Case Report

Persistent complement-dependent anti-AnWj in a lymphoproliferative disorder: a case study and review

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AnWj is a high-incidence antigen present on the red blood cells (RBCs) of greater than 99 percent of the general population. A 58-year-old man underwent autologous hematopoietic stem cell transplantation (HSCT) for stage IVa mantle cell lymphoma. This procedure was complicated by failure to engraft, necessitating ongoing support with blood components. After a 2-month period of uneventful transfusion support, the patient experienced increasingly severe reactions with fever and evidence of intravascular hemolysis, including hemoglobinuria. Testing revealed a complement-dependent anti-AnWj. Phenotyping confirmed the AnWj– phenotype. Anti-AnWj was persistent despite immunosuppression, including treatment with allogeneic HSCT. Of interest, the pathogenesis of the downregulation of the graft AnWj in this patient is unclear. ***Immunohematology* 2011;27:83–88.**

Key Words: anti-AnWj, lymphoma, hemolysis

AnWj is a high-incidence antigen present on the red blood cells (RBCs) of greater than 99 percent of the population. Its molecular basis is not yet known. An antibody to this antigen, anti-AnWj, has been described in extremely rare serum samples as a result of an acquired AnWj– phenotype in individuals with lymphoproliferative disorders.[1–5] In several cases, successful treatment of the underlying disease has resulted in disappearance of the antibody.[1,4] Since it was first recognized, there have been several reports that have addressed transfusion in the presence of anti-AnWj, with a range of clinical outcomes.[1,4–9] We report a case of persistent acquired complement-dependent anti-AnWj in the context of an underlying lymphoproliferative disorder unresponsive to immunosuppression including allogeneic hematopoietic stem cell transplantation (HSCT).

Case Report

A 58-year-old man diagnosed with stage IVa mantle cell lymphoma achieved complete remission after chemotherapy and proceeded to an autologous HSCT. Unfortunately, the autologous HSCT was complicated by failure to engraft that necessitated transfusion support with both RBCs and platelets. Bone marrow biopsy revealed a hypoplastic marrow with features consistent with severe aplasia without evidence of residual lymphoma. The cause of the aplasia was likely iatrogenic, although a stromal defect could not be excluded. Three months later, episodes of hemoglobinuria after transfusion of RBCs and intermittent mild transfusion reactions with evidence of biochemical hemolysis and a positive hemosiderin test were noted (Fig. 1); however, posttransfusion testing performed on four donor units, transfused on June 20, July 4, July 30, and August 24, 2008, and on antibody screening cells was unremarkable. These reactions culminated in a severe hemolytic transfusion reaction on August 24, when transfusion of 150 mL of blood resulted in chills,

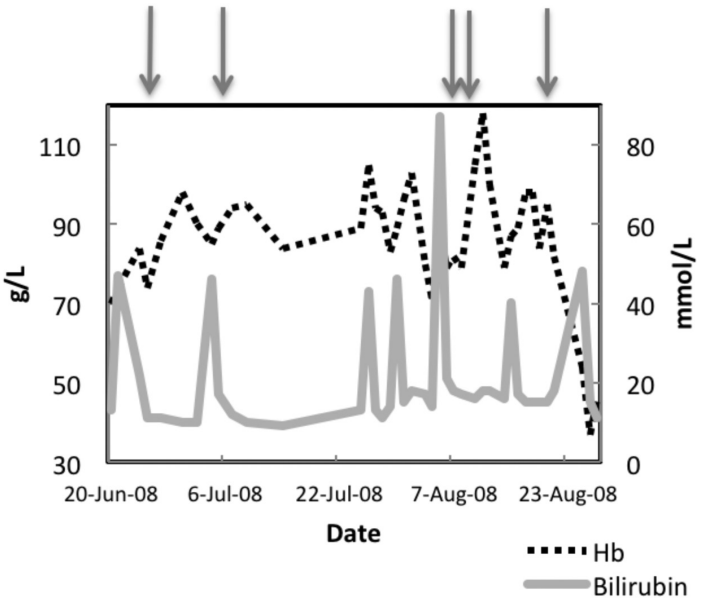

Figure 1. Results of laboratory testing including bilirubin (μmol/L) and hemoglobin (Hb; g/L) that demonstrate evidence of biochemical hemolysis (bilirubin peaks) without a unsustained increase in Hb following red blood cell (RBC) transfusion with AnWj+ units. Interspersed are transfusions with AnWj+ units (arrows) that did result in an incremental increase in Hb without overt evidence of hemolysis. The severity of the transfusion reactions increased over this time period. Posttransfusion testing was unremarkable (see text).

fever, tachycardia, and dyspnea. Visible hemoglobinuria and hemoglobinemia were present in posttransfusion samples; both were negative before transfusion. Initial testing at the hospital blood bank demonstrated that the direct antiglobulin test (DAT) was positive on posttransfusion samples: IgG = 1+; C3d = 1+. Compatibility testing with the donor unit and antibody screening cells by the low ionic strength saline (LISS) indirect antiglobulin test (IAT) did not demonstrate any reactivity. Tests for paroxysmal cold hemoglobinuria and paroxysmal nocturnal hemoglobinuria were negative.

Materials and Methods

RBC samples used in the investigation at the Australian Red Cross Blood Service–Victoria Reference Laboratory were from donors to the Blood Service and the Serum, Cells and Rare Fluids (SCARF) international exchange program. Most had been stored frozen at −80°C. Antiglobulin reagents were obtained from commercial sources. Serologic tests were performed by standard tube methods and by gel (DiaMed Micro Typing System, DiaMed, Cressier, Switzerland). Elutions were performed using an elution kit (Gamma ELU-KIT, Immucor, Norcross, GA).

Results

Testing performed at the Blood Service Reference Laboratory on September 1, 2008, revealed a complement-dependent anti-AnWj that reacted with all cells except those of the dominant form of Lu(a–b–). The antibody was detected only when serum was used and a polyspecific antiglobulin reagent containing anti-C3d was present. Phenotyping confirmed by the International Blood Group Reference Laboratory (IBGRL) in Bristol, United Kingdom, revealed the patient to be group B, D+, Lu(a–b+), AnWj–.

Clinical Outcome

The patient was supported during this time with limited stocks of locally available Lu(a–b–) RBC units. No further hemolytic transfusion reactions were reported. An attempt to address the underlying aplastic anemia and AnWj– phenotype with concurrent anti-AnWj was managed with various immunosuppressive regimens that included intravenous immunoglobulin, rituximab, antithymocyte globulin, mycophenolate, prednisolone, and cyclosporine, all without any effect. He was symptomatic of his profound anemia (hemoglobin [Hb] ~ 40 g/L) and unable to walk on his own. There was a significant decline in his functional status that resulted in a prolonged hospital admission. In addition, he was thrombocytopenic, had episodes of rectal bleeding, and was refractory to platelet transfusions. Given the limited availability of compatible RBCs, severe aplastic anemia necessitating ongoing platelet transfusions, and constant risk of neutropenic sepsis, possible residual disease, and the persistent presence of anti-AnWj, the patient proceeded to have a reduced-intensity sibling-matched allogeneic HSCT with a conditioning regimen that included alemtuzumab and total body irradiation. The use of alemtuzumab as part of the conditioning regimen was expected to destroy the antibody-producing B cells. The anti-AnWj was most likely IgM in immunoglobulin class, and with the half-life of IgM being 5 to 10 days, it was hypothesized that if the antibody-producing cells were eradicated then the antibody would have been cleared in 25 to 50 days.

The stem cell donor was group A, D+, Lu(a–b+), AnWj+, and therefore blood component support through the period of conditioning and until the point of engraftment was a major challenge, requiring additional collections from the limited number of Australian donors, use of the small number of locally available cryopreserved units, and also units specially collected and provided by international blood service partners.

The peritransplant period was unremarkable, and the patient engrafted by day 30. Despite the donor of the HSCT being group A, there was no hemolysis in the peritransplant period. Molecular and cytogenetic studies demonstrated 100 percent engraftment with donor cells. On engraftment the patient's RBC requirements were reduced transiently for 2 months; however, there was evidence of compensated hemolysis (Fig. 2). Given the limited availability of RBCs and the patient's symptomatic anemia, a clinical decision was made to transfuse with random donor units, and from January 16 to 27, 2009, he had received 3 units of allogeneic blood with an increase in Hb from 54 to 94 g/L (Fig. 2). On January 27 he experienced a reaction to an unscreened unit of RBCs, which was possibly attributed to a hemolytic reaction (Fig. 2). The patient experienced a further transfusion reaction on February 6, without a sustained increment in Hb but with biochemical evidence of hemolysis (Fig. 2). Subsequently, the patient was only transfused with screened donor units that were essentially AnWj–. Phenotyping of the patient at 8 weeks after transplantation demonstrated downregulation of AnWj expression. The AnWj typing was always weakly positive from December 15, 2008, onward. In addition, the antibody to AnWj and positive DAT persisted throughout this period. The results are summarized in Table 1. The patient's condition deteriorated, and a decision was made for palliation.

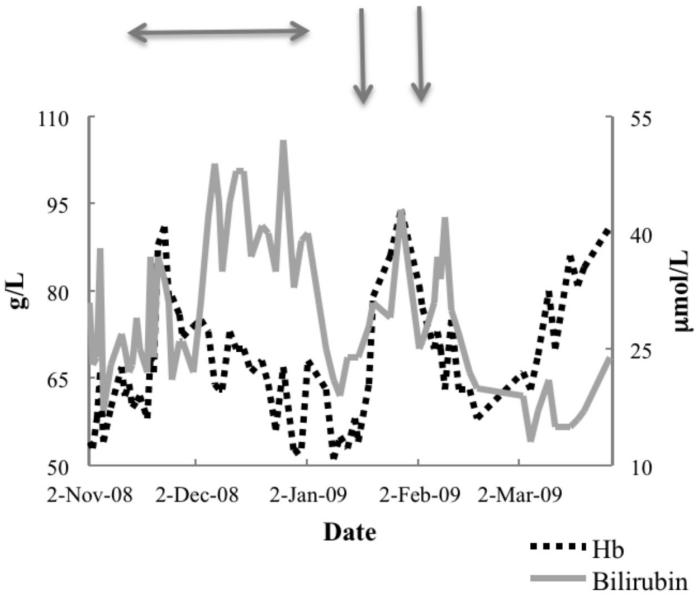

Figure 2. Results of laboratory testing including bilirubin (µmol/L) and hemoglobin (Hb; g/L) after allogeneic hematopoietic stem cell transplantation that demonstrates compensated hemolysis (double-headed arrow) after engraftment associated with reduced RBC transfusion requirements. Subsequent transfusions on January 27 and February 6, 2009 (arrows), with unscreened donor units resulted in biochemical evidence of hemolysis without a sustained increment in Hb.

Discussion

We report a case of intravascular hemolysis when incompatible blood was transfused to a patient with a complement-binding, allo-anti-AnWj. This is similar to the report by Fitzsimmons and Caggiano,[7] but in contrast to that of Marsh et al.,[6] who described uneventful transfusion with incompatible blood in the presence of an auto-AnWj that did not bind complement. In our case the patient had a prolonged period of active hemolysis not responsive to profound immunosuppression, including treatment with allogeneic HSCT. Various immunosuppressive regimens were attempted in our patient with limited success.

AnWj is known as a high-incidence antigen present on RBCs of greater than 99 percent of the population. An antibody to this antigen, anti-AnWj, has been described extremely rarely, most often transiently, and frequently in individuals with lymphoproliferative disorders.[1–5] The AnWj blood group antigen has been shown to be the RBC receptor for *Haemophilus influenzae* type A.[10] It was originally described as Anton in 1972 by Boorman and Tippett, who studied an antibody made by a pregnant woman, and included in the para-Lutheran antigens by Race and Sanger.[11] A second example of anti-Anton that failed to react with RBCs of persons who had inherited *In(Lu)* was described by Daniels in 1980.[12] The term Lu15 was reserved for the antigen involved.[12] In 1983 Marsh and colleagues[6] first described anti-Wj, an autoantibody that reacted with all RBCs and could be demonstrated in persons with *In(Lu)* only by adsorption-elution methods. It subsequently became apparent that anti-Anton and anti-Wj were antibodies detecting the same determinant.[1,13] In 1986, the new name AnWj was given to both Anton and Wj.[14] The number 901009 has been assigned to the high-incidence antigen AnWj by the International Society of Blood Transfusion (ISBT).[14]

The para-Lutheran antigens are a heterogeneous group of public antigens characterized by their absence from Lutheran-negative RBCs. Most RBCs of adults are AnWj+; however, cord RBCs, RBCs of the Lu (a–b–) phenotype caused by

Table 1. Results of testing performed at the Australian Red Cross Blood Service–Victoria Reference Laboratory

Date	Sample type	ABO	DAT (grade)*	AnWj typing	Antibody	Comment
Sept 1, 2008 (original referral)	Clotted	B	C3d (1+)	Not done	Anti-AnWj	
Sept 10, 2008	EDTA	B/O	IgG (±)	Not done	Nil (plasma)	Eluate negative
Sept 25, 2008	EDTA	–	Neg	AnWj neg	Very weak anti-AnWj	Tested by IBGRL
Oct 3, 2008	Serum	–	–	–	Anti-AnWj	
Oct 30, 2008	4 units group O Lu (a–b–) imported from the United States					
Oct 31, 2008 (posttransfusion)	Clotted	B/O	IgG (±), C3d (1+)	–	Anti-AnWj	
Nov 10, 2008 (postallograft)	Clotted	A/B/O	Neg	–	Anti-AnWj	
Nov 27, 2008	Clotted	A/O	C3d (1+)	AnWj neg	Anti-AnWj	
Dec 15, 2008	Clotted	A/O	C3d (1+), trace IgG	AnWj+^{w}	Anti-AnWj	
Dec 22, 2008	EDTA	A (some O)	C3d (1+), trace IgG	AnWj+^{w}	Nil (plasma)	Eluate contains anti-A
Dec 23, 2008	EDTA	–	C3d (weak)	AnWj+^{w}	–	Tested by IBGRL
March 12, 2009	Clotted	A/O	C3d (1+)	AnWj+^{w}	Anti-AnWj	

*The DAT was done using EDTA blood samples.

DAT = direct antiglobulin test; EDTA = ethylenediaminetetraacetate; IBGRL = International Blood Group Reference Laboratory.

the dominant suppressor gene *In(Lu),* and RBCs from rare individuals with anti-AnWj in their serum essentially type as AnWj–.[15] Poole and Giles[16] in 1982 demonstrated that the Anton "para-Lutheran" antigen is associated with the Lutheran blood group system only by *In(Lu),* a gene independent of the Lutheran locus. Several examples of anti-AnWj have been documented to be autoantibodies; their appearance was associated with transient depression of AnWj on the RBC.[1,6,7] The AnWj– phenotype is usually considered to be an acquired phenomenon. In 1991, Poole and colleagues[17] described a family showing inheritance of the Anton blood group antigen, AnWj, and independence of AnWj from Lutheran.

Although hemolytic transfusion reactions have been attributed to anti-AnWj, predicting events for individual patients is difficult. Given the limited availability of AnWj– RBCs, predicting the hemolytic potential by in vitro and in vivo assays in each case may be considered in the event of a requirement for transfusion. Several reports have addressed transfusion in the presence of anti-AnWj.[1,4–9] In these reports, the antibody was an autoantibody, with the results of transfusing incompatible blood varying from asymptomatic to fever, chills, and intravascular hemolysis with hemoglobinuria and hemoglobinemia. Whitsett et al.,[4] using chromium studies, revealed that allogeneic AnWj+ RBCs had shortened survival with two components identified; 24 percent of RBCs were eliminated rapidly (within 24 hours), with an additional 24 percent of RBCs being eliminated more slowly (6 days). This pattern is usually observed when there are both IgG and IgM antibodies present, or when the IgG antibodies fix complement.[4] The shortened survival of allogeneic blood of similar phenotype may explain the report of transfusion reactions explained by autoanti-AnWj. Erythrocyte survival studies may be useful in cases of anti-AnWj to assist in predicting acute hemolytic reactions,[5,9,18] especially when antigen-negative donor units may not be available; however, these studies are not routinely accessible and often are not available in the timeframe required for urgent transfusion decision making.

The molecular basis for the high-incidence AnWj is not yet known. The acquired form of the AnWj– phenotype is a transient depression of AnWj, usually with a concomitant alloanti-AnWj, and is extremely rare. It has been described in individuals with lymphoproliferative disorders. The pathogenesis of the downregulation of AnWj is unclear; however, in several cases successful treatment of the underlying disease resulted in disappearance of the antibody and reinstatement of AnWj.[1,4] Transient depression of RBC antigens has been described; such cases include loss of Kell and Lutheran during consecutive relapses of autoimmune thrombocytopenia.[19] Various mechanisms have been postulated and include antigen shedding, defective insertion of the antigen into the RBC membrane, glycosylation, or blocking with downregulation of antigen copy number.[19] Further work needs to be done to identify the mechanism of this downregulation.

CD44 is postulated to be the location of AnWj. CD44 is part of the collagen family of receptors and has an important role in hyaluronic acid binding, lymphocyte homing, leukocyte activation, and cell adhesion.[20] It is ubiquitously expressed.[20] Spring et al.[21] demonstrated that the Indian blood group antigen is located on CD44. The molecular basis of the In^a/In^b polymorphism is a result of a single point mutation in the CD44 gene.[22] A case report of a 9-year-old with a novel form of congenital dyserythropoietic anemia associated with a deficiency of erythrocyte CD44 lacked In antigens and happened to be Co(a–b–) and AnWj–.[23] A phenotypic association of AnWj with CD44 was described, and unlike In^a/In^b, AnWj is resistant to trypsin, chymotrypsin, neuraminidase, and papain.[24] The protease cleavage sites on CD44 were localized by monoclonal anti-CD44 antibodies that predict that AnWj is located in a region near the lipid bilayer.[15] This region provides several sites for potential posttranslational modification.[15] The lack of AnWj on CD44-deficient cells and the appearance of AnWj on Jurkat cells transfected with wild-type CD44 cDNA lends support to AnWj being located on CD44.[25] It has been suggested that the structure of the antigen depends on posttranslational modification of the protein by a particular glycosylation.[15] This may explain the conversion in newborn infants from AnWj– to AnWj+ within the first 50 days of life. This conversion was seen to take only 1 day to complete. This was not attributable to replacement of AnWj– cells with AnWj+ cells from the bone marrow but caused by an extrinsic factor.[26] However, no such factor could be isolated from the serum of the neonates. The mechanism that enables a RBC population to express antigen within days is not understood but may in part be caused by the potential for posttranslational modification as discussed earlier.

Interestingly, AnWj is the putative receptor for fimbriae-bearing strains of *H. influenzae*.[10] Elucidation of the nature of AnWj is of considerable interest because as a receptor for *H. influenzae,* a major cause of respiratory infections worldwide, it may serve as a putative therapeutic target. *H. influenzae* agglutinated RBCs of all types except those from individuals with *In(Lu)*-acquired AnWj– phenotype or from cord blood samples.[10] Epithelial cells from an individual with inherited AnWj– phenotype failed to bind *H. influenzae,* but cells from

individuals with the acquired phenotype, in which cells other than erythrocytes express AnWj, were capable of binding *H. influenzae*.[27] It is postulated that the low levels of AnWj in the acquired AnWj– phenotype, *In(Lu),* and cord blood are sufficient for binding of *H. influenzae*.[27]

The few cases in the literature that describe anti-AnWj describe it as a transient phenomenon, typically in the setting of an underlying lymphoproliferative disorder. In our case the patient had a prolonged period of active hemolysis unresponsive to profound immunosuppression that included an allogeneic HSCT. The pathogenesis of the downregulation of AnWj in this setting remains unclear. These situations present a major management challenge for transfusion support in patients who have antibodies to high-incidence antigens whose clinical significance is unclear. Caution is required in their transfusion management.

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