Case Report

Major non-ABO incompatibility caused by anti-Jk^a in a patient before allogeneic hematopoietic stem cell transplantation

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A 49-year-old white man with blood group AB, D+ was found to have alloanti-Jk^a and -K when he developed a delayed hemolytic transfusion reaction before allogeneic hematopoietic stem cell transplant (HSCT). Given that his stem cell donor was blood group O, D+, Jk(a+), K–, rituximab was added to his conditioning regimen of fludarabine and melphalan to prevent hemolysis of engrafting Jk(a+) donor red blood cells. The patient proceeded to receive a peripheral blood stem cell transplant from a matched unrelated donor with no adverse events. To our knowledge, this is the first case of successful management of major non-ABO incompatibility caused by anti-Jk^a in a patient receiving an allogeneic HSCT reported in the literature. ***Immunohematology*** **2013;29: 11–14.**

Key Words: anti-Jk^a, major incompatibility, hematopoietic stem cell transplantation

ABO mismatch is well characterized in the setting of hematopoietic stem cell transplant (HSCT). Because of the disparate genetic locations of HLA and ABO genes, ABO mismatch occurs in 30 to 50 percent of transplants.[1] Red blood cell (RBC)–incompatible transplantation does not seem to have an adverse effect on transplant outcomes, such as engraftment, graft-versus-host disease (GVHD), relapse, or survival.[2] However, it does carry the risk of hemolytic transfusion reactions (HTR), and it must be managed appropriately with interventions such as graft processing and proper blood component support.

RBC incompatibility can be classified into two categories. Major incompatibility is when the recipient has antibodies directed against donor RBC antigens. Minor incompatibility is defined as a donor having antibodies directed against recipient RBC antigens. Bidirectional incompatibility, usually in a group A donor with a group B recipient or vice versa, is when there is both major and minor incompatibility. Major incompatibility carries the risk of acute HTR and also delayed RBC recovery after transplant. Minor incompatibility can result in "passenger lymphocyte syndrome," in which the donor B lymphocytes produce antibodies against recipient RBCs that cause a delayed hemolytic transfusion reaction 7 to 12 days after transplantation.

Patients undergoing HSCT require frequent RBC transfusions owing to both the underlying disease and treatment with chemotherapy or radiation.[3] Frequent transfusions predispose patients to developing alloantibodies to non-ABO RBC antigens. RBC alloantibodies may become undetectable over time and with restimulation can cause a delayed HTR if their past existence is not known before transfusion.[4] Non-ABO antigens such as those in the Rh, Kell, and Kidd blood group systems have been implicated as targets for passenger lymphocyte syndrome after transplant.[5–8]

We present a patient who developed an unusual form of bidirectional incompatibility in the form of minor ABO incompatibility, being a group AB recipient with a group O donor, and major non-ABO incompatibility, with preformed anti-Jk^a directed against the donor's Jk(a+) RBCs.

Case Report

A 49-year-old man was diagnosed with primary myelofibrosis in 2007. He was treated with hydroxyurea until May 2011, when he developed worsening anemia and thrombocytopenia that did not improve after hydroxyurea was discontinued. Bone marrow biopsy revealed advanced reticulin fibrosis. The patient had massive splenomegaly of 26 cm. His blood group was AB, D+, and he had a negative screening test for RBC alloantibodies. He had received 38 units of group AB, D+ RBC transfusions over a period of 8 months. Because his antibody screening test was negative, neither the patient nor any of the 38 RBC units were phenotyped for antigens beyond the standard A, B, and D. At this point it was decided that the patient needed an allogeneic HSCT.

The patient was admitted to our hospital for a matched unrelated donor HSCT. He received reduced-intensity conditioning with fludarabine 25 mg/m^2/day given intravenously from Day –9 to Day –5 and melphalan 140 mg/m^2 given intravenously on Day –4. GVHD prophylaxis was initiated with tacrolimus 0.02 mg/kg/day continuous infusion starting on Day –3 and sirolimus 12 mg on Day –3 and 4 mg

daily thereafter. Peripheral blood stem cells were mobilized from the donor with granulocyte colony-stimulating factor, and 8×10^6 CD34+ cells/kg were infused into patient. Patient and donor were 10 of 10 HLA matched, with minor ABO incompatibility as the donor was group O, D+.

On admission to our hospital, the patient's screening test for unexpected RBC antibodies was positive, but no specific antibody could be identified. Because of the positive antibody screen and because the prospective stem cell donor was group O, RBC units selected for transfusion were group O, D+ and crossmatched using an indirect antiglobulin test. On pretransplant Day −8, the patient was transfused with one RBC unit as his hemoglobin was 7.3 g/dL. On pretransplant Day −6, the patient received an additional two units of RBCs as his hemoglobin was again low, 6.8 g/dL. On pretransplant Day −5, the patient was found to have an anti-Jk^a. On further review it was found that one of the RBC units transfused on Day −6 was Jk(a+). At this point the patient's direct antiglobulin test (DAT) was positive with IgG and C3 coating his RBCs. An eluate prepared from his RBCs contained anti-Jk^a. On Day −4, the patient's hemoglobin dropped from 8.4 g/dL to 6 g/dL, his lactate dehydrogenase (LDH) rose to 942 IU/L, and his total bilirubin rose to 2.8 mg/dL, all consistent with a delayed HTR (Fig. 1). He required five units of RBCs to maintain an adequate hemoglobin level over the next 3 days. Given that the donor was Jk(a+), one dose of rituximab 375 mg/m^2 intravenously was added on Day −3 to prevent hemolysis after donor cell engraftment, as well as to reduce likelihood of passenger lymphocyte engraftment given the minor ABO incompatibility.

On pretransplant Day −2, the patient was also found to have an anti-K. No RBC units transfused during this admission were positive for K and the donor was K–, so this was not investigated further.

Materials and Methods

The patient's ABO/D status was verified using monoclonal anti-A, -B, and -D reagents (BioClone, Ortho Clinical Diagnostics, Inc., Raritan, NJ). Initial screens for RBC antibodies were performed using the gel test (MTS Anti-IgG Card, Micro Typing Systems, Inc., Pompano Beach, FL). Monoclonal anti-Jk^a typing reagent (BioClone) was used to detect circulating Jk(a+) RBCs. Monoclonal anti-IgG,

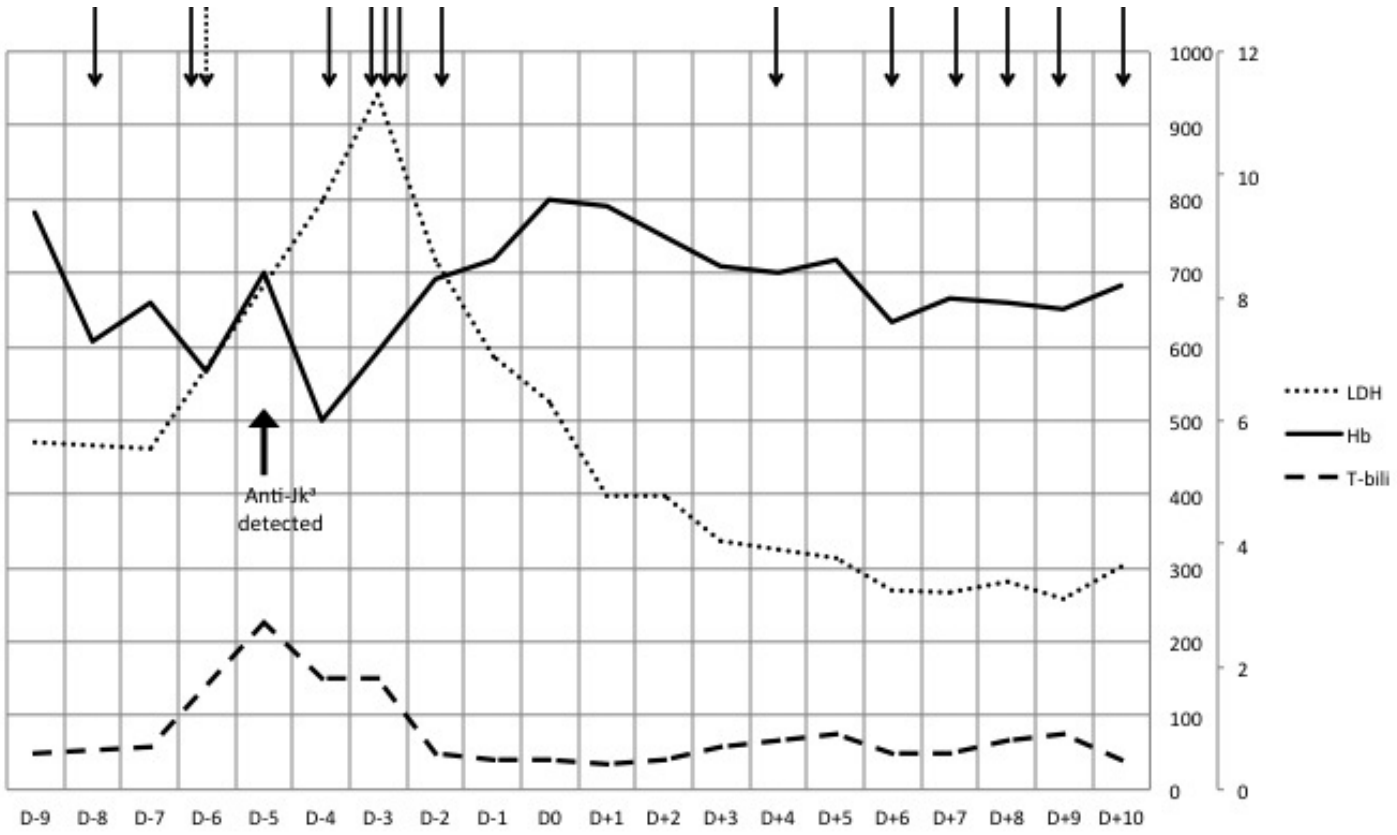

Fig. 1 Patient's hemoglobin (Hb), lactate dehydrogenase (LDH), and bilirubin (T-bili) trend during transplant (occurring on Day 0). Each arrow at the top of the graph represents one transfused unit of red blood cells (RBCs). Solid arrows represent group O, D+, Jk(a–) RBC units; dashed arrow represents the one group O, D+, Jk(a+) RBC unit the patient received on Day −6. Anti-Jk^a was detected on Day −5.

-C3d, polyspecific AHG (BioClone) were used to perform the DAT. Monoclonal Anti-AB containing the ES4 clone (anti-A,B Murine Monoclonal Blend Series 1, Immucor Gamma, Norcross, GA) was used to type the patient's ABO status after transplant. Both the gel test (MTS, Anti-IgG card, Ortho) and automated solid-phase capture assay (Galileo Echo; Immucor Gamma) were used to serially monitor the patient's anti-Jk^a.

Results

By the day of transplantation, the patient's hemoglobin had stabilized, and LDH and bilirubin were back to baseline. After transplantation, the patient received daily RBC transfusions with group O, D+, Jk(a–), K– RBCs from Day +4 to Day +10; during this time there was no increase in markers of hemolysis, and the DAT remained negative. The patient achieved neutrophil engraftment on Day +11. On the same day, the patient first typed macroscopically as group O, D+ Jk(a–), a reflection of the 11 units of matched RBCs transfused during the preceding 2 weeks. On Day +21, the patient typed as O+, Jk(a+), indicating successful RBC reconstitution with donor erythropoiesis. Of note, with standard anti-A and anti-B typing, the patient forward typed as group O; however, with anti-A/B from an ES4 clone, the patient continued to show 1+ reactivity.

The patient's anti-Jk^a was initially detected at a titer of 2 on Day –5. On serial monitoring, the titer decreased to 1 on Day +14 and became undetectable using gel-column assay (MTS Anti-IgG card, Ortho) on Day +17. Using solid-phase adherence assay (Capture, Immucor) the antibody was still detected until Day +40, converted to negative on Day +49, and remained so thereafter. Despite the persistence of detectable antibody, the patient required only two additional units of RBCs, one each on Day +24 and Day +40, and his posttransplant course remained uneventful otherwise.

Discussion

In this case, we report a patient who developed alloantibodies to both Jk^a and K before allogeneic HSCT. Neither of these antibodies was detectable during the time leading up to his admission for HSCT, during which period his transfusion support was provided outside of our institution. Although we do not know the Jk^a status of his prior transfusions, the prevalence of Jk^a and K suggest that at least 15 of the 20 units he received in the 3 months before admission would have been Jk(a+), and 2 units would have been K+. Unfortunately, the patient had almost completed his conditioning regimen for HSCT when the anti-Jk^a and -K were identified. This example of anti-Jk^a detected in our patient was clinically significant as evidenced by its ability to cause a delayed HTR, necessitating aggressive RBC transfusion support for a short time before transplant. There is scant literature available about the management of this type of situation.

The paucity of data regarding this issue likely is because patients undergoing HSCT have a low incidence of alloantibodies relative to the number of RBC transfusions they receive.[9] The lack of alloimmunization is thought to be related to the intense chemotherapy given to patients for their underlying hematologic disease before transplant. Patients like ours constitute a minority of HSCT candidates who did not receive chemotherapy other than hydroxyurea before transplant, and this may have increased his likelihood of developing RBC alloantibodies.

Even when patients have alloantibodies, the traditional conditioning regimen before HSCT with chemotherapy or radiation will usually prevent these antibodies from being able to act effectively against donor RBCs. In one study, of 14 patients who were found to have alloantibodies before transplant, all but one no longer had detectable antibodies after transplant,[9] supporting the hypothesis that RBC alloantibodies can be eradicated in patients with HSCT.

However, as the indications for HSCT expand to include nonmalignant diseases such as thalassemia and sickle cell disease, and the use of reduced-intensity conditioning regimens continues to increase, non-ABO incompatibility issues may become a more prevalent problem in the future. For example, Borge et al.[10] reported a patient with sickle cell disease who also had anti-Jk^a and received HSCT from a Jk(a+) donor. In this case the recipient had delayed RBC engraftment that persisted for more than 180 days after transplant. A nonmyeloablative conditioning regimen was used, consisting of alemtuzumab 1 mg/kg and a single dose of total body irradiation 300 cGy, while oral sirolimus was used for prevention of GVHD.[11]

Although we also used a reduced-intensity conditioning regimen, we believe that in our patient the immunosuppressive agents, fludarabine and rituximab, were key in preventing potential problems. Fludarabine, a purine nucleoside analog, is a highly immunosuppressive agent that causes myelosuppression and prolonged reduction of CD4+ lymphocytes.[12] Rituximab is an anti-CD20 monoclonal antibody that selectively depletes circulating B cells, thus preventing antibody production.[13] These two agents in combination effectively target all the immune cells required to generate an HTR. Eventually, with achievement of complete donor chimerism,

the donor immune cells would be expected to completely eliminate any residual alloantibody-producing cells.

For ABO-incompatible HSCT, delayed RBC engraftment has been associated with the length of time for the isohemagglutinin titers to decrease to clinically insignificant levels (1+ or lower in strength).[14] In our case, the initial anti-Jk^a titer was 2 and became 1+ on Day +14, while donor erythropoiesis was established on Day +21. Thus, it may be useful to monitor antibody titers in the non-ABO–incompatible HSCT setting as an early indicator of whether successful donor erythropoiesis may be achieved. Whether high initial titers or persistent antibody detection warrants further intervention has yet to be determined.

Of note, the increased sensitivity of laboratory methods used in the detection of RBC antigens and antibodies may also cause RBC compatibility issues in HSCT to become more prevalent in the future. For instance, the anti-Jk^a in our patient could be detected on solid-phase capture assay up to 32 days after the gel-column assay became negative. Also, although the patient first typed as blood group O on Day +11, he continued to have detectable A and B on his RBCs when using the more sensitive assay with the ES4 clone.

In conclusion, we present the case of a patient with minor ABO and major non-ABO mismatch who successfully received matched unrelated donor HSCT. We believe that major non-ABO mismatch is an underreported phenomenon that usually does not lead to clinically significant consequences, and clinicians should not be discouraged from using a major non-ABO–incompatible donor if such an incompatibility is discovered before HSCT. However, close monitoring of the recipients with markers for serum hemolysis should be performed during HSCT because severe hemolysis can occur in such situations. Monitoring antibody titers may also be helpful in predicting its clinical relevance. Depending on the level of concern, rituximab may be an additional strategy to use in this situation to avoid hemolysis and ensure successful RBC engraftment. We feel that this is a clinical situation that may become more common in the future given changing patterns of HSCT and the increased sensitivity of assays used to detect RBC antigens and antibodies.

References

1. Mielcarek M, Leisenring W, Torok-Storb B, Storb R. Graft-versus-host disease and donor-directed hemagglutinin titers after ABO-mismatched related and unrelated marrow allografts: evidence for a graft-versus-plasma cell effect. Blood 2000;96:1150–6.
2. Rowley SD, Donato ML, Bhattacharyya P. Red blood cell-incompatible allogeneic hematopoietic progenitor cell transplantation. Bone Marrow Transplant 2011;46:1167–85.
3. Schonewille H, Haak HL, van Zijl AM. Alloimmunization after blood transfusion in patients with hematologic and oncologic diseases. Transfusion 1999;39:763–71.
4. Ramsey G, Larson P. Loss of red cell alloantibodies over time. Transfusion 1988;28:162–5.
5. Leo A, Mytilineos J, Voso MT, et al. Passenger lymphocyte syndrome with severe hemolytic anemia due to an anti-Jk^a after allogeneic PBPC transplantation. Transfusion 2000;40:632–6.
6. Young PP, Goodnough LT, Westervelt P, Diersio JF. Immune hemolysis involving non-ABO/RhD alloantibodies following hematopoietic stem cell transplantation. Bone Marrow Transplant 2001;27:1305–10.
7. Adams BR, Miller AN, Costa LJ. Self-limited hemolysis due to anti-D passenger lymphocyte syndrome in allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant 2009;45:772–3.
8. López A, de la Rubia J, Arriaga F, et al. Severe hemolytic anemia due to multiple red cell alloantibodies after an ABO-incompatible allogeneic bone marrow transplant. Transfusion 1998;38:247–51.
9. Perseghin P, Balduzzi A, Galimberti S, et al. Red blood cell support and alloimmunization rate against erythrocyte antigens in patients undergoing hematopoietic stem cell transplantation. Bone Marrow Transplant 2003;32:231–6.
10. Borge PD, Stroka-Lee AH, Hsieh MM, et al. Delayed red blood cell chimerism in an HSC transplant for sickle cell disease associated with a non-ABO alloantibody (abstract). Transfusion 2010;50(Suppl 2):155A.
11. Hsieh MM, Kang EM, Fitzhugh CD, et al. Allogeneic hematopoietic stem-cell transplantation for sickle cell disease. N Engl J Med 2009;361:2309–17.
12. Fenchel K, Bergmann L, Wijermans P, et al. Clinical experience with fludarabine and its immunosuppressive effects in pretreated chronic lymphocytic leukemias and low-grade lymphomas. Leuk Lymphoma 1995;18:485–92.
13. Zecca M, Nobili B, Ramenghi U, et al. Rituximab for the treatment of refractory autoimmune hemolytic anemia in children. Blood 2003;101:3857–61.
14. Bolan CD, Leitman SF, Griffith LM, et al. Delayed donor red cell chimerism and pure red cell aplasia following major ABO-incompatible nonmyeloablative hematopoietic stem cell transplantation. Blood 2001;98:1687–94.

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