Immunohematology

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Red blood cell transfusion in a patient with anti-AnWj: a case report

R.E. Stowers, E.M. Richa, J.R. Stubbs, and S.B. Moore

Anti-AnWj (Anton) has been associated with clinically significant hemolytic transfusion reactions. More than 99 percent of studied populations have RBCs that express the antigen. Reported here is a patient with anti-AnWj who was transfused with antigen-positive RBCs without adverse reaction. ***Immunohematology* 2007; 23:55–58.**

Key Words: RBC, anti-AnWj

The AnWj antigen is located in the ISBT 901 series of high-incidence antigens. It has a more than 99 percent occurrence in all populations.[1] As such, the clinical significance of an anti-AnWj has to be interpreted carefully as RBC units negative for this antigen are extremely rare. Limited evidence exists to help guide transfusion practice in patients with this antibody; we describe a single case in which a patient whose serum contained anti-AnWj was successfully transfused with serologically incompatible RBCs without an adverse reaction. The signs and symptoms of an adverse reaction include fever or chills or both, nausea or vomiting, pain (at infusion site or flank), dyspnea, hypotension or tachycardia or both, renal failure, disseminated intravascular coagulation, jaundice, hemoglobinemia, hemoglobinuria, inadequate increase in posttransfusion Hb, increase in bilirubin (e.g., indirect bilirubin), increase in LDH, and decreased haptoglobin.

Case Report

The subject was a 57-year-old Caucasian male (of German, Irish, and French descent) with a history of autoimmune hemolytic anemia (AIHA) that was diagnosed at a different institution in March 2005 and was treated with prednisone and azathioprine. He was admitted on July 25, 2005, to the coronary care unit at our institution with a 40-pound weight loss and unstable angina. He was diagnosed with a myocardial infarction, and he was found to be anemic with a Hb of 8.3 g/dL. Cardiac catheterization showed three-vessel coronary artery disease with high-grade stenosis of the mid left anterior descending coronary artery. During hospitalization, the following complications occurred: hyperglycemia—probably related to steroid therapy—*Aspergillus fumigatus* infection of the lower lobe of the right lung, and bronchial bleeding during bronchoscopy. The bronchial hemorrhage was associated with a drop in Hb to 7.2 g/dL; RBC transfusion was considered to be clinically indicated owing to the patient's coronary artery disease (CAD), chest pain (rated at 5 of 10), pain on inspiration, and electrocardiographic changes.

Materials and Methods

Historical serologic investigation

The American Red Cross (ARC) North Central Blood Services (St. Paul, MN) had identified an anti-AnWj in this patient's serum in March 2005. They documented it as reactive by IAT in saline and albumin. According to the ARC report, the serum of the patient reacted weakly using PEG-IAT with all RBCs tested, including those that were either AnWj– or Lu(a–b–) and the patient's own RBCs. This panagglutinin was detectable only in PEG-IAT. The ARC testing excluded antibodies to common blood group antigens by using test RBCs that were negative for AnWj or were Lu(a–b–) by using both saline-IAT and albumin-IAT. The DAT was positive (ARC did not specify strength) with anti-human IgG and negative with anti-human C3b, C3d. The ARC performed an elution because of the positive DAT with anti-IgG and the resulting eluate reacted with all RBCs tested, including those that were AnWj– or Lu(a–b–). The patient's RBC phenotype performed by the ARC before any transfusion was as follows: group O, D+, C+E–c–, M+N+S–s+, P1–, K–k+

Kp(b+), Fy(a–b+), Jk(a–b+), AnWj–; the ARC did not provide the Lutheran phenotype of the patient. These results were interpreted to indicate the presence of an antibody to the high-prevalence antigen AnWj plus a warm autoantibody. The immune stimulus for developing the anti-AnWj was unknown.

In light of observed evidence of the association with delayed hemolytic transfusion reactions,[2] the ARC (St. Paul, MN) recommendation was to transfuse AnWj– RBCs (if available) or RBCs with the dominant Lu (a–b–) phenotype encoded by *In(Lu)*.

Current serologic investigation

The patient arrived at our institution on July 25, 2005, after receiving two units of phenotypically similar [E–, K–, Lu(a–b–)] group O, D+ RBCs provided by the ARC and infused at another institution. Three additional group O, D+ RBC units were transferred to our facility by the ARC. These latter three units were phenotypically similar (E–, K–) but the units were Lu(a–b+) and thus AnWj+. Units were negative for E and K as a precaution.

It is known that Lu(a–b–) RBCs have poor expression of the AnWj antigen.[1] Therefore, reagent RBCs negative for AnWj were used in conjunction with Lu(a–b–) reagent RBCs to evaluate for the presence of antibodies to other major blood group antigens. Testing in the immediate spin phase using the patient's plasma (collected in EDTA) revealed no reactivity with reagent RBCs and 1+ reactivity against the autologous RBCs. Testing was then carried into the PEG-IAT phase, which showed 2+ and 3+ reactivity with AnWj+ and Lu(a–b+) RBCs, respectively, w+ reactivity with AnWj– and Lu(a–b–) RBCs, and 3+ reactivity with autologous RBCs. A DAT showed 2+ reactivity using monoclonal anti-human IgG (Ortho-Clinical Diagnostics, Raritan, NJ) and was negative when tested with anti-human C3b, C3d (Ortho-Clinical Diagnostics). Using the acid/EDTA method, an elution was performed. The eluate showed no reactivity at PEG-IAT against the same antibody identification panel of RBCs described earlier. Although our eluate results were puzzling, the rest of our results were consistent with those reported by the ARC in St. Paul when testing in PEG-IAT.

The rarity of the antibody also presents problems when trying to locate antisera for typing the patient's RBCs. The AnWj typing sera at our institution failed to react with the control RBCs, and we were unable to locate additional antisera from outside sources. A phenotype was performed for the common blood group antigens, and, where indicated, the patient's RBCs were treated with acid/EDTA to remove antibody that could interfere with antigen typing. The phenotype performed in our facility was in agreement with the ARC with the following additional antigen typings: e+, CW+, Le(a–b+).

It was concluded through RBC antibody identification testing and antigen typing that the patient lacked alloantibodies to the major blood group antigens, and it was inferred through testing performed at our facility and the ARC that the patient had made anti-AnWj. As previously mentioned, we could not confirm the AnWj because of a lack of antisera, but the ARC typed the patient's RBCs and determined that they were AnWj–. Autoanti-AnWj has been reported as a result of transient suppression of the AnWj antigen, and therefore we could not definitively rule out that possibility.[1] Testing in LISS decreased the panreactive autoantibody's strength to nonreactive macroscopically. An allogeneic adsorption was performed on the patient's serum using intact RBCs in an attempt to remove the anti-AnWj to rule out all antibodies to common blood group antigens using our test methodology of choice, PEG-IAT. The adsorption was performed on a phenotypically similar (matched for antigens as described earlier—except AnWj) RBC aliquot. The allogeneic adsorption was successful, and there was no evidence of alloantibodies against any of the major blood group antigens using PEG-IAT. Left behind in the absorbate was an autoanti-e. We can speculate that a warm autoantibody with specificity other than anti-e exists, as well as one with anti-e specificity.

The clinical service was made aware of the difficulty in obtaining serologically compatible, antigen-negative RBC units. Based on assessment of clinical need by the patient's service, an order for one unit of RBCs was received. A unit known to be Lu(a–b–) was crossmatched. This unit was serologically incompatible (2+) using our routine PEG-IAT methodology, presumably because of the warm autoantibody (as it reacted 2+) but was compatible using LISS-IAT. This unit was transfused without clinically evident complications. Samples from the patient's available siblings (a total of four) were also crossmatched with the patient's plasma and were found to be incompatible using both PEG-IAT and LISS-IAT methods. RBC antigen typing was not performed on the samples from the patient's siblings as a result of this incompatibility.

Requests for Lu(a–b–) RBC units could not be filled through the American Rare Donor Program (ARDP). Because of this, it was considered necessary to assess the likely clinical significance of the patient's anti-AnWj. A sample of the patient's serum was sent to the National Reference Laboratory for Blood Group Serology of the ARC for performance of a monocyte monolayer assay (MMA). Concurrently, an in vivo radiolabeled (In-111) RBC survival study was performed at our institution.

The MMA is an in vitro diagnostic evaluation of the hemolytic potential of an antibody. It has been shown previously through multiple studies to be a predictor of RBC antibody clinical significance.[3-5] It was performed using group O AnWj– RBCs and group O AnWj+ RBCs. Pooled monocytes from two normal blood donors were used. The AnWj+ RBCs reacted strongly positive by DAT after incubation with the patient's serum. AnWj– RBCs were negative by the DAT after incubation with the patient's serum. The percentages of reactive monocytes, after incubation with the patient's serum, with and without fresh complement, were 19.0 percent and 37.5 percent, respectively, with AnWj+ RBCs. AnWj– RBCs, after incubation with the patient's serum, with and without fresh complement, displayed 0.5 percent and 0.3 percent of reactive monocytes, respectively. Between 0 percent and 3 percent of reactive monocytes is considered within the normal range. Values above this range suggest that the antibody in question may cause accelerated clearance of antigen-positive RBCs. Again, the ARC recommendation was to transfuse AnWj– RBCs.

In the radiolabeled survival study,[6,7] an intravenous infusion of 10 mL of indium 111-labeled Lu(a–b+) RBCs (2+ crossmatch incompatible at PEG-IAT and w+ crossmatch incompatible at LISS-IAT) was administered, and samples were obtained at specified intervals to determine the percentage of radiolabeled RBCs persisting in the circulation. The intervals were 3 hours, 20 hours, 47 hours, and 70 hours after infusion. The results at the specified intervals were 95 percent, 87 percent, 81 percent, and 80 percent, respectively. Data from this particular test suggested that RBCs of this phenotype were unlikely to undergo a clinically relevant degree of hemolysis within 3 days of transfusion.

Clinical outcome

Because of clinical necessity and despite the conflicting predictive test results, a decision was made to transfuse two available units of Lu(a–b–) RBCs that were 1+ and 2+ crossmatch incompatible in PEG-IAT phase but compatible in LISS-IAT phase of testing. The transfusions were without any clinically apparent adverse outcomes, and 2 days later the patient's Hb level had risen from 7.2 g/dL to 8.6 g/dL. Subsequent to that transfusion, no more Lu(a–b–) units were available at our institution, and the ARDP was unable to supply additional units. The patient required further transfusion and was transfused two (1+ incompatible at PEG-IAT and compatible at LISS-IAT) units of Lu(a–b+) RBCs, again without any clinical adverse reaction. Our transfusion service recommended premedication with IVIG (1 g/kg) and IV hydrocortisone to reduce the possibility of an acute hemolytic reaction and its clinical effects. The patient's Hb increased from 8.1 g/dL before transfusion to 11.2 g/dL after transfusion. Owing to the nature of his warm AIHA, anti-AnWj, and compounding myocardial infarction and CAD, the patient's clinical service limited blood loss from laboratory testing and phlebotomy. Because of this, there were few biochemical markers for hemolysis ordered. Results from those that were are summarized in Figure 1.

At another hospital, the patient later underwent coronary artery bypass graft surgery. He presented to our institution 10 months later, in September 2006, with gastrointestinal bleeding associated with the use of aspirin and other nonsteroidal anti-inflammatory medications. His Hb level on admission was 6.1 g/dL and dropped to 5.1 g/dL the next day. In addition to the anti-AnWj, an anti-Fy^a was found, further complicating the patient's serologic workup. No serologically compatible units or units that were Lu(a–b–), Fy(a–) were available. Two units of Lu(a–b+), Fy(a–) RBCs were transfused (reactions were 2+ incompatible at

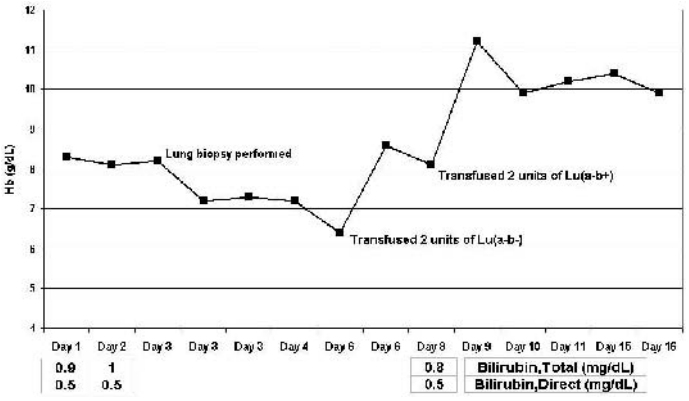

Fig. 1. Results of hemoglobin and bilirubin measurements for the patient during his hospital stay. RBC transfusions (and Lutheran phenotype) are indicated.

PEG-IAT and 1+ incompatible at LISS-IAT) without adverse reaction and his posttransfusion Hb stabilized at 6.1 g/dL.

Discussion

Although anti-AnWj has been reported to cause significant clinical hemolysis, there is clearly insufficient experience to reliably predict its potential for hemolysis in any particular case.[8] The MMA and in vivo RBC survival testing in our case gave conflicting information, and the RBC survival study seemed to more accurately predict the clinical result in this case. It should be noted that, although it is unlikely, the MMA could have been influenced by an antibody to an unidentified low-prevalence antigen. Although RBC units negative for AnWj are virtually impossible to locate in the general population, it is slightly less difficult to find Lu(a–b–) units. The patient's clinical condition led to a decision that RBC transfusion was necessary despite the inability to locate AnWj– or Lu(a–b–) RBC units for him. Based on the objective evidence provided by the in vivo survival study, we deemed Lu(a–b+) RBC units, in spite of serologic incompatibility, to be a relatively safe choice for transfusion. The patient suffered no apparent adverse clinical events after transfusion. One may wish to consider that this occurred because the initially reported alloanti-AnWj may, in fact, have been an autoanti-AnWj. However, this is only one case, and it is certainly possible that quite different results might be found in other cases. Similarly, caution should be taken with patients who have antibodies to high-prevalence antigens whose clinical significance is unknown. This case report should not be applied generally to all antibodies to high-prevalence antigens.

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Application of gel technology in the serologic characterization of autoantibody in DAT-positive autoimmune diseases

S.S. Das and R.K. Chaudhary

Gel tests are now available for the determination of immunoglobulin classes and subclasses and complement fractions coating RBCs. These tests simplified serologic characterization of autoantibodies in various autoimmune diseases. The aim of this study was to evaluate the use of gel cards in the serologic characterization of autoantibody with regard to the immunoglobulin classes, complement fractions, and IgG subclasses, and the influence of these characteristics on hemolysis. Gel cards were used to characterize the RBC-bound autoantibodies in 66 DAT-positive patients. Hematologic and biochemical parameters such as Hb, reticulocyte count, serum bilirubin, and serum LDH were obtained from the patient files. Of the 49 patients carrying IgG on their RBCs, 21 (42.8%) were suffering from connective tissue disorders and another 16 patients (32.7%) had autoimmune hemolytic anemia. A total of 19 of these 49 patients had evidence of hemolysis. Thirteen of the 17 patients (76.5%) whose RBCs were coated with more than one type of immunoglobulin and complement were experiencing hemolysis ($p < 0.05$). Seventy-five percent (21 of 28) of patients having IgG1, IgG3, or both on their RBCs showed hemolysis ($p < 0.05$). Thus, it is important to serologically characterize autoantibodies in autoimmune disorders to effectively predict the prognosis and disease outcome. This characterization can be performed effectively with the gel test, which can be introduced in blood centers as a replacement to the conventional tube technique.
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Key Words: autoimmune hemolytic anemia, direct antiglobulin test, gel test

Autoimmune hemolytic diseases are characterized by the formation of antibody against the individual's own RBCs.[1] These autoantibodies, detected by the DAT, at times cause hemolysis that adds to the increased risk of morbidity and mortality of patients. The pathogenicity of autoantibodies depends on several factors, of which classes, subclasses, and titer of autoantibody and the associated complement activation are of serologic importance.[2–5] Although the conventional tube technique (CTT) is still the gold standard for DAT evaluation, the use of gel technology (GT) is growing gradually because of its simplicity, reproducibility, avoidance of RBC washing, and ease of detecting immunoglobulin classes and subclasses and complement fractions.[6,7] GT has been found to be as sensitive as flow cytometry for the detection of RBC–bound IgG.[8] GT uses the gel filtration media impregnated with polyspecific or monospecific anti-human globulin (AHG) reagents to bring about agglutination.[9] Gel cards are now available to assess the strength of the DAT (DAT IgG dilution cards) or to determine the immunoglobulin subclasses of antibodies. These cards have simplified the serologic characterization of autoantibody. Moreover, with the use of these cards clinical hematologists may not have to depend on immunohematology laboratories for the evaluation of their patients as these tests can be performed without extensive infrastructure and trained manpower.

The purpose of this study was to evaluate DAT-positive autoimmune diseases using special gel cards from DiaMed AG, Cressier sur Morat, Switzerland.

Note: The special gel cards used in this study may not be available to all gel users as they have not been approved by the FDA for the U.S. market.

Materials and Methods

The study was carried out at the immunohematology laboratory of the Department of Transfusion Medicine, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India, from January 2004 to June 2006. After seeking approval of the institute research committee and consent of the patients, EDTA blood samples from 164 patients clinically suspected of suffering from various autoimmune disorders were evaluated for DAT profile and autoantibody characterization.

Gel technology

Fifty microliters of a 1% RBC suspension in LISS was added to each microtube of the labeled ID cards (LISS-Coombs card, DiaMed) containing gel matrix impregnated with polyspecific AHG containing anti-IgG and anti-C3d. The cards were then centrifuged at 70 g for 10 minutes in the dedicated DiaMed ID centrifuge (DiaMed). The DAT reactions were graded according to the manufacturer's instructions. A discrete RBC button at the base of the column indicated a negative reaction. Appropriate controls were tested along with the samples following the manufacturer's instructions.

Samples that tested positive by the DAT with polyspecific AHG were further tested for immunoglobulin class using gel cards containing monospecific AHG (anti-IgG, anti-IgM, anti-IgA, anti-C3c, and anti-C3d). If the antibody detected was IgG in nature, gel cards impregnated with monospecific anti-IgG1 and anti-IgG3 were used to determine the IgG subclass. Both IgG1 and IgG3 may activate complement, so this test algorithm was intended to estimate the risk and severity of hemolysis for the underlying disease. The strength of the DAT was determined using a gel card with anti-IgG in serial dilutions (1:1, 1:10, 1:30, 1:100, 1:300, and 1:1000). This test not only indicates the clinical importance of a DAT-positive result but also helps in assessing the relative amount of IgG sensitizing the RBCs and further helps in estimating the risk of hemolysis in an autoimmune disease.[10,11] An anti-IgG titer of more than or equal to 300 was considered clinically relevant in accordance with the guidelines furnished by the manufacturer.[10] In all the techniques, the findings of the agglutination reactions were graded as 4+, 3+, 2+, 1+, weak, and negative and documented accordingly.

Four variables were used to determine hemolysis: a decrease in Hb (< 9 g/dL), an increase in reticulocytes (> 2 %), a bilirubin level of more than 2 mg/dL, and an LDH concentration of more than 500 IU.

Statistical analysis

A two-tailed Student's t test using computer software (SPSS, Chicago, IL) was used to analyze the relationship between IgG classes and subclass with hemolysis. A probability value of less than 0.05 was considered as significant.

Results

Of the 164 samples evaluated, 66 were reactive by the polyspecific DAT using the GT. The median age of these DAT-positive patients was 31 years, with a female predilection (M/F = 1:3.5). The majority (88%) of them had autoantibodies reactive at 37°C. Of the 66 patients, 24 (36.4%) were clinically and serologically diagnosed with autoimmune hemolytic anemia (AIHA), 20 (30.3%) with systemic lupus erythematosus (SLE), and 6 (9.1%) with autoimmune hepatitis; the rest were diagnosed as having various other autoimmune diseases such as rheumatoid arthritis (RA; n = 2), glomerulonephritis (n = 3), Hashimoto's thyroiditis (n = 2), inflammatory myositis (n = 1), pancreatitis (n = 1), or lymphoproliferative disorders such as leukemias and lymphomas (n = 7).

Table 1 depicts the prevalence of different immunoglobulin classes and complement fractions in the various autoimmune diseases we encountered. In 74.2 percent (49 of 66) of the patients, IgG alone was present ($p < 0.05$). Of these 49 patients, 21 (42.8%) were suffering from connective tissue disorders such as SLE, RA, inflammatory myositis, etc., and another 16 patients (32.7%) had AIHA. Multiple immunoglobulin classes were detected in 16 patients, of which the majority (50%) had AIHA. The RBCs of one 65-year-old patient with Hodgkin's lymphoma were found to be coated with only C3c and C3d.

Association of immunoglobulin classes, IgG subclasses, and complement fractions with clinical hemolysis is shown in Table 2. Of the 49 patients with RBCs coated with only IgG, 19 (38.8%) had evidence of hemolysis. Thirteen of the 17 patients (76.5%) having multiple immunoglobulin classes or complement or both were experiencing hemolysis ($p < 0.05$).

Table 1. Immunoglobulin class and complement fractions in autoimmune diseases (N = 66)

Type of autoantibody	AIHA (N = 24)	CTD (N = 23)	AIH (N = 6)	LPD (N = 7)	Others (N = 6)
IgG (n = 49)	16	21	3	3	6
IgG + IgM (n = 2)	–	1	–	1	–
IgG + IgA (n = 1)	1	–	–	–	–
IgG + C3 (n = 7)	3	1	2	1	–
IgG + IgA + C3 (n = 1)	1	–	–	–	–
IgG + IgM + IgA + C3 (n = 5)	3	–	1	1	–
C3c + C3d (n = 1)	–	–	–	1	–

AIHA = autoimmune hemolytic anemia.

Connective tissue disorders (CTD) include systemic lupus erythematosus, rheumatoid arthritis, and inflammatory myositis.

AIH = autoimmune hepatitis.

Lymphoproliferative disorders (LPD) include leukemias and lymphomas.

Others include glomerulonephritis, Hashimoto's thyroiditis, and pancreatitis.

Table 2. Correlation of IgG classes, subclasses, and complement fractions of autoantibodies with hemolysis in autoimmune disease (N = 66)

Antibody type	Hemolysis n (%)	No hemolysis n (%)
Ig class / complement (N = 66)	32 (48.5)	34 (51.5)
IgG (n = 49)	19 (38.8)	30 (61.2)
IgG + IgM (n = 2)	1 (50)	1 (50)
IgG + IgA (n = 1)	1 (100)	–
IgG + C3 (n = 7)	5 (71.4)	2 (28.6)
IgG + IgA + C3 (n = 1)	1 (100)	–
IgG + IgA + IgM + C3 (n = 5)	5 (100)	–
C3 (n = 1)	–	1 (100)
IgG/C3 subclass (N = 66)	32 (48.5)	34 (51.5)
IgG1 (n = 21)	14 (66.7)	7 (33.3)
IgG3 (n = 2)	2 (100)	–
IgG1 + IgG3 (n = 5)	5 (100)	–
C3c/C3d (n = 1)	1 (100)	–
No IgG1/IgG3 (n = 37)	10 (27)	27 (73)

$p < 0.05$ comparing IgG1, IgG3, or both vs. no detectable IgG1 or IgG3 and their association with hemolysis.

Subclasses of IgG such as IgG1, IgG3, or both were detected in 28 patients, of whom 21 (75%) were associated with hemolysis compared with only 27 percent of patients experiencing hemolysis when these were not demonstrated on the RBCs ($p < 0.05$).

Of the 21 patients exhibiting hemolysis as a result of IgG1, IgG3, or both, 12 had a titer more than or equal to 300. However, the values of the hemolysis determinants in these patients were not statistically significant when compared with an autoantibody titer of less than 300 causing hemolysis in the other 9 patients.

Discussion

The advent of GT has tremendously improved RBC serologic investigations and has replaced the CTT in many blood centers.[6,9] Introduction of specialized gel cards, such as monospecific immunoglobulin cards, IgG subclass cards, and IgG dilution cards, have made it possible to characterize RBC-bound antibodies in a simplified and rapid way. Detailed investigation of these autoantibodies helps in predicting the severity of hemolysis and disease outcome and in planning therapy in autoimmune disorders.[11]

We determined serologic characteristics of autoantibodies in patients with autoimmune diseases with regard to immunoglobulin class, subclass, and titer of IgG using specialized gel cards provided by DiaMed, Switzerland. Of the 49 patients having IgG as the sole autoantibody, 21 (42.8%) had connective tissue disorders and another 16 patients (32.7%) had AIHA. Multiple immunoglobulin classes were detected in 16 patients, of which the majority (50%) had AIHA. Similarly, Sokol et al.[12] and Issitt et al.[13] found 63 percent and 43.7 percent, respectively, of their AIHA patients had IgG as the sole autoantibody. In a study of samples from different autoimmune patients, IgG and C3d were seen in 43.7 percent, IgG only in 50 percent, and C3d sensitization in 6.3 percent of patients.[14] All of these results approximated to the findings of the present study.

We observed association of class and subclass of autoantibody with clinical hemolysis. In 74.2 percent (49 of 66) of our patients, IgG was the solitary immunoglobulin and of these, 38.8 percent had in vivo hemolysis (Table 2). However, more than 76 percent (13 of 17) of those patients whose RBCs were coated with multiple immunoglobluins or complement or both experienced hemolysis. Similar observations were made by Sokol et al.[15] and Fabijanska-Mitek et al.,[16] who reported that RBCs coated with multiple immunoglobulins undergo hemolysis more readily compared with RBCs coated with IgG alone. IgG molecules cause hemolysis mainly by complement activation[17]; however, the presence of regulatory RBC proteins (DAF or CD55, MIRL or CD59) restrict such activation, and clinical hemolysis with IgG alone is less common. Contrary to this, multiple immunoglobulins readily activate the classic complement pathway and cause significant hemolysis.

We found that in vivo hemolysis was more pronounced when RBCs were coated with IgG1, IgG3, or both (Table 2). Sokol et al.[15] observed IgG1 as the predominant IgG subclass in 98 percent of the total cases. In contrast to this, only 31.8 percent (21 of 66) of our patients had IgG1 alone bound to their RBCs, and this difference could be attributed to different methods of detection. The gel cards used in the present study were impregnated with only serially diluted anti-IgG1 and anti-IgG3, so the presence of IgG2 and IgG4 could not be ruled out.

The strength of the DAT was determined in all patients using IgG dilution gel cards (DiaMed). However, no correlation of DAT titer with in vivo hemolysis was observed, although an increased value (> 300) was associated with abnormal hematologic and biochemical values.

The results of our study demonstrate that GT is effective for the characterization of RBC-bound

autoantibodies with regard to their classes, subclasses, and titer and its correlation with in vivo hemolysis in autoimmune diseases. Dittmar et al.[8] in 2001 reported that laboratories should not rely on a single method to characterize autoantibodies, especially in conditions with clinical evidence of hemolysis. Considering the various advantages of GT and its recent developments, it would be prudent to introduce the technique in blood centers either as a replacement or as an additional parallel assay to the CTT in the serologic characterization of autoimmune hemolysis. In addition, the clinicians can become "self-reliant" with regard to complete serologic evaluation of patients with autoimmune diseases.

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Labeling of cell therapy products: a review of the past and a look into the future

N.R. Haley and J.P. Miller

In early bone marrow transplantation, the cells given were thought to be simple replacements of diseased cells after myeloablative therapies. Today human cell populations are often used as agents of therapy. As the uses and cell varieties have multiplied, so has the need grown for a labeling system that works both inside institutions as well as between widely separated sites of collection and infusion.

The pioneers in cell therapy were Nobel Prize winner E. Donnall Thomas and colleagues.[1,2] These bone marrow transplanters reasoned that if the bone marrow was diseased with leukemia, and an HLA-matched sibling could be found, the diseased bone marrow could be eradicated by total body irradiation and replaced with donated, matched, healthy bone marrow. Their success in the late 1970s was a phenomenal innovation for treating a disease that had been commonly fatal. The work was highly experimental, and the bone marrow product was usually labeled simply with the name of the donor and that of the patient for whom it was intended. Distance, time, and processing were not major factors. The matched sibling was brought to the place where the patient was being treated, the cells were collected at that site, and the transplant product was given fresh with minimal processing.

Further expansion of cell therapy soon brought new labeling requirements. In the early 1980s, oncologists at the University of Nebraska reasoned that if they could get a reduced-disease autologous bone marrow (or later, peripheral blood that was likely to contain engrafting bone marrow cells), they could give the patient the same kind of high-dose chemotherapy that the allogeneic transplant patients were receiving and achieve a similar improvement or cure.[3,4] Because the patient was the donor, there were no problems with finding a match or with graft-versus-host disease. This simplicity, however, led to a more complex labeling and tracking situation. The cellular products were collected, processed, and frozen for the patient's future use in the same storage space with other patients' grafts. The labeling consisted mainly of the patient's name and Social Security or hospital number. Because multiple collections of cells from each patient had to be made over time, there was a proliferation of cells to be frozen and stored together. Sometimes cells from patients with similar names were mixed up.

When blood centers began to collect apheresis products for transplantation, it was their practice to put unit numbers on the label in addition to the autologous patient information. These unique numbers allowed the centers to use their regular routine for typing and infectious disease testing. As automation in blood banking progressed, centers started using bar-coded numbers.

The success of autologous and related donor bone marrow transplantation led to an expanded search for donors. The related, matched-sibling approach could serve only a small number of patients; only about 30 percent of patients had an HLA-matched sibling. In 1987, the National Marrow Donor Program (NMDP) began to make HLA matches between volunteer donors and patients for unrelated bone marrow transplantation.[5] The labeling problems were now magnified. Cell products had to be collected and shipped, often across countries and continents, yet donors were promised anonymity. The NMDP constructed a system in which a unique number was handwritten on the label and the label was placed on the product immediately after collection. The information connecting the number to the donor and to the matched patient was held by the NMDP.

The limitations of handwritten numbers soon became apparent. Many laboratories around the world

discovered that the way numbers are written, especially the numbers 1 and 7, can be very different and very difficult to read. Fortunately, because there were seldom multiple products traveling to any one location at the same time, and because each product was transported by a courier, this did not usually pose a problem.

Purpose of Labeling System

A labeling system has several essential tasks. These include the ability to:

- name and uniquely identify each product,
- state the contents (other than the product) and conditions of storage, and
- state the status of the product, namely to
  - state whether the product is fully screened and tested for allogeneic use or is for autologous use only, and
  - state other circumstances limiting use, including donor ineligibility, as determined by the FDA.

A labeling system should also include:

- standard names for standard products,
- a machine-readable labeling process, recognized worldwide, that is also eye-readable (allowing maximum safety and control of the process where computerized management is possible, but not leaving out locations that do not have access to those sophisticated systems), and
- labels that meet FDA requirements (at least in the United States).

Unique electronically readable identifiers can be incorporated into data systems for continuity of product identity through processing. These same data systems can be accepted into patient charts and electronic identification systems in laboratories and clinics so there can be no inadvertent substitution of the wrong product at the time of shipping or infusion.

Current thinking among FDA speakers is that cell therapy products, when they are more than minimally manipulated or are from unrelated donors (are other than autologous or related products), should be approved by the FDA as biologic products. Thus they would require either an FDA-approved investigational new drug application or a biological product application. The FDA published a rule in February 2004 that human drug and biologic products would be required to be labeled with a National Drug Code (NDC) number (21 CFR 201.05). The final rule requires that product labels must have a linear bar code that contains, at a minimum, the drug's NDC number. The rule also requires machine-readable information on blood and blood component labels. In direct questions to FDA officials concerning the applicability of this rule to cell therapy products, they confirmed that this requirement would also apply to cell therapy products.

Product Names

In the pioneer days of bone marrow transplantation, the collected early progenitor cells were called hematopoietic stem cells, whether they came from bone marrow or from peripheral blood. Product names were quite freeform in those days, but usually contained the name of the source material, such as bone marrow or peripheral blood by apheresis, and the designation stem cells. Sometimes this designation was more hopeful than factual.

Culturing of colony-forming units was introduced in research laboratories in the late 1980s to assess the regenerative power of the cells collected. These assays, however, were (and still are) difficult to perform in a standardized manner. Also, the cultures took 2 to 3 weeks to grow out and be read, making these assays impractical for a graft quality measurement.

In the early 1990s, CD34 surface antigen testing of cells by flow cytometry made it possible to predict engraftment potential. As a result, the term stem cell became suspect for these products, because the testing found cells that could speed engraftment but did not always find the earliest cells that assured long-term hematopoietic engraftment.[6] This led to use of the term hematopoietic progenitor cell. The AABB[7] and the Foundation for the Accreditation of Cellular Therapy (FACT) standards[8] required that products be labeled with their product name as well as with identifying numbers or names. The FACT standards of the day required that the word *Human* also be included in the label. This led to quite complex and lengthy names, such as "Human Peripheral Blood Hematopoietic Progenitor Cells." Although very descriptive, such long names took up a lot of real estate on the label, especially if the font size was large enough to be readable.

Another problem was that different institutions had different names for the same cell therapy products. It was difficult to know whether products with similar names met the same specifications or were collected and stored in the same way. They needed a common language.

Circular of Information Product Names

A group of cell therapy leaders drawn from the professional groups involved in writing standards for the field met in 2001 to create a circular of information for cell therapy products (published in 2002). Although circulars of information had been previously required by the standard-setting organizations, they were quite variable in content and complexity. This new group agreed that the basic elements should include general descriptions of the products, their common uses, and possible adverse consequences that patients might experience, be observed for, and be treated for, if necessary.

An updated Circular of Information for the Use of Cell Therapy Products was published in 2005. It was prepared jointly by the AABB, America's Blood Centers (ABC), American Association of Tissue Banks, American Red Cross (ARC), American Society for Blood and Marrow Transplantation, FACT, International Council for Commonality in Blood Bank Automation (ICCBBA), International Society for Cellular Therapy (ISCT), and NMDP. In this document, the interested parties accepted a simplified system of mutually agreed-upon names, using the abbreviation HPC in place of hematopoietic progenitor cells. The most commonly collected and labeled products were given the names HPC, Apheresis; HPC, Marrow; and HPC, Cord.

Stimulated peripheral blood did not merit a separate product name, as it could only be collected by apheresis procedures to get sufficient numbers for transplantation. Because apheresis procedures could not be used to collect marrow or cord blood, the group reasoned that it was sufficient to use HPC, Apheresis as the name of this product. Similarly, Human Umbilical Cord Blood Hematopoietic Progenitor Cells was considered too long a name for the label; HPC, Cord was accepted.

This kind of agreement was timely because cord blood banking and transplantation opened a whole new area of cell therapy.[9] Many features were similar to more traditional blood banking, such as the need to perform screening and infectious disease testing on unrelated, anonymous donors and mothers before the units could be banked and offered for general use. But the old system of using name, hospital number, or both was clearly inadequate, especially inasmuch as large allogeneic, public cord blood banks were being set up worldwide.[10] Establishing agreed-upon product names was a very good direction for a field already exploding with new uses for cell therapies and new cell products to be named. For example, doses of cells rich in lymphocytes from the allogeneic bone marrow donor were found to be useful after transplantation to treat leukemia relapse and facilitate more complete engraftment of the donor immune system in the recipient.[11] These collections targeted T lymphocytes, not HPC. Should they be named Donor-specific Lymphocyte Infusions (DLI), Therapeutic T Cells, or Therapeutic Leukocyte Infusions, because one could not guarantee that all the cells collected were T lymphocytes? Should the name reflect the content of the collection or the intent or probable use of the collection? This debate continues.

FDA Regulation and Guidance

As the field of cell therapy and transplantation progressed from a few research centers to much larger operations, potentially affecting more of the population of the United States, the FDA became increasingly involved. In May 2005, it established labeling regulations pertaining to cell therapy products defined in 21 CFR §1271 and covered by the Public Health Service Act (PHSA) in §361.[12] 21 CFR §1271(c) requires that human cells, tissues, and cellular and tissue-based products (HCT/P) have:

- a distinct identification code, e.g., alphanumeric, that relates to the donor and all associated records that assist in tracking the product from the donor to the recipient (except in the case of autologous or directed family donations, cannot be the person's name, Social Security Number, or medical records number),
- a description of the type of HCT/P,
- an expiration date, if any, and
- warnings, if applicable, as defined in
  - 21 CFR §1271.60. If the donor has not had evaluation for infectious disease completed, the product must be held or shipped in quarantine. If the product is released for urgent medical need before the evaluation of the donor can be completed, the product must be prominently labeled with "NOT EVALUATED FOR INFECTIOUS SUBSTANCES" and "WARNING: Advise patient of communicable disease risks."
  - 21 CFR §1271.65. If an HCT/P is from an ineligible donor, but is intended for use under special circumstances, such as use in a first-degree or second-degree blood relative; reproductive cells or tissue from a reproductive donor who knows and is known by the specific

recipient; or there is an urgent medical need. In this case the product must bear the Biohazard legend and the statement: "WARNING: Advise patient of communicable disease risks."

The following information must accompany the HCT/P either on the label or in accompanying information:

- name and address of the establishment that determined that the HCT/P meets release criteria and makes the HCT/P available for distribution,
- storage temperature,
- other warnings, where appropriate, and
- instructions for use when related to the prevention of the introduction, transmission, or spread of communicable disease.

ISBT 128 Bar-coded Product Labels and Unique Identifiers

Although there are any number of possible solutions to the internationally readable bar-coded label requirements, one system under consideration in the United States is already internationally accepted. The Working Party on Automation and Data Processing of the International Society of Blood Transfusion (ISBT) proposed the ISBT 128 system in 1989. The AABB, ABC, and ARC established the Council for Commonality in Blood Banking Automation (CCBBA) to implement ISBT 128 in the United States. The standard, data identifiers, and application specification were developed between 1990 and 1994. In 1994, the ISBT council approved the ISBT 128 application specification and established the office for the ICCBBA from the CCBBA to ensure that any new standard designed around code 128 would be maintained. In 1995, ICCBBA was incorporated (not-for-profit) in Virginia. Facilities collecting blood, HPC, and tissue and manufacturers of equipment or software that use ISBT 128 are required to register with ICCBBA, Inc. For further information, visit the ICCBBA, Inc., Web site at http://www.iccbba.com.

ISBT 128 specifies the following:

- use of a unique donation identifier worldwide,
- data structures for important information on the product label,
- the assignment of product codes by ICCBBA,
- a data structure for software developers to interface necessary input and output messages, and
- a standard label format that ensures a consistent layout of critical product information.

ISBT 128 has gained widespread acceptance. As of 2005, facilities in 40 countries on five continents and 44 worldwide vendors for software, bags, and labels had registered with ICCBBA to use ISBT 128. The number of registrants increases each year. International organizations, including the AABB, European Plasma Fractionators Association, European Blood Alliance, and FDA, have endorsed ISBT 128. In June 2000, the FDA issued a guidance document recognizing ISBT 128 as an acceptable standard for uniform labeling. Despite this acceptance, adoption of the system has been slow with cost cited as the reason. However, now that it is being accepted and slowly put into use around the country, it seems unlikely that hospitals and centers would be anxious to adopt multiple systems to take care of each cell and tissue type.

Minimally Manipulated Products

The cell therapy minimally manipulated, homologous-use cell preparations are described in PHSA §361, and the regulations for these products are found in 21 CFR §1270 and §1271. A simple, general bar-coding system as described in a previous section for minimally manipulated, homologous-use products covered under PHSA §361 has been constructed by a coalition of industry groups and presented to the FDA as an official recommendation. The Office of Cell and Gene Therapy (OCGT) at the FDA Center for Biologic Evaluation and Research has considered the naming of the minimally manipulated products, and the issue is as yet undecided. The FDA has asked for public comment on whether NDC codes, the same type of bar codes as those required for pharmaceuticals and highly processed cell products, are appropriate considering that bar coding is currently required.

More Than Minimally Manipulated Products

What happens if the cell therapy products are more complex in their preparation and production and are covered under PHSA §351? They are covered in regulations by 21 CFR §211 and §600. These more-than-minimally manipulated products will be named by a new plan devised especially for them. A naming plan was devised and accepted by the Cellular Therapies Working Group from the American Medical Association and the OCGT. These products will be covered under PHSA §351,13 meaning that these products must be produced under full good manufacturing practices and regulatory oversight.

In this plan, the name of a product is created using the following components to construct the name.

- Prefix: established by the United States Adopted Names council to provide uniqueness
- Infix: may be derived from the following:
  - manipulation
    - -gen = transduced;
    - -pul = pulsed with peptide or other agent;
    - -fus = fused with peptide, cells, or other agent
  - cell type, e.g.,
    - -myo = myoblast
    - -isle = islet cell
    - -den = dendritic cell
- Suffix
  - -cel (stem for cell therapies); used for all cell therapies
  - -imut (stem for immunomodulators); used for all noncellular cell therapy products, which include cell lysates, peptides, or proteins used for cancer vaccines. Substems for these vaccines are as follows:
    - -lisimut (stem for cell lysates)
    - -pepimut (stem for peptides)
    - -protimut (stem for protein)
- Qualifiers: letter after hyphen at the end of name
  - T = autologous
  - L = allogeneic
  - X = xenogenic

Following these guidelines, a product name would be composed of a prefix, infix 1, infix 2, and a suffix with a qualifier. For example, the name for a neuronal stem cell would be derived from neuro (infix for the cell), prog (second infix for progenitor), and cel (stem of the product) followed by a qualifier letter T, L, or X to indicate the source. Similarly, a dendritic cell that has been fused with a tumor cell would be a fusdencel.

The remainder of this plan, including more examples, can be found at Web site http://www.ama-assn.org/ama/pub/category/15395.html.

View of the Future

The starting point to solve the current label puzzle is the consensus established by the two circulars of information from the professional societies. A label design task group is now meeting to construct ISBT 128 bar-coded labels for cell therapy products currently covered by 21 CFR §1271 and by professional standards. This is a critically important task because there are no standard label formats and no widely accepted label designs although these are required by regulation. An international standard is desperately needed. For example, one-half of all of the allogeneic bone marrow transplantation products from unrelated donors in Australia come from international sources as reported by Dr. Kerry Atkinson at the 2006 ISCT meeting in Berlin.

In the United States, if industry does not agree on and present bar codes and labels to the FDA, the FDA will provide an issued bar code for only those products that are nationally recognized; these will be structured the same as the bar codes issued for pharmaceutical products. This action will limit the computerized control of both domestic and international products that might be required for hard-to-match patients. Because blood transfusion services are accepting ISBT 128 coding for transfusion components, hospital computer systems are being modified to accept this electronic language internationally.

Cell therapy laboratories are rightfully concerned about the expense of installing a new labeling system when they have not been required to have one in the past. This is a positive aspect because, to date, large sums of money have not been spent on competing and incompatible systems. By extending the system that the transfusion service will have in place, the regulatory requirements can be met with a system that may have extended utility. It is clear that a unique, machine-readable as well as eye-readable system will have to be put in place. Using this opportunity to install an internationally recognized system will make future labeling steps more useful in identifying and tracking products through the shipping process, integrating the cell therapy products into patient charting, and promoting the patient's safety by using the checks and balances available to assure certainty of identification that can eventually be extended to both cell therapy product and patient.

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Serologic and molecular characterization of the B(A) blood group in the Chinese population

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B(A) phenotype individuals have normal B antigen and a small amount of A antigen on the RBCs with anti-A in the plasma. Some highly potent monoclonal anti-A reagents are capable of agglutinating B(A) RBCs, which therefore usually results in a discrepancy between RBC and plasma ABO grouping. To date, five B(A) alleles *(ABO*B(A)01, B(A)02, B(A)03, B(A)04,* and *B(A)05)* have been defined by nucleotide sequences. To get a more complete picture of B(A) phenotypes found in the Chinese population and resolve blood donor typing problems caused by B(A) alleles, a serologic and molecular study of nine unrelated Chinese individuals and three families carrying B(A) alleles was conducted. Allele *B(A)02* with a 700C>G mutation, allele *B(A)04* with a single 640A>G substitution, and allele *B(A)05* with a 641T>C mutation were detected in multigenerational families and unrelated blood donors. Neither the *B(A)01* nor *B(A)03* alleles with 703A>G substitutions were observed in this study. In addition, a polymerase chain reaction with a sequence-specific primer genotyping assay was developed for rapid identification of *B(A)02, B(A)04,* and *B(A)05* alleles using genomic DNA samples. ***Immunohematology* 2007;23:69–74.**

Key Words: ABO subtype, B(A) phenotype, B(A) allele, PCR-SSP genotyping

Owing to the naturally overlapping substrate specificities of the A and B glycosyltransferases, the presence of small amounts of A antigen on certain B group RBCs was observed in the 1980s by using certain highly potent monoclonal anti-A reagents; these were designated as the B(A) phenotype.[1-4] The B(A) phenotype is attributable to specific mutations in the B gene that cause the B transferase to synthesize small amounts of A antigen. B(A) individuals carry normal B antigens and a small amount of A antigens on their RBCs, whereas their sera contain anti-A. The molecular basis of the first B(A) phenotype was determined by Yamamoto et al.[5] To date, five B(A) alleles (*ABO* B(A)01, B(A)02, B(A)03, B(A)04,* and *B(A)05*) have been defined by nucleotide sequences according to the Blood Group Antigen Gene Mutation Database (http://www.ncbi.nlm.nih.gov). Compared with *B101* alleles, the *B(A)01* allele has one silent substitution (657T>C) and one replacement substitution (703A>G), resulting in an amino acid change from serine to glycine at amino acid position 235. The *B(A)02* allele was originally identified in an Asian individual with a single 700C>G substitution, which predicts the alteration of proline to alanine at position 234.[6] The allele *B(A)03* differs from *B(A)01* by only a single silent substitution of 657C>T.[7] Both the alleles *B(A)04* and *B(A)05* were recently found in Chinese populations with 640A>G and 641T>C substitutions, respectively.[8-10] A further analysis of B(A) phenotype found in Chinese individuals was carried out. This study involved nine unrelated $A_{weak}B$ phenotype blood donors who had ambiguous ABO typing results. The study was then extended to three families, followed by identification of three B(A) alleles: *B(A)02, B(A)04,* and *B(A)05.*

Materials and Methods

Sample collection

Nine B(A) samples were collected from unrelated individuals from 2000 to 2005. Five individuals carried the *B(A)02* allele, three carried the *B(A)04* allele, and one carried the *B(A)05* allele. Three samples were obtained from Shanghai city, two from Zhejiang province, and the other four from Sichuan, Fujian, Anhui, and Henan provinces, respectively.

Serologic studies and DNA-based ABO genotyping

Peripheral blood samples were collected using ACD anticoagulant tubes. The participants in this study were all Chinese, and informed consent was obtained. Serologic characteristics were determined by use of hemagglutination and adsorption and elution methods, according to the *AABB Technical Manual,* with the exception of reverse ABO grouping.[11] In our reverse ABO grouping, 1 drop of plasma instead of 2 to 3 drops of serum was added to each tube. The reagents used

for this study were murine monoclonal anti-A (BIRMA-1), anti-B (LB-2), and anti-A,B (ES4+ES15; Serological Corporation, Livingston, UK) and human polyclonal anti-A, anti-B, and anti-A,B (Blood Reference Laboratory, Shanghai, China), in addition to anti-A_1 (*Dolichos biflorus*) and anti-H (*Ulex europaeus*) lectins (Gamma Biologicals Inc., Houston, TX). Genomic DNA was prepared from whole blood using a DNA blood mini kit (QIAamp, Qiagen, Hilden, Germany). Preliminary genotyping for *A101, A2, B,* and *O* alleles was carried out using the duplex polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method, which allows detection of single-nucleotide polymorphisms (SNP) at nucleotide (nt) 261, nt 467, nt 703, and nt 1096, as described by Olsson and Chester.[12] In brief, a 252-base pair (bp) fragment of exon 6 and an 843-bp fragment of exon 7 were amplified by PCR in the same tube using primer pairs mo57/mo46 and mo101/mo71 (Table 1). Each PCR reaction was carried out with a final volume of 25 µL containing 2.5 µL of PCR buffer, 20 mM $MgCl_2$, 0.2 mM dNTP, 1 U *Pfu* DNA polymerase (Bio Basic Inc., Markham Ontario, Canada), 0.2 µM primers, 10% (vol/vol) glycerol, and 50 ng of genomic DNA. After denaturation for 10 minutes at 95°C, samples were subjected to 35 cycles of PCR in a DNA thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, Foster City, CA). The first 10 cycles consisted of 94°C for 60 seconds, 63°C for 90 seconds, and 72°C for 60 seconds. The remaining 25 cycles consisted of 94°C for 60 seconds, 61°C for 90 seconds, and 72°C for 60 seconds followed by a final extension at 72°C for 10 minutes. Ten microliters of PCR product was digested with *Kpn*I and *Msp*I at 37ºC for 2 hours according to the manufacturer's protocols (New England Biolabs, Ipswich, MA). Then, 7 µL of the digestion mixes was separated electrophoretically for 1.5 hours at 150 V on 12% nondenatured polyacrylamide gels. The gel was stained using ethidium bromide.

Cloning and sequencing

The entire exons 6 and 7 of the ABO gene were amplified by PCR using two pairs of primer, mo57/mo46 and mo101/mo71, respectively. The blunt-end PCR products of *Pfu* DNA polymerase were purified using a gel extraction kit (Qiagen). Adenine tails were added artificially. The PCR products were cloned into pGEM-T easy vector (Promega, Madison, WI), and then transformed into competent DH5α *E. coli.* After transformation, four to six colonies were randomly selected, and plasmid DNA was extracted using Wizard Minipreps' DNA purification system (Promega). For each sample, at least two positive clones were sequenced with a BigDye Terminator Cycle Sequencing kit on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems).

PCR-SSP genotyping

An easy polymerase chain reaction with a sequence-specific primer (PCR-SSP) assay was developed to detect SNP at nt 640, nt 641, and nt 700. This approach relies on the sequences of the primers used in the PCR. When the 3′ nucleotide of a primer perfectly matches the sequence at the site of a given allele, the sample DNA, as a template for PCR, will be amplified. However, when the 3′ nucleotide of the primer is mismatched, amplification will not occur. For the detection of each allele, two pairs of primers are included in the PCR mixes; one is an allele-specific primer to amplify a specific allele and the other is a common primer to amplify a common gene to control the efficiency of the PCR. The sequence-specific oligonucleotide primers were designed according to published sequence data (Table 1). Six PCR-SSP mixes (B640A, B640G, B641T, B641C, B700C, and B700G) were prepared, to detect nt 640A, nt 640G, nt 641T, nt 641C, nt 700C, and nt 700G, respectively. Control primers that amplified a 427-bp fragment of the human growth hormone (HGH) gene were included in all reactions. The initial PCR was carried out with 1 µL of purified DNA sample (0.05 to 0.10 µg), 1 µL of diluted Taq polymerase (0.25–0.33 U), and 8 µL of PCR mix (10 mM Tris-HC1 [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% [wt/vol] gelatin, 0.2 mM dNTPs, 0.5 µM of each forward and reverse primer, 0.2 µM of each internal control primer) in a final 10-µL reaction volume. After denaturation for 5 minutes at 95°C, samples were subjected to 30 cycles of PCR in a DNA thermal cycler. Each cycle consisted of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1.5 minutes, followed by a final extension at 72°C for 5 minutes. PCR products were analyzed by electrophoresis on a 2% agarose gel containing 0.5 µg of ethidium bromide/mL and visualized with ultraviolet transillumination.

Classification and nomenclature

The *ABO* allele names used in this paper conform to the unofficial nomenclature used in the Blood Group Antigen Gene Mutation Database.[13]

Table 1. Primers used for B(A) blood group genotyping by PCR-SSP

Primer*	Sequence (5′-3′)	Nucleotide position	GenBank accession no.	Primers: PCR product size (bp)
mo57(F)	CGGGATCCATGTGGGTGGCACCCTGCCA	20069–20089	AY268591	mo57/mo46:252
mo46(R)	CGGAATTCACTCGCCACTGCCTGGGTCTC	20286–20308	AY268591	
mo101(F)	CGGGATCCCCGTCCGCCTGCCTTGCAG	21292–21311	AY268591	mo101/mo71:843
mo71(R)	GGGCCTAGGCTTCAGTTACTC	22108–22127	AY268591	
640A(F)	GTGTGCGTGGACGTGGACA	622–640	AF134412	640A/703A:100
640G(F)	GTGTGCGTGGACGTGGACG	248–266	DQ124679	640G/703A:100
641T(F)	TGTGCGTGGACGTGGACAT	623–641	AF134412	641T/703A:99
641C(F)	TGTGCGTGGACGTGGACAC	249–267	DQ124678	641C/703A:99
703A(R)	GGCTGCTTCCGTAGAAGCT	6142–6160	AJ536135	
526G(F)	CTGTCAGTGCTGGAGGTGG	893–912	AJ536135	
700C(R)	TGCTTCCGTAGAAGCTGGG	1319–1298	F134412	526G/700C:215
700G(R)	TGCTTCCGTAGAAGCTGGC			526G/700G:215
HGH(F)	GCCTTCCCAACCATTCCCTT	5947–5965	M13438	
HGH(R)	TCACGGATTTCTGTTGTGTTTC	700–718	M13438	HGHF/HGHR:427

*(F) indicates forward primer; (R), reverse primer. Primers mo57, mo46, mo101, and mo71 were prepared according to Olsson and Chester, 1995. The sequences underlined are artificial. Primer 700G was modified from the primer originally reported by Yu et al.[6] Primer pair 640A/703A detects nt 640A and nt 703A; 640G/703A detects nt 640G and nt 703A; 641T/703A detects nt 641T and nt 703A; 641C/703A detects nt 641C and nt 703A; 526G/700C detects nt 526G and nt 700C; 526G/700G detects nt 526G and 700G.

Table 2. Serologic characteristics and genotypes of B(A) phenotype

			Reaction of RBCs with					Reaction of plasma with		
Samples*	Phenotype	Genotype	Anti-A	Anti-B	Anti-A,B	Anti-A_1	Anti-H	A Cells	B Cells	O Cells
Family A										
1	B†	*B(A)04/B*	0	12	12	0	5	5	0	0
2	O†	*O/O*	0	0	NT	NT	10	8	10	0
3	B†	*B/O*	0	12	12	0	5	8	0	0
4	B(A)	*B(A)04/O*	8	12	12	0	8	8	0	0
5	B(A)	*B(A)04/O*	5	12	12	0	8	8	0	0
6	A	*A/O*	12	0	NT	NT	8	0	12	0
7	B(A)	*B(A)04/O*	8	12	12	0	10	8	0	0
Family B										
1	B(A)†	*B(A)05/O*	5	12	12	NT	10	5	0	0
2	O†	*O/O*	0	0	NT	NT	12	8	8	0
3	B(A)	*B(A)05/O*	8	12	12	0	12	8	0	0
4	B(A)	*B(A)05/O*	8	12	12	0	12	8	0	0
5	B(A)†	*B(A)05/O*	5	12	NT	NT	8	NT	NT	NT
Family C										
1	O†	*O/O*	0	0	NT	0	12	10	10	0
2	B†	*B(A)02/B*	0	12	NT	0	5	10	0	0
3	B(A)	*B(A)02/O*	8	12	NT	0	8	8	0	0
4	B(A)	*B(A)02/O*	8	12	NT	0	8	10	0	0
5	B	*B/O*	0	12	NT	0	5	10	0	0
6	B(A)	*B(A)02/O*	10	12	NT	0	8	8	0	0
7	B	*B/O*	0	12	NT	0	5	8	0	0
8	B(A)	*B(A)02/O*	8	12	NT	0	8	8	0	0
Unrelated										
1	B(A)	*B(A)02/O*	10	12	NT	0	12	8	0	0
2	B(A)	*B(A)02/O*	10	12	NT	0	12	8	0	0
3	B(A)	*B(A)02/O*	12	12	NT	0	12	10	0	0
4	B(A)	*B(A)02/O*	12	12	NT	0	8	10	0	0
5	B(A)	*B(A)04/O*	8	12	12	0	12	12	0	0
6	B(A)	*B(A)04/O*	5	12	12	0	12	12	0	0

*Sample numbers are the same as used in Figure 2.
† No. 5 in family B is a baby. All others marked with dagger are older than 60 years of age.
NT = not tested.

Results and Discussion

Some monoclonal anti-A reagents used in our routine donor blood grouping are capable of agglutinating B(A) RBCs; therefore, this results in a discrepancy between RBC (forward) and plasma (reverse) ABO grouping. In Table 2, a discrepancy between the forward and reverse ABO grouping was observed in nine blood donors (Family A No. 7, Family B No. 4, Family C No. 6, and six unrelated individuals from No. 1 to No. 6). B(A) RBCs showed a trace reaction with human polyclonal anti-A and stronger agglutination with murine monoclonal anti-A. Some samples carrying the *B(A)02* allele scored as high as 12 when they were reacted with monoclonal anti-A. In addition, these sera contained only anti-A antibody. Our observation is in concordance with the study performed by Yu et al.[6] The presence of A antigens on the RBCs was confirmed by adsorption and elution methods, whereas only B and H substances were detected in their saliva (data not shown). The phenotype could be serologically distinct from classic cis-AB. In classic cis-AB, the B antigen on RBCs is weakly expressed, and most of them are represented serologically by A_2B_3.[14] The preliminary genotypes of all nine B(A) donors were B/O heterozygous,

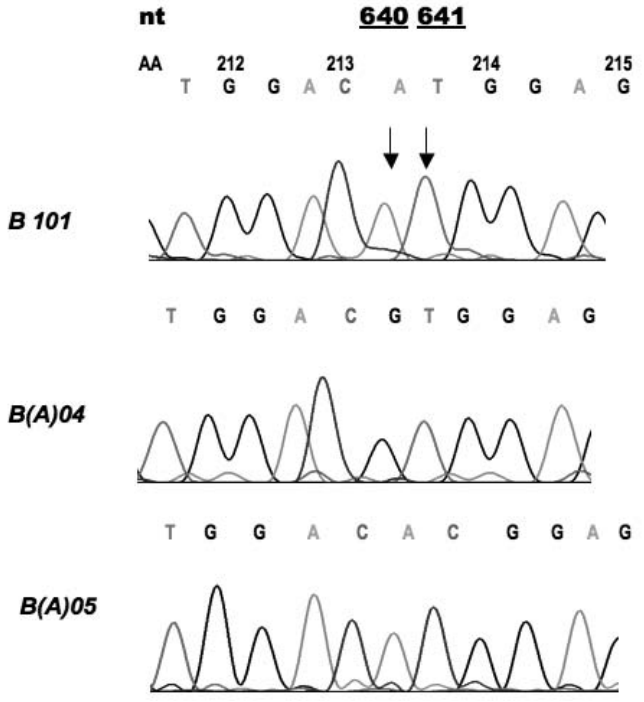

Fig. 1. Sequencing chromatograms. The region from codon 212 to 215 (number on top) in exon 7 of *B101, B(A)04,* and *B(A)05* alleles is shown. The arrows indicate the position for nucleotide 640 and 641, respectively.

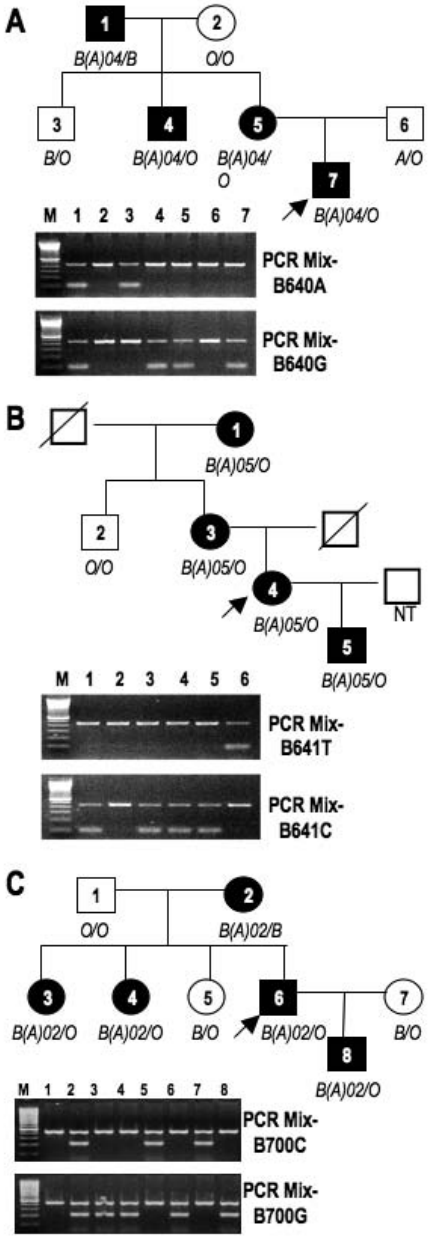

Fig. 2. Family pedigree and detection of SNP by PCR-SSP assay. The arrows indicate the propositi. ABO genotypes are noted in the symbols. Filled squares and circles represent *B(A)02, B(A)04,* or *B(A)05* allele carriers; open squares and circles indicate those family members who do not carry the B(A) alleles; NT = not tested; / = deceased. The numbers used for the family members are the same as those used on the gel electrophoretogram. Lane M shows a 100-bp DNA ladder. The top band is the housekeeping gene (HGH) as an internal positive control. **(A)** Segregation and inheritance of *B(A)04* allele in family A. Genotypes at nt 640 were determined by two PCR mixes, B640A and B640G, in two separate PCR amplifications. **(B)** Inheritance of *B(A)05* allele in family B. Two PCR mixes, B641T and B641C, were used in two separate PCR amplifications to determine genotypes at nt 641. An unrelated donor with genotype *B101/B101* was used (number 6 on the gel) as a positive control for PCR mix B641T. **(C)** Segregation and inheritance of *B(A)02* allele in family C. Two PCR mixes, B700C and B700G, were used in two separate PCR amplifications to determine genotypes at nt 700.

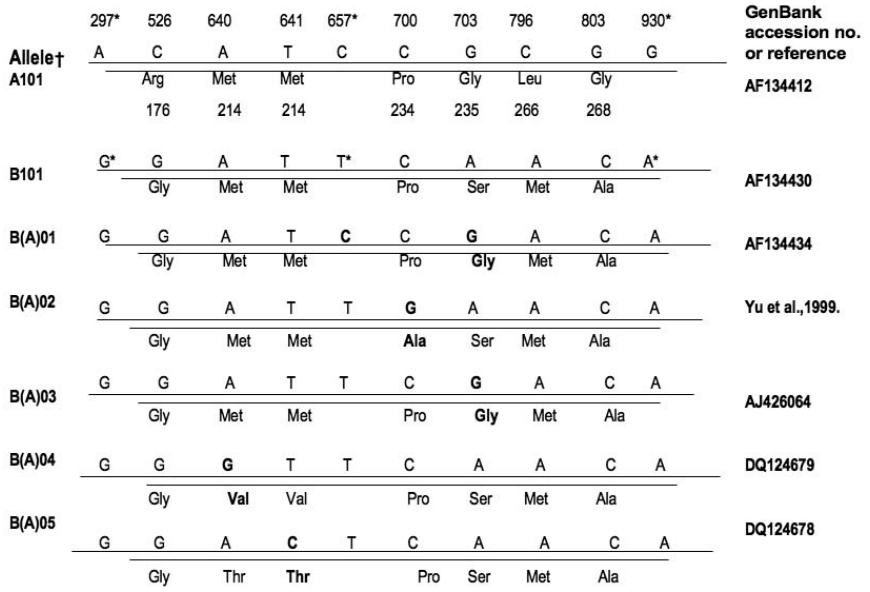

Fig. 3. Variation of nucleotides and deduced amino acid in exon 6 and exon 7 of *ABO* gene. Dagger signifies allele nomenclature according to Blood Group Antigen Gene Mutation Database.[13] The letter marked with an asterisk indicates silent substitution.

determined by performing a PCR–RFLP genotyping assay (Table 2).

The genotypes of the family propositi were determined using sequence analysis. Exons 6 and 7 of the *ABO* gene were cloned and then sequenced in both forward and reverse directions. Nucleotide sequence alignment revealed that the exon 7 sequence of donor No. 7 in family A differs from the *B101* allele by a single 640A>G substitution, which changes codon 214 from methionine to valine (ATG → GTG; Figure 1). Sequence analysis of another donor (No. 4 in family B) indicated a single 641T>C substitution in exon 7 of *ABO* gene, which results in an amino acid replacement of methionine by threonine at codon 214 (ATG → ACG; Figure 1). Donor No. 6 (in family C) was found to carry a 700C>G mutation that had been reported previously as the *B(A)02* allele by Yu et al.[6]

B(A) alleles with 640A>G or 641T>C mutations were designated as *B(A)04* and *B(A)05,* respectively. Family studies indicated that both the *B(A)04* and *B(A)05* alleles were inherited as expected (Figure 2). The nucleotide sequences of these two alleles have been deposited in GenBank with accession number DQ124679 for *B(A)04* and DQ124678 for *B(A)05,* respectively. To rapidly detect *B(A)02, B(A)04,* and *B(A)05* alleles using genomic DNA, an easy PCR-SSP method was developed. As shown in Figure 2, six possible genotypes at nt 640, nt 641, and nt 700 could be clearly distinguished from each other. To check the reliability and specificity of the typing method, a total of 34 DNA samples, including 19 B(A), 5 A, 5 B, and 5 O phenotypes, were repeatedly tested and a concordance rate of 100 percent was observed. In addition, the validity of this method was verified by sequence analysis.

Most ABO variants arise from mutations in exons 6 and 7 of the *ABO* gene. The products of *A* and *B* genes differ by four amino acid substitutions at 176, 235, 266, and 268. It was thought that the amino acid residues at these four positions were critical for determination of the specificities of the A and B glycosyltransferase, and that an overlapping specificity results in the formation of B(A) phenotype.[5,14] Both the *B(A)01* and *B(A)03* alleles have a Ser235Gly substitution, whereas the *B(A)02* allele encodes a Pro234Ala substitution just before position Ser235. As shown in Figure 3, the *B(A)04* and *B(A)05* alleles encode a Met214Val and a Met214Thr substitution, respectively, at the same position 214. This suggests that the residue at 214 might also be involved in the determination of the enzyme specificity.

Acknowledgments

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Transfusion of multiple units of Js(b+) red blood cells in the presence of anti-Js^b in a patient with sickle β-thalassemia disease and a review of the literature

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Js^b is a high-frequency antigen. Anti-Js^b is a rare alloantibody, and its clinical significance is poorly documented. We report a case in which a 12-year-old boy of Nigerian descent with sickle β-thalassemia presented with multiple alloantibodies, including a panagglutinin and acute chest syndrome, necessitating the emergent transfusion of five units of phenotype-similar, crossmatch-incompatible RBCs, four of which were given during an exchange transfusion. The patient was later found to have anti-Js^b. In addition to routine serologic methods to study the patient's RBCs and plasma, a monocyte monolayer assay (MMA) was performed on the patient's samples obtained 2 and 9 days after transfusion of the Js(b+) RBCs to determine the potential clinical significance of the anti-Js^b. Various laboratory parameters including quantitative hemoglobin fraction analyses were used to monitor for increased RBC destruction. The MMA reactivity of the patient's anti-Js^b increased from 2.3 percent on day 2 after transfusion to strongly positive at 88 percent and 66.5 percent (with and without the addition of fresh serum) 1 week later. MMA reactivity of greater than 5 percent is associated with increased RBC destruction. There was no clinical or laboratory evidence of increased hemolysis above baseline. However, decreased RBC survival was suggested by the relatively brisk decrease of the HbA_1 fraction after the transfusions. The current case and others reported in the literature suggest that anti-Js^b may have limited potential for causing overt hemolysis. However, in patients with underlying hematologic disease, even mildly increased RBC destruction may pose problems clinically, and thus transfusion of Js(b+) RBCs should be avoided. In emergent situations, the potential of adverse effects of transfusing incompatible units should be balanced against the risk of withholding transfusion. Family members, especially siblings, should be considered as potential designated donors for patients with antibodies directed against high-frequency antigens. Available reports on anti-Js^b in the literature are also reviewed. ***Immunohematology* 2007;23:75–80.**

Key words: red blood cell, antibody, crossmatch-incompatible, transfusion, anti-Js^b

Js^b is a member of the Kell blood group system. It is a high-frequency antigen that is found on the RBCs of nearly 100 percent of Caucasians and more than 99 percent of individuals of African descent.[1]

The antithetical antigen Js^a, first detected in 1958,[2] was found to be present on the RBCs of about 20 percent of African Americans tested in Seattle. None of the individuals of Caucasian or Asian descent studied possessed this antigen. Js^b was subsequently detected in 1963 by Walker et al.,[3] when anti-Js^b was found in the serum of a Js(a+b–) African American woman from Memphis. Later that year, another African American woman in Chicago was found to have anti-Js^b.[4] Like the woman in the first case report, she also had an extensive transfusion history. In their efforts to locate compatible units for her, it was determined that only 0.65 percent of the 460 African Americans tested were Js(b–). Included in their study were the incompatible cord blood samples from three African American infants, which demonstrated reactions with anti-Js^b as strong as those samples obtained from adults. In 1968, Marshall[5] reported another example of anti-Js^b in which the sensitization was likely induced by pregnancy. When the patient presented later with multiple fractures and considerable blood loss, they were able to transfuse her with previously frozen autologous units or compatible Js(b–) allogeneic units.

There have been only a handful of reports of anti-Js^b in the setting of HDN,[6–9] with severity ranging from not requiring treatment to hydrops fetalis and death. The outcomes of patients with anti-Js^b transfused with Js(b+) RBCs are even less well documented, with only two abstracts[6,10] and one case report that could be found in the literature,[11] reporting no obvious clinical reaction[6,10] or delayed hemolytic reaction.[11]

We describe a case of anti-Js^b, in the setting of simple and exchange transfusions with a total of five units of incompatible Js(b+) RBCs, in a patient with sickle β-thalassemia disease.

Case Report

A 12-year-old boy of Nigerian descent with a history of sickle cell β-thalassemia disease and multiple hospitalizations owing to complications of his disease presented with rapidly progressive pulmonary infiltrates and increasing oxygen requirements. He had recently begun routine monthly RBC transfusions because of recurrent episodes of acute chest syndrome despite treatment with hydroxyurea. His last transfusion had been given 12 days before presentation. On admission, his Hb and Hct were 6.8 g/dL and 21.2 percent, respectively. The patient had a previous history of anti-E and a warm autoantibody. The current serologic investigation initially revealed anti-C, anti-S, and a pan-reactive antibody that could not be immediately identified. The previously identified anti-E and warm autoantibody were not demonstrable. The DAT at this time was negative with anti-C3, but microscopically positive with anti-IgG. Because of the patient's critical clinical condition, he was transfused with one unit of RBCs, and a single-volume exchange transfusion was performed the same day with four units of RBCs. All five units were crossmatch-incompatible, but phenotype-matched to the patient, and thus negative for C, E, S, K, Fy^a, and Fy^b. Subsequently, the pan-reactive antibody was identified to be anti-Js^b by the Reference Laboratory at the American Red Cross (ARC), Southern California Region. All five units transfused were presumably Js(b+) owing to the high frequency of this antigen and the reactivity of each unit with the patient's serum. The patient, however, tolerated all units well without clinical signs of hemolysis and showed clinical improvement of his symptoms. His Hb and Hct were 8.7 g/dL and 25.4 percent, respectively, immediately after the exchange transfusion. Gel electrophoresis showed HbA_1 fraction of 65.7 percent and HbS fraction of 29.5 percent at this time. Various laboratory para-meters were used to monitor the extent of hemolysis and showed no evidence of increased hemolysis compared with his baseline levels throughout the remainder of his hospitalization and at his two follow-up visits, occurring 9 and 15 days after the exchange transfusion. The patient also remained clinically well without overt signs of hemolysis.

Since his initial presentation, frozen units of Js(b–)C–E–S– RBCs had been located through the ARC, Southern California Region. These were transfused on the day of his discharge from the hospital, at his second follow-up appointment, and roughly every 2 weeks thereafter. One unit was transfused each time. The patient received these units without incident.

His previous transfusion history was reviewed retrospectively. It was revealed that about 2 weeks before admission, he was noted to have HbS fraction of 88.6 percent and HbA_1 of 0 percent on quantitative hemoglobin electrophoresis only 1 month after his previous transfusion of two RBC units. This was felt by his primary care physician to represent markedly decreased RBC survival compared with his baseline, and in retrospect was likely caused by the anti-Js^b, as well as the anti-S, which were not detectable at the time. Previous phenotyping studies on both units transfused at that time had shown that they were negative for C and E. However, they were likely Js(b+) based on the high frequency of this antigen. Retention segment was available only on one of the two units, and additional phenotyping showed that this unit was also positive for S. The microscopically positive DAT result at the time of his admission provided further evidence of ongoing delayed hemolytic transfusion reaction.

Materials and Methods

Antibody detection at the admitting hospital was performed by tube testing using a LISS method (ImmuAdd, Immucor Inc., Norcross, GA). Initial antibody identification was performed using untreated and enzyme-treated RBCs (Panocell, Immucor). The DAT was performed using monospecific antihuman reagents (Anti-IgG and Anti-C3, Immucor).

Because of the complexity of testing involved, the patient's blood sample was sent to the ARC Reference Laboratory. The patient's phenotype was determined using HbS-containing RBCs harvested by hypotonic saline wash method using 0.9 percent normal saline (Blood Bank Saline, pH 7.0-7.2, Nerl Diagnostics, East Providence, RI) diluted 1 to 3 with deionized water, which lysed the HbA-containing transfused RBCs but left the patient's own HbS-containing RBCs intact. Patient RBCs were phenotyped for ABO, D, and common antigens using commercial reagents (Immucor, and Ortho-Clinical Diagnostics, Inc., Raritan, NJ) and conventional tube methods. Rare antisera were prepared in-house or provided through SCARF exchange.

DATs with monospecific rabbit, heavy chain specific antihuman reagent (Anti-IgG, Ortho-Clinical Diagnostics), and monoclonal anti-complement reagent (Anti-C3b,d Gamma Biologicals Inc., Houston, TX) were performed by standard tube method.

An eluate was prepared from the patient's RBC sample using a commercial acid elution kit (Elu-kit II, Gamma Biologicals) and tested by conventional PEG tube IAT method.

Antibody detection and identification were performed by gel technology (ID-Micro Typing System, Ortho-Clinical Diagnostics) according to the manufacturer's written instructions and by conventional tube method, using commercial reagent RBCs (Ortho-Clinical Diagnostics and Immucor) and rare RBCs prepared in-house or provided through SCARF. Two potentiators, 20 percent PEG and LISS (both prepared in-house), were used to enhance antibody detection in tube tests. Ficin (0.01%)- and DTT (2 M, pH 8)-treated RBCs were prepared and used during the antibody identification by conventional tube methods. The ficin and DTT reagents (Sigma Aldrich, St. Louis, MO) were prepared in-house. Antibody titer was determined by serial dilution of the patient's plasma with 6% bovine serum albumin (prepared in-house from 22% albumin from Immucor) using normal saline (Blood Bank Saline, pH 7.0-7.2, Nerl Diagnostics) IAT. Allogeneic adsorption studies were performed using ficin (0.01%)-treated RBCs of known phenotype to deliberately adsorb the anti-Js^b from the patient's serum to allow for exclusion and confirmation of other common alloantibodies.

The monocyte monolayer assay (MMA) was performed as described elsewhere[12] by the Research Laboratory of the ARC, Southern California Region.

Results

On the patient's admission, workup by the admitting hospital's blood bank showed the patient's RBCs to be group O, D+, and weakly positive by the DAT with anti-IgG and negative with anti-C3. The antibody screen was positive with all RBCs tested. The sample was referred to the Research Laboratory of the ARC, Southern California Region, for further antibody identification.

The reference laboratory confirmed the DAT results. Because of the patient's recent transfusion history and sickle β-thalassemia diagnosis, a hypotonic wash method was performed on the patient's RBC sample to lyse HbA-containing transfused RBCs, isolating the patient's own HbS-containing RBCs for phenotyping. The isolated RBCs were negative by the DAT with anti-IgG, and the common antigen phenotype was determined to be D+C–E–c+e+, S–s+, K–, Fy(a–b–), and Jk(a+b+). The patient's plasma reacted variably (1+ to 3+) with all RBCs tested. RBCs that were phenotype-matched to those of the patient with regard to the above common phenotype were also weakly reactive (1+) with the patient's plasma, 2+ reactive after ficin treatment, and nonreactive after treatment with 2 M pH 8 DTT. A titration was performed using the phenotype-matched RBCs, which yielded a titer of 16. Several other examples of rare frozen RBCS that, in addition to being phenotype-matched to the patient's RBCs, also lacked various high-frequency antigens were tested against the patient's plasma, including Sl(a–), McC(a–), Yk(a–) Cs(a–), Hy(–), Lu(b–), and Js(b–) RBCs. Only the Js(b–) RBCs were nonreactive. The patient's RBCs harvested by hypotonic wash method were then typed as Js(a+b–). Additional Js(b–) and Js(b+) RBCs were thawed and tested as necessary, to allow for the confirmation of anti-Js^b, as well as anti-C and anti-S. These studies, however, could not detect the previously identified anti-E and warm autoantibody, and could not exclude anti-K, in part because of inadequate sample.

A subsequent sample was obtained immediately after the emergent transfusion with five units of crossmatch-incompatible, Js(b+) RBCs. This sample was moderately positive by the DAT with IgG only. An acid eluate of these RBCs reacted strongly (3+) with all Js(b+) RBCs and weakly (1+) with all Js(b–) RBCs by PEG IAT. This weak reactivity with Js(b–) RBCs may represent the warm autoantibody that had been previously detected, and also subsequently confirmed in a later sample. The plasma was confirmed to contain anti-Js^b, anti-C, and anti-S. Because of the unavailability of C–S–Js(b–)K+ reagent RBCs, anti-K was excluded by performing an allogeneic adsorption onto ficin-treated D–C–E–K– group O RBCs, then testing the absorbed serum against K+ RBCs. The previously identified anti-E and warm autoantibodies were again not demonstrable at this time.

Various clinical laboratory studies, including Hb and Hct, corrected reticulocyte count, LDH, total bilirubin, DAT, quantitative hemoglobin gel electrophoresis, and urinalysis were performed to monitor for laboratory signs of hemolysis, which remained essentially unremarkable. However, somewhat decreased RBC survival was suggested by the relatively rapid decrease in the HbA_1 fraction on quantitative hemoglobin gel electrophoresis studies, especially

between day 9 and day 15 after the exchange transfusion, when the HbA_1 fraction dropped from 55.6 percent to 37.2 percent in 6 days. These laboratory values, along with pertinent clinical events, are tabulated in Table 1.

The MMA was performed on the patient's samples obtained 2 and 9 days after transfusion of the Js(b+) RBCs to determine the potential clinical significance of the anti-Js^b. The MMA reactivity on the earlier sample was negative (2.3 %), but became strongly positive on the sample obtained 9 days after the transfusions, with reactivity scores of 88.0 percent and 66.5 percent, with and without the addition of frozen normal serum as a source of complement, respectively. Reactivity more than 5 percent is considered capable of causing decreased RBC survival.

Discussion

Because of the rarity of anti-Js^b, its clinical significance is not clear and reports in the literature are scarce. In this report we present the case of a 12-year-old boy of Nigerian descent with β-thalassemia sickle cell disease, who received one unit of crossmatch-incompatible RBCs, followed by a single volume transfusion with four units of crossmatch-incompatible RBCs. All five units were Js(b+). The patient tolerated these units without clinical or laboratory signs of increased hemolysis above the patient's hemolytic baseline, although decreased survival of transfused RBCs was suggested by the relatively brisk decrease in the HbA_1 fraction between 9 and 15 days later as determined by the quantitative hemoglobin analyses by gel electrophoresis.

A review of the literature shows that anti-Js^b may be clinically significant in a pregnant woman and cause HDN.[6-9] In some reports, the disease was severe,[6,8,9] and it resulted in hydrops fetalis and death of the infant in one case.[6] Two separate groups of investigators documented the outcomes of the two pregnancies in the same woman with anti-Js^b.[7,8] In the first report, the HDN was fairly mild despite a high maternal anti-Js^b titer, and the infant did not require an exchange transfusion.[7] In her subsequent pregnancy 10 years later, the HDN was more severe, and an exchange transfusion with frozen, compatible RBCs in addition to phototherapy was performed. Maternal anti-Js^b titer remained essentially the same during both pregnancies.[8]

The clinical consequences of transfusing Js(b+) RBCs in the presence of anti-Js^b are even less well documented. In one case, a single unit (275 mL) of RBCs was given to a female patient postpartum, which did not result in any clinically obvious reaction.[5] However, as mentioned above, this patient's infant suffered from severe HDN with hydrops fetalis, and died 5 hours after receiving 100 mL of incompatible RBCs. In the case reported by Huestis et al.,[4] the patient received only a few milliliters of incompatible RBCs, yet the patient developed shaking chills 1.5 hours later. It is unclear, however, whether her symptoms were manifestations of a hemolytic reaction or the result of her underlying disease. The investigators performed RBC survival studies with radioactively labeled RBCs, which suggested a dosage effect of Js^b, with Js(a+b+) RBCs destroyed less rapidly than Js(a–b+) RBCs in the presence of the alloantibody. As expected, Js(a+b–) RBCs had the best survival in their study. More recently, Blue-Hnidy et al.[10] reported in an abstract a man with multiple alloantibodies, including anti-Js^b, who received repeated transfusions of Js(b+) RBCs in the face of ischemic changes seen on the electrocardiogram. He tolerated all transfusions well without clinical evidence of acute hemolysis.

The only known report of well-characterized hemolytic reaction in this setting is the case reported by Waheed and Kennedy,[11] in which a 39-year-old woman received four units of crossmatch-compatible RBCs, and within 15 days developed a mixed field positive DAT; her Hb dropped from 12.0 g/dL to 5.7 g/dL, requiring admission to the hospital. Anti-Js^b was found in her serum and in the eluate of her RBCs. After receiving two units of Js(b–) RBCs, she was able to maintain a stable Hb and Hct, and the DAT became both macroscopically and microscopically negative 36 days later. Interestingly, the patient's pretransfusion sample typed as Js(b+) with five different anti-Js^b sera. When her DAT became negative after transfusion, her RBCs still typed as Js(b+). However, her pretransfusion RBCs were nonreactive with her own posttransfusion serum and eluate. These serologic results suggest that this was a case of an allo-anti-Js^b in a Js(a+b+) patient rather than an autoantibody, pointing to the heterogeneity of the Js^b locus and antigen structure. It appears that the patient possessed a variant Js^b antigen, and then formed anti-Js^b against an epitope that was absent on her own Js^b antigen. It is unclear whether the fact that her anti-Js^b had limited specificity mitigated the extent of the hemolytic transfusion reaction.

The MMA has been proposed as a valuable tool for predicting the clinical significance of alloantibodies,

Table 1. Summary of pertinent clinical events and laboratory data

Day	Clinical event	Hb (g/dL)	Hct (%)	Total bilirubin (mg/dL)	LDH (units/L)	Reticulocyte % (corrected)	HbA_1	HbS	MMA*	Other
12 days before admission	~1 month after transfusion of two units of C–E–K–HbS–, presumably Js(b+) RBCs. Received another unit of C–E–K–HbS–, presumably Js(b+) RBCs	7	21.4	2	1284	13.8	0	88.6	–	
Day of admission (Day 0)	Presentation with acute chest syndrome	6.8	21.2	2.3	–	12.2	–	–	–	DAT microscopically positive with IgG, negative with C3
Day 0	Immediately after simple transfusion and exchange transfusion with 5 C–E–S–K–Fy(a–b–) Js(b+) HbS– units	8.7	25.4	2.9	1246	–	65.7	29.5	–	Urinalysis normal. Anti-Js^b, -C, and -S identified in pretransfusion sample. Anti-Js^b titer = 16.
Day 2	One unit of C–E–S–Js(b–) HbS– RBCs given	9.0	26.5	3.7	1212	–	–	–	2.3%	
Day 5	Discharged	8.6	25.6	2.1	1115	–	–	–	–	
Day 9	First follow-up	8.8	26.9	1.9	–	–	55.6	39.2	66.5%/ 88%	Urinalysis normal. DAT positive.
Day 15	Second follow-up	8.1	25.3	1.8	–	15.3	37.2	56.6	–	

*MMA reactivity is reported as the percentage of monocytes phagocytosing or adherent to test RBCs. Reactivity scores of the assay with and without the addition of fresh-frozen serum as a source of complement are reported. Alloantibodies with scores more than 5% are considered capable of causing RBC destruction.

especially when antibodies are known to sometimes be clinically significant and other times not. In a retrospective review of 20 years of MMA data,[12] it was shown that only alloantibodies with reactivity greater than 5 percent are potentially clinically significant. One third of the patients with results between 5.1 percent and 20 percent had clinical symptoms of hemolysis, such as jaundice, fever, chills, change in blood pressure, vomiting, hemoglobinuria, and back pain; two thirds of patients with greater than 20 percent MMA reactivity had such symptoms. When clinical signs of hemolysis were absent, laboratory signs of hemolysis such as decreased Hb or Hct, decreased haptoglobin, rising bilirubin, and increased LDH were seen in 67 percent of those with reactivity between 5.1 percent and 20 percent, and in 75 percent of patients with reactivity greater than 20 percent. In the same review, only one of three cases of anti-Js^b showed a positive MMA result, with reactivity greater than 20 percent. However, the pertinent clinical information for these three cases in this study is not available.

Given the lack of information in the literature, it is not possible to say whether the lack of hemolysis after transfusion of incompatible RBCs in the presence of anti-Js^b is the rule or the exception. It is well known that an alloantibody can change its biologic behavior with repeated sensitizations.[7,8,13] Because the MMA reactivity is strongly positive and there is evidence of increased destruction of Js(b+) RBCs, the prudent approach is to transfuse fully compatible RBCs whenever possible. Increased RBC destruction alone may not be of much clinical significance for most patients, but for our patient, and others with hematologic diseases and increased RBC destruction at baseline, transfusion of RBCs with decreased survival is best avoided. However, this may not always be possible, considering that such patients may present emergently and there is simply not enough time to locate compatible units. The potential of adverse events associated with transfusion of incompatible RBCs must be balanced against the risk of withholding transfusion in such circumstances. Our patient does not have siblings or suitable family members who can serve as his donor. In similar patients, available siblings should certainly be considered as potential donors. Even when the siblings do not have the Js(a+b–) phenotype, Js(a+b+) RBCs from siblings and other family members may be destroyed less rapidly,

inasmuch as the Js[b] antigen appears to demonstrate a dosage effect.[4] Needless to say, close follow-up with clinical and laboratory monitoring is required whenever Js(b+) RBCs are transfused in these situations.

Authors' Note

At the time of this writing, the patient is undergoing a chronic transfusion regimen, still requiring one unit of RBCs every 2 to 4 weeks. We have been fortunate enough to be able to locate compatible units for him thus far. Needless to say, it has been and will be extremely challenging to provide Js(b–) and HbS– units on a continued basis for this patient, who has multiple additional alloantibodies and will be transfusion-dependent in the foreseeable future. Efforts have been made to recruit designated donors for him. We hope that with the publication of this report, we can publicize this very difficult case, and hopefully be able to identify additional units and donors for our patient with the help of our colleagues.

Acknowledgment

The authors would like to acknowledge and commend the serology skills of the Senior Reference Specialists at the ARC, Southern California Region, who were instrumental in the identification of this patient's serum antibodies, and the Research Laboratory at the ARC, Southern California Region, for the performance of the MMA. We wish to thank the American Rare Donor Program for their continued assistance in locating the RBC units required for the care of this patient. Also many thanks to Dr. George Garratty for his assistance with the clinical management of this patient and his valuable input for this manuscript.

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Consortium for Blood Group Genes (CBGG): Miami 2006 report

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The Consortium for Blood Group Genes (CBGG) is a worldwide organization whose goal is to have a system to interact, establish standards, operate a proficiency program, and provide education for laboratories involved in DNA and RNA testing for the determination of blood group, platelet, and neutrophil antigens. The purpose of this report is to summarize the CBGG meeting held in Miami in October 2006. *Immunohematology* 2007;23:81–84.

The Consortium for Blood Group Genes (CBGG) was started by a group of people interested in DNA analyses for blood groups who recognized that there was a growing need for establishing standard protocols and proficiency testing. The current coordinator is Marion Reid, and there are three liaisons: Lilian Castilho for Brazil, Gregory Denomme for Canada, and Connie Westhoff for the United States. All members are expected to interact and participate. The background and progress, including CBGG logo, history, and mission, have been published.[1,2] The CBGG is a nonprofit organization whose purpose is to provide a means for members to interact, educate, and help each other. Exchange of information is mainly accomplished through electronic mailings and the AABB Special Interest Group (SIG) Molecular Forum. (To access this SIG, log on to the AABB website as an AABB member, choose "AABB Forums," and then scroll down to "Hot Topics Section").

Discussion Document

A discussion document was circulated to all members before the meeting and was addressed by the group at the meeting. The information given in the descriptive history, structure, language, membership, mission, and goals sections was accepted with minor changes. The key areas of focus that had been identified by CBGG for discussion at the 2006 Miami meeting were discussed and are summarized here. The 2006 Discussion Document will be archived for reference purposes. Suggestions from the group present and from those who sent electronically mailed comments will be incorporated into the document. The updated 2007 version will be distributed to CBGG members before the 2007 meeting and is available to nonmembers on request.

Establish a uniform terminology

Of major interest to the group is a uniform terminology for alleles encoding blood group antigens and phenotypes. Standards exist for the terminology of nucleic acid and amino acid changes (http://www.hgvs.org/mutnomen/). Also, as a detailed and consistent system for naming blood group alleles is being addressed by an ad hoc group of the International Society of Blood Transfusion Working Party on Terminology for Red Cell Surface Antigens, of which several CBGG members are also members, it was decided to wait for and adopt their recommendations.

Develop template disclaimers

Disclaimer statements used by CBGG members when reporting results of home-brew DNA analysis for blood groups were solicited before the meeting. A draft generic disclaimer statement for use in clinical reports was developed by the group, which members then submitted to their legal departments for suggestions and approval. It reads:

> These in vitro diagnostic tests were developed and their performance characteristics established by <<facility name>>. The tests have not been cleared or approved by the Food and Drug Administration (FDA). <<Facility name>> is certified under the Clinical Laboratory Improvement Amendment (CLIA) of 1988 as qualified to perform high complexity clinical testing. These results are not intended as the sole means for clinical diagnosis or patient management decisions.

* a full listing of the members of the CBGG can be found in Table 1 on page 83.

> There are situations where the genotype of a person may not reflect the red cell phenotype and not all performance characteristics have been determined. Mutations that inactivate gene expression or rare new variant alleles may not be identified in these assays.

Comments by legal counsel will be shared at the 2007 meeting, and a consensus disclaimer prepared for discussion before distributing the final version.

Identify sources of funding

Sources of funding were discussed. The group agreed that a nominal membership fee is appropriate, the amount and format (e.g., institution versus individual) to be determined. The funds will be used for meeting room rentals and refreshments. Other sources of funding that were identified included a fee for participating in the proficiency program and for shipping DNA from the repository (see discussion in a later section). The possibility of obtaining education money through the AABB or meeting funds through the National Institutes of Health R13 funding mechanism needs to be investigated, although it was thought they may not be appropriate. It remains to be decided in which country or state the treasury should reside. To minimize expenses, the 2007 meetings will be held in conjunction with an International Immunohematology Symposium in Sao Paulo, Brazil, and the International Conference of Investigative Immunohematologists meeting in North Carolina. The 2008 meeting will be held before the AABB meeting in Montreal.

Develop standards of practice

Before the meeting, a survey was circulated about standards. All responders, except one abstention, voted for the CBGG to set standards, and all, except one abstention, voted that the CBGG should not accredit laboratories. At the meeting, there was discussion about the role of CBGG in setting standards, and it was agreed that although this is not an "official" organization, a consensus was reached that it would be useful to members of the CBGG to have standards, at least in a self-help capacity. Because of the international membership, draft standards have been written in International Standards Organization format, and after the Miami meeting, they were disseminated to CBGG members for input.

Expand and operate a proficiency program

A simple exchange of samples between several laboratories performing home-brew DNA assays is in effect. Samples are exchanged in the spring and fall. DNA from one sample is sent for testing for one defined single nucleotide polymorphism for which the predicted antigen had been confirmed by hemagglutination, with the caveat that the proficiency exercise would not involve rare alleles and should be straightforward. Results obtained by the testing laboratories are returned to the laboratory that sent the sample(s), and the shipping laboratory confirms (or not) the results and interpretation. Currently, the New York Blood Center (NYBC) is the central coordinating facility.

Establish a DNA repository of well-characterized DNA

Members agreed that the best source of DNA for reference samples for proficiency, validation, and control samples is from transformed cell lines. It was recognized that not all members have the ability to establish cell lines. Currently, NYBC in New York, American Red Cross Blood Services in Philadelphia, and Puget Sound Blood Center in Seattle have access to the required technology and would make DNA available to those who do not. Ultimately, a comprehensive collection of samples would be established, which would include common and rare samples for new assay development and validation. Until suitable rare donors can be obtained for the preparation of cell lines, DNA isolated from blood samples could be available for validation purposes. DNA samples should be those in which the genotype has been confirmed (preferably by sequencing) by the source laboratory. The criteria for establishing the validity of these samples will be developed. Institutional review board (IRB) approval may be required for obtaining blood samples for this purpose and is certainly required if publishing any results related to this function. The cost to maintain the repositories is to be determined.

Regulatory and informed consent issues

Predicting the presence or absence of a blood group antigen using a DNA assay is not genotyping for a disease; it is considered an alternative method to serologic testing. When such testing is performed for patient care, which includes typing patients and donors, IRB approval and informed consent are typically not required.

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A representative of the FDA attends CBGG meetings. In September 2006, the FDA held a meeting entitled, "Molecular Methods in Immunohematology." Transcripts from talks given at this meeting are available online at: http://www.fda.gov/cber/minutes/workshop-min.htm#immuno and slides from presentations are at: http://www.fda.gov/cber/summaries.htm#stats.

Consensus panel of markers

The value of developing a consensus panel of DNA assays, based on ranking clinically significant markers for minor blood groups and developing a "compatibility score," was discussed.

Conclusion

The CBGG is a self-help, nonprofit, organization designed for members to learn from each other. Anyone interested in molecular testing for blood groups and willing to contribute intellectually is welcome to join. To become a member, contact Marion Reid (mreid@nybloodcenter.org), Lilian Castilho (castilho@unicamp.br), Greg Denomme (greg.denomme@bloodservices.ca), or Connie Westhoff (WesthoffC@usa.redcross.org).

Acknowledgments

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Monoclonal antibodies available at no charge:
The New York Blood Center has developed a wide range of monoclonal antibodies (both murine and humanized) that are useful for donor screening and for typing RBCs with a positive DAT. These include anti-A1, -M, -s, -U, -D, -Rh17, -K, -k, -Kp^a, -Js^b, -Fy3, Wr^b, -Xg^a, -CD99, -Do^b, -H, -Ge2, -CD55, -Ok^a, -I, and anti-CD59. Most of the antibodies are murine IgG and require the use of anti-mouse IgG for detection (Anti-K, k, -Kp^a, and -Fy^a). Some are directly agglutinating (Anti-M, -Wrb and -Rh17) and one has been humanized into the IgM isoform (Anti-Js^b). The antibodies are available at no charge to anyone who requests them. Please visit our Web site for a complete list of available monoclonal antibodies and the procedure for obtaining them.

For additional information, contact: Gregory Halverson, New York Blood Center, 310 East 67th Street, New York, NY 10021; e-mail: ghalverson@nybloodcenter.org; or visit the Web site at http://www.nybloodcenter.org >research >immunochemistry >current list of monoclonal antibodies available.

Meetings!

September 20 National Institutes of Health, Department of Transfusion Medicine, Symposium

The National Institutes of Health, Department of Transfusion Medicine, 26th Annual Symposium: Immunohematology and Blood Transfusion, co-hosted by the Greater Chesapeake and Potomac Region of the American Red Cross, will be held on September 20, 2007. The symposium is free of charge; advanced registration is encouraged. **Contact** Karen Byrne at NIH/CC/DTM, Bldg. 10/Rm 1C711, 10 Center Drive MSC 1184, Bethesda, MD 20892-1184 or: KByrne@mail.cc.nih.gov or visit the Web site: www.cc.nih.gov/dtm >education

Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

Instructions to the Authors

I. GENERAL INSTRUCTIONS

Before submitting a manuscript, consult current issues of *Immunohematology* for style. Double-space throughout the manuscript. Number the pages consecutively in the upper right-hand corner, beginning with the title page.

II. SCIENTIFIC ARTICLE, REVIEW, OR CASE REPORT WITH LITERATURE REVIEW

A. Each component of the manuscript must start on a new page in the following order:

1. Title page
2. Abstract
3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables
8. Figures

B. Preparation of manuscript

1. Title page
   a. Full title of manuscript with only first letter of first word capitalized (bold title)
   b. Initials and last name of each author (no degrees; all CAPS), e.g., M.T. JONES, J.H. BROWN, AND S.R. SMITH
   c. Running title of ≤40 characters, including spaces
   d. Three to ten key words
2. Abstract
   a. One paragraph, no longer than 300 words
   b. Purpose, methods, findings, and conclusion of study
3. Key words
   a. List under abstract
4. Text (serial pages): *Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Survey results and review papers may need individualized sections*
   a. Introduction
      Purpose and rationale for study, including pertinent background references
   b. Case Report (if indicated by study)
      Clinical and/or hematologic data and background serology/molecular
   c. Materials and Methods
      Selection and number of subjects, samples, items, etc. studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer's name, city, and state. Do not use patient's names or hospital numbers.
   d. Results
      Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable
   e. Discussion
      Implication and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction
5. Acknowledgments: *Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.*
6. References
   a. In text, use superscript, Arabic numbers.
   b. Number references consecutively in the order they occur in the text.
7. Tables
   a. Head each with a brief title; capitalize the first letter of first word (e.g., Table 1. Results of . . .) use no punctuation at the end of the title.
   b. Use short headings for each column needed and capitalize first letter of first word. Omit vertical lines.
   c. Place explanation in footnotes (sequence: *, †, ‡, §, ¶, **, ††).
8. Figures
   a. Figures can be submitted either by e-mail or as photographs (5″ × 7″ glossy).
   b. Place caption for a figure on a separate page (e.g. Fig. 1 Results of. . .), ending with a period. If figure is submitted as a glossy, place first author's name and figure number on back of each glossy submitted.
   c. When plotting points on a figure, use the following symbols if possible: ○ ● △ ▲ □ ■.
9. Author information
   a. List first name, middle initial, last name, highest degree, position held, institution and department, and **complete** address (including ZIP code) for **all** authors. List country when applicable.

III. EDUCATIONAL FORUM

A. All submitted manuscripts should be approximately 2000 to 2500 words with pertinent references. Submissions may include:

1. An immunohematologic case that illustrates a sound investigative approach with clinical correlation, reflecting appropriate collaboration to sharpen problem solving skills
2. Annotated conference proceedings

B. Preparation of manuscript

1. Title page
   a. Capitalize first word of title.
   b. Initials and last name of each author (no degrees; all CAPs)
2. Text
   a. Case should be written as progressive disclosure and may include the following headings, as appropriate
      i. Clinical Case Presentation: *Clinical information and differential diagnosis*
      ii. Immunohematologic Evaluation and Results: *Serology and molecular testing*
      iii. Interpretation: *Include interpretation of laboratory results, correlating with clinical findings*
      iv. Recommended Therapy: *Include both transfusion and nontransfusion-based therapies*
      v. Discussion: *Brief review of literature with unique features of this case*
      vi. Reference: *Limited to those directly pertinent*
      vii. Author information (see II.B.9.)
      viii. Tables (see II.B.7.)

IV. LETTER TO THE EDITOR

A. Preparation

1. Heading (To the Editor)
2. Title (first word capitalized)
3. Text (written in letter [paragraph] format)
4. Author(s) (type flush right; for first author: name, degree, institution, address [including city, state, Zip code and country]; for other authors: name, degree, institution, city and state)
5. References (limited to ten)
6. Table or figure (limited to one)

Send all manuscripts by e-mail to immuno@usa.redcross.org

Becoming a Specialist in Blood Banking (SBB)

What is a certified Specialist in Blood Banking (SBB)?

- Someone with educational and work experience qualifications who successfully passes the American Society for Clinical Pathology (ASCP) board of registry (BOR) examination for the Specialist in Blood Banking.
- This person will have advanced knowledge, skills, and abilities in the field of transfusion medicine and blood banking.

Individuals who have an SBB certification serve in many areas of transfusion medicine:

- Serve as regulatory, technical, procedural, and research advisors
- Perform and direct administrative functions
- Develop, validate, implement, and perform laboratory procedures
- Analyze quality issues, preparing and implementing corrective actions to prevent and document issues
- Design and present educational programs
- Provide technical and scientific training in blood transfusion medicine
- Conduct research in transfusion medicine

Who are SBBs?

Supervisors of Transfusion Services	Managers of Blood Centers	LIS Coordinators Educators
Supervisors of Reference Laboratories	Research Scientists	Consumer Safety Officers
Quality Assurance Officers	Technical Representatives	Reference Lab Specialist

Why be an SBB?

Professional growth Job placement Job satisfaction Career advancement

How does one become an SBB?

- Attend a CAAHEP-accredited Specialist in Blood Bank Technology Program **OR**
- Sit for the examination based on criteria established by ASCP for education and experience

Fact #1: In recent years, the average SBB exam pass rate is only 38%.

Fact #2: In recent years, greater than 73% of people who graduate from CAAHEP-accredited programs pass the SBB exam.

Conclusion:

The **BEST** route for obtaining an SBB certification is to attend a CAAHEP-accredited Specialist in Blood Bank Technology Program

Contact the following programs for more information:

Program	Contact Name	Contact Information
Walter Reed Army Medical Center	William Turcan	202-782-6210; William.Turcan@NA.AMEDD.ARMY.MIL
Transfusion Medicine Center at Florida Blood Services	Marjorie Doty	727-568-5433 x 1514; mdoty@fbsblood.org
Univ. of Illinois at Chicago	Veronica Lewis	312-996-6721; veronica@uic.edu
Medical Center of Louisiana	Karen Kirkley	504-903-2466; kkirkl@lsuhsc.edu
NIH Clinical Center Department of Transfusion Medicine	Karen Byrne	301-496-8335; Kbyrne@mail.cc.nih.gov
Johns Hopkins Hospital	Christine Beritela	410-955-6580; cberite1@jhmi.edu
ARC-Central OH Region, OSU Medical Center	Joanne Kosanke	614-253-2740 x 2270; kosankej@usa.redcross.org
Hoxworth Blood Center/Univ. of Cincinnati Medical Center	Catherine Beiting	513-558-1275; Catherine.Beiting@uc.edu
Gulf Coast School of Blood Bank Technology	Clare Wong	713-791-6201; cwong@giveblood.org
Univ. of Texas SW Medical Center	Barbara Laird-Fryer	214-648-1785; Barbara.Fryer@UTSouthwestern.edu
Univ. of Texas Medical Branch at Galveston	Janet Vincent	409-772-4866; jvincent@utmb.edu
Univ. of Texas Health Science Center at San Antonio	Bonnie Fodermaier Linda Smith	SBB Program: 210-358-2807, bfodermaier@university-health-sys.com MS Program: 210-567-8869; smithla@uthscsa.edu
Blood Center of Southeastern Wisconsin	Lynne LeMense	414-937-6403; Irlemense@bcsew.edu

Additional information can be found by visiting the following Web sites: www.ascp.org, www.caahep.org, and www.aabb.org

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