Transfusion policy: when to stop the use of extremely rare blood for an allogeneic hematopoietic progenitor cell transplant recipient with a history of red cell alloimmunization

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BACKGROUND: Decisions for when to select, and when to discontinue, antigen-negative blood in hematopoietic progenitor cell transplantation (HPCT) recipients with red blood cell (RBC) antibodies can be confusing. In HPCT performed for sickle cell anemia patients who require extremely rare antigen-negative blood, the balance of caution and practicality is further complicated.

CASE REPORTS: Four sickle cell anemia patients with current or historic RBC antibodies underwent allogeneic HPC transplantation. One required extremely rare (group O D–, hrB–) blood. None of the antibodies caused significant hemolysis after transplant. In the case requiring rare blood, antigen-negative blood was requested after donor RBC engraftment because of incomplete donor white blood cell (WBC) chimerism.

CONCLUSIONS: RBC antibodies derived from a recipient of allogeneic HPCT rarely cause significant hemolysis, in contrast to the more severe picture sometimes seen with donor-derived antibodies. When donor WBC chimerism is delayed past the time of donor RBC engraftment, there can be concern for the possibility of future recipient-type antibody production. Even 100 percent donor lymphocyte chimerism is no guarantee of total host plasma cell ablation. Immunoglobulin allotyping, when informative, can suggest chimerism for several years. Recipient-type blood, when extremely rare, may not be available for that duration.

For peripheral blood hematopoietic progenitor cell (PBHPC) transplant recipients with red blood cell (RBC) antibodies, recipient-type antigen-negative RBC products are transfused immediately after transplantation, to reduce the risk of hemolytic reactions, delayed engraftment, and pure RBC aplasia (PRCA). Once the RBCs from the patient are typing as donor, appropriate transfusion practice is not well established. For a recipient with a history of RBC alloantibodies, at what point after transplant may donor-type RBCs be used for transfusion? A conservative view is to wait until lymphocytes type 100 percent donor, as residual recipient lymphocytes may resume production of RBC alloantibodies with anti-donor specificity. Alternatively, one might switch to donor-type blood when a set period of time has elapsed since the transplant, or when RBC engraftment has occurred, during or after the period of mixed-field typing. We report four cases of sickle cell patients who had RBC antibodies before PBHPC transplant and examine the evidence for various transfusion strategies. One of these patients (Case 1) required extremely rare blood, which produced a dilemma for providing antigen-negative RBC products and thereby

ABBREVIATIONS: ABO-M = mismatched; HPBPCT = hematopoietic peripheral blood progenitor cell transplantation; HPCT = hematopoietic progenitor cell transplantation; PBHPC = peripheral blood hematopoietic progenitor cell; PRCA = pure red cell aplasia.

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accelerating the switch to donor-type RBC products. In transplantation for sickle cell anemia, RBC antibodies may engender more caution than in stem cell transplantation for malignancies or other conditions not involving RBCs, because the remote possibility of PRCA could represent a serious complication.

MATERIALS AND METHODS

Hematopoietic PC source and conditioning regimen

All four patients underwent bone marrow transplants (BMTs) from HLA-identical siblings as treatment for sickle cell disease. Fully myeloablative regimens were used for cytoreduction. The chemotherapy-only regimens included busulfan and cyclophosphamide or busulfan and fludarabine and graft-versus-host disease (GVHD) prophylaxis included tacrolimus or cyclosporine and methotrexate or prednisone.

Donor chimerism was assessed in sex-mismatched donor-recipient pairs by metaphase karyotype analyses and fluorescent in situ hybridization of the X and Y chromosomes in interphase cells. Restriction fragment length DNA polymorphisms (RFLPs) were used for chimerism monitoring for sex-matched pairs.

CASE REPORTS

Patient 1

A 13-year-old hemoglobin (Hb) SS girl, group O, D−E−hrB− received a BMT from a group O, D+E+hrB+ sibling. The patient had numerous transfusions during her hip surgery at age 2. Seven years before transplant, anti-hrB was identified in the patient’s serum sample. This antibody became undetectable in subsequent samples during the 6 years before transplant. The patient was frequently hospitalized for vasocclusive crises but rarely received transfusions. Anti-E was identified 3 years before transplant and also subsequently became undetectable. Four months before transplant, the patient received two partial exchange transfusions for a frontal lobe infarct. She did not receive transfusions from family members.

After transplant, the patient required 10 units of RBC products, the last product on Posttransplant Day +38 just after an urgent appendectomy. All 10 units were D−, hrB− from frozen rare blood stores. On Posttransplant Day +41, the patient’s RBCs typed mixed field with anti-D. On Day +45, the patient typed O D+ with no unagglutinated RBCs. The patient’s Hb level stabilized and there were no signs of hemolysis. Peripheral blood white blood cell (WBC) chimerism by RFLP showed 50 percent donor on Day +15, 90 percent donor on Day +30, 50 percent donor on Day +82, and 50 percent donor on Day +131. Marrow chimerism was 72 percent donor on Day +131 (not shown).

RBC phenotype was assessed by RBC agglutination to anti-D. The patient typed D− before Day +41, then mixed-field with anti-D on Day +41, and fully D+ beginning on Day +43. The arrows represent 10 RBC transfusions that took place on Days +4, +5, +10, +13, +19, +24, +35, +38, and +38.

Patient 2

A 19-year-old Hb SS male, group B D+, received a BMT from a group O D+, HLA-identical sibling. Both recipient and donor were negative for the presence of K. The recipient had a previous history of four transfusions and had anti-K (2+ strength) before transplant. He received 4 additional units of K− RBCs just before transplant. Anti-K persisted at 2+ to 3+ strength, through Posttransplant Day +31, after which antibody screens were not performed. He required 7 RBC products between Posttransplant Days 0 and +18. The day of switch from group O to group B was not documented, because no ABO typing
was performed after transplant. Engraftment studies revealed him to have 100 percent donor cells by fluorescent in situ hybridization performed on peripheral blood. He required no additional transfusions, and his antibody screen was not followed until 1.5 years after transplant, when both indirect antiglobulin test (IAT) and direct antiglobulin test (DAT) were negative.

**Patient 3**

An 11-year-old SS girl, group O D+C-, received a BMT from a group O D+C+, HLA-identical sibling. The recipient had a remote history of anti-C, along with a warm autoanti-e and a cold autoantibody. She had been on a hypertransfusion protocol, which was discontinued due to her reactions to chelation therapy. Her antibody screen was negative at the time she was evaluated for transplant, and the screen remained negative throughout follow-up. She received 10 units of C– RBCs after transplant, in the face of severe hemorrhagic cystitis. Full engraftment—with 100 percent donor chimerism on peripheral blood—took place, and the patient was well 1 year after transplant.

**Patient 4**

An 8-year-old S Lepore girl, group A D–, received a BMT from a group A D+ HLA-identical sibling. The recipient had a history of one RBC transfusion and received 2 additional units of RBCs in the months just before transplant. Her DAT and IAT were positive, and a panagglutinin with some autoanti-e specificity was identified. No alloantibodies were apparent. After transplant, she received 9 units of RBCs. Her IAT became negative on Posttransplant Day 16. Her RBCs typed mixed-field with anti-D on Posttransplant Day 75 and subsequently typed as D+. That patient developed chronic GVHD and remains on immunosuppressive treatment. Her chimerism studies have shown 100 percent donor engraftment by RFLP performed on peripheral blood.

**DISCUSSION**

Patient 1 required group O blood negative for the presence of both D and hr\textsuperscript{a} antigens. This phenotype is extremely rare, comprising less than 0.01 percent of donors. The alloantibodies were no longer detectable before transplant and did not resurface after transplant. RBC engraftment occurred successfully, making the patient’s RBCs type D+, hr\textsuperscript{a+}. The transplant was ABO-matched. The rarity of the blood (<0.01% of donor units) made it extremely difficult to provide antigen-negative blood to this recipient. Although RBC engraftment occurred around Day +41, the patient developed mixed WBC chimerism (Fig. 1).

In a study by Walters and coworkers,\textsuperscript{1} approximately 25 percent of children with sickle cell disease developed stable mixed chimerism after HLA-identical sibling PBPC transplant. None of these patients experienced clinical complications related to sickle cell disease after transplantation, and only one patient required RBC transfusion beyond 90 days after transplantation. Several factors were evaluated for their association with developing stable mixed chimerism and/or rejection after myeloablative transplantation. These included RBC transfusion history, patient age, patient sex, donor sex, donor Hb genotype, incidence of RBC alloimmunization, and presence or absence of chelation therapy. Of these factors, only one achieved significance: patients younger than 10 years were more likely to develop mixed chimerism than those who were older than 10 years. There was a trend suggesting that patients receiving chelation therapy for transfusional iron overload had an increased risk of recurrent sickle cell disease. In the patients who developed mixed chimerism, there was no subsequent graft rejection or disease recurrence. This observation contrasts with observations after transplantation for cases of \(\beta\)-thalassemia major, in which mixed chimerism was associated with an increased risk of disease recurrence.\textsuperscript{2}

The immunologic basis for development of stable donor-host chimerism after myeloablative hematopoietic progenitor cell transplantation (HPCT) for sickle cell disease remains incompletely understood. Some individuals with stable chimerism after HPCT for thalassemia had oligoclonal representation of V\(\beta\) family T-cell populations, suggesting that the emergence of selected T-cell clones may be responsible for establishing bidirectional donor-host tolerance.\textsuperscript{3}

For Patient 1, WBC chimerism status was assessed by molecular methods, with granulocytes and lymphocytes together. After Day +45, she had 100 percent donor RBC chimerism status by agglutination. Even so, she could be a partial chimera for the erythroid lineage. Kean and others\textsuperscript{4,5} noted an enrichment of donor RBCs compared to donor WBCs in the peripheral blood of sickle mice after nonmyeloablative transplantation. There was no apparent selective advantage of donor RBC precursors in the marrow. Indeed, Wu and associates\textsuperscript{6} demonstrated higher expression of donor-derived \(\beta\)-globin RNA relative to the level of donor-derived genomic DNA in patients with sickle cell disease who developed stable mixed chimerism after transplant. Analysis of chimerism in immature and mature erythroblasts confirmed the intramedullary loss of SS erythroblasts with progressive maturation. Ineffective erythropoiesis of SS progenitors thus provides a maturation advantage for AA or SA donor erythroid precursor cells.

Case 2 is an ABO minor mismatch, which requires clinical vigilance at the time of transplantation. Donor-derived anti-A or anti-B can cause severe hemolysis. In
ABO minor mismatch, group O RBCs should be selected after transplantation even though the patient typing does not appear to require this.\(^7\) In contrast, selection of blood for Cases 3 and 4 is more straightforward, because recipient-type blood is continued as long as the patient types negative for C, and for Case 4, units negative for the presence of D are used while the patient types as D−. After RBC engraftment, usual practice is to switch to donor-type units.

Major ABO-mismatched (ABO-M) allogeneic PBPC transplantation can be complicated by delayed erythroid engraftment,\(^9\) ineffective erythropoiesis,\(^12\) and/or alloimmunization to the Rh system occurred in none of 10 of 150 BMT patients (7%). Three of 78 D− recipients of D− grafts (4%) developed anti-D after receiving D+ platelets (PLTs; not RBCs).\(^16\) None of these patients experienced complications. Most authors do not recommend prophylaxis with RhIG during transfusion of D+ PLTs to D− recipients of D− grafts.\(^17\)

A mother whose serum sample contained anti-D, anti-C, and anti-E, however, received a PBPC transplant from her son, who had suffered from hemolytic disease of the newborn.\(^18\) She experienced hemolysis of all RBCs transfused. Perseghin and associates\(^20\) reported that 10 of 218 patients (6%) developed alloantibodies, and all but one of these alloantibodies were anti-Jka.\(^2\) The need to provide D− blood to the patient in Cases 3 and 4 is more straightforward, because the conditioning regimen appears to be less toxic to host plasma cells. In one study, 4 of 24 nonmyeloablative major ABO-M transplant recipients (29%) experienced PRCA.\(^13\) Of the 4 PRCA patients, one had anti-K and another had anti-K, anti-C, and anti-e, which were present before transplant and remained so for 5 months afterward. They resolved within 5 weeks from the time that anti-A/anti-B titers declined. One group comprising 23 nonmyeloablative major ABO-M transplant recipients found no delay in RBC engraftment, but described 2 cases of transient PRCA.\(^14\) In another series of 8 nonmyeloablative major ABO-M transplant recipients, 1 patient experienced PRCA in the presence of alloanti-Jka.\(^9\) Whether the anti-Jk\(^a\) contributed to the development of PRCA is unclear. Because PRCA caused by non-ABO alloantibodies has been reported only in association with major ABO-M, specifically in the nonmyeloablative setting, this complication is of theoretical concern in Case 1.

In Cases 1 and 4, the recipients are D− and the donors are D+. The need to provide D− blood to the patient in Case 1 made the case much more difficult. Patients with anti-hr\(^b\) antibodies who are D+ can usually receive D+, e−, units, which are not as rare as D−, hr\(^a\)− units. Surprisingly, alloimmunization to the Rh system occurred in none of 9 D− recipients of D− nonmyeloablative grafts despite having been transfused with between 7 and 500 mL of D+ RBCs.\(^15\) None of these patients experienced complications. Most authors do not recommend prophylaxis with RhIG during transfusion of D+ PLTs to D− recipients of D− grafts.\(^17\)

Table 1 reviews the incidence of non-ABO RBC alloantibodies formed after PBHPC transplant. Abou-Elella and coworkers\(^19\) found that 4 of 193 BMT patients (2.1%) developed RBC alloantibodies from the time of hospital admission for BMT to hospital discharge. This alloimmunization rate was only 0.1 percent per unit of RBCs transfused. Persoghin and associates\(^20\) reported similar rates for the post-HPBPCT period. In the pre-HPBPCT period, 10 of 218 patients (6%) developed alloantibodies, and all but one of these alloantibodies disappeared after HPBPCT. Ting and colleagues\(^21\) found that 10 of 150 BMT patients (7%) formed non-ABO RBC alloantibodies. In 7 of these 10 cases, the antibodies were likely produced by cells of the engrafted marrow.\(^1\) Two patients with a previous anti-D did not form anti-D after transplant of PBHPCs from a D+ donor.\(^23\) In another series, 8 of 217 HPBPCT recipients (3.7%) developed alloantibodies.\(^22\) Of these, 4 were ABO-M transplants; the

<table>
<thead>
<tr>
<th>Type of transplant</th>
<th>Number of patients immunized</th>
<th>Alloimmunization rate per unit of RBCs transfused</th>
<th>Specificities to the following antigens</th>
<th>Length of follow-up</th>
<th>Day of antibody formation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMT</td>
<td>4 of 193 (2.1%)</td>
<td>0.1%</td>
<td>E, Jk(^a), M, Lu(^14)</td>
<td>Median, 4 months (range, 1-18 months)</td>
<td>Median, Day 32 (range, Day 12 to 11 months)</td>
<td>Abou-Elella(^17)</td>
</tr>
<tr>
<td>HPBPCT</td>
<td>3 of 215 (1%)</td>
<td>1.2/1000</td>
<td>E, M</td>
<td>58.7 ± 25.9 days</td>
<td>+58, +90, +210</td>
<td>Persoghin(^20)</td>
</tr>
<tr>
<td>BMT</td>
<td>10 of 150 (7%); 3 likely due to host B cells</td>
<td></td>
<td>Jk(^a), E-like, K-like, M, Le(^a), N, Jk(^b), Le(^e)</td>
<td></td>
<td>Median, Day 23</td>
<td>Ting(^21)</td>
</tr>
<tr>
<td>HPBPCT</td>
<td>8 of 217 (3.7%); 2-4 likely due to host B cells</td>
<td></td>
<td>D, K(2), Jk(2), M(2), Le(^a)</td>
<td></td>
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<td>de la Rubia(^22)</td>
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association of ABO mismatch and RBC alloimmunization was significant. In 2 cases, 1 anti-D and 1 anti-Jk<sup>a</sup>, the antibody was directed against the donor’s RBC antigens. In 4 cases, the antibody was against an antigen absent in both donor and recipient. Only 2 patients developed severe immune hemolytic anemia in the early posttransplant period.23

In addition to the above case series, there are several reports of recipient RBC alloantibodies persisting after HPCT.23-26 In these cases, hemolysis was generally mild and transient. In the most severe case of this group, the recipient had anti-E and anti-c and was successfully transplanted from an E+c+ donor.26 The anti-E and anti-c remained detectable even 20 months after BMT, and the DAT was still positive. This patient experienced chronic hemolytic anemia until receiving prednisolone on Day 221.

Transfusion practices in major ABO-M PBHPC transplantation vary among centers. Frequency of monitoring the RBC typing varies, and the endpoint for providing recipient-type (usually O) RBCs also varies. According to one survey, 73.5 percent of centers provided O (or recipient-type) RBCs until recipient RBCs are no longer detectable, whereas 14.7 percent switched after 1 month posttransplant, 2.9 percent after 2 months, 5.8 percent after 3 months, and 2.9 percent after 6 months post-transplant.27

What methods for chimerism studies can give a physician confidence that host plasma cells have been ablated? In one case, polymerase chain reaction (PCR)-based analysis of short-tandem repeat microsatellite regions from immunomagnetically isolated peripheral B cells showed 100 percent donor origin at Day 216, but the patient had detectable anti-donor RBC antibodies even at Day 360.28 Griffith and coworkers28 performed PCR-based analysis of short-tandem repeats on peripheral blood B and T cells and on aspirated marrow plasma cells as well. Recipient plasma cells were present in all of six cases of PRCA after nonmyeloablative ABO-M transplants, despite 100 percent donor B cells in two cases with high anti-donor isohemagglutinins.

Immunoglobulin allotyping is another technique for detecting B-cell chimerism. Van Tol and coworkers29 found immunoglobulin G (IgG) allotype differences to be much more sensitive than other methods for detecting B-cell chimerism. More than a year after BMT, IgG of recipient origin persisted in 15 of 18 informative recipients (83%), despite the fact that the circulating B cells appeared to be entirely of donor origin at that time. Other investigators have reported similar results (reviewed in Petz and Garratty8). Immunoglobulin allotypes show a low polymorphism and are noninformative in the majority of donor-recipient couples.29 Thus, lymphocyte chimerism studies cannot guarantee a lack of host plasma cells.

RBC alloantibodies are rare in the PBHPC transplant setting. Those which are formed are usually transient, and existing alloantibodies usually disappear during or after the conditioning regimen. Indeed, this occurred with all four cases reported here. In a situation where a RBC alloantibody is detectable in patient serum, standard practice is to give antigen-negative blood until the IAT becomes negative for that alloantibody.7 Checking that an eluate is also negative could provide an additional measure of confidence. When, however, the RBC alloantibody is historical and currently undetectable, antigen-negative blood may be transfused as an extra precaution against delayed RBC engraftment and/or RBC aplasia, until donor RBC engraftment occurs successfully. Every case is unique. When a patient is transplanted not for malignancy but rather for sickle cell disease, even a remote risk of RBC aplasia cannot be tolerated. Indeed, delayed lymphocyte chimerism has been seen after nonmyeloablative transplant in sickle cell patients (J. Tisdale, oral communication). This observation has prompted additional concern about the potential for reactivation of RBC alloantibody production, particularly in the setting of major ABO-M nonmyeloablative transplantation.

In summary, Case 1 required extremely rare group O blood, negative for the presence of both D and hr<sup>b</sup> antigens, comprising less than 0.01 percent of donors. The alloantibodies were no longer detectable before transplant and did not resurface after transplant. RBC engraftment occurred successfully, making the patient’s RBCs type D+, hr<sup>b+</sup>. The transplant was ABO-matched. The rarity of the blood (<0.01% of donor units) made it extremely difficult to provide antigen-negative blood to this recipient. In the face of a negative IAT and negative DAT, we believe that it is appropriate to give D+, hr<sup>b+</sup> blood for this particular patient after donor RBC engraftment, regardless of the WBC chimerism status. Even 100 percent lymphocyte chimerism is no guarantee of total host plasma cell ablation. Immunoglobulin allotyping, if informative, would likely suggest chimerism for several years, and continuing to transfuse extremely rare recipient-type units for this duration would not be practical.

To conclude, antigen-negative RBCs are used after HPCT until erythroid engraftment appears complete by agglutination testing. When feasible, antigen-negative RBCs can be used in cases of stable mixed WBC chimerism. In the case of historic, undetectable alloantibodies, the risk of developing late hemolysis is low. If the antigen-negative RBCs are extremely rare, continuing the antigen-negative units would unnecessarily deplete resources. Fortunately, the likelihood of needing further transfusion after erythroid engraftment is extremely low. In the study where 13 of 50 patients with clinically successful allotransplants developed stable mixed chimerism after myeloablative HPCT,3 only 1 patient required RBC trans-
fusion beyond 90 days after transplantation, and that patient subsequently became transfusion-independent.

REFERENCES


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