This Issue of
*Immunohematology*
Is Supported by
a Contribution From

[Ortho-Clinical Diagnostics](https://www.ortho.com/)
a Johnson & Johnson company

Dedicated to
Education in the Field of
Blood Banking
CONTENTS

101
Prophylactic phenotype matching of donors for the transfusion of nonalloimmunized patients with sickle cell disease
I. A. SHULMAN

103
Transfusion practices for patients with sickle cell disease at a major academic medical center
A. AFENYI-ANNAN and N. BANDARENKO

108
Partners for Life: the transfusion program for patients with sickle cell disease offered at the American Red Cross Blood Services, Southern Region, Atlanta, Georgia

112
The Charles Drew Program in Missouri: a description of a partnership among a blood center and several hospitals to address the care of patients with sickle cell disease
E. J. ISAAK, B. LECHIEN, T. LINDSEY, AND M. R. DEBAUN

117
Children’s National Medical Center’s transfusion protocol for sickle hemoglobinopathies
C. P. MINNITI, T. KRATOVL, AND N. L. C. LUBAN

121
Transfusion support of patients with sickle cell disease at the Children’s Hospital of Philadelphia
D. A. SESOK-PIZZINI, D. F. FRIEDMAN, K. SMITH-WHITLEY, AND S. J. NANCE

126
Review: clinical transfusion management in sickle cell disease
Z. R. ROGERS

132
Serologic and molecular genetic management of a pregnancy complicated by anti-Rh18
R. L. HASPEL, S. VEGE, D. MICHELLE, R. M. KAUFMAN, AND C. M. WESTHOFF

136
In search of red blood cells for alloimmunized patients with sickle cell disease
C. FLICKINGER

143
Molecular characterization of GYPB and RH of donors in the American Rare Donor Program
S. VEGE AND C. M. WESTHOFF

148
IN MEMORIAM
Robin Coombs

150
CLASSIFIED AD

151
ERRATUM

152
ANNOUNCEMENTS

154
INSTRUCTIONS FOR AUTHORS

155
ADVERTISEMENTS
Immunohematology is published quarterly (March, June, September, and December) by the American Red Cross, National Headquarters, Washington, DC 20006.

Immunohematology is indexed and included in Index Medicus and MEDLINE on the MEDLARS system. The contents are also cited in the EBASE/Excerpta Medica and Elsevier BIOBASE/Current Awareness in Biological Sciences (CABS) databases.

The subscription price is $30.00 (U.S.) and $35.00 (foreign) per year.

Subscriptions, Change of Address, and Extra Copies:
Immunohematology, P.O. Box 40325
Philadelphia, PA 19106
Or call (215) 451-4902
Web site: www.redcross.org/pubs/immuno

Copyright 2006 by The American National Red Cross
ISSN 0894-203X
Prophylactic phenotype matching of donors for the transfusion of nonalloimmunized patients with sickle cell disease

I.A. Shulman

Sickle cell disease (SCD) affects approximately 72,000 people in the United States (approximately 1 in 500 African Americans and 1 in 1000 to 1400 Hispanics). During the past three decades, the survival of patients with SCD has improved significantly, partly because transfusion therapy has played a role as a disease-modifying strategy for several manifestations of the disease, including acute chest syndrome, acute intrahepatic sequestration, acute multiorgan failure syndrome, acute splenic sequestration, acute symptomatic anemia, aplastic crisis, bacterial and malarial infections, chronic organ failure, complicated pregnancy, eye surgery (e.g., scleral buckle procedure), frequent pain episodes, priapism, primary and secondary prevention or treatment of stroke, and surgery requiring general anesthesia.

Depending on clinical circumstances, patients with one or more of the aforementioned manifestations may receive simple or exchange RBC transfusions that are administered episodically or chronically. However, each transfusion of a unit of RBCs poses a risk of RBC antigen alloimmunization. Studies show that 25 percent or more of chronically transfused SCD patients may experience this complication unless precautions are taken. When alloimmunization occurs, the most common clinically significant antibodies to develop in transfused SCD patients include antibodies to Rh and Kell antigens.

It is preferable to avoid alloimmunization to RBC antigens because this complication makes obtaining compatible units for future transfusions difficult and places the patient at risk for development of hemolytic transfusion reactions, the symptoms of which can mimic or trigger serious complications of SCD. In addition, hemolytic transfusion reactions can be serious and potentially life-threatening complications in their own right. Studies have shown that the transfusion of ABO- and D-compatible donor RBCs that lack C, E, or K when the same antigens are absent from the recipient’s RBCs can reduce the rate of alloimmunization in patients with SCD as well as the occurrence of hemolytic transfusion reactions.

According to a National Institutes of Health (NIH) publication the antigenic phenotype of the RBCs (at least the common antigens belonging to ABO, Rh, Kell, Duffy, Kidd, Lewis, Lutheran, P, and MNS) should be determined in all patients older than 6 months. A permanent record of the phenotype results should be maintained in the blood bank to optimize matching and a copy of the record should be given to the patient or family. Furthermore, limited matching of donor RBCs for E, C, and K is usually performed, unless patients have antibodies. The transfusion of phenotype matched RBCs may be accomplished by typing the RBCs of the patient before transfusion, if not previously performed, for Rh and Kell, in particular E, C, and K and performing more extensive matching for antigens in systems such as Duffy, Kidd, and MNS for those patients who are already alloimmunized. Donor RBCs lacking the antigens that the patient’s RBCs lack would then be selected for transfusion.

Although NIH publication 02-2117 was not the result of a formalized consensus process, it does represent the efforts of those who have dedicated their professional careers to the care of individuals with SCD. In spite of this publication, transfusion practices in SCD remain disparate in the United States, as illustrated by two recent national surveys.
Afenyi-Annan and Brecher surveyed 50 academic medical centers to determine the extent of the use of phenotype-matched donor RBCs in the transfusion of patients with SCD. Of 37 academic centers that responded to a questionnaire, 27 (73%) reported that they provide antigen-matched donor RBCs. Although the most common antigens matched were C, E, and K (24 of the 27 sites or 89%), there were multiple variances among those 27 centers as to which antigens were selected for phenotype matching. The authors concluded that, despite a common approach by some of the academic centers, there was no single standard of care with regard to RBC component selection.

A survey by the College of American Pathologists (CAP) examined 1182 labs in North America to determine the extent of RBC antigen testing of nonalloimmunized patients with SCD as well as the use of phenotype-matched donor RBCs in the transfusion of these patients. The CAP survey, unlike the one by Afenyi-Annan and Brecher, did not specifically target academic medical centers. The CAP survey results indicated that approximately 63 percent of North American laboratories did not routinely phenotype patients with SCD for antigens other than ABO and D. Of the 37 percent of the laboratories that did perform additional antigen testing beyond ABO and D, only 75 percent actually selected phenotype-matched RBCs for patients with SCD. On the other hand, more than three-quarters of the respondent laboratories reported that for patients with SCD they use RBCs that are leukocyte reduced and nearly two-thirds of these laboratories reported that they use RBCs that test negative in a sickle cell screening test. The authors of the CAP survey concluded that the majority of North American hospitals do not determine the RBC phenotype of nonalloimmunized patients with SCD (beyond ABO and D) and the laboratories that do perform RBC antigen testing of these patients beyond ABO and D most commonly match for C, E, and K when phenotype-similar RBCs are transfused.

The authors in this issue of *Immunohematology* describe various transfusion management protocols used for patients with SCD for whom they care in their institutions: those designated as comprehensive sickle cell centers and others around the country. All of these centers actively treat patients with SCD. Given the lack of consensus in the United States regarding the need to perform RBC phenotyping of patients with SCD, and the use of phenotypically-matched donor RBCs for their transfusions, it will be interesting to read the articles that follow with regard to alloimmunization and to the use of leukocyte-reduced, irradiated, storage duration limited, and HbS negative RBC components.

References


Ira A. Shulman, MD, Professor and Vice Chair of Pathology/Director of Transfusion Medicine Keck School of Medicine at the University of Southern California Department of Pathology; LAC+USC Medical Center, 1200 N. State Street, Los Angeles, CA 90033.
Transfusion practices for patients with sickle cell disease at a major academic medical center

A. Afenyi-Annan and N. Bandarenko

The University of North Carolina at Chapel Hill (UNC) is a tertiary-care, academic university hospital and a major referral center for patients across the state of North Carolina. This 700-bed, Level 1 trauma center transfuses more than 22,000 RBC units to patients annually. Clinical services and areas of the hospital which rely most heavily on transfusion support for their activities are transplantation (bone marrow and solid organ), hematology, critical care (medical and surgical intensive care units), cardiothoracic surgery, pediatrics, the operating room, the emergency department, labor and delivery, dialysis, and outpatient services. UNC is recognized for its expertise in coagulation, transfusion medicine, and hematology, particularly in sickle cell disease (SCD). The sickle cell center at UNC, which began in 1980 and continues today, in conjunction with our neighboring institution, Duke University Medical Center, is designated as part of a National Institutes of Health comprehensive sickle cell center. Several of the physicians are dedicated to the care of pediatric and adult patients with SCD, as well as to research on transfusion management of these patients and recruitment of African American blood donors. This article describes the practices of this institution for transfusion management of patients with SCD, as well as some of its efforts related to this challenging area of transfusion medicine. Immunohematology 2006;22:103–107.

As a comprehensive sickle cell center (CSSC), the University of North Carolina at Chapel Hill (UNC) encounters patients of all ages with a variety of HbS hemoglobinopathies; the most commonly treated are patients with Hb SS, Hb SC, and Hb Sβ-thal.

On the basis of the CSSC 2005 census, there were 586 active patients with SCD, or approximately 50 per month, treated at our institution. Of these, 14 to 15 or approximately one-third were transfused each month. This frequency reflects the fact that many patients with SCD who have complex transfusion needs are ultimately referred to UNC for management.

Transfusion Therapy for Patients with SCD

Delivery of RBC transfusions to patients with SCD varies by method (simple vs. exchange) and frequency (episodic vs. chronic). In evaluating the need for simple versus exchange transfusion, a number of important factors should be considered. These include the acuity of anemia, the patient's baseline Hct prior to presenting for treatment, and the urgency of the need for oxygen-carrying capacity. Simple transfusions are often helpful as a first step to increase the oxygen-carrying capacity in a stable patient who presents with symptomatic anemia when such transfusions would increase the patient's Hct to a maximum of 33 percent.

The rheology of SCD is such that, above this threshold, complications from hyperviscosity may become a problem. When hyperviscosity or volume overload or both are a concern, or when a patient presents with life- or organ-threatening disease, exchange transfusion is the preferred method. Emergent automated exchanges are therefore performed for a variety of the indications in SCD, including stroke, acute chest syndrome, and hepatic sequestration. The goal of transfusion is to reduce HbS to 10 to 30 percent, depending on the clinical setting.

Also in place at UNC is a chronic RBC exchange program that has enrolled about 40 patients cumulatively since 1996 and currently has 20 active patients, the majority of whom are pediatric (65%). These patients are managed with apheresis RBC exchanges. Exchange transfusions and iron chelation therapy are the only two methods known to prevent iron overload patients receiving chronic transfusion therapy. At our institution, pediatric patients with abnormal transcranial Doppler studies who are at risk for stroke and those with a history of stroke receive exchange transfusion in accordance with the STOP trials. These patients will potentially receive life-long RBC exchange transfusions as new data indicate that their risk of stroke reverts back to baseline if transfusion therapy is stopped. We are also part of the multi-institutional, ongoing clinical trial that uses exchange transfusion for pediatric patients with SCD and silent infarcts (silent infarct and transfusion trial: SIT). Although the use of
chronic transfusion therapy in adults after stroke has not been systematically studied, we perform RBC exchanges prophylactically in this setting. Other uses of chronic RBC exchange in our population include recurrent priapism and pulmonary hypertension in adults.

Other circumstances in which transfusion therapy is used include preoperative management of patients and pregnancy. In the former case, manual exchange is performed by the phlebotomy of 1 unit of RBCs followed by the simple transfusion of 1 to 2 units of RBCs to achieve a Hct of 33 percent. In the latter, transfusions are only used when a pregnancy is at risk because of SCD complications, with goals similar to those for a nonpregnant patient. We typically do not perform transfusions for patients with SCD who have controversial indications, such as recurrent nonhealing leg ulcers and debilitating pain, and before the use of IV contrast media.\(^5\)

Transfusion Protocol

Because of the expertise of our health care providers and the frequency with which patients with SCD are transfused at our facility, the overall environment is favorable for reliable communication and consistent transfusion practice for these patients. Familiarity with the special requirements and transfusion complications in this patient population makes clear the need for appropriate identification of these patients before transfusion.

The blood component order form is an important tool that allows a physician to indicate the diagnosis of SCD and request special transfusion needs. Verbal communication of patient diagnosis or component attributes may also occur. This information is crucial for encounters with new patients without a prior history at our institution or blood bank. The blood bank history review, which occurs before patient testing, will also identify samples from patients with SCD in the laboratory. Some patients are recognized by name by virtue of the frequency of their visits or the complexity of their serologic testing.

Patient testing

Routine serologic testing on samples from patients with SCD is the same as that for any potential transfusion recipient; this testing includes ABO and D typing and antibody detection and identification, if necessary. Typing of the RBCs of these patients for C, E, c, e, K, Ky\(^a\), Ky\(^b\), M, N, S, s, P1, Le\(^a\), and Le\(^b\) antigens is routinely performed when they are initially seen at our facility. Records from a referring facility may be helpful in instances where phenotyping is not possible or if transfusion of RBCs is urgently needed. If RBC transfusion has occurred recently, a hypotonic saline washing procedure can be performed to lyse allogeneic RBCs containing HbA to allow phenotyping of autologous RBCs.

Selection of RBC units for transfusion

All units of RBCs are prestorage leukocyte-reduced and ABO and D compatible with the patient's blood type. As inventory allows, units are selected that have been stored 14 days or less. Pretransfusion prophylactic phenotype matching is routinely and consistently performed for C, E, and K. If other clinically significant antibodies are present in the patient's plasma, or are historically known, appropriate antigen-negative RBCs are selected in addition to those typed as C–, E–, and K–. HbS testing of RBCs (Sickle Dex, Ortho-Clinical Diagnostics, Raritan, NJ) is performed when requested by the clinician or automatically for apheresis RBC exchange procedures. To calculate accurate RBC replacement volumes for these exchanges, quantitative Hb electrophoresis is performed.

Rationale

The rationale for pretransfusion prophylactic phenotype matching for C, E, and K is related to the immunogenicity of these antigens. The overall alloimmunization rate for chronically transfused patients with SCD is much higher than that for other similarly transfused populations, estimated at 25 to 30 percent.\(^5\)–\(^9\) While the majority of these patients will not form antibodies, those that do may experience complications including hemolytic transfusion reactions, stimulation of sickle cell crises, and increased difficulty of finding compatible RBCs when needed in the future. A general cautious approach at our institution has been shaped by the clinical experience of managing more severe transfusion complications in patients with SCD, such as hyperhemolysis. Because it is relatively easy to select C–, E–, and K– units from our general inventory, we can effectively prevent alloimmunization to these most immunogenic antigens.\(^5\)–\(^9\) Although alloimmunization rates of these patients at our institution have not been specifically measured, the occurrence of new antibodies is infrequent and certainly less than 25 percent for those patients transfused exclusively at our facility. However, newly
identified antibodies directed against low-incidence antigens, such as Js, and warm autoantibodies do occur. On occasion, patients are transfused elsewhere without partial phenotype matching and then transferred to our facility. In this setting, we have seen sensitization to Rh and Kell antigens and, less commonly, delayed transfusion reactions because of anti-S, anti-Jk$^a$, and anti-Jk$^b$. Sensitization to the latter antigens is also a possibility at our facility because we do not routinely provide RBCs negative for these antigens unless the plasma had a broadly reactive warm autoantibody or there was a previously identified alloantibody.

**Special considerations**

Extended phenotype matching is performed whenever possible in patients with SCD with warm autoantibodies, especially when the autoantibody is causing hemolysis. If the warm-reactive autoagglutinin is not associated with hemolysis, phenotype matching of RBCs is limited to C, E, and K and those indicated by antibody identification. Units for transfusion are selected on the basis of compatibility tests showing reactivity no stronger than those with the autologous RBCs.

For patients with antibodies to low-incidence antigens, RBCs for transfusion are selected on the basis of their compatibility by the IAT. If the antibody is no longer detectable and has an identified specificity, antigen-negative RBCs are requested from our blood supplier in advance. If appropriate antisera are not available to type units, RBCs are accepted from donors on the basis of historic antigen typing. If there is insufficient time for these pretransfusion steps, the ordering physician signs a conditional release card that provides documentation of his recognition of a possible, albeit unlikely, anamnestic immune response if the patient is re-exposed to the low-incidence antigen. This ensures that a proper risk and benefit assessment is made and also provides the opportunity for the blood bank physician to communicate with and educate the ordering physicians.

Our interest in blood bank practices for patients with SCD prompted systematic inquiry at the state and national level. We conducted a cross-sectional survey of North Carolina hospitals with blood bank facilities to assess hospital and patient demographics, as well as the services, testing, and blood components offered to patients with SCD. Data from 76 of 106 (70%) hospitals showed the majority to be community hospitals (90%) whose blood banks saw a wide range of patients with SCD each month (0 to 30; median = 1). While all provided simple transfusion services, only 16 percent performed exchange transfusions, and 7 percent provided chronic transfusion programs. Less than one-third (29%) of these hospital blood banks had specific policies or procedures for patients with SCD. Less than one-half routinely provided HbS-negative RBCs (42%). With respect to the use of phenotype-matched RBCs, 38 percent provided antigen-matched RBCs, but only one-half (17%) did this prophylactically. Those providing phenotype-matched RBCs were more likely (p < 0.001) to have a trauma center (84% vs. 25%), to have policies for work-ups for patients with SCD (77% vs. 19%), to perform routine RBC phenotyping (50% vs. 4%), and to provide HbS-negative RBCs (65% vs. 16%).

We also conducted a national survey of major academic medical centers to ascertain current practices in selecting RBCs for the transfusion of patients with SCD. Thirty-seven of 50 (74%) centers responded. The majority did perform partial phenotype matching of RBCs; however, a specific standard of care was not apparent. The most common antigens matched prophylactically were: E (73%), K (70%), and C (68%), followed by c (41%), and e (41%). There were also differences in transfusion practices for pediatric versus adult patients with SCD. Because of our experience, published data, and the results of these surveys, we continue to advocate the standardization of SCD transfusion practice to guide health care providers at both major academic medical centers as well as community-based hospitals.

**Blood Supply Issues**

Providing blood to this population can be challenging. Although the majority of patients do not form antibodies, those that do can present a problem in finding compatible RBCs. As a group, patients with SCD are the largest users of the national rare blood inventory, using up to one-third of the rare blood supply. Therefore, excellent communication between our hospital transfusion service and our regional blood supplier is essential. To fill that need, we have developed several strategies with our blood supplier. These include on-demand 24-hour access to units with special attributes for emergent indications and the ability to discuss special needs with either a reference laboratory specialist or the blood supplier’s medical director.
RBCs for scheduled automated exchanges are ordered in advance and arrive in time for HbS testing and crossmatching at our facility. Our blood supplier provides reliable and safe blood resources and, with the added service of an advanced reference laboratory, even our most complex transfusion recipients can be managed appropriately. Nonetheless, the need for more active recruitment of donors within the African American community still exists.

A few centers that specialize in treating patients with SCD in conjunction with their regional blood collection facilities have begun programs to actively recruit and retain African American donors for their patients with SCD. These programs have mostly focused on matching these donors with the transfusion needs of their pediatric population. African American donors are asked to donate up to four times a year for the patients with SCD with whom they are matched. The degree of matching varies. Most of these centers provide C-, E-, and K-matched RBCs for these patients and have a readily available inventory in which to find rare RBCs. Others have attempted to provide extended, phenotypically matched RBCs for all of the transfusion needs of these patients. These types of programs require extensive commitment on the part of the blood center to coordinate donor collections with patient transfusion needs; staffing, donor recruitment, and donor retention are critical. The success of such programs in improving transfusion care to patients with SCD appears anecdotally to be good, decreasing alloimmunization rates in the short term. However, long-term effects have not been studied.

In our region, no specific donor recruitment process exists except on a local level. One of our institutional goals is to develop an active recruitment and retention program to match African American donors and patients with SCD, focusing our recruitment on donors at the 11 historically black colleges and universities (HBCUs) in our state. The HBCUs are a potentially rich source of African American donors that to date have been underused. Currently, one such program at a local HBCU has been widely successful in recruiting and retaining African American donors and has been cited by the American Red Cross as “a model for colleges and universities nationwide.”13 Active research is ongoing with this institution in an attempt to learn and understand the key components that have made it successful, and to adopt these components into a workable program that can be used at other institutions in our state.14

Conclusions

Patients with SCD provide many challenges for the field of blood banking and transfusion medicine. Our institution has taken a number of steps to ensure that practices here are consistent with national recommendations. We are fortunate to have a reliable blood supplier to facilitate the standard of care adopted at this center and we are pleased with the strides to examine SCD transfusion practices at the local, state, and national levels. There remains a need to standardize practices to minimize morbidity associated with transfusion in this population. Efforts need to be directed at academic medical centers, but must also reach community-based hospitals where many of these patients present during crises. Accomplishing this goal will ultimately require consensus within the blood banking community and recruitment of donors who can best meet the RBC phenotype needs of these patients.

References


Araba Afenyi-Annan, MD, MPH; and Nicholas Bandarenko, MD, University of North Carolina Hospitals, 101 Manning Drive, 1021 East Wing, Blood Bank, CB#7600, Chapel Hill, NC 27514.

Phone, Fax, and Internet Information: If you have any questions concerning Immunohematology, Journal of Blood Group Serology and Education, or the Immunohematology Methods and Procedures manual, contact us by e-mail at immuno@usa.redcross.org. For information concerning the National Reference Laboratory for Blood Group Serology, including the American Rare Donor Program, please contact Sandra Nance, by phone at (215) 451-4362, by fax at (215) 451-2538, or by e-mail at snance@usa.redcross.org.
Partners for Life: the transfusion program for patients with sickle cell disease offered at the American Red Cross Blood Services, Southern Region, Atlanta, Georgia

K.L. Hillyer, V.W. Hare, C.D. Josephson, S.B. Harris, and C.D. Hillyer

The American Red Cross Blood Services (ARCBS), Southern Region, located in Atlanta, Georgia, currently serves 128 hospitals, primarily in the state of Georgia, with a service area of 60,000 square miles. The region distributed approximately 350,000 units of RBCs to its customers in 2005. Georgia has a population of 8.2 million, with 4.5 million residing in the metropolitan Atlanta area. Of the four major counties encompassing this metro area, African Americans make up the majority of the population in two of these (DeKalb and Fulton Counties). In metropolitan Atlanta and throughout Georgia, there are many patients, mostly African Americans, with sickle cell disease (SCD), who are in need of RBC transfusions.

Since January 1993, the ARCBS Southern Region has offered a phenotype-matching transfusion program for patients with SCD called Partners for Life (PFL). The physicians and staff at the ARCBS Southern Region Immunohematology Reference Laboratory (IRL) have worked closely with the faculty of the Departments of Adult and Pediatric Hematology and Oncology at the Emory University School of Medicine as well as at the Morehouse School of Medicine to develop and further refine the PFL program. Most of the patients with SCD enrolled in the PFL program are treated by these same faculty physicians at the Georgia Comprehensive Sickle Cell Center, consisting of three major children’s hospitals in Atlanta: Children’s Healthcare of Atlanta at Egleston Hospital, Children’s Healthcare of Atlanta at Scottish Rite Hospital, and the Hughes Spalding Hospital of the Grady Health System.

In February 2006, Children’s Healthcare of Atlanta also assumed responsibility for the management of services at Hughes Spalding Hospital. Today, Children’s Healthcare of Atlanta is the second largest pediatric healthcare system in the United States. The Children’s Healthcare of Atlanta system comprises 430 licensed beds, 63 of which are dedicated to hematology and oncology. Each year, Children’s Healthcare of Atlanta manages approximately 450,000 patient visits, 21,400 hospital admissions, 33,800 surgical procedures, 211,300 emergency department visits, and 8900 specialty clinic visits.

The American Family Life Assurance Company of Columbus (AFLAC) Cancer Center and Blood Disorders Service of Children’s Healthcare of Atlanta treats approximately 1000 pediatric patients per year who have a diagnosis of SCD. Approximately 300 of the Children’s Healthcare of Atlanta patients with SCD are enrolled in the PFL program, and of these, nearly 175 are chronically transfused, using the simple transfusion, phenotype-matching protocol of the PFL program. Many of these pediatric patients also require one or more acute RBC exchange procedures each year, due to various complications of SCD.

The PFL phenotype-matching protocol has been essentially identical since its inception in 1993. Patients with SCD are extensively phenotyped by the
Partners for Life

ARCBS Southern Region IRL upon entry into the PFL program, before their first transfusion. Patients are separated into two categories: Category I patients have no history of prior antibody formation; Category II patients have already made one or more antibodies when they enter the PFL program.

Category I patients receive RBCs that are phenotypically matched for C, E, c, e, and K. Category II patients receive RBCs that are phenotypically matched for C, E, c, e, K, Fy², Jk², and Jkª, as well as for any other RBC antigens to which the patients have already made antibodies.

All PFL patients receiving simple transfusions are provided with RBCs that are HbS negative, leukocyte-reduced, and (if at all possible) less than 14 days old. For patients with SCD undergoing acute RBC exchange, every attempt is made to procure the appropriate phenotypically matched RBCs that are less than 10 days old.

RBCs that are CMV negative or irradiated are not routinely provided to PFL patients. On rare occasions, such as for transfusion of those patients preparing for or undergoing marrow or stem cell transplants for SCD, irradiated or CMV-negative components may be requested and provided.

From 1993 until 2000, the PFL program was a limited-donor, phenotype-matching program, with each PFL patient being assigned between eight and ten partially phenotypically matched blood donors. PFL blood donors would commit to donating RBCs for their “partner” patients with SCD, according to the chronic simple transfusion schedule set for each of the patients.

Although the PFL program was successful in reducing the alloimmunization rate in this group of children during that 7-year period to less than 7 percent, down from the previous rate of more than 20 percent, only 6 percent of PFL patients received all of their RBC transfusions exclusively from their PFL donors. The vast majority of PFL patients received a combination of RBCs from their PFL donors and RBCs from phenotypically matched units (using PFL Category protocols) from the general blood donor pool. The reasons for this lack of adherence to protocol are many, and have to do with the complicated logistics of matching a specific donor’s blood unit with a specific patient’s transfusion schedule.

Another component of the difficulty in having only PFL units go to each PFL patient is caused when PFL patients present to nonparticipating hospitals in (typically) emergent situations. Although the patients’ guardians have always been given PFL cards stating their children’s RBC phenotypes and any RBC antibodies already made, many of these children received RBC units at nonparticipating hospitals that were not phenotypically matched, contributing to the 7 percent alloimmunization rate observed in PFL patients.

Given the complicated logistics and higher costs associated with the special recruitment of partner donors, the constant and often confusing communication with hospitals about each PFL patient’s transfusion schedule, the difficulties in scheduling partner donors’ collections to meet each separate patient’s transfusion schedule, the “no-show” rate for donors and patients, and the time-sensitive shipping of the PFL donor units, the ARCBS Southern Region converted, in 2000, to the use of phenotypically matched RBCs from the general donor pool for its PFL program patients. This change instantly created a larger pool of donors from which to screen units for rapid provision to SCD patients.

This change also made the logistics of the PFL program more efficient, especially as regards effective communication between the blood center and its partner hospitals. Each week, the hospitals now electronically send the ARCBS Southern Region IRL a list of all PFL patients who are scheduled for chronic transfusion during the following week. The IRL then tests for and determines the appropriate RBCs needed for each patient scheduled for transfusion, and ships the freshest units possible prior to the anticipated date of transfusion.

The alloimmunization rate in the PFL program is currently around 5 percent. It is believed that the rate has not dropped closer to 0 because many PFL patients continue to present to nonparticipating hospitals for emergency transfusions and often receive non-phenotypically matched RBC units at those hospitals.

There is currently no specific telerecruitment strategy for recruiting blood donors into the PFL program. The ARCBS Southern Region does have an active African American blood donor recruitment campaign; the donor recruiters are knowledgeable about SCD and the importance of African American donations to the care and well-being of patients with SCD.

A unique and special contribution to the success of the PFL program in building an appropriate blood inventory has been the region’s nationally recognized
Minority Donor Recruitment Advisory Board, comprising African American leaders in government, business, nonprofit, religious, and other organizations throughout the State of Georgia. These board members have truly embraced the cause of increasing blood donations by African Americans; they emphasize the importance of blood donation daily within their places of business, their communities, their churches, and in other settings as well. They personally host or support blood drives at churches, businesses, and other organizations comprising primarily African Americans, including the Historically Black Colleges and Universities of the Atlanta University Complex. These college campuses have strongly supported the ARCBS Southern Region in holding successful blood drives on several occasions each year.

The success of this recruitment campaign in raising awareness about the importance of blood transfusion within the large African American population in Georgia has greatly contributed to the region’s ability to maintain the appropriate inventory with which to supply partially phenotypically matched RBCs to all patients with SCD in the region’s service area.

A special communication process occurs daily among the collections staff, the donor information databases, the data entry employees, the manufacturing laboratory personnel, and the IRL staff within the region, to identify appropriate units for testing and phenotype matching. Approximately 13 percent of all donors in the ARCBS Southern Region self-identify as African American on their blood donation records. RBCs from these donors are initially screened by the IRL for C and E, using automated RBC typing equipment. When additional serologic testing identifies a donor with the correct phenotype for a PFL patient, a “flag” is entered by IRL staff into the computer system, so that any future donations from that donor will be sent directly to the IRL after RBC processing and testing.

After initial identification, donors are informed of their special RBC phenotype status via a letter, explaining the importance of their donations to patients with SCD; a special PFL wallet-size card is included in the letter. This card may be presented by the donor to collections staff members at blood collection sites, to further identify these special units for patients with SCD. Of note, approximately 400 PFL donors from the former limited-donor pool program remain actively involved in the “new” PFL program, and they donate at regular intervals to provide RBCs for any PFL (or SCD) patient in need.

Approximately 5500 phenotypically matched RBCs were distributed last year for acute and chronic transfusions from the ARCBS Southern Region to patients with SCD who were actively enrolled in the PFL program. It is estimated that at least 4000 blood donors with the correct phenotypes are needed each year to support the ARCBS Southern Region’s PFL phenotype-matching program. This estimate includes many variables, such as the predicted number of units to be distributed for PFL patients during the year, the rate of unsuccessful donations (as a result of “quantity not sufficient,” donor deferrals, etc.), the RBC discard and donor “no-show” rates, and the number of times each year each donor gives blood. Of note, the majority of PFL donors donate more than once per year.

It is important to note that many phenotypically matched RBCs are supplied by the ARCBS Southern Region to patients with SCD who are not officially enrolled in the PFL program; most adults at the academic center’s hospitals (as well as at other large hospitals throughout the state) who require transfusion for SCD receive phenotypically matched units from the ARCBS Southern Region, most often using the same phenotype-matching protocol as for PFL. Many of these adults are on chronic transfusion protocols; many often require acute RBC exchange transfusion (erythrocytapheresis) as a result of acute chest syndrome, cerebrovascular accidents, multisystem organ failure, and other complications of their disease.

Each year, approximately 12,000 phenotypically matched RBCs are distributed to meet the transfusion needs of all adult and pediatric patients treated for SCD within the 60,000 square-mile service area for which the ARCBS Southern Region is the primary blood supplier. Using the same variables as were considered above (discard rate, no-show rate, etc.), it is roughly estimated that at least 10,000 blood donors with the appropriate phenotypes are needed each year to support the transfusion needs of all patients with SCD in the State of Georgia.
Acknowledgments

The authors would like to thank Alexander Watkins, III, AMT(HEW), Reference Technologist II in the ARCBS Southern Region IRL, for his daily efforts in supporting and coordinating the PFL program and for the important information he contributed to this summary.

Krista L. Hillyer, MD, (corresponding author) Assistant Professor, Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Emory University Hospital, 1364 Clifton Road NE and Chief Medical Officer, American Red Cross Blood Services, Southern Region, 1925 Monroe Drive NE, Atlanta, Georgia 30324; Virginia W. Hare, MT(ASCP)SBB, Reference Laboratory Technical Customer Liaison, American Red Cross Blood Services, Southern Region; Cassandra D. Josephson, MD, Assistant Professor, Departments of Pathology and Laboratory Medicine and Pediatric Hematology/Oncology, Emory University School of Medicine; Shealynn B. Harris, MD, Assistant Medical Director, American Red Cross Blood Services, Southern Region; and Christopher D. Hillyer, MD, Professor, Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Emory University Hospital, 1364 Clifton Road NE, Atlanta, Georgia 30322.

IMPORTANT NOTICE ABOUT MANUSCRIPTS FOR IMMUNOHEMATOLOGY

Please e-mail all manuscripts for consideration to Marge Manigly at mmanigly@usa.redcross.org
Sickle cell disease (SCD) is an inherited blood disorder which can be complicated by stroke in infancy and childhood. The primary and secondary prevention of stroke in this patient population is regular RBC transfusion therapy at least every three weeks, but there is no consensus on the ideal RBC transfusion therapy. The Charles Drew Program, a partnership among a blood center and several hospitals affiliated with academic medical centers in Missouri, provides RBCs for the care of patients with SCD. There are three basic aims: the RBC components are phenotypically matched on three minor RBC antigens, the units are less than 7 days old, and each patient has a limited number of dedicated donors, so that the donor exposure is minimized. This report describes the operational phases of this program and summarizes its performance with respect to each of these aims.

Program History and Protocol

The Drew Program began in 1999 as a partnership among the Missouri/Illinois Region of the American Red Cross (ARC) and several hospitals in Missouri specializing in the care of pediatric patients. The program has three objectives. The first objective is to provide phenotypically matched, hemoglobin S (HbS)-negative, leukoreduced RBCs to all pediatric patients with sickle cell anemia who require chronic transfusion therapy and are participating in the program. All patients receive units with a limited phenotype match (C, E, and K in addition to ABO and D). Studies supporting this practice have been published in the scientific literature. One transfusion service requests that the units also be matched for Fy. Any patient who develops an antibody receives units which are negative for the antigenic determinant against which the antibody is directed. The second objective is to ensure
that these units are fresh with the goal of limiting the interval from collection to transfusion to a period of five days. The third objective is to limit the number of donors to which each patient is exposed.

**Recruitment Process**

Blood drives are held in conjunction with donor groups with a significant African American base. When this base accounts for 30 percent of the donor group membership, the drive is designated as a Drew drive. Donor groups with this type of demographic make-up consist of churches, corporations, and schools. Before a Drew drive, a dedicated donor recruiter educates the group about the sickle cell program, its purpose, and the requirements for participation. At the time of the drive, this dedicated recruiter, or one of a few hand-picked designees, has a one-on-one interview with any donor expressing an interest in becoming a member of the program. A realistic picture of the program requirements is presented to the donor, who can then make a more informed decision about whether a commitment to the stringent requirements of the program can be made. If the donor still expresses an interest after the interview, the unit which they donate is specially tagged, so that it can be phenotyped. On the basis of the phenotype, the donor is matched with one of the patients in the program and is placed on a recruitment list for that single patient. When the patient has an upcoming appointment, the donors on the patient’s list are called and asked to donate within a short window of time (commonly 5 days) before the scheduled treatment date. Donors accepting the responsibility of joining the program are expected to donate a minimum of three times per year.

**Communication between Transfusion Service and Blood Center**

Each transfusion service sends a list of scheduled transfusions along with the expected number of units needed for each patient to the blood center every month. This list is essential to the two departments at the blood center which coordinate most of the program activities: donor recruitment and the reference lab. The recruiters use these patient schedules to guide the selection of donors and to select an appropriate appointment date. All units intended for patients in the program are channeled to the blood center reference lab, which is ultimately responsible for appropriate distribution of the units to the transfusion services. With the first donation from a given donor, two tags are affixed to the unit at the time of distribution. The first tag indicates for whom the unit is intended; the second tag lists the confirmed antigens. Subsequent donations from the same donor are only tagged with the name of the recipient and a fax is sent to the hospital indicating the historical phenotype of the donor. The reference lab maintains an open dialogue with the hospital transfusion services during this distribution and during the period which follows. Patient issues and product issues are resolved through this open dialogue. At the conclusion of each month, the reference lab sends a complete report of all units issued during that month to every transfusion service. Next to each listed unit, the transfusion service notes whether the unit was transfused to the intended recipient and when it was transfused. The transfusion service also notes any units the patient may have received which came from a source outside of the program. The report is then returned to the reference lab, which collects all the information and generates a complete transfusion history on each of the patients in the program.

**Donor Profiles**

The Missouri/Illinois Region of the ARC is a large blood center that collected an average of 287,097 productive units per year (range 259,160-310,614) between July 1998 and June 2005 (Table 1). During the same period, the average number of those productive units donated by individuals identifying themselves as African Americans was 11,088 per year (range 8,616-13,420). The difference between the number of donations from African Americans from July 1998 to June 1999 and from July 2000 to June 2001 was attributed to increased minority recruitment efforts and to increased community awareness concurrent with the initiation of the Drew Program. Donation

**Table 1.** Productive RBC units collected by the Missouri/Illinois Region of the ARC from July 1998 through June 2005

<table>
<thead>
<tr>
<th>Fiscal year</th>
<th>Units from all donors</th>
<th>Units from African American donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/98–6/99</td>
<td>285,894</td>
<td>8,616</td>
</tr>
<tr>
<td>7/99–6/00</td>
<td>285,645</td>
<td>12,821</td>
</tr>
<tr>
<td>7/00–6/01</td>
<td>298,526</td>
<td>13,420</td>
</tr>
<tr>
<td>7/01–6/02</td>
<td>310,614</td>
<td>12,966</td>
</tr>
<tr>
<td>7/02–6/03</td>
<td>282,579</td>
<td>10,848</td>
</tr>
<tr>
<td>7/03–6/04</td>
<td>287,261</td>
<td>9,615</td>
</tr>
<tr>
<td>7/04–6/05</td>
<td>259,160</td>
<td>9,333</td>
</tr>
<tr>
<td></td>
<td>2,009,679</td>
<td>77,619</td>
</tr>
</tbody>
</table>
frequency within the dedicated donor base (those donating three times per year or more) is illustrated in Table 2.

### Transfusion Service and Patient Characteristics

There are four pediatric hospitals serving patients with sickle cell anemia. Among the four hospitals, there are three that participate in the Drew Program. The remaining hospital receives phenotyped units without restriction on the age of the units or the number of donor exposures. Because the three hospitals participating in the Drew Program are all affiliated with academic centers, many of the patients in the program came as referrals because of complications at outside institutions, and some of these patients had already formed antibodies before they joined the program. The current phenotypes of the patients in the program and the antibodies formed by the patients are listed in Table 3.

In addition to the special requirements with respect to phenotype, age, and donor exposure, the donors in the program are placed on treatment protocols involving some form of exchange RBC transfusion, either partial manual exchange or RBC apheresis, to prevent the issues associated with iron overload. The distribution of patients receiving each form of treatment is outlined in Table 4.

### Program Performance

From 1999 to January 2005, the program concentrated on the first objective of the protocol; implementation of the second and third objectives required a critical mass of dedicated donors. Beginning in January 2005, modifications in program design were made so that the operational progress of the program could be monitored and assessed through the use of a monthly productivity report. The productivity report requires documentation of the requested number of units, the number of scheduled donations to fill that request, the number of calls required to make those scheduled donations, and the outcome of those scheduled donations for each patient requiring transfusion therapy in that month. For all successful donations, the day of collection and the day of distribution are documented. This allows the program to maintain data on the number of patients transfused, the number of units each patient requires, the number of scheduled appointments needed to meet that commitment, the number of calls needed to make the requisite number of scheduled appointments, the number of deferrals and cancellations, the number of

---

**Table 2. Donation frequency of donors in Charles Drew Program from July 2003 through June 2005**

<table>
<thead>
<tr>
<th>Frequency</th>
<th>7/03-6/04</th>
<th>7/04-6/05</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 times per year</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6 times per year</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>5 times per year</td>
<td>58</td>
<td>69</td>
</tr>
<tr>
<td>4 times per year</td>
<td>91</td>
<td>147</td>
</tr>
<tr>
<td>3 times per year</td>
<td>229</td>
<td>229</td>
</tr>
<tr>
<td>2 times per year</td>
<td>408</td>
<td>345</td>
</tr>
<tr>
<td>once per year</td>
<td>668</td>
<td>520</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,469</td>
<td>1,324</td>
</tr>
</tbody>
</table>

**Table 3. Patient phenotypes and antibodies produced in patients in the Charles Drew Program**

<table>
<thead>
<tr>
<th>Patient phenotype</th>
<th>Number of patients with phenotype</th>
<th>Number of patients who produced antibody</th>
<th>Antibodies produced*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Common</td>
<td>Low frequency</td>
</tr>
<tr>
<td>C-, E-, K-</td>
<td>15</td>
<td>E-, K-, S-, Jk*</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-, K (2)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>-Co*, Go*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Kp*</td>
</tr>
<tr>
<td>C-, E-, K-, Fy(a-)</td>
<td>12</td>
<td>E-, K-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fy*</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Js*</td>
</tr>
<tr>
<td>C-, K-</td>
<td>7</td>
<td>K (2)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Yi*</td>
</tr>
<tr>
<td>E-, K-, Fy(a-)</td>
<td>7</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Go*</td>
</tr>
<tr>
<td>E-, K-</td>
<td>5</td>
<td>Jk*</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D</td>
</tr>
<tr>
<td>C-, K-, Fy(a-)</td>
<td>3</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>D-, C-, E-, K-</td>
<td>2</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>K-</td>
<td>2</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

*Each antibody was produced once unless otherwise noted.

**Table 4. Transfusion protocols used to treat patients in the Charles Drew Program**

<table>
<thead>
<tr>
<th></th>
<th>Simple</th>
<th>Partial</th>
<th>RBC exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital 1</td>
<td>0</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Hospital 2</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Hospital 3</td>
<td>0</td>
<td>15</td>
<td>11</td>
</tr>
</tbody>
</table>

*Currently, two patients have had their transfusions suspended.
no-shows, and the age of the units at the time of distribution. The performance of the program with respect to fill rate and age of units at distribution is illustrated in Table 5.

Because the program is still actively accruing donors, the orders from the hospitals are not always filled with units from donors who have been accepted into the program. Until the program has accrued a sufficient number of donors to meet this requirement, the balance of the order is filled with random allogeneic units which meet all the phenotyping requirements. The element missing when these random units are used is the restriction on the number of donors. For those units coming from donors participating in the program, the operational procedures are usually successful in ensuring that the units are fresh.

Beginning in February 2005, a pilot project was performed as a feasibility study to determine how successful the program could be at fulfilling the third objective of the protocol. An attempt was made to limit the donor exposure for 12 of the patients at two of the hospitals. The results of this pilot project have been submitted for publication. Overall, within the first year, donor exposure was reduced by an average of 45 percent based on the performance of the blood center. Because of additional logistic variables at the hospitals, this resulted in an overall reduction in donor exposure of 32 percent at one hospital and 37 percent at the other.

**Discussion**

Since its inception in 1999, the Drew Program has tried to meet three objectives in the delivery of care to the patients with sickle cell anemia who require chronic transfusion therapy. The first objective has been met, and all patients in the program receive HbS-negative, leukoreduced RBCs which are phenotypically matched. Two protocols are used. In one of the protocols, the patients receive units that are phenotypically matched for C, E, and K. In the second protocol, the units are also matched for Fy<sup>a</sup>. On the basis of the antibody profile of the patients in the program as indicated in Table 5 and consistent with previous reporting in other publications, these two matching protocols would have prevented the majority of clinically significant antibody-related complications.

The program regularly meets the second objective and the average age of the units is often 5 days. The rationale for this objective is the belief that the frequency of transfusion therapy may be reduced if the transfused RBCs are closer to the beginning of their shelf life. Since the patients require regular transfusion therapy, this objective may help to minimize the disruptions that therapy causes in the lives of these patients. While this reasoning sounds plausible, this assumption has not been investigated and validated.

The program has been working on the third objective since February 2005. The rationale behind this objective is that minimizing donor exposure may reduce the rate of alloimmunization. Transfusion is a recognized form of immunization, but in some instances, it may induce immunologic tolerance. The capacity of transfusion to induce immunologic tolerance has been used to treat women with recurrent abortions and patients receiving solid-organ transplants. Tolerance may be the result of induction of CD4+ regulatory T cells, and there is evidence that this effect may depend on the degree of HLA-DR matching between donor and recipient, the presence of co-stimulatory molecules, and possibly on the presence of leukocytes within the transfused units.

### Table 5. Effectiveness of the Charles Drew Program

<table>
<thead>
<tr>
<th>Date</th>
<th>Hospital 1</th>
<th>Hospital 2</th>
<th>Hospital 3</th>
<th>Hospital 1</th>
<th>Hospital 2</th>
<th>Hospital 3</th>
<th>Hospital 1</th>
<th>Hospital 2</th>
<th>Hospital 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug 2005</td>
<td>68</td>
<td>15</td>
<td>67</td>
<td>57</td>
<td>7</td>
<td>65</td>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Sep 2005</td>
<td>73</td>
<td>6</td>
<td>63</td>
<td>54</td>
<td>6</td>
<td>63</td>
<td>5</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Oct 2005</td>
<td>58</td>
<td>6</td>
<td>60</td>
<td>42</td>
<td>6</td>
<td>59</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Nov 2005</td>
<td>64</td>
<td>12</td>
<td>68</td>
<td>46</td>
<td>4</td>
<td>66</td>
<td>7</td>
<td>N/A</td>
<td>6</td>
</tr>
<tr>
<td>Dec 2005</td>
<td>70</td>
<td>11</td>
<td>82</td>
<td>40</td>
<td>6</td>
<td>82</td>
<td>6</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Jan 2006</td>
<td>64</td>
<td>12</td>
<td>83</td>
<td>50</td>
<td>14</td>
<td>81</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Feb 2006</td>
<td>58</td>
<td>27</td>
<td>74</td>
<td>45</td>
<td>12</td>
<td>73</td>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Mar 2006</td>
<td>64</td>
<td>25</td>
<td>93</td>
<td>65</td>
<td>12</td>
<td>89</td>
<td>7</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Apr 2006</td>
<td>60</td>
<td>17</td>
<td>76</td>
<td>52</td>
<td>17</td>
<td>76</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Totals</td>
<td>579</td>
<td>131</td>
<td>666</td>
<td>451</td>
<td>84</td>
<td>654</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
At this point the phenomenon is incompletely understood, and the determination of the clinical value in reducing donor exposure merits investigation and clarification. The recently completed pilot project showed that donor exposure can be significantly reduced; the clinical value of this reduction may take several years to document.

**Acknowledgments**

The authors would like to acknowledge that the Drew Program and this publication are partially supported with funding from grant 5R01DK062619-03 (TL and MD).

**References**


Children’s National Medical Center’s transfusion protocol for sickle hemoglobinopathies

C. P. Minniti, T. Kratovil, and N. L. C. Luban

Children’s National Medical Center (CNMC) is located in the nation’s capital of Washington, DC; this area has a large international population because of its embassies and chanceries; the World Bank, and other government and international agencies; and the presence of Howard University and its graduate schools with a large international student body. As of July 2006, CNMC has a roster of 1058 patients, aged 0 to 21 years, with sickle cell disease (SCD) or another hemoglobin S (HbS) hemoglobinopathy. Patients are followed in one of several SCD clinics, which include several specialized treatment sites with programmatic emphasis on the newborn infant, hydroxyurea treatment, chronic pain, pulmonary disease, sleep apnea, and pulmonary hypertension. Through our participation in a number of National Heart Lung and Blood Institute (NHLBI)-funded clinical trials, Stroke Prevention in Sickle Cell Anemia (STOP II), Stroke with Transfusions Changing to Hydroxyurea (SWiTCH), Silent Cerebral Infarct Trial and Hydroxyurea Trials (SITT), special sessions for the education of parents and children, and screening of potentially eligible patients for stroke prevention have also been developed. We currently treat 47 patients with chronic transfusion protocols. Our 26-bed hematology oncology unit has an average inpatient census of 8 to 10 patients with SCD. In fiscal year (FY) 2005, there were 530 inpatient hospitalizations and 3300 outpatient visits. Among inpatients, approxi-mately 40 percent were transfused for complications related to acute chest syndrome, acute splenic sequestration, or acute stroke, or in preparation for a surgical procedure, most often cholecystectomy.

CNMC developed its own blood donor center (BDC) in 1992, initially in concert with the American Red Cross, but it is now free-standing and independent. Blood components are provided to our patients exclusively from these two sources. In FY 2006, CNMC drew 2389 and transfused 1797 units of RBCs to 2536 patients, a portion of whom were patients with SCD or other hemoglobinopathies. An active therapeutic apheresis, and donor platelet and plasma donor pheresis, program is integral to the BDC. In FY 2006, 51 therapeutic erythrocytapheresis procedures were performed for acute complications of SCD or for patients on chronic transfusion regimens. Limitations on staffing, space, and equipment, and an increase in blood component requirements, along with competition for hematopoietic stem cell (HSC) collections preclude a greater therapeutic apheresis program for patients with SCD at the present time.

SCD Transfusion Protocol

Since 1976, under the supervision of one of the authors (Luban), the CNMC Blood Bank has followed the same protocol. All new patients are identified weekly by the patient care team and a full RBC phenotype is completed during the patient’s first hospitalization. This information is recorded in the laboratory information system (now computerized) along with a restriction for HbS negative RBCs. Three modes of transfusion are currently used. These include simple transfusion for acute symptomatic anemia exacerbation and in preparation for a surgical procedure, partial exchange transfusion, and erythrocytapheresis. Among the 47 patients on chronic transfusion regimen, 6 are on partial exchange transfusion as required by specific protocol, 6 are on erythrocytapheresis, and 35 are on simple transfusion. The goal for patients on chronic transfusion is to ensure a quantitative HbS concentration of less than 30 percent as measured by a pretransfusion quantitative HbS. The patient’s Hb and Hct are used to determine the volume of RBCs to be used at each transfusion. Additive anticoagulant RBCs are used unless the child is in florid renal or hepatic failure.
Phenotype matching is performed for patients on protocols when such matching is required. Patients not on research protocols do not receive phenotypically matched RBCs until they develop their first clinically significant antibody (-Le\(^a\), -Le\(^b\), and -M excluded). After development of their first antibody, they receive units phenotypically matched for C, E, and K. After the development of a second clinically significant antibody, they receive fully phenotypically matched units.

CNMC began bedside leukocyte-reduction of RBCs in 1988 and has had a fully prestorage leukocyte-reduced RBC supply since 1998. All patients, regardless of diagnosis, receive leukocyte-reduced RBC components. CMV seronegative units are reserved for those children who are to undergo HSC transplant; they are placed on this additional restriction after ensuring the CMV seronegativity status of their serum and that of the donors during the pretransplant evaluation. Irradiation restrictions for children with SCD are applied once the patient is listed for HSC transplant.

Our protocol of exchange transfusion with erythrocytapheresis is a modification of the aforementioned protocol. When an emergency erythrocytapheresis is indicated and time permits, RBCs phenotypically matched for C, E, and K are selected. The decision to forego phenotype matching is made by the blood bank director, who is also a practicing pediatric hematologist, and the medical supervisor of apheresis. Decisions are based on the clinical condition of the patient’s cardiorespiratory status, neurological presentation, and potential for deterioration.

Age of the components to be used for partial and full exchange is determined by institutional protocol, paralleling the concepts inherent in both neonatal exchange and massive transfusion, and incorporates principles of physics. Older units (> 14 days old) are used initially as they will be diluted by the patient’s blood volume and will be preferentially removed during the procedure. Fresher units (5–7 days old) are used toward the end of the procedure as this represents the major (70%) fraction of RBCs remaining in the patient. These concepts are detailed by mathematical formula.

We have established clinical pathways for acute chest syndrome (ACS), stroke, fever, pain, and presurgical preparation, some of which include transfusion. For example, our ACS pathway includes a simple transfusion in children with an infiltrate on chest x-ray, an O\(_2\) requirement, and an arterial blood gas with pO\(_2\) less than 70 percent. This early intervention, coupled with aggressive pulmonary toilet, has significantly reduced the number of exchange transfusions for ACS and PICU admissions. The stroke pathway involves input from representatives of neurology, neuroradiology, cardiology, ICU, hematology, transfusion medicine, and laboratory medicine who are alerted when a new patient presents with an acute neurologic event. Erythrocytapheresis is performed as soon as logistically possible and within 12 hours of presentation to CNMC emergency department.

**CNMC “Buddy” Program**

Our desire to exclusively use leukocyte-reduced RBC components, and the recognition that donors with sickle cell trait could not provide RBCs for prestorage leukocyte-reduction because of filter failures,\(^9\) prompted the change in the direction of our Buddy Program. Initiated in 1998 and expanded in 2002, our program was established to increase the number of African American donors who were phenotyped and committed to frequent donations for our patients. We contact all parents of children on chronic transfusion and those being prepared for surgical admissions. We work with the family and friends of these patients to encourage blood donation for the broad community of children we serve. There is particular emphasis on the program for those with preexisting antibodies requiring fully phenotypically matched RBCs and for those children on protocols or on chronic transfusion. Blood samples from donors are initially tested for HbS and other hemoglobinopathies; if found to be of the AA hemoglobin phenotype, their RBCs are fully antigen-typed and their demographic, contact, blood group, and RBC phenotype are entered into a computer program shared with the BDC. On a weekly basis, an updated listing of patients for transfusion for the month is provided by the patient care team and donors are recruited by the BDC, or backfilled by the blood supplier. There are only 6 children whose complex allo- and autoantibody status precludes us from finding donors except through the rare donor registry of our blood supplier. These intermittently transfused patients include one with an anti-C, -E, -N, -V, -Fy\(^a\), Js\(^a\), cold and warm autoantibody and another with anti-C, -K, -V, Js\(^a\), and -Go\(^a\). For these and selected other cases, the transfusion service places the orders with the blood supplier. In 2006, the Buddy Program supplied all or part of the transfusion needs of 53 patients.
the establishment of a blood mobile in the first quarter of this fiscal year, focused donor recruitment and collection efforts should enable provision of more units for each patient.

Ensuring Communication Concerning Allo- and Autoantibody Formation

Of 190 children transfused more than twice, 51, or 27 percent, have clinically significant antibodies. These include 5 with only autoantibodies and 46 with alloantibodies, of whom 9 also have autoantibodies. None have been pregnant. This rate of antibody formation is similar to what has been previously reported.6,7,8 As soon as an alloantibody, additional alloantibody, or autoantibody is identified, a series of events occur. These include sending a letter to the parents detailing the antibody(ies), the appropriate choice of RBCs, and a need to share this information with any health care provider; requesting that the child wear an identification bracelet (MedicAlert bracelet, MedicAlert Foundation, Turlock, CA); updating information in the electronic medical record; placing restrictions in the computerized laboratory information system with paper card file backup; and providing a copy of the patient letter to the patient convenience chart and patient care team.

While most children with SCD and other hemoglobinopathies in the DC metropolitan area are cared for at CNMC, international and regional travel, including summer camp experiences, can put children with antibodies at risk should they need transfusions. Our solution is to encourage wearing an identification bracelet (MedicAlert bracelet, MedicAlert Foundation) or similar national system with instant access to this critical information. When teenagers from CNMC make the transition to adult care, their allo- and autoantibody information is included as part of the detailed transitional medical history. Detailed letters with full phenotype and antibody history are prepared for families returning to their home country or on travel.

Rationale for the CNMC Phenotype Matching Policy

CNMC participated in the Cooperative Study of Sickle Cell Disease (CSSCD), one of the very first multi-institutional NHLBI-sponsored data collection and intervention studies. While contributing to the CSSCD study design, it became obvious that CNMC had a larger number of non-American born children with HbS hemoglobinopathies and that these children tended to have a greater number of antibodies per child and more unusual antibodies, and occasionally became non-transfusible unless blood was obtained through the rare donor registry. This prompted a CNMC-published study on differences in alloantibody development, and served as the basis of studies by others that attempted to identify the pathophysiology of antibody development, looking at HLA and the impact of phenotype matching on antibody development.7-11 CSSCD demonstrated an alloimmunization rate of 18.6 percent and noted that the chances of sensitization continue to rise with increasing number of transfusions.6 Based on our experience and clinical expertise, it is thought that the CNMC protocol ensures rapid availability of RBCs when needed for acute complications of SCD, and for acute erythrocytapheresis, while overburdening neither the blood supplier nor the CNMC BDC or transfusion service. The protocol also protects the chronically transfused children whose risk may increase with subsequent transfusion through use of phenotypically matched RBCs from a donor pool more racially matched to the child.

Unanswered Questions

The advantages of initiating complete phenotype matching at inception of first transfusion have never been demonstrated. Whether there is genetic predisposition to allo- and autoantibody formation is also unanswered.12 The reason that certain RBC antigens are more likely to elicit sensitization is also unknown although antigen density and other factors have been implicated. No criteria backed with pathophysiological measures have been established regarding the timing of acute erythrocytapheresis for patients with frank ischemic stroke, transient ischemic attacks, or incipient or frank ACS. While erythrocytapheresis with or without hemodilution but with aggressive chelation can decrease the transfusional iron burden, the timing, cost, and effect of the increase in blood donor exposure and the risk of catheter placement must be kept in mind when suggesting these modalities as standard of care. Future clinical and translational studies detailing the pathophysiologic mechanisms of allo- and autosensitization are needed.
References


Caterina P Minniti, MD, Director, Red Cell Disorders, Division of Hematology and Oncology, Children's National Medical Center, Washington, DC, and Associate Professor of Pediatrics, The George Washington University Medical Center; Tonya Kratovil, MD, Assistant Professor, Uniformed Services University of Health Sciences (USUHS), Bethesda, MD; and Naomi L. C. Luban, MD, Chief, Laboratory Medicine & Pathology, Senior Attending Hematologist, Children's National Medical Center, 111 Michigan Avenue, NW, Washington, DC and Professor, Pediatrics and Pathology, The George Washington University Medical Center, Washington DC 20010.
Transfusion support of patients with sickle cell disease at the Children’s Hospital of Philadelphia

D.A. SESOK-PIZZINI, D.F. FRIEDMAN, K. SMITH-WHITLEY, AND S.J. NANCE

The American Red Cross (ARC), Penn-Jersey Region, Cooperative Sickle Cell Donor Program (CSCDP) began in September 1997 at the request of one of the clinical hematologists treating patients with sickle cell disease (SCD) at the Children’s Hospital of Philadelphia (CHOP). The ARC Penn-Jersey Region now collects more than 1000 productive units of blood per month from donors who voluntarily participate in this program designed to attract African American blood donors. This program is successful because of the combined efforts of CHOP physicians, the ARC Penn-Jersey Region, the Philadelphia/Delaware Valley Chapter of the Sickle Cell Disease Association of America, and generous blood donors. The program currently serves two children’s hospitals in Philadelphia. The keystone of the program is the blue tie tag that was developed by CHOP and ARC Penn-Jersey Region staff used to identify units in the program.

Over 1000 children with SCD are enrolled in the comprehensive sickle cell center (CSCC) at CHOP where they receive medical care during routine and acute illness visits. Approximately 115 children receive chronic transfusion therapy and have ongoing needs for “blue tag” units from the CSCDP. Primary and secondary stroke prevention is the most common indication for transfusion, followed by prevention of acute chest syndrome and acute splenic sequestration recurrences. Many children receive acute RBC transfusions to decrease the morbidity of SCD complications, such as acute chest syndrome and acute exacerbation of anemia, or as preoperative therapy to decrease the risk of acute chest syndrome following general anesthesia.

Transfusion Protocol

The CHOP protocol for transfusion of RBCs for patients with SCD consists of prospective phenotype matching for C, E, and K, and issuance of prestorage leukocyte-reduced “CMV safe,” irradiated RBCs that are HbS negative. The HbS testing is done at CHOP using a rapid solubility test. In the event that a blue tag unit is HbS positive, it is transferred to another institution for use in their general inventory.

The practice of matching for C, E, and K, when phenotype-matched donor RBCs are needed for patients with SCD, is consistent with other hospitals’ practices. At CHOP, to determine the need for matching, each patient with SCD has an extended RBC phenotype performed using an untransfused specimen. If the patient has been recently transfused, other methods may be needed to determine the phenotype of the patient’s RBCs. While a hypotonic wash method can be used to isolate Hbs-positive RBCs (the patient’s RBCs), molecular methods are useful for the detection and identification of variants of e and D that occur at a high rate in this population. In the event the antigen profile is unknown, RBCs are released that are negative for C, E, and K until a true phenotype can be determined.

For patients on chronic transfusion programs, many of whom are patients with SCD, our protocol calls for transfusing RBCs less than 21 days old at the time of transfusion. The rationale for this practice is that the in vivo recovery and survival of transfused RBCs decline with storage age and chronically transfused patients will have higher Hb values, longer transfusion intervals, and ultimately less transfusion iron loading, if fresher RBCs are used. It is not always possible to meet this
requirement if there are other constraints such as multiple antibodies or limited supplies. In addition, this freshness protocol is not applied to patients with SCD that have acute or one-time indications for transfusion.

Our SCD transfusion protocols support a very large RBC exchange program. We currently treat more than 50 patients who return to the apheresis unit for partial or complete RBC exchange every 3 to 4 weeks. Many of these patients are on prophylactic protocols for primary or secondary stroke prevention and require the percentage of HbS to be maintained at either less than 30 percent or less than 50 percent (for secondary stroke prevention in selected patients). Patients receiving RBC exchange usually have peripheral access. In the event that peripheral access fails, a port that is suitable for use in apheresis is placed for access. Automated RBC exchange transfusion is preferred at our institution for patients with SCD requiring chronic transfusions to decrease total body iron burden, thereby preventing or forestalling iron overload.

**Selection of Protocol**

Eighteen to 25 percent of all patients with SCD are alloimmunized because of RBC antigen frequency disparities between African American patients with SCD and European American blood donors. Because of the high frequency of alloimmunization in this patient population, all children with SCD have an extended RBC antigen phenotype performed in their first year of life, before their first RBC transfusion or at entry into the CHOP CSCC. With approximately two-thirds of the antibodies formed having specificities for antigens in the Rh or Kell systems, we adopted the practice of transfusing RBCs that are C, E, and K-matched. Multi-institutional studies conducted to determine whether RBC transfusions could decrease or prevent neurological and respiratory complications in patients with SCD have also adopted transfusion guidelines using phenotype-matched RBCs. Those antibodies that did develop had specificities in the Duffy, Kidd, and MNS systems. Presently, we are analyzing the number of patients with SCD and alloimmunization and the number of delayed hemolytic transfusion reactions since the CSCDP began in 1997.

**Summary of Data from the Program**

To provide an overview of this program, we reviewed the transfusion experiences of patients with SCD at our institution from 2002 to 2005. On the average, more than 3800 RBCs with blue tags were received per year, or 73 units per week. This represents 36 percent of all the RBCs received over this time period. These RBCs provided more than 16,000 transfusions over the 4-year period, about 58 percent of the total RBC transfusions. Of the blue tag units received, 92 to 93 percent were transfused. Interestingly, only 51 percent of these were transfused to patients with SCD; the remaining units were transferred into the general inventory and transfused to patients with other diagnoses. At CHOP, blue tag units are also routinely ordered and used for patients with thalassemia.

The range of transfusion exposures among the patients with SCD in this 4-year period was broad, with 66 percent of these patients receiving no transfusions, 19 percent receiving 1 to 9 transfusions, 9 percent receiving 10 to 99 transfusions, and 5.5 percent receiving more than 99 transfusions. About 70 percent of the transfusions, blue tag or general inventory, went to this latter group of very heavily transfused patients. These figures do not represent these patients' total lifelong transfusion history, only the experience of the 4 years reviewed.

Overall, only about 60 percent of RBCs transfused to patients with SCD came from the blue tag inventory, the remainder came from general inventory. Viewed from the patient's perspective, 137 of the 300 patients with SCD who were transfused in the 4 years reviewed, or 46 percent, received 90 percent or more of their transfusions from the blue tag inventory; 93 patients or 31 percent received all of their transfusions from the blue tag inventory. No one patient of these 93 who were entirely supported with the blue tag inventory received less than 50 transfusions or more than 10. On the other extreme, there were 47 patients, or 16 percent of those transfused, who received 0 to 10 percent of their transfusions from the blue tag inventory, 37 of them receiving no blue tag units at all. This group of patients, who received no blue tag units over 4 years, included 3 patients who received a total of more than 100 units.

We also investigated 14 patients with SCD who, although they received more than 10 RBC transfusions, less than 10 percent of their blood support was provided with blue tag units. In nine of these cases,
additional antibodies including anti-S, -Jk\(^a\), -Jk\(^b\), -Js\(^a\), -V, -f, -Co\(^a\), and -Wr\(^a\) made it difficult to provide the patient’s RBC needs from within the blue tag inventory. In two cases, the patient’s RBCs were group B, D-; in two cases, R\(_2\)R\(_2\) RBCs were needed because of auto-anti-e, and in one case, extended phenotype matching had been prescribed because of unexplained hemolytic transfusion reactions. It is possible that the diagnosis of SCD was not known to the blood bank in one case.

**Donor Recruitment Process**

There are more than 10,000 donors who have self-identified for the CSCDP. Demographic analysis of the program reveals a younger age group than the general donor population at the ARC in Philadelphia with the majority of donors between 19 and 29 years of age. In addition, because of the distribution of ABO groups, one-half of the donated RBCs are group O. When a donor walks into any collection site in the Penn-Jersey Region, there is a poster display showing a bright young child with SCD and the distinctive blue tie tags. The names and logos for CHOP and the ARC are displayed on the front of the tag as co-branding for the CSCDP. The back of the tie tag has information about SCD and the need for African American donors. A donor who decides to be an “African American Hero” takes a tie tag and hands it to the phlebotomist. Once the donor meets all of the usual donor criteria, the collection staff attaches the blue tie tag to the primary collection bag. The tie tag remains with the unit while other components are made from the donation. Plasma and platelets are not labeled with a tie tag. The tie tag is the marker to ensure that each RBC is captured as a CSCDP unit. The RBCs are placed in storage locations according to the C, E, and K phenotype of the component, thus making the units easily accessible when the order for transfusion is being completed.

Since the donors are very likely to be African Americans, extended typing for antigens in the Kidd, Duffy, and MNS systems are not performed, as the population frequencies of these antigens are similar in the donors and patients. However, segments are retained for testing for rare blood types. African Americans are the population of interest for antigens such as hr\(^a\), U, Js\(^b\), and Hy, as this population has the highest chance (albeit low) to be negative for these
antigens. If a rare blood type is found, the unit is selected for long-term storage in the frozen state and the donor is invited to be listed in the American Rare Donor Program.

**Communication Process Between the Transfusion Service and Blood Supplier**

Our transfusion protocol for patients with SCD requires some effort on the part of the blood bank technologist. Ideally, for each patient’s blood order, units must be obtained from the blue tag inventory that are leukocyte-reduced, irradiated, less than 21 days old, AB0 and D type-specific, crossmatch compatible, phenotype matched for C, E, and K, as well as antigen negative for other clinically significant antibodies present in the patient’s plasma. This protocol is the same for all patients with SCD, whether they are inpatients or outpatients, scheduled or emergent, except that the freshness criterion is not applied for acute transfusions. The expectation for outpatient transfusions, which comprise the majority of these transfusions, is that patients will have their blood bank specimens drawn 1 to 3 days before the transfusion so as not to wait for blood availability when they arrive for the transfusions.

To meet these demands, there must be good coordination between the clinical service, the blood bank, the blood supplier, and the regional and national reference laboratories. Each week, the schedule of outpatient transfusions for the following week is compiled for both simple transfusions and RBC exchanges and is transmitted by fax to the blood bank. This schedule shows the anticipated date of transfusion and the anticipated number of units or volume of RBCs needed. A blood bank technologist reviews this schedule with the patient’s AB0 blood group, D type, C, E, and K phenotype, and antibody history, and then places a blood order with the ARC for each patient’s needs, typically two days after the initial fax. The technologist will sometimes order 1 or 2 additional units if the patient has a history of clinically insignificant antibodies that interfere with cross-matching. In addition, the technologist will review the existing inventory of blue tag units and will order additional units to accommodate unscheduled blood orders for patients with SCD. This inventory of extra units typically consists of 5 group A and 5 group O, all negative for C, E, and K; this selection maximizes flexibility in using them for unscheduled needs. Finally, the technologist identifies blue tag units that are older than 21 days of storage, removes the blue tag, and transfers them to the general RBC inventory.

The ARC IRL must then find units to fill these orders, using units that will be less than 21 days old on the date of transfusion and selected from the blue tag inventory, if possible. Since the CSCDP provides a generous supply of RBCs from a predominantly African American background, the IRL is typically able to fill the majority of orders, amounting to 60 to 80 units each week, within 1 to 2 days. Orders for patients with complex antibody problems may take several more days. When the appropriate units are identified, they are shipped to the hospital blood bank where these units are stored separately from the rest of the available inventory.

**Unique Features of the Program**

Although this program is very successful in supporting our patients with SCD, there are some challenges when the patient does not exhibit one of the most common African American blood types. For example, for a patient who is e–, and develops an anti-e or autoanti-e, there may be difficulty in locating an e– blue tag RBC unit each time a transfusion is needed. Similarly, patients who form other antibodies, such as anti-S or -Jk^a, or -Jk^b, may not be able to be supported with blue tag units. If blood cannot be found in the blue tag inventory, the decision whether to delay transfusion or go outside the program must be made.

Blue tag units in our CSCDP are available for use for patients with elective and acute needs for blood transfusions to reduce the morbidity and mortality associated with SCD-related complications. Other programs that use directed or selected donations cannot often provide RBCs to patients with acute, nonelective needs for RBC transfusions. Our program continues to provide phenotype-matched RBCs for all patients with SCD and, when possible, attempts to transfuse patients using RBCs exclusively from this unique donor program.

**Acknowledgments**

This is to acknowledge Pamala Blair, MT(ASCP), Carmelita Savage, MT(ASCP)BB, and Kecia Smith, MT(ASCP)BB, for their daily supervisory support of the blue tag CSCDP at CHOP, and Qiana Riley, MLT, for donor recruitment efforts for the CSCDP. We also acknowledge Cynthia Majzner and the ARC Penn-Jersey IRL staff, who ensure that every unit is used judiciously.
References


Deborah Sesok-Pizzini, M.D., M.B.A, Assistant Professor; Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine and Medical Director, Blood Bank and Transfusion Medicine, Children’s Hospital of Philadelphia, 34th Street and Civic Center Blvd, Philadelphia, PA 19104-4399; David F. Friedman, M.D., Assistant Professor, Department of Pediatrics, University of Pennsylvania School of Medicine, and Associate Medical Director, Blood Bank and Transfusion Medicine, Children’s Hospital of Philadelphia, Philadelphia, PA; Kim Smith-Whitley, M.D., Associate Professor, Department of Pediatrics, University of Pennsylvania School of Medicine, and Associate Director, Clinical Sickle Cell Program, Children’s Hospital of Philadelphia, Philadelphia, PA; Sandra J. Nance, MS, MT(ASCP)SBB, Director, IRL, Atlantic Division IRL Director, American Red Cross, Philadelphia, PA.


IMMUNOHEMATOLOGY IS ON THE WEB!

www.redcross.org/pubs/immuno

For more information or to send an e-mail message “To the editor”

immuno@usa.redcross.org
Sickle cell disease (SCD) is the most common genetic disorder in the United States, affecting individuals of sub-Saharan African, south Asian, and Mediterranean ancestry. The abnormality is a mutation in the sixth amino acid residue of the beta globin chain from glutamic acid to valine ($\beta^s$); resulting in a hemoglobin tetramer that may polymerize during normal oxygen carriage, causing the characteristic crescent or sickle-shaped RBCs in the peripheral blood for which the disorder is named. The abnormal RBCs are fragile, causing chronic hemolysis that results in anemia as well as a vasculopathy that causes ischemic damage to many body organs, including the spleen, kidneys, lung, and brain.

SCD includes a number of syndromes of variable frequency and severity. Sickle cell anemia (HbSS—homozygous for two $\beta^s$ genes) is the most common, occurring in about 1 in 350 African American births. Symptomatic double heterozygote states for hemoglobins that interact with $\beta^s$ are less common and include sickle C disease (HbSC), and sickle beta thalassemia (HbS-thalassemia, with the designation $\beta^o$ when no hemoglobin A is produced and $\beta^+$ when some A is present). While patients with HbSS experience the majority of the complications requiring RBC transfusion, such conditions are all experienced albeit less frequently by persons with other forms of SCD.

RBC transfusion, either to quickly increase the oxygen carrying capacity from acute exacerbation of anemia or to chronically suppress the production of $\beta^s$-containing RBCs, is a mainstay of treatment for SCD. Although stable compensated anemia, which patients with SCD have at baseline and during uncomplicated acute painful crisis, does not require transfusion, the majority of adult patients have required transfusion at least once in their lives. This article will review the commonly used transfusion methods, the indications for transfusion, and the current management of iron overload in patients with SCD.1,2

Red Blood Cell Product Selection and Methods of Administration

Immunologic considerations

Alloimmunization, as a result of antigenic discrepancy between patients of African ancestry and predominantly Caucasian blood donors, has historically affected 5 to 50 percent of patients with SCD. Previously undetected alloimmunization, a problem made worse by multiple sites of care and transfusion center ignorance of complete transfusion history, may increase the occurrence of delayed hemolytic transfusion reactions (DHTR). Further, the clinical signs of DHTR may mimic those of sickle cell crisis: pain, low-grade fever, and exaggerated anemia, so diagnosis may be further delayed.1,2 Once such a hemolytic reaction begins, patients with SCD may undergo hemolysis of autologous as well as transfused RBCs, an autohemolysis-hyperhemolysis syndrome which may be persistent and severe.3,4 During such events, further transfusion of even crossmatch-compatible RBCs should be avoided, if possible. The recommended treatment is usually corticosteroids and intravenous gamma globulin; erythropoietin is added if reticulocytopenia is present.1

Product selection

Because of the increased, lifelong need for RBC transfusion as well as the increased probability and consequences of alloantibody formation in persons with SCD, most centers that care for a large number of these patients perform extended RBC antigen typing before the first transfusion. RBC components, known to be phenotypically matched for ABO and Rh (Cc, D, Ee) and K can then be crossmatched. Theoretical calculations suggest that the use of a limited extended-phenotype matching for these antigens would prevent 53 percent of antibodies in SCD patients.5 Extended-phenotype matching for ABO, Rh, and K has been shown in a multicenter trial to reduce the
alloimmunization rate in patients with SCD from 3 percent to 0.5 percent per unit transfused and to reduce DHTR by 90 percent. While this is recommended, it may not be practical in all sites. Further, since the majority of the units will be used in chronic transfusion programs described below, units less than 7 to 10 days old are selected for transfusion to SCD patients, whenever possible. Selected units should be screened and found to be hemoglobin S (HbS) negative.

In our center, we attempt to obtain an extended RBC phenotype on all HbSS and HbS-βthal patients at a routine clinic visit before their second birthday. The phenotype is stored in the blood center records as an “antibody” so that when a clinical order is received for RBCs, phenotype-matched RBCs can be provided. Our hospital blood bank maintains an inventory of several units of group O, D– (extended antigen phenotype known), K– units to be used for urgent transfusion of patients with SCD; more can be obtained from the regional blood center within 2 to 4 hours. When an extended phenotype has not been performed before the request for crossmatch, the clinician is given the option of awaiting limited antigen typing from the regional blood center that may take 8 to 12 hours or accepting crossmatch compatible group O, D–, K– units already on site. For chronically transfused patients, who use the majority of the extended-phenotyped units, the transfusion is scheduled with the blood bank in advance so additional units with the required phenotype may be moved to the hospital blood inventory the day before the planned outpatient use.

To ensure that sufficient extended-phenotype compatible RBCs are available, our sickle cell program has paired with the regional blood center to establish a Special Care Team: patients volunteer to tell their stories in donor recruitment literature branded with the logos of the team, the hospital, and the donor center. Volunteer donors are asked to self-identify as African American and to indicate that they are willing to be recalled for the program. RBC samples from identified donors are then extended-antigen typed and thereby linked to the program, not to a specific patient. Units collected under this program receive a special tag at the time of release for transfusion, raising awareness of the need for special donors and of the program. In the first 18 months of program operation, the donor center recorded a 3.3 percent increase in African American donors and a 7.6 percent increase in units donated.

Many other centers have established similar programs.

**Transfusion Methods**

*Acute versus chronic transfusion*

Acute transfusion is given for an urgent problem, usually accelerated anemia in SCD patients, and is the standard form of transfusion. Infusion of the bank RBCs raises the hemoglobin and restores circulating intravascular volume and oxygen carrying capacity. Although blood substitutes have been tried in patients with SCD during acute events, currently the short half-life of such products and concerns about repeated administration limit their usefulness.

Chronic or long-term transfusion is managed through a program in which patients receive RBCs every 3 to 6 weeks to suppress their own HbS production and to ameliorate intravascular sickling and further organ damage. Within 1 to 3 planned monthly transfusions, the reticulocyte count is usually suppressed to less than 5 percent, the goal being to maintain a patient at a desired hemoglobin level and below a specific percentage of HbS indefinitely with continued transfusion.

*Chronic transfusion—simple versus exchange*

The usual volume for simple transfusion is 2 to 3 units for an adult and 10 to 15 ml/kg for a pediatric patient, administered by peripheral or central venous access over 4 hours. Such volumes are the easiest to administer and reduce the concentration of HbS by dilution. If the patient is particularly anemic (below 5–6 gm/dL) it may be possible to decrease the percentage of HbS to below 30 percent with serial, simple transfusions. However care must be taken to prevent volume overload particularly if more than one transfusion is planned in a short period of time. Similarly, it is important to avoid a Hb of more than 11 gm/dL and its attendant hyperviscosity, which may precipitate a painful crisis or stroke, unless the percentage of HbS is less than 30 percent.

Exchange transfusion can be performed manually or by mechanical erythrocytapheresis. In acute situations, exchange transfusion can rapidly decrease the percentage of HbS while maintaining euvolemia and avoiding hyperviscosity. Chronic exchange transfusion, which usually requires two good sites of intravenous access, can maintain the benefits of low HbS percentage while avoiding iron overload. If isovolemic hemodilution methods are used, transfusional iron burden may even be reduced by subsequent procedures.
Alternatives to Transfusion—Hydroxyurea

Hydroxyurea therapy reduces the frequency of painful events and acute chest syndrome episodes in patients of all ages with SCD. It also has a clear benefit on increasing survival. Similar results have been seen in pediatric patients with minimal toxicities, most commonly reversible myelosuppression. This chemotherapeutic agent is well tolerated orally, but does require at least monthly monitoring of the blood count and evaluation of other potential toxicities such as gastrointestinal upset, splenic enlargement and blood chemistry abnormalities. This requirement for frequent monitoring in addition to the theoretical risk of teratogenicity and leukemogenicity have limited hydroxyurea’s acceptance by many patients.

Indications for Transfusion

Central nervous system

1. Secondary prevention of thrombotic stroke

Acute infarctive stroke is the most common neurologic disorder in patients with SCD, occurring in 7 to 10 percent of HbSS patients during childhood. These events occur as the result of a progressive intracranial vasculopathy caused by sickle cell induced damage to the endothelium. Chronic RBC transfusion is the gold standard in the prevention of recurrent events, having been reported to reduce the rate of these events from 47 to 93 percent to 10 to 20 percent. However, transfusions must be continued lifelong as discontinuation is well documented to result in 50 percent recurrence of ischemic central nervous system (CNS) events up to 12 years later. Initially the intent is to transfuse to maintain a pretransfusion Hb of 9 to 10 gm/dL, HbS less than 30 percent, and reticulocyte count less than 5 percent. After four years of continuous transfusion without recurrent neurologic events, some centers change the pretransfusion goal to a Hb of 8 to 8.5 gm/dL and a HbS of less than 50 percent to decrease transfused iron and lengthen the interval between transfusions. Intracranial hemorrhage is also seen in up to 25 percent of adult patients, but the utility of chronic transfusion in secondary prevention of hemorrhagic stroke is less well established.

In a single-institution trial, Ware reported that patients on chronic transfusion for secondary stroke prevention can overlap treatment with concomitant hydroxyurea therapy while gradually reducing the intensity of transfusion and still maintain protection again recurrent CNS events. Once they are stable on hydroxyurea, phlebotomy can then be used to resolve the transfusional iron overload. The currently enrolling NIH-NHLBI-funded Stroke with Transfusions Changing to Hydroxyurea (SWiITCH) study is designed to test this approach in a multicenter setting. At this time, however, the use of hydroxyurea to prevent primary or recurrent CNS events must be considered investigational.

2. Primary prevention of stroke

With the high-occurrence risk of stroke in children with HbSS, a great deal of interest has been focused on prediction of which children are at risk. Risk factors for thrombotic stroke identified in the Cooperative Study of Sickle Cell Disease include a prior transient ischemic event, low steady-state Hb, elevated systolic blood pressure as well as recent frequency of acute chest syndrome. The Stroke Prevention in Sickle Cell Anemia (STOP I) trial demonstrated that, in children aged 2 to 16 years with a time average mean velocity of more than 200 m/s on transcranial Doppler study, chronic transfusion could decrease the risk of initial stroke by 90 percent. In the subsequent STOP II study, it could not be defined when it was safe to stop transfusions, reinforcing the recommendation that they continue indefinitely for both the primary and secondary prevention of stroke.

Multiorgan failure syndrome

In this life-threatening complication, which transpires during a severe painful crisis, generalized vaso-occlusion occurs, resulting in a rapid fall in Hb and platelet counts, encephalopathy, and evidence of renal and hepatic dysfunction. Prompt exchange transfusion to a HbS less than 30 percent and a Hb of about 10 gm/dL has been associated with improved survival and recovery of organ function.

Acute chest syndrome and pulmonary hypertension

Acute chest syndrome is a unique pulmonary event in patients with SCD defined as a new lobar or segmental infiltrate on chest radiograph, with fever, hypoxia, and respiratory symptoms, and is frequently associated with an acute decline in Hb of 2 gm/dL or more. Acute chest syndrome is the leading cause of death of patients with SCD and recurrent events have been implicated in the development of pulmonary hypertension. There are no direct data that simple transfusion can hasten the resolution of acute chest
syndrome. Most clinicians advise simple transfusion if there is a significant supplemental oxygen requirement and the Hb is below 7 to 8 gm/dL. Exchange transfusion is reserved for patients with rapidly progressive courses, those in whom adequate oxygenation cannot be maintained on 50 percent supplemental oxygen, and others who might experience dramatic improvement during the procedure. During the STOP I trial, data accumulated that compliance with chronic transfusion reduced the incidence of acute chest syndrome in pediatric patients from 15.7 to 2.2 events per 100 person years (p=.0001). Clinicians will often advise a short term (6 months or less) of chronic transfusion therapy for patients with unusually severe or frequently recurrent acute chest syndrome. Treatment with hydroxyurea is also appropriate, following or instead of the short term chronic transfusion program for prevention of recurrent acute chest syndrome. About one-third of adults with HbSS are reported to have pulmonary hypertension, defined by a tricuspid regurgitant jet velocity of equal to or more than 2.5 m/s, a diagnosis associated with premature mortality. Chronic transfusion has also been proposed as a treatment to stop the progression of or reverse early pulmonary hypertension in small pilot studies. However, the true role for chronic transfusion in management of patients with pulmonary hypertension may depend, as in primary stroke prevention, on screening for early risk groups. Pain There are no data that transfusion can hasten the resolution of a painful event once it has started. However, the STOP trial did confirm that aggressive chronic transfusion can reduce the frequency of painful events from 27.1 to 9.7 events per 100 patient-years (p=.014). Again clinicians frequently offer a short-term chronic transfusion program for recurrent painful events; however hydroxyurea has also been demonstrated to reduce the frequency of this complication. Exaggerated acute anemia During the course of many complications of SCD, patients may become more anemic than is usual for them. RBC transfusion is indicated when there is evidence of tissue hypoxia or end-organ stress. The most common cause of acute anemia is transient RBC aplasia caused by human parvovirus B19. This virus induces RBC production arrest for 4 to 14 days, half of the RBC life span in some forms of SCD. The Hb falls and the reticulocyte count is usually below 1 percent. RBC transfusion to attain a Hb of 8 to 9 gm/dL is indicated and close follow-up required until the reticulocyte count returns to normal. Acute splenic sequestration and hepatic sequestration HbS-contained RBCs may become trapped in the small vessels and sinusoids of the spleen and liver, resulting in rapid organ enlargement and dysfunction. Patients present with anemia and pain over the enlarged organ, and thrombocytopenia is frequently seen in severe events. Acute splenic sequestration is most common in children with HbSS and HbS-βthal between 6 months and 5 years of age, where it can be rapidly fatal if not promptly diagnosed and managed. RBC transfusions both reverse the symptoms of acute anemia and promote release of the sequestered cells. Care must be exercised to prevent over-transfusion and a rise in Hb to more than 11 gm/dL, at which point the patient is at risk for hyperviscosity and sludging, particularly within the intracranial vessels. Chronic, sometimes painful, splenomegaly, which can be seen in patients with all forms of SCD but particularly in adolescents and young adults with HbSC, may also be observed. This does not usually require acute transfusion but may place the patient at increased risk of exaggerated acute anemia during intercurrent illness. For recurrent splenic sequestration episodes requiring transfusion, splenectomy should be considered. A short-term chronic transfusion program may be used to foster involution of the spleen or temporize until the clinician or the family is comfortable with splenectomy. Hepatic sequestration is marked by a 3- to 4-fold increase in transaminases and bilirubin in association with anemia and painful hepatomegaly. Acute transfusion is required when anemia is severe and over-transfusion should be avoided as outlined above. Priapism Priapism, a prolonged painful erection of the penis, is a very common complication of SCD, occurring most commonly in patients with HbSS beginning at 2 to 3 years of age. Although anecdotal response to RBC therapy has been reported, there has never been a randomized controlled trial of simple or exchange
transfusion in management of either prolonged or recurrent priapism. Recent research has demonstrated the frequent success of alternative medical and surgical strategies to relieve the acute prolonged (more than 4-hour) episodes of priapism that may lead to penile ischemia, fibrosis, and impotence. Further, an association between SCD, priapism, exchange transfusion, and neurologic events dubbed the ASPEN syndrome has been reported. As a result, most centers reserve transfusion for single episodes unresponsive to alternative management that have persisted for more than 24 hours. Limited chronic RBC transfusion has also been used to prevent recurrent priapism in patients with frequently recurrent prolonged episodes.

Pregnancy
There are conflicting data regarding the benefit of regular “chronic” transfusion during pregnancy. Instead, most centers provide selective transfusion targeted to address clearly identifiable medical and obstetric complications such as hypoxemia, progressive symptomatic anemia, acute chest syndrome, splenic sequestration, or pre-eclampsia during pregnancy.

Leg ulcers
Leg ulcers occur on either side of the malleolus spontaneously or following minor trauma, often becoming infected, and are very slow to heal. While higher Hb levels are thought to benefit wound healing, there is a paucity of clinical data to support use of chronic transfusion in SCD-related leg ulcers.

Preparation for general anesthesia
The need for surgical intervention is common in patients with SCD and general anesthesia is associated with painful crisis, acute chest syndrome, and excess mortality within the week. Routine preoperative and often exchange transfusions have been the standard practice for patients undergoing major surgery, particularly where upper abdominal incisions may predispose to hypoventilation, but the practice is based on little firm data.

The preoperative transfusion study found no difference in outcome between routine preoperative transfusion to about 10 gm/dL by aggressive exchange to less than 30 percent HbS and simple transfusion to the target Hb regardless of HbS percentage. However, the simple transfusion group had the advantage of reduced transfusion-related complications. In addition, this trial was limited by enrollment of few patients more than 21 years of age with known cardiopulmonary dysfunction (recurrent acute chest syndrome or pulmonary hypertension) so some authors continue to advocate exchange transfusion for selected high-risk patients. Other authors have demonstrated that for low-risk cases transfusion is not required. Thus the decision to transfuse must be individualized. In our center, patients with SCD with a steady-state Hb less than 8.5 gm/dL (hemoglobinopathies other than HbSS and HbS-β0 thal), not undergoing upper abdominal surgery (any case other than cholecystectomy and splenectomy), and without a history of recent or recurrent acute chest syndrome would be less likely to require preoperative transfusion.

Iron overload
The obligate burden of recurrent acute or chronic transfusion is iron overload. Before 2006, iron overload necessitated chelation with subcutaneous or intravenous deferoxamine (Desferal, Novartis Pharmaceuticals Corp., East Hanover, NJ). The rigorous demands of subcutaneous deferoxamine infusion, 10 to 12 hours a night, 5 or 6 nights a week, invited poor adherence, leaving patients protected from the complications of SCD but at risk for hepatic and cardiac damage from the transfused iron.

The oral iron-chelator deferasirox (Exjade, Novartis Pharmaceuticals Corp.) was just licensed in the United States for treatment of transfusional hemosiderosis in patients 2 years of age and older. This oral dispersible tablet is taken on an empty stomach 30 minutes before eating daily and causes chelated iron to be excreted in the stool. In head-to-head studies iron excretion equivalent to the iron removed with deferoxamine was observed. Deferasirox has a significant side effect profile with pruritic rash, abdominal pain, and elevations in both creatinine and transaminases being seen in 6 to 38 percent of patients. Most toxicities respond to suspension of the medication and reintroduction at a lower dose. While there are other oral iron-chelators in advanced clinical trials, deferasirox is the only one approved for use in the United States.

The availability of this oral iron-chelator will likely increase the willingness of clinicians to use RBC transfusion therapy. However, the efficacy of this medication to remove all concerns of iron overload from patients with SCD who require chronic or repetitive transfusion has not yet been demonstrated.
and given the significant side effects observed in the licensure trials and in early clinical use, significant concerns still exist.

References

Antibodies, such as anti-Rh18 (Hr/Hr\(^S\)), that react with the common products of RHCE can cause HDN as well as severe hemolytic transfusion reactions. Individuals with anti-Rh18 antibodies can have different RHCE genetic backgrounds; therefore, sera and RBCs from these individuals may cross-react. In these situations, genotyping may be the best method to determine compatibility. We report a 26-year-old pregnant Puerto Rican woman who presented at 31 weeks' gestation with anti-E and anti-Rh18 in her serum. No potential donors were identified among family members or within the American Rare Donor Program; therefore, a unit of the patient's RBCs was collected one week before her planned caesarian section. To improve our ability to supply blood for this patient in the future, molecular testing was performed. The patient was found to be homozygous for an RH haplotype in which a variant RH*DAR, is linked to a variant RH*ceAR. The DAR-ceAR haplotype has been described in Dutch-African populations, but this is the first report of an individual self-identified of Hispanic ethnicity. This case report demonstrates the clinical importance of molecular testing of patients with rare Rh phenotypes. *Immunohematology* 2006;22:132–135.

**Key Words:** RH18, RH19, Hr, hr\(^S\), Shabalala, molecular testing, hemolytic disease of the newborn

Anti-Rh18 (anti-Hr, anti-Hr\(^S\)) reacts with a high-frequency antigen on the RhCE protein and was first reported in 1960 in a pregnant South African woman with the surname Shabalala.\(^1\) Adsorption studies identified two antibodies. One reacted with all common Rh phenotypes and was called anti-Hr because of its similarity with antibodies made by individuals with a phenotype produced by an Rh deletion. The other had anti-e-like specificity and was called anti-Shabalala (anti-Rh19, anti-hr\(^S\)).

Anti-Rh18 has been implicated in HDN and hemolytic transfusion reactions.\(^1\)\(^-\)\(^3\) Serologic identification of these variant e phenotypes is difficult because of the paucity of serum and lack of standardization of reagents.\(^4\) In addition, sera and RBCs from individuals with anti-Rh18 are not all compatible with each other, as several different RHce genes may be associated with a single variant e serologic phenotype.\(^3\)

Although a great deal has been learned in the last decade concerning the molecular basis of variant Rh phenotypes (as summarized by Reid and Lomas-Francis\(^5\)), it is not yet commonplace to request blood group antigen molecular testing on a patient, or to base transfusion recommendations on the findings.\(^6\) This report describes the serologic and molecular management of a pregnant woman with anti-Rh18 and highlights the value of molecular testing in patient care.

**Case Report**

A 26-year-old, group O, D+, pregnant Puerto Rican woman (gravid-6, para-2, with two therapeutic and one spontaneous abortion) presented at 31 weeks' gestation with anti-E and -Rh18 in her serum. She had no history of prior transfusions or children with HDN. Because of the risk of HDN with the current pregnancy, a search was initiated for potential blood donors. With no family members available for donation and no compatible donors registered in the American Rare Donor Program (ARDP), a unit of the patient's RBCs was collected one week before her planned caesarian section. For collection, she was brought to the labor and delivery floor and attached to a fetal heart monitor. She tolerated the donation well and there was no evidence of fetal distress. At birth, the baby's RBCs were positive by the DAT but there was no clinical evidence of hemolysis.

**Materials and Methods**

**Serologic testing**

Antibody detection was initially performed using a column agglutination test (Ortho-Clinical Diagnostics, Raritan, NJ) followed by additional tube testing with either PEG or LISS enhancement (ImmucorGamma, Norcross, GA). Common antigen typings were
performed with commercially available cells and antisera (ImmucorGamma; Ortho-Clinical Diagnostics). Anti-hr⁺ and hr⁻ RBCs were obtained through SCARE.

RBCs used for adsorption were treated with in-house prepared ZZAP made with DTT and papain; eluates were prepared using an acid elution method from RBCs used in the first pass of adsorption (Elu-kit II, ImmucorGamma). Indirect antiglobulin testing of adsorbed plasma and eluates was performed using PEG (Gamma PeG, ImmucorGamma) and murine IgG-specific antihuman reagent (Anti-IgG, ImmucorGamma).

Triturations were performed using doubling dilutions of plasma in 0.9% saline. DATs were performed using murine polyspecific, IgG, C3b, and C3d component specific antihuman globulin reagents (Anti-IgG, Anti-C3bC3d, and AntiHuman Globulin, ImmucorGamma). RBCs positive by the DAT were prepared for phenotyping using an EDTA glycine-acid kit (EGA, ImmucorGamma).

**Molecular testing**

Genomic DNA was isolated from the patient's WBCs through the use of a DNA extraction kit (QIAamp Blood Mini Kit, QiAGEN, Hilden, Germany). A previously published method employing multiplex PCR was performed to test for the presence of RHD exons 4 and 7, the inactivating pseudogene, and for C and c genotyping.⁷ RHD zygosity was determined by PCR detection of the hybrid Rhesus box, which is a marker for the RHD deletion, and with a PCR-RFLP method.⁸ PCR products were separated on 2% agarose gels and visualized with ethidium bromide staining. RNA was isolated from the patient's RBCs with a specialized reagent (Trizol, Invitrogen, Carlsbad, CA) and cDNA was synthesized with a kit (First Strand cDNA Synthesis kit, Invitrogen,). The cDNA was amplified with primers flanking the coding regions of RHD and RHCE. PCR products were ligated into pCR 2.1-TOPO TA vector and transformed into chemically competent E. coli cells (TOPO TA cloning kit, Invitrogen). Positive clones were determined by color selection with X-Gal. Plasmids were isolated with the use of a kit (QIAprep Spin Mini kit, Qiagen) and analyzed with restriction enzymes. Six plasmids representing different Rh transcripts were sequenced (Children's Hospital of Philadelphia). Sequences were aligned and compared using Clustal X.

**Results**

**Serologic investigation**

The patient's RBCs initially typed as group O, D⁺ and her plasma reacted with all RBCs tested using column agglutination testing and tube testing with PEG (3-4+) and using tube testing with LISS (1-3+). The DAT and autocontrol were negative. Staff at the American Red Cross (ARC) reference laboratories in Dedham, Massachusetts, and in Philadelphia, Pennsylvania, phenotyped the patient's RBCs as: group O, D, C⁻, E⁻, c⁺, c++. They also identified anti-E, -Hr, and -hr⁻. Her serum did not react with -D− RBCs.

To confirm the serologic findings, aliquots of the patient's plasma were adsorbed onto rr and Rr RBCs. Adsorbed plasma and the eluates were tested against E⁺e⁻, E⁻c⁺, E⁻e⁺hr⁻ RBCs. After adsorption onto rr RBCs, the patient's plasma reacted only with E⁺e⁻ RBCs, confirming the presence of anti-E. The eluates prepared from rr RBCs used for adsorption reacted with E⁺c⁻ RBCs confirming the presence of anti-Hr. After adsorption onto Rr RBCs, the patient's plasma reacted with only E⁺e⁺ RBCs and not with E⁺e⁻,hr⁻ RBCs, confirming the presence of anti-hr⁻.

Trituration studies were performed on the patient's serum in the third trimester of pregnancy. The titer against E⁺,c⁺,hr⁻ RBCs was 4 with a score of 16. The titer against E⁻,c⁺ RBCs was 0 with a score of 0. At birth, the infant's RBCs typed as E⁻, hr⁻ and the DAT was positive with anti-IgG. The Hct was 40.2 percent with a total bilirubin of 2.1 g/dL.

**Molecular investigation**

Testing on genomic DNA indicated the presence of RHD exons 4, 7, and 10 and intron 4 and was negative for the inactivating RHD pseudogene found in the Black population. The absence of the hybrid Rhesus box indicated a RHD homozygote.⁹ The PsI PCR-RFLP assay was inconclusive as has been reported previously for some ethnic groups.¹⁰

The cDNA sequence analysis identified two different transcripts, a variant RHce, ceAR, and a variant RHD, DAR (Fig. 1). The ceAR allele is characterized by polymorphisms 48G>C (W16C), 712A>G (M238V), 733C>G (L245V), 787A>G (R263G), 800T>A (M267K), and 916A>G (306V). This allele is associated with hr⁻ and VS− phenotypes. The DAR allele is characterized by changes 602C>G (T201R), 667T>G (F223V), and 1025T>C (I342T).¹¹ The DAR allele, also termed weak D type 4.2, is associated with a weak D phenotype and the production of anti-D.
Anti-Rh18 is known to cause HDN. The antibody was first discovered in the serum of a South African woman whose newborn had severe jaundice requiring exchange transfusion. Moores has since reported seven additional cases of HDN related to anti-Rh18 requiring exchange transfusion.

This report describes the use of serologic and molecular methods in the management of a pregnant woman with anti-Rh18. With the risk of HDN, a search was initiated for an Rh:-18 unit. The uncommon nature of this phenotype is evidenced by the lack of an available compatible donor in the ARDP. Procurement of overseas blood was deemed too risky regarding infectious disease testing, and the decision was made to collect an autologous unit from the patient one week before her planned caesarian section. This report and others demonstrate that blood can be donated safely even late in pregnancy.

Anti-Rh18 has caused fatal hemolytic transfusion reactions but identification of compatible donors is hampered by the lack of reagents and poor standardization of serum used for identification of rare RHCE phenotypes. The uncertain nature of reactivity between these samples is emphasized by a report from 2002. Two patients identified by serologic testing to have anti-Rh18 did not have compatible serum and RBCs. In these patients with variant Rh phenotypes, compatible donors are best located through RH genotyping.

Noizat-Pirenne identified three RHCE alleles, ceAR, ceEK, and ceMO, in Afro-Caribbean patients associated with hr– phenotype that can lead to formation of anti-Rh18. The patient described here was homozygous for variant RHCE, ceAR, linked to variant RHD, DAR which explained production of anti-Rh18 and -hr as well as her weak D phenotype. In a group of 326 Black South African blood donors, DAR was present in 4.9 percent of these individuals, ceAR in approximately 6.1 percent and DAR/ceAR in 4.3 percent. This patient stated her ethnic background as Hispanic, demonstrating that groups other than those that identify themselves as Black have variant Rh haplotypes.

Unfortunately, since hr– phenotypes can have varying molecular backgrounds, it is extremely difficult to find compatible units for transfusion of these patients. Also, as this case illustrates, many of these variant RHce are inherited with variant RHD genes that are associated with the formation of anti-D. As more units are RH molecularly characterized throughout the country, it will become easier to find compatible blood for future transfusion support. In addition, the serum from this patient is valuable for large-scale donor screening and will be more informative because of the well-characterized RH genotype. This will contribute to better understanding of the reactivity patterns among variants.

There is clearly a role for molecular testing in transfusion medicine. This report shows the feasibility of RH genotyping and its potential for improving management of patients with uncommon phenotypes. In the future, increased RH genotyping of blood donors and recipients, as well as distribution of rare sera and genotyped RBCs, will increase the availability of rare blood and improve patient care.

References

Discussion

Noizat-Pirenne identified three RHCE alleles, ceAR, ceEK, and ceMO, in Afro-Caribbean patients associated with hr– phenotype that can lead to formation of anti-Rh18. The patient described here was homozygous for variant RHCE, ceAR, linked to variant RHD, DAR which explained production of anti-Rh18 and -hr as well as her weak D phenotype. In a group of 326 Black South African blood donors, DAR was present in 4.9 percent of these individuals, ceAR in approximately 6.1 percent and DAR/ceAR in 4.3 percent. This patient...


Richard L. Haspel, MD, PhD, Department of Pathology, Beth Israel Deaconess Medical Center, Boston, Massachusetts; Dawn Michelle and Richard M. Kaufman, MD, Department of Pathology, Brigham and Women’s Hospital, Boston, Massachusetts; Sunitha Vege, MS and Connie M. Westhoff, SBB, Ph.D, Molecular Blood Group and Platelet Testing Laboratory, American Red Cross Penn-Jersey Region, Philadelphia, Pennsylvania, 19123.

Attention SBB and BB Students: You are eligible for a free 1-year subscription to Immunohematology. Ask your education supervisor to submit the name and complete address for each student and the inclusive dates of the training period to Immunohematology, P.O. Box 40325, Philadelphia, PA 19106.
Patients with sickle cell disease (SCD) typically require transfusions with RBC components, which exposes them to numerous, possibly foreign antigens and potentially causes them to produce an antibody or antibodies to the antigens they lack. As transfusion of these patients increases, the likelihood that they will produce an initial antibody or additional antibodies increases. Once a clinically significant antibody is produced, units of RBCs that lack the associated antigen should be transfused. Often patients with SCD present to the transfusion service with numerous antibodies in their serum, making the search for compatible RBCs a challenge. The American Rare Donor Program (ARDP) has been used to search for RBCs to meet the transfusion needs of this patient population. Between January 2005 and June 2006, approximately 33 percent of the requests to the ARDP for RBC components were for alloimmunized patients with SCD. Of these requests, 94.9 percent were completely or partially filled; requests for r,r, Hy–, and E–, hr– units of RBCs were among the most difficult to fill. This article will discuss the use and effectiveness of the ARDP and testing laboratories associated with the National Reference Laboratory for Blood Group Serology at the American Red Cross in obtaining compatible RBCs for alloimmunized patients with SCD. Immunohematology 2006;22:136–142.

**Key Words:** sickle cell disease, American Rare Donor Program, rare blood donors

Patients with sickle cell disease (SCD) typically require RBC transfusions for treatment of the severe anemia indicative of the disorder and to alleviate the clinical symptoms or prevent the complications of the disease.\(^1\)\(^-\)\(^7\) Patients with SCD who require chronic transfusion support have increased exposure to foreign RBC antigens; this exposure increases not only the likelihood that antibodies will be produced, but also the number of specificities.\(^8\)\(^-\)\(^9\) The incidence of alloimmunization to RBC antigens within this population has been reported as 5 to 40 percent.\(^8\)\(^-\)\(^12\)

Alloimmunization can be reduced by transfusing phenotypically matched RBCs.\(^13\)\(^-\)\(^15\) Such units may be found in ethnically matched donors, from directed donors, or by phenotyping donor RBC components.\(^9\)\(^,\)\(^13\)\(^-\)\(^14\)\(^,\)\(^17\) Although there is a 93 percent probability that RBCs of the E–, C–, Fy(a–), K–, Jk(b–) phenotype would be from an African American donor, only about 10 percent of African Americans in an urban population donate, making it difficult to support the transfusion needs of patients with SCD with these RBCs alone.\(^16\) On the other hand, although about 90 percent of Caucasian donors in this same setting donate, there is only a 7 percent probability that their RBCs would be of this phenotype. This disparity in phenotype increases the time and testing resources needed to obtain phenotype-matched RBCs which may not be cost-effective.\(^9\)\(^,\)\(^14\)\(^,\)\(^17\)

Many transfusion services have defined protocols by which they attempt to reduce the alloimmunization of this patient population, while minimizing testing time and resources. These protocols range from providing limited phenotype-matched RBCs for only C, c, E, and e to providing extended phenotype-matched RBCs for C, c, E, e, K, S, Fy\(^a\), and Jk\(^b\). Castro et al. concluded that limited phenotype matching for C, c, E, e, and K would have prevented alloimmunization in 53.3 percent of the patients in their study and extended phenotype matching would have prevented 70.8 percent.\(^15\) While it would be beneficial to always provide extended phenotype-matched RBCs, these phenotypes are 22.7 times less prevalent among random blood donors, making the testing costly and labor intensive.\(^15\)\(^,\)\(^17\) In addition, considering the emergent nature of sickle cell crisis\(^13\) and the transient nature of patients with SCD who may be treated at various facilities with differing transfusion protocols, it may not be practical to think that all patients with SCD could receive extended phenotype-matched RBCs. Transfusing such patients with nonphenotype-matched RBCs may become necessary, negating the positive effects of previous adherence to a phenotype-matched transfusion protocol.\(^17\)
Although transfusion services may establish protocols to reduce alloimmunization, patients with SCD who receive RBC transfusions often do produce antibodies.\textsuperscript{1,8,12} Specificities of the antibodies produced may be as few as one or as many as more than ten and may include an antibody to a high-incidence antigen.\textsuperscript{8} Finding compatible RBCs is often a challenge for the routine transfusion service.

**Use of the American Rare Donor Program**

**Overview and rare donor criteria**

The American Rare Donor Program (ARDP), formed in 1998 as a merger of the donor databases of the AABB and the American Red Cross (ARC), is a source for obtaining units of rare RBCs. Currently, the ARDP database has phenotype information on more than 35,000 active rare donors in the United States, Puerto Rico, and Milan, Italy. RBCs collected from these rare donors are available to the 81 ARDP member facilities upon request. In addition, nonmember facilities with a transfusion request for rare RBCs may access the ARDP by contacting a member facility, making the services of the ARDP available to all transfusion services and to all patients, both nationally and internationally.

The ARDP is governed by a standard operating procedure (SOP) approved by members of the ARDP advisory committee. This SOP provides guidelines for membership and procedural steps for accessing the ARDP for donor submissions and patient requests.

Demographic and rare phenotype information on donors is submitted by ARDP members, reviewed for appropriateness and completeness, and entered into the database. According to the current ARDP SOP, a donor RBC phenotype is considered rare if it meets one of the following criteria:

1. Group O and group A; R_{1}R_{1}, R_{2}R_{2}, R_{0}R_{0}, or rr; and K:–1; and Fy(a–) or Fy(b–); and Jk(a–) or Jk(b–); and S– or s–

2. Group O and group A; R_{1}R_{1}, R_{2}R_{2}, or rr; and K:–1; and Fy(a–b–)

3. All ABO groups; negative for a high-incidence antigen (1/10,000), such as U, Js\textsuperscript{b}, Kp\textsuperscript{b}, Yt\textsuperscript{b}, or Ge:-2

**Searching for rare units of RBCs**

Patient phenotype requests are entered into the ARDP database and matched with member facilities having registered donors. These facilities are then contacted to determine RBC component availability. If components are not available, the ARDP system manager will expand the search to include recruiting donors, collecting autologous units, testing family members, or, if both RBC components and donors are not available in the United States, initiating an international search.

In addition, if an antibody to a high-incidence antigen is demonstrable in the patient’s serum and antigen-negative RBCs are not available, the transfusion service may be advised to send the patient’s blood sample to the National Reference Laboratory for Blood Group Serology at the ARC for a monocyte monolayer assay (MMA). MMA results can determine the probable clinical significance of that antibody and may allow the transfusion of RBCs that are antigen-positive, if transfusion is imminent.\textsuperscript{18}

Although somewhat effective at procuring rare RBCs or easing phenotype requirements, these additional search efforts are no substitute for having rare RBCs available in inventory when needed. Recruitment efforts are never a guarantee that a transfusable unit will be obtained. The recruiters may be unable to contact the donor, the donor may be temporarily deferred, unwilling, or unable to donate, or the frequency of the transfusion requests may infringe on the 56-day wait requirement between donations. Autologous donations depend on the clinical condition of the patient and family member testing relies on the availability and willingness of family members to be tested as well as the likelihood that their RBC phenotype will be a match.

Importing rare RBCs through an international search is regulated by the FDA with strict guidelines requiring that the physician and patient acknowledge the unlicensed nature of all imported units of RBCs. In addition, imported RBCs cannot be transfused to any other patient, leaving them to be discarded if not transfused to the designated patient.

**Effectiveness of the ARDP**

Although the database currently stores information on more than 35,000 active rare donors, RBCs are not always available at the member facilities when needed to fill a transfusion request. Table 1 shows the effectiveness of the ARDP in filling all patient requests and in filling requests for patients with SCD from January 1, 2005 through June 30, 2006. Approximately 33 percent (351/1070) of the total requests were for patients with SCD; of these, 94.9 percent were completely or partially filled compared with an overall
ARDP fill rate of 93.6 percent. On the basis of the rare donor criteria defined in the ARDP SOP, 210 (59.8%) of the 351 requests were for multiple common antigen-negative RBCs and 141 (40.2%) were for high-incidence antigen-negative RBCs.

Of the 210 requests for multiple common antigen-negative RBCs, 209 (99.5%) were filled (Table 2). The one unfilled request (0.5%) was for rr RBCs, an expected statistic considering that only 6.8 percent of the RBCs within the African American population are of the rr phenotype and only 8.0 percent of the RBCs within the Caucasian population are of the K−, Fy(a−), Jk(b−) phenotype.

Of the 141 requests for high-incidence antigen negative RBCs, 124 (87.9%) were filled (Table 3). The most difficult RBCs to find for this patient population included the phenotypes Jo(a−), Hy−, and r∗r∗ (The request for r∗r∗ also required the RBCs to be Jk[b−]). In addition, requests for Ge−2, and for E−, hrb− RBCs were completely unfilled. (The RBCs for the Ge−2 request had to be C−, E−, K−, Fy(a−), and S− and those for the E−, hrb− request had to be C−; there were no registered phenotype-matched donors in the database for either of these requests.) It is interesting to note that the four requests for E−, hrb− RBCs were completely or partially filled. This will be discussed in a later section.

Impact of antibodies to high- and low-incidence antigens on the fill rate

Although not typically considered clinically significant and the associated antigens are not included in the rare phenotype criteria defined in the ARDP SOP, antibodies against M, N, P1, Lea, or Leb antigens may be clinically significant. If determined as such by the ARDP member facility, these antigens would be added to the RBC search. In addition, requests for RBCs negative for low-incidence antigens would add an extra challenge to the component search; antisera resources are limited and most facilities do not routinely type donor RBCs for these antigens before submitting them to the ARDP. Segments from liquid RBCs or deglycerolized RBC segments would need to be tested. If frozen segments are not available, the requesting facility may need to determine if the risk of waiting for another component of the needed phenotype is worth that of receiving the frozen RBC unit and typing it after it is deglycerolized. This

### Table 1. ARDP requests for rare RBCs (January 1, 2005 to June 30, 2006)

<table>
<thead>
<tr>
<th></th>
<th>Total number of requests</th>
<th>Requests completely filled</th>
<th>Requests partially filled</th>
<th>Requests unfilled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>All patients</td>
<td>1070</td>
<td></td>
<td>912</td>
<td>85.2</td>
</tr>
<tr>
<td>Patients with SCD</td>
<td>351</td>
<td></td>
<td>303</td>
<td>86.3</td>
</tr>
</tbody>
</table>

### Table 2. ARDP requests for common multiple antigen-negative RBCs for patients with SCD (January 1, 2005 to June 1, 2006)

<table>
<thead>
<tr>
<th>Requested phenotype</th>
<th>Total number of requests (n = 210)</th>
<th>Requests completely filled</th>
<th>Requests partially filled</th>
<th>Requests unfilled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>R0*</td>
<td>101</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rr†</td>
<td>53</td>
<td>45</td>
<td>84.9</td>
<td>7</td>
</tr>
<tr>
<td>R1†</td>
<td>41</td>
<td>39</td>
<td>95.1</td>
<td>2</td>
</tr>
<tr>
<td>R2†</td>
<td>15</td>
<td>15</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
<td></td>
<td>200</td>
<td>95.2</td>
</tr>
</tbody>
</table>

*K−; and Fy(a−) or Fy(b−); and Jk(a−) or Jk(b−); and S− or s−
†K−; and Fy(a−) or Fy(b−); and Jk(a−) or Jk(b−); and S− or s− OR K−1; and Fy(a−b−) only

### Table 3. ARDP requests for high-incidence-negative RBCs for patients with SCD (January 1, 2005 to June 1, 2006)

<table>
<thead>
<tr>
<th>Requested phenotype</th>
<th>Total number of requests (n = 141)</th>
<th>Requests completely filled</th>
<th>Requests partially filled</th>
<th>Requests unfilled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>U−, D+</td>
<td>62</td>
<td>47</td>
<td>75.8</td>
<td>8</td>
</tr>
<tr>
<td>Js(b−)</td>
<td>42</td>
<td>40</td>
<td>95.2</td>
<td>1</td>
</tr>
<tr>
<td>U−, D−</td>
<td>8</td>
<td>5</td>
<td>62.5</td>
<td>2</td>
</tr>
<tr>
<td>r∗r*</td>
<td>7</td>
<td>1</td>
<td>14.3</td>
<td>4</td>
</tr>
<tr>
<td>Hy−</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
<td>3</td>
</tr>
<tr>
<td>Jo(a−)</td>
<td>5</td>
<td>2</td>
<td>40.0</td>
<td>1</td>
</tr>
<tr>
<td>E−, hrb−</td>
<td>4</td>
<td>3</td>
<td>75.0</td>
<td>1</td>
</tr>
<tr>
<td>Lu(b−)</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>k−</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>I−</td>
<td>1</td>
<td>0</td>
<td>−</td>
<td>1</td>
</tr>
<tr>
<td>Ge−2</td>
<td>1</td>
<td>0</td>
<td>−</td>
<td>0</td>
</tr>
<tr>
<td>E−, hrb−</td>
<td>1</td>
<td>0</td>
<td>−</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>103</td>
<td>73.0</td>
<td>21</td>
</tr>
</tbody>
</table>
presents a challenge to the transfusion service and to the medical staff caring for the patient.

Table 4 shows the fill rates for requests for rare RBCs required to be negative for multiple common antigens as well as for additional antigens within the M, N, P1, Le\textsuperscript{a}, or Le\textsuperscript{b} group, for one or more low-incidence antigens, or for both. Of the 210 requests, 108 (51.4%) required that the RBCs be negative for additional antigens such as M,N,P1,Le\textsuperscript{a}, or Le\textsuperscript{b}, or low-incidence antigens, such as C\textsuperscript{w}, Jsa, Kp\textsuperscript{a}, V, or VS or both; only 1 of the 210 (0.5%) requests was unfilled.

The fill rates for the requests for high-incidence antigen-negative RBCs shifted toward an increase in the need for additional antigens to be negative as well as an increase in unfilled requests (Table 5). Of the 141 requests, 130 (92.1%) also required that the RBCs be negative for some or all of the common multiple antigens as defined in the ARDP SOP (86 requests) or that additional antigens be negative (44 requests). Of the 141 requests, 18 (12.8%) were unfilled.

The ARDP was able to provide RBCs for 99.5 percent of the requests for patients with SCD needing multiple common antigen-negative RBCs, reflecting the fact that these phenotypes are indicative of the rare criteria defined in the ARDP SOP, are common among the African American donor population, and although not prevalent, are found in the predominantly Caucasian donor population. Obtaining RBCs became more difficult when the requests switched to high-incidence antigen-negative RBCs more characteristic of the African

<table>
<thead>
<tr>
<th>Requested phenotype</th>
<th>Total number of requests (n = 210)</th>
<th>Requests completely filled</th>
<th>Requests partially filled</th>
<th>Requests unfilled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Multiple common antigens\textsuperscript{*}</td>
<td>92</td>
<td>87</td>
<td>94.6</td>
<td>5</td>
</tr>
<tr>
<td>Multiple common antigens\textsuperscript{*} and M- or N- or Le\textsuperscript{a}- or Le\textsuperscript{b}- and low-incidence antigen</td>
<td>44</td>
<td>43</td>
<td>97.7</td>
<td>1</td>
</tr>
<tr>
<td>Multiple common antigens\textsuperscript{*} and low-incidence antigen</td>
<td>43</td>
<td>42</td>
<td>97.7</td>
<td>1</td>
</tr>
<tr>
<td>Multiple common antigens\textsuperscript{*} and M- or N- or Le\textsuperscript{a}- or Le\textsuperscript{b}-</td>
<td>31</td>
<td>28</td>
<td>90.3</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>210</td>
<td>200</td>
<td>95.2</td>
<td>9</td>
</tr>
</tbody>
</table>

\textsuperscript{*}R\textsubscript{1}R\textsubscript{1}, R\textsubscript{2}R\textsubscript{2}, R\textsubscript{0}R\textsubscript{0}, or rr; and K-1; and Fy\textsubscript{(a-b)}; and Jk\textsubscript{(a-b)}; and S- or s- OR R\textsubscript{1}R\textsubscript{1}, R\textsubscript{2}R\textsubscript{2}, or rr; and K-1; and Fy\textsubscript{(a-b)}

<table>
<thead>
<tr>
<th>Requested phenotype</th>
<th>Total number of requests (n = 141)</th>
<th>Requests completely filled</th>
<th>Requests partially filled</th>
<th>Requests unfilled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>High-incidence antigen and multiple common antigens\textsuperscript{*}</td>
<td>86</td>
<td>67</td>
<td>77.9</td>
<td>12</td>
</tr>
<tr>
<td>High-incidence antigen and multiple common antigens\textsuperscript{*} and M- or N- or Le\textsuperscript{a}- or Le\textsuperscript{b}-</td>
<td>16</td>
<td>15</td>
<td>93.8</td>
<td>1</td>
</tr>
<tr>
<td>High-incidence antigen and multiple common antigens\textsuperscript{*} and M- or N- or Le\textsuperscript{a}- or Le\textsuperscript{b}- and low-incidence antigen</td>
<td>14</td>
<td>7</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>High-incidence antigen and multiple common antigens\textsuperscript{*} and low-incidence antigen</td>
<td>12</td>
<td>7</td>
<td>58.3</td>
<td>2</td>
</tr>
<tr>
<td>High-incidence antigen</td>
<td>11</td>
<td>5</td>
<td>45.4</td>
<td>3</td>
</tr>
<tr>
<td>High-incidence antigen, M- or N- or Le\textsuperscript{a}- or Le\textsuperscript{b}-</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>High-incidence antigen and M- or N- or Le\textsuperscript{a}- or Le\textsuperscript{b}- and low incidence</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>103</td>
<td>73.0</td>
<td>20</td>
</tr>
</tbody>
</table>

\textsuperscript{*}R\textsubscript{1}R\textsubscript{1}, R\textsubscript{2}R\textsubscript{2}, R\textsubscript{0}R\textsubscript{0}, or rr; and K-1; and Fy\textsubscript{(a-b)}; and Jk\textsubscript{(a-b)}; and S- or s- OR R\textsubscript{1}R\textsubscript{1}, R\textsubscript{2}R\textsubscript{2}, or rr; and K-1; and Fy\textsubscript{(a-b)}
American population, for example, U- or Js(b-) (87.2%). For these requests, it may have been useful to perform additional testing, such as the MMA, or to initiate an international search for the desired RBCs.

**Expanded search efforts**

As presented earlier, 94.9 percent of the transfusion requests for patients with SCD were filled. This fill rate was achieved, in part, by the ARDP system manager’s expanded search efforts. Generally, such efforts to obtain RBCs for these patients are limited. These patients are not candidates for autologous transfusion; the genetic abnormality of their RBCs creates the clinical symptoms and transfusion needs characteristic of the disease. MMA testing may not be useful in assessing the clinical significance of an antibody to a high-incidence antigen; the typical antibodies produced by these patients are historically known to be clinically significant, such as anti-U, -Js and -Kp. Family member testing may not be effective; family members may have HbS trait or SCD and would not be suitable donors. Donor recruitment and international searches may be the main mechanisms for procuring RBCs for this patient population.

In addition, the variant nature of the genotypes within the Rh blood group system does not always ensure that antigen-negative RBCs will be serologically compatible. In particular, e variants, hr8- and hr4-, common in individuals of African descent, are notorious for this disparity and have created additional challenges for the ARDP to provide RBCs for patients with SCD and to safeguard against the unnecessary shipment of rare components. To help reduce the risk to rare components, ARDP works with the molecular testing laboratory at the Penn-Jersey Region of the ARC in Philadelphia to provide RH molecular characterization of patient and potential donor samples to determine compatibility. Donor testing is especially beneficial for creating a database of molecularly characterized units that can then be genotypically matched to future patients’ needs, ensuring compatibility and reducing the alloimmunization of these patients.

**Challenging phenotypes**

Since April of 2000, 29 requests for rare RBCs of the phenotype group O, r1r1, K:-1, Jk(b–) have been submitted to the ARDP. Only 12 donors with this phenotype are in the ARDP database, 3 of whom are more than 75 years old. With only nine potential donors, it was often unlikely that components of this phenotype were available when needed and recruitment efforts were typically undertaken. As stated previously, recruitment efforts are never a guarantee that a transfusable unit will be obtained. Interestingly, RBCs of this phenotype were obtained for 24 of the 29 requests, attributing much to the ARDP, its members, and its donors.

Another difficult phenotype request to fill was that for group O, R1R1, U– RBCs. Since October 2002, 11 requests have been submitted to the ARDP for this phenotype. Only 7 donors with this RBC phenotype were in the database and again, RBCs were not available and recruitment efforts were undertaken. However, in this case, only 4 of the requests resulted in units of RBCs; recruitment efforts were not as effective.

Although not made as often as those for RBCs negative for multiple common antigens, requests for e variant RBCs, such as hr8- or hr4-, do occur (Table 2). These requests present additional challenges to the ARDP because of the low number of donors with these phenotypes and of the diversity of the variants. Even if antigen-negative RBCs are located, they may be incompatible with the serum of the patient. In addition, the patient may have other antibodies, narrowing the search for and the availability of compatible RBCs. Requests for RBCs began in March 1996 for the following phenotype: group O, R1, K:–1, Fy(a–b–), S–, and hr8-. Although there were no registered donors in the database, antigen-negative RBCs were located in the United States and were transfused. Additional requests in April 1996 and July 1996 also resulted in compatible RBCs within the United States. Requests for rare RBCs of this phenotype were not submitted to the ARDP again until July 2002, at which time RBCs were not located in the United States nor were components located for the same request in December 2003. In July 2005, the requesting facility requested that an international search be initiated. Contact with the South African National Blood Service in South Africa procured two units in July 2005 and again in March 2006.

In April 2005, the ARDP received a request for RBCs of the phenotype group O, R1R1, Jk(b–), and hr8-. Five donors with RBCs of that phenotype were in the ARDP database; all were contacted. One donor no longer donated because of age, but the other four donors responded to the recruitment effort. One donor was deferred; the other three donated at various ARDP member facilities across the United States. Samples from...
Use of the ARDP for patients with SCD

each of the donors and from the patient were sent to the molecular testing lab at the American Red Cross in Philadelphia for genotyping. Once the donors with the best genotype match were determined, their liquid units of RBCs were shipped to the transfusing site.

Summary

Patients with SCD present challenges to transfusion services because of the numerous antibody specificities they may produce, the numerous times that they may be transfused, the disparity between the RBC phenotype of the African American patient population and that of the predominantly Caucasian donor population, and the variability within the genetic makeup of their RBC antigens. Some transfusion services institute protocols for providing antigen-negative RBCs for these patients to decrease alloimmunization while others match only for ABO and D. Donor populations, staffing, and testing resources all influence the transfusion protocols chosen by these services. Regardless of the selected protocol, the ARDP serves as a critical source for rare RBCs needed for patients with SCD.

References


Source:
Molecular characterization of GYPB and RH in donors in the American Rare Donor Program

S. Vege and C. M. Westhoff

Transfusion of patients with sickle cell disease (SCD) has been a challenge in clinical transfusion medicine, especially when the required donor RBCs must be U− and negative for high-prevalence RH phenotypes (hrB, hrS). It is now possible to genotype donors to identify or confirm Uvar and U− phenotypes, as well as Rh hrA− and hrH− phenotypes, and to characterize the different RH backgrounds found in these donors. In a preliminary study of donors registered in the American Rare Donor Program, twelve different RH backgrounds were identified in eighteen hrB− or hrS− donors. These results, summarized in the current report, confirm the heterogeneous nature of these phenotypes and are relevant for selection of donor units for patients with antibodies to high-prevalence Rh antigens. Not all phenotypically similar units will be compatible, and matching the Rh genotype of the donor to the patient is important to prevent further Rh sensitization. Most donors referred were hrB− and carry at least one hybrid RHCE*CE(3-7)-D gene that encodes a variant C antigen linked to RHCE*ce that encodes the VS+V− phenotype. Surprisingly, the majority of donors were heterozygous, some even carrying conventional alleles, suggesting that the loss of expression of the hrH− epitopes on RBCs is a dominant phenotype. Although antigen-matching of patients with SCD with donors for C, E, and K antigens has decreased the incidence of alloimmunization, some patients still become sensitized to Rh antigens. Not all antibodies that are called anti-hrB or -hrS have identical specificities. Neither hrB− nor hrS− is associated with one specific genetic polymorphism. These phenotypes encompass multiple different Rh protein polymorphisms encoded by numerous RHCE*ce genes, and the structural determinants that define these specificities are not yet known.

An important consideration for transfusion in patients with antibodies to these high-prevalence antigens is that not all donors with these phenotypes will be compatible. Additionally, because they occur on very diverse Rh backgrounds, the complete Rh genotype of the donor and patient should be considered to prevent further Rh alloimmunization. In the United States, because these phenotypes are more common in Blacks, the search for potential donors in the United States targets African American groups. However, the lack of well-characterized serologic reagents hampers screening efforts.

Genotyping is an important new tool, both for screening donors and for typing patients, to provide appropriate units for transfusion when antibodies to high-prevalence antigens are present. Within the Rh system, the clinically significant anti-hrB and -hrS have caused transfusion reactions and fatalities. Transfusion in these situations is not straightforward because not all antibodies that are called anti-hrB or -hrS have identical specificities. Neither hrB− nor hrS− is associated with one specific genetic polymorphism. These phenotypes encompass multiple different Rh protein polymorphisms encoded by numerous RHCE*ce genes, and the structural determinants that define these specificities are not yet known.

An important consideration for transfusion in patients with antibodies to these high-prevalence antigens is that not all donors with these phenotypes will be compatible. Additionally, because they occur on very diverse Rh backgrounds, the complete Rh genotype of the donor and patient should be considered to prevent further Rh sensitization.

With recent advances in genotyping techniques and knowledge of the molecular basis of expression of variant antigens, it is now feasible to genotype donors at the GYPB and RH loci to identify U− and Rh variant RBC donors. Here we propose an approach to genotype ARDP donors to identify or confirm Uvar and U− phenotypes, and we illustrate the power of genotyping to confirm hrA− or hrH− RBC phenotypes and to characterize the different RH gene backgrounds found in these donors.
**GYPB Testing to Characterize U– and U⁺var**

In Black ethnic groups, the S–s– phenotype is usually characterized by complete deletion of the GYPB gene and absence of the U antigen, but the S–s– phenotype is also associated with weak expression of U (U⁺var). The antisera used for phenotyping are not always well characterized and S–s–U⁺var donors may be misidentified as U–. This is problematic when searching for U– units for GYPB-deleted patients with anti-U. The GYPB gene is not deleted when the RBCs have a U⁺var phenotype. The molecular basis of U⁺var involves changes in or around exon 5 of the GYPB gene. The GYPB(P2) allele has a splice site mutation in intron 5 (+5 g>τ) that causes complete skipping of exon 5, while GYPB(NY) results from a point mutation (230C>T) in exon 5 that causes partial exon skipping. Testing for the presence of these mutations discriminates S–s–U– donors from U⁺var donors. Molecular genotyping is encouraged to confirm the U status of donors entered into the ARDP registry as U– or as S–s–U⁺var.

**RH Testing to Characterize hr⁺ and hr⁻**

The two highly homologous genes RHHD and RHCHE encode proteins that carry D, and C or c, and E or e antigens, respectively. However, more than 50 Rh serologic specificities are known; these are the result of point mutations or hybrid RHHD and RHCHE. More than 120 different RHHD variants, and approximately 50 different RHCHE variants, have been reported to date. The numerous genetic polymorphisms potentially encode different antigenic forms of the Rh proteins. Altered C, e, or D antigens are not uncommon in patients with SCD. The altered C antigen is encoded by a hybrid RHHD-CE(3-7)-D gene that does not encode D epitopes but encodes a C antigen that differs from that found in Europeans. Altered e antigen expression is encoded by many different genes more commonly found in Blacks, and altered D antigen is associated with numerous gene mutations found in many different ethnic groups. Altered Rh antigens are associated with the absence of the high-prevalence antigens hr⁺ and hr⁻ on RBCs.

To investigate the RH genes in potential hr⁺ or hr⁻ donors referred to the ARDP, DNA was isolated from WBCs. Fresh RBCs, if available, were tested with the anti-hr⁺-like monoclonal antibody FOR-2E3. Characterization of the RH genes was performed with a combination of PCR and RFLP or allele specific (AS)-PCR techniques in addition to amplification and sequencing of RH-specific exons. RHD zygosity testing was determined by direct detection of the hybrid Rhesus box.

**Summary**

Table 1 summarizes the hr⁺ or hr⁻ status of the donor RBCs determined by the referring laboratory, the results of testing with the anti-hr⁺-like FOR-2E3 monoclonal antibody by the American Red Cross National Reference Laboratory for Blood Group Serology (NRLBGS), and the Rh phenotype of the eighteen donors studied.

Fifteen of the donors were referred to the ARDP as apparent hr⁺–, two as hr⁻– (donors 6 and 18), and one was both hr⁺– and hr⁻– (donor 9). Fresh RBCs were available from 12 of the 18 donors for testing with anti-hr⁺-like FOR-2E3 monoclonal antibody. RBCs from only two of the ten hr⁺– donors tested did not react (donors 7 and 12). The RBCs from one apparent hr⁺– donor (donor 6) and the hr⁻–/hr⁻ (donor 9) also did not react. In total, the monoclonal FOR-2E3 antibody gave negative reactions with only four of twelve donor RBCs that lack high-prevalence Rh antigens.

Six donors were D– and twelve were D+. RBCs from all donors phenotyped as C+, c+, E–, e+ with the exception of one, donor 9, whose RBCs were C– and also c+, E–, and e+.

<table>
<thead>
<tr>
<th>Donor</th>
<th>hr⁺–/hr⁻–</th>
<th>FOR-2E3</th>
<th>Rh phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>hr⁺–</td>
<td>-</td>
<td>D– C+c+E–e+</td>
</tr>
<tr>
<td>2</td>
<td>hr⁺–</td>
<td>-</td>
<td>D– C+c+E–e+</td>
</tr>
<tr>
<td>3</td>
<td>hr⁺–</td>
<td>+w</td>
<td>D– C+c+E–e+</td>
</tr>
<tr>
<td>4</td>
<td>hr⁺–</td>
<td>-</td>
<td>D– C+c+E–e+</td>
</tr>
<tr>
<td>5</td>
<td>hr⁺–</td>
<td>-</td>
<td>D– C+c+E–e+</td>
</tr>
<tr>
<td>6</td>
<td>hr⁺–</td>
<td>0</td>
<td>D– C+c+E–e+</td>
</tr>
<tr>
<td>7</td>
<td>hr⁺–</td>
<td>0</td>
<td>D+ C+c+E–e+</td>
</tr>
<tr>
<td>8</td>
<td>hr⁺–</td>
<td>-</td>
<td>D+ C+c+E–e+</td>
</tr>
<tr>
<td>9</td>
<td>hr⁺–/hr⁻–</td>
<td>0</td>
<td>D+ C+c+E–e+</td>
</tr>
<tr>
<td>10</td>
<td>hr⁺–</td>
<td>2+</td>
<td>D+ C+c+E–e+</td>
</tr>
<tr>
<td>11</td>
<td>hr⁺–</td>
<td>-</td>
<td>D+ C+c+E–e+</td>
</tr>
<tr>
<td>12</td>
<td>hr⁺–</td>
<td>0</td>
<td>D+ C+c+E–e+</td>
</tr>
<tr>
<td>13</td>
<td>hr⁺–</td>
<td>1+s</td>
<td>D+ C+c+E–e+</td>
</tr>
<tr>
<td>14</td>
<td>hr⁺–</td>
<td>1+</td>
<td>D+ C+c+E–e+</td>
</tr>
<tr>
<td>15</td>
<td>hr⁺–</td>
<td>-</td>
<td>D+ C+c+E–e+</td>
</tr>
<tr>
<td>16</td>
<td>hr⁺–</td>
<td>1+s</td>
<td>D+ C+c+E–e+</td>
</tr>
<tr>
<td>17</td>
<td>hr⁺–</td>
<td>2+</td>
<td>D+ C+c+E–e+</td>
</tr>
<tr>
<td>18</td>
<td>hr⁺–</td>
<td>2+</td>
<td>D+ C+c+E–e+</td>
</tr>
</tbody>
</table>

*sample was positive with one source and negative with another.
Table 2. Summary of twelve different RH backgrounds identified in eighteen donors

<table>
<thead>
<tr>
<th>Donor</th>
<th>Rh phenotype</th>
<th>RHD Genes</th>
<th>RHCE Genes</th>
<th>Total # of donors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D-CE(3-7)-D</td>
<td>D-CE(3-7)-D</td>
<td></td>
</tr>
<tr>
<td>1, 2</td>
<td>D- C+c+E-e+</td>
<td>D-CE(3-7)-D</td>
<td>D-CE(3-7)-D</td>
<td>*ce 2</td>
</tr>
<tr>
<td>3, 4</td>
<td>D- C+c+E-e+</td>
<td>D-CE(3-7)-D</td>
<td>D-CE(3-7)-D or Deleted D</td>
<td>Ce 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>D- C+c+E-e+</td>
<td>D-CE(3-7)-D</td>
<td>Deleted D</td>
<td>Ce (48C) 1</td>
</tr>
<tr>
<td>6</td>
<td>D- C+c+E-e+</td>
<td>D-CE(3-7)-D</td>
<td>Deleted D</td>
<td>Ce 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>D+ C+c+E-e+</td>
<td>D-CE(3-7)-D</td>
<td>DIII type 5</td>
<td>Ce (48C) 1</td>
</tr>
<tr>
<td>8</td>
<td>D+ C+c+E-e+</td>
<td>D-CE(3-7)-D</td>
<td>D</td>
<td>Ce (48C) 1</td>
</tr>
<tr>
<td>10, 11, 12, 14</td>
<td>D+ C+c+E-e+</td>
<td>D-CE(3-7)-D</td>
<td>DAU</td>
<td>Ce 4</td>
</tr>
<tr>
<td>13</td>
<td>D+ C+c+E-e+</td>
<td>D-CE(3-7)-D</td>
<td>DAU</td>
<td>Ce (48C) 1</td>
</tr>
<tr>
<td>15</td>
<td>D+ C+c+E-e+</td>
<td>D-CE(3-7)-D</td>
<td>DAU type 1</td>
<td>Ce 1</td>
</tr>
<tr>
<td>16, 17</td>
<td>D+ C+c+E-e+</td>
<td>D-CE(3-7)-D</td>
<td>D</td>
<td>Ce 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>D+ C+c+E-e+</td>
<td>DAU-0</td>
<td>ceMO</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DAR D</td>
<td>ceAR Ce</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>D+ C+c+E-e+</td>
<td>DAR D</td>
<td>ceAR Ce</td>
<td>1</td>
</tr>
</tbody>
</table>

Molecular characterization of donors in the ARDP

All of the D- donors and ten D+, hrB- donors had at least one RHDC-CE(3-7)-D hybrid. The RBCs of all phenotyped as C+ but the samples genotyped as RHDC- by multiplex PCR assay. RHDC/c genotyping results are discordant in samples that carry the hybrid RHDC-CE(3-7)-D gene that encodes an altered C antigen.

Only three of the donors had a conventional RHDC (donors 8, 16, and 17). Seven had partial D, which included five DAU, one DIII type 5, and one DIva type 1. Partial D was also present in the hrB-/hrB- donor 9, who was homozygous for DAU-0, and in the hrB- donor 15 who had a DAR allele and a conventional RHDC. DAR is known to be linked to the ceAR allele.15

Conclusion

This report summarizes the preliminary results of genotyping eighteen ARDP donors identified as negative for the high-prevalence Rh antigens hrB or hrB. Twelve different RH backgrounds were found in the eighteen donors phenotyped as hrB- or hrB-, confirming the heterogeneous nature of these phenotypes.3

The majority of donors referred were confirmed to be hrB-. They all had at least one hybrid RHDC-CE(3-7)-D gene encoding altered C linked to a ceB allele that encodes the VS+ V- phenotype. These samples phenotyped as hrB- or hrB-, confirming the heterogeneous nature of these phenotypes.3

The amino acid changes Leu245Val and Gly336Cys may exert a dominant influence on expression of the hrB epitopes on RBCs from the donor homozygous for D-CE(3-7)-D or ceB donors (12) were heterozygous. A hybrid RHDC-CE(3-7)-D gene encoding altered C expression is linked to the ceB allele.14

Indeed, the majority of the ceB alleles were found with the hybrid RHDC-CE(3-7)-D gene. However, in donors 7 and 8, RHDC-DIII type 5 or conventional RHDC was also found with the ceB allele.

One of the predicted hrB- donors (donor 6) had an RH genotype more consistent with a hrB- phenotype, i.e., a hybrid RHDC-CE(3-7)-D gene with ceB. In addition, the RBCs did not react with the anti-hrB-like FOR-2E3 monoclonal antibody. The hrB- and hrB- donor (donor 9) was homozygous 48C (16Cys) and 667T (223Phe), characteristic of ceMO alleles.2 Donor 18 was heterozygous for RHDC-CEAR (16Cys, 238Val, 245Val, 263Gly, 267lys, 306Val)15 and for a conventional RHDCB.
Transfusion of patients with SCD represents a significant challenge in clinical transfusion medicine. SCD may be the single disease for which transfusion therapy may increase in the next decade as a result of the stroke prevention trial in sickle cell anemia.\textsuperscript{16,17} Complications of chronic transfusion include iron overload and alloimmunization. The recent availability of oral iron chelation agents is predicted to make transfusion a more acceptable treatment option. To address the problem of alloimmunization, many programs transfuse patients with RBCs that are phenotype-matched for D, C, E, and K, and some programs attempt to supply RBCs from African American donors to SCD patients whenever possible. Although transfusion of antigen-matched units reduces the incidence of alloantibody production, some patients with SCD will still become sensitized to Rh antigens, indicating units were not truly Rh antigen matched. The prevalence in the sickle cell population of RH alleles that encode altered e, C, or D explains why these patients become immunized despite conventional antigen-matching.

The hr\textsuperscript{a–} and hr\textsuperscript{a–} donors are an important resource for the management of alloimmunized patients with SCD. With the use of RH genotyping, patients with SCD who are homozygous for variant alleles and who are at risk for production of “apparent auto” and alloantibodies to high-prevalence Rh antigens can now be identified. RH genotyping of these patients, partnered with RH genotyping of donors, would have a positive impact on patient care because it allows the selection of both compatible units and units that eliminate the risk of further Rh alloimmunization. This approach would also optimize the use of donations from members of minority groups.

Our goal is to characterize the RH genes in these rare donor units. Patients with SCD who make antibodies to high-prevalence Rh antigens will then be RH genotyped and blood for transfusion will be based on an RH “genetic” match. It is anticipated that the implementation of molecular genetic methods for transfusion in SCD will move transfusion practice into the age of molecular medicine.

Acknowledgments

Many thanks to Kathy Weber and Sue Johnson at the BloodCenter of Wisconsin, John Ochsenfeld at Northern California ARC Region, Florida’s Blood Center, Florida Blood Services, and ITxM (Institute for Transfusion Medicine) for referring the donors. We are especially thankful to the NRLBGS reference laboratory staff for performing the FOR-2E3 monoclonal testing.

References


Sunitha Vege, MS, Supervisor; and Connie M. Westhoff, MT (ASCP)SBB, PhD, Scientific Director, Molecular Blood Group and Platelet Testing Laboratory, American Red Cross Penn-Jersey Region, 700 Spring Garden Street, Philadelphia, Pennsylvania, 19123.
Robert Royston Amos (Robin) Coombs

Robin Coombs, the renowned Cambridge University immunologist, who in the 1940s devised the critical diagnostic test that bears his name, died on January 25, 2006, after a long illness. He is the last survivor of the distinguished group of British immunologists who were responsible for the renaissance of British immunology after the Second World War.

Robert Royston Amos Coombs was born in London, January 9, 1921, and grew up in Cape Town, South Africa. He returned to study veterinary medicine at the Royal Veterinary College, Edinburgh, Scotland. It was while working at the Veterinary Research Center, Weybridge, England, on the serodiagnosis of glanders, a horse disease caused by Burkholderia mallei infection, with the sensitive serodiagnostic test, the complement-dependent conglutination reaction, and reading the early immunologic literature about this reaction that he became interested in immunology (particularly in antibodies and red cells).

In 1944 he went to Cambridge University as a PhD student in the Department of Pathology, where he remained until his retirement in 1988. Soon after obtaining his PhD in 1947, he became assistant director of research, and in 1966, became the Quick Professor of Biology. He was the prime mover in the development of clinical immunology, developed new critical tests, and trained many of the world’s leading immunologists. He was the author of 299 scientific papers and three books. He retired in 1988 and spent the rest of his life in Cambridge. He was a Stringer Fellow at Kings College and later a Fellow at Corpus Christi College.

During the Second World War, Professor R.A. Fisher’s Galton Laboratory Serum Unit of the National Medical Research Council was relocated to the Department of Pathology at Cambridge University. Coombs came in contact with Rob Race and Arthur Mourant, who were working on the recently discovered clinical important rhesus (Rh) blood group system incompatibility between mother and fetus that caused hemolytic disease of the newborn. The immunology and genetics of the system showed that, in addition to the normal “complete” form of the anti-Rh antibody, which agglutinated Rh-positive red cells directly, there existed an “incomplete” antibody that could only be detected by Race’s very involved so-called “blocking antibody test.”

In a discussion over afternoon tea one day with Race and Mourant, Race turned to Coombs and stressed there was a real need for a simpler, better test to measure these so-called “incomplete” antibodies. According to a tale that has become something of an immunologic legend, Coombs developed the principle behind the antiglobulin test while traveling to Cambridge from London that evening on an ill-lit wartime train. Coombs reflects, in a 1988 article, how “unable to read, I was pondering how to measure these antibodies on red cells.” Reflecting on Ehrlich’s side-chain theory, he deduced that when these incomplete antibodies reacted with the red blood cells, the red cells would become coated with anti-Rh immunoglobulin and that a further antibody against the globulin fraction of the serum would then agglutinate the cells. In subsequent series of experiments conducted with Mourant and Race, this technique proved to be extremely useful in detecting Rh antibodies and other incomplete IgG antibodies. The description of the method and application to various diseases was published in The Lancet and British Journal of Experimental Pathology in 1945 and 1946. Within a very short time, the antiglobulin
(Coombs) test was adopted by virtually every hematology laboratory and blood transfusion service worldwide.

He preferred the test not to be called the Coombs test. Because of simplicity, however, the antiglobulin test is almost universally referred to as the Coombs test. Despite the stellar career, he continually demonstrated personal humility. He was a self-effacing man who didn’t seek fame.

The work with which the name of Coombs will always be associated, the discovery while still a graduate student of the antiglobulin reaction, is only a small part of his enormous contribution to immunology. He played a leading role in the development of the clinical immunology. His research interests were varied, involving such unrelated areas as asthma and allergy, transplantation surgery, rheumatology, and autoimmunity. Clinicians and scientists worldwide came to exchange ideas on disease mechanisms and to work and study with him. He was always involved in multiple scientific investigations simultaneously.

He was devoted to his laboratory work and training of a large number of PhD students who came to his laboratory from many parts of the world. He was a person of towering intellect with a remarkably wide range of scientific interests and a talent for technical innovation. He was a careful experimentalist; everything had to be brought to the highest attainable level of excellence; he was a perfectionist, a hard taskmaster, and a very inspirational teacher. Those of us fortunate enough to come under his influence were deeply affected by his infectious enthusiasm. He was especially generous in including us in visits with the many outstanding internationally renowned scientists who came into his laboratory. He cared enormously about those who trained with him and followed our careers with affectionate interest.

He was elected to the Royal Society in 1965 and in 1973 was elected as Honorary Fellow of the Royal College of Physicians, a rare honor for a nonmedically qualified person. He had an impressive list of honorary degrees and awards for his work from the Universities of Guelph, the Netherlands, and Edinburgh. He received many prizes and awards, including the AABB Karl Landsteiner Memorial Award. It was the great respect and affection he earned from all those who passed through his laboratory, however, that gave him the greatest satisfaction.

Angelyn A. Konugres, PhD (retired)
Director of Immunohematology Laboratory
Brigham & Women’s Hospital
Principal Associate in Obstetrics & Gynecology and Reproductive Biology, Harvard Medical School
Boston, MA

Reprinted with permission from Transfusion, Volume 46, October 2006.
DEPARTMENT OF CLINICAL LABORATORY SCIENCES
SCHOOL OF ALLIED HEALTH PROFESSIONS
VIRGINIA COMMONWEALTH UNIVERSITY

Faculty Position

The Department of Clinical Laboratory Sciences at Virginia Commonwealth University invites applications for a full-time, 12 month, tenure-track faculty position. The Department, located on the MCV Campus of VCU, is one of nine departments in the School of Allied Health Professions. VCU is a large urban, research-extensive institution with a richly diverse university community and commitment to multicultural opportunities. The Department offers both B.S. and M.S. degree programs in Clinical Laboratory Sciences and provides the CLS specialty track in the Ph.D. program in Health Related Sciences.

The successful candidate will be responsible for teaching clinical immunology and immunohematology (blood banking) courses on campus and on line at the undergraduate and graduate levels, interacting with clinical faculty at affiliated clinical sites, and student mentoring. Also expected are scholarly activities and research, university service responsibilities, and professional activities.

Applicants must have a master's degree (Ph.D. preferred), national certification as a generalist in the clinical laboratory, clinical or college teaching experience, excellent interpersonal and written and oral communication skills, and demonstrated scholarly productivity. Preference will be given to applicants with specialist certification in blood banking and a record of active participation in professional societies.

Salary and rank will be commensurate with education and experience.

Review of applications will begin immediately and continue until the position is filled. Send a letter of interest, curriculum vita, and the names of three references to: William Korzun, Ph.D., Department of Clinical Laboratory Sciences, Virginia Commonwealth University, P O Box 980583, Richmond, VA 23298-0583.

“Virginia Commonwealth University is an equal opportunity/affirmative action employer. Women, minorities and persons with disabilities are encouraged to apply.”
ERRATUM

Vol. 22, No. 2, 2006; page 47

With many thanks! Dedication to Marilyn K. Moulds

A reader has informed the editors of Immunohematology that there is an error on page 47, fifth paragraph, third sentence. The sentence should read “She has also been an active member of organizations such as the AABB, South Central Association of Blood Banks (SCABB)...”

Free Classified Ads and Announcements

Immunohematology will publish classified ads and announcements (SBB schools, meetings, symposia, etc.) without charge. Deadlines for receipt of these items are as follows:

Deadlines
1st week in January for the March issue
1st week in April for the June issue
1st week in July for the September issue
1st week in October for the December issue

E-mail or fax these items to Cindy Flickinger, Managing Editor, at (215) 451-2538 or flickingerc@usa.redcross.org.

Manuscripts: The editorial staff of Immunohematology welcomes manuscripts pertaining to blood group serology and education for consideration for publication. We are especially interested in case reports, papers on platelet and white cell serology, scientific articles covering original investigations, and papers on new methods for use in the blood bank. Deadlines for receipt of manuscripts for consideration for the March, June, September, and December issues are the first weeks in November, February, May, and August, respectively. For instructions for scientific articles, case reports, and review articles, see “Instructions for Authors” in every issue of Immunohematology or on the Web. Include fax and phone numbers and e-mail address with your manuscript.
**ANNOUNCEMENTS**

**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, -Js, -Fy3, -Wr, -Xg, -CD99, -Do, -H, -Ge2, -CD55, -Ok, -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, and -Fy). Some are directly agglutinating (Anti-M, -Wr and -Rh17) and one has been humanized into the IgM isoform (Anti-Js). 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 website at http://www.nybloodcenter.org >research >immunochemistry >current list of monoclonal antibodies available.

**March 23-25 AABB Immunohematology Reference Laboratory (IRL) Conference 2007**
The AABB Immunohematology Reference Laboratory (IRL) Conference 2007 will be held March 23 through 25, 2007, at the Hyatt Regency in Albuquerque, New Mexico. Continuing education credits will be provided. Registration will begin in January 2007. For more information, contact the AABB Department of Meetings and Programs at (301) 215-6480 or, visit the Web site at http://www.aabb.org >meetings and events >national and regional conferences.
ANNOUNCEMENTS CONT’D

MSc in Transfusion and Transplantation Sciences

Are you working in NHS or National Blood Service and are looking for training? This course could be for you.

Applications are invited from medical or science graduates to study for the MSc in Transfusion and Transplantation Sciences. The course is run jointly by The Department of Cellular & Molecular Medicine, University of Bristol and the Bristol Institute of Transfusion Sciences.

The course starts in October 2007 and can be studied full-time for 1 year or part-time over 2 or 3 years by block release.

The course aims to develop your interest, knowledge and understanding of the theory, practical techniques and research relating to the science of transplantation and transfusion medicine.

For example,
- How is blood processed?
- When do we give platelet transfusions?
- How is tissue engineering being used to replace heart valves?
- What causes haemolytic anaemia?
- How do we reduce the transfusion related risk of HIV and vCJD?

Teaching combines informal lectures, tutorials, practical laboratory experience and a research project with the bias on transfusion.

The lecture units are: Haemopoiesis, Immunology, Platelets and coagulation, Blood groups, Haematological diseases, Blood donation, Blood components, Clinical transfusion, Transfusion transmitted infections, Stem cell transplantation, Solid organ transplantation and Tissue engineering.

The course is accredited by The Institute of Biomedical Sciences and directed by Professor David Anstee and Dr Tricia Denning-Kendall.

For further details visit:
http://www.blood.co.uk/ibgrl/MSc/MScHome.htm
or contact:
Dr Tricia Denning-Kendall,
University of Bristol, Geoffrey Tovey Suite,
TEL 0117 9912093, E-MAIL P.A.Denning-Kendall@bristol.ac.uk
Instructions for Authors

SCIENTIFIC ARTICLES, REVIEWS, AND CASE REPORTS
Before submitting a manuscript, consult current issues of Immunohematology for style. Type the manuscript on white bond paper (8.5" × 11") and double-space throughout. Number the pages consecutively in the upper right-hand corner, beginning with the title page. 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—see 7 under Preparation
8. Figures—see 8 under Preparation

Preparation of manuscripts
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 and J.H. BROWN
   C. Running title of ≤ 40 characters, including spaces
   D. 3 to 10 key words
2. Abstract
   A. One paragraph, no longer than 300 words
   B. Purpose, methods, findings, and conclusions of study
3. Key words—list under abstract
4. Text (serial pages)
   Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Results of surveys and review papers are examples that may need individualized sections.
   A. Introduction
      Purpose and rationale for study, including pertinent background references.
   B. Case Report (if study calls for one)
      Clinical and/or hematologic data and background serology.
   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 patients’ names or hospital numbers.
   D. Results
      Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable.
   E. Discussion
      Implications 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.
   C. Use inclusive pages of cited references, e.g., 1431–7.
   D. Refer to current issues of Immunohematology for style.
7. Tables
   A. Head each with a brief title, capitalize first letter of first word (e.g., Table 1. Results of ...), and 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 explanations 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 academic degree, position held, institution and department, and complete address (including zip code) for all authors. List country when applicable.

SCIENTIFIC ARTICLES AND CASE REPORTS SUBMITTED AS LETTERS TO THE EDITOR
Preparation
1. Heading—To the Editor:
2. Under heading—title with first letter capitalized.
3. Text—write in letter format (paragraphs).
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. One table and/or figure allowed.

Send all manuscripts by e-mail to:
Marge Manigly at mmanigly@usa.redcross.org
NATIONAL REFERENCE LABORATORY FOR BLOOD GROUP SEROLOGY

Immunohematology Reference Laboratory
AABB, ARC, New York State, and CLIA licensed
(215) 451-4901—24-hr. phone number
(215) 451-2538—Fax

American Rare Donor Program
(215) 451-4900—24-hr. phone number
(215) 451-2538—Fax
ardp@usa.redcross.org

Immunohematology
(215) 451-4902—Phone, business hours
(215) 451-2538—Fax
immuno@usa.redcross.org

Quality Control of Cryoprecipitated-AHF
(215) 451-4903—Phone, business hours
(215) 451-2538—Fax

Granulocyte Antibody Detection and Typing
• Specializing in granulocyte antibody detection and granulocyte antigen typing
• Patients with granulocytopenia can be classified through the following tests for proper therapy and monitoring:
  —Granulocyte agglutination (GA)
  —Granulocyte immunofluorescence (GIF)
  —Monoclonal Antibody Immobilization of Granulocyte Antigens (MAIGA)

For information regarding services, call Gail Eiber at: (651) 291-6797, e-mail: eiber@usa.redcross.org,
or write to:
Neutrophil Serology Reference Laboratory
American Red Cross
St. Paul Regional Blood Services
100 South Robert Street
St. Paul, MN 55107

National Platelet Serology Reference Laboratory
Diagnostic testing for:
• Neonatal alloimmune thrombocytopenia (NAIT)
• Posttransfusion purpura (PTP)
• Refractoriness to platelet transfusion
• Heparin-induced thrombocytopenia (HIT)
• Alloimmune idiopathic thrombocytopenia purpura (AITP)

Medical consultation available

Test methods:
• GTI systems tests
  —detection of glycoprotein-specific platelet antibodies
  —detection of heparin-induced antibodies (PF4 ELISA)
• Platelet suspension immunofluorescence test (PSIFT)
• Solid phase red cell adherence (SPRCA) assay
• Monoclonal antibody immobilization of platelet antigens (MAIPA)
• Molecular analysis for HPA-1a/1b

For information, e-mail: immuno@usa.redcross.org or call:
Maryann Keashen-Schnell
(215) 451-4041 office
(215) 451-4205 laboratory

Sandra Nance
(215) 451-4362

American Red Cross Blood Services
Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594
IgA/Anti-IgA Testing

IgA and anti-IgA testing is available to do the following:
• Monitor known IgA-deficient patients
• Investigate anaphylactic reactions
• Confirm IgA-deficient donors

Our ELISA assay for IgA detects antigen to 0.05 mg/dL.

For information on charges and sample requirements, call (215) 451-4909, e-mail: flickingerc@usa.redcross.org, or write to:

American Red Cross Blood Services
Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594
ATTN: Cindy Flickinger

CLIA LICENSED

National Neutrophil Serology Reference Laboratory

Our laboratory specializes in granulocyte antibody detection and granulocyte antigen typing.

Indications for granulocyte serology testing include:
• Alloimmune neonatal neutropenia (ANN)
• Autoimmune neutropenia (AIN)
• Transfusion related acute lung injury (TRALI)

Methodologies employed:
• Granulocyte agglutination (GA)
• Granulocyte immunofluorescence by flow cytometry (GIF)
• Monoclonal antibody immobilization of neutrophil antigens (MAINA)

TRALI investigations also include:
• HLA (PRA) Class I and Class II antibody detection

For further information contact:

Neutrophil Serology Laboratory
(651) 291-6797
Randy Schuller
(651) 291-6758
schullerr@usa.redcross.org

American Red Cross Blood Services
Neutrophil Serology Laboratory
100 South Robert Street
St. Paul, MN 55107

CLIA LICENSED
Donor IgA Screening

- Effective tool for screening large volumes of donors
- Gel diffusion test that has a 15-year proven track record:
  - Approximately 90 percent of all donors identified as IgA deficient by are confirmed by the more sensitive testing methods

For information regarding charging and sample requirements, call Kathy Kaherl at:
(860) 678-2764, e-mail: kaherlk@usa.redcross.org
or write to:

Reference Laboratory
American Red Cross
Connecticut Region
209 Farmington Ave.
Farmington, CT 06032

CLIA LICENSED

Reference and Consultation Services

Antibody identification and problem resolution
- HLA-A, B, C, and DR typing
- HLA-disease association typing
- Paternity testing/DNA

For information regarding our services, contact Mehdizadeh Kashi at (503) 280-0210, or write to:

Pacific Northwest Regional Blood Services
ATTENTION: Tissue Typing Laboratory
American Red Cross
3131 North Vancouver
Portland, OR 97227

CLIA LICENSED, ASHI ACCREDITED
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:

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

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

A Comprehensive Laboratory Manual

Featuring—

• Over 100 methods—just about every method used in a reference lab.

• Eleven chapters discussing problems faced by blood group serologists and the procedures and methods that can be used to solve them.

• An extra set of the methods to use at the bench, printed on durable waterproof paper.

• See business reply order card enclosed in this issue or order on the Web at redcross.org/immunohematology
2006 Subscription Application

☐ United States—$30 per year*  ☐ Outside United States—$35 per year*

SBB/BB students free for 1 year with letter of validation

NAME ________________________________________________________________ DEGREE(S) ______________________

ORGANIZATION __________________________________________________________________________________________

DEPT./DIV. ____________________________________________________________________________________________

STREET __________________________________________________________________________________________________

CITY, STATE, ZIP CODE ____________________________________________________________________________________

☐ Check if home address used  ☐ Check enclosed

☐ VISA Acct. No. __________________________ Exp. Date: ________________

☐ MasterCard Acct. No. ____________________ Exp. Date: ________________

*Make check payable in U.S. dollars to THE AMERICAN RED CROSS. Mail this card in an envelope addressed to: American Red Cross, Musser Blood Center, 700 Spring Garden Street, Philadelphia, PA 19123-3594. THIS FORM MUST ACCOMPANY PAYMENT.
<table>
<thead>
<tr>
<th>NAME</th>
<th>DEGREE(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORGANIZATION</td>
<td></td>
</tr>
<tr>
<td>DEPT./DIV.</td>
<td></td>
</tr>
<tr>
<td>STREET</td>
<td></td>
</tr>
<tr>
<td>CITY</td>
<td>STATE</td>
</tr>
<tr>
<td>ZIP CODE</td>
<td></td>
</tr>
</tbody>
</table>

- Check enclosed.
- □ VISA Acct. No. ____________________________ Exp. Date: __________
- □ MasterCard Acct. No. ______________________ Exp. Date: __________

*Make check payable in U.S. dollars to THE AMERICAN RED CROSS. Mail this card in an envelope addressed to: American Red Cross, Musser Blood Center, 700 Spring Garden Street, Philadelphia, PA 19123-3594. **THIS FORM MUST ACCOMPANY PAYMENT.**