demonstrated the presence of anti-Co^a in both. Based on clinical and laboratory evaluation this patient appears to have had an AHTR due to anti-Co^a. This is the first known reported case of an AHTR caused by anti-Co^a.
***Immunohematology* 2001;17:45–49.**

Key Words: blood groups, transfusion reaction, hemolysis, anti-Co^a, Colton A (Co^a) antigen

Co^a is a high-frequency blood group antigen expressed on red blood cells (RBCs) of 99.8 percent of Caucasians and may be more frequent in other ethnic-racial groups.[1] The Colton protein has been identified as aquaporin 1, a red cell water-selective transport protein that regulates water homeostasis.[2] The first examples of anti-Co^a were described by Heisto et al. in 1967.[3] Anti-Co^a has been reported to cause delayed hemolytic transfusion reactions,[4] hemolytic disease of the newborn,[5] and accelerated clearance of RBCs in an in vivo survival study.[6] Acute hemolytic transfusion reactions (AHTRs) due to anti-Co^a have not previously been reported. The case presented here concerns a patient who developed an apparent AHTR due to anti-Co^a. We believe that this case is the first reported case of an AHTR caused by anti-Co^a.

Case Report

A 58-year-old group A, D+ man with longstanding venous insufficiency was admitted to the hospital on February 18, 2000 for left leg valve transplantation. His medical history was significant for a 38-year history of pain, edema, and discomfort in his left lower extremity due to venous insufficiency, ulcers, and thromboses. He had received multiple red cell transfusions during surgery for ulcer excisions followed by skin grafts, multiple nerve blocks, and a sympathectomy. He had a history of moderate-to-severe aortic stenosis and of gastrointestinal bleeding in 1993. At that time he was also noted to have anti-Fy^a. Medications on admission included oxycodone, amitriptyline, zolpidem, and warfarin. Admission hemoglobin was 11.9 g/dL. Initial blood bank evaluation confirmed anti-Fy^a. The patient's RBC phenotype was reported as Fy (a–), Jk (a), and S–. The patient underwent a left lower extremity venous valve transplant on February 18, 2000. A left deep venous thrombosis necessitating heparin therapy complicated the patient's postoperative course.

Subsequently, the patient developed left thigh and popliteal hematomas that were evacuated on February 28, 2000. During this phase of the patient's hospitalization, and prior to this second operation, the patient received 6 units of RBCs. An anti-E was detected for the first time. A type and crossmatch just prior to the patient's second surgery revealed no compatible units and the specimen was sent to the reference laboratory. Additional studies including alloadsorptions revealed the presence of anti-E, -Fy^a, a cold autoantibody, and an apparent warm autoantibody. Further phenotyping revealed that the patients RBCs also were E– and K–. Eight units of least-incompatible RBCs were sent to the patient's hospital. During the second surgery, the patient was transfused with one of the units and the other seven were returned. The transfused unit was E–, Fy (a–), K–, and Jk (a–). In the postanesthesia recovery unit, within 60 minutes from the start of the transfusion, the patient was noted to develop rigors, increased temperature, and hemoglobinuria. The hematocrit was also noted to have decreased. There was no clinical evidence of bleeding

to account for the decline in hematocrit. Due to the clinical impression of an AHTR, the pre- and immediate postreaction samples were re-evaluated in the reference laboratory. A panel of RBCs to test for the presence of antibodies to high-frequency antigens demonstrated anti-Co^a. A posttransfusion sample revealed a new anti-Jk^a. The patient's RBCs were Co(a–) and Jk(a–).

Materials and Methods

Polyspecific anti-IgG, -C3d, murine monoclonal anti-IgG (Gamma Biologicals, Houston, TX) and murine monoclonal anti-C3b, C3d (Ortho-Clinical Diagnostics, Raritan, NJ) were used for the direct antiglobulin test (DAT). Testing was performed at immediate spin and after a 10-minute incubation.

The following monoclonal reagents were used for the initial reference laboratory RBC phenotyping: anti-A, -B, -D, -C, -c, -E, and -e (Ortho); the following indirect antiglobulin reagents were used: anti-K, -Fy^a, -S (Ortho) and -Jk^a (Gamma). The patient's ABO serum grouping was performed using A_1 and B cells (Ortho).

Initial panels used in the reference laboratory investigation included select cells from Panocell 16 (Lot # 09280, Immucor, Norcross, Georgia) and Panel One (Lot numbers 0222 and 0118, Gamma). Panels were read at immediate spin, followed by the addition of two drops of N-Hance (Gamma). Each tube was incubated at 37°C for 10 minutes, followed by centrifugation and reading. Each tube was washed x 4 with saline, two drops of anti-IgG (Gamma) were added, followed by centrifugation and reading. Negative antihuman globulin reactions were checked using Coombs Control Cells (Ortho).

A cold autoantibody, one of the suspected antibodies, interfered with the patient's ABO serum grouping. The test was repeated and resolved using two drops of the patient's serum plus A_1 and B cells, and O cord cells. A prewarmed panel was performed, using the same cells as the original select cell panel. The following were warmed separately at 37°C for 5 to 10 minutes: one drop of each panel cell to be tested, enough N-Hance to add two drops to each tube, and the patient's serum. Two drops of prewarmed serum and N-Hance were added to each panel cell tube. All tubes were incubated at 37°C for 10 minutes. All tubes were washed x 4 with warm saline, followed by the addition of two drops of anti-IgG (Gamma) to each tube.

An acid eluate was performed using Elu-Kit II (Gamma). As a control, the last wash was tested against two selectogen cells (Ortho). Polyethylene glycol (PEG) adsorptions were performed to remove the apparent warm autoantibody from the serum. Raw PeG (Sigma Chemicals, St. Louis, MO) was obtained and prepared as a reagent for use in our laboratory using the following method: 20 grams of PEG dissolved into 100 mL of phosphate-buffered saline. Three different donor's cells (Bonfils Blood Center, Denver, CO) lacking the following antigens were used to perform the adsorptions:

- Donor 1 (O, D+) lacking E, c, Fy^a, Jk^b, and K antigens
- Donor 2 (O, D+) lacking C, E, Fy^a, Fy^b, Jk^b, K, and S antigens
- Donor 3 (O, D–) lacking E, Fy^a, Jk^a, K, and s antigens

The cells from the three donors were tightly packed, with as much donor plasma as possible removed. Equal volumes of packed adsorbing RBCs, PEG, and patient's serum were incubated at 37°C for 15 minutes. Following centrifugation, the adsorbed serum was harvested and tested against a select cell panel from the initial panels used in the investigation.

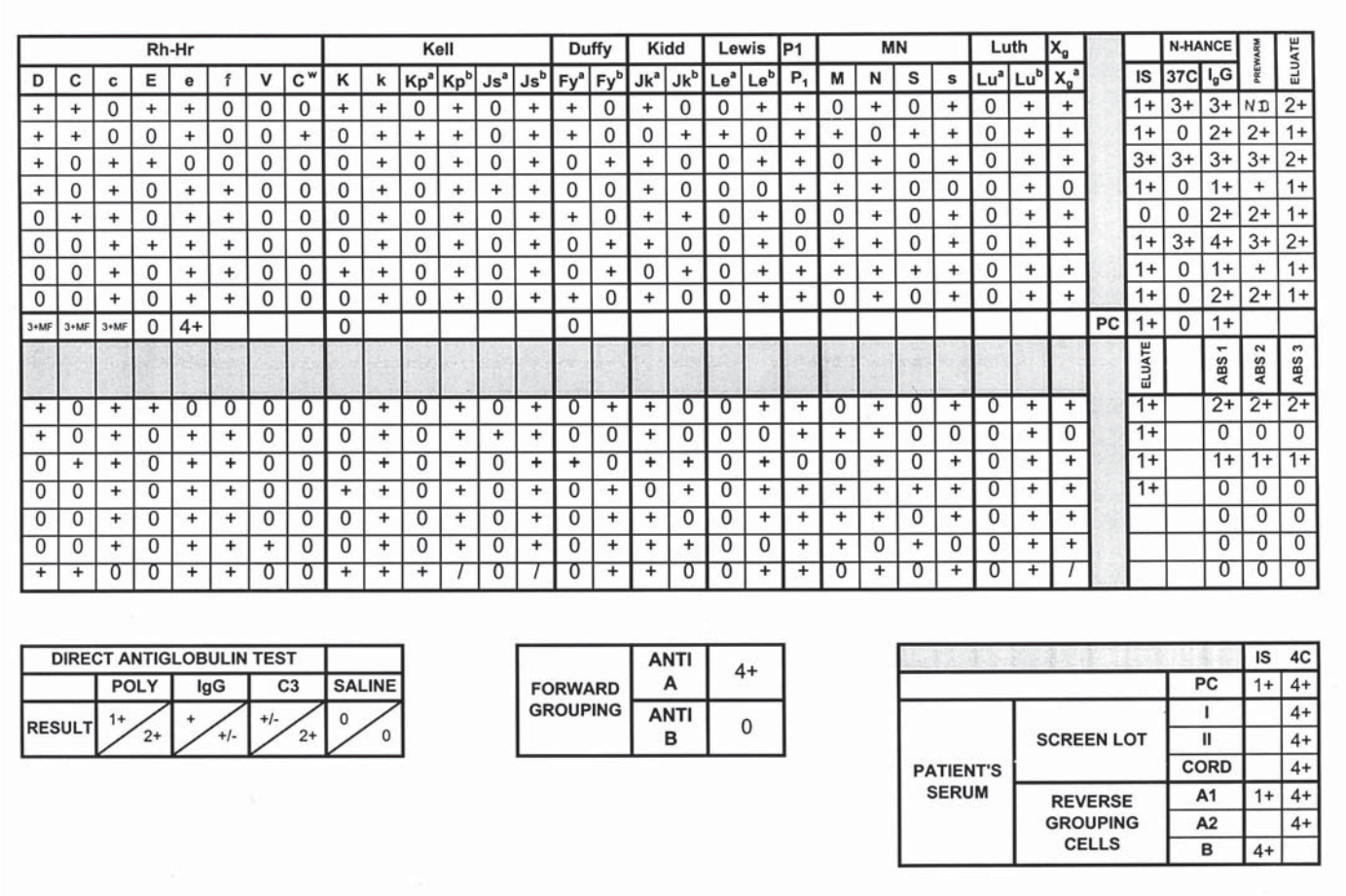
Upon report of the suspected transfusion reaction, the patient's pretransfusion serum was tested with the following cells: one Rh_{null} / Fy (a+), one Rh_{null} / Fy (a–), three E– / Fy (a–), and one Co (a–). The pretransfusion eluate was also tested against the Rh_{null}/Fy (a–) cell. The patient's RBCs were further phenotyped for the following antigens: Vel, Kp^b, I, Co^a, Lu^b, Yt^a, PP_1P^k, Ge, and LAN. A select cell panel was run using methods already described, testing at least one cell that was negative for each of the high-frequency antigens listed above. Seven more Co(a–), E–, Fy (a–) RBCs were tested with the patient's serum. Six of these cells were Jk (a+). All rare cells and antisera were obtained from either the Serum, Cells, and Rare Fluids (SCARF) program or Bonfils Blood Center donors.

Results

Pretransfusion antibody study

As the patient's type and crossmatch prior to his second surgery revealed no compatible units, the specimen was sent to the reference laboratory for further evaluation. This evaluation revealed an anti-E reactive at 37°C with N-Hance and anti-Fy^a plus -E at the antiglobulin test using anti-IgG. A cold autoantibody at immediate spin (IS) and an apparent warm autoantibody were also present. The serologic evaluation is shown in Figure 1. All negative anti-human globulin reactions showed agglutination with check cells.

An eluate showed a panagglutinin at IS. At the antiglobulin test, anti-E plus a panagglutin was present and the adsorbed serum contained only anti-E plus -Fy^a (Fig. 1).



Rh-Hr								Kell						Duffy		Kidd		Lewis		P1	MN				Luth		X_g		N-HANCE		PREWARM	ELUATE
D	C	c	E	e	f	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	$X_g{}^a$		IS	37C	I_gG	
+	+	0	+	+	0	0	0	+	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	+		1+	3+	3+	ND	2+
+	+	0	0	+	0	0	+	0	+	+	+	0	+	+	0	0	+	+	0	+	+	0	+	+	0	+	+		1+	0	2+	2+	1+
+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+	+		3+	3+	3+	3+	2+
+	0	+	0	+	+	0	0	0	+	0	+	+	+	0	0	+	0	0	0	+	+	+	0	0	0	+	0		1+	0	1+	+	1+
0	+	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	0	+	0	+	+		0	0	2+	2+	1+
0	0	+	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	+		1+	3+	4+	3+	2+
0	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	+		1+	0	1+	+	1+
0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	+		1+	0	2+	2+	1+
3+MF	3+MF	3+MF	0	4+				0						0														PC	1+	0	1+		
																													ELUATE		ABS 1	ABS 2	ABS 3
+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+	+		1+		2+	2+	2+
+	0	+	0	+	+	0	0	0	+	0	+	+	+	0	0	+	0	0	0	+	+	+	0	0	0	+	0		1+		0	0	0
0	+	+	0	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	0	0	+	0	+	0	+	+		1+		1+	1+	1+
0	0	+	0	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	+		1+		0	0	0
0	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	+	+	0	+	0	+	+				0	0	0
0	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	+	0	0	+	+				0	0	0
+	+	0	0	+	+	0	0	+	+	+	/	0	/	0	+	+	0	0	+	+	0	+	0	+	0	+	/				0	0	0

DIRECT ANTIGLOBULIN TEST				
	POLY	IgG	C3	SALINE
RESULT	1+ / 2+	+ / +/-	+/- / 2+	0 / 0

FORWARD GROUPING	ANTI A	4+
	ANTI B	0

			IS	4C
		PC	1+	4+
PATIENT'S SERUM	SCREEN LOT	I		4+
		II		4+
		CORD		4+
	REVERSE GROUPING CELLS	A1	1+	4+
		A2		4+
		B	4+	

Fig. 1. Pretransfusion evaluation performed by reference laboratory. Antibodies identified included anti-E, anti-Fy^a, and "cold" and "warm" autoantibodies.

Transfusion reaction clinical, laboratory, and serologic evaluation

Following transfusion of one of the units of RBCs, the patient developed signs and symptoms suggestive of an AHTR. The results of the hospital's transfusion reaction evaluation are shown in Table 1. The sample was drawn within 3 hours from the time of the suspected AHTR. Most notable were the laboratory values and an increase in the strength of the DAT.

Identification of anti-Co^a

Due to the AHTR, the reference laboratory re-evaluated the patient's pretransfusion sample in an attempt to identify an antibody to a high-frequency antigen. The results of this evaluation are shown in Figure 2. An antibody to Co^a by LISS IgG was identified. All negative anti-human globulin reactions showed

Table 1. Vital signs, laboratory, and blood bank evaluation pre- and post-AHTR*

	Pre	Post	Unit
Vital signs			
Temperature (C)	38.4	39.2	
Blood pressure (mmHg)	155/95	150/95	
Pulse	115	105	
Respirations	16	16	
Laboratory evaluation			
Hematocrit (%)	22.3	18.3	
Creatinine (mg/dL)	1.1	1.8	
Bilirubin (mg/dL)	—	3.9	
Urinalysis	—	hemoglobinuria	
Blood bank evaluation			
ABO and Rh	A+	A+	A+
Serum	normal	icteric	normal
DAT (IgG)	+/–	$1+^w$	
Clerical check	OK	OK	

*Acute hemolytic transfusion reaction

Rh-Hr								Kell						Duffy		Kidd		Lewis		P1	MN				Luth		Co		
D	C	c	E	e	f	V	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Co^a		LISS/IgG
+	+	+	0	+				0						0	+	0	+	0	+	+	0	+	0	+			+		3+
+	+	+	+	+				0						0	+	+	0				+	+	0	+			+		3+
0	0	+	0	+			0	+	+	0		0		0	+	0	+	0	+	+	+	0	0	+			0		0
+	+	+	0	+			0	0	+	0		0		0	+	+	0	0	+	0	+	0	+	0			0		0
+	+	0	0					0	+					0	+	+	0	0	+		+	+	0	+			0		0
0	0	+	0	+				+	+					0	+	0	+	0	+	+	+	0	0	+			0		0
+	+	+	0	+			0	0	+	0		0		0	+	+	0	0	0	+	0	+	0	+			0		0
+	+	0	0	+			0	0	+					0	+	+	0	0	+	0	+	+	+	+			0		0
0	0	+	0	+				0						0	+	+	+	0	+	0	+	+	0	+			0		0
Patient's Cells																													
			0					0						0		0							0				0		

Fig. 2. Re-evaluation performed by reference laboratory on the pretransfusion sample following the report of an AHTR. Anti-Co^a was identified. Patient's phenotype was Co (a–).

agglutination with check cells. Phenotyping of the patient's RBCs revealed the patient was Co (a–).

In further testing, Rh_{null} / Fy(a+) cells tested 2+ positive, whereas Rh_{null} / Fy (a–) cells tested ± and antibodies to Vel, Kp^b, I, Lu^b, Yt^a, PP_1P^k, Ge, and LAN were ruled out. A posttransfusion sample, in addition to anti-Co^a, -E, and -Fy^a, also revealed the presence of an anti-Jk^a.

Discussion

An AHTR is defined as the rapid destruction of RBCs that occurs during or shortly after a blood transfusion (< 24 hours). The most common cause of AHTR is transfusion of ABO-incompatible RBCs with formation of antigen-antibody complexes that induce activation of the complement cascade and lead to intravascular hemolysis. Most often, these antibodies are IgM and are naturally acquired; however, antibodies may be IgG and can be acquired due to a previous pregnancy or blood transfusion. The clinical features of an AHTR are variable and depend on the quantity of incompatible RBCs transfused, the type of antibody and its antigen specificity, the thermal amplitude of the antibody, complement activation, and the clinical condition of the patient. The most frequent presentation is fever (greater than a 1°C rise in temperature) with or without chills. Other signs and symptoms include hemoglobinemia, hemoglobinuria, hypotension, chest pain, dyspnea, flushing, oliguria or anuria, and disseminated intravascular coagulation. Laboratory diagnosis usually includes a decline in hematocrit, hemoglobinemia, hemoglobinuria, and a positive DAT that may show a mixed-field appearance. An unconjugated hyperbilirubinemia, abnormal coagulation studies, and worsening renal function may also be identified.[7] Our patient developed an increased temperature (although not greater than 1°C), a decrease in hematocrit, hemoglobinuria, and increased strength of the DAT consistent with the clinical impression of an AHTR. Re-evaluation of the patient's serum samples identified an antibody to the high-frequency antigen Co^a and a new anti-Jk^a. Phenotyping of the patients RBCs revealed the patient to be Co (a–). Because Co (a–), Jk (a+) cells did not show agglutination during repeat testing of the pretransfusion sample, we feel that it is highly likely that the transfusion reaction was caused by anti-Co^a. In addition, the reaction was most consistent with a preformed antibody and the anti-Jk^a was identified only after the transfusion reaction. The only Jk (a+) units that were transfused were given 1 month prior to the reaction and this length of time is not consistent with a delayed transfusion reaction. Our patient did not receive any further transfusions during his hospital stay so we cannot document an increase in hematocrit and a lack of reaction to a transfusion of Co(a–) RBCs.

The differential diagnosis for an AHTR includes thermal destruction of RBCs by heating and cooling devices, concomitant administration of drugs or

nonisotonic fluids, bacterial contamination, or a hemolytic condition within the recipient (e.g., autoimmune hemolytic anemia).[7] In our patient, no other causes for the AHTR were identified.

This case describes what we believe to be the first reported case of an AHTR caused by anti-Co^a. Previous reports have implicated anti-Co^a in delayed hemolytic transfusion reactions[4] and hemolytic disease of the newborn.[5] In addition, accelerated clearance of Co(a+) RBCs has been identified in patients with anti-Co^a during in vivo survival studies.[3,6] In their initial report, Heisto et al.[3] described a shortened T_{50} when Co(a+) RBCs were injected into a patient with anti-Co^a. These findings were later confirmed by Kurtz et al.[6] who found a T_{50} of only 5 minutes in a 56-year-old G3P2 woman with a history of four previous transfusions. She was identified as having anti-Co^a and rapidly cleared ^{51}Cr-labeled Co(a+) RBCs within 24 hours. The authors suggested that Co(a–b+) RBCs be used for transfusion in patients with anti-Co^a. Although anti-Co^a is most often IgG, some of these antibodies have been shown to bind complement and examples of IgM antibodies have been reported.[6] Even though Co^a has not previously been reported to cause an AHTR, Lee and Bennett[8] reported a case of a 74-year-old man who developed an AHTR due to anti-Co^b.

In our case, the anti-Co^a was initially diagnosed as a warm autoantibody (WAA) due to its reactivity with all test cells. The differentiation between a WAA and a high-frequency antigen can be difficult. Cash et al.[9] reported similar difficulty with a 60-year-old female who was initially suspected to have a warm autoantibody. After transfusion of two units of RBCs, she developed a delayed hemolytic transfusion reaction. The antibody was subsequently identified as anti-At^a.

We believe the clinical presentation and laboratory evaluation is consistent with an AHTR due to anti-Co^a.

References

1. Issitt PD, Anstee DJ. Applied blood group serology. 4th ed. Montgomery Scientific Publishers, 1998.
2. Mudad R, Telen MJ. Biologic functions of blood group antigens. Curr Opinion Hematol 1996; 3: 473–9.
3. Heisto H, Van Der Hart M, Grethe M, et al. Three examples of a new red cell antibody, anti-Co^a. Vox Sang 1967; 12:18–24.
4. Montanaro-Kitzke H, Julius H, Delaney M, Studnicka L, Landmark J. Anti-Co^a implicated in delayed hemolytic transfusion reaction. Transfusion 1982; 22:407.
5. Simpson WKH. Anti-Co^a and severe haemolytic disease of the newborn. S Afr Med J 1973; 47: 1302–4.
6. Kurtz SR, Kuszaj T, Ouellet R, Valeri CR. Survival of homozygous Co^a (Colton) red cells in a patient with anti-Co^a. Vox Sang 1982; 43:28–30.
7. Jeter EK, Spivey MA. Noninfectious complications of blood transfusion. Hematol Oncol Clin North Am 1995; 9:187–204.
8. Lee EL, Bennett C. Anti-Co^b causing acute hemolytic transfusion reaction. Transfusion 1982; 22:159–60.
9. Cash KT, Brown T, Sausais L, Uehlinger J, Reed, LJ. Severe delayed hemolytic transfusion reaction secondary to anti-At(a). Transfusion 1999; 39:834–7.

Randal B. Covin, MD, University of Colorado Health Sciences Center, Department of Pathology, Denver, Colorado; Karen S. Evans, MT(ASCP)SBB, Bonfils Blood Center, Denver, Colorado; Richard Olshock, MD, Lutheran Medical Center, Department of Pathology, Wheat Ridge, Colorado; and Hannis W. Thompson, MD (corresponding author), Director, Transfusion Service, Department of Pathology, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Box A022, Denver, Colorado 80262.