Case report

Delayed transfusion reaction associated with multiple antibodies

Usha Srinivasan, M.B., B.S., M.D. (Path.)*
John W. King, M.D., PhD.

Department of Blood Banking

Technical improvements and special training of blood bank personnel have led to decreasing frequency of transfusion reactions, and most hemolytic reactions are now preventable. However, certain blood group incompatibilities not detectable before transfusion can be responsible for delayed destruction of transfused erythrocytes. Rarely such reactions can be severe enough to induce life threatening sequelae. It is generally not appreciated that a major transfusion reaction can occur without incompatibility being demonstrated either before or immediately after transfusion. This report is an example of such a problem.

Case report

A 59-year-old woman was admitted for total left hip reconstruction on September 24, 1973. In 1959 a right hip cup arthroplasty was performed and during the operation she had received several blood transfusions. Her only delivery had been by abdominal caesarean section because of a large fetus about 30 years before, and at that time she had several transfusions. The baby was born jaundiced, but the patient did not know the cause. She had also received several blood transfusions during an abdominal hysterectomy about 20 years before. No adverse effects had been observed after the transfusions.

Physical examination was essentially unremarkable except from the orthopedic point of view. Her blood group was O Rh positive and hemoglobin value 13.8

* *Resident, St. Vincent Charity Hospital.*

g/100 ml. The serum was screened for antibodies against pooled cells by saline, albumin, and indirect Coombs' techniques. On September 27, a left total hip reconstruction operation was performed. During the operation she received four units of blood that were crossmatched and found compatible by saline, albumin, and indirect Coombs' methods. Her condition during surgery was satisfactory, but in the immediate postoperative period she required the transfusion of a fifth unit of blood. Her blood pressure remained stable after this. On the day following surgery, hemoglobin value was 9.4 g/100 ml with a hematocrit reading of 33%. The hematocrit reading dropped to 24%; hemoglobin value, 8.3 g/100 ml on the third postoperative day when her temperature rose to 102 F. She was given two units of packed cells on the fourth postoperative day. On the sixth day after surgery jaundice was noticed clinically. The temperature was 100.4 F and hemoglobin value 6 g/100 ml. The liver edge was palpated 2 cm below the costal margin. The spleen was also palpable. Serum bilirubin level was 6.5 mg/100 ml, lactic dehydrogenase (LDH) 1200 Wacker units, and the serum glutamic oxalacetic transaminase (SGOT) 500 Karmen units. On the seventh postoperative day, jaundice deepened, temperature rose to 102.8 F, hemoglobin value dropped to 4 g/100 ml, bilirubin rose to 8 mg/100 ml and gross hematuria developed. There was also slight disorientation. Request for more units of blood could not be met because of difficulty in cross matching. Both direct and indirect Coombs' tests were positive. Multiple antibodies were detected on antibody screening. After trying a number of donor units, one unit was found compatible and was transfused without any adverse effects. At this stage, a peripheral blood smear showed many fragmented and nucleated erythrocytes and leukocytosis. Urine examination revealed many red blood cells

Table 1. Hospital course of patient

	Hemoglobin g/100 ml	Reticulocyte count %	Jaundice	Direct Coombs' test	Indirect Coombs' test
Preoperative	13.8			−	−
Day of surgery (5 units of whole blood)	9.7				
Postoperative day					
1	9.4				
2	8.3				
4	10.0				
5	9.9			−	−
(2 units of packed cells)					
6	6.0		+	+	+
7	4.0	2.9	++	−	+
8	6.9		+	−	+
9	6.8		±	−	+
10	7.8		−	−	+
11	8.9		−	−	+
12	8.9	9.0	−	−	+
18	10.9	4.1	−	−	+
21	10.2	3.3	−	−	+
26	9.9	3.0	−	−	+

and hemosiderin (2 plus). Serum haptoglobins were absent. The possibilities of drug induced hemolysis and Coombs' positive hemolytic anemia were considered in addition to transfusion reaction. The patient's urine output was adequate throughout; blood urea nitrogen (BUN) and creatinine remained normal.

All transfusions were discontinued at this point. The jaundice cleared gradually, hemoglobin level rose steadily, and her general condition improved. She was discharged on October 28, 1973. The salient features of her hospital course are outlined in *Table 1*.

Immunological studies

The patient's pretransfusion serum showed no detectable antibodies on screening against pooled cells (Hemantigen) using standard saline, albumin, and indirect Coombs' methods. During the hemolytic episode after transfusion, the serum showed the presence of multiple antibodies as demonstrated by commercial red cell panels employing saline and albumin and indirect Coombs' technics at room temperature and at 37 C. The direct Coombs' test was positive only transiently on the sixth postoperative day. Because of the presence of multiple antibodies, problems were encountered in their exact identification. Using several red cell panels, anti-c, anti-E, and anti-Jk^b were identified tentatively (*Table 2*). Specificity of these antibodies was confirmed by testing a number of red blood cells known to have these antigens and by demonstrating the absence of these antigens in the patient's red blood cells (*Table 3*). Absorption and heat elution techniques were also employed.

The antigenic makeup of the husband, son, and siblings of the patient were also studied. The son also lacked

Table 2. Reactions given by three antibodies: anti-c anti-E, and anti-Jk^b in serum specimens taken during 1-month period

Day	Anti-c				Anti-E				Anti-Jk^b			
	Saline 20 C	Saline 37 C	Alb.	IAG	Saline 20 C	Saline 37 C	Alb.	IAG	Saline 20 C	Saline 37 C	Alb.	IAG
1	−	−	−	−	−	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	+	+	−	−	−	+
	+	+	+	+	+	+	+	+	−	−	−	+
	+	+	+	+	+	+	+	+	−	−	−	+
14	+	+	+	+	+	+	+	+	−	−	−	+
	+	+	+	+	+	+	+	+	−	−	−	+
21	+	+	+	+	+	+	+	+	−	−	−	+
	+	+	+	+	+	+	+	+	−	−	−	+
30	+	+	+	+	+	+	+	+	−	±	−	+

Table 3. Antigenic makeup of the patient, family, and the donors

Person	Group	C	D	E	c	e	Jk^b
Patient	O	+	+	−	−	+	−
Son	A	+	D^u	−	−	+	−
Husband	A	+	D^u	−	−	+	−
Brother	O	+	+	−	−	+	
Sister	O	+	+	−	−	+	
Donor 4636 (transfused compatible)	O	+	+	−	−	+	
Donor 3909 (incompatible)	O	+	+	−	+	+	
Donor 3910 (incompatible)	O	+	+	−	+	+	
Donor 3992 (incompatible)	O	+	+	−	+	+	
Donor 4558 (compatible)	O	+	+	−	−	+	
Donor 4559 (compatible)	O	+	+	−	−	+	
Donor 4828 (incompatible)	O	+	+	+	+	+	
Donor 4609 (incompatible)	O	+	+	−	+	+	
Donor 4196 (incompatible)	O	+	+	+	+	+	
Donor 4129 (incompatible)	O	+	+	−	+	+	
Donor 4627 (incompatible)	O	+	+	+	−	+	
Donor 5697 (incompatible)	O	+	+	+	+	+	
Donor 5665 (incompatible)	O	+	+	+	+	+	
Donor 5595 (incompatible)	O	+	+	−	+	+	

the E, c, and Jk^b antigens, thus ruling out pregnancy as the cause of primary sensitization. Antibody studies on the serum on three different occasions showed the same antibodies. Titers of antibodies were determined using albumin and Coombs' techniques for E and c, and indirect Coombs' for Jk^b (*Table 4*). There was no significant change in the titer of E and c over a period of two weeks, but anti-Jk^b antibody did show some reduction in titer. The presence of anti-P was suspected in the initial sample, but this could not be confirmed in later samples.

Discussion

It appears likely that the transfusions the patient received during the intraoperative and postoperative periods triggered an anamnestic response based on sensitization in the past by multiple transfusions. The resulting production of multiple antibodies brought about rapid destruction of the transfused cells. Antibodies that were

Table 4. Antibody titration on serum specimens of patient

Day*	Anti-c	Anti-E	Anti-Jk^b
7	1:256	1:256	1:32
14	1:256	1:256	1:16
21	1:256	1:256	1:16
30	1:256	1:256	1:16

* Days after first transfusions were given. All titrations done using indirect Coombs' technique.

responsible for intravascular hemolysis appear to be of anti-Jk^b specificity. These are lytic antibodies *in vitro* and *in vivo*. The other antibodies (namely anti-E and anti-c) may have contributed in part to the reaction, since they were present in high titers and *in vitro* tests with red cells with E and c antigens showed very strong agglutination with the patient's serum.

In this case, the initial cross match before transfusion by all three accepted techniques were compatible. No irregular antibodies were demonstrable by the usual screening procedures. However, a full-fledged picture of intravascular hemolysis developed after several transfusions which evoked no immediate untoward effects. Several such delayed reactions to apparently compatible blood transfusions have been described (*Table 5*). There are at least three possible reasons why antibodies are not detectable in pretransfusion screening: (1) The antibodies may be fixed to the tissues, especially the spleen, and thus may be absent in detectable amounts in the blood.[1] (2) The antibodies may be present in titers too low to be detected by present techniques but enough to evoke reaction leading to extensive hemolysis *in vivo*. This is especially possible with anti-Kidd antibodies which are known to be difficult to detect. It is often difficult to de-

Table 5. Review of cases showing multiple antibodies

Author	History	Present transfusions	Reaction first detected	Antibodies detected
Joseph et al 1964[9]	Pregnancy, 20 yr before single unit of transfusion	5 units	7th day	Anti-c, E, Jk^b
Kissmeyer-Nielsen et al 1961[14]	10 units, 3 mo before	18 units	5th day	Anti-Fy^a, C, and possibly anti-Jk^b
Croucher et al 1967[6]	2 units, 20 yr before 2 pregnancies	10 units	10th day	Anti-Ce, Fy^a, and e
	1 unit, 7 yr before	5 units	9th day	Anti-c, M
	Transfusion, 7 yr before	6 units	16th day	Anti-E, K
	6 pregnancies	14 units	19th day	Anti-Fy^a, Lu^a
Kurtides et al 1966[7]	3 units, 18 yr before	3 units	8th day	Anti-C, D, Jk^b
Meltz et al 1971[5]	Pregnancy, 6 yr before	2 units	5th day	Anti-U, Le^a, U
Present case	Pregnancy, 30 yr before	5 units	6th day	Anti-E, c, Jk^b
	Transfusions, 20 and 13 yr before			

tect sensitization with anti-IgG Coombs' serum; but the use of anti-complement globulin serum facilitates this detection. Simultaneous appearance of other antibodies may make detection of anti-Kidd antibodies even more difficult. They are, however, very potent *in vivo* and appear to produce more rapid destruction of red cells than might be expected from their rather weak reaction *in vitro*.[2] (3) The antibodies may be present in very low titers so that they are undetectable by present techniques. However, an anamnestic response to recent transfusions brings about rapid destruction of transfused cells.[3]

Delayed transfusion reactions are recognized and reported infrequently. Posttransfusion anemias are usually blamed on continued internal or external blood loss, autoimmune hemolytic anemia, or acute hemolytic anemia caused by sepsis or drugs. Clinical manifestations of delayed reactions range from asymptomatic development of positive indirect Coombs' test on repeated cross matches to overt acute hemolytic anemia with fever, jaundice, hemoglobinuria, spherocytosis, loss of serum haptoglobins, and positive direct Coombs' test. Acute renal failure associated with delayed transfusion reaction is rare, but there are at least two reported cases.[4, 5] Croucher et al[6] described three cases which were all tentatively diagnosed as acute acquired hemolytic anemia. However, the correct diagnosis was made later by identification of isoantibodies that appeared at various intervals after transfusion. These cases deserve special mention, because the possibility of delayed transfusion reaction is often overlooked in the differential diagnosis.

In published cases of reactions after the transfusion of apparently compatible blood, antibodies could not be demonstrated in 50% of cases.[7] In some posttransfusion anemias, hemolysis can be demonstrated only by shortened red cell survival using ^{51}Cr studies with no antibodies detectable. These cases usually show a "collapse curve pattern" of red cell survival indicating the activity of antibodies with delayed appearance.[8]

Delayed transfusion reaction may be due to either a primary response or an anamnestic response after a variable delay after transfusion. Usually a primary response is so mild that it goes virtually unnoticed and occurs 10 to 20 days after transfusion. An anamnestic response occurs commonly 3 to 10 days after transfusion with much more rapid destruction of red cells. Sensitization in these cases occurs either from previous transfusions or pregnancies.

Reported cases of delayed hemolytic reaction have been due to anti-c,[6, 9, 10] anti-E,[6, 9, 11-13] anti-Jk^a,[3, 6] anti-Jk^b,[4, 9] anti-Fy^a,[2, 6] anti-U, anti-V and anti-Le^b.[5] Delayed hemolytic reactions associated with multiple antibodies are rare, and only a few such cases have been reported (*Table 5*).

The antibodies that are detectable during the hemolytic phase of the delayed reaction may not be detectable a few weeks to a few months later.[2, 3, 9] These patients may thus regain compatibility (by cross matching) with blood having the same antigenic components against which antibodies were formed earlier. It is advisable for these patients to wear a bracelet showing the specificity of irregular antibodies that were detected. It may be extremely difficult to find compatible

blood for these patients with multiple antibodies, especially if the antigens involved are of relatively high frequency in the population. In some instances, it may be safer for these patients to keep their own blood frozen for emergency use. At present, the only available means of avoiding reactions of this type are to pay strict attention to weak reactions in conventional cross matching techniques, to use more sensitive methods for antibody screening, and to do complete blood typing in suspected cases.

Summary

A severe delayed hemolytic transfusion reaction associated with the production of multiple antibodies after transfusion of apparently compatible blood in a woman who had received a transfusion previously is reported. The antibodies detected were anti-c, anti-E, and anti-Jk^b.

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