Passenger lymphocyte syndrome after ABO-incompatible allogeneic hematopoietic stem cell transplantation; dynamics of ABO allo-antibody and blood type conversion

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ABSTRACT
Passenger lymphocyte syndrome (PLS) is a specific subtype of graft versus host disease (GVHD) following allogeneic hematopoietic stem cell transplantation (allo-HSCT) characterized by an immune-mediated hemolysis caused by donor-derived B cells. However, precise nature of PLS has not been well characterized due to its rarity. We herein report two cases of PLS following ABO-incompatible HSCT whose clinical course and dynamics of anti-ABO allo-antibody and blood type conversion were closely examined. Both cases demonstrated acute hemolysis upon engraftment, and the presence of high titer allo-antibody against recipients' red blood cells (RBCs) helped us to reach the diagnosis of PLS. Hemolysis in both cases showed spontaneous improvement with prednisolone and supportive therapy including transfusion and fluid support. In one case with blood type O, the patient recursively developed PLS in the second and the third HSCT from ABO-mismatch donors, leading to a hypothesis that original blood type O may serve as a background for acute elevation of serum anti-ABO antibody and therefore a risk for developing PLS in multiple ABO-incompatible HSCTs. When hemolysis is noted following ABO-incompatible HSCTs, PLS should be considered and measurement of anti-ABO antibodies is warranted.

KEYWORDS
Passenger lymphocyte syndrome; hemolysis; hematopoietic stem cell transplantation; graft-versus-host disease

Introduction
Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is widely performed as a curative treatment for various hematological diseases. Passenger lymphocyte syndrome (PLS), a rare subtype of graft-versus-host disease (GVHD), is a hemolysis following minor or bidirectional ABO-incompatible HSCT [1–5]. In PLS, B lymphocytes derived from the donor graft produce antibodies against the recipient’s red blood cells (RBCs), which then leads to hemolysis 1–3 weeks after allo-HSCT [4–7]. Hemolysis occasionally becomes critical with organ dysfunctions and disseminated intravascular coagulation (DIC) [2,7]. However, due to its rarity, the risk factors and prophylaxis for PLS have not been well characterized, and optimal management of PLS remains obscure. Here we report two cases of PLS, whose clinical course, dynamics of anti-ABO antibody titers and blood type conversion were closely monitored and characterized.

Case reports
Case 1: A 38-year-old woman with blood group O, Rh+ underwent the first allogenic bone marrow transplantation (BMT) from an unrelated Human Leucocyte Antigen (HLA)-matched male donor with blood group B, Rh+ for mixed phenotype acute leukemia. The conditioning regimen was cyclophosphamide 120 mg/kg and total body irradiation (TBI) 12 Gy, and the prophylaxis for GVHD was tacrolimus and methotrexate (10 mg/m² on day 1 and 7 mg/m² on days 3 and 6). She had remained in complete remission (CR) until two years after the first HSCT, when her leukemia relapsed. She received re-induction chemotherapy followed by the second BMT from another unrelated HLA-matched male donor with group A, Rh+. Before the second BMT, her blood type had been converted to B, Rh+. The conditioning regimen for the second BMT consisted of fludarabine 180 mg/m², intravenous busulfan 12.8 mg/kg, and TBI 2 Gy, and the prophylaxis for GVHD was tacrolimus and methotrexate. Serum was removed from the graft before infusion. Engraftment was achieved on day 14, however, her hemoglobin (Hb) levels dropped suddenly from 7.8 g/L (day 16) to 3.8 g/L (day 19). Serum lactate dehydrogenase (LDH) and indirect bilirubin levels were elevated to 739 IU/L and 1.6 mg/dL, respectively, and
Figure 1. Clinical course of case 1. (A) Clinical course of the second HSCT for case 1. Massive hemolysis requiring RBC transfusion coincided with an acute increase of anti-B antibody titer several days following engraftment on day 14. Blood type converted from B (recipient type) to A (donor type), and the titer for anti-A antibody became weak after hemolysis. (B) Clinical course of the third HSCT for case 1. Massive hemolysis requiring RBC transfusion presented simultaneously with increased titers of anti-A and anti-B antibodies upon engraftment on day 17. Blood type converted from A (recipient type) to O (donor type) with a resolution of hemolysis.
serum haptoglobin was undetectable (<1 mg/dL) (Figure 1(A)). The direct globulin test (DAT) was negative on day 19, however, testing for anti-RBC antibody showed a high titer of anti-B antibodies (4+) on and after day 19. Based on these clinical data, the patient was diagnosed with PLS, an acute hemolytic reaction caused by donor lymphocyte-derived antibodies directed against the recipient’s RBCs. Of note, there were no findings suggestive of transplanted thrombotic microangiopathy (TA-TMA) after allo-HSCT, such as increased fragmented red blood cells (schistocytes) in peripheral blood smears, proteinuria, the change of blood pressure and neurological symptoms, thus excluding the possibility of hemolytic anemia associated with TA-TMA. She had no organ dysfunction and the hemolysis improved by day 26 without additional immunosuppressive therapy. As shown in Figure 1(A), type-B RBCs disappeared on day 19 and the blood type converted to type-A on day 26. Hemolysis resolved by day 26 with a clearance of type-B RBCs and lowering of anti-A antibody titer.

Twelve months after the second allo-HSCT, leukemia relapsed again and she received re-induction chemotherapy. After achieving CR, she went on to receive the third BMT from an unrelated HLA-DR 1 locus mismatch donor with blood type O, Rh+. The conditioning regimen consisted of fludarabine 125 mg/m², melphalan 140mg/m² and TBI 2 Gy, and the prophylaxis for GVHD was the same as those in the first allo-HSCT. RBCs and serum were separated and removed from the graft before infusion. She achieved engraftment on day 17. However, she again presented moderate hemolysis on day 17 with acute decline of Hb levels (3.9 g/dL), elevation of LDH (310 IU/L) and indirect bilirubin (3.0 mg/dL) levels, and high serum titers of anti-A and anti-B antibodies, which were compatible with PLS (Figure 1(B)). Interestingly, however, DAT was negative on day 17, possibly due to almost complete destruction of the recipient’s RBC. TA-TMA-induced hemolytic anemia was ruled out because there were no laboratory and clinical findings supporting TA-TMA. PLS was mild and resolved without additional immune suppression with full conversion of blood type from A to O.

Case 2: A 20-year-old male with blood group B, Rh+ underwent bidirectional ABO-incompatible BMT from an HLA-matched unrelated male donor with blood group A, Rh+. The conditioning therapy was myeloablative, consisting of oral busulfan 16 mg/kg and cyclophosphamide 4500 mg/m², with tacrolimus and methotrexate (10 mg/m² on day 1 and 7 mg/m² on days 3 and 6) for GVHD prophylaxis. RBCs and serum were separated and removed from the graft before infusion. The patient achieved engraftment on day 14 without adverse events. However, his Hb level dropped acutely from 8.4 g/L (day 19) to 6.6 g/L (day 21), with concomitant elevations in LDH (326 IU/L) and serum creatinine (Cre) levels (1.07 mg/dL) (Figure 2). Further tests revealed normal reticulocytes, a prominent decrease in haptoglobin levels (2 mg/dL), a positive DAT, and a high titer of anti-B antibodies (3+ to 4+). These findings were compatible with PLS. TA-TMA-induced hemolytic anemia was ruled out because of the lack of findings supporting TA-TMA such as increased schistocytes in peripheral blood smears, proteinuria, high blood pressure and neurological symptoms. Although 0.5 mg/kg of oral prednisolone (PSL) was started on day 21, his LDH continued to increase to 2667 IU/L, and renal function exacerbated (Cre 1.50 mg/dL) on day 24. We subsequently increased PSL dosage to 1.0 mg/kg and stopped tacrolimus. His LDH and Cre levels improved on day 28, and he became completely independent of RBC transfusion. Blood typing and anti-ABO antibody monitoring showed that, on day 21, peripheral RBCs were a mixture of type-A and -B with an extremely high titer (3+) of anti-B antibody (Figure 2). Recipient’s type-B RBCs disappeared and donor-derived type A RBC became dominant on day 28 when hemolysis improved, although anti-A antibody still existed at low levels (1+).

Discussion

In PLS, it is considered that B lymphocytes in the allograft recognize recipients’ RBCs, leading to the production of anti-recipient ABO antibodies and hemolysis [4,5,7]. PLS also occurs following solid organ transplantation [4,8], and the amount of lymphocytes transplanted with the organ appears to be associated with the incidence of PLS [9]. In allo-HSCT, risk factors for PLS are: (1) transplantation from an ABO-minor mismatched donor (especially, in the paring of group A recipients and group O donors), (2) the use of peripheral blood stem cells as a donor source rather than bone marrow (no PLS has been reported following cord blood transplantation), (3) the absence of methotrexate for GVHD prophylaxis, and (4) reduced intensity conditioning therapy before transplantation [1,3,6–8]. It should be noted that reduced-intensity conditioning regimens differ widely in their myeloablative and immunosuppressive capacities and thus data from the literature on PLS may not be entirely comparable. In our cases, both patients did not have obvious risk factors except ABO-minor mismatched transplantation. Although such risk assessment may be helpful in some cases, prediction of PLS is still difficult in the current practice. It has been reported that the titer of anti-recipient ABO antibodies in the donor serum before transplantation does not serve as a predictor of the incidence or severity of PLS [5]. In contrast, close monitoring of anti-recipient ABO antibodies after allo-HSCT may be helpful for predicting PLS. Abe et al. analyzed the results of anti-ABO
antibody testing in 61 cases following allo-HSCT from minor or bidirectional ABO-incompatible donors. They found 6 cases with transiently elevated anti-recipient ABO antibodies and all cases had hemolytic findings [10]. From these data, the authors suggested that PLS could be predicted by monitoring anti-recipient ABO antibodies after transplantation. Notably, it was reported that anti-recipient ABO antibodies are associated with acute GVHD and may be useful to predict acute GVHD and poor outcome [11,12].

It is noteworthy that Case 1 experienced recurrent PLS both after the second and the third HSCT. It is well known that ABO antigens are expressed not only on RBCs, but also on a wide variety of human tissues, leading to the hypothesis that a significant amount of anti-ABO allo-antibody can be trapped by systemically expressed ABO antigens. If this hypothesis is correct, systemic absorption of anti-ABO allo-antibody will not occur in type O recipients, which may exacerbate the elevation of donor-derived anti-ABO allo-antibody in the patients’ serum, aiding a development of PLS. We speculate this is one of the mechanisms why Case 1 demonstrated recurrent PLS after the second and the third HSCT. This hypothesis must be confirmed by further investigation of similar cases.

Preventive means and standard treatment for PLS have not been established. A previous study proposed that effective prophylaxis for PLS involves reducing the lymphocyte number in the marrow products [7]. However, this is not always possible in routine practice. The management of PLS has been essentially supportive [7]. For severe cases of massive hemolysis and organ dysfunction, immunosuppressive therapies, such as prednisolone and calcineurin inhibitor, and rituximab have been proven to be effective [3,5,7,13]. Despite these observations, standard management of PLS, including practical approaches and timing and duration of treatment, is not clear and it remains to be established in clinical research.

Our cases are intriguing in that the titer of anti-ABO antibodies and conversion of blood types were closely monitored during the course of PLS. In case 1, serum concentration of tacrolimus decreased to less than 10 mg/dL on days 9-16, before the onset of PLS in the second HSCT (Figure 1(A)). Calcineurin inhibitors suppress humoral immunity by acting on naive B cells, and we speculate that suboptimal concentration of tacrolimus may lead to the production of anti-recipient’s ABO antibodies through insufficient suppression of B cell activation [14]. Another interesting point in case 1 is that the DAT was negative in both episodes of PLS, possibly due to massive destruction of antibody-sensitized recipient’s RBCs.

Conclusion

We report 2 cases with PLS after ABO-incompatible allo-HSCT. When hemolytic anemia is noted following
ABO-incompatible allo-HSCT, anti-recipient ABO antibodies should be measured at an early point. In addition, type O patients who underwent the prior HSCT with type A or type B donor may have a higher risk for developing PLS in the following HSCTs from ABO-mismatch donors. Additional investigation is required to further reveal the clinical course and mechanism of PLS and to establish its appropriate management.

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