Autoantibody formation after alloimmunization inducing bystander immune hemolysis

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The development of RBC autoantibodies resulting from or associated with allogeneic blood transfusions is not an easily determined complication of RBC transfusions. This report discusses one patient who developed RBC autoantibodies in association with an allogeneic blood transfusion and alloimmunization leading to a temporary bystander immune hemolysis. A 72-year-old woman was hospitalized as a result of severe anemia and received two units of ABO- and D-compatible RBCs. She had a history of two pregnancies 40 years before, but no history of RBC transfusion, and her antibody screen was negative. On the tenth day after transfusion her hemoglobin dropped, and alloanti-c was identified in her serum and eluate. At this time she received another two units of compatible blood according to her phenotype (group O, R_1R_1, K:-1). After 48 hours, she developed joint pain, pyrexia, and hemoglobinuria, and her Hb dropped from 9.2 g/dL to 5.3 g/dL. The direct antiglobulin test was positive, an IgG autoantibody was present in the eluate, and the antibody investigation revealed the presence of anti-Jk^b in addition to the previously identified alloanti-c. Her genotype was determined, and, based on the findings, two additional units were selected, found to be compatible, and transfused without incident. Transfusions were discontinued, and she was treated with IVIG and corticosteroids. Her Hb increased to 9.7 g/dL, and the patient made an uneventful recovery. It was concluded that transfusion of incompatible RBCs induced the formation of an autoantibody in this patient, resulting in lysis of bystander RBCs. The need for additional blood transfusion was successfully avoided by treatment with IVIG, steroid therapy, and rituximab. ***Immunohematology* 2009;25:9–12.**

Key Words: bystander hemolysis, autoantibody, alloimmunization, RBC transfusion

The presence of alloantibodies in chronically transfused patients and pregnant women is a well-recognized complication of RBC transfusions and pregnancies and, as such, is unrelated to the concomitant autoantibodies. In contrast to alloimmunization, the risk of autoimmunization resulting from or associated with the development of alloantibodies is poorly understood; however, RBC autoimmunization and the development of autoimmune hemolytic anemia (AIHA) should be recognized as a complication of allogeneic RBC transfusion.[1]

Recent studies of patients with multiple transfusions, such as those with sickle cell anemia, have drawn attention to the association between autoimmunization and alloimmunization.[2–5] RBC autoimmunization has also been described in both animal and human experimental models in which introduction of incompatible RBCs induced both alloantibody and autoantibody formation.[6–10]

The development of AIHA concurrent with or shortly after alloimmunization from blood transfusions has also been reported, and one possible mechanism to explain this phenomenon is that alloantibody binding to transfused RBCs could lead to conformational changes in antigenic epitopes that then stimulate production of an autoantibody.[1] The term *bystander immune hemolysis* is applied when AIHA occurs after exposure to alloantigens.[11]

This phenomenon, which has been recognized in the medical literature for many years, seems to receive little attention although it is clinically very important and it touches on some basic immunologic principles that might have broader implications than has been previously realized.[12]

We report one case of severe, life-threatening bystander immune hemolysis associated with the development of an autoantibody in association with allogeneic blood transfusions and alloimmunization.

Case Report

A 72-year-old white woman with a history of coronary artery disease, hypertension, diabetes mellitus, and angiodysplasia was hospitalized as a result of severe anemia, weakness, chest discomfort, dyspnea, and acute lower gastrointestinal bleeding. On physical examination, the patient was pale, tachycardic, and normotensive with normal oxygen saturation by pulse oximetry. Her admission laboratory evaluation revealed an Hb of 7.9 g/dL, Hct of 24%, reticulocyte count of 3.1%, WBC count of $4.53 \times 10^3/\mu L$, platelet count of $343 \times 10^3/\mu L$, blood urea nitrogen of 21 mg/dL, and creatinine of 0.7 mg/dL. She received two units of ABO- and D-compatible blood. No RBC alloantibodies were detected, and her RBCs did not react in the DAT before this transfusion. No symptoms occurred during the transfusion. Ten days later, the patient was readmitted with slight signs and symptoms of hemolysis, and there was no evidence of blood loss. The clinical investigation revealed no history of previous RBC transfusion, but she had a history of two pregnancies 40 years ago. The patient's Hb level was 8.2 g/dL, LDH was 1509 IU/L, nonconjugated bilirubin was 1.5 mg/dL, and hemoglobinemia was 8.9 mg/dL; alloanti-c was identified in her serum and eluate. Clinical and serologic results demonstrated a delayed hemolytic transfusion reaction (DHTR) attributable to anti-c. At this time she received two additional units of compatible blood according to her phenotype (group O, R_1R_1, K:-1). After 48 hours, she

exhibited generalized musculoskeletal pain, pyrexia, and hemoglobinuria, and her Hb dropped from 9.2 g/dL to 5.3 g/dL. The DAT was 4+; a warm IgG autoantibody that reacted with her RBCs and all panel cells by the IAT was present in the serum and eluate. A differential absorption method revealed the presence of the known alloanti-c coexisting with a newly identified alloanti-Jk^b in her serum. Molecular analysis was performed, and she received two units of compatible blood according to her genotype *(RHD, RHCE*C/C, RHCE*e/e, KEL*2/2, JK*A/A, FY*A/B, GYPB*S/s)* without clinical or laboratory response. A clinical diagnosis of AIHA was made, and treatment was started on day 8 after the diagnosis with IVIG (0.4 g/kg for 5 days) and IV methylprednisolone (0.5 g for 5 days). Her Hb increased to 9.8 g/dL, but a chest discomfort persisted. She received two RBC units, and 48 hours after the transfusion her Hb dropped to 7.7 g/dL. Transfusions were discontinued, and she was treated with rituximab (500 mg one time weekly for 1 month). After 2 months, there was no evidence of hemolysis, her Hct remained stable, and the laboratory tests revealed alloanti-c and alloanti-Jk^b in her serum. No RBC autoantibody was detectable.

Materials and Methods

Serologic studies

Direct and indirect antiglobulin tests were performed by hemagglutination in tubes and in gel cards (DiaMed AG, Cressier sur Morat, Switzerland). Antisera and reagent RBCs were obtained from a variety of commercial companies (DiaMed AG; Gamma Biologicals Inc., Houston, TX; Fresenius Hemocare, São Paulo, Brazil). Eluate was performed using Gamma Elu Kit II (Gamma Biologicals Inc.) from patient RBCs. Differential absorptions were performed on the patient's serum to remove the autoantibody, allowing the identification of the alloantibodies.

Molecular studies

DNA was extracted from blood samples by using a kit (Easy DNA kit, Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. Allele-specific PCR (ASPCR) and PCR-RFLP were used for *RH, KEL, FY, JK,* and *Ss* genotyping. The primers and the amplification conditions used for genotyping have been previously published.[13]

Results

Patient's samples

Before the first hemolytic transfusion reaction, the alloanti-c was not detected; most probably the antibody titer was very low and the antibody was undetectable by serologic techniques. After the second hemolytic transfusion reaction following the transfusion of compatible R_1R_1 RBC units, alloanti-c and alloanti-Jk^b were found.

The two alloantibodies were identified in the serum samples with PEG-IAT, GEL-LISS, and GEL-PAPAIN. After the first hemolytic episode, the patient's RBCs were weakly reactive in the DAT (tube and gel) and alloanti-c was identified in the eluate, but after the second hemolytic episode, the DAT became strongly positive. The eluate from the posttransfusion sample demonstrated the presence of a warm IgG autoantibody that reacted with her RBCs and all panel cells, including all c– panel cells, by the IAT. Her serum was positive with all RBCs tested, and allogeneic adsorptions using selected RBCs with the same patient's antigen profile according to the predicted phenotype from genotype results *(RHD, RHCE*C/C, RHCE*e/e, KEL*2/2, JK*A/A, FY*A/B, GYPB*S/s)* were performed to identify underlying alloantibodies. After allogeneic adsorptions on patient's serum, alloanti-c and alloanti-Jk^b were identified.

After the third transfusion episode, even receiving two units of compatible blood according to her genotype, the DAT was still strongly positive (4+), and a panagglutinin was found in the eluate, suggesting the presence of the autoantibody.

RBC units

A total of three transfusion episodes, during which six different RBC units were transfused, were analyzed retrospectively. In the first transfusion episode, crossmatches performed with serum from a pretransfusion sample and two units of ABO- and D-compatible RBCs were found to be repeatedly negative with PEG-IAT. In the second transfusion episode, crossmatches performed with the absorbed serum of the posttransfusion sample and two units of compatible blood according to her phenotype (group O R_1R_1, K:-1) were negative, and in the last transfusion episode, crossmatches performed with the absorbed serum of the posttransfusion sample and two units of compatible blood according to her phenotype (group O R_1R_1, K:-1, Jk[b–]) were also negative. RBCs from the first donor unit were found to be c+ and Jk(b+), but the other five RBC units were c– and Jk(b–). Retrospectively, the transfusion that was most probably responsible for the immune response–induced alloantibody and autoantibody formation could be traced back to the first transfusion episode, when the c+ and Jk(b+) RBC units were administered.

Discussion

This case demonstrates the development of AIHA after alloimmunization from blood transfusion and hemolytic reaction. The autoimmune hemolysis was initially associated with the appearance of an alloantibody; anti-c was detected in an eluate prepared from the patient's RBCs after the first DHTR. However, 10 days after the last compatible transfusion, the DAT became strongly positive and an eluate prepared from the patient's RBCs indicated the presence of a warm IgG autoantibody, suggesting that it was responsible for the bystander hemolysis. The nadir levels of Hb and Hct during acute hemolysis were substantially lower than the

pretransfusion levels of Hb and Hct, indicating the destruction of autologous RBCs. Based on these observations, we believe that there is clinical evidence of autoimmune hemolysis in the present case.

The development of RBC autoantibodies and AIHA after transfusion was first described by Dameshek, in 1965,[6] when he observed a number of patients who exhibited a positive antiglobulin test after several transfusions. He suggested that some of these reactions had the appearance of an autoimmune reaction, possibly because of cross-reacting antibodies. However, he stated that because the stimulation of exogenous antigen is required to set off the reaction and it is not self-perpetuating, such reactions are actually alloimmune reactions resulting in a temporary state of pseudo-auto immunity.

There is a significant body of literature on the development of RBC autoantibodies and AIHA after transfusion and it is of interest to keep in our minds the possibility of autoimmunity and bystander immune hemolysis. Different reports describe the temporary presence of autoantibodies and AIHA, with either spontaneous resolution in weeks or months or complete response to initial therapy. Some reports describe acute fatal hemolysis, and only a few describe AIHA or autoantibodies persisting for an indefinite time.[14,15]

Data from animal experiments[16–18] show the induction of RBC autoantibodies in mice immunized with rat RBCs. The mice developed autoantibodies, as demonstrated by a positive IAT and DAT. Some mice exhibited AIHA. It is of great interest that persistence of the positive DAT required that the mice be repeatedly immunized with rat RBCs. After immunization was interrupted, the serologic tests returned to normal. When the mice were immunized again with rat RBCs, there was a burst of autoantibody production, and then after 4 to 5 weeks, autoantibody production ceased.

There are several published reports on humans. Lalezari et al.[19] published a report of a 40-year-old woman who had a partial D phenotype and had been transfused with several units of D+ blood, and 10 years later, received three additional units. She had a high titer of anti-D, and this anamnestic response was associated with the development of an autoantibody. The DAT, which had been negative before transfusion, became positive and remained so for 6 months. Chaplin et al.[20] described 5 patients with sickle cell disease who exhibited AIHA. All patients had made alloantibodies in response to transfusion before they made autoantibodies. The patients with AIHA experienced severe anemia and had increased reticulocyte counts. All patients responded to steroid therapy. After discharge the DATs became negative. Argiolu et al.[21] reported 4 patients with thalassemia major in whom they diagnosed AIHA on the basis of an increase in transfusion requirement, associated with the presence of RBC autoantibodies. All the patients were treated with IVIG therapy, and 3 responded as indicated by normalization of the blood consumption. Wodzinski et al.[22] described an extraordinary case of paroxysmal cold hemoglobinuria (PCH) after an ABO-incompatible transfusion. The authors suggested that the ABO HTR triggered or exacerbated an autoimmune response manifesting as PCH. Dameshek and Levine[23] reported a case in which successive transfusions resulted in extreme, almost fatal, hemolysis. In contrast to their later conclusions, Dameshek and Levine[23] at that time suggested that an irreversible autohemolytic process had been established by the multiple transfusions and that this report[6] was published well before the description of autoabsorption or alloadsorption of sera containing autoantibodies to detect the presence of alloantibodies, which could have been the cause of the hemolysis.

Young et al.[1] drew attention to the association between autoimmunization and alloimmunization that occurs commonly but only manifests obvious clinical ill effects on rare occasions. The phenomenon deserves much more attention because it involves basic immunologic mechanisms that have broad implication. Our case reinforces that RBC autoimmunization and the development of AIHA could be recognized as an adverse effect of allogeneic RBC transfusion. A strategy that minimizes exposure to allogeneic blood transfusion would reduce risks of RBC autoimmunization and development of new alloantibodies.

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