Report

Alloimmunization of patients by blood units harboring distinct DEL variants

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The alloimmunization potential of many *RHD* variants is unknown, and it can be explored by lookback and traceback studies. Héma-Québec (HQ) investigated the *RHD* status of 3980 D– repeat blood donors. Thirteen were found to be *RHD* positive: 4 *RHD*ψ,* and 1 *RHD*487delACAG,* which show a D– phenotype; and 1 *RHD*885T* and 7 *RHD*(93–94insT)* causing a DEL phenotype when C antigen is present. Lookback studies were done to verify the alloimmunization potential of these eight DEL donors. Coincidentally, Canadian Blood Services (CBS) performed a traceback study by investigating the *RHD* status of donors after a D– recipient developed anti-D after transfusion of two D– red blood cell (RBC) units. Donor genotyping was done either manually (HQ) or using the Progenika Bloodchip platform (CBS). Donations were traced through computer records. Letters were sent to hospital blood bank physicians to verify the presence of anti-D in recipients and to donors to request repeat samples. A total of 118 RBC units were transfused, 82 to D– recipients. Anti-D was found in three patients transfused with *RHD*(93–94insT)* DEL red blood cells. One donor presenting the same DEL variant was involved in the traceback study. Even without strong evidence clearly demonstrating the alloimmunization potential of DEL variants, whenever HQ or CBS identifies a donor harboring a DEL phenotype, his or her D status will be changed from D– to D+ to protect against the potential alloimmunization risk. ***Immunohematology* 2013;29:136–140.**

Key Words: DEL, *RHD* variant, alloimmunization, transfusion reaction

RH blood group D antigen is the second most immunogenic, after ABO antigens. Antibodies to D are considered clinically significant because they have been associated with hemolytic transfusion reactions and hemolytic disease of the newborn.[1–3] The D antigen expression is polymorphic and varies among populations. In whites, the D– phenotype is observed in 15 to 17 percent compared with 5 percent in black Africans and less than 3 percent in Asians. Many variants accounting for the D– phenotype have been observed and they vary among populations.[1,4]

The D antigen's polymorphic nature is reflected by a wide range of partial or weak antigen expression, which led to the classification of variant D antigens: partial and weak. Partial D–expressing individuals could be alloimmunized when transfused with normal D+ red blood cells (RBCs), because some D epitopes are absent from their RBC membrane. On the other hand, weak D antigens are thought to cause less alloimmunization because the polymorphisms are located within transmembrane domains or intracellular regions, although these changes might alter the overall protein structure.[3,5,6]

A weak Ds subcategory consists of extremely weak *RHD* variants, termed DEL. A small amount of anti-D can be eluted from DEL RBCs after incubation with anti-D, although there is no agglutination by indirect antiglobulin test (IAT).[7] Normal D individuals might have as many as 20,000 sites per erythrocyte,[6] whereas DEL individuals have between 20 and 40.8 DEL individuals are mainly found in the Far East (10–30% in China and Japan), although DEL phenotypes resulting from different *RHD* variants have been observed in whites. These variants are caused by *RHD* missense mutations, splice site mutations, or deletion of *RHD* exon(s).[8–19]

It is well established that some weak Ds and most DEL units are mistyped as D– by routine automated serologic methods used to type blood donors, posing a potential risk of anti-D immunization when transfused to D– recipients.[4,7,14,17,20] To address this potential risk, blood centers may implement testing of every apparently D– donor. This can be accomplished by adapting the adsorption-elution technique used to detect the DEL phenotype to a higher throughput setting or by using molecular testing.[14,20,21] A *Vox Sanguinis* International Forum published in 2006 summarized the practices of several blood centers regarding the use of molecular testing to detect *RHD* variants missed by IAT.[22] The majority of centers did not routinely use molecular typing, but would consider it for C+ or E+ donors, implying that all D– donors would have to be typed for C and E before molecular analysis. Molecular typing was also considered when a discrepancy was observed between monoclonal reagents on serologic testing.

As for demonstrating the risk of alloimmunization, very few studies have been reported to date.[4,7,9,15,22] Yasuda and collaborators published a clear case of secondary immunization after transfusion of DEL RBCs.[7] Shao's and Kim's groups published alloimmunization cases involving East Asians.[14,20] In one of the cases, the recipient showed an anti-D after only 9

days. Other studies have focused on D immunization in weak D types.[15]

Alloimmunization potential can be identified by two different means: lookback and traceback studies. Lookbacks start by molecular typing of donors and subsequently investigate whether D– recipients are alloimmunized after a transfusion involving D variant donors. On the other hand, traceback studies are initiated when a D– recipient develops an anti-D after an apparent D– transfusion. Identification of the donors and molecular typing are performed.

In 2007, Héma-Québec (HQ) launched a large genotyping project for repeat whole blood donors.[23–25] Four *RHD* variants were found among the D– donors: *RHD*(93–94insT), RHD*ψ, RHD*(487delACAG),* and *RHD*885T*.

*RHD*ψ* and *RHD*(487delACAG)* alleles have always been associated with a D– phenotype. The other two alleles, *RHD*(93–94insT)* and *RHD*885T,* are considered DEL in the presence of the C antigen. Nothing is known about their alloimmunogenicity.

Interestingly, the most frequent DEL phenotype found to date in Canadian blood donors is the result of variant allele *RHD*(93–94insT)* (7 of 3980 D– donors, 0.18%). All donors with this allele are likely of French Canadian ancestry. This allele was first reported in 2006 in one German donor,[26] and examples reported since then include three in Spain,[16] two in Germany,[17] and one in Denmark.[19] However, there is no information on the clinical importance of the allele in terms of alloimmunization potential.

The additional T in *RHD*(93–94insT)* causes a frameshift mutation that leads to a stop codon at amino acid position 35. Such mutation early in a protein would be predicted to completely suppress antigen expression, but replication slippage may explain traces of functional protein.[17]

Preventing alloimmunization is a daily challenge for blood banks. However, little is known about the antigen density sufficient to cause an anti-D alloimmunization. Many D– recipients transfused with weak D type 1 and 2 were alloimmunized.[27–29] The density of these weak Ds has been estimated to be between 400 and 1200.[28,30] Gassner and colleagues published an alloimmunization case involving a weak D type 26 with an antigen density of 29 to 70.[15]

To evaluate the risk of alloimmunization from the eight DEL blood donors described earlier, HQ undertook a lookback study going back to 2000. Coincidentally, a traceback study was performed at Canadian Blood Services (CBS) to investigate anti-D alloimmunization in an elderly female recipient, reported by a hospital blood bank, involving two D– donors.

Materials and Methods

Donor Testing

Genotyping was originally done using the SNPstream Genotyping System (Beckman-Coulter, Fullerton, CA) and is described in detail elsewhere.[23] Donor serologic typing was initially done using the Olympus PK7200 (Beckman-Coulter, anti-D P3X61+P3X21223B10+P3X290+P3X35 and PK2, Diagast, Loos, France). The D– status was confirmed using standard serologic methods (e.g., IAT). The DEL status of the eight HQ suspected DEL donors was confirmed by adsorption-elution, following manufacturer's instructions (Elukit Plus, Dominion Biologicals Limited, Dartmouth, Nova Scotia, Canada; anti-D D175–2+D415 1E4, Novaclone, Dominion Biologicals Limited).

Lookback Study

For D– recipients, hospital blood banks were asked to verify the presence of anti-D, and if it was identified before the transfusion of interest. Finally, they were asked whether any other blood products were transfused during the same period. Standard serology methods were used to identify anti-D.

Traceback Case

An 88-year-old woman with no previous transfusions and one pregnancy had a negative antibody screen on her admission at the hospital with an abdominal aortic aneurysm. Four weeks after she received two O, D– RBC units, her antibody screen tested positive and anti-D was identified. Both transfused units typed D– C+E-c+e+. An investigation was initiated including extended serologic D antigen testing by IAT using reagents from three different manufacturers (Novaclone; Gamma-clone, Immucor, Norcross, GA; and Bioclone, Ortho Diagnostic Systems, Markham, Ontario, Canada; using ALBAclone Advanced Partial RhD Typing kit, Penicuik, UK), and adsorption-elution (Elukit Plus, Dominion Biologicals Limited). *RHD* genotyping was performed by Progenika using the BLOODCHIP system (Novartis, Cambridge, MA).

Results

Lookback Study

Blood donations made by HQ's eight DEL donors were traced through Progesa (Mak-System, Paris, France) as far as year 2000. Hospital blood banks that received the RBC units were notified in writing of the unusual D status of these donors. They were asked whether or not the RBC units had been transfused, and if so, to D+ or D– recipients.

Table 1. Red blood cell units from the three *RHD*(93–94insT)* donors possibly involved in antigen D alloimmunization

Donor	Total RBC units delivered to hospitals	Available information for transfused units	Units transfused to D– recipients	Number of recipients with anti-D	Number of recipients without anti-D	Recipients lost to follow-up
1	34	23	19	1	12	6
2	33	21	14	1	9	4
3	28	21	14	1	7	6
Total	95	65	47	3	28	16

RBC = red blood cell.

Letters were sent to 27 hospitals that received the 171 traced RBC units. Information was available for 118 units that were transfused to 36 D+ recipients and 82 D– recipients. No further follow-up was pursued for the D+ recipients.

Antibody screening done several weeks after transfusion showed anti-D in three patients transfused with three units from different donors bearing the same *RHD*(93–94insT)* variant (Table 1). Recipients with anti-D also received other blood products during the same transfusion episode (Table 2). These patients were hospitalized for surgery (abdominal aortic aneurysm [n = 2] and cardiac surgery in a case of Marfan syndrome). Some of these products were platelets from D+ donors. None of the blood donors had anti-D, and we are not aware of their transfusion or pregnancy history.

Little information is available on the alloimmunization potential of *RHD*11* RBC units in a CDe haplotype.[31] This donor gave 11 units. We have information for seven of these units. Two were transfused to D– patients. The first patient had no anti-D, and the second was lost to follow-up.

Traceback Case

Of the two donors involved in this possible alloimmunization event, one was confirmed D– by serologic methods and by genotyping. The other typed D– by IAT and the ALBA kit, but was determined serologically to be DEL by adsorption-elution. His genotype was found to be *RHD*(93–94insT)*. This donor lives in Nova Scotia (Canada) and is of Acadian descent, 17th-century French colonists in Canada. The lookback showed that three other RBC units from earlier donations were transfused. Two recipients remained antibody negative, and one was lost to follow-up.

Discussion

This study demonstrates the difficulties inherent in determining immunization potential in clinical settings. Investigators in the four cases reported could not completely determine the definitive cause of the observed alloimmunization because DEL RBCs were involved as well as apheresis platelets from D+ donors and a possible D+ pregnancy. At the very least, this DEL variant appears able to trigger a secondary immune response. Additionally, the frequency of alloimmunization appears higher in a traceback study, which starts with a potentially alloimmunized patient, compared with a lookback study, which starts with genotyped donors. As a precaution, HQ and CBS have adopted the policy that all known DEL donors will be considered as D+. They will be notified, and explanations will be provided.

Table 2. Alloimmunized recipients and blood products received

Recipients	Sex/Age	Pregnancy	*RHD*(93–94insT)* RBCs	Whole blood platelets	Apheresis platelets (year)*
A	F/87	Yes	1	0	1 D+ (2006)
B	F/88	Yes	1	0	1 D+ (2004)
C	F/44	Unknown	1	6	1 D+ (2006)
CBS case	F/88	Yes	1	0	0

*The apheresis process was modified at Héma-Québec in 2006. Before 2006, the Spectra system was used. After 2006, the Trima Accel apparatus (Terumo BCT) was implemented.

RBCs = red blood cells, CBS = Canadian Blood Services.

From this study, we might speculate about the potential risk of DEL to immunize D– individuals. Even with a density of 20, a DEL RBC unit might contain on average 4×10^{13} D antigens, equivalent to 200 to 400 µL of normal D+ blood. Ogasawara et al. observed that anti-D–sensitized DEL erythrocytes were not phagocytosed in a monocyte phagocytosis assay, suggesting a low alloimmunization risk.[32]

Studies have shown that 200 mL of D+ blood could alloimmunize D– healthy individuals when booster doses were injected (500–1000 µL) after a 6-month rest period.[33]

The first three alloimmunization cases described in this work were also transfused with apheresis platelets. A study done at HQ showed an equivalent of 3 µL or 1.8×10^7 residual RBCs in apheresis platelets prepared on the Trima apparatus (Dr. Louis Thibault, personal communication). Recipient B received platelets before 2006; these platelets were prepared with the Spectra system. They might have contained more residual RBCs, but data are not available for our center.

As for the CBS' case, the antibody screen was positive 4 weeks after transfusion. The earliest antibody response reported by Frohn and collaborators was 14 days[33] and for Wagner et al., it might be as fast as 11 days.[9] This patient gave birth to one child more than 40 years previously. The child's D status is unknown to us; therefore, we are unsure whether this case represents primary alloimmunization or an anamnestic response.

Even without strong evidence clearly demonstrating the alloimmunization potential of DEL variants, whenever a donor harboring a DEL phenotype is identified, HQ and CBS practices will be to change the donor's D status from D– to D+ to prevent the potential alloimmunization risk.

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