Warm autoimmune hemolytic anemia with mimicking anti-c and -E specificities

H.-Y. Hsieh, D. Moroney, D. Naumann, J. Hata, N. Vosnidou, R. Kessinger, N. Shahab, N. Hakami, and D. Smith

An 18-month-old male was admitted to a hospital with a hemoglobin of 4.1 g/dL and a reticulocyte count of 53 percent. There was no history of prior transfusion. Serologic evaluation revealed the presence of both a positive direct antiglobulin test (DAT) and an indirect antiglobulin test (IAT). The patient's red blood cells (RBCs) typed as group A, C–D–E–c+e+ (cde/cde). Evaluation of the IAT revealed the presence of anti-c and anti-E. All other major antibodies were ruled out. Upon adsorption of the patient's serum with ficin-treated Cde/Cde RBCs, both antibody specificities were adsorbed; however, the antibodies were not adsorbed with native (untreated) Cde/Cde RBCs. Furthermore, the autoantibody was not adsorbed by Rh_{null} cells, thereby suggesting Rh specificity. The serum was incompatible with cde/cde RBCs and compatible with Cde/Cde RBCs. The patient was successfully transfused with Cde/Cde RBCs followed by resolution of his anemia, as evidenced by an increased and stable hemoglobin. It was concluded that the autoantibody had mimicking anti-c and -E specificities. This is a report of an unusual case of autoimmune hemolytic anemia because the Rh autoantibody appeared to have dual mimicking specificities, and the patient's RBCs were antigen negative for one of the antibody specificities, i.e., anti-E. ***Immunohematology*** **2002;18:19–22.**

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Patients with warm autoimmune hemolytic anemia often require transfusion. These transfusions may be complicated by hemolytic reactions secondary to undetected alloantibodies or the autoantibody proper.[1,2] For these reasons it is critical to perform appropriate tests to obtain compatible or most compatible units for transfusion.

Autoantibodies with Rh specificity commonly cause autoimmune hemolytic anemia.[3] Rh antibodies usually do not fix complement,[4,5] however, one such case describing a complement-binding anti-D in a D^u variant woman was reported.[6] Some of these antibodies are detected by routine serologic methods while others are detected by polybrene or polyethylene glycol techniques.[7,8] Autoantibodies mimicking alloantibodies have been described by others.[9–11] Some of these have Rh specificity[12,13] and several examples of patients with multiple Rh autoantibodies have been reported.[14,15] IgA and IgM autoantibodies with Rh (anti-e) specificity have also been described.[16,17]

In this report we describe a previously untransfused infant with autoimmune hemolytic anemia after a viral illness. The serum antibody had a unique specificity reacting with c and E antigens. Although the serum failed to react with native (untreated) Cde/Cde red blood cells (RBCs), both specificities could be adsorbed with ficin-treated Cde/Cde RBCs. These findings suggest a more complex Rh autoantibody exhibiting mimicking anti-c and -E specificities.

Case Report

An 18-month-old nontransfused male was admitted to a hospital with symptoms of fatigue and fever. The patient had been exposed to several family members with upper respiratory symptoms. Pertinent physical findings included pallor, elevated temperature, and jaundice. A blood count was obtained and revealed a hemoglobin of 4.1 g/dL (normal range 10.5 to 14.0 g/dL), a hematocrit of 13.4 percent (normal range 32 to 42%), a reticulocyte count of 53 percent (normal range 1.0 to 3.0%), a white blood cell count of 27.1×10^9/L (normal range 6 to 17.5×10^9/L), and a platelet count of 331×10^9/L (normal range 150 to 400×10^9/L). The total bilirubin was elevated at 5.3 mg/dL (normal range 0.1 to 1.5 mg/dL) with a direct of 0.3 mg/dL (normal range 0.0 to 0.3 mg/dL). Both the direct antiglobulin test (DAT) and the indirect antiglobulin test (IAT) were 3+. The patient's RBCs typed as group A, C–D–E–c+e+ (cde/cde) with anti-E and -c reactivity detected both in serum and in eluate.

The patient was treated with intravenous prednisolone and then transfused with the least incompatible RBCs available at that time (group A, cde/cde). There was an improvement in the hemoglobin

concentration. Compatible Cde/Cde RBCs were obtained within 8 hours of admission. The transfusion of both cde/cde and Cde/Cde packed RBCs occurred within 30 hours of the hospitalization. A 1.0g/dL increment in hemoglobin was obtained with 42 mL of the packed cde/cde RBCs; however, a similar increment in hemoglobin concentration was obtained with every 36 mL of Cde/Cde RBCs transfused. The patient experienced no complications, was converted to oral prednisone, and was discharged thereafter with a hemoglobin of 10.2 g/dL. Follow-up 3 weeks later revealed complete resolution of his anemia with a hemoglobin of 13.9 g/dL. An additional serum specimen was not available for further serologic studies.

Materials and Methods

The standard immunohematology techniques used are described in the American Association of Blood Banks (AABB) *Technical Manual.*[18] RBC panels were obtained from Immucor, Inc. (Norcross, GA). Phenotyping antisera were acquired from either Immucor or Gamma Biologicals (Houston, TX). Antibody screens and panels were performed in low-ionic-strength saline (LISS). Anti-C (Gamma) and anti-c, -E, and -e (Immucor) were monoclonal reagents requiring incubation at 37°C for 5 to 15 minutes. Positive and negative controls were run with each monoclonal Rh typing reagent. Antibody elution was performed using the Gamma Elu-Kit II. Polyspecific and monospecific anti-C3d and anti-IgG were obtained from Gamma, as were ficin and GammaZyme-S. Adsorptions were performed at 37°C for 30 minutes with ficin-treated or untreated Cde/Cde RBCs. Tests with Rh_{null} cells were done by the Gamma Biologicals Reference Laboratory, Houston, Texas. All specimens used for serologic studies were obtained from the patient within the first days of hospitalization. All other routine patient laboratory data were obtained by standard laboratory procedures using reagents and instruments according to the manufacturers' instructions.

Results

The patient typed as C–D–E–c+e+ (cde/cde) with Rh monoclonal typing sera. The positive and negative controls run with each monoclonal Rh reagent reacted as expected. Those results excluded interference with the patient's Rh typing by the positive DAT. The initial specimen had a 3+ DAT with monospecific anti-IgG and was negative with anti-C3d. The IAT was 3+ with cDE/cDE RBCs and 2+ with cde/cde screening RBCs using a polyspecific antiglobulin reagent. The CDe/CDe screening cell was nonreactive. Evaluation of the serum with a polyspecific antiglobulin reagent and an eluate from the patient's RBCs with a monospecific anti-IgG revealed anti-c and -E reactivities (Table 1). Three antigen negative and three antigen positive RBCs were used to confirm each reactivity. In both the serum and the eluate, anti-E reactivity was weak and anti-c strong in the IAT phase using monospecific anti-IgG. Antibodies to other antigens were ruled out. Since the serum and eluate had identical specificities, the patient's serum was first adsorbed with untreated Cde/Cde RBCs (37°C for 30 minutes). An eluate was prepared from the Cde/Cde RBCs used for adsorption. No antibody was detected in the eluate (Table 2), illustrating that the autoantibody was not adsorbed by untreated RBCs lacking the corresponding antigens. Another aliquot of serum was adsorbed × 4 with ficin-treated Cde/Cde RBCs and reactivity of the adsorbed serum determined (Table 3). The weaker anti-E specificity was readily adsorbed. However, anti-c reactivity remained after three adsorptions, albeit

Table 1. Evaluation of the serum and eluate in LISS at IS and 37°C and by the indirect antiglobulin test (IAT)

Panel Cell #	Rh-Hr Phenotype								Agglutination Results LISS		IAT		
	D	C	c	E	e	f	V	C^w	IS	37°C	Serum	Eluate	CC
1	+	+	0	+	+	0	0	0	0	0	w±	w±	
2	+	+	0	+	+	0	0	0	0	0	w±	+	
3	+	+	0	+	+	0	0	0	0	0	w±	w±	
4	+	0	+	+	0	0	0	0	0	0	+++	+++	
5	+	0	+	0	+	+	0	0	0	0	++	++	
6	0	+	+	0	+	+	0	0	0	0	w±	+	
7	0	0	+	+	+	+	0	0	0	0	+++	+++	
8	0	0	+	0	+	+	0	0	0	0	++	++	
9	0	0	+	0	+	+	0	0	0	0	++	++	
10	+	+	0	0	+	0	0	+	0	0	0	0	++
11	+	+	0	0	+	0	0	0	0	0	0	0	++
12	+	+	0	0	+	0	0	0	0	0	0	0	++

Note: Immediate spin (IS) and 37°C reactions were performed in LISS. Cells were washed and polyspecific antihuman globulin was added to the serum and monospecific anti-IgG was added to the eluate. All negative reactions were confirmed using check cells (CC).

Table 2. Results of testing an eluate prepared from Cde/Cde RBCs used to adsorb patient's serum

Cell #	Rh-Hr Phenotype								Agglutination Results IAT*	
	D	C	c	E	e	f	V	C^w	IgG	CC†
1	+	+	0	+	+	0	0	0	0	++
2	+	0	+	0	+	+	0	0	0	++

All adsorptions were performed at 37°C for 30 minutes with native (untreated) Cde/Cde RBCs. An eluate was prepared from the cells and tested for reaction with selected panel cells in LISS at 37°C for 30 minutes and by the indirect antiglobulin test* using monospecific anti-IgG. All negative reactions were confirmed using check cells.†

Table 3. Results obtained with the indirect antiglobulin test (IAT) after the serum was adsorbed × 4 with ficin-treated Cde/Cde red blood cells (RBCs).

Cell #	Rh-Hr Phenotype								IAT Results Adsorption #				
	D	C	c	E	e	f	V	C^w	#1	#2	#3	#4	CC*
1	+	+	0	+	+	0	0	0	w±	0	0	0	+
2	+	0	+	0	+	+	0	0	++	+	w±	0	+

All serum adsorptions with ficin-treated Cde/Cde RBCs were performed at 37°C for 30 minutes. Adsorbed sera were incubated in LISS with a selected panel of RBCs for 15 minutes, then washed, and monospecific IgG antiglobulin reagent was added. All negative reactions were confirmed with check cells.* Reactivity of w± to ++ denotes residual antibody in serum after the respective serial absorption.

markedly decreased, and was removed by the fourth. Furthermore, the unadsorbed serum did not react with Rh_{null} cells nor was reactivity reduced by adsorption with Rh_{null} cells. All crossmatches with Cde/Cde RBCs were compatible.

Discussion

The anti-c and -E activities could be a single autoantibody (anti-cE) reacting with both antigens or with a common determinant expressed on the protein carrying c and/or E antigen. A less likely explanation would be two separate autoantibodies produced by distinct populations of IgG-secreting plasma cells—one producing anti-c and the other producing anti-E. Because additional serum specimens were not available, it is difficult to differentiate between the two possibilities. The data clearly imply that whatever the apparent specificity was, the antibody reacted with an epitope or epitopes exposed on ficin-treated antigen-negative Cde/Cde RBCs. This makes the true nature of the antibody difficult to determine. It is perhaps best described as an autoantibody with mimicking anti-c and -E specificity.

The clinical significance of this autoantibody is not debatable as it was associated with profound hemolytic anemia. Whether the mimicking specificities proper were clinically significant may be questioned. Since the transfusions occurred within a short period of time and immediately after initiation of intravenous prednisolone therapy, the effect of prednisolone on antibody titer and transfused RBC clearance is questionable. However, since the Cde/Cde RBCs resulted in only a 117 percent better increment than the cde/cde RBCs in hemoglobin concentration, the clinical significance of the mimicking specificities is uncertain.

Rh autoantibodies are common, and some that react with more than one Rh antigen have been described. Schonitzer and Kilga-Nolger reported an autoanti-DE in a 16-year-old girl with a cystic ovarian teratoma.[19] Fudenberg et al. were the first to report autoantibodies of apparently the wrong specificity, i.e., to antigens absent on the subject's erythrocytes.[20] Others have also reported autoantibodies to Rh epitopes absent on the patient's RBCs. Such was the case described by Issitt et al. in a Cde/Cde patient with myelofibrosis who developed an autoanti-E.[21] This may not be an uncommon phenomenon, as others have described similar individuals with RBCs that have a positive DAT and yield an eluate that reacts with antigens they phenotypically lack. In this report, an autoanti-E was eluted from the patient's cde/cde cells. This antibody would not react with RBCs with a homozygous expression for the e antigen; however, the autoantibody reacted with RBCs with a homozygous expression for the C antigen and a heterozygous expression for the E antigen. Unfortunately, reagent RBCs with homozygous expression for C and E antigens (CDE/CDE) were not available to further characterize the autoantibody, but one would expect the anti-E component of the autoantibody to react more strongly with RBCs with a homozygous expression for the E antigen. Thus, this autoantibody had dual mimicking specificity with one component of the autoantibody directed to an antigen absent on the patient's RBCs. This unusual case of autoimmune hemolytic anemia exemplifies the complexity of the Rh system.

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Hsin-Yeh Hsieh, PhD, Department of Pathology and Anatomical Sciences, University of Missouri, Columbia, MO; Diana L. Moroney, MT, American Red Cross, Missouri-Illinois Region, Columbia, MO; Deanne E. Naumann, MT, SBB, American Red Cross, Missouri-Illinois Region, Columbia, MO; D. Jane Hata, PhD Candidate, Department of Pathology and Anatomical Sciences, University of Missouri, Columbia, MO; Nancy C. Vosnidou, MS, Division of Biological Sciences, University of Missouri, Columbia, MO; Rovenna L. Kessinger, MD, Department of Pathology and Anatomical Sciences, University of Missouri, Columbia, MO; Nassir Shahab, MD, Department of Internal Medicine, University of Missouri, Columbia, MO; Nasrollah Hakami, MD, Department of Internal Medicine, University of Missouri, Columbia, MO; and Daniel S. Smith, MD (corresponding author), Department of Pathology and Anatomical Sciences, M263 Medical Sciences Building, University of Missouri, Columbia, MO 65212.

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