Vox Sang. 49: 336–339 (1985)


0042–9007/85/0495–0336 $ 2.75/0

# Delayed Hemolytic Transfusion Reaction Associated with Rh Antibody Anti-f: First Reported Case

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**Abstract.** A delayed hemolytic transfusion reaction (DHTR) occurring in a 30-year-old Caucasian man is described. His blood groups were B, CDe/cDE ($R_1R_2$, f-) K-, S-s+, Fy (a + b-), and Jk (a + b +). Eight days after receiving two units of packed red blood cells, his urine was strongly positive for hemoglobin; serum-free hemoglobin was 125 mg/dl. Serum contained a clearly reactive Rh antibody of anti-f specificity and a weakly reactive anti-Kell. The DHTR was probably caused by an anti-f alloantibody appearing as an anamnestic response to transfusion of seemingly compatible, but f-positive blood.

The Rh antibody with the specificity called anti-f (anti-ce) was first discovered in the serum of a hemophilic who had received numerous transfusions of blood products [1]. An alloantibody of anti-f specificity was also found in the serum of a much transfused patient with acquired hemolytic anemia [2]. The clinical significance of anti-f is sparse. Autoantibodies of anti-f specificity have been found in the sera of 3 patients with autoimmune hemolytic anemia (AIHA) [3–5]. Three patients with hemolytic disease of the newborn due to anti-f antibody have been described [6–8]. We are reporting a patient in whom a delayed hemolytic transfusion reaction (DHTR) to seemingly compatible blood developed as a result of a delayed anamnestic alloantibody response of apparent anti-f specificity. No previous reports of a DHTR due to an alloantibody of anti-f specificity could be found in the literature.

## Case Report

A 30-year-old Caucasian man was admitted to the hospital because of a dark stool and vomiting of blood for 1 day. At 16 years of age, the patient underwent splenectomy for his traumatically ruptured spleen and was transfused with 12 units of whole blood to replace losses.

Physical examination showed orthostatic changes in blood pressure. The hematocrit was 41% on entry and decreased to 28% over the next 36 h. Gastroscopy revealed a duodenal ulcer. The patient was transfused with two units of packed red blood cells (RBC) that were compatible on routine crossmatching, i.e., the

patient's serum was incubated with donor RBC in low ionic strength medium enhanced with bovine albumin (EM-X, Biological Corporation of America) at 37°C for 10 min, followed by an indirect antihuman globulin (AHG) test with polyspecific Coombs reagent (Gamma Biological Corporation). Gastrointestinal bleeding ceased quickly, but low back pain developed on the fourth hospital day. On the eighth hospital day, the patient awoke with severe back pain and noted 'dark bloody' urine.

The patient appeared healthy and had slight scleral icterus as the only positive finding on physical examination. The hematocrit was 40%. Plasma was yellow-red in color; free hemoglobin was 125 mg/dl (normal <3) and haptoglobin 5 mg/dl (normal 40–140). A smear of the peripheral blood had a marked number of siderocytes and Howell-Jolly bodies, typical of the post-splenectomy state. Reticulocytes were 4.9%, or 204,000/μl. Serum bilirubin was 0.2 mg/dl direct and 1.8 mg/dl total; iron was 253 μg/dl and the total iron-binding capacity was 356 μg/dl. Urine was dark brown and strongly positive for hemoglobin and protein but contained no RBC, white blood cells, casts, myoglobin, or bilirubin. The serum creatinine concentration was 1.1 mg/dl, and the urine output was over 100 ml/h at all times measured. Direct and indirect AHG tests were negative. On the next day, the ninth hospital day, direct AHG test of RBC was negative, but indirect AHG test of serum was positive. The clinical course was without complications, and the hematocrit remained stable at 40%. Blood samples for special studies were obtained on hospital days 9 and 15, and were sent to the Immunohematology Reference Laboratory of the Central California Region of the American Red Cross Blood Services.

The blood groups of the patient were B, CDe/cDE ($R_1R_2$, f-), K-, S-s+, Fy (a+b-), and Jk (a+b+). No antibodies were found in a dichloromethane eluate prepared from the patient's cells. Serum on days 9 and 15 contained an Rh antibody of anti-f specificity that was clearly reactive by albumin-AHG at 37°C, ficin-AHG at 37°C, low ionic strength saline-AHG at 37°C, and 'manual' polybrene. Serum on both days also contained an anti-Kell (K) antibody that was weakly reactive by albumin-AHG at 37°C and by manual polybrene. The anti-f antibody was equally reactive by all the testing methods used; the titer of the anti-f antibody was 1:16 by the ficin-AHG method. RBC from both units of blood that had been transfused to the patient were retested and found to be Kell-negative and to have the Rh phenotypes of CDe/Ce (f-), and cDe/ce (f+). Thus, the second unit contained homozygously f-positive RBC. The crossmatch with the f-positive unit of transfused RBCs was no longer compatible, so that the DHTR seems to have been caused by the anti-f antibody as an anamnestic response to the transfusion of seemingly compatible f-positive blood. Tests for survival of donor RBC were not done, nor was a $^{51}Cr$ survival of transfused f-positive RBC attempted. Six months after the DHTR, a repeat antibody screen of the patient's blood was negative; neither anti-f nor anti-Kell antibodies were detectable.

## Discussion

The expression of some Rh antigens is dependent on the arrangement of their gene complex; the f-antigen is produced only by humans bearing the Rh gene complexes cDe or cde [3]. This f-antigen (Rh6) is considered to be a compound antigen found in about 64% of the population that is expressed only when the c and e genes occur on the same gene complex [9]. Furthermore, the anti-f antibody does not react with separate c or e antigens. When humans lack the f-antigen, they can produce alloantibodies with anti-f specificity when transfused with f-positive RBC.

The first two patients described with an anti-f antibody as well as the patient reported herein all had the Rh genotype CDe/cDE [1, 2]. Because these 3 patients were f-negative, they could be immunized by transfusion of f-positive RBC. The f-antigen shows a gene dosage effect: anti-f antibody reacts more strongly with homozygous f-positive RBC than with heterozygous [3, 10]. The primary immunization of this patient with f-positive blood seems to have occurred 14 years earlier at the time of his ruptured spleen, and the secondary immunization occurred 8 days before the DHTR at the time of his bleeding

duodenal ulcer. A weakly reactive anti-Kell antibody was also detected in the patient's serum and, although related in some unknown manner to the anamnestic response to the f-antigen, it was unrelated to the DHTR. The long delay between the primary and secondary immunizations and the seeming compatibility of the blood administered to the patient at the time of the secondary immunization are typical of DHTR. They underscore the difficulties in the prevention of DHTR [11].

The patient reported herein first presented with hemoglobinuria. Although rhesus antibodies usually are not associated with the fixation of complement [12], patients have been reported with DHTR and intravascular hemolysis associated only with Rh antibodies perhaps as a result of erythrophagocytosis [13].

Various approaches have been taken in the prevention of DHTR. Total matching of the RBC antigens of the blood administered to multiply transfused patients is impractical [14]. Even a more sensitive test for the pretransfusion crossmatch may not prevent DHTRs that result from secondary immune responses [15]. Warning measures for patients known to have DHTR are important: informational bracelets or wallet cards for the patients, written warnings in the patient's hospital chart, and addition of the patient's name to a central registry of blood banks [16]. Although these safeguards are useful, DHTR due to the anamnestic rise of an alloantibody by secondary immunization with seemingly compatible blood are difficult to eliminate completely [17]. This irreducible hazard of DHTRs provides a sober warning of the ever present dangers of any therapeutic transfusion with blood and its products until more effective compatibility tests are devised.

## Acknowledgment

The authors thank Dr. *R.E. Rosenfield* for his confirmatory serological tests and many helpful suggestions.

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Received: October 30, 1984
Revised manuscript received: March 1, 1985
Accepted: March 18, 1985

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