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【 CASE REPORT 】

Persistent Expression of Recipient-type ABH Antigen after ABO-incompatible Allogeneic Hematopoietic Stem Cell Transplantation

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Abstract:

In general, the recipient's ABO blood type changes to the donor's ABO blood type after ABO-incompatible allogeneic hematopoietic stem cell transplantation (HSCT). However, we experienced a 26-year-old male with acute myelogenous leukemia (AML) who underwent ABO-incompatible HSCT twice and persistently showed his original blood type even after demonstrating complete donor-type chimerism. Based on the results of various examinations, we considered that the antigen of the recipient's original blood type persistently synthesized in the recipient's non-hematopoietic organs was secreted and adsorbed on the surface of donor-derived RBCs. We should therefore perform detailed examinations to determine the precise blood type after ABO-incompatible HSCT.

Key words: allogeneic HSCT, blood type, ABO blood group antigen, ABO-incompatibility, transfusion

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Introduction

The plasma membranes of red blood cells (RBCs) mainly consist of lipids and proteins, which are covered with carbohydrate chains. ABO blood types are determined by the type of sugars according to the enzymes encoded by the ABO gene and they are used to build these carbohydrates on the surface of RBCs. In the setting of allogeneic hematopoietic stem cell transplantation (HSCT), RBCs are produced by donor-derived hematopoietic stem cells after HSCT, which express carbohydrates including sugars based on the donor's ABO gene. Therefore, in general, the recipient's ABO blood type ultimately changes to the donor's ABO blood type after ABO-incompatible HSCT (1-3). However, we experienced a 26-year-old male patient with acute myelogenous leukemia (AML) who underwent ABO-incompatible HSCT twice and persistently showed his original blood type even with AML in complete remission (CR) and proven complete donor-type chimerism. We performed detailed tests for his blood type, and herein report the results of these tests along and also suggest a possible mechanism for the persistence of his original blood type during his complex clinical course.

Case Report

A 26-year-old male was referred to our hospital 7 months before a second HSCT for bradycardia. He had undergone his first HSCT for AML with RUNX1-RUNX1T1 from a human leukocyte antigen (HLA)-mismatched unrelated female donor with ABO incompatibility (the recipient's ABO blood type was A and the donor's ABO blood type was O) 11 years previously and had remained in molecular CR. Two months before he was referred to our hospital, he began to

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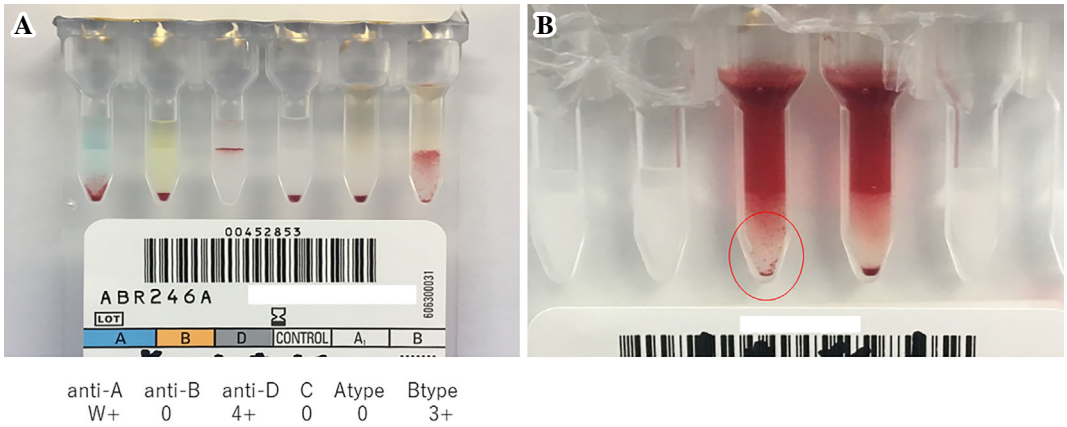

Figure 1. **A: Results of column agglutination testing for the RBCs of the patient in complete remission with complete donor-type chimerism after his 1st allogeneic transplantation. Column agglutinin testing showed that the RBCs of this patient still weakly expressed type A antigen. Reversed typing showed agglutination not with type A RBCs, but rather with type B RBCs. B: An adsorption-elution study using anti-A antibody for the RBCs of the patient in complete remission with donor-type chimerism after 1st allogeneic transplantation. An adsorption-elution study for the RBCs of the patient using anti-A antibody was performed after a healthy donor's type O RBCs were mixed with the plasma of this patient. The solution including dissociated antibody showed weak agglutination with type A RBCs.**

experience chest pain and dyspnea. At our hospital, his white blood cell (WBC) count was found to be 6,000/μL with 18.5% blast cells, and echocardiography showed a bulky mass (59×20 mm) on the right side of the atrial septum, which was considered to be responsible for the bradycardia. He was diagnosed to have a relapse of AML. Initially, he denied any treatment other than palliative care. However, masses also appeared in his spine at Th8, Th10-12 and L5-S1, which caused lower-limb paralysis and bladder rectal dysfunction. At that time, he decided to receive treatments aiming for a cure, and started to undergo intensive chemotherapy and radiation therapy for the masses in Th8, Th10-12 and L5-S1. After 2 courses of chemotherapy, he achieved molecular CR, and complete first donor-type chimerism was confirmed by sex chromosome fluorescence in situ hybridization (FISH) in his bone marrow cells. However, in an ABO typing test using antibodies against type A and type B blood by the tube method, mixed field agglutination was observed with anti-A antibody. This result suggested that his RBCs still expressed small amounts of antigen of type A, which was his original ABO-type. This result was also confirmed by column agglutinin testing (Fig. 1A). Reversed typing showed agglutination not with type A RBCs, but instead with type B RBCs.

In order to exam what kind of type A RBCs existed, we performed a red blood cell flow cytometry analysis. First, the RBC fraction fixed with glutaraldehyde was incubated with anti A and B monoclonal antibodies (Ortho Diagnostic System) for 30 minutes at 4°C, and then was washed twice with phosphate-buffered saline (PBS). Next, RBCs were stained with fluorescein isothiocyanate (FITC)-conjugated anti-human immunoglobulin FITC by incubation for 30 minutes at 4°C, and then was washed twice with PBS. ABO antigen on the surface of RBCs was analyzed by a flow cytometer (BECTON DICKINSON; FACSCalibur). This analysis showed that there was only one peak of RBCs with a weak expression of type A antigen (Fig. 2). We hypothesized that type A antigen from the recipient's non-hematopoietic cells was secreted and adsorbed onto the surface of the donor's type O RBCs.

To confirm our hypothesis, we performed an adsorption-elution study (4) in the following way. A healthy donor's type O RBCs were mixed with the plasma of this patient, and stored overnight at 4°C. After being washed with saline, anti A and B monoclonal antibodies (Ortho Diagnostic System) were added to the RBC fraction. This was again stored overnight at 4°C for the adsorption of antibodies by antigens on RBCs, and then excess antibodies were removed by washing with saline. For the elution of antibodies from antigens on RBCs, 200 μL of saline was added, and then cells were incubated at 50-52°C for 10 minutes, and centrifuged. The solution including dissociated antibody was reacted with various types of RBCs to identify the antibodies in the solution by confirming hemagglutinin. In the present patient, a solution including dissociated antibodies showed weak agglutination with type A RBCs (Fig. 1B). This demonstrated that the solution included anti-A antibodies which were combined with type A antigen on RBCs, and thus type A antigen existed in this patient's plasma and was adsorbed onto type O RBCs.

The patient underwent a 2nd HSCT from an unrelated male donor whose blood ABO type was B in his 2nd CR. Twenty-five days after HSCT, bone marrow aspiration showed persistent CR, and a chimerism analysis using the

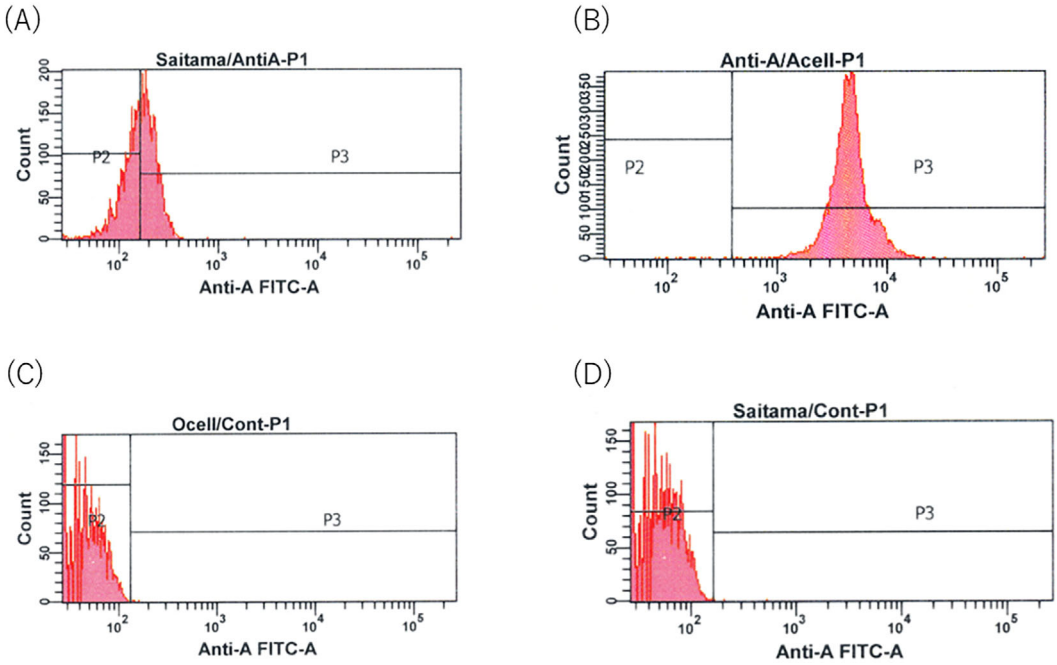

Figure 2. Expression of type A antigen on the surface of the RBCs of the patient in complete remission with donor-type chimerism after his 1st allogeneic transplantation. A flow cytometry analysis showed only a peak of RBCs, which express type A antigen weakly (A), compared with normal type A RBCs (B). Type O RBCs (C) or the RBCs from another patient whose blood type was originally A and who underwent allogeneic transplantation from a donor whose blood type was O (D).

short tandem repeat method in bone marrow cells showed complete 2nd donor-type chimerism. However, the microplate method showed weak positivity on anti A antibody serum, but strong positivity on anti B antibody.

Although we did not exam the ABO genotype or A and B glycosyltransferase activity, this result suggests that the recipient's type A antigen was absorbed again on type B RBCs that were derived from the donor's hematopoiesis.

Discussion

In the present patient whose original blood type was A, small amounts of type A antigen were detected on his RBCs after the 1st HSCT from a donor whose blood type was O, and small amounts of type A antigen and type B antigen were detected on his RBCs after the 2nd HSCT from a donor whose blood type was B, while complete donor-type chimerism was confirmed in both situations. These results showed that type A antigen existed on the surface of his RBCs, and additional examinations suggested that type A antigen secreted from his non-hematopoietic cells into his serum was absorbed on the surface of donor-derived RBCs. Anti-A antibodies were not detected after both the 1st and 2nd HSCT. Absorption of anti-A antibodies which were produced by donor-derived cells to recipient-derived non-blood tissues was considered to be the main reason, but part of the anti-A antibodies might have combined with the type A antigen in the serum.

Previous studies, including one from de Vooght et al. (5), reported that recipient-type ABH antigen can be detected on the surface of RBCs after ABO-incompatible HSCT even after complete donor-type chimerism was confirmed. Kishino et al. (4) reported that RBCs which express recipient-type ABH antigen can be detected after ABO-incompatible HSCT, because the recipient's ABH antigen which was produced by the recipient's mucous cells or gastrointestinal cells can persist (6). These antigens are considered to shed into the intestinal lumen, and thereafter they are digested, absorbed and transported to the serum (7-9). ABH antigens are then absorbed on the surface of the donor-derived RBCs. In our patient, *in vitro* studies suggested that the recipient's type A antigen was also present on the surface of donor-derived RBCs after HSCT by a similar mechanism. However, the amount of the recipient's ABH antigen on the surface of RBCs after HSCT was small, and the reaction to ABH antibody was too weak in routine laboratory tests to determine the blood type.

In our patient, an FCM analysis showed the uniform weak expression of type A antigen on the surface of RBCs after HSCT, and an absorption-elution study was effective for detecting this weak surface antigen on RBCs.

Based on previous reports and various tests that we performed for this patient, we considered that the recipient's type A antigen that was synthesized in the recipient's non-hematopoietic cells was secreted and adsorbed on RBCs derived from the donor, and as a result, RBCs after HSCT also showed type A antigen on their surface.

This report is associated with several limitations. First, we

confirmed the RBC phenotype but did not confirm their genotype. We also did not confirm any other type of blood group; for instance, Rh, Kell, Duffy, Kidd and MNS. Therefore, our categorizing of the blood group might not be perfect. Secondly, because of the limitations associated with a chimerism analysis, the possibility some residual red blood cells of the recipient type still remain cannot be ruled out.

In conclusion, it is sometimes difficult to precisely determine the ABO blood type after ABO-incompatible HSCT. Since this can be a problem when transfusion is planned, it is important to perform detailed examinations to determine the precise blood type, at least when equivocal results are obtained in routine tests.

The authors state that they have no Conflict of Interest (COI).

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