Case Report

A delayed and acute hemolytic transfusion reaction mediated by anti-c in a patient with variant RH alleles

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The Rh system is the most complex of the human blood groups. Of the 55 antigens that have been characterized, the system's principal antigens D, C, E, c, and e are responsible for the majority of clinically significant Rh antibodies. In the last few years, advancements in molecular testing have provided a wealth of information on the genetic diversity of the Rh locus. This case report describes a patient with variant *RHD*DAR* alleles inherited in conjunction with two compound heterozygote *RHCE*ceEK/RHCE*ceAR* alleles. The patient was previously alloimmunized to D, C, and E and possibly hr^S. Further transfusion of D−C−E−K− RBCs resulted in a suspected acute hemolytic transfusion reaction and the subsequent identification of anti-c. Monocyte monolayer assay testing suggests clinical significance with a range of 29.5–38.5 percent reactive monocytes. ***Immunohematology* 2018;34:109-112.**

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The Rh system is the most complex of the human blood groups. Of the 55 antigens that have been characterized, the system's principal antigens D, C, E, c, and e are responsible for the majority of clinically significant Rh antibodies. Antibodies to antigens in the Rh system have been implicated in hemolytic disease of the fetus and newborn (HDFN) as well as acute and delayed hemolytic transfusion reactions (HTRs). In the last few years, advancements in molecular testing have provided a wealth of information on the genetic diversity of the Rh locus. The Rh system is a very polymorphic and immunogenic blood group system that is second only to the ABO system in its clinical significance for blood transfusion. Nucleotide changes can result in qualitative and quantitative changes in Rh antigen expression.[1] Individuals who express a partial D on their red blood cells (RBCs) can be immunized to make anti-D. Similarly, people who are homozygous or hemizygous for alleles encoding partial c and/or partial e can also produce alloantibodies.[2] In addition to the common antigens of the Rh system (D, C, c, E, e) there are 50 additional antigens that constitute the Rh blood group system.[3] The Rh locus consists of a pair of adjacent homologous genes, *RHD* and *RHCE,* that respectively encode for D and for C or c and E or e antithetical pairs. The antigenic diversity present in this blood group system is attributed to a variety of molecular mechanisms including nucleotide substitutions and gene conversion.[4] These molecular mechanisms lead to the creation of unique protein sequences with resultant unique antigens and an increase in the number of antigens in the Rh system relative to other blood group systems. Further adding to the complexity, variant *RHD* and *RHCE* alleles can encode qualitative and/or quantitative changes in the Rh antigen expression.[5] Individuals with such variants, when exposed to the conventional antigen during transfusion, will recognize those epitopes that they lack as foreign, and an immune response may be elicited. Routine serologic RBC phenotyping does not differentiate between conventional and variant Rh antigens. Within the Rh blood group system, partial D, C, and e variants and their corresponding alloantibodies have been well described. Alloanti-c in a c+ individual was first reported by Moulds et al.[6] in 1982, with a number of additional cases reported since that time.[7–9]

Peyrard et al.[10] reported the first case of alloanti-c/ce in a patient with the *RHCE*ceAR* allele that encodes a partial c antigen. Hipsky et al.[11] also described the identification of alloanti-c in the plasma of an African American patient with sickle cell disease carrying the *RHCE*ceAR* allele.

We report a case of a 72-year-old multi-transfused African American woman with altered *RHD* and *RHCE* alleles predicting partial D, partial c, and partial e antigens who developed a clinically significant alloanti-c.

Case Report

The patient is a 72-year-old African American woman with a past medical history of multiple sclerosis, congestive heart failure, and recurrent gastrointestinal (GI) bleeding secondary to diverticulosis. The patient presented to the

emergency department with acute GI bleeding and received fluid support and 2 units of packed red blood cells (PRBCs). She was admitted to the hospital for further evaluation and treatment. A colonoscopy was performed that revealed a diverticular bleed in the ascending colon; this bleed was successfully cauterized. During this time, her hemoglobin (Hgb) was observed to fall to 4.9 g/dL with a hematocrit (Hct) of 16 percent (normal 12.0–15.5 g/dL and 37–48%, respectively); she was emergently transfused 4 units of PRBCs. Initial serologic results indicated the patient to be group A, weak D positive with a positive antibody detection test. The patient's previous serologic history included identification of anti-D, -C, -E, warm autoantibody, and cold autoantibody. The initial antibody screening was performed by the hospital, and the presence of additional RBC antibodies was suspected. The patient's sample was referred to the Immunohematology Reference Laboratory (IRL) (American Red Cross, Charlotte, NC) for further serologic evaluation and antibody identification.

Results

Initial serologic testing performed by the IRL indicated that the patient was group A, weak D positive with a negative direct antiglobulin test (DAT). Serologic phenotype testing was performed and the results were C–E–c+e+; K–; Fy(a–b–); Jk(a+b+); S+s–. The patient's plasma exhibited panreactivity with D– phenotypically similar RBCs in the presence of a negative autocontrol. This reactivity suggested the presence of an additional antibody (ies) unrelated to the previously identified alloanti-D, -C, and -E. Plasma studies showed 1+ reactivity with D– phenotypically similar cells using low-ionic-strength saline solution in the indirect antiglobulin test (IAT) and 3+ reactivity with polyethylene glycol (PEG) in the IAT. The reactivity was resistant to ficin treatment as well as to 0.2 M dithiothreitol. Given the patient's history of warm and cold autoantibodies, adsorption studies were performed at 37°C using ZZAP-treated autologous cells. No reduction in strength was observed with the autoadsorbed plasma. Allogeneic adsorptions were performed using papain-treated R_0 RBCs. Clinically significant antibodies to common RBC blood group antigens were excluded in the alloadsorbed plasma. An acid eluate was prepared from the adsorbing cells to ascertain the identity of the panreactivity. The acid eluate demonstrated an anti-e–like antibody. The eluate was reactive with three sources of D– e+ RBCs and negative with three sources of D– hr^B– RBCs.

The facility was contacted to report the anti-e–like reactivity, and molecular genotype testing was recommended for further characterization. No compatible units negative for D, C, E, e, and K were available for immediate transfusion. Therefore, after consultation with the facility, units negative for D, C, E, and K were recommended in the event emergent transfusion was needed.

Unfortunately, there was insufficient time to perform a monocyte monolayer assay (MMA) before transfusion of incompatible blood.[12] An MMA can be performed to help assess the clinical significance of an antibody when antigen-negative units are rare and not likely to be readily available. Given the patient's clinical situation (GI bleed with a low Hgb level), the facility transfused 2 crossmatch-incompatible units of PRBCs negative for D, C, E, and K over a 48-hour time period. No adverse reactions were noted after transfusion of e+ units in the presence of the anti-e–like antibody.

Molecular testing was performed by the American Red Cross National Molecular Laboratory. Results indicated the patient carried a *RHD*DAR* variant allele inherited with compound heterozygote *RHCE*ceEK/RHCE*ceAR* alleles, predicting partial D, partial c, partial e, and the lack of the high-prevalence antigens, hr^S and Hr (Table 1).

Table 1. Molecular testing results

	Allele name	Antigens encoded by allele
Probable *RHD* genotype	*RHD*DAR* (hemizygous or homozygous)	Partial D
Probable *RHCE* genotype	*RHCE*ceEK/*	Partial c, partial e, Hr–, hr^S–
	*RHCE*ceAR*	Partial c, partial e, Hr–, hr^S–, $V+^w$, VS–
Predicted phenotype		Partial D+C–E– partial c+ partial e+ Hr– $V+^w$ VS– hr^S–

The molecular results confirmed suspected serologic reactivity. *RHD*DAR* allele is associated with D typing discrepancies and the production of alloanti-D. In addition, the patient had two altered *RHCE* alleles predicting the hr^S– phenotype and the production of alloanti-e and/or anti-hr^S. The facility was contacted to report the additional probable anti-hr^S reactivity in the presence of previously identified anti-D, -C, and -E. Consultation was made with the American Rare Donor Program, and no RH allele–matched units were available for further transfusion needs. Despite the presence of the probable anti-hr^S, the patient tolerated the previous 2 units of PRBCs well, with no adverse effects. The patient's Hgb

increased to 7.7 g/dL with an Hct of 23 percent, and she was discharged.

Five days after the initial transfusion, the patient was readmitted to the hospital for a suspected delayed HTR. The patient presented with dark urine, Hgb of 6.3 g/dL, total bilirubin of 2.6 mg/dL (normal 0.1–1.2 mg/dL), lactate dehydrogenase 1034 IU (normal 100–190 IU), and haptoglobin less than 15 mg/dL (normal 30–200 mg/dL). The patient then received 2 additional units of D−C−E−K− PRBCs.

On completion of transfusion of the second unit, the patient began to display symptoms of a possible acute HTR. The patient's temperature increased from 98.0 to 99.5°F, with accompanying symptoms of rigors, dyspnea, and severe back pain. Blood pressure increased from 149/68 mmHg to 166/94 mmHg, with an increase in pulse from 85 bpm to 93 bpm. Oxygen saturation remained unchanged at 99 percent.

The facility began an initial transfusion reaction investigation. The post-transfusion sample was severely hemolyzed, but the DAT remained negative. Gram stain was performed and was negative for any microorganisms. A current blood sample was drawn and sent to the local IRL for additional serologic testing. The DAT on both the pre- and post-transfusion samples was negative.

Because of the recent transfusion and suspected transfusion reaction, an eluate was prepared despite the negative DAT. Expecting the eluate to contain the previously identified probable anti-hr^S, the eluate was tested against a panel of e+ and e− cells. All cells were reactive 4+ at PEG-IAT, and the patient's EDTA glycine acid–treated autologous cells were negative, as was the last wash. All cells tested with the eluate were positive except for Rh_{null} RBCs. Two Rh_{null} RBCs were tested and found to be negative with the patient's neat eluate. Additionally, one RBC with the Rh:−46 phenotype was available for testing and was significantly weaker in reactivity and displayed only a 1+ reaction. This cell was negative for c and had a weakened expression of e. Given that the patient's molecular results indicated an altered expression of c, the possibility of anti-c or anti-Hr was suspected. The eluate was tested against a panel of hr^S− cells to investigate the presence of anti-Hr, but all cells were 3+ reactive with the neat eluate. The eluate was adsorbed using R_1R_1 cells. Anti-c was demonstrated in the adsorbed eluate at PEG-IAT. Additionally, the previously identified anti-hr^S was also shown to be demonstrating in the neat eluate when tested with a c−e+ RBC.

Plasma studies were also performed on the post-transfusion sample. The plasma was reactive 4+ with two phenotypically similar cells [D−C−E−s−K−Fy(a−b−)]. The autocontrol remained negative. Adsorption studies were performed using aliquots of R_1R_1 cells. The adsorbed plasma demonstrated the presence of anti-c. To further confirm the anti-c specificity, an additional aliquot of the plasma was adsorbed with R_2R_2 cells. An acid eluate was then prepared from the adsorption cells. This eluate reacted with D−c+E− RBCs and was nonreactive with Rh_{null} RBCs. Clinically significant antibodies to other major blood group antigens were excluded in the allogeneic adsorbed eluate and plasma. The presence of anti-c in conjunction with anti-D, -C, -E, and probable anti-hr^S presented significant challenges in the possibility of locating any compatible blood for this patient. An MMA was performed by the American Red Cross National Reference Laboratory for Blood Group Serology in an attempt to assess the clinical significance of the alloantibody to the c antigen. Two random sources of group O, D−C−E−K− RBCs and autogeneic cells were used. Results are listed in Table 2. Because the patient had antibodies to both c and e as well as to D, C, and E, phenotypically similar cells (D−C−E−) were chosen for the MMA. Cells that could further distinguish the significance of the reactivity with regard to the partial anti-c or probable anti-hr^S were not available. MMA testing using genotypically similar RBCs would have been ideal, but none are currently available in the United States.

Table 2. Monocyte monolayer assay results

	IgG DAT*	% Reactive monocytes†
Random #1	4+	30.3 with fresh complement
D−C−E−K−Fy(a−b−)s−	4+	34.0 without fresh complement
Random #2	4+	38.5 with fresh complement
D−C−E−K−	4+	29.5 without fresh complement
Autologous cells	Negative	0.3 without fresh complement

*Results of coated test red blood cells after 37°C incubation.

†The normal range for the monocyte monolayer assay is 0–3 percent reactive monocytes. Values above 3 percent suggest that the antibody may cause accelerated clearance of antigen-positive red blood cells.[18]

DAT = direct antiglobulin test.

Discussion

We report the serologic and molecular findings on a 72-year-old multi-transfused African American female patient. The presence of anti-c in conjunction with anti-D, -C, -E, and probable anti-hr^S posed significant challenges in the identification and provision of compatible blood for this patient. The MMA results, the patient's clinical status, and post-transfusion laboratory and serologic results indicate that the alloanti-c is clinically significant.

Variants of c are much less frequent than variant forms of the other major Rh antigens.[12] Westhoff et al.[13] postulate that the less frequent occurrence of c variants in comparison with other Rh antigens is because the two proline residues involved in c expression form a stable structure that is resistant to the changes that often occur with *RHCE*. Individuals of African descent have a much higher incidence of variant Rh antigens as compared with individuals of European descent, with the latter constituting the largest percentage of blood donors by ethnicity.[14,15] The high prevalence of variant antigens in African American patients thereby increases the risk of alloimmunization when these patients are exposed to the conventional antigen during transfusion.[16,17]

The detection of the presence of a variant antigen, identification of the corresponding alloantibody, determination of its clinical significance, and the logistics of finding compatible blood can be quite challenging. Rh_{null} RBCs were recommended for any future transfusions for this patient, until such time that a possible RH allele–matched donor unit could be identified for further compatibility testing and evaluation. This case demonstrates the increasingly important role of molecular testing in the overall clinical/laboratory assessment of alloimmunized patients with partial RBC antigens and for subsequent transfusion recommendations and guidance.

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