Case report: immune anti-D stimulated by transfusion of fresh frozen plasma

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FFP has occasionally been reported to generate an immune response to RBC antigens (e.g., anti-D and anti-Fy^a). The Council of Europe requires that each unit of FFP have less than 6×10^9/L RBCs. However, there is considerable variation internationally in the method of production and the level and assessment of RBC contamination of FFP. This study reports the case of a 63-year-old group B, D– man who received multiple transfusions of D– blood products over a 4-month period. Seven months later the patient's antibody screen remained negative and he was transfused with seven units of D– RBCs and six units of FFP, four of which were D+. Two months later anti-D, -E, and -K were detected in his plasma. Although the anti-E and anti-K could have resulted from transfusion of antigen-positive RBCs, the anti-D could have resulted only from transfusion of the D+ FFP. The D status of FFP is currently not considered when selecting products for transfusion even though the D antigen is highly immunogenic and the level of RBC contamination of FFP is not always known. This case highlights that transfusion of FFP is a stimulus for RBC antibodies and that when a patient has had a recent transfusion of FFP, consideration should be given to obtaining a sample for pretransfusion testing within 3 days before a scheduled RBC transfusion. In addition, the D status of FFP should be considered before administering FFP to premenopausal D– women. ***Immunohematology* 2005;21:149–51.**

Key Words: red blood cell, antibody, anti-D, contamination, fresh frozen plasma, FFP

On rare occasions FFP can generate an immune response to RBC antigens. The literature includes reports of anti-D,[1–3] -Fy^a,[3] -E,[4] and -Jk^a [4] being identified or increasing in titer after transfusion of as few as two units of FFP. The Council of Europe (CE) set guidelines in 2004[5] for maximum levels of RBC contamination in FFP in an attempt to circumvent immunization by RBC antigens. However, there is considerable variation internationally in the method of production and the level and assessment of RBC contamination of FFP.[6] We describe a patient who developed anti-D after the transfusion of D+ FFP.

Case Report

A 63-year-old man was diagnosed in May 2003 with locally extensive adenocarcinoma of the esophagus. Laser therapy and single-fraction radiotherapy were applied to the esophagus for control of dysphagia and bleeding. Cytotoxic chemotherapy with fluorouracil plus epirubicin and cisplatin from June to December 2003 resulted in significant reduction in the size of the tumor. The patient's warfarin therapy, administered after aortic valve replacement in 2002, was maintained when possible. The patient's RBCs typed as group B, D– and from March to August 2003 he was transfused with a total of 21 units of group B, D– or group O, D– RBCs for anemia. No atypical RBC antibodies were detected during this time. On February 12, 2004, the patient presented to the emergency department with acute gastrointestinal hemorrhage. The patient's Hb was 6.0 g/dL and the International Normalized Ratio was 8.9. No atypical RBC antibodies were detected in the patient's plasma and he was transfused over a 15-hour period with seven units of D– RBCs and six units of FFP, four of which were D+ (Table 1). Endoscopy and computerized tomography scan showed regrowth and progression of the tumor, with extensive upper mediastinal lymphadenopathy and a persisting pulmonary deposit. No further blood product support was required during this admission and the patient was discharged for palliative care. When the patient's sample was tested 2 months later, on April 8, the antibody screen was positive. Anti-D, -E, and -K were identified, with reaction strengths of 4+, 3+, and 2+, respectively, and remained detectable on subsequent testing two months later.

Materials and Methods

Antibody identification was performed by LISS-IAT gel cards (DiaMed AG, Switzerland) using commercial reagent RBCs (Commonwealth Serum Laboratories, Melbourne, Australia) diluted to 1 percent in Diamed-ID Diluent 2. Reactions were graded from 0 (no agglutination) to 4+ (maximum agglutination).

Table 1. Blood products transfused on 2/12/2004*

	Product	ABO/Rh	Antigen typing	
			E	K
1	FILRBC	O Neg	NT	Neg
2	FILRBC	O Neg	NT	Pos
3	FILRBC	O Neg	Neg	Neg
4	FILRBC	B Neg	Neg	Neg
5	FILRBC	B Neg	NT	NT
6	FILRBC	B Neg	Neg	Neg
7	FILRBC	B Neg	Neg	Neg
8	FFP	B Neg	Neg	Neg
9	FFP	B Neg	Neg	Neg
10	FFP	B Pos	Pos	Neg
11	FFP	B Pos	Neg	Neg
12	FFP	B Pos	NT	Neg
13	FFP	B Pos	Neg	Neg

* FILRBC = leukodepleted RBCs, NT = not tested

Transfusion history of the patient was obtained from the laboratory information systems at two health care institutions. All other Western Australian transfusion services confirmed the absence of any transfusion history.

Discussion

This report describes the development of anti-D in a multiply transfused D– patient after stimulation by residual RBC antigens in D+ FFP. Anti-K and anti-E also developed, most likely due to the transfusion of antigen-positive RBCs (Table 1). One of the transfused units of RBCs was K+ and, although the E phenotype of some of the transfused units was unknown and the incidence of the E antigen in D– Australian donors is only 4 percent,[7] the possibility that anti-E resulted from transfusion of E+ RBCs cannot be excluded.

The D antigen is highly immunogenic: historic experiments demonstrated primary immunization after a cumulative dose of only 0.03 mL or 0.24×10^9 RBCs.[3] For this dose, injections of 0.01 mL of whole blood (approximately 0.005 mL RBCs) were given at 2-week intervals. A total of six injections was administered.

The minimum volume of a single dose of RBCs required to stimulate primary antibody sensitization is unproved and will vary among subjects. Similarly the number and volume of RBCs required to stimulate an anamnestic response are uncertain, though they will be considerably less. In an attempt to circumvent immunization by RBC antigens, CE guidelines require that each unit of FFP contain less than 6×10^9/L RBCs.[5] Compliance would allow up to 1.5×10^9 RBCs in a 250-mL unit of FFP, although there are often less than 0.18×10^9 RBCs.[8,9] RBC stroma, as found in FFP, is less immunogenic than intact RBCs.[3] However, this and previously reported cases[1,2] implicating FFP indicate that there is sufficient antigenicity to stimulate a response after frozen storage and subsequent thawing and infusion. As few as two units of FFP have been shown to stimulate a secondary anti-D response.[1,2] It is not known whether the anti-D produced in this case was a primary or secondary response. Although no D+ RBCs had been transfused since 2002, the possibility of prior sensitization to D antigen cannot be completely discounted.

Current transfusion guidelines[10,11] do not require the D status of FFP to be considered before transfusion and CE Guidelines[5] do not require FFP products to be labeled with the Rh type. The FFP transfused in this case was prepared from whole blood. In some countries FFP may be subjected to pathogen reduction with methylene blue (MB) or solvent detergent (SD). RBC stroma is present in untreated FFP and in MBFFP, but not in SDFFP, because it is removed by the solvent.[12] This case report highlights that the D status of FFP is relevant in transfusion practice. Specifically, a patient who has had a recent transfusion of FFP may be sensitized to RBC antigens, indicating the need for an antibody screen within three days before RBC transfusion. In addition, transfusion of D+ FFP to D– premenopausal women may have significant implications, and it has been reported that D– girls and premenopausal women requiring FFP transfusion should receive D– FFP only if SDFFP is not available.[12]

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