Primary anti-D Immunization by DEL Red Blood Cells

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Extremely weak D variants called DEL are serologically detectable only by adsorption-elution techniques. A nucleotide change of exon 9 in RHD gene, RHD (K409K, 1227G>A) allelic variant is present in almost all the DEL individuals of East Asians. No DEL phenotype has yet been shown to induce a primary alloanti-D immunization in East Asia. A 68-yr-old D-negative Korean man was negative for anti-D at admission, and he developed alloanti-D after transfusion of red blood cells (RBC) from 4 apparently D-negative donors. Four donors who typed D-negative by routine serologic test were analyzed by real-time PCR for RHD gene and RHD (K409K). One donor was found to have RHD (K409K). This is the first case in which DEL RBCs with RHD (K409K) induced a primary alloanti-D immunization in Asian population. Because the DEL phenotype can induce an anti-D immunization in D-negative recipients, further discussion is needed whether RhD negative donors should be screened by molecular method and what an efficient genotyping method is for detecting the RHD gene carriers in Korea. (Korean J Lab Med 2009;29:361-5)

Key Words : RHD, DEL RBC, Anti-D antibody, Alloimmunization

INTRODUCTION

Anti-D immunization may cause hemolytic transfusion reactions and hemolytic disease of the newborn. For the transfusion recipient who has D–negative red blood cells (RBC), D antigen–negative blood units should be given. The frequency of D–negative varies widely among different ethnic groups. Fifteen percent of Caucasian populations, 3 to 7 percent of African populations and only 0.5 percent of Chinese and Japanese populations are D–negative phenotype [1–4].

The D–negative frequency in Korean persons is 0.15 percent, about 1/100th of that in Caucasian individuals [5]. Recently the molecular basis of D–negative in Korean donors was characterized by two separate studies [6, 7]. Among Korean D–negative persons, nearly three–fourths showed RHD total deletion, 13 to 16 percent had RHD (K409K) allele, and 9 to 10 percent had the RHD–CE–D hybrid form, RHD (K409K, 1227G>A) allelic variant, a substitution of the last nucleotide of exon 9 in RHD gene.
DEL phenotype and most DEL donors are typed as D-negative because routine serologic typing does not distinguish D-negative from the DEL phenotype. According to the national transfusion guidelines of many other countries, RBCs from persons with the DEL phenotype are not differentiated from truly D-negative and therefore are used as D-negative blood for D-negative recipients. So far, two cases of anti-D immunization by DEL red blood cells have been reported in Austria and Japan [8, 9]. This report describes a case of primary anti-D immunization induced by transfusion of RBCs from a Korean donor with DEL phenotype, carrying RHD (K409K) allele.

CASE REPORT

1. Immunohematology

A 68-yr-old group O, D-negative (ce) Korean man was admitted to Dong-A University Hospital for operation of atherosclerosis of abdominal aorta. He received 2 units of crossmatch-compatible group O, D-negative RBC during an aortobifemoral bypass surgery on February 21, 2008. He had no history of prior transfusions or transplantations. Antibody screening test before the transfusion was negative (ID-DiaCell I+II, DiaMed AG, Cressier, Morat, Switzerland). Additionally, he received 2 units of crossmatch-compatible group O, D-negative RBC because of low hemoglobin level on day 5 post-operation. On March 6, 2008, the antibody screening became positive and anti-D was identified in the patient’s serum by gel technique (ID-DiaPanel & ID-DiaPanel-P, DiaMed AG). The autologous control was negative. There was no evidence of acute or delayed hemolytic transfusion reactions after those transfusions and no remarkable changes in the levels of hemoglobin, indirect bilirubin, and lactate dehydrogenase.

The anti-D developed 9 days after last RBC transfusion in the recipient was unexplained, so analytical checks for errors including sample labeling, patient and donor units identification as well as transfusion procedure were performed. Indication of an error was not detected. He did not receive Rh immunoglobulin. Forty days later the patient’s anti-D was persistently detected and showed similar strength of agglutination.

2. Molecular biology

Stored blood samples transfused on Feb 26 were used to confirm the status of RHD gene at the molecular level. The blood samples transfused on Feb 21 were not available for molecular study because the documented interval for storage of those samples was outdated. For these 2 units of RBC, we investigated RHD gene analysis using plasma stored in Korean Red Cross. Informed consent was obtained from the patient for the RHD genotype analysis.

DNA was isolated from plasma or peripheral blood with QIAamp blood mini kit (QIAGEN, Hilden, Germany). RHD genes were determined by real-time PCR using the LightCycler 2.0 (Roche, Penzberg, Germany).

1) Detection of intron 4 and exon 7 by real-time PCR

Plasma from 4 donors and peripheral blood from the patient were examined for the presence of RHD-specific polymorphisms located in RHD intron 4 and RHD exon 7 by real-time PCR and samples negative in these real-time PCR tests were further tested for the RHD promoter region and RHD exon 10. Negative results were interpreted as the complete deletion of RHD gene. The real-time PCR procedures for RHD intron 4 and RHD exon 7 were as follows: 50 ng of DNA template mixed together with 2 μL LC FastStart DNA Master SYBR Green I (Roche, Penzberg, Germany), 0.4 μM each primer (Table 1), and 3.0 mM MgCl₂ in a final volume of 20 μL. The following PCR program was used: 10 min at 95°C and 33 cycles of 5 sec at 95°C, 3 sec at 58°C, and 10 sec at 72°C. The program for analytical melting was 5 sec at 95°C and 30 sec at 65°C and an increase to 99°C at a 0.2°C per second ramp rate. The melting temperature (Tm) for intron 4 was 87.0 ± 0.5°C and that of exon 7 was 86.5 ± 0.5°C.

2) Detection of promoter region and exon 10 by real-time PCR

The real-time PCR procedure for promoter region was
as follows: 50 ng of DNA template mixed together with 2 μL LC FastStart DNA Master SYBR Green, 0.3 μM each primer (Table 1), 1 μL 10% dimethyl sulfoxide (DMSO), and 3.0 mM MgCl₂ in a final volume of 20 μL. The following PCR program was used: 10 min at 95°C and 33 cycles of 3 sec at 95°C, 2 sec at 60°C, and 10 sec at 72°C. The program for analytical melting was 5 sec at 95°C and 15 sec at 65°C and an increase to 99°C at a 0.2 °C per second ramp rate. The Tm for promoter region was 82.5 ± 0.5°C.

The real-time PCR procedure for exon 10 was as follows: 50 ng of DNA template mixed together with 2 μL LC FastStart DNA Master SYBR Green I, 0.4 μM each primer (Table 1), and 4.0 mM MgCl₂ in a final volume of 20 μL. The following PCR program was used: 10 min at 95°C and 33 cycles of 5 sec at 95°C, 5 sec at 60°C, and 10 sec at 72°C. The program for analytical melting was 5 sec at 95°C and 15 sec at 65°C and an increase to 99°C at a 0.2 °C per second ramp rate. The Tm for exon 10 was 84.5 ± 0.5°C.

3) Detection of RHD (K409K) by real time PCR

A sample positive in RHD intron 4 and RHD exon 7 real-time PCR was further investigated for RHD (K409K) by real-time PCR. The real-time PCR procedure for RHD (K409K) was as follows: 50 ng of DNA template mixed together with 2 μL LC FastStart DNA Master HybProbe 2 μL (Roche, Penzberg, Germany), 0.3 μM each primer (Table 2), 0.3 μM each probe (Table 2), and 4.0 mM MgCl₂ in a final volume of 20 μL. The following PCR program was used: 10 min at 95°C and 40 cycles of 5 sec at 95°C, 10 sec at 60°C, and 10 sec at 72°C. The program for analytical melting was 30 sec at 95°C and 2 min at 40°C and an increase to 80°C at a 0.4°C per second ramp rate. The RHD (K409K) variant was analyzed by lower Tm of 61.5 ± 0.5°C and wild type with higher Tm of 66.5 ± 0.5°C.

By real-time PCR analysis, one of four donors was found to have RHD (K409K) and the patient and three donors showed complete deletion of RHD gene. The blood positive for RHD (K409K) was transfused on February 26, 2008 and alloanti-D was detected after 9 days of transfusion.

**DISCUSSION**

The Rh blood group is the second most clinically significant antigen of the blood group system in human. It also is the most polymorphic of the blood groups with variations due to deletions or missense mutations. These molecular variations lead to reduced antigen density [13]. DEL is serologically designated as a quantitative variant of D antigen. A small amount of anti-D can be eluted from DEL red blood cells after incubation with anti-D, although there is no agglutination by the indirect antiglobulin test procedure of D typing. By molecular studies, DEL phenotype was expressed by several different mutations such as RHD (M-295I, 885G>T), RHD (K409K, 1227G>A), RHD (IVS3+1G>A), RHD (IVS5-38del4), RHD (X418L), and RHD (delEx8) [8, 12, 14, 15]. These DEL variants can induce an alloanti-D in D-negative recipients because most DEL donors are typed as D-negative by serologic method. We could find two reports of anti-D alloimmunization in D-negative recipients after the transfusion of DEL variants. A 67-yr-old D-negative Japanese woman with a history of allosensitization to D antigen developed a secondary alloanti-D immunization induced by RHD (K409K) RBCs [9], and a 58-yr-old D-negative Austrian woman developed a primary
alloanti-D by another DEL variant, RHD (IVS5–38del4) [8]. In our case, interestingly, a 68-yr-old D-negative Korean man developed a primary alloanti-D by RHD (K409K) RBCs.

The molecular basis of D-negative is different among different ethnic populations. Most of D-negative Caucasian populations have complete RHD deletion but populations from China, Japan, and Korea have rather diverse mutation variants. The frequency of RHD (K409K, 1227G>A) allele in apparent D-negative Korean donors is about 13 to 16 percent, which is the most prevalent mutation variant for DEL phenotype in Korea [6, 7]. Recently, Taiwan researchers developed a real-time PCR-melting curve analysis to detect a nucleotide change at 1227 position of RHD exon 9. Since almost all the DEL type in East Asians have the characteristic 1227A mutation, this real-time PCR method for single nucleotide polymorphism showed high sensitivity (100%) and high specificity (98.75%) [16].

The routine RHD genotyping in donor screening program was adopted in Germany and Upper Austria. Samples of first-time D-negative donors by serology were tested in pools of 20 for the RHD-specific polymorphic sites by PCR but the target sites of RHD gene were different in each country. Within 6 yr of testing, the prevalence of RHD gene carriers detected was 0.21 percent and DEL phenotype was approximately one-half of the RHD gene carriers in Germany. Ninety-four samples of 23,330 pretyped D-negative samples carried at least one RHD marker in Upper Austria. These two studies suggested that the integration of RHD genotyping into routine screening was practical and it would be necessary to adapt a proper RHD genotyping strategy in each population [17, 18]. Our report is the first case in which DEL RBCs with RHD (K409K) induced a primary alloanti-D immunization in Asian population. Because the DEL phenotype can induce an anti-D immunization in D-negative recipients, further discussion is needed whether D-negative donors should be screened by molecular method and what an efficient genotyping method is for detecting the RHD gene carriers in Korea.

REFERENCES


