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Asia-Pacific Journal of Blood Types and Genes

2017, 1(2):71-74

APJBG

The best strategy of blood transfusion in patients with Rh_{null} syndrome

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ABSTRACT

Rh_{null} syndrome is a very rare disease. Patients with this syndrome present with negative serological Rh typing of E, e, C, c, and D antigens. Only one study has previously discussed Rh_{null} syndrome in Chinese individuals. We experienced two patients with Rh_{null} syndrome in China, Rh genotypes being CcDEe in the first patient and CCDee in the second patient. The first patient was a pregnant woman (gravida 2, para 1) with a negative red blood cell (RBC) antibody screen test. The second patient was a middle-aged man, transfused with ccdee, ccdEe, and ccdee RBC products, the pre-transfusion specimen was negative and post-transfusion specimen was anti-c,e, respectively. The hemoglobin level continued to increase in the second patient after being transfused with ccdEe RBC products. In the first patient, the result of the antibody screen test was still negative after artificial abortion. In patients with Rh_{null} syndrome, RBC products that have the same Rh genotype as the patient can be safely transfused.

Keywords: Rh_{null} syndrome, Rh associated glycoprotein, transfusion strategy

INTRODUCTION

Individuals with Rh_{null} syndrome are negative for RhD and RhCE genotypes[1], which is caused by mutations of the RhCE/RhD gene (Rh_{null} amorph type) and mutations of the Rh-associated glycoprotein (RhAG) (the regular Rh_{null} type)[2] . Previous studies have demonstrated that individuals with Rh_{null} syndrome usually have the anti-Rh29 antibody, making transfusion a challenge[3,4]. We described one patient with Rh_{null} syndrome, who was immune to different Rh types of red blood cell (RBC) products and presented with pregnancy, and another patient transfused with different Rh types of RBC products. This report aimed to suggest the best transfusion strategy for patients with the rare Rh_{null} syndrome.

METHODS

Antibody screen test, identification and phenotyping

Screen and panel cells used at the Blood Group Laboratory (BGL) of the Beijing Red Cross Blood Center (BRCBC) cover antigen systems, including Rh, MNS, Duffy, Kidd, Kell, Lewis, P1, Xg, Lutheran, Dia and Mur (Bio-Rad Laboratories GmbH, Germany; Jiangsu LiBio Biotech, China), which have a relatively high prevalence in Asia. Detection methods include conventional tube techniques in room temperature and gel column tests (Bio-Rad Laboratories GmbH, Germany; Jiangsu LiBio Biotech, China). The Rh phenotype was confirmed in column agglutina-

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tion testing (Bio–Rad Laboratories GmbHMunchen, Germany; Jiangsu LiBio Biotech, China). Other procedures, such as sample reception, report documentation, and report writing were all based on regulations and standard procedures specified by the BGL of the BRCBC.

Genotyping and molecular analysis

Polymerase chain reaction with sequence–specific primers was performed for the Rh blood group genotyping by using commercial kit (Multi–erythrocyte antigen genotype, Jiangsu LiBio Biotech, China). The sequence primers and amplification protocol for RhD exons 1–10 and RhCE exons 1–10 were designed using the Sanger sequence method by a commercial kidd gene kit (RhD and RhCE whole exons sequence, Jiangsu LiBio Biotech, China). Sequencing of PCR purified products was done by Sangon Biotech (Beijing, China) and the results were analyzed using sequence analysis software (Geneious R9, New Zealand). The RhD and RhCE allele sequence (GenBank ID: No. 6006 and 6007) template was used as reference to analyze and mark the mutations.

RESULTS

Case 1

A 21–year–old Chinese woman, gravida 2, para1, presented at 18 weeks gestation, with a history of chronic anemia. She had been diagnosed as being Rh serological results showing negative during an antenatal examination, and phenotyping results demonstrated that she was negative for RhCE and RhD antigens (***Fig.1 A***, ***Fig.2 A***). The result of the antibody screen test was negative, and the Rh genotype was CcDEe. We examined the Rh genotype by sequencing the exons of RhD and RhCE, and the results showed CcDEe.

Case 2

A 53–year–old man was referred for mitral and aortic valve replacement to treat rheumatic heart disease in 2002. In 2009, a Doppler echocardiogram showed tricuspid regurgitation, and drug treatment was prescribed with continuous follow–up until March 2017. Then he underwent tricuspid valve replacement with mechanical valves and was hospitalized in April 2017. The routine preoperative antibody screen test result was negative, and the serological blood type was A and negative for D (***Fig.1 B, Fig.2 B***). Genotyping was conducted, and all RhCE and RhD antigens were negative; thus, we initially planned to transfuse RhD–negative RBC products. Since the hemoglobin (Hb) level was 119 g/L, we obtained autologous whole blood that was stored preoperatively. EPO and iron supplements had been given since 2 weeks before phlebotomy was performed.

The patient received auto–transfusion with autologous scavenged RBCs(1,300 mL) intraoperatively.

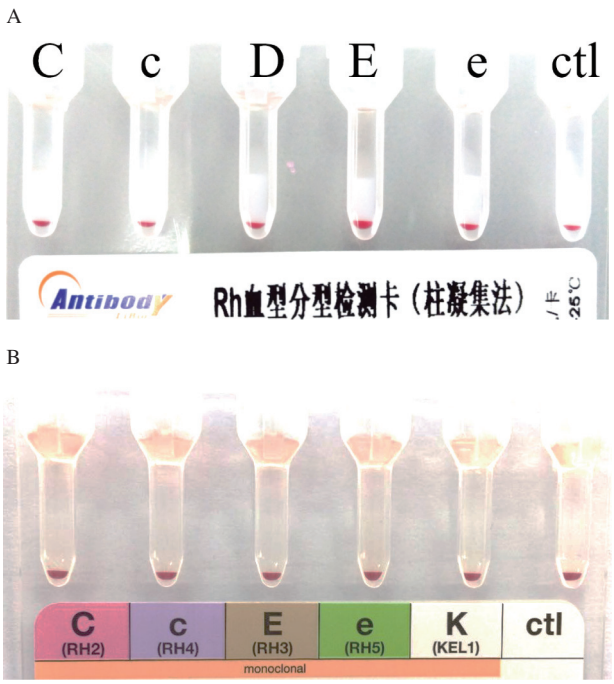

Fig.1. **The serological Rh typing for the Rh_{null} syndrome.** A showed C, c, D, E, e phenotyping negative in first patient with R_{hnull} syndrome. B showed D, C, c, E, e phenotyping negative in secone patient with Rh_{null} syndrome and control result showed negative.

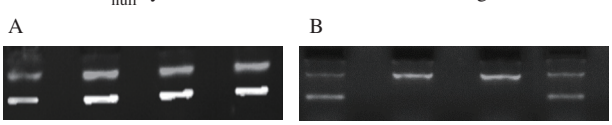

Fig.2. **The Rh genotyping for the Rh_{null} syndrome.** The upper white band human growth hormone receptor(hGHR) gene fragment as internal control and lower white band index positive antigen for C, c, E, or e(from left to right). A showed the first patient was CcDEe in genotyping; B showed second patient was CCDee in genotyping.

After tricuspid valve replacement was finished, autologous pre–stored whole blood (2×200 mL collected from 7 and 14 days preoperatively) was transfused. In addition, suspended ccdee RBCs (2 units) were given on day 1, and fresh–frozen ccdee RBCs (2 units) were transfused on day 2. Later, suspended ccdEe RBCs (2 units) were transfused again on day 8, and fresh–frozen ccdee RBCs (1.5 units) were transfused on day 11. The antibody screen test result was negative at every pre–transfusion examination until day 13 post–operatively. The antibody screen test identified the anti–f (anti–c,e) in the patient´s plasma on day 13, and the direct agglutination test showed a weak positive

result. Other data, including the Hb level, are shown in ***Table 1***. The Rh genotyping and sequence of Rh exons confirmed the CCDee genotype, so we decided to use CCDee RBC products instead of serologically RhD–negative RBC products. Since the Rh genotype of blood products corresponded to the patient´s genotype, the Hb level continued to increase from 64 g/L on day 11 to 82 g/L on day 18.

Table 1 **The Hb level, unexpected antibody screen test and transfusion record of case 2**

Date	Hb(g/L)	Blood products	Blood type	Unexpected antibody screen test
2 weeks before surgery	119	draw autologous pre–stored whole blood, 200 mL		
	115, 118	draw autologous pre–stored whole blood, 200 mL		
Surgery, Day 0	97	autologous scavenged whole blood, 400 mL	patient´s blood	Negative
		washed autologous scavenged red cell, 400 mL	patient´s blood	
		washed autologous scavenged red cell, 500 mL	patient´s blood	
		autologous pre–stored whole blood, 400 mL	patient´s blood	
Day 1	82, 79	red cells suspension, 2 units	A, ccdee	Negative
Day 2	78, 70	frozen red blood cells, 2 unit	A, ccdee	Negative
Day 8	69	red cells suspension, 2 units	A, ccdEe	Negative
Day 11	64	frozen red blood cells, 1.5 unit	A, ccdee	Negative
Day 13				anti–f
Day 16		red cells suspension, 2 units	A, CCDee	
Day 17		red cells suspension, 2 units	A, CCDee	
Day18	82			
Day21	77			
Day24	87			

DISCUSSION

In patients with the Rh_{null} phenotype, no Rh antigens are found on the surface of the RBC membrane, and the morphologic changes of the RBCs include spherocytes, stomatocytes, and an isocytosis[5,6]. Rh_{null} syndrome induces abnormal organization of RBC membrane phospholipids, increased activity of Na^+K^+ pump and subsequential imbalance of water and ions in the cells[7]. Rh_{null} cells usually reduce the survival period, lower resistance to osmolality, increase the reticulocyte count, and cause varying degrees of hemolysis because of the abnormal morphology and cell membrane contents. Patients with Rh_{null} syndrome require transfusion during operations or instances of massive bleeding. Therefore, the strategy of blood transfusion is an important issue.

Several immunized patients with Rh_{null} syndrome with anti–Rh29 (allo–antibodies reactive with total Rh antigens) and other immunized patients with the Rh_{null} syndrome have been reported to have anti–Hr0 (allo–antibodies reactive with E, e, C, and c antigens) or anti–e[3,4,8,9]. In our study, we observed a pregnant woman with a history of artificial abortion and a cardiac patient immunized to different Rh types of RBC products. The second patient produced anti–c,e by ccdee RBCs, and was transfused with CCDee RBCs, which was the same genotype as his suspended RBCs; this was effective for increasing the Hb level. Several patients with Rh_{null} syndrome with unexpected antibodies have undergone repeated blood exchange transfusion successfully, including the pregnant woman and cardiac patient in our study.

Although the D antigen frequently occurs (99.6%) in China, allo–antibodies against Rh antigens are still the most frequent challenge of blood transfusions in China[10]. Rh phenotyping is routinely conducted at several hospitals in China, and prophylaxis in patients to ensure transfusion matching could effectively reduce the occurrence of the unexpected antibody. For some patients, e.g. those with auto–control positive samples, a Ce deficiency, and Rh_{null} syndrome, genotyping of RBC groups is an advanced tool for solving the challenge of transfusion.

We observed two patients with Rh_{null} syndrome, immunized to different Rh types of RBC products. We also demonstrated unexpected results of the antibody screening test (i.e. a negative result can become positive for anti–c,e after ccdee RBC transfusion). Rh genotyping matched RBCs may be the best strategy of blood transfusion in patients with Rh_{null} syndrome to use.

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Received 28 April 2017, Revised 18 May 2017, Accepted 31 May 2017