Primary immune response to blood group antigens in burned children

N. BACON, E. PATTEN, AND J. VINCENT

Delayed hemolytic transfusion reactions (DHTRs) are generally attributed to an anamnestic immune response. Case reports of DHTRs due to a primary immune response are rare. Transfusion reactions occurring in patients on the pediatric burn unit from 1981 to September 1988 were reviewed, and additional information was obtained for patients for whom a DHTR was documented. Of 62 transfusion reactions, 11 were classified as a primary immune response (DHTR), with either a positive antibody screen, a positive direct antiglobulin test (DAT), or both. None of the 11 patients included in the study had been previously transfused or pregnant. The average number of units transfused prior to antibody identification was 19. The average time elapsed between the first transfusion and antibody identification was 3.6 weeks. Anti-K and anti-E were the most frequently identified. Three patients had a decrease in hemoglobin (average 1.5 g/dL) and hematocrit at the time that a positive DAT was detected. Such changes could not be demonstrated for the remaining eight patients. The conclusion was that a DHTR may be caused by a primary immune response in burned children more often than expected, but DHTR signs and symptoms are often not apparent due to the complications of burn trauma. ***Immunohematology*** 1991;1:8–11.

Blood tranfusion with an expected rise in hematocrit usually precedes the appearance of a delayed hemolytic transfusion reaction (DHTR). Three to 14 days after the transfusion there is a decrease in hematocrit, and other laboratory and clinical findings may develop, including fever, jaundice, hemoglobinuria, spherocytes on the peripheral smear, and a positive direct antiglobulin test (DAT). The DAT may remain positive until all incompatible transfused red cells have been cleared from the circulation. The responsible antibody should then be detectable in the patient's serum or eluate and will remain detectable in the serum from weeks to years.[1,2,3,4]

The diagnosis of a DHTR is often missed if clinical symptoms are mild or absent. One researcher reported that among 40 cases of DHTRs encountered over a 5-year period, there were 34 in which a positive DAT was the first clue to correct diagnosis. In the remaining six cases, the first indication of a DHTR was a positive indirect antiglobulin test (IAT), the presence of spherocytes in the blood film, or an unexpected decrease in hemoglobin concentration.[5]

In studies conducted at the Mayo Clinic from 1978–1980, DHTRs were reported at the rate of one per 1,500 transfusions.[6] The Mayo Clinic investigators proposed the following criteria for the diagnosis of a DHTR: (1) the pretransfusion crossmatch was compatible at all phases; (2) the pretransfusion antibody screen of the recipient's serum was negative; (3) a repeat crossmatch of donor cells with posttranfusion recipient serum was incompatible; (4) DAT and IAT on the posttransfusion recipient sample were positive; (5) antibody could be eluted from the recipient's red cells after transfusion; (6) history of pregnancy or transfusion was available; (7) at least two of the following clinical and laboratory tests suggestive of hemolysis were present: elevated levels of serum indirect bilirubin, decreased blood hemoglobin level by 1 g/dL or more, decreased serum haptoglobin level by 50 percent or more from pretransfusion level, hemoglobinuria, or hemosiderinuria.

DHTRs are generally attributed to an anamnestic immune response and only rarely result from a primary immune response.[1,2,3,4] One case of a DHTR caused by a primary immune response was reported by Patten et al.[7] The patient had received blood after complications of a vaginal delivery and presented with hemoglobinuria and anemia (hemoglobin 8.0 g/dL) 4 weeks posttransfusion. The causative antibody, anti-C, could not be identified for another 4 weeks. The length of time before the antibody could be identified and the patient's history of no prior transfusion or pregnancy led to the assumption that this DHTR was due to a primary immune response.

Another case of a DHTR caused by a primary immune response was reported by Solanki and McCurdy.[8] The 17-year-old sickle cell disease patient had not been transfused previously. He received two units of red cells, which resulted in the expected rise in hemoglobin and hematocrit. Between 9 and 12 days later, the hematocrit value dropped to the pretransfusion level. The blood

bank was unable to demonstrate a positive DAT or the existence of an antibody. The anemia was then treated with an additional transfusion, which resulted in an anamnestic-like response. Symptoms included fever, hemoglobinemia, hemoglobinuria, and transient renal failure. Anti-C, -E, -Jk^b, and -Fy^a were eluted from his red cells 24 hours after the acute reaction. Included in the report were two other cases of DHTRs occurring in patients with a negative transfusion history. A 38-year-old female developed a reaction 10–15 days after an initial transfusion of two units of red cells. Three days later, anti-E and -Wr^a were identified. A 48-year-old nontransfused female developed an anti-M and -H 9–12 days after transfusion of two units. Pregnancy history was not given in either case.

We decided to study patients in our hospital to determine whether a primary immune response could be documented and whether it was responsible for DHTRs. A chart review was done on pediatric burn patients. This population was chosen because the group consisted of previously healthy children who had no history of prior transfusions or pregnancy. As burn patients, these children were multiply transfused, could be followed in the hospital for weeks, and frequently developed red cell alloantibodies.

Materials and Methods

Regardless of type, all transfusion reactions from 1981 to September 1988 in patients on the pediatric burn unit were reviewed and then sorted according to etiology. Sixty-two suspected transfusion reactions had been reported and evaluated by the blood bank protocol (consisting of a DAT, inspection of the serum for icterus or hemolysis, and clerical check). Eight reactions were not considered to meet criteria and therefore were not classified. There were 11 DHTRs, 25 febrile reactions, 17 allergic reactions, and one case of an ABO incompatible plasma transfusion. The diagnosis of DHTR was based on serological evidence only. When a recently transfused patient demonstrated a new antibody and/or the DAT was positive, a transfusion reaction report was generated and categorized as a DHTR. If possible, the donor unit segments were typed for the corresponding antigen. Only the reactions classified as DHTR were included for chart review. The data collected included age, sex, degree and percent of burn, ABO and Rh type, identity of the antibodies found in the serum or eluate, results of the DAT, the time interval between the first transfusion and antibody detection, prior medical history, and whether the reaction was detected by the primary care physician or by the laboratory during routine pretransfusion testing. Clinical and laboratory symptoms of DHTR such as fever, unexplained fall in hemoglobin or hematocrit, hemoglobinemia, and hemoglobinuria were also particularly noted.

Antibody identifications and elutions were performed by standard blood bank procedures.

Results

All 11 DHTRs due to primary immune response had been detected in the blood bank during routine pretransfusion testing. All had a negative DAT and IAT before transfusion. The DATs became positive in 8 of the patients, but the antibody screen became positive in all 11 (Table 1). The average time interval between the first transfusion and antibody detection was 3.6 weeks (range 10 days to 16 weeks). The average number of units transfused until antibody detection was 19 (range 10–57). All patients were Rh positive—five As, four Os, and two Bs. Caucasian, black, and Hispanic persons were represented. Ages ranged from 22 months to 18 years.

The most frequently identified antibodies were anti-E and -K. Three patients had anti-E and -K in their serum, but each had only one or the other in the eluate. Other antibodies identified were anti-C, -S, and -Jk^a, all occurring singly. In all but cases #1, 3, and 4, the corresponding antigens were found on the red cells of some of the units transfused. Those three cases received 28, 39, and 38 units, respectively, and antigen exposure could be inferred from the expected frequency of the antigen in the donor population. Clinical symptoms and laboratory parameters indicative of DHTR were difficult to demonstrate because of the nature of burn trauma. These patients were febrile most of the time, and many were suffering from multiple bacterial infections. Although daily hematological parameters were available, many could not be correlated to the DHTR, because the patients had had repeated surgeries for skin grafts and/or orthopedic repair. No serum haptoglobin results were found on the charts. Liver enzymes, bilirubin, and coagulation studies were not done routinely.

Extensive family and medical history had been obtained on each patient. None of these 11 patients had prior transfusion documented on their charts, nor was there anything in their histories that made prior transfusion suspect. Two females, ages 16 and 18, denied a history of pregnancy.

Table 1. Serological and clinical findings in children with a primary immune response

Case #	Age/sex	Number of units transfused prior to detection of antibody	Time interval*	Anti-body	Direct anti-globulin test†	Eluate	Laboratory data
1	10/F	28	4.0	E,K	m+,IgG,poly	K	Hgb,Hct ↓
2	2/M	16	2.3	E,K	m+,poly	E	Hgb,Hct ↓
3	10/M	30	16.0	K	m+IgG,poly	K	Hgb,Hct ↓
4	16/F	38	4.0	K	neg	not done	‡
5	14/M	57	8.0	S	m+,poly,C3,IgG	neg	
6	18/F	14	1.9	C	m+,IgG,poly	C	
7	13/M	26	11.0	Jk^a	m+,poly,C3	neg	
8	13/M	38	9.0	E	m+,IgG	E	
9	2/M	10	2.1	E,K	m+,C3	pos/too weak to identify	
10	7/F	13	2.4	E	neg	neg	
11	22 mo./F	12	1.4	E	neg	not done	
	Average	19	3.6				

*Weeks between transfusion and antibody detection. Transfusions in other hospitals before transfer to this institution were included.
†Microscopic reaction (m+)
Positive with polyspecific antihuman globulin (poly)
Positive with monoclonal anti-IgG antihuman globulin (IgG)
Positive with anticomplement fraction in antihuman globulin (C3)
‡Unable to correlate clinical symptoms such as anemia (decrease in hemoglobin, hematocrit, fever, etc.), because of surgery on that day or constant febrile state.

Three patients had a decrease in hemoglobin and hematocrit close to the time that the blood bank reported a positive DAT. The primary care physician did not suspect a DHTR in any of the 3 patients. In case #1, the patient was found to have a positive DAT 2 days after his hemoglobin decreased from 11.0 g/dL to 9.8 g/dL. There was no surgery or documented bleeding that would have explained the decrease, and the hemoglobin had been stable for several days before the decrease. In case #2, the patient's hemoglobin decreased from 10.7 g/dL to 9.0 g/dL on the day that the blood bank reported a positive DAT. No apparent cause for the decrease was documented on the chart. Case #3 had a decrease in hemoglobin from 13.3 g/dL to 12.5 g/dL on the day that the positive DAT was reported, and it continued to decrease to 11.7 g/dL the next day. This patient's last surgery and transfusion had been three months before the discovery of the antibody. The average decrease in hemoglobin for these three patients was 1.5 g/dL.

Only case #5 had experienced several febrile reactions probably due to leukoagglutinins before the development of the red cell alloantibody.

Discussion

The accepted time frame for an anamnestic response ranges from 2–14 days but usually occurs within 7 days.[4,9,10] Antibody production due to a primary response, on the other hand, occurs no earlier than 7–10 days and may not be detectable for several weeks.[4,9,11] Absence of prior red cell exposure and the average time interval of 3.6 weeks (range 10 days to 4 months) from the first transfusion until the antibody detection lead to the conclusion that the antibodies detected in our patients were probably due to a primary immune response. The mild or absent clinical symptoms are also compatible with this conclusion. Unfortunately, the severity of the patients' injuries made clinical symptoms difficult to relate to their blood transfusions.

The frequency with which anti-E and -K were implicated should be expected on the basis of the antigenicity of the Rh and Kell antigens.[4] Several studies have shown anti-Jk^a to be the most commonly found antibody in DHTR due to an anamnestic immune response,[3,4,6] because most Kidd antibodies become undetectable a few weeks or months after stimulation. The only Kidd antibody found in our study was not detected until 11 weeks after the initial transfusion, a length of time that would not be indicative of an anamnestic response.

These children were atypical in that their immune systems had been hyperstimulated by such factors as trauma, bacterial infections, skin grafts, and multiple transfusions. With the exception of the 18-year-old female who developed an anti-C in 13 days, the fastest antibody producers were the youngest patients in our population. The 22-month-old produced anti-E in 10 days, and two 2-year-olds produced anti-K and -E in

16 and 15 days, respectively. Further study is indicated to demonstrate whether burned children become sensitized to red cell antigens more frequently than other pediatric patients or adults.

At least one author believes that documentation of nonsymptomatic DHTRs is unnecessary.[3] Many of these nonsymptomatic DHTRs are probably due to a primary immune response. Therefore, detection and identification of red cell alloantibodies as soon as possible after initial transfusion would be the best means of avoiding anamnestic DHTRs in the future.

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Nancy E. Bacon, MT(ASCP)SBB, Permian Basin Blood Center, 2200 West Illinois, Midland, TX 79701; Ethel D. Patten, MD, and Janet L. Vincent, MS, Blood Bank, University of Texas Medical Branch, Galveston, TX.