Life-threatening delayed hyperhemolytic transfusion reaction in a patient with sickle cell disease: effective treatment with eculizumab followed by rituximab

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BACKGROUND: Hyperhemolysis in sickle cell disease is a rare and potentially life-threatening complication of transfusion.

STUDY DESIGN AND METHODS: In this article we report a case of delayed hemolytic transfusion reaction with resultant hyperhemolysis triggered by an anti-IH autoantibody with alloantibody behavior.

RESULTS: The anti-IH was reactive at room temperature as well as 37°C, but only weakly reactive with autologous red blood cells. Initial cold agglutinin titer was 512. The profound, life-threatening, intravascular hemolysis was rapidly and dramatically reduced with the Complement 5 (C5) inhibitory antibody, eculizumab. The auto/allo cold agglutinin was subsequently suppressed with rituximab treatment.

CONCLUSIONS: Eculizumab, a potent C5 inhibitory antibody, can be a rapid and effective therapy for hyperhemolytic transfusion reactions when given in a sufficient dose to fully block the activation of complement C5.

Delayed hemolytic transfusion reactions (DHTRs) are a known complication of transfusion in patients with sickle cell disease (SCD).[1] However, DHTR in SCD patients may exhibit unusual clinical, immunologic, and hematologic findings not observed in other individuals. Despite transfusion of antigen-matched blood products, SCD patients can still develop hemolytic transfusion reactions.[1-6] This may be explained, in part, by the changes in the red blood cell (RBC) cytoskeleton and membrane that render the SCD RBC more sensitive to both mechanical and complement destruction.[7] We report a rare case of a life-threatening DHTR with hyperhemolysis syndrome (HHS) in a SCD patient triggered by an anti-IH autoantibody with alloantibody behavior. The intravascular hemolysis was successfully inhibited with eculizumab therapy. The autoantibody was later suppressed with rituximab treatment.

ABBREVIATIONS: ARC = American Red Cross; C5 = Complement 5; DHTR(s) = delayed hemolytic transfusion reaction(s); HHS = hyperhemolysis syndrome; PNH = paroxysmal nocturnal hemoglobinuria; SCD = sickle cell disease; SPRCA = solid-phase red blood cell adherence.

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CASE REPORT

The patient was a 35-year-old African American female with SCD and an extensive transfusion history. Two weeks before presentation she was given 2 RBC units before undergoing a laparoscopic cholecystectomy for gallstones. Her pretransfusion records demonstrated blood type Group A, D+ with detection of known alloantibodies which included anti-C, anti-E, anti-K, anti-S, anti-Fy^a, anti-Jk^a, and anti-Sd^a. The transfused units were ABO, Rh-matched and negative for antigens to her known alloantibodies and cross-match compatible. After surgery she discharged with hemoglobin (Hb) of 8.2 g/dL.

Two weeks later she presented with severe fatigue, worsening jaundice, and total body pain. Physical examination was notable for scleral icterus, pale conjunctivae, and a well-healed surgical scar on her abdomen. Hb levels and absolute reticulocyte count were 7.4 g/dL and 211 $\times$ 10^9/L, respectively, with a lactate dehydrogenase (LDH) of 1174 IU/L (normal, 120-240 IU/L) on initial presentation. Vital signs were unremarkable. She was admitted for the management of a possible pain crisis and received intravenous (IV) fluids, pain medication, and IV antibiotic.

One day after admission, she had a fever of 39°C and her oxygen saturation dropped to 85% on 10 L of oxygen using a nonrebreather mask. Hb precipitously decreased to 3.8 g/dL (from 7.4 g/dL) with laboratory values suggestive of acute hemolysis with LDH of more than 5000 IU/L, total bilirubin of 9.8 mg/dL, and haptoglobin of less than 20 mg/dL. Acute kidney injury was also present with serum creatinine increasing to 1.29 mg/dL from 0.77 mg/dL on admission. She did not have overt hemoglobinuria. Multiple blood cultures were negative for an acute infection.

An EDTA sample sent to the blood bank demonstrated brown cloudy plasma by visual inspection consistent with intravascular hemolysis. Pretransfusion testing demonstrated a positive antibody screening test with panreactivity in all panel cells. Cross-matching of the patient's plasma with segments from the two previously transfused units now showed incompatibility, indicating newly formed alloantibodies. The direct antiglobulin test (DAT) was weakly positive for complement only. Additional testing performed at the American Red Cross (ARC) Immunohematology Reference Laboratory identified anti-IH as the cause of the DTHR. No other new alloantibodies were detected.

She was then transfused with five additional type-matched, antigen-negative RBC units over the next 3 days. Despite transfusion, her Hb further declined to 3.6 g/dL. Reticulocyte count also decreased to 36 $\times$ 10^9/L and renal function further deteriorated (creatinine, 2.08 mg/dL). Because of the aggressive complement-mediated intravascular hemolysis, eculizumab was given on a compassionate use protocol after approval from the USC Investigational Review Board. Meningococcal vaccination was given before beginning eculizumab and the patient received 14 days of ciprofloxacin for meningococcal prophylaxis. The initial dose of eculizumab, 1200 mg, was given weekly for 4 weeks for the induction phase beginning on the third hospital day (Treatment Days 1, 8, 15, and 22), followed by every 2 weeks maintenance starting on Treatment Day 29. With eculizumab treatment, the LDH decreased rapidly from more than 5000 IU/L to 1626 IU/L by Treatment Day 8, 747 IU/L on Day 22, and 467 IU by Treatment Day 29 (Fig. 1). The patient's Hb stabilized at 5.4 g/dL by Day 7 of eculizumab treatment without further need for transfusion (Fig. 1). Immunosuppressive therapy with rituximab, 375 mg/m^2, was given weekly for 4 weeks starting on Treatment Day 3, after the initiation of eculizumab. By Day 8, her Hb returned to baseline levels and the serum creatinine normalized (0.54 mg/dL). The cold agglutinin titer, initially noted to be 512, remained elevated until Day 84 (256 on Treatment Day 42, 4 on Treatment Day 84).

MATERIALS AND METHODS

Blood bank evaluation

All serologic testing was performed on EDTA patient samples according to procedures outlined in the AABB Technical Manual.[8] Screening studies were performed by solid-phase RBC adherence (SPRCA; Neo Galileo Immucor SPRCA platform, Immucor, Norcross, GA). Panel studies were performed by SPRCA and confirmed with gel microcolumn method (gel system, Ortho-Diagnostics, Raritan, NJ) using Resolve Panel A and Panel B reagent RBCs. The DAT was performed with reagents from Immucor, using both tube and SPRCA (Echo Immucor SPRCA platform, Immucor) methods. Reactivity with a polyspecific reagent was further characterized by testing with monospecific anti-IgG and a blend of anti-C3b and anti-C3d. Titration studies using serial dilutions of the patient's plasma were performed in normal saline at 4°C with group O indicator RBCs. Additional indirect antibody tests, acid elution studies, and thermal amplitude studies were performed at the ARC Reference Laboratories.

On this presentation, the antibody screen revealed the presence of a panagglutinin that reacted with all panel cells with 4+ strength. The DAT by tube method was weakly positive for C3 only. DAT by SPRCA, a method less sensitive to IgM- and complement-mediated coating of RBCs, was negative. This posttransfusion sample was no longer compatible when again cross-matched with rider segments from the previously transfused RBC units. The patient's sample was further tested at ARC Reference Laboratories. Anti-IH was identified in the patient's serum that was reactive at room temperature and 37°C by strict prewarm technique. Strong (4+) reactivity was observed

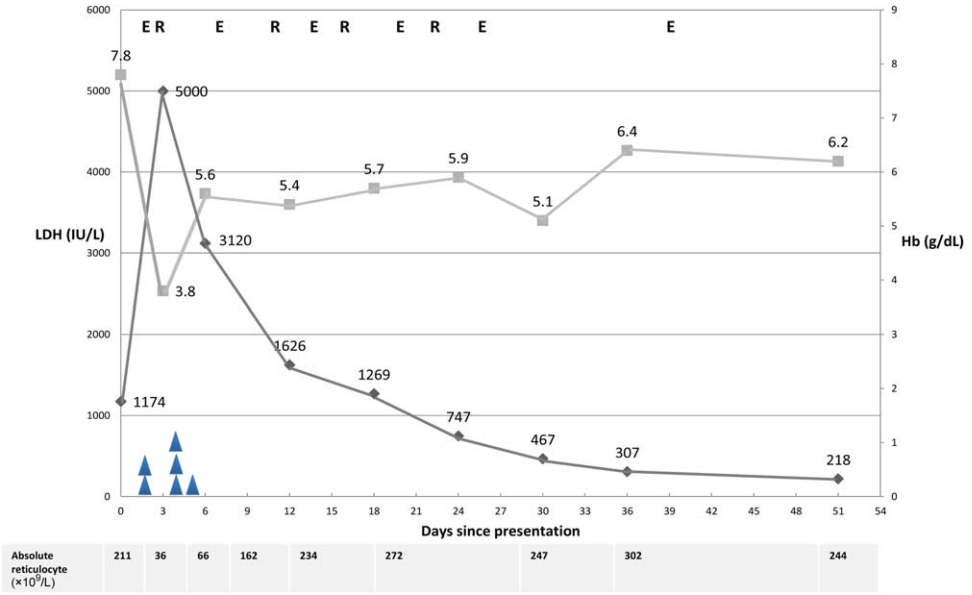

Fig. 1. Effects of eculizumab and rituximab therapy in a SCD patient with hyperhemolysis. Levels of hemolysis measured by Hb (g/dL, top line) and LDH (IU/L, bottom line). Arrowheads represent days 1 unit of RBCs were given. E and R represent days when eculizumab and rituximab were given. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with A2, B, and O RBCs while weak (1+) reactivity was seen with autologous and A1 RBCs. An acid eluate showed a similar reactivity pattern, demonstrating strong reactivity with A2 and O RBCs and no reactivity with autologous and A1 RBCs. Initial 4°C cold agglutinin titer was 512. Dithiothreitol-treated plasma and thermal amplitude studies showed that the high titer anti-IH was of IgM subtype with reactivity with H antigen–rich RBCs (O and A2).

DISCUSSION

DHTRs are well-known complications of transfusion reported to occur in 0.04% to 0.1% of transfused patients.[1] These reactions classically present when transfused RBCs express antigens foreign to the recipient, prompting alloantibody formation. Clearance of donor RBCs manifests as a decrease in Hb due to hemolysis 3 to 15 days later. However, SCD patients may have atypical DHTR with unusual clinical, immunologic, and hematologic findings, including hemolysis despite receiving transfusion of antigen-matched blood products.[2,3,5] In addition to alloantibody formation, autoantibodies or the lack of any detectable antibody has been observed.[3-5] The diagnosis of a DHTR is often delayed or overlooked as patients frequently present, like our patient, with symptoms attributed to a vasoocclusive crisis.[3,4,9] Approximately 11% of SCD patients with DHTR may develop a life-threatening anemia known as the HHS, characterized by a decrease in Hb below the pretransfusion Hb level.[1]

In this case, we report an unusual DHTR induced HHS that was triggered by a newly formed anti-IH cold agglutinin. The anti-IH cold agglutinin was a weak autoagglutinin with broad thermal amplitude. IH cold agglutinins are a rare cause of hemolytic transfusion reactions.[10-13] The anti-IH found in our patient also had characteristics of an alloantibody, mediating hemolysis of donor RBCs. The anti-IH reacted very weakly with autologous RBCs and did not correlate with observed degree of hemolysis. Most anti-IH have activity at 25°C and rarely induce significant RBC destruction. In rare cases, anti-IH may demonstrate reactivity at 37°C may cause hemolysis.[13] The anti-IH observed in this patient had characteristics of an alloantibody reactive at 37°C and induced significant hemolysis.

Anti-IH has complex specificities to both I and H antigen and is generally found in the sera of individuals with weak RBC expression of H antigen.[12] Because H antigen is a substrate that is converted to A and B blood groups, its degree of expression varies among ABO groups. It is most highly expressed in group O, followed by A2, B, A2B, A1, and A1B. Subgroup A2 RBCs have significantly higher expression of H than A1 RBCs because their enzyme responsible for converting H to A is less efficient.[10] When a patient has a high-titer anti-IH with a broad thermal range and is transfused with group O or A_2 RBCs rich in H antigens, clinically significant hemolysis can occur. Three reported cases have described an acute hemolytic transfusion reaction after the administration of

either group O or group A_2 RBCs to patients with anti-IH.[10,12,13] Read and colleagues[11] reported a group A_1 patient who was transfused with group A (subtype undetermined) RBCs and subsequently developed an anti-IH–mediated DHTR 14 days later. Of note, two of these cases responded to subsequent transfusion of group-specific, H-antigen–poor RBCs, indicating that the anti-IH was primarily mediating hemolysis of group O or A2 donor RBCs rather than autologous cells.[10,12] Although 80% of group A donor units are subgroup A_1, it is possible that our patient received an A_2 unit that triggered the formation of anti-IH with ensuing lysis of A2 donor cells. Regrettably, the transfused unit was not available for subtyping. A 1 g/dL decrease in Hb is consistent with the hemolysis of 1 unit of transfused A_2 RBCs. However, the subsequent precipitous decrease in Hb and increase in LDH within 24 hours is unlikely to be due only to the destruction of donor RBCs alone. Anti-IH, behaving as an alloantibody, resulted in the hemolysis of donor RBCs, which served as the trigger for HHS. Although acute hemolytic transfusion reactions due to anti-IH have been reported in SS disease, this is the first case of HHS attributable to anti-IH.

The pathophysiology of HHS remains unclear. HHS is characterized by alloantibody (or sometimes unknown RBC antibody) formation leading to hemolysis of transfused and autologous RBCs.[1,6] Several mechanisms have been proposed to explain this hemolytic phenomenon including "innocent bystander" hemolysis,[1,5] suppression of erythropoiesis,[3] and macrophage-mediated destruction of RBCs.[6] Transfusion reactions, such as in this case, are associated with complement activation on the RBCs. Most of the reports of HHS have occurred in SCD patients. Because of membrane distortion and membrane loss due to sickle Hb tactoid formation, sickle cells may lose the membrane glycosylphosphatidylinositol-anchored complement regulatory proteins CD55 and CD59.[7] This can result in an increased sensitivity to complement-mediated destruction similar to that seen in paroxysmal nocturnal hemoglobinuria (PNH).[14] In addition, recent reports suggest that free heme in the plasma may induce increased complement activation through increased alternative pathway activation.[15] While C3b opsonization results in extracellular elimination of RBCs in the liver and spleen, terminal complement activation, Complement 5 (C5)b-C9 formation of the membrane attack complex, results in intravascular hemolysis. As a result of the acquired "PNH" phenotype, intravascular hemolysis may occur due to increased C5b-C9 deposition on the RBC membrane.[7] It is likely that increased complement activity accelerates the destruction of the SCD RBCs causing the HHS. This phenomenon may explain why, in our patient, the DAT was negative for IgG and showed only weak reactivity with C3b reagent despite massive intravascular hemolysis.

Reticulocytopenia is often observed during HHS. Petz and colleagues[3] suggested that transfusion-induced suppression of erythropoiesis is responsible for the decrease in autologous red production cells and emphasized that withholding further transfusion with steroid treatment can result in reticulocyte recovery. However, steroids should have little or no effect on a cold agglutinin–mediated HHS. RBC precursors and reticulocytes may also be susceptible to HHS complement destruction. On day 1 of presentation, our patient had an absolute reticulocyte count of 211×10^9/L, which plummeted to 36×10^9/L after transfusion of RBCs (Fig. 1) without improvement in the Hb. Additional mechanisms could include RBC precursor and reticulocyte destruction in the marrow by contact lysis via activated macrophages.[5] Due to oxidative stress, sickle cells readily expose phosphatidylserine on their outer membranes, further enhancing macrophage clearance.[6] Sickle cell reticulocytes may also adhere via integrin $\alpha 4\beta 1$ to vascular cell adhesion molecule-1 on the macrophage as an additional mechanism of macrophage mediated destruction.[5,16] However, a complement-mediated destruction of RBC precursors may have also occurred suggested by the rapid increase in the patient's reticulocyte count after starting treatment with eculizumab.

Definitive treatment of HHS has not been established. Transfusion is generally avoided unless absolutely clinically necessary as it may exacerbate further hemolysis as observed in our patient.[3,5] Steroids are ineffective in cold agglutinin hemolysis and would have no effect on RBC membrane complement reactions. IVIG has shown efficacy in one report of SCD HHS in children, although the mechanism by which it would modulate the disease cannot be readily explained.[17] Rituximab, an anti-CD 20, has been used to treat cold agglutinin hemolysis and also utilized as a prophylactic agent to prevent DHTR in SCD patients.[18-21] In cases of HHS that require transfusion support, rituximab administered before transfusion has prevented further alloantibody or autoantibody production[20] and reduced hemolysis.[20,21] Rituximab has also been shown to bind macrophages expressing Fcγ receptors, resulting in decreased cell recruitment.[22] Thus, rituximab may prevent macrophage-dependent contact-lysis destruction of RBCs and reticulocytes. Rapid increases in reticulocyte counts have been reported after rituximab administration in HHS.[20,21] However, reduction of cold agglutinin antibody to rituximab is slow and does not occur for a median of 1.5 months.[18,19] In our patient, it took 42 days (6 weeks) for the agglutinin titer to decrease from the initial 512 to 256 and an additional 42 days (6 weeks; 84 days/12 weeks in total) for the titer to decrease to 4, too long to explain the rapid improvement experienced by the patient.

The use of eculizumab had not previously been reported in the treatment of DHTR or HHS in sickle cells. A highly humanized monoclonal antibody to C5, eculizumab is approved for the treatment of two complement-mediated diseases, PNH and atypical hemolytic uremic

syndrome.[23,24] By inhibiting C5, eculizumab prevents the cleavage of C5 to C5b, which leads to the downstream generation of the terminal complement complex, thus inhibiting intravascular hemolysis. It also inhibits the generation of C5a, a potent inflammatory protein. It has been reported to be effective in the treatment of cold antibody-mediated hemolysis.[25] A recent case report of ABO-incompatible transfusion reaction in a PNH patient was successfully treated using eculizumab.[26] In a case report of hyperhemolysis due to DHTR in a HIV-positive patient without a hemoglobinopathy, the authors stated that eculizumab was ineffective in treating the hemolysis. However, in that case the patient received only a single dose of 600 mg. The failure to improve the Hb with transfusion, rather than changes in the LDH, was used by the authors as the measure of the effectiveness of the treatment.[27] It is highly likely that the dose of eculizumab was insufficient to cause complete C5 blockade. In addition, ongoing hemolysis due to persistence of the antibody and extravascular clearance both in the spleen and in the liver may have prevented a significant increase in the Hb. In the report of the successful treatment with eculizumab of the hemolytic transfusion reaction in the PNH patient, the patient received a 900-mg initial dose followed by weekly dosing for 4 weeks.[26] In our patient, to ensure complete complement blockade, a 1200-mg dose for 4 weeks was used for the induction.

Because of the aggressive complement-mediated intravascular hemolysis, our patient's rapidly worsening anemia, and deteriorating renal function, eculizumab was given to the patient. After initiation of eculizumab therapy our patient's LDH levels decreased by 40% by Day 8, 70% by Day 15, and 90% on Day 22. Her Hb stabilized without transfusion at 5.6 g/dL within the first week of treatment without the need for additional transfusion. Hematologic improvement occurred well before the cold agglutinin titer decreased (7 days vs. 84 days).

This is the first reported case of life-threatening HHS, triggered by anti-IH, successfully treated with eculizumab. Anti-IH with characteristics of an alloantibody is a rare potential cause of DHTR and HHS in SCD. Eculizumab rapidly reduced the acute intravascular hemolysis in this patient and may have utility in the treatment of other cases of severe DHTR, alone or as in our patient combined with rituximab.

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CONFLICT OF INTEREST

ICW is part of the Alexion Pharmaceutical speaker bureau and HAL is married to ICW. The other authors have disclosed no conflicts of interest.

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