Development of warm auto- and allo-antibodies in a 3-year old boy with sickle cell haemoglobinopathy following his first transfusion of a single unit of red blood cells

Megan E. McNerney[1], Beverly W. Baron[1], Samuel L. Volchenboum[2], Mona Papari[1], Monica Keith[3], Kristina Williams [4], Elie Richa[1]

[1]University of Chicago, Medical Center, Biological Sciences Division, Department of Pathology, Chicago IL; [2]Department of Pediatric Hematology and Oncology, University of Chicago, Chicago IL; [3]Immunohematology Reference Laboratory, American Red Cross Heart of America Region, Peoria, IL; [4]Immunohematology Reference Laboratory Operational Support Manager, American Red Cross Biomedical Headquarters,Washington, USA.

Introduction

Alloimmunisation is a very common problem for blood banks; foreign red blood cell (RBC) antigens induce the formation of antibodies in a manner that depends on the immunogenicity of the antigen as well as on genetic and environmental factors. Alloimmunisation is estimated to occur in 0.3% to 38% of the population[1], but it can occur in almost 50% of multiply transfused patients[2], such as those with sickling haemoglobinopathies and thalassaemia. Autoantibodies, which are antibodies directed against a patient's self-antigens, are less widely recognised as a complication of multiple transfusions[3] but may be clinically significant. Autoantibodies may make it difficult to identify co-existing alloantibodies, delaying the identification of appropriately matched blood products, since most warm autoantibodies react with most donor RBC. In addition, autoantibodies can be associated with clinically significant warm autoimmune haemolytic anaemia[3]. In this report, we describe the case of a child with sickle cell anaemia who developed both auto- and alloantibodies following a transfusion of a single unit of packed RBC.

Case report

A 3-year old African-American male with a history of sickle cell anaemia presented to the emergency room with a 2-day history of bilateral leg pain and 1-day history of abdominal and chest pain. He was admitted for a presumed sickle cell anaemia-associated vaso-occlusive crisis. His past medical history included two episodes of pneumonia and several pain crises usually managed with pain medication. Four months previously, he had been admitted for sickle cell anaemia-associated leg pain and acute chest syndrome, at which time he received one unit of packed RBC selected to be negative for K and E antigens, since the patient was K and E negative. He was typed as having group A, D+ blood, and his antibody screen was negative at that time. This was the only transfusion the patient had ever received prior to his current admission.

Laboratory values on admission were as follows: white blood cell count 13.7 x 10^9/L, haemoglobin (Hb) 7.4 g/dL, haematocrit 22.6%, and platelet count 380 x 10^9/L. Electrophoresis of the patient's haemoglobin indicated a Hb S level of 83.1%, Hb F of 11.4%, Hb A2 of 3.7%, and Hb A <2%. The antibody screen and direct antiglobulin test (DAT) were positive. The day following admission, the patient developed increased dyspnoea and became hypoxic. His chest X-ray demonstrated new bilateral basilar airspace opacities. He was transferred to the intensive care unit and placed on a ventilator. His Hb dropped to 6.6 g/dL the same day, at which time the paediatric service consulted the blood bank for possible RBC exchange transfusion as a method to decrease his Hb S level. As a temporising measure, the paediatric team then decided to transfuse him with one unit of Hb S-negative leucodepleted, least incompatible packed RBC negative for K and E antigens. This transfusion proceeded without any complications, and his Hb S level consequently decreased to 53.6%, with Hb F of 7.5%, Hb A2 of 3.6% and Hb A of 35.7%, and his Hb increased to 8.9 g/dL. During the succeeding days, his Hb S level remained at 50-60% and his Hb level was 7.8 g/dL. He was also treated with ceftriaxone and azithromycin for suspected pneumonia and was extubated 4 days later.

Three days later, he developed acute stridor and

laryngeal oedema of unclear etiology. He was transferred back to the paediatric intensive care unit where he responded to intravenous epinephrine and steroids as well as inhaled albuterol. Four days later, he was discharged home, as he was clinically stable. That same night at home, he was found unresponsive. Emergency medical services were called, and the patient was found to be pulseless and apnoeic. Despite efforts to resuscitate the patient, the boy was pronounced dead upon arrival at the hospital. A post-mortem examination revealed necrotising herpes simplex virus pneumonia in both lungs, though the aetiology of the patient's sudden death remains unclear.

As far as immunohaematology tests are concerned, antibody screens were performed using the gel method (Ortho ProVue®, Ortho Clinical Diagnostics, Rochester, NY, USA). For antibody elutions, the Gamma Elu-Kit II (Immucor Gamma, Norcross, GA, USA) was employed. Three donor cell lines (R_1R_1, R_2R_2, and rr) were used for allogeneic adsorptions. Each of the three cell lines was pre-treated with the enzyme papain (freeze-dried, Immucor Gamma) and all cell lines were negative for the K antigen. Three 37ºC adsorptions were performed with each cell line. LISS and PEG (both from Immucor Gamma) were used as enhancers in the tests with adsorbed plasma.

The patient's antibody screen was found to be positive at the time of admission, a 2+ pan-reactive agglutination pattern was noted on a 10-cell panel, and the autocontrol was also 2+. The DAT was 1+ using monospecific IgG antiglobulin reagent and negative with anti-C3. Red cell acid elution revealed a broad spectrum, warm autoantibody. Additional specimens from the patient were sent to the American Red Cross (ARC) Reference Laboratory, Heart of America Region (Peoria, IL, USA) for evaluation of the presence of underlying red cell alloantibodies.

The patient's RBC phenotype was as follows: E^-; K^-; Fy(a^+b^+); Jk(a^+b^+); and M^-. The ARC Reference Laboratory reported a positive DAT (2+) using monospecific IgG reagent, and no anti-C3 reactivity was observed. The elution results demonstrated pan-agglutination. Following an allogeneic absorption test, the following alloantibodies were demonstrated: anti-E, anti-K and anti-M. Two of the adsorbing cell lines (R_1R_1 and rr) were negative for the E, K, and M antigens; therefore, antibody specificities to these antigens were not adsorbed by these two cell lines lending support to classifying these antibody specificities as alloimmune in nature rather than attributing the reactivity to antibodies with mimicking specificities.

Discussion

The cause of this child's sudden death remains unclear. Necrotising HSV pneumonia has been reported only once in the literature as an unusual and rare cause of mortality in a patient with sickle cell anaemia with acute chest syndrome[4]. We would like to focus on the transfusion medicine issues in this case, particularly the development of the patient's red cell antibodies.

Autoantibodies may develop after the formation of multiple alloantibodies as a result of immune system activation[5], and they tend to be directed toward Rh blood group antigens, frequently the e antigen[3]. Autoantibody formation following alloantibody formation was first described by Dameshak and Levine[6]. Similar findings were described by Worlledge, who stated that persistent alloimmunisation might lead to autoimmunisation after multiple transfusions[7]; this hypothesis was corroborated by Chaplin and Zarkowsky[8] and Sosler[9]; however, patients often develop autoantibodies without preceding alloantibodies[3]. Interestingly, there are some reports that transfusion-associated autoantibodies tend to be transient as compared to the more common idiopathic autoantibodies, though it is not known whether associated autoimmune haemolytic anaemia is also more transient[5].

Until a study by Castellino *et al*.[3], most reports on autoantibodies were either case reports or small series studied retrospectively. Castellino *et al*. reviewed the records of 184 paediatric patients who had received multiple transfusions for sickle cell anaemia; 14 patients (7.6%) developed IgG autoantibodies. The median number of transfusions was 24 (range, 3-341). The autoantibodies were panagglutinins in 11 cases and showed anti-e specificity in three cases. Four patients had significant haemolysis, all of whom had both anti-IgG and anti-C3 positivity. Similarly, Aygun *et al*.[10] found that 6 out of 78 (8%) of their paediatric patients with sickle cell anaemia and 6 out of 62 (9.7%) adults with sickle cell anaemia developed autoantibodies. Most patients had prior alloantibodies. Five of the paediatric patients had panagglutinins, and one had anti-e specificity.

The pathophysiological mechanism for transfusion-induced autoantibody formation is still unclear. Many theories and models have been described in the literature to explain this phenomenon. One likely explanation is that alloantibody binding to transfused RBC leads to a conformational change in the antigen; this neoantigen then stimulates the production of antibodies that cross-react with self-

antigens[3]. Epitope-spreading may also stimulate production of autoantibodies[5]. Patients with sickle cell anaemia may be particularly susceptible to autoantibody formation due to chronic deformation of the RBC membrane, leading to RBC neoantigen exposure in an inflammatory intravascular milieu during a vaso-occlusive crisis[3]. Of note, there is evidence that infection may increase the risk of alloantibody formation, possibly by further activating the immune system[11]. Our patient did have pan-sinusitis and fever at the time of his first transfusion, and he also had HSV pneumonia, which might have contributed to antibody formation, although HSV has not been specifically reported in the literature to induce auto- or alloimmunisation to RBC antigens. Another possible explanation for antibodies in some patients might be that they represent naturally occurring antibodies, such as anti-M in infants[12] or anti-K as described by Marsh[13] or anti-E[12], but these naturally occurring antibodies are usually IgM and not IgG as in our case. The aetiology of our patient's alloantibodies remains unclear, since, according to the history obtained from his mother, he had only received one prior unit of packed RBC, which was negative for K and E antigens. It is conceivable that there was an error in the typing of the patient or donor RBC unit that led to antibody formation, however this seems unlikely, as the patient had been typed several times and no discrepancies were identified. The RBC units are also typed twice before being released for transfusion and again no discrepancies were reported. Also, the patient did not receive any intravenous immunoglobulins or packed RBC from a donor positive for a red cell antibody. Thus, there is no supporting evidence that the antibody specificities detected were passively acquired. To our knowledge, there is only one prior case reported in the literature of a child developing auto- and alloantibodies after receiving only one unit of RBC[14].

Key words: Sickle cell haemoglobinopathy, warm autoantibody, alloantibodies.

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Received: 23 April 2009 - Revision accepted: 1 September 2009
Correspondence: Elie Richa
Blood Bank and Transfusion Medicine, University of Chicago,
Biological Sciences Division, Department of Pathology
5841 S. Maryland Ave MC0007,
Chicago, IL 60637 - USA
e-mail: Elie.Richa@uchospitals.edu