

Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

VOLUME 17, NUMBER 2, 2001



American Red Cross

Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

VOLUME 17, NUMBER 2, 2001

C O N T E N T S

Intravenous Rh immune globulin prevents alloimmunization in D- granulocyte recipients but obscures the detection of an alloanti-K

D.F. STRONCEK, J.L. PROCTER, L. MOSES, C. BOLAN, G.J. POMPER, C. CONROY-CANTILENA, H.L. MALECH, H.G. KLEIN, AND S.F. LEITMAN

Rapid genotyping of the major alleles at the Duffy (*FY*) blood group locus using real-time fluorescence polymerase chain reaction

F.ARAÚJO, C. PEREIRA, A. ALEIXO, I. HENRIQUES, F. MONTEIRO, E. MEIRELES, P. LACERDA, AND L.M. CUNHA-RIBEIRO

Acute hemolytic transfusion reaction caused by anti-Co^a

R.B. COVIN, K.S. EVANS, R. OLSHOCK, AND H.W. THOMPSON

Tube and column agglutination technology for autocontrol testing

J.E. COURTNEY, J.L. VINCENT, AND A.J. INDRIKOV

Comparison of three low-ionic diluents for dilution and storage of reagent A₁ and B cells for testing in gel technology

E.A. STEINER AND L. DAKE

Intravenous Rh immune globulin prevents alloimmunization in D- granulocyte recipients but obscures the detection of an alloanti-K

D.F. STRONCEK, J. L. PROCTER, L. MOSES, C. BOLAN, G.J. POMPER, C. CONROY-CANTILENA,
H.L. MALECH, H.G. KLEIN, AND S.F. LEITMAN

Rh immune globulin (RhIG) has been used to prevent alloimmunization in D- recipients of apheresis platelet transfusions from D+ donors that may contain up to 5 mL of D+ red blood cells (RBCs). Granulocyte concentrates contain approximately 30 mL of RBCs and it has been necessary to give D- recipients granulocyte transfusions from D+ donors. Intravenous RhIG has not yet been demonstrated to be effective in preventing D alloimmunization with granulocyte transfusions. Four D- recipients received multiple D+ granulocyte transfusions from D+ donors and multiple injections of intravenous RhIG at a standard dose of 600 µg for each D+ transfusion. Two D- males with chronic granulomatous disease were given 32 and 13 daily granulocyte transfusions, 18 and 2 of which, respectively, were D+. After the first dose of intravenous RhIG, both patients exhibited circulating anti-D that was undetectable 3 to 4 years later. Two patients with severe aplastic anemia were given 5 and 14 granulocyte transfusions, 4 and 7 of which, respectively, were D+. Both patients died before the effectiveness of RhIG could be assessed. In one of these patients the indirect and direct antiglobulin tests became positive after the first dose of intravenous RhIG, which required that subsequent granulocyte transfusions from D+ donors be crossmatched by immediate spin (IS) testing only. A delayed hemolytic reaction attributed to alloanti-K occurred after granulocytes from a K+ donor were given to this patient. These results suggest that intravenous RhIG can be used to prevent alloimmunization to D in D- patients receiving large quantities of RBCs from D+ granulocyte transfusions. However, anti-D and other passive antibodies from RhIG prohibit the use of the antiglobulin crossmatch with antigen-positive granulocyte donor samples. It may be important to frequently collect new samples to screen for newly formed alloantibodies when IS crossmatches are used in place of the antiglobulin crossmatch. *Immunohematology* 2001; 17:37-41.

Key Words: granulocytes, granulocyte transfusions, intravenous immune globulin, Rh immune globulin, Rh alloimmunization, Rh D, transfusion reactions

The therapeutic use of granulocyte concentrates has increased as a result of the large quantity of cells that can be collected from donors mobilized with granulocyte colony-stimulating factor (G-CSF).

Transfusion of granulocytes from D-incompatible donors is more problematic than transfusion of platelets from D-incompatible donors because of a higher contamination of red blood cells (RBCs) in granulocyte concentrates. Granulocyte concentrates contain an average of 27 mL of RBCs¹ and may contain more than 40 mL of RBCs, compared with a maximum of 5 mL of RBCs permitted in apheresis platelet concentrates or 0.5 mL in platelet concentrates prepared from a whole blood donation.² As donors for granulocyte collections must be willing to receive an injection of G-CSF, the pool of available granulocyte donors is of limited size. The number of D- granulocyte donors is limited further by the need to find donors who are ABO compatible with the recipient and, in some cases, who are seronegative for cytomegalovirus.

Preventing alloimmunization to the D antigen in women of childbearing age has been the goal of transfusion services and obstetricians for many years because anti-D has been implicated in fetal death and in severe hemolytic disease of the newborn. Preventing alloimmunization to the D antigen in other individuals would allow for the transfusion of D+ blood in an emergency, continued support with D+ granulocyte concentrates, and possibly prevent the concomitant formation of autoantibody.³ The timely administration of appropriately dosed intramuscular or intravenous Rh immune globulin (RhIG) can prevent alloimmunization of D- women during pregnancy. The incidence of alloimmunization to the D antigen in women receiving antepartum and postpartum prophylactic intramuscular RhIG was reduced to 0.1 percent.⁴ A similar incidence of D alloimmunization of 1 in 1122 (0.09%) in women

receiving antepartum and postpartum prophylactic intravenous RhIG was reported by Bowman et al.⁵

The transfusion of blood products that contain D+ RBCs also can cause alloimmunization in D- individuals. Timely and appropriate doses of intramuscular RhIG can prevent alloimmunization to the D antigen in nearly 100 percent of D- recipients of D+ RBCs.⁶⁻⁸ The use of intravenous RhIG has been approved by the Food and Drug Administration to treat children and adults with acute and chronic immune thrombocytopenia purpura and for suppression of D alloimmunization in pregnancy. The calculated dose of RhIG for the WinRho SDF™ product (WinRho SDF™, Cangene Corporation, Winnipeg, Canada) can be administered by either the intramuscular or intravenous route. Dosage is based on 5 IU being equivalent to 1 µg and 18 µg is required for each mL of D+ RBCs. The product is available in 600 IU (120 µg) and 1500 IU (300 µg) vials. It has been shown to be effective in the prevention of D alloimmunization for recipients of D+ platelet products.⁹ There is evidence provided by Jouvenceaux et al.¹⁰ that the intravenous route is more effective than the intramuscular route for protection against transfusion of D+ RBCs. The WinRho SDF™ package insert recommends the administration of more intramuscular RhIG (120 IU or 24 µg per mL RBCs) than intravenous RhIG (90 IU or 18 µg per mL RBCs) for exposure to D+ RBCs. Recently, intravenous RhIG has been successfully used to prevent D alloimmunization in a D- child inadvertently transfused with D+ blood.¹¹

Materials and Methods

Study design

We evaluated the administration of intravenous RhIG to four D- pediatric patients transfused with granulocytes from D+ donors. Two of the children had chronic granulomatous disease (CGD) and two had severe aplastic anemia. Intravenous RhIG was chosen because repeated high doses were required, and the children being treated had a limited muscle mass and in some cases were thrombocytopenic. Our pharmacy routinely supplies intravenous RhIG.

RBC antibodies

Plasma from EDTA-anticoagulated whole blood was screened for atypical antibodies and, when indicated, antibody identification was performed using a low-ionic-strength solution (LISS) antiglobulin gel technique (Micro Typing Systems, Inc., Pompano Beach, FL).¹²

The direct antiglobulin test (DAT) was performed by standard tube technique using patient RBCs from EDTA-anticoagulated whole blood and polyspecific, mono-specific anti-IgG, and monospecific anti-C3d (BCA, West Chester, PA) antiglobulin reagents. Titrations, adsorption studies, and antigen typings were performed using standard tube techniques. Eluates were prepared from RBCs using Elu-Kit™ II (Gamma Biologicals, Houston, TX). The patients were monitored for the presence of circulating anti-D using the antibody screen.

Mobilization and collection of granulocytes

Granulocyte concentrates were collected from donors only given dexamethasone, 8 mg PO, approximately 12 hours prior to collection; G-CSF (Filgrastim, Amgen, Thousand Oaks, CA) only, 5 µg/kg SQ, approximately 18 hours prior to the collection; or both dexamethasone and G-CSF. Granulocyte concentrates were collected using a CS3000 blood cell separator (Fenwal, Baxter Healthcare Corporation, Round Lake, IL). A granulocyte separation chamber was used and 7 liters of whole blood were processed per procedure, using 30 mL of 46.7% of trisodium citrate anticoagulant (tirCitrasol; Citra anticoagulants, Braintree, MA) in 500 mL of hydroxyethyl starch (Baxter). The interface offset was 33. The final volume of the concentrates was approximately 250 mL.

Administration of intravenous RhIG

For patients 1, 2, and 3, 3000 IU (600 µg) of WinRho SDF™ were given intravenously for each D+ granulocyte concentrate transfused. RhIG was given within 48 hours following the incompatible transfusion. Because patient 4's clinical condition was unstable, her antibody screen was monitored for circulating anti-D and she received a reduced dose of RhIG (600 µg for every 2 D+ granulocyte concentrate; Table 1).

Table 1. Number of granulocyte concentrates and doses of RhIG given to each patient

	Patient			
	1	2	3	4
Gender	Male	Male	Male	Female
Age (years)	15	6	9	11
Diagnosis	CGD*	CGD	SAA†	SAA
Blood type	B -	O -	B -	A -
Number of granulocyte concentrates given	32	13	5	14
Number of D+ concentrates	18	2	4	7
RhIG doses (600 mg)	18	2	4	4

* Chronic granulomatous disease

† Severe aplastic anemia

Results

Patient 1, a 15-year-old male with CGD, received 32 granulocyte concentrates collected from dexamethasone-treated donors over 49 days. Eighteen of the granulocyte concentrates were from D+ donors. Circulating anti-D was detected after intravenous RhIG was administered, but the DAT was negative. Following the last intravenous RhIG infusion, his antibody screen was strongly reactive with D+ screening cells (3+). The antibody screen was repeated regularly for several weeks following the last infusion; 3 months after the last infusion, the antibody screen remained weakly reactive with D+ cells. The next antibody screen, which was performed 4 months later, was negative. More than 65 antibody screens were performed for anti-D over the next 4 years, all of which were negative (Table 2).

Table 2. Results of the antibody screen (AS) and the direct antiglobulin test (DAT) in granulocyte concentrate recipients before, during, and 4 months after the administration of RhIG

	Patient			
	1	2	3	4
Before RhIG administration				
AS	Negative	Negative	Negative	Negative
DAT	Negative	Negative	Negative	Negative
During RhIG administration				
AS	Anti-D	Anti-D	Anti-D	Anti-D, -C, -E
DAT	Negative	Positive*	Not done	Positive†
After RhIG administration				
AS	Negative	Negative	Not done	Not done
DAT	Negative	Negative	Not done	Not done

*The DAT was reactive with anti-IgG; an eluate detected anti-D

†The DAT was reactive with anti-IgG and anti-C3d; an eluate detected anti-D and -A

Patient 2, a 6-year-old male with CGD, was given 13 dexamethasone-mobilized granulocyte concentrates over 25 days, 2 of them from D+ donors. After intravenous RhIG was administered, anti-D was detected in his serum and the DAT was positive. Anti-D was eluted from his RBC sample, which contained D+ transfused RBCs. No additional granulocyte concentrates were transfused. His DAT remained positive for 2 weeks following the last granulocyte transfusion. The antibody screen was strongly reactive with D+ cells (2+) during the 2 weeks following the infusion of 6000 IU of intravenous RhIG but was only weakly reactive at 3 weeks. Titration studies with R₁R₁ RBCs revealed an endpoint of w+ at 1, 4, and 4 for samples drawn on days 12, 15, and 19, respectively, of postinfusion intravenous RhIG. Four

months after the infusion of RhIG, his antibody screen and DAT were negative, as were three assays performed during a 3-year follow-up period.

Patient 3, an 11-year-old male with severe aplastic anemia, was given five G-CSF plus dexamethasone-mobilized granulocyte concentrates over 9 days for a disseminated aspergillus infection of the lung and *Enterobacter enterococcus bacteremia*. Four of the granulocyte concentrates were from D+ donors. He was given four doses of 3000 IU (600 µg) of intravenous RhIG after receiving four granulocyte concentrates from D+ donors, which he tolerated without complications. Anti-D was detected 2, 6, and 9 days postinfusion of RhIG. A DAT was not performed. He died from the infections 10 days after the first granulocyte concentrate was given.

Patient 4, an 11-year-old group A girl with severe aplastic anemia, was treated with antithymocyte globulin and cyclosporin A. She developed an aspergillus infection of her sinuses despite treatment with amphotericin and was transfused with 14 G-CSF and dexamethasone-mobilized granulocyte concentrates, 7 of which were from D+ donors, over 23 days. She was given four infusions of 3000 IU (600 µg) of intravenous RhIG. Antibody screen results were negative prior to the administration of intravenous RhIG. Circulating anti-C and -D were detected after two doses of RhIG and infusion of two D+ granulocyte concentrates. Over the next 6 days, she received three D+ granulocyte transfusions and a third dose of RhIG. This lot of RhIG was shown to contain anti-D, -C, and -E at titration levels of >4096, 64, and 4, respectively, by indirect antiglobulin test (IAT). Subsequently, circulating anti-E as well as anti-D were detected in the patient's plasma. She received multiple granulocyte and platelet transfusions from group O donors. The DAT was positive with both IgG (1+) and C3d (1+) antiglobulin. Anti-D and -A were eluted from her RBCs.

A fourth dose of RhIG and two more D+ granulocyte concentrates were given over the next 8 days. The last D+ granulocyte transfusion was associated with a pulmonary reaction due to a newly formed HLA antibody and the granulocyte transfusions were discontinued. The next day, the patient's lactate dehydrogenase level (LDH) increased to 1257 U/L from 132 U/L the previous day. Her bilirubin level increased from 13.4 mg/dL to 17.0 mg/dL and her hematocrit fell from 24% to 19%. Repeat testing of her plasma detected a new anti-K in addition to passively acquired Rh antibodies. An aliquot of the RhIG lot reacted microscopically positive by tube LISS/IAT with

one K:1,2 cell. The patient's plasma reacted 2+ with the same cell by the gel antiglobulin test. The DAT remained positive with anti-IgG (1+) and anti-C3d (1+) and a weak panagglutinin was eluted from her RBCs along with anti-D and -K, which were demonstrable after removal of the autoantibody by adsorption.

Phenotyping of RBCs collected from Patient 4, 1 week before the onset of hemolysis, showed mixed-field reactivity with the A, E, and K antigen typings. During the 2 weeks before the hemolytic episode, three units of A- RBCs were transfused. Two units were C-, E+, K+, and one was C-, E-, K+. Two days after the onset of the hemolytic reaction, a titration of her serum against a K+ RBC revealed an endpoint of w+ at 32, which was indicative of a newly formed alloantibody. The presumed passively acquired anti-E and -D showed titration result endpoints of w+ at 2 and 8, whereas the anti-C was not demonstrable.

All further RBC transfusions were negative for the D, C, E, and K antigens. The DAT remained positive for the next 6 days. The aspergillus infection progressed and she died 15 days after the last granulocyte transfusion. At the time of death, her LDH had fallen to 514 U/L, and there were no other signs of hemolysis.

Discussion

Four D- recipients were given repeated granulocyte concentrates from D+ donors containing approximately 30 mL of RBCs and were treated with intravenous RhIG to prevent alloimmunization. Two patients with CGD were followed for more than 3 years after the transfusion of D+ granulocyte concentrates and neither produced anti-D. The effectiveness of RhIG could not be assessed in two other patients with severe aplastic anemia who died within weeks of their last granulocyte transfusion, but both patients tolerated the infusions of RhIG.

Although this was not a controlled study, the results suggest that intravenous RhIG is effective in preventing D alloimmunization in granulocyte transfusion recipients. CGD patients have defects in granulocyte respiratory burst but otherwise have normal immune systems. These results are similar to a previous study of 10 D- volunteers who received 2.5 mL of D+ RBCs and 120 µg doses of intravenous RhIG up to 48 hours after the RBCs¹³ and case reports on four D- women who were inadvertently transfused with one to three units of D+ RBCs and received 3000 to 7750 µg intravenous RhIG.¹⁴ The preventative dose of intravenous RhIG suggested by Mollison and colleagues is 10 to 15 µg per mL of RBCs.¹⁴ Hemoglobinuria was

the only untoward effect listed for the administration of such large doses of intravenous RhIG in the presence of a significant amount of D+ RBCs.

The manufacturer of intravenous RhIG indicates that 18 µg will suppress the immunizing potential of 1 mL of D+ RBCs. Because a typical granulocyte concentrate contains approximately 30 mL of RBCs, initially we elected to give 600 µg of intravenous RhIG after each granulocyte transfusion. The number of RBCs in each granulocyte concentrate is variable, but when 600 µg of intravenous RhIG was given after each transfusion, circulating anti-D was readily detectable after one or two intravenous RhIG infusions, suggesting that the dose was adequate. In fact, Patient 4 received only four doses of intravenous RhIG to treat seven D+ granulocyte transfusions and passively infused anti-D, and -E were detected after the last D+ granulocyte transfusion. Although the optimum dose of intravenous RhIG for recipients of D+ granulocyte transfusions is not known, we recommend that initially 600 µg of intravenous RhIG be given for each D+ transfusion. However, if the antibody screen remains reactive with D+ cells after multiple doses of RhIG, it is likely that giving 600 µg for every two D+ granulocyte transfusions will be sufficient. The infusions of RhIG were well tolerated by the children in this study.

The antiglobulin crossmatch cannot be used for compatibility testing of granulocyte products with D+ RBCs in patients with passive anti-D. Circulating anti-D was detected in all four patients following the administration of intravenous RhIG. In addition, passively acquired anti-C and -E were detected in one patient. Because the antiglobulin crossmatch is of limited use, it is important to follow the recipient's antibody screen closely and frequently analyze samples for the presence of newly developed alloantibodies. This is especially difficult when RhIG contains multiple antibody specificities. Passive transfer of anti-D, -C, -E, -Le^b, and -Bg has been reported with RhIG prophylaxis following D+ platelet transfusions.¹⁵ Differentiating passively acquired antibodies from newly developed alloantibodies is not always possible. Therefore, it may be necessary to provide antigen-matched RBC units that are crossmatched by immediate spin to check for ABO compatibility between the donor and recipient. If possible, it may be prudent to perform extended RBC phenotyping on D- granulocyte transfusion recipients before transfusion of products containing RBCs. The phenotype information could be helpful in determining the antigens to which the recipient can produce alloantibodies.

In conclusion, intravenous RhIG prophylaxis was successful in two D- patients receiving granulocyte transfusions containing D+ RBCs. However, it is not known if these individuals were at risk for alloimmunization (i.e., immune responders) and further studies are needed. The use of intravenous RhIG was well tolerated and avoids the problems associated with multiple intramuscular injections. Approximately 15 percent of Caucasians are D-; therefore, the ability to transfuse D- patients with granulocyte concentrates from D+ donors increases the pool of donors available to these patients more than fivefold. This is particularly important because granulocyte concentrates must be collected from ABO compatible and, in some cases, CMV-negative donors. However, anti-D and other passive antibodies from RhIG prohibit the use of the antiglobulin crossmatch with antigen-positive granulocyte donors. Thus, for granulocyte concentrate recipients treated with RhIG to prevent D alloimmunization, it may be necessary to screen the recipient's serum more frequently than every 3 days to prevent the transfusion of an incompatible product.

Acknowledgment

We thank Ellen Lazarus, MD, for reviewing the manuscript and for her suggestions.

References

1. DePalma L, Leitman SF, Carter CS, Gallin JI. Granulocyte transfusion therapy in a child with chronic granulomatous disease and multiple RBC antibodies. *Transfusion* 1989; 29: 421-3.
2. Vengelen-Tyler V, ed. Technical manual. 13th ed. Bethesda, MD: American Association of Blood Banks, 1999: 458.
3. Rosse WF, Gallagher D, Kinny TR, et al. Transfusion and alloimmunization in sickle cell disease. The cooperative study of sickle cell disease. *Blood* 1990; 76: 1431-7.
4. Pollack W, Gorman JG, Freda, VJ, et al. Results of clinical trials of RhoGAM in women. *Transfusion* 1968; 8: 151-3.
5. Bowman JM, Albert D, Friesen M, Pollock JM, Taylor WE. WinRho: Rh immune globulin prepared by ion exchange for intravenous use. *CMA Journal* 1980; 123: 1121-5.
6. Ennker J, Maas DHA, Schneider J. Immunoprophylactic anti-Rho(D) treatment after mismatched transfusions. *Eur J Obstet Gynecol Reprod Biol* 1979; 9: 117-24.
7. Bowman HS. Effectiveness of prophylactic Rh immunosuppression after transfusion with D-positive blood. *Am J Obstet Gynecol* 1976; 124: 80-4.
8. Hartwell EA. Use of Rh immune globulin. ASCP practice parameter. *Am J Clin Pathol* 1998; 100: 281-92.
9. Zeiler T, Wittmann G, Zingsem J, Weisbach V, Zimmerman R, Eckstein R. A dose of 100 IU intravenous anti-D gamma globulin is effective for the prevention of RhD immunization after RhD-incompatible single donor platelet transfusion. *Vox Sang* 1994; 66: 243.
10. Jouvenceaux A, Adenot N., Berthoux F, Revol L. Gamma-globuline anti-D lyophilisée intra-veineuse pour la prévention de l'immunisation anti-Rh. *Rev Franc Transfus* 1969; 13 (Suppl 1): 341.
11. Anderson B, Shad AT, Gootenberg JE, Sandler SG. Successful prevention of posttransfusion Rh alloimmunization by intravenous Rho (D) immune globulin (WinRho SD). *Am J Hematol* 1999; 60: 245-7.
12. Kupferman MJ, Cipolone KM, Procter JL, Stroncek DE. Comparison of tube and gel red cell agglutination techniques in detecting chimeras after a major ABO-mismatched allogeneic hematopoietic stem cell transplantation. *Immunohematology* 1998; 14: 68-71.
13. Bowman, JM. The advantages of intravenous Rh-immune globulin. *Clin Obstet Gynecol* 1982; 25: 341-7.
14. Mollison PL, Engelfriet LJ, Contreras M, eds. *Blood transfusion in clinical medicine*. 9th ed. Boston, MA: Blackwell Science, 1993: 241-2.
15. Wright J, Freedman J, Lim FC, Garvey MB. Crossmatch difficulties following the prophylactic use of Rh immune globulin. *Can Med Assoc J* 1979; 120: 1235-8.

D.F. Stroncek, MD, Department of Transfusion Medicine, 10 Center Drive MSC-1184, Building 10, Room 1C711, Bethesda, MD, 20892; J.L. Procter, MD, MT(ASCP)SBB, L. Moses, MT(ASCP)SBB, C. Bolan, MD, G.J. Pomper, MD, and C. Conry-Cantilens, MD, Department of Transfusion Medicine, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD; H.L. Malech, MD, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; and H.G. Klein, MD, and S.F. Leitman, MD, Department of Transfusion Medicine, National Institutes of Health, Bethesda, MD.

Rapid genotyping of the major alleles at the Duffy (*FY*) blood group locus using real-time fluorescence polymerase chain reaction

F. ARAÚJO, C. PEREIRA, A. ALEXIO, I. HENRIQUES, F. MONTEIRO, E. MEIRELES, P. LACERDA, AND L.M. CUNHA-RIBEIRO

The Duffy blood group system has clinical importance due to involvement in transfusion reactions and hemolytic disease of the newborn. Recently, the molecular basis of the two alleles, *FY**A and *FY**B (125G>A), and the mutation situated in the promoter region of the *FY* gene (-33T>C), have been elucidated. In order to develop an accurate, easy, and rapid genotyping method, we describe a procedure using the LightCycler®. Samples from 53 Caucasian Portuguese blood donors and 7 black, healthy, European individuals were phenotyped with commercial antisera. DNA was extracted from blood samples and the relevant sequences were amplified with the same cycling conditions, using real-time polymerase chain reaction. The melting point of the *FY**A allele was 63°C and of the *FY**B allele, 55°C. The allele without mutation at the promoter region had a melting point at 64°C and the *FY**B silent allele at 58°C. The results in Caucasian individuals were similar to those found in European and American populations. When *FY* genotyping techniques are necessary, the methodology described is preferable to conventional methods as it is reliable, high speed, and uses small volumes, providing a highly competitive technology for use by a routine laboratory. *Immunohematology* 2001;17:42-44.

Acute hemolytic transfusion reaction caused by anti-Co^a

R.B. COVIN, K.S. EVANS, R. OLSHOCK, AND H.W. THOMPSON

Co^a is a high-frequency blood group antigen in the Colton blood group system expressed on red blood cells (RBCs) of approximately 99.8 percent of random persons. Anti-Co^a has been reported to cause delayed hemolytic transfusion reactions, hemolytic disease of the newborn, and accelerated clearance of RBCs in vivo. Acute hemolytic transfusion reactions (AHTRs) have not previously been reported. A 58-year-old man was hospitalized for vascular surgery. Initial blood bank evaluation revealed anti-Fy^a. The patient received six units of RBCs during his initial hospitalization and developed anti-E. A subsequent sample was sent to the reference laboratory when all units of RBCs appeared incompatible. Additional studies, including alloabsorptions, revealed the presence of anti-E, anti-Fy^a, and an apparent warm autoantibody. One unit of least-incompatible RBCs was transfused during surgery. The patient had an increase in temperature. Hemoglobinuria and a decrease in hematocrit were also noted. Due to the clinical impression of an AHTR, the pre- and postreaction samples were re-evaluated in the reference laboratory and demonstrated the presence of anti-Co^a in both. Based on clinical and laboratory evaluation this patient appears to have had an AHTR due to anti-Co^a. This is the first known reported case of an AHTR caused by anti-Co^a. *Immunohematology* 2001;17:45-49.

Tube and column agglutination technology for autocontrol testing

J. E. COURTNEY, J.L. VINCENT, AND A.J. INDRIKOV

The incidence of positive autocontrol test results with column agglutination technology is a concern. This study investigates the incidence and significance of positive autocontrols in the ID Micro Typing System™ (gel) and the Gamma ReACT™ (ReACT). The study encompassed a total of 1021 randomly selected samples from patients and 95 samples from donors collected during 1 month. The autocontrol testing was carried out according to the manufacturer's instructions for the column agglutination tests. The tube method was carried out using low-ionic-strength solution (LISS). The direct antiglobulin test (DAT) was performed using the tube method, and further investigated with elution studies if warranted. Seventy-nine patient's samples (7.74%) had a positive autocontrol: the gel test, 72 (91.13%); ReACT, 21 (26.58%); and the tube method, 27 (34.18%). Of the 79 positive autocontrols, 44 samples had a negative DAT. Of the samples with positive DAT results, only one possessed a clinically significant antibody, anti-D. Moreover, the same sample also tested positive in all three methods. Column agglutination techniques have increased sensitivity for a positive autocontrol beyond the conventional tube method. However, ReACT and gel tests differ significantly in their frequency of positives. Investigation of the significance of a positive autocontrol in column agglutination technology when the conventional tube method is also positive is suggested. *Immunohematology* 2001;17:50–52.

Comparison of three low-ionic diluents for dilution and storage of reagent A₁ and B cells for testing in gel technology

E. A. STEINER AND L. DAKE

Currently, ABO serum grouping performed by gel technology employs a red cell diluent containing EDTA (MTS Diluent 2 Plus™) that does not permit extended storage of the red cell suspensions. A diluent currently used for suspension and long-term storage of reagent red cells for antibody detection and identification (Ortho 0.8% Red Cell Diluent™) was evaluated for use with A₁ and B cells. Because this diluent does not contain EDTA, testing was limited to EDTA samples. As a comparison, a Micro Typing Systems (MTS) diluent not containing EDTA (MTS Diluent 2™) was also tested. MTS-suspended red cells were maintained for 24 hours and compared with Ortho-Clinical Diagnostics 0.8% suspended red cells maintained for 7 days. ABO serum grouping was performed on 144 EDTA plasma samples using all three cell suspensions. Acceptable results were noted in all aspects of testing. *Immunohematology* 2001;17: 53–56.