CONTENTS

1
Dedication in memoriam
R.R. Vassallo

2
HPA-1a/b(Pl^A/J/A2, Zw^a/b): the odyssey of an alloantigen system
R.H. Aster and P.J. Newman

9
Neonatal alloimmune thrombocytopenia: a 50-year story
C. Kaplan

14
Platelet additive solutions: current status
H. Gulliksson

20
ABO and platelet transfusion therapy
L. Cooling

34
Scott Murphy’s contribution in the early years of posttransfusion purpura: a remembrance
M. Keasen-Schnell

38
Differences in ABO antibody levels among blood donors: a comparison between past and present
Japanese, Laotian, and Thai populations
T. Mazda, R. Yabe, O. NaThalang, T. Thammapong, and K. Tadokoro

42
COMMUNICATION
Missing Scott Murphy, a platelet “maestro”
P. Rebulla

43
IN MEMORIAM
Katherine M. Beattie

44
ERRATUM

45
ANNOUNCEMENTS

46
ADVERTISEMENTS

50
INSTRUCTIONS FOR AUTHORS
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This issue of *Immuno-hematology* is a very special one. It is dedicated to Dr. Scott Murphy, a uniquely gifted physician renowned for his equanimity, who passed away last year after a long and courageous battle with lymphoma. After lengthy service on *Immunohematology*’s editorial board, Scott became its senior medical editor in 2003, serving in that capacity for almost 3 years, until his untimely death in April 2006. He was a driving force behind a bold new direction for the journal, beginning with its 20th anniversary celebration in 2004, marked by four special issues devoted to review articles. As a result of Scott’s efforts, the journal has a new look. Every issue is packed with important reviews from leaders in transfusion medicine along with the primary research reports that have always made the journal a valuable resource for blood group serologists and molecular biologists.

After an internal medicine internship and residency at the Peter Bent Brigham Hospital in Boston, Scott returned to his native Philadelphia to complete a research fellowship in hematology at the Presbyterian-University of Pennsylvania Medical Center. He published his very first paper in the *New England Journal of Medicine* the following year. A landmark report, this research launched warm platelet storage, making routine platelet support feasible and ushering in an era of aggressive cancer treatment and complex surgical procedures. Scott served on the medical staffs of the Presbyterian-University of Pennsylvania Medical Center and the Thomas Jefferson University Hospital, finally accepting the position of chief medical officer at the Penn-Jersey Region of the American Red Cross Blood Services in 1994. Throughout his career, he guided international efforts in platelet container design, storage medium development, and platelet viability assessment. Most recently, he developed “Murphy’s Rule,” a proposal for an objective quality standard for stored platelets adopted by the Food and Drug Administration. The importance of Scott’s work has been recognized with numerous honors, including the AABB’s Karl Landsteiner Award and the American Red Cross’ Charles R. Drew Award for Lifetime Achievement. He was a member and past chairman of the Biomedical Excellence for Safer Transfusion (BEST) Collaborative, a prolific group of international transfusion medicine leaders, an accomplishment of which Scott was quite proud.

This issue, written by his peers and collaborators, contains reviews of interest to platelet serologists and hospital-based cellular therapists. Richard Aster and Peter Newman recount the noteworthy history of the discovery of the HPA-1 polymorphism that has resulted in a better understanding of platelet alloimmunity and improvements in the support of alloimmunized patients. Cecile Kaplan takes this further with a skillful description of the consequences of platelet alloimmunization in fetuses and neonates, reviewing the common syndrome of fetomaternal/neonatal alloimmune thrombocytopenia. Hans Gulliksson provides a fascinating perspective on platelet additive solutions that promise to improve storage conditions and reduce the allogeneic plasma content of apheresis and whole blood-derived platelets. Laura Cooling writes elegantly about the basic science underlying major and minor ABO mismatches in platelet transfusion as well as the clinical consequences of the use of out-of-group platelets. Maryann Keashen-Schnell shares a personal perspective as one of Scott’s coworkers, investigating a perplexing case of posttransfusion purpura.

Finally, an original article and several noteworthy communications complete this issue, dedicated to the life and work of a dearly missed collaborator, mentor, leader, and friend, Scott Murphy.

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HPA-1a/b (PlA1/A2, Zwα/b): the odyssey of an alloantigen system

R.H. Aster and P.J. Newman

The HPA-1a/b (PlA1/A2, Zwα/b) alloantigen system was one of the first such systems to be identified on a cell other than the RBC. From the discovery of HPA-1a in 1958 to the present day, studies of this antigen and its allele, HPA-1b, have led to a remarkable number of "firsts" in immunobiology. In this review, we shall trace the history of the HPA-1a/b system and will highlight selected observations made during the past 48 years that have contributed not only to the field of platelet immunology but also to immunohematology and transfusion medicine in general.

Serologic Characterization of HPA-1a and 1b, the First Recognized Platelet-Specific Alloantigen System

It is likely that an HPA-1a-specific alloantibody was first identified by Zucker and colleagues in 1956 or 1957 in studying a patient who developed profound thrombocytopenia and hemorrhagic symptoms 5 days after receiving a blood transfusion. The patient's serum contained a strong antibody that produced agglutination (Fig. 1) and induced complement-dependent lysis of platelets from four normal subjects but not with the patient's own platelets obtained after recovery, about 4 weeks after the acute episode. Although the antigen recognized by this antibody was not characterized further, this case was almost certainly the first example of a syndrome that was later designated "posttransfusion purpura" (PTP) associated with an antibody specific for HPA-1a. Shortly thereafter, van Loghem and colleagues identified a platelet isoagglutinin in serum from a transfused patient and showed that it was specific for a marker they designated "Zwα" that was present in about 98 percent of the Dutch population and was inherited as a dominant trait. Their studies were facilitated by the fact that van Loghem himself possessed the rare Zwα-negative phenotype. At about the same time, Shulman and colleagues at the National Institutes of Health in Bethesda used platelet agglutination and quantitative complement fixation to characterize two examples of an alloantibody specific for a high-frequency alloantigen they called "platelet antigen 1" (PlA1). It was soon found that PlA1 and Zwα were identical. The expected allele of Zwα (Zwβ) was identified by van der Weerdt et al. as the target for an IgM platelet isoagglutinin found in a transfused patient. When a unified nomenclature was developed for platelet-specific alloantigens, Zwα/PlA1 and Zwβ/PlA2 were designated "HPA-1a" and "HPA-1b," respectively.

Analysis of the allelic frequencies of this and other platelet alloantigen systems suggests that the HPA-1a epitope was present in the primordial allele of platelet membrane glycoprotein (GP) IIIa (discussed in a later
section), traveled with early humans out of Africa during migrations to Northern Europe approximately 40,000 years ago, and finally came to North America with European colonists about 500 years ago (Fig. 2). Today, the approximate gene frequencies of HPA-1a and HPA-1b in the latter two populations are 0.86 and 0.14, respectively. However, HPA-1b is much less common in Amerindians and African Americans and is extremely rare in Asians.

Early Studies Led to Identification of Two Thrombocytopenic Syndromes Associated With Sensitization to HPA-1a

The two examples of anti-HPA-1a studied by Shulman and his colleagues came from patients who had received a blood transfusion and, 1 week later, developed profound thrombocytopenia lasting about 3 weeks (Fig. 3). They coined the term “posttransfusion purpura” (PTP) to define the combination of clinical and serologic findings typical of this condition. By 1986, at least 75 well-documented cases of PTP, nearly all associated with anti-HPA-1a antibodies, had been described. Not long after their description of PTP, Shulman et al. identified two more examples of anti-HPA-1a in women who had given birth to infants with severe neonatal alloimmune thrombocytopenia (NATP), and provided the first description of that condition involving maternal-fetal incompatibility for a platelet-specific antigen. It is now recognized that NATP occurs in about one of approximately every 1000 newborns, and that maternal-fetal incompatibility of HPA-1a is responsible in the majority of cases.

HPA-1a-Negative Platelets Were Used for the First “Antigen-Compatible” Platelet Transfusions

The recognition of NATP as a clinical entity raised the issue of whether HPA-1a-negative (HPA-1b-positive) platelets could be transfused successfully to infants with thrombocytopenia caused by maternal anti-HPA-1a. Because only 2 percent of the general population is homozygous for HPA-1b and few blood banks were capable of performing platelet typing in the 1960s, platelets from HPA-1a-negative donors were not generally available. However, Adner and colleagues showed that washed maternal platelets produced satisfactory posttransfusion increments in an infant with this condition. Because a mother’s platelets are invariably compatible with her own antibody, this approach has subsequently been used for treatment of many infants born with NATP, regardless of the specificity of the mother’s alloantibody.

Localization of HPA-1a to GPIIIa (the Integrin β3 Subunit)

From studies done using techniques available in 1960, Shulman and colleagues concluded that HPA-1a might reside on a relatively abundant platelet membrane protein. An important clue to the actual localization of the antigen came from the finding by Kunicki and Aster that platelets from patients with type I Glanzmann thrombasthenia, known to lack glycoproteins IIb and IIIa, were invariably HPA-1a negative. Kunicki and Aster then used immunochemical methods to isolate GPIIIa, showed that it was the carrier protein for HPA-1a, and speculated that the
antigen was held in a configuration suitable for antibody recognition by one or more disulfide bonds. Newman and colleagues soon thereafter localized the HPA-1a epitope to a 17-kd tryptic fragment of the GPIIIa and provided evidence that oligosaccharide moieties were not required for immunogenicity. Recognition that GPIIIa was one of the subunits of the integrin \( \alpha_{IIb}\beta_3 \) enabled others to show that HPA-1a/b alloantigens are not restricted to platelets, but can also be expressed as part of the \( \alpha\beta_3 \) complex on endothelial cells, macrophages, and fibroblasts.

**Genetic Basis for the HPA-1a/b Polymorphism**

Discovery of PCR in the mid-1980s made it possible for the first time to identify nucleotide and amino acid polymorphisms assumed to be responsible for the creation of platelet alloantigens. Initially, it appeared that this approach might not be applicable to “platelet molecular biology” as platelets both lack a nucleus and contain only vanishingly small amounts of mRNA. This problem was circumvented by showing that mRNA could be extracted from circulating platelets, converted into cDNA, cloned, and then sequenced. Using this approach, Newman and colleagues then sequenced cDNAs encoding GPIIIa from serologically identified HPA-1a-positive and HPA-1a-negative platelets and found a single nucleotide that accounted for the generation of Leu33 (PlA1 = HPA-1a) and Pro33 (PlA2 = HPA-1b) allelic forms of GPIIIa. That the Leu33Pro polymorphism was not only associated with this alloantigen system but was directly responsible for creating the alloantigenic epitope was later shown by expressing the Leu33 and Pro33 forms of GPIIIa in Chinese hamster ovary cells and demonstrating that anti-HPA-1a human alloantisera bound only the Leu33 allelic isoform, whereas anti-HPA-1b sera reacted with only the Pro33 form of the glycoprotein. This combined approach was subsequently used by us and others to characterize nucleotide and amino acid substitutions responsible for the creation of other clinically important platelet-specific alloantigens, making possible routine DNA typing for most of the platelet antigen systems, an application that is now commonplace in transfusion medicine.

**PlA1 at Last! Structural Characterization of a Platelet-Specific Alloantigen**

The discovery that the HPA-1a and -1b alloantigen system was controlled by a polymorphism at amino acid 33 of GPIIIa, together with a complex biochemical analysis of disulfide bond assignments within GPIIIa, facilitated generation of several models of the HPA-1a and HPA-1b epitopes. One such early model is shown in Figure 4, which depicts a circular peptide loop held together by cysteine residues 26 and 38 that was predicted to form the alloantigen. Frustratingly, however, when cyclic peptides expected to contain HPA-1a/b were synthesized, they were found to be incapable of reacting with HPA-1a- and HPA-1b-specific antibodies. An explanation for this apparent anomaly was finally provided by Springer and colleagues, who determined the actual crystal structure of the N-terminal plextrin-semaphorin-integrin (PSI) homology domain of GPIIIa—the domain that encompasses polymorphic residue 33. In addition to providing the correct disulfide bond assignments, this landmark study provided the coordinates of the PSI domain at the N-terminus of \( \beta_3 \) integrin and made it possible to visualize for the first time the region of GPIIIa that controls formation of the HPA-1a and HPA-1b epitopes (Fig. 5)—some 45 years after the initial description of this platelet alloantigen system! More work remains, however, because the actual surface in GPIIIa recognized by HPA-1a-specific antibodies appears to be complex, as Valentin and coworkers found that...
certain mutant forms of GPIIIa are recognized by some, but not all, HPA-1a-specific antibodies, and devised the terms "type 1" and "type 2" to describe those that require a cysteine residue at amino acid residue 435 and those that do not, respectively. It seems likely that type 1 antibodies recognize a peptide sequence containing Leu33 plus a noncontiguous peptide sequence near Cys435.

The Immune Response to HPA-1a is Unique

As blood banks and other laboratories acquired the ability to detect HPA-1a-specific antibodies, it soon became apparent that not all HPA-1a-negative individuals challenged with HPA-1a-positive platelets became sensitized to this antigen. In a series of studies begun in the 1980s, it was found that HPA-1a is unique among blood group antigens in inducing an immune response that is almost invariably linked to the class II HLA marker DRB3*0101 or DQB1*02. In the case of NATP, the relative risk for a fetus that is HPA-1a incompatible with its mother is approximately 141 if the woman is positive for DRB3*0101, roughly the same as the risk of developing ankylosing spondylitis in HLA-B27-positive individuals. The allele HPA-1b induces antibodies much less often than HPA-1a, and the response to this marker is not HLA-linked.

A potential explanation for the remarkable association between the immune response to HPA-1a and HLA was provided by Gorski and associates. In one series of studies, they showed that T cells from two women who had produced anti-HPA-1a antibodies are specifically stimulated by a cyclic peptide containing the polymorphism that controls HPA-1a expression (residues 27–37 of the HPA-1a allele of GPIIIa, β3 integrin) but not by the same set of peptides from the HPA-1b allele (Leu to Pro at amino acid residue 33). Interestingly, a common complementarity determining region motif was identified in responding T cells from two different women, despite use of genes from different V beta families to encode the peptide recognition domain. These findings showed that the response to HPA-1a is unusual in that the same peptide is recognized by both B-cell and T-cell receptors. The same group then found that a peptide containing Leu33 bound tightly to recombinant DRB3*1010 whereas the one containing Pro33 was nonreactive, thus providing a molecular explanation for the unidirectional nature of the immune response to HPA-1 antigens.

The HPA-1a/b Alloantigen System in Hemostasis

A new and still evolving chapter in the HPA-1a/b story began with observations by Weiss and coworkers that persons admitted to an intensive care unit with myocardial infarction were more likely to be positive for HPA-1b than individuals from the general population. This association appeared to be particularly significant in younger individuals. This report set off a series of epidemiological and biochemical studies that are remarkable for lack of consensus in nearly 100 published reports. For example, one study found that fibrinogen binds more readily to GPIIb/IIIa on activated HPA-1b-positive platelets than on HPA-1b-negative platelets, but no difference was found by two other groups. Various reports suggested that HPA-1b-positive individuals are at higher risk for thrombosis or premature atherosclerosis and even renal transplant rejection. However, a retrospective analysis of DNA samples from almost 15,000 individuals found no association between HPA-1b and thrombosis, atherosclerosis, or stroke. HPA-1b-positive platelets were found to be hypersensitive to the agonist ADP in one study and resistant to activation by thrombin receptor activating peptide in another. Recent work has provided a certain amount of objective biochemical evidence that HPA-1b-positive
platelets may be “hyperfunctional” in several respects.48,49 Perhaps further work along these lines will provide an explanation for apparently discordant observations made at the clinical level.

Conclusion
Since the alloantigen system now designated HPA-1a/b was discovered about 1960, serologic, biochemical, epidemiologic, and molecular biological studies of this diallelic alloantigen system have contributed importantly to our understanding of alloimmune thrombocytopenia, the cellular and humoral basis of the alloimmune response, the structural basis of alloantigenicity, and platelet pathophysiology. As more is yet to be learned, it is to be hoped that further examination of this remarkable alloantigen system will be similarly rewarding.

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References


Richard H. Aster, MD, and Peter J. Newman, PhD, Blood Research Institute of BloodCenter of Wisconsin, Inc., and Departments of Medicine and Cell Biology, Medical College of Wisconsin, Milwaukee, Wisconsin.
Neonatal alloimmune thrombocytopenia: a 50-year story

C. Kaplan

Since the first description of the immunologic mechanisms in neonatal and thrombocytopenic purpura1 and the first report of a maternal antibody directed against a platelet alloantigen inherited from the father,2 in the 1950s, much has been learned concerning fetal and neonatal alloimmune thrombocytopenia (FNAIT), but questions are still unanswered. FNAIT has been regarded as the platelet counterpart of HDN, but, in contrast to HDN, the first infant is affected in 50 percent of cases. This condition, which has been estimated to have an incidence of 1 in 800 to 1 in 1000 live births,3,4 can cause severe bleeding in the fetus and newborn, and all incompatible fetuses will be at risk for subsequent pregnancies. Therefore, it is important to diagnose FNAIT and to manage the subsequent pregnancies to prevent the consequences of severe fetal thrombocytopenia.

1953–2007: the Platelet Alloantigen Story

Over the years, considerable progress has been made in the characterization of platelet-specific alloantigens. Improvements in serologic methods for the detection of maternal alloantibodies—including the use of antigen-capture assays, the monoclonal antibody-specific immobilization of platelet antigens technique,3 or monoclonal antigen capture ELISA6—the development of immunochemical techniques, and the advent of molecular biological techniques have led to the description of 24 platelet-specific alloantigens. A human platelet antigen (HPA) nomenclature was adopted in 1990 to replace the lab-specific nomenclature that was used previously. The antigenic systems are numbered in order of the date of discovery; the high-incidence allele is called “a,” and the low-incidence allele is called “b” in the original population in which the alleles were identified.7 Under the auspices of the International Society of Blood Transfusion and the International Society of Thrombosis and Haemostasis, a Platelet Nomenclature Committee has published tables that will be updated; these include the list of the HPA antigens, the antigen genetic basis, and the platelet antigen alleles defined by sequencing. The HPA nomenclature will still be used for clinical and scientific purposes.8 The reader is referred to the table of numbered HPAs, their glycoprotein location, and approximate Caucasian phenotype frequencies in “Scott Murphy’s contributions in the early years of posttransfusion purpura: a remembrance” in this issue of Immunohematology:

HPA antigens are expressed on different integrins playing a role in cellular interactions. \( \alpha_{\text{IIb}}\beta_3 \) (GPIIb–IIIa) is the major platelet integrin and is restricted to platelets, whereas the \( \alpha_v\beta_3 \) integrin is expressed more widely. \( \alpha_{\text{IIb}}\beta_1 \), also known as GPIa-IIa, is the second most important platelet integrin and is also found on lymphocytes. Antigens located on \( \beta_3 \) (GPIIIa) have been found on other cells, such as endothelial cells, and on activated T lymphocytes when located on \( \alpha_2 \); this may play a pathophysiologic role.

Frequencies of platelet antigens vary among different populations. In Caucasians, HPA-1a is by far the most common antigen implicated in FNAIT,9 followed at much lower frequency by HPA-5b,10 then HPA-3.11 In contrast, in Asians, FNAIT is essentially associated with HPA-4 and HPA-5b. FNAIT has been reported involving rare or private antigens.8 Recent studies have shown that these low-frequency antigens are not restricted to single families,12 especially anti-HPA-9bw, which could account for up to 2 percent of confirmed cases13,14; therefore they must not be ignored in the screening for FNAIT with a negative initial laboratory investigation. The humoral maternal response is not uniform in this condition and must be taken into account when undertaking serologic diagnosis.15 Further study of the characteristics of the maternal alloantibodies and their relevance to the clinical condition would be of interest.16
The genetic basis for maternal alloimmunization has been investigated. Alloimmunization to the HPA-1a antigen appears to be associated with HLA class II alleles: \textit{DRB3*0101} and \textit{DQB1*0201} (odds ratio 24.9 and 39.7, respectively).\textsuperscript{17,18} An anti HPA-1b response was not associated with either \textit{DRB3*0101} or any known HLA class II molecules.\textsuperscript{19} This finding implies the Leu33/Pro33 substitution on the platelet glycoprotein IIa plays a role in antigen presentation. Data have shown that the binding of peptides from the Leu33/Pro33 dimorphic region to HLA-\textit{DR3*0101} is allele specific with stimulation of specific T cells\textsuperscript{20,21} providing help to B cells for generating alloantibodies; however, the positive predictive value is only 35 percent, and utility for screening is therefore limited.

The immune response to HPA-5b antigen is strongly associated with a particular \textit{DRB1} gene sequence encoding residues Glu-Asp at positions 69 and 70 of the DRβ chain.\textsuperscript{22} For other antigens, because of the low number of cases, statistical analyses are not significant when compared with the general population, as seen for HPA-6b and \textit{DRB1*1501, DQA1*0102, DQB1*0602} haplotypes shared by immunized mothers.\textsuperscript{23}

\section*{1953–2007: the Fetal and Neonatal Alloimmune Thrombocytopenia Story}

\subsection*{Natural history}

FNAIT has been known for decades as “neonatal alloimmune thrombocytopenia” (NAIT). The usual presentation is a full-term neonate exhibiting petechiae or widespread purpura at birth, or a few hours after birth, to a healthy primiparous mother. Otherwise, this infant is well with no clinical signs of infection (hepatosplenomegaly) or malformation (hemangioma, absence of radii). Visceral hemorrhages such as gastrointestinal bleeding or hematuria are less common than purpura or hematoma. The thrombocytopenia is isolated. Coexisting anemia is caused by hemorrhage.

Anti-HPA-1a and anti-HPA-3a immunization induce severe neonatal thrombocytopenia with platelet counts less than 50 \times 10^9/L in most cases.\textsuperscript{9,11} NAIT linked to HPA-5b incompatibility seems to be less severe than HPA-1a NAIT.\textsuperscript{10} The most serious complication is fetal or neonatal intracranial hemorrhage (ICH; 25.5\% of cases for HPA-1a, 24\% for HPA-3a, 15\% for HPA-5b)\textsuperscript{24} leading to death in up to 10 percent or neurologic sequelae in up to 20 percent of reported cases.

The risk of life-threatening hemorrhage necessitates prompt diagnosis and effective therapy. Phenotyped platelet transfusion is the best postnatal management. Because of the logistic difficulties in obtaining such platelets in emergency situations, random platelet transfusions with or without IVIG have been proposed.\textsuperscript{25} However, compatible platelets give better results.\textsuperscript{26} On the other hand, thrombocytopenia may be asymptomatic and pass unnoticed unless there is a routine platelet count performed. Therefore, unexpected or unexplained neonatal thrombocytopenia or severe early onset thrombocytopenia in both preterm and term babies should raise the possibility of NAIT and guide investigations accordingly. The fetal thrombocytopenia tends to worsen in subsequent incompatible pregnancies.

\subsection*{Fetal blood sampling}

In 1983, a major advance in the diagnosis of NAIT was the use of ultrasound-guided fetal blood sampling (FBS), which led to a better understanding of the fetal status.\textsuperscript{27} The mean platelet count has been evaluated to be more than 150 \times 10^9/L by the end of the first trimester of pregnancy in healthy fetuses and similar to the adult platelet count later on. Therefore, thrombocytopenia has been defined in the fetus and the neonate as a platelet count less than 150 \times 10^9/L, irrespective of the gestational age.\textsuperscript{28}

In 1984, the first fetal alloimmune thrombocytopenia case was documented with FBS at 32 weeks of gestation, and in utero maternal platelet transfusion before delivery was proposed as therapy to avoid perinatal ICH.\textsuperscript{29} Severe fetal thrombocytopenia was then documented early during pregnancy when FBS, as part of the antenatal management protocol, was carried out at 21 weeks of gestation for subsequent pregnancies in women with a previously affected infant.\textsuperscript{30} A retrospective survey of 5194 fetal blood samplings showed that fetal thrombocytopenia resulting from maternal alloimmunization was the most severe thrombocytopenia observed among the different disorders encountered, including chromosomal malformations, infections, or maternal autoimmune thrombocytopenia.\textsuperscript{31}

\subsection*{Development of antenatal management}

The rationale for antenatal management is the high rate of recurrence for subsequent incompatible fetuses who usually experience more severe thrombocytopenia. The first attempts to prevent severe
thrombocytopenia in the preterm period and thus ICH during delivery were in utero platelet transfusions before delivery. However, this management could not prevent in utero ICH, which has been reported primarily before 30 weeks of gestation. Because of the short survival of transfused platelets, weekly fetal platelet transfusions have been proposed and shown to be effective in preventing ICH in a number of cases. The risks of in utero platelet transfusion, bleeding, and fetal loss have been estimated to be 1 to 2 percent per procedure and 8 percent per pregnancy. Fetal cardiac arrhythmia (prolonged bradycardia) has also been reported. Less invasive therapy has also been proposed, involving maternal administration of IVIG, steroids, or both.

The mechanism of action of IVIG is complex, including inhibition of the transplacental passage of maternal alloantibodies and modification of the immune response. Corticosteroids may modulate the maternal immune response. Different IVIG protocols have been developed and differences in results were reported mainly because of the definition of a successful response. Low-dose corticosteroids, as sole therapy, have been given to a limited number of patients, and response to therapy appears highly variable.

The optimal management remains to be determined, and an international forum in 2003 demonstrated the absence of standardization. The decision regarding therapy depends on different factors, among which the fetal status plays a central role. There is no reliable and sensitive parameter for predicting which fetuses will be severely affected and which ones will respond to therapy. The only way to assess the fetal status is to perform FBS, but the risk of serious adverse events from this technique is high, up to 10 percent. Most protocols favor maternal therapy with less invasive strategies with stratification according to the previously affected sibling’s status.

Serial in utero platelet transfusions are considered only as salvage therapy after failure of maternal treatment.

Antenatal screening

No systematic screening for FNAIT exists at the moment, and this condition is still underdiagnosed. Reduction of neonatal death and disability is a public health issue, and recent progress in the prevention of current causes of neonatal disorders has been efficient at reducing the risk of neonatal disorders going undiagnosed and untreated. Prospective studies have shown that although 2 to 3 percent of women are at risk for developing anti-HPA immunization, only 1 in 800 to 1000 newborns will be affected. It has been found that 26 percent of infants born to immunized mothers are nonthrombocytopenic. Until procedures are found that predict which women will have an affected fetus, maternal screening has a low sensitivity. Only identifying thrombocytopenic newborns at birth increases the sensitivity but will miss fetuses severely affected during pregnancy who should have been treated. Cost-effectiveness analysis indicated screening newborns was more cost-effective than screening primiparous women. A consensus on routine investigation and optimal antenatal management has yet to be reached.

Future Directions

Although real progress has been achieved in the more accurate diagnosis and management of FNAIT, further studies must focus on improvements in antenatal management and on mechanisms of maternal sensitization. A recent study has shown that high maternal anti-HPA-1a alloantibody concentrations may provide an indication of severely affected fetuses and this may contribute to a less invasive antenatal therapeutic strategy. However, the follow-up of maternal anti-HPA-1a concentration during pregnancy should not be considered as an indicator of therapeutic effectiveness.

A murine model has also been recently developed, which may contribute to a better understanding of this condition in the future.

References


Neonatal alloimmune thrombocytopenia


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Platelet additive solutions: current status

H. Gulliksson

During the last two decades, there has been great interest in developing and using platelet additive solutions (PASs) for the storage of platelets. At present, such additive solutions are in use for transfusion in several countries. PAS is generally used as a substitute for plasma to (1) reduce the amount of plasma transfused with platelets and to recover plasma for other purposes, primarily fractionation into plasma products; (2) avoid transfusion of large volumes of plasma with possible adverse reactions and circulatory overload; (3) make possible photochemical treatment for the inactivation of bacteria and other pathogens in platelets; and (4) improve storage conditions as was discussed in a previous review.

A basic principle is that aging of platelets after in vitro storage at 22°C is significantly slower than aging of platelets in vivo at 37°C, a situation that may make extended storage of platelets possible. Three approaches were suggested to be of specific importance to improve storage conditions of platelets: (1) reducing the activation of platelets during collection of blood and the preparation and storage of platelets; (2) reducing the metabolic rate in terms of glucose consumption and lactate production; and (3) ensuring that glucose will be available in the storage medium during the entire storage period. The activation of platelets can be counteracted either by the addition of platelet activation inhibitors or of certain components such as magnesium to the PAS.

The use of PAS offers the possibility of including components with specific effects on platelets in the storage solution that are not present in plasma or in the anticoagulant. A number of effects have been observed that can be assigned to certain components. Reducing platelet activation and inclusion of key components in the platelet storage environment, such as acetate, citrate, glucose, potassium, and magnesium, were suggested to be useful tools to optimize platelet storage conditions. The compositions of some present PASs are presented in Table 1. The purpose of this review is to describe some events of the last several years as a complement to the knowledge presented in previous reviews.

In Vivo Characteristics

Results from a number of in vivo studies using PASs have been published during the last decade. In the first patient transfusion studies in the 1990s using PASs such as PAS-II (T-Sol, Baxter) or Plasma Lyte A (Table 1), the results were not consistent. In some studies, satisfactory CCIs were found in patient transfusion studies,9,11 in some studies significantly lower CCIs were observed than for platelets stored in plasma.12,14 In a recent study, encouraging platelet recovery and survival data were found, comparing platelets prepared by apheresis and stored in PAS-II for 1 versus 7 days.15 Mean recovery was 69 percent and 53 percent, and survival was 8.2 and 5.1 days at Days 1 and 7, respectively. The ratios of Day 7 to Day 1 were 0.80 and 0.65 for recovery and survival, respectively. A proposal by Murphy of a new standard of efficacy for the evaluation of platelets for transfusion has created considerable interest. This concept implies that acceptably stored platelets on the last day of storage would demonstrate at least two-thirds the recovery and one-half the survival of platelets collected from the same subject and then retransfused as reference “fresh” platelets. The ratios in this study met the proposed criteria for 7-day storage, although the use of a Day 1 control may not totally have fulfilled the requirements of the reference indicated.

Comprehensive patient transfusion studies using pathogen-reduced platelets have added significant knowledge about platelets stored in more recent PASs, particularly PAS-III (InterSol, Baxter). A photochemical treatment method using a novel psoralen, amotosalen HCl, in combination with ultraviolet light has been developed to inactivate viruses, bacteria, protozoa, and WBCs in platelets. Two major studies were conducted in Europe and in the United States, namely
the euroSPRITE trial in Europe and the SPRINT trial in the United States. In the euroSPRITE trial, comparable platelet CIs and CCIs and a comparable safety profile for photochemically treated and reference untreated pooled buffy-coat (BC) platelet units in 103 oncology patients with thrombocytopenia were demonstrated. In the SPRINT study, which included 645 transfused patients, the hemostatic efficacy of photochemically treated and reference untreated platelets was found to be equivalent.19 Transfusion reactions were significantly fewer, and adverse events and overall safety profiles were comparable for treated and reference platelets.20 In a second evaluation of data from the euroSPRITE patient transfusion study using BC-derived platelets, reference groups with untreated platelets were compared.22 Reference platelets were stored in either T-Sol (n=35) or plasma (n=16). The results indicated that 1-hour and 24-hour mean platelet CIs and posttransfusion hemostatic scores were not significantly different for patients receiving platelet components suspended in 100 percent plasma and patients receiving platelets in a T-Sol environment. In a review in 2003, the preliminary conclusion had been that additional in vivo data, from studies of clinical outcome after transfusion of platelets stored in either PAS or plasma, were needed/required.6 Today, comprehensive clinical data support the routine use of PAS as an equivalent alternative to plasma to suspend platelets.

### In Vitro Platelet Aging at 22°C Versus In Vivo Aging at 37°C

Because the average survival of human platelets in the circulation, after release from megakaryocytes, is known to be 9 to 10 days, it may be questioned whether it is at all meaningful to extend the shelf life of platelet concentrates up to or even beyond 7 days.23

Removal of platelets from the circulation is believed to be age-dependent and is probably associated with failure to maintain normal hemostasis. Transfusion of aging platelets may be of little help to stop bleeding because they would be removed from the circulation.

Studies comparing aging of platelets in vivo with in vitro storage at 22°C, using isotope labeling, suggested that aging of platelets after in vitro storage for 5 days at 22°C was similar to aging of platelets during 2.1 days in vivo at 37°C.7 The relative aging factor was found to be 0.42 (2.1 days divided by 5.0 days). In a subsequent study, a similar relative aging factor was found (0.44).7 These differences were associated with a much higher turnover rate of ATP, the major energy carrier in platelets, at 37°C than at 22°C. The calculated ATP turnover ratio of 0.48 at those two temperatures was of the same magnitude as the calculated relative aging factor, indicating that the relative decrease in aging of platelets at 22°C compared with that at 37°C is similar to the relative decrease in metabolic rate at this temperature. The rate of aging of platelets during storage at 22°C may be less than half of that found in vivo at 37°C. If this ratio is applied to the normal lifespan of platelets, 9 to 10 days in the circulation would correspond to at least 18 to 20 days of in vitro storage at 22°C. These data may provide meaningful objectives to develop methods and storage environments for extended storage of platelets.

### In Vitro Platelet Aging at 22°C Versus In Vivo Aging at 37°C

Because the average survival of human platelets in the circulation, after release from megakaryocytes, is known to be 9 to 10 days, it may be questioned whether it is at all meaningful to extend the shelf life of platelet concentrates up to or even beyond 7 days.23 In general, the various PASs can be used for apheresis as well as for BC platelets. The storage medium normally is composed of a mixture of plasma (generally 20–40%) and PAS (60–80%). The main difference is that in apheresis platelets, ACD is often preferred to CPD anticoagulant, because resuspension of platelets after preparation is facilitated. In some apheresis equipment, platelets are kept in the centrifugation chamber during the entire apheresis cycle; in other equipment, platelets are continuously transferred to a platelet storage container. These differences may result in significant variation in platelet activation. Although apheresis equipment originally may have been designed for the preparation of platelets suspended in plasma, most equipment can be
used for platelet collection in a small final resuspension volume of plasma, which is necessary to allow the addition of PAS. Satisfactory results were obtained by Ringwald et al.\textsuperscript{24,25} when evaluating the suspension of high concentrations of apheresis platelets (4000–5000 $\times 10^9$ platelets/L) in PAS.

BC-derived platelets suspended in PAS are generally prepared in pools from several donors. There are primarily variations in the number of BCs included in the pools (generally 3–7 BCs) and in the time of holding of whole blood preceding the preparation of BCs. Either BCs can be prepared on the day of collection, generally within 8 hours, and stored overnight for the preparation of platelets on the following day, or whole blood can be stored overnight for the preparation of BCs and platelets on the following day.

The platelet rich plasma (PRP) method is generally not used for the routine preparation of platelets in PAS. However, in a recent study by Sweeney et al.,\textsuperscript{26} platelets were prepared from individual donors by the PRP method in a first step and then pooled as platelets from several donors and suspended in either plasma or PAS. Platelets were stored for 7 days for in vitro evaluation. Good preservation of platelet quality in both environments was reported.

**Effects on Metabolism and Platelet Function Associated With Components in PAS**

The results of early studies by Holme et al.\textsuperscript{27} and Gulliksson et al.\textsuperscript{28} on the effects of PAS indicated that the presence of glucose during the entire platelet storage period is crucial for platelet metabolism. Effects observed after depletion of glucose involved rapid decrease in adenine nucleotide levels, cessation of lactate production, and finally disintegration of platelets. Depletion of glucose is generally associated with an increased rate of platelet metabolism and fall in pH levels. The results suggested that depletion of glucose, not the pH level alone, is detrimental to platelets during storage. In contrast to plasma, the fall in pH during storage of platelets in PAS will stop at a significantly higher level than pH 6.0, often at about pH 6.5 as a result of the limited amount of glucose generally available in PAS.\textsuperscript{28} Because the buffering capacity of PAS is approximately half that of plasma, PAS is more susceptible to increased production of lactic acid by platelets.\textsuperscript{29,30} On the other hand, metabolism of acetate present in PAS significantly stabilizes the pH level. In parallel with glucose, acetate is used as a substrate for the oxygen-dependent platelet metabolism, and enters into the tricarboxylic acid cycle, and is further oxidized in the respiratory chain.\textsuperscript{29–32} The end products are carbon dioxide from the first step and water from the second step. By formation of bicarbonate from the carbon dioxide produced by acetate, very stable pH levels are maintained during platelet storage.\textsuperscript{29,30,32} A possible third substrate for platelet metabolism may be fatty acids.\textsuperscript{33}

Generally, phosphate has two possible roles during storage of platelets, as a stimulant of platelet glycolysis to increase production of lactic acid and as a buffer to prevent a fall in pH. These two effects theoretically compete and may neutralize each other. There are no indications of net utilization or production of phosphate during storage of platelets.\textsuperscript{29}

The new PASs designated Composol and PAS-IIIM as well as the early Plasma Lyte A all contain magnesium and potassium. Composol and PAS-IIIM also contain citrate, in contrast to Plasma Lyte A. The three components: magnesium, potassium, and citrate are associated with complexity of effects and interdependence. Effects on platelet membrane function and platelet activation as well as rate of glycolysis have been described and the various effects may even be combined.

Increased concentration of extracellular magnesium ions significantly inhibits exposure of P-selectin, decreases binding of fibrinogen to ADP-activated platelets, and significantly decreases ADP-induced platelet aggregation.\textsuperscript{34} Magnesium may also modify calcium influx into the platelets. In addition, magnesium, calcium, and the concentration of citrate have an effect on potassium permeability of the platelet membrane and the intracellular concentration of potassium.\textsuperscript{35} Citrate also heightens platelet responsiveness to some activating agents such as ADP.\textsuperscript{36} In addition, effects on the rate of glucose consumption and lactate production related to the concentration of citrate and the presence of potassium and magnesium in PAS have been observed. Platelets stored in medium with a citrate concentration of 8 mmol/L produced only half the quantity of lactate produced by platelets in a similar medium with a citrate concentration of 14 to 26 mmol/L.\textsuperscript{37} Inasmuch as no negative effects on adenine nucleotide levels were observed, the results suggested that synthetic media preferably should include citrate at low concentrations to avoid excessive lactate production and an acid pH. On the other hand,
increased production of lactate associated with higher concentrations of citrate can be neutralized by the addition of acetate.37 Again, this situation illustrates the complexity of effects and interdependence associated with those components. In parallel, the combination of magnesium and potassium ions present in PAS has a similar effect on platelets, i.e., significantly reduced metabolic rate in terms of glucose consumption and lactate production and also reduced platelet activation.38–40

When platelets are shipped between different centers, agitation may be interrupted for a considerable period of time. A previous study using a plasma environment suggests that possible storage time without agitation is strongly affected by platelet concentration.41 The level of pH should be kept above 6.5 to avoid negative effects on functional in vitro factors such as hypotonic shock response (HSR). In a recent study, the effects of interruption of agitation on platelets stored in two PAS alternatives, namely PAS-IIIM and Composol, were evaluated.42 At a platelet concentration of approximately 1000 $\times$ $10^9$/L, platelets could be stored for 4 days in PAS-IIIM with maintenance of HSR and pH. This was not possible with Composol, suggesting that the presence of phosphate is of importance to maintaining pH and other in vitro characteristics during interruption of agitation. Similar effects were not observed during continuous agitation.

**Future Perspectives**

To conclude, present knowledge and experience support platelet storage only at 22°C. The use of PAS instead of plasma as the platelet storage environment would provide benefits to patients and facilitate the inclusion of certain components with proven favorable effects on platelets that are present in neither plasma nor anticoagulant. The present knowledge of effects associated with different approaches discussed above suggests that reducing platelet activation in combination with the inclusion of key components in the platelet storage medium, such as acetate, citrate, glucose, potassium, magnesium, and additional possible future ingredients, should be the tools to optimize the storage conditions and maintain the function of platelets. Additional in vivo studies of recovery and survival as well as count increments in patients with thrombocytopenia will be needed.

**References**


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ABO and platelet transfusion therapy

L. Cooling

It is appropriate to take steps to optimize the efficacy and safety of transfusion therapy. Perhaps we should study the role of ABO compatibility in platelet transfusion therapy more carefully so that further improvements may be made.

Scott Murphy, Transfusion Editorial 1988

The relative importance of ABO compatibility and platelet transfusion has been a matter of debate for more than 50 years. Since the early studies of Richard Aster, it has been recognized that transfusion of ABO-incompatible platelets can be associated with decreased platelet increments after transfusion. In the last 10 to 15 years, there has been increasing discussion and concern regarding minor-incompatible platelet transfusions that have been linked to acute hemolytic transfusion reactions, venoocclusive disease, and increased morbidity in allogeneic transplants. This review will briefly summarize the biochemistry, regulation, clinical practice, and considerations surrounding ABO compatibility and platelet transfusions.

ABO Glycoconjugates on Platelets

ABO antigens are expressed on several endogenous platelet glycoproteins and glycolipids. Platelet-specific glycoproteins (GP) known to express ABH antigens include GPIIb/IIIa, GPIb/IX, GPIa/IIa, GP1c, GPIV, GPV, CD31 (PECAM), and CD109. In particular, appears to be a significant contributor and determinant of ABO expression on platelets. The GPIIb/IIIa complex numbers nearly 250,000 molecules per platelet and possesses between five and eight bi- and triantennary N-glycans capable of displaying ABH epitopes. GPIIb/IIIa is the major target of human anti-A and anti-B in vitro. Likewise, a linear correlation between A antigen on GPIIb and intact platelet membranes (R = 0.91) was reported by Cooling et al. Translocation and ongoing synthesis of GPIIb/IIIa may also contribute to the increased antigenicity of platelets during in vitro storage. Julmy et al. reported a nearly 50 percent increase in ABH expression on platelets during routine storage, accompanied by evidence of platelet activation and translocation of α-granule proteins (including GPIIb/IIIa) to the platelet membrane. More recent studies in platelet proteomics and the platelet transcriptome suggest ongoing translation and synthesis of GPIIb/IIIa during storage as well.

Platelets also express a small population of glycosphingolipids with ABH activity. Early studies showed the ability of platelets to adsorb and elute soluble type 1 chain AB and Lewis antigens from plasma, leading to the speculation that all ABH-active glycolipids were entirely of exogenous origin. Subsequent studies using chain-specific monoclonal antibodies showed that platelets expressed type 2 chain glycolipids and glycoproteins, indicating that most ABH-active glycolipids were of megakaryocyte or endogenous origin. This was confirmed by Cooling et al. who identified ABH-active glycolipids in platelets from Le(a+b–) individuals who should lack soluble type 1 chain antigen. The ABH-active glycosphingolipids of platelet are predominantly simple, linear structures (6–8 oligosaccharides) although a few complex sialylated structures are present. In group A donors, type 4 chain or globo-ABH structures have also been identified. This is in sharp contrast to RBCs, which express a rich variety of ABH-active glycolipids, including complex, highly branched polyglycosylceramides. The contribution of ABH-active glycolipids to overall ABH expression on platelets appears to be minor.

ABO Expression on Megakaryocytes

Although recent studies indicate some residual protein synthesis in young platelets, the majority of ABH synthesis occurs at the level of the megakaryocyte. ABH antigens have been identified on immortalized...
megakaryoblastic cell lines and cultured bone marrow megakaryocytes. In the latter, ABH exhibits developmental and distinct clonal variation, with individual megakaryocyte colonies differing widely in the amounts of A, B, and H antigens expressed. Clonal variation may underlie the heterogeneity observed on circulating platelets. Cooling et al. subsequently showed a direct, linear relationship between H and A antigens on circulating platelets by two-color flow cytometry (R = 0.95). On the basis of their findings, Dunstan et al. and Cooling et al. speculated that H antigen synthesis is a rate-limiting step regulating ABH expression in megakaryocytes and platelets.

H antigen appears to be developmentally regulated in early hematopoiesis. H, LeY, and FUT1 mRNA are expressed by CD34+ cells and likely early megakaryocyte progenitors. During megakaryocytic differentiation, both H and LeY antigens are progressively lost with increasing ploidy and proplatelet formation. The presence of H antigen on early megakaryocyte progenitors may facilitate adhesion to stromal fibroblasts, a necessary step for megakaryocytic preservation and expansion. Schmitz and colleagues were able to inhibit megakaryocyte adhesion to stromal fibroblasts with fucosylated bovine albumin, soluble H antigen, and fucose-specific lectins. Although the precise lectin-ligand pair involved in fucose-mediated adhesion is not yet identified, several megakaryocyte proteins critical to megakaryocyte development and adhesion can express ABH antigens, including αβ1, αβ5β1 GPⅡb/Ⅲa, GPⅡb, and PECAM. H antigen on integrins may be particularly important: H and LeY expression are associated with increased fibronectin binding, cell adhesion, and resistance to apoptosis in epithelial tissues. An increase in fucose-mediated adhesion, with delayed megakaryocyte maturation, may account for delayed platelet engraftment in some group O autologous transplant patients.

**Platelet ABO and Genetic Variation**

There are dramatic differences in the amount of ABO expressed on platelet membranes between individual donors. Genetic differences in ABO glycosyltransferase alleles underlie some of these differences. In group A donors, expression of A antigen on platelet membranes, glycoproteins, and glycolipids is linked to an A1 RBC phenotype. In contrast, A1 donors express little or no A antigen and can be considered group O compatible. Lewis and secretor phenotype appear to play little role in either the presence or strength of ABO antigens on platelets: A1, Le(a–b+) donors are also negative for A antigen by flow cytometry. Because they lack A antigen on platelets, A2 individuals can develop an anti-A, and ABH-specific refractoriness.

Among group A1 donors, there is a wide variation in platelet A expression (Fig. 1E). In individual donors, the percentage of circulating platelets positive for A antigen by two-color flow cytometry (CD41+, HPA+) can range from less than 5 percent to 87 percent (population average = 40%). Even within a single donor, there is distribution of positive and negative platelets (Fig. 1B). Despite the latter, the average percentage of platelets positive for A antigen is relatively stable over time and may represent a unique, donor-specific characteristic. As shown in Figure 1F, the percentage of A antigen–positive platelets, in paired samples (S1, S2) collected from the same donor at two separate times, is nearly identical.

Unlike A1 donors, most group B donors weakly express ABO antigens on platelets. In our own studies, only 20 percent of platelet donors were positive for B antigen by flow cytometry. Similar findings are observed with platelet crossmatching, in which 83 to 100 percent of group B platelet donors were crossmatch-compatible with group O and group A serum, respectively. Despite these results, group B antigen can be identified on platelet glycoproteins and glycosphingolipids in most group B donors (80–90%). The density or antigenicity on individual platelet glycoproteins, however, may be decreased relative to most A1 donors (< 50%), on the basis of solid phase and radioimmunoassay studies with human anti-B.

**High-Expressor Phenotype**

Approximately 4 to 8 percent of A1 and B donors are ABO high expressors (HXP), defined as a 2- to 3-fold increase in platelet ABO expression (≥ 2 SD). ABO HXPs can be further subclassified as type 1 and type 2 on the basis of their flow cytometry profiles. Type 1 donors demonstrate a bimodal population of strongly positive and moderately to weakly positive platelets, akin to the bimodal population of positive and negative platelets observed in most A1 donors (Fig. 1B). In contrast, type 2 donors have an essentially uniform population of strongly positive platelets (Fig. 1C).
Transfusion of ABO-HXP platelets to a group O recipient has resulted in profound transfusion failures, even with HLA-matched platelets. Platelets from ABH-HXP donors should be reserved for ABO-identical recipients only.

The molecular origin of the ABO-HXP phenotype is still unknown. Family studies indicate that the trait is autosomal dominant. There is no relation between the ABO-HXP and a secretor phenotype. Elevated ABO glycosyltransferase activity has been noted in the sera of some donors; however, sequencing studies of the ABO gene have not revealed any mutations. Cooling et al. suggested that the ABO-HXP might represent clonal upregulation of the $H$ or $FUT1$ gene. Future studies focusing on transcriptional regulation of $H$ and ABO genes in megakaryocytes, including epigenetic phenomena, may eventually unravel the genetic mystery behind the ABO-HXP phenotype.

**ABO Compatibility and Platelet Transfusion Response**

The influence of platelet ABO and the post-transfusion response is highly variable, reflecting both donor and recipient factors. Recipient factors that may influence the transfusion response include ABO type, sex, and parity; isohemagglutinin titers; and immunosuppressive drugs. Platelet and donor factors can include ABO type (A1, A2, B), donor-specific differences in ABO strength, platelet activation, and storage time. As noted in the previous section, ABO expression varies widely between individual donors and ABO types.
The earliest studies examining the effect of ABO and platelet transfusion were performed by Richard Aster in 1965.\(^2\) Using radiolabeled platelets, Aster et al. reported a 60 to 90 percent decrease in the 1-hour posttransfusion platelet increments after transfusion of group A and AB platelets to group O recipients. This was substantiated in an elegant study by Jimenez et al.,\(^3\) who compared the posttransfusion recovery in paired recipients of split apheresis products from the same donor. The authors found similar platelet recoveries when both recipients were ABO compatible with the donor (R = 0.8); however, transfusion to an ABO-incompatible recipient was accompanied by marked, significant decreases in posttransfusion recovery at 1 (p < 0.001), 4 (p < 0.004), and 24 hours (p < 0.04). Improved platelet recovery with ABO-identical and ABO-compatible platelets has also been observed in large clinical trials, including the Trial to Reduce Alloimmunization to Platelets.\(^3\)\(^4\),\(^5\)

There are several examples of platelet refractoriness caused by ABO in the literature.\(^3\)\(^4\),\(^1\)\(^2\),\(^4\)\(^1\)\(^2\) In a small randomized trial of ABO-identical and ABO-unmatched platelets, the vast majority of patients receiving ABO-unmatched platelets had a decrease in posttransfusion recovery, with 37 percent having clear evidence of ABO-specific refractoriness.\(^4\) In a second study, clinical refractoriness was significantly higher in patients receiving ABO-mismatched platelet transfusions (69% vs. 8%) and was typically heralded by a sudden acute rise in isoagglutinin titers.\(^4\) In a more recent study, ABO-incompatible platelet transfusions stimulated isohemagglutinin titers in 40 to 50 percent of patients after only one to two transfusions.\(^4\) Not surprisingly, patients with documented ABO refractoriness often have high titer isohemagglutinins (\(> 1:500–1:1000\)). Platelet-associated antibody in these patients can approach 30,000 molecules of IgG per platelet after transfusion of an ABO-incompatible platelet.\(^7\) In contrast, IgM binding to platelets is minimal (\(< 350 \text{ molecules/platelet}\)).\(^7\)

Although most case reports and studies have focused on ABO major-incompatible transfusions, poor platelet recoveries and clinical refractoriness can also be observed with ABO minor-incompatible or out-of-group transfusions.\(^3\)\(^4\),\(^6\),\(^7\) According to one study, plasma-incompatible transfusions were associated with an 18 percent decrease in posttransfusion recovery when compared with ABO-identical transfusions.\(^9\) The adverse effect of incompatible-plasma infusion may be caused by the formation of immune complexes composed of donor ABO antibodies and soluble ABO substances in blood.\(^5\)\(^6\),\(^7\) These immune complexes may then bind to either complement or Fc receptors on platelets, leading to accelerated immune clearance.\(^6\) Among refractory patients, 40 percent have elevated levels of circulating immune complexes. Among group A patients transfused with ABO-mismatched platelets, 80 percent had evidence of immune complexes containing IgG anti-A of donor origin.\(^7\)

**Alloimmunization**

Routine transfusion of ABO-incompatible platelets can promote HLA alloimmunization. In randomized trials, patients routinely transfused with ABO-mismatched platelets were more likely to develop both HLA and platelet-specific antibodies, accompanied by an earlier onset and higher incidence of clinical platelet refractoriness.\(^4\)\(^4\),\(^8\) ABO incompatibility can also act synergistically with HLA and crossmatch compatibility to further decrease the posttransfusion response.\(^3\)\(^4\),\(^9\)

In a retrospective study of 51 refractory patients, Blumberg and colleagues\(^3\)\(^9\) demonstrated decreased platelet recoveries in 60 percent of all ABO-incompatible transfusions, regardless of platelet crossmatch results. Among patients receiving crossmatch-compatible platelets, an ABO mismatch was associated with a 40 percent decrease in posttransfusion recovery. The impact was more dramatic with ABO-mismatched, crossmatch-incompatible platelets (85% decrease). Overall, ABO-identical platelets were clinically equivalent or better than ABO-incompatible, HLA-matched, or crossmatch-compatible platelets.

The synergism between ABO, HLA, and clinical refractoriness can have direct economic consequences. The University of Rochester reported a 20 percent decrease in platelet utilization and a 40 percent decrease in HLA-matched platelets after instituting a policy requiring ABO-identical platelets for their leukemic patients.\(^50\)\(^5\)\(^2\) This policy has led to improved survival and decreased morbidity based on the results of a small randomized trial (n = 40 patients). Leukemia patients who received only ABO-identical platelets had longer remissions and improved survival relative to patients transfused with ABO-nonidentical platelets.\(^53\) There was also a decrease in major bleeding episodes (5%) with 70 percent of patients having no evidence of clinical bleeding.\(^54\)

The impact and cost effectiveness of ABO compatibility in other patient populations is still
debated. In a retrospective study of cardiovascular surgery patients, Blumberg et al. found that patients transfused with ABO-identical platelets had decreased morbidity and mortality, as measured by fever, antibiotic usage, transfusion support, and hospital admission days. These differences corresponded to an average savings of $10,000 to $15,000 in direct costs and patient charges. These findings have been challenged by Lin et al., who found no significant differences in either morbidity or mortality in a similar group of cardiovascular patients. In the latter study, the authors concluded that the use of ABO-nonidentical platelets is “an acceptable and safe practice” in surgical patients.

ABO, Platelets, and Transplantation

There is increasing concern regarding platelets and ABO compatibility in bone marrow transplant patients, particularly in patients receiving ABO-incompatible allogeneic transplants who have complex transfusion needs. Because of the adverse effect of ABO incompatibility on erythroid engraftment, it is a practice to transfuse plasma and platelets that are compatible with the stem cell or marrow donor. In most instances, this may require transfusion of an ABO major-incompatible or minor-incompatible platelet. Several studies have linked increased platelet transfusions and transfusion of plasma-incompatible platelets with increased morbidity, venoocclusive disease, multiorgan failure, and death. Organ dysfunction usually follows a 1- to 2-week period of rising platelet transfusion requirements, suggesting a causal relationship.

It is hypothesized that donor ABO antibodies may result in immune complex formation in the host, leading to systemic inflammation. Alternatively, passively infused antibodies may recognize and bind ABO antigens on endothelial cells, leading to an increase in treatment-related toxicity and microvasculature damage. Some transplant centers provide ABO-compatible, plasma-reduced products for their ABO-mismatched, allogeneic transplant patients.

Little is known regarding the effect of ABO-incompatible platelets in the setting of solid organ transplantation, particularly ABO-incompatible kidney and heart transplants. Current regimens require several rounds of plasmapheresis or immunoadsorption combined with immunosuppression to decrease isoagglutinin titers. To avoid passive transfusion of isoagglutinins harmful to the graft, platelets should be plasma compatible with the donor in the immediate perioperative period. However, transfusion of ABO major-incompatible platelets could also present problems, particularly postoperatively. As discussed in a previous section, ABO-incompatible platelets can stimulate isoagglutinin titers in some recipients. In one small study, ABO-incompatible platelet transfusions from the intended kidney donor were used to test the immune responsiveness of the recipient. Of seven patients, one developed HLA antibodies and three demonstrated a rise in isohemagglutinins titers. Only patients with little or no response to platelet transfusion underwent transplantation with good outcomes 4 to 7 years after transplant.

Acute Hemolytic Transfusion Reactions

Currently, the greatest discussion around ABO and platelet transfusion is the risk of acute hemolytic transfusion reactions (HTR) with ABO minor-incompatible or out-of-group platelets (Table 1). Although most queried transfusion services provide ABO-identical platelets or plasma-compatible platelets when available, it is estimated that 10 to 40 percent of transfusions are plasma incompatible with the recipient. The most common reasons for transfusing out-of-group platelets are limited inventory of ABO type-specific platelets and HLA-matched platelets and to minimize product wastage. To date, only a handful of severe HTRs with platelet transfusion have been reported in the literature and Internet chat rooms.

Despite the transfusion of more than 2 million platelet concentrates per year (including potentially 200,000 to 400,000 out-of-group transfusions), the true risk and incidence of hemolysis with plasma-incompatible platelet transfusions is still a matter of conjecture. Facility-specific rates for platelet-associated HTR range from less than 1 in 1000 to less than 1 in 25,000 for all platelet transfusions and less than 1 in 100 to less than 1 in 9000 for minor-incompatible platelet transfusions. This wide range may reflect heterogeneity in the type of platelet products transfused (apheresis vs. random), index of clinical suspicion, and severity of the reaction. On the basis of the yearly number of transfusions in the United States, it is clear that severe HTRs after out-of-group platelet transfusions are relatively rare events. However, mild hemolysis may be more common than appreciated. Oza et al. found mild to moderate evidence of hemolysis with elution of ABO antibodies in 1 to 2
Table 1. Acute hemolytic transfusion reactions with platelets

<table>
<thead>
<tr>
<th>Reference</th>
<th>ABO type</th>
<th>Platelet donor and product</th>
<th>Outcome†</th>
<th>Donor ABO type</th>
<th>Donor(s)</th>
<th>Saline</th>
<th>AHG</th>
<th>Product</th>
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</tr>
<tr>
<td>Murphy71</td>
<td>AML</td>
<td></td>
<td>NR</td>
<td>A O</td>
<td>O</td>
<td>512</td>
<td>2048</td>
<td>Apheresis-HLA</td>
<td>225 mL</td>
<td>4.0 (40%)</td>
</tr>
<tr>
<td>Chow72</td>
<td>AML-M4</td>
<td></td>
<td>NR</td>
<td>AB O</td>
<td>O</td>
<td>1024</td>
<td>520‡</td>
<td>Apheresis, pooled</td>
<td>1200§</td>
<td>5.5 (50%)</td>
</tr>
<tr>
<td>Mair73</td>
<td>Neuroblastoma</td>
<td>Apheresis</td>
<td>NR</td>
<td>A O,AB</td>
<td>O</td>
<td>128</td>
<td>1024</td>
<td>Apheresis</td>
<td>225</td>
<td>2.6 (31%)</td>
</tr>
<tr>
<td>McManigal74</td>
<td>Cardiac surgery</td>
<td>Apheresis-HLA</td>
<td>NR</td>
<td>AB O,B,A</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Apheresis</td>
<td>3380§</td>
<td></td>
</tr>
<tr>
<td>Larsson75</td>
<td>AML</td>
<td>Anti-A:16384</td>
<td>NR</td>
<td>A O</td>
<td>O</td>
<td>4096</td>
<td>NR</td>
<td>Apheresis</td>
<td>371</td>
<td>2.3 (30%)</td>
</tr>
<tr>
<td>Duguid76</td>
<td>Cardiac surgery</td>
<td>Apheresis</td>
<td>NR</td>
<td>A O</td>
<td>O</td>
<td>NR</td>
<td>NR</td>
<td>Apheresis</td>
<td>100</td>
<td>4.5 (33%)</td>
</tr>
<tr>
<td>Valbonesi77</td>
<td>Breast cancer</td>
<td>Double apheresis</td>
<td>NR</td>
<td>A O</td>
<td>O</td>
<td>&gt;8000</td>
<td>NR</td>
<td>Double apheresis</td>
<td>37</td>
<td>3.5 (40%)</td>
</tr>
<tr>
<td>Sauer-Heilborn78</td>
<td>Trauma</td>
<td>Apheresis</td>
<td>NR</td>
<td>A O</td>
<td>O</td>
<td>1024</td>
<td>1024</td>
<td>Apheresis</td>
<td>260</td>
<td>NR</td>
</tr>
<tr>
<td>Anonymous82</td>
<td>RAEB</td>
<td>Apheresis</td>
<td>NR</td>
<td>A O</td>
<td>O</td>
<td>NR</td>
<td>NR</td>
<td>Apheresis</td>
<td>600</td>
<td>5.7 (36%)</td>
</tr>
<tr>
<td>Yeast83</td>
<td>NAIT</td>
<td>Apheresis</td>
<td>NR</td>
<td>B O</td>
<td>O</td>
<td>NR</td>
<td>NR</td>
<td>Apheresis</td>
<td>15</td>
<td>10 (83%)</td>
</tr>
<tr>
<td>Anonymous84</td>
<td>Leukemia</td>
<td>Apheresis</td>
<td>NR</td>
<td>AB O</td>
<td>O</td>
<td>NR</td>
<td>NR</td>
<td>Apheresis</td>
<td>NR</td>
<td>5.3 (53%)</td>
</tr>
<tr>
<td>Josephson85</td>
<td>Leukemia</td>
<td>Apheresis</td>
<td>NR</td>
<td>A O</td>
<td>O</td>
<td>256</td>
<td>8192</td>
<td>Apheresis</td>
<td>50</td>
<td>3</td>
</tr>
<tr>
<td>Angiolillo86</td>
<td>Histiocytosis</td>
<td>Apheresis-HLA</td>
<td>NR</td>
<td>A O</td>
<td>O</td>
<td>128</td>
<td>NR</td>
<td>Apheresis</td>
<td>107</td>
<td>3.5 (47%)</td>
</tr>
<tr>
<td>Sapatnekar87</td>
<td>Medulloblastoma</td>
<td>Apheresis</td>
<td>NR</td>
<td>A O</td>
<td>O</td>
<td>2048</td>
<td>16384</td>
<td>Apheresis</td>
<td>145</td>
<td>3.9 (32%)</td>
</tr>
<tr>
<td>Sadani87</td>
<td>AML-M0</td>
<td>Apheresis</td>
<td>NR</td>
<td>A O</td>
<td>O</td>
<td>160</td>
<td>1280</td>
<td>Apheresis</td>
<td>NR</td>
<td>3.8 (49%)</td>
</tr>
</tbody>
</table>

Mean 32.5
Range 0–72

AHG = antihuman globulin; ALL = acute lymphocytic leukemia; AML = acute myelocytic leukemia; NAIT = neonatal alloimmune thrombocytopenia; NR = not reported; RAEB = refractory anemia with excess blasts.

* Age in years.
† Fall in Hb by g/dL and percent decrease (%) from pretransfusion levels.
‡ Isoagglutinin titers in the recipient after transfusion of ABO minor-incompatible platelets.
§ Same HLA-matched donor, transfusions 1 month apart.
¶ Total volume of incompatible plasma infused.
|| Active bleeding.
** Intrauterine transfusion.
percent of all ABO-mismatched apheresis platelets, which accounted for 6.7 percent of all transfusion reactions associated with platelets. An increased rate of adverse reactions with ABO minor-incompatible transfusions was also noted by Wagner and Adamo.93 Out-of-group platelet transfusions, however, have a minimal impact on RBC utilization. Two studies have found no difference in the level of anemia or RBC utilization in oncology patients receiving plasma-incompatible transfusions.73,94

A summary of severe HTRs associated with plasma-incompatible platelets is shown in Table 1. In nearly all cases, hemolysis followed the transfusion of group O platelets to a non-group O patient. Clinically, the unfortunate recipient displayed classic symptoms of an acute HTR immediately or shortly after transfusion, leading to rapid recognition and diagnosis. In a few instances, the diagnosis was only suspected hours or days later after the onset of hemoglobinemia, hemoglobinuria, or unexplained anemia.66,68,75,77,85,87 Although most patients recovered with hydration and supportive care, a handful of deaths are known, including at least five deaths in the United States.68,78 In very rare cases, plasma exchange, RBC exchange with group O RBCs, or both were performed to stem the hemolysis.77

Laboratory studies in these patients were all consistent with an acute HTR. Patients' RBCs typically became strongly positive in the DAT (IgG, C3) with elution of ABO antibodies from the RBCs. Laboratory evidence of intravascular hemolysis was always present, including an abrupt drop in hemoglobin, hemoglobinemia, hemoglobinuria, decreased haptoglobin, spherocytes, hyperbilirubinemia, and elevated lactate dehydrogenase. Disseminated intravascular coagulation has been reported in two cases with fatal outcomes.68,78 The degree of hemolysis can be impressive, with a drop in hemoglobin ranging from 2 to 10 g/dL. In many cases, the patient will show an appropriate increment in platelet count after transfusion.70,74,81 An investigation of the implicated platelet donors revealed unusually high isoagglutinin titers in most cases. Several of the group O donors in published reports were women, and included a directed donation from a group O mother to her 2-year-old daughter.68,77 Women with a history of a prior ABO-incompatible pregnancy are at increased risk for developing high-titer isoagglutinins.95

Several factors may raise the risk for an HTR after an out-of-group platelet transfusion. Apheresis platelets, which contain 200 to 400 mL of plasma from a single donor, carry a higher risk of causing hemolysis because of a high-titer donor than do pooled platelets. This is substantiated by a review of the literature, in which 23 of 28 (82%) platelet-associated HTRs were associated with apheresis platelets. This risk is substantially lessened with pooled platelets because of the smaller volume of plasma per donor (50 mL), which is subsequently diluted 4- to 6-fold in the final product. The use of pooled platelets does not eliminate the risk of an HTR: four HTRs caused by plasma-incompatible pooled random platelets have been reported, in two neonates75 and two adults.55,66 In the latter, two high-titer donors were pooled and infused.66 The total volume of ABO-incompatible plasma must also be considered, including residual plasma present in transfused group O RBCs.74,87,96 McManigal et al.74 reported severe hemolysis in a patient who received multiple out-of-group platelet transfusions, resulting in the transfusion of 3380 mL of incompatible plasma (106% of patient’s blood volume) during a period of 4 days.74

Patient factors can also contribute to the risk of an HTR after an ABO-incompatible transfusion. As shown in Table 1, there is an increased risk of hemolysis when high-titer isoagglutinins are transfused to group A and AB recipients (23 of 28)—a finding not surprising to blood bank staff. Very young children may also be at increased risk because of their relatively small blood volume.75,81,85,87 It has been surmised that young children and nonsecretors may also have an increased susceptibility because ABO substances capable of adsorbing and neutralizing ABO antibodies are decreased in or absent from their blood. Among published cases, only two investigated the Lewis type of the recipient.65,78 One recipient was a nonsecretor [Le(a+b–)]65; however, the second patient was Le(a–b+),78 suggesting that soluble ABO antigens may provide only limited protection. Finally, other ill-defined patient factors could also play a role. Look-back investigations of two high-titer group O apheresis platelet donors failed to identify any additional HTRs in 70 prior transfusion recipients.76,78

Approaches to Minimize Hazards of Plasma-Incompatible Platelet Transfusions

The increased use of apheresis platelets, in which all the plasma is from a single donor, has heightened awareness and concern regarding out-of-group platelet transfusions. Several strategies have been
implemented. Most transfusion services have policies dictating the transfusion of ABO-identical or plasma-compatible platelets when available. More proactive strategies are discussed in the following sections.

Identification of “dangerous donors”

Although only 2 percent of U.S. centers routinely screen group O donors, the practice is relatively common in Europe. With rare exceptions, donors identified as high titer are still permitted to donate; however, their products are segregated and labeled as high titer with a warning to only transfuse to group O patients. The cost effectiveness of prospective screening is still unproven, given the relative rarity of HTR with out-of-group platelets. Sadani et al. recently reported a severe HTR in a group A patient after transfusion of group O apheresis platelets that initially tested as low titer by automated testing. Subsequent testing revealed a high-titer anti-A IgG (Table 1). In contrast, the New York Blood Center, which routinely screened group O apheresis donors until 1998, has had no reports of acute HTRs in the 5 years, and more than 25,000 platelet transfusions, since screening was discontinued.

A persistent problem that plagues widespread donor screening is the absence of a recognized standard method for testing. Several manual and automated methods are currently used for donor screening (tube, gel, and solid phase) with a variety of end point measurements: saline titers, AHG titers, or hemolysis. Even with a single method, there is a wide range in results (Fig. 2A). Improvements and increased use of automated blood group testing, such as gel and solid phase methods, may eliminate much of the perceived variation in donor isoagglutinin testing with minimal add-on cost to the final product. This has been demonstrated in England, where high-throughput testing is performed using a single plasma or serum dilution (1 in 100) on an automated blood analyzer. A cost analysis by Emory University, which implemented testing of all group O apheresis donors by gel method, estimated an additional cost of $1.20 per apheresis platelet.

A second problem with donor screening is determining a critical titer for “safe” versus high-titer, “dangerous” donors. As shown in Figure 2A, the percentage of donors classified as high titer can range from 3 to 50 percent, depending on the population tested and the method and critical titer used. A method and critical titer that classifies 40 to 50 percent of group O donors as high titer could significantly hamper platelet availability to non-group O patients, particularly during times of severe shortages. A survey of current practice shows a cutoff titer of 32 to 200 for saline testing and 250 to 512 for AHG (Table 2).
this review, we compared the distribution of isoagglutinin titers from normal group O apheresis donors and group O donors implicated in HTRs (Table 1). As shown in Figures 2B and 2C, many of the cutoffs used (32–64) are quite conservative.

Plasma reduction and platelet additive solutions

A popular method to reduce the risk of ABO HTR caused by plasma-incompatible transfusions combines plasma reduction with resuspension in either additive solutions or group AB donor plasma. In the most common method, platelet concentrates are prepared and stored in plasma (30–40%) diluted with a platelet additive storage solution. Although not licensed in the United States, a selection of storage solutions are commercially available or undergoing trials in Europe. The use of additive solutions has several attractive advantages for increasing transfusion safety including a decreased risk of HTRs as well as TRALI and allergic and febrile reactions caused by residual proteins, cytokines, and antibodies present in donor plasma.

In Germany, Wagner and Alamo noted a marked decrease in transfusion reactions with plasma-reduced platelet concentrates (0%) compared with routine pooled platelets (27%). Plasma-reduced platelets reportedly have equivalent posttransfusion recovery at 1 hour although there is reduced 24-hour survival.

Plasma reduction and use of platelet additive solutions may reduce, but will not completely eliminate, the risk of an acute HTR caused by a high-titer donor. An enlightening case was recently published by Valbonesi et al., who described severe HTRs in two recipients of a split, group O apheresis unit (Table 1). Apheresis platelets were collected “dry” with only minimal plasma carryover (74 mL), followed by resuspension in 400 mL of a platelet additive (T-Sol). An investigation identified extremely high isoagglutinin titers (> 8000) in the female donor. The authors noted that this was the first severe HTR encountered in more than 16,000 “dry” platelet collections in the last 10 years.

Limiting plasma infusion

Some transfusion services monitor and limit the total volume of incompatible plasma that a recipient can receive. Policies vary between institutions, ranging from 300 to 500 mL of plasma per day to 1000 mL of plasma per week (Table 3). In the United States, approximately 10 percent of polled institutions (255) limited the amount of incompatible plasma transfused to adult patients.

In neonates and young children with small blood volumes, there is a greater concern regarding the risk of out-of-group platelets. As a consequence, most U.S. hospitals serving neonatal populations provide only ABO-identical or plasma-compatible platelets (60%). Very few institutions (1%, 29 institutions) have policies limiting incompatible plasma infusion to neonates and children.

Identification of A₂ donors as “universal platelet donors”

Because A₂ donors have little or no A antigen on platelet membranes, they are compatible with group A and O donors. Donor screening to identify group A₂ donors could increase the inventory of group O compatible from 44 to 52 percent, increasing the number of apheresis platelets available for platelet crossmatching, which requires ABO compatibility between donor and patient. It could also benefit selection and survival of HLA-matched platelets. To our knowledge, only Norway has policies for identifying A₂ platelet donors as “universal donors.” A₂ platelets are also an ideal component for patients undergoing ABO-mismatched allogeneic bone marrow transplantation. In ABO major-incompatible transplants, present guidelines recommend that transfused platelets be plasma compatible with the marrow or stem cell donor to minimize delays in erythroid engraftment. As a consequence, platelets will often be ABO incompatible with the patient’s isohemagglutinins. In group A (donor) to group O (patient) mismatched

### Table 2. Strategies for screening apheresis platelet donors for high-titer isoagglutinins

<table>
<thead>
<tr>
<th>Country</th>
<th>Screen donors</th>
<th>Method</th>
<th>Critical titer</th>
<th>% Donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>No (2%)³⁸</td>
<td>Tube, gel</td>
<td>1:50–1:200</td>
<td>3–28%</td>
</tr>
<tr>
<td>England</td>
<td>Yes Automated</td>
<td>Tube</td>
<td>1:100</td>
<td>3–10%</td>
</tr>
<tr>
<td>Scotland</td>
<td>Yes</td>
<td>Tube</td>
<td>1:50</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>Yes Gel</td>
<td>IAT</td>
<td>1:64</td>
<td>2:56</td>
</tr>
<tr>
<td>Germany</td>
<td>Yes Tube, saline</td>
<td>1:64</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Czech Republic</td>
<td>Yes Tube, saline</td>
<td>1:64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>Yes IAT</td>
<td>1:250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>Yes tube, saline</td>
<td>IAT</td>
<td>1:100</td>
<td></td>
</tr>
<tr>
<td>Switzerland</td>
<td>Yes Hemolysis</td>
<td>1:16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finland</td>
<td>Yes Tube, saline</td>
<td>1:32</td>
<td>5.7%</td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>Yes IAT</td>
<td>1:512</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
transplants, this incompatibility can be overcome by transfusion of A₂ platelets, which are ABO compatible with the patient’s isoagglutinins and plasma compatible with the donor graft. A₂ platelets might also be a product of choice in group O (donor) to group A (patient) minor-mismatch transplants. Likewise, A₂ platelets are an ideal product for group O patients receiving group A-incompatible solid organ transplants. In these patients, transfusion of incompatible plasma and platelets should be avoided because of the risk of acute humoral rejection and immune stimulation, respectively. It is my fervent wish that A subtyping of apheresis platelet donors become a standard of practice by blood collection centers.

**Summary**

Platelets express ABO antigens on a large number of platelet glycoproteins and glycolipids. The amount of ABO antigen expressed on individual donor platelets is heterogeneous and determined by genetic and epigenetic factors. Routine transfusion of ABO-incompatible platelets can be associated with cumulative adverse effects including decreased posttransfusion recovery, increased platelet utilization, incompatible platelet crossmatches, HLA alloimmunization, and ABH-specific refractoriness. Out-of-group platelet transfusion can also be associated with adverse effects including decreasing platelet increments and, rarely, severe HTRs. Out-of-group platelet transfusion may have more severe consequences in ABO-mismatched bone marrow transplant patients, who are at increased risk for significant morbidity and mortality because of ABO incompatibility.

ABO-identical platelets should be provided whenever possible, particularly for patients requiring long-term transfusion support. In patients undergoing ABO-mismatched transplants, provision of A₂ platelets may be preferable. In patients requiring HLA-antigen negative and HLA-matched platelets, HLA matching has priority; however, the ABO compatibility of HLA platelets should be recorded and considered when assessing the success or failure of HLA platelets. ABO compatibility should always be considered when evaluating and monitoring patients with clinical refractoriness, particularly group O patients who have higher mean isohemagglutinin titers.

In neonates and young children, only ABO-identical or plasma-compatible platelets should be transfused. In surgical and other patients with short-term transfusion needs, ABO compatibility is less of a concern. ABO-identical or plasma-compatible platelets should be transfused if available; however, an actively bleeding patient should never be denied a platelet transfusion because of ABO compatibility. The risks of bleeding and the need to achieve hemostasis outweigh the potential adverse consequences of transfusing an ABO-incompatible platelet. One potential exception is ABO-incompatible organ transplantation, in which plasma containing donor-reactive isoagglutinins should be avoided. In the postoperative period, donor-compatible platelets may be considered to minimize immune stimulation of recipient isoagglutinins.

**Table 3. Transfusion strategies for minimizing HTR caused by incompatible-plasma infusion**

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Adults</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABO-identical platelets</strong></td>
<td>4 plasma-incompatible platelet transfusions per week&lt;sup&gt;79&lt;/sup&gt;</td>
<td>&gt;40 kg: 600 mL per week</td>
</tr>
<tr>
<td>1000 mL incompatible plasma per week&lt;sup&gt;79&lt;/sup&gt;</td>
<td>25–39 kg: 400 mL per week</td>
<td></td>
</tr>
<tr>
<td>2 plasma-incompatible apheresis platelets per 72 hours&lt;sup&gt;80&lt;/sup&gt;</td>
<td>5–24 kg: 100 mL per week</td>
<td></td>
</tr>
<tr>
<td>300–500 mL incompatible plasma per day&lt;sup&gt;101&lt;/sup&gt;</td>
<td>Neonates: 0 mL, plasma-compatible only</td>
<td></td>
</tr>
</tbody>
</table>

**References**

ABO and platelets


98. de Wildt-Eggen J, Gulliksson H. In vivo and in vitro comparison of platelets stored in either synthetic media or plasma. Vox Sang 2003;84:256-64.


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Scott Murphy’s contribution in the early years of posttransfusion purpura: a remembrance

M. KEASHEH-SCHNELL

Dr. Scott Murphy always enjoyed a medical mystery and his passion for the syndrome of posttransfusion purpura (PTP) was one of his favorite topics. This “perfect storm” of immunologic events is characterized by profound thrombocytopenia approximately 7 days after transfusion and includes the development of a platelet-specific alloantibody which destroys any antigen-positive transfused platelets as well as the patient’s own platelets, which lack the corresponding antigen. Dr. Murphy’s interest in this syndrome began in the early years of his research career. He also provided our laboratory with the first antiserum, which we used to develop testing procedures and to investigate numerous cases of PTP. In the next few pages of this edition dedicated in his honor, I would like to provide a brief trip down memory lane to remember his valued contribution to the Platelet Serology Laboratory at the Penn-Jersey Region of the American Red Cross (ARC) in Philadelphia, to outline some of the many advances that have taken place in the investigation of platelet antibodies, and to summarize the results of our laboratory investigation of PTP at Penn-Jersey.

I first had the pleasure of meeting Scott Murphy in the late 1970s while working as a research technologist at the Penn-Jersey Region of the ARC. At that time, we were in the process of developing a procedure to type donor platelets for the $\text{Pl}^{A1}$ antigen. Dr. Miriam Dahlke, our medical director, informed me that Dr. Murphy had offered to share a supply of anti-$\text{Pl}^{A1}$, which was the result of a plasma exchange performed on a patient diagnosed with PTP. Not wanting to wait for him to change his mind, I headed to his laboratory at the other end of town on that very hot, late spring day, armed with a box and a small supply of ice.

I was greeted by a bearded 1970s version of our own Scott Murphy, complete with heavy cotton lab coat emblazoned with his name and “Cardeza” in bright red embroidery. He appeared supremely happy in his crowded research lab as he presented me with a bag containing nearly a full liter of plasma. I remember his response after I thanked him and asked if our laboratory could do anything to return the favor. He said, “just type a lot of platelets for our patients.” I would later learn that this was his way, always a gracious gentleman who held his patients and others’ as his first priority.

As I headed back with my precious cargo, I noticed a small split in the bag and was faced with the dilemma of making it back to the ARC building before it thawed (and leaked all over Center City Philadelphia). The just-in-time arrival of an available taxi cab allowed me to beat the clock and the weather. I have to admit that this was, unquestionably, the only time in my career that, while working late into the evening, the usually boring task of preparing a thousand tiny 1-mL vials was pure pleasure.

In 1975, while on the staff of the Hematology Research Laboratory at the Presbyterian-University of Pennsylvania Medical Center in Philadelphia, Dr. Murphy coauthored a seminal publication calling attention to the variety of responsible antibodies and patient profiles in the relatively newly described and clinically mysterious syndrome of PTP. Before the publication of this paper in 1975, all of the 14 described cases of PTP were attributed to the presence of anti-$\text{Pl}^{A1}$ produced by $\text{Pl}^{A1}$-negative female patients. Two of the three patients described in this paper (one $\text{Pl}^{A1}$-negative man who produced anti-$\text{Pl}^{A1}$ and a $\text{Pl}^{A1}$-positive woman with a demonstrable anti-platelet antibody of unknown specificity) served to expand the definition of PTP to include both male and female patients with antibody specificities not limited to anti-$\text{Pl}^{A1}$.
PTP is a relatively rare, but well-defined, syndrome first described by Shulman et al. in 1961.2 The classic patient profile describes an older, multiparous woman with a precipitous, antibody-mediated drop in platelet count occurring 7 to 10 days after transfusion. Unlike the maternal antibodies responsible for neonatal alloimmune thrombocytopenia, the antibodies produced by patients diagnosed with PTP cause severe thrombocytopenia in the antibody producer. PTP is self-limiting, but carries a significant risk of fatal hemorrhage. Similarities have been noted between PTP and the hemolysis of autologous RBCs during delayed hemolytic transfusion reactions caused by some RBC antibodies.3 To date, there is no definitive explanation for the destruction of autologous cells in either situation.

In the years after Dr. Murphy’s 1975 publication and since the first description of a platelet-specific antigen by van Loghem in 1959,4 much has been learned about the nature of platelet antigens. The nomenclature for platelet-specific antigens was standardized by a Working Party on Platelet Serology5 (Table 1) and adopted by the International Society of Blood Transfusion. In this more orderly, numeric system, the platelet antigens are designated as HPA (human platelet antigens) and are numbered (with Arabic numbers) according to discovery date, followed by a lower case “a” or “b,” which denotes the high (a) or low (b) frequency member of the antigen pair. As an example of the need for simplification, the first antigen reported was referred to as Zw in Europe and PlA1 in the United States. Under the new nomenclature, it is now called HPA-1a. Its lower frequency allele (PlA2 or Zw) is now HPA-1b.

We now know that six platelet-specific antigen systems (HPA-1 through 5 and HPA-15) are biallelic with one high frequency and one low frequency antigen or allele.6 For the remaining 10 antigens (HPA-6 through 14 and HPA-16), alloantibodies against the low frequency but not the high frequency antigen have been observed. The molecular basis and location on the platelet membrane has been resolved for antigens listed in Table 1. With the exception of HPA-14, the differences in the alleles are the result of a single amino acid substitution at a specific location on the gene encoding for the membrane glycoprotein. The isoantigen, Naka,7 is now known to be located on GPIV (CD36).8,9

These advances, as well as the availability of monoclonal antibodies, which recognize specific glycoprotein locations on the platelet surface, have led to the development of serologic testing techniques with increased sensitivity10–14 as well as to the development of molecular techniques15 for platelet antigen typing.

Other specificities have since been identified as pathogenic antibodies in PTP using a variety of investigative techniques. These include anti-PlA2 (HPA-1b),16 anti-Baka (HPA-3a),17 anti-Bakb (HPA-3b),18 anti-Pena (HPA-4a),19 anti-Bra (HPA-5b),20 and anti-Brb (HPA-5a).21,22

In 1992, Dr. Murphy became the medical director of the Penn-Jersey Region, and we had the honor of working with him for the next 14 years. His fascination with the syndrome of PTP and his concern for the patients involved never waned. He was never too busy to make time in his schedule to learn the details of each new case. He would then consult directly with the patient’s clinician to ensure that our center could provide appropriate testing, consultation, and transfusion support. Options for transfusion support, the availability of monoclonal antibodies, which recognize specific

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**Table 1. Human platelet antigen nomenclature**

<table>
<thead>
<tr>
<th>New nomenclature</th>
<th>Old nomenclature</th>
<th>Glycoprotein location</th>
<th>Phenotypic frequency (approx. % Caucasians)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPA-1a</td>
<td>PlA1 (Zw)</td>
<td>IIIa (CD61)</td>
<td>98</td>
</tr>
<tr>
<td>HPA-1b</td>
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when needed, would vary from case to case and might include antigen-negative single-donor platelets or washed or deglycerolized RBCs.

Since 1983, our laboratory has investigated 115 patient samples submitted to rule out PTP. Samples were tested for the presence of platelet-specific and HLA antibodies using a variety of methodologies. Of 115, 33 (28.6%; 31 from women, 2 from men) contained platelet-specific antibody(ies) and these patients were diagnosed with PTP based on this finding along with clinical history. Of these, 23 demonstrated anti-HPA-1a, 4 anti-HPA-1b, 3 anti-HPA-3a, 1 anti-HPA-1b and anti-HPA-3a, 1 anti-HPA-5b, and 1 anti-HPA-5a (Fig. 1). Molecular testing was used to confirm the antigen-negative status in five cases. Nineteen were confirmed using serologic techniques on postrecovery platelet samples. Nine were not submitted or were unavailable for confirmation. Of note is the fact that 23 of the 33 (69.6%) sera also demonstrated HLA class I antibodies in combination with platelet-specific antibodies, indicating the necessity to use glycoprotein-based as well as standard whole-cell methods as part of the investigative protocol to ensure detection of coexisting antibodies. Of the remaining 82 samples, the majority either were negative or demonstrated HLA antibody only. These results, along with other published data, support Dr. Murphy’s early report of the heterogeneous nature of PTP.

Thank you, Scott Murphy, for your support, sustained enthusiasm, and undiminished desire to help patients in need. You are missed by everyone here at the ARC. As I anticipate further advances in the challenging fields of platelet testing and transfusion, I will not forget that happy, bearded researcher of many years ago, and I am delighted to report that the plasma handed to me 29 years ago remains in use in our laboratory and continues to help so many of “our patients.”

References


Maryann Keasen-Schnell, BS, Supervisor, Platelet/ Neutrophil Serology Laboratory, Manager, Process Improvement, American Red Cross Blood Services, Heritage Division, Penn-Jersey Region Department of Technical Services, 700 Spring Garden Street, Philadelphia, PA 19123.
Passively transfused blood group antibodies cause clinical problems. High titers of anti-A and anti-B seem to be one reason for hemolytic transfusion reactions and for ABO HDN. In Japan, anti-A and anti-B titers notably decreased in the 15 years between 1986 and 2001. At present, titers of more than 100, as measured using the saline method, are rare. Differences in the level of anti-A and anti-B among ethnic populations have been reported; these differences were found to be the result of environmental factors rather than hereditary factors. In the present study, the anti-A and anti-B titers of random donors in three Asian populations are compared. In Thailand, the IgM anti-A and anti-B titers are low and are similar to the Japanese titers reported in 2001, but the IgG anti-A and anti-B titers are high and are similar to the Japanese titers reported in 1986. In the Lao People’s Democratic Republic, both the IgM and the IgG anti-A and anti-B titers are high and are similar to those reported in Japan in 1986. In addition, anti-A and anti-B titers of different sex donors and of various age groups were also compared. High titers were found in 8.8 percent of the female donors in the younger than 30 age group and in 36.7 percent of the female donors in the older than 50 age group. 

**Key Words:** ABO blood groups, anti-A, anti-B

Passively transfused blood group antibodies cause clinical problems. The effects of anti-A and anti-B in ABO-mismatched platelet transfusions, especially in HLA-matched platelet transfusions, have long been debated. Although reports are rare, intravascular hemolytic transfusion reactions have been known to occur. These hemolytic transfusion reactions seem to be caused by high titers of anti-A and anti-B. Such reactions have been seen in groups A, B, and AB recipients receiving group O plasma.

Anti-A and anti-B levels have been reported to differ among ethnic populations. Many previous studies have suggested that ethnicity is a risk factor for ABO HDN. However, the differences among populations were found to be the result of environmental factors rather than hereditary factors. Redman et al. addressed the question of variations in ABO antibody levels in persons with different ethnic backgrounds by studying antibody levels in Asian, Caucasian, and African blood donors, all of whom were living in the north London area of the United Kingdom. Although the highest levels of IgG anti-A and anti-B titers were found among group O African female donors, these levels were not significantly higher than those in the other group O donors who were tested.

In this report, we investigated the changes in the anti-A and anti-B titers in the Japanese population during the last 19 years. Both anti-A and anti-B titers decreased greatly during this period. We also compared these titers with the anti-A and anti-B titers in Laotian and Thai populations.

**Materials and Methods**

Sera were obtained from Japanese blood donors in 1986 (n = 106), 2001 (n = 107), and 2005 (n = 93) in Tokyo, Japan. In 2001, sera were obtained from blood donors in Vientiane, Lao People's Democratic Republic (Lao PDR; n = 58); in 2005, sera were obtained from blood donors in Bangkok, Thailand (n = 93). All serum samples were obtained randomly from the general population of volunteer unremunerated blood donors. The sera were stored at −30°C until tested.

Sera from group O Japanese blood donors classified according to age (16–29 years old and 51–69 years old)
ABO antibody levels in Asians and sex were also collected for the present study. These serum samples were separate from those collected from the general population in 1986, 2001, and 2005.

The sera were titrated using a 1 to 1 dilution with 0.01M PBS, pH 7.2, containing 2% BSA. The IgM antibody titers were determined using the saline method with incubation at room temperature for 15 minutes. The IgG antibody titers were determined by an IAT using LISS and an incubation of 30 minutes at 37°C. Before the IAT, the sera were treated with 2-ME for 15 minutes at 37°C using the method described in the AABB Technical Manual; this treatment is used to destroy IgM antibodies in the sera.

Testing was performed by the same person to avoid variations in technique and test interpretation.

**Results**

In the Japanese population, anti-A and anti-B titers in 1986 were distributed between 64 and 1024 (mode 256) for IgM (Table 1) and between 64 and 2048 (mode 256-512) for IgG (Table 2). In 2001, they markedly decreased to between 16 and 512 (mode 32–64) for both IgM and IgG. In 2005, the IgM titers further decreased to between 2 and 64 (mode 8–16). In the Laotian population in 2001, the IgM titers were similar to those of the 1986 Japanese population; the distribution was between 128 and 1024 (mode 256), whereas the IgG titers were slightly higher than those of the 1986 Japanese population.

### Table 1. Distribution of anti-A and anti-B IgM titers in three populations

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* Data include group O and nongroup O donors

### Table 2. Distribution of anti-A and anti-B IgG titers in three populations

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* Data include group O and nongroup O donors
population: between 256 and 4096 (mode 512–1024). In the Thai population in 2005, IgM titers were similar to those of the 2001 Japanese population: the distribution was between 4 and 256 (mode 16), and IgG titers were similar to those of the 1986 Japanese population: the distribution was between 8 and 2048 (mode 128–256).

In the Japanese group O blood donors, IgM titers of anti-A and anti-B were low and no differences were noted between the sexes. However, IgG titers of anti-A and anti-B showed differences between the sexes and an increase with donor age. High titers (> 512) of anti-A, anti-B, or both were found in 8.8 percent (9 in 102) of the female donors in the younger than 30 age group and in 36.7 percent (18 in 49) of the female donors in the older than 50 age group, determined by the antihuman globulin test. No male donors were found with titers more than 256.

Discussion

In the Japanese population, the anti-A and anti-B titers markedly decreased within the 15-year period between 1986 and 2001. At present, the Japanese anti-A and anti-B titers are relatively low, compared with those seen in the Laotian and Thai populations. Schwartz et al.8 showed that only 3.3 to 3.6 percent of American group O blood donors had high anti-A and anti-B titers (> 100). People with high ABO system antibody titers are now rare among the Japanese population, and the mean titers are lower than those of blood donors in New York.

The mechanisms responsible for the reductions in Japanese anti-A and anti-B titers are unknown. However, previous studies have suggested that environmental factors may influence anti-A and anti-B titers.5,6 Enteric bacteria and other parasites may affect the production of anti-A and anti-B.4,6 Environmental factors can affect the prevalence, counts, and susceptibility of enteric bacteria.9,10 Because environmental factors are thought to affect anti-A and anti-B titers, we compared the anti-A and anti-B titers in three Asian countries: Japan, Lao PDR, and Thailand. Japan has long been a developed country, Lao PDR is a developing country, and Thailand has recently been given developed country status.

The environment in Japan continues to change. Recently, the Japanese lifestyle has become more westernized, especially with regard to food.11 The prevalence of allergic diseases, heart disease, diabetes, and cancer has been increasing in Japan. In contrast, Lao PDR remains relatively undeveloped, and Laotians usually eat natural foods, whereas Thais and the Japanese eat more processed foods. These environmental differences may explain, at least in part, why the Laotian anti-A and anti-B titers were similar to the titers observed in Japanese donors in 1986, whereas the IgM and IgG antibody titers among the Thais were similar to those observed in the Japanese donors in 2001 and 1986, respectively. Our data support the concept that anti-A and anti-B titers are affected by environmental factors. Lao PDR is rapidly developing and becoming more westernized. We plan to continue our comparison of Laotian and Thai antibody titers in the future.

Acknowledgment

We thank Dr. W.L. Gyure, Seton Hall University, for his support in the preparation of this manuscript.

References


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COMMUNICATION

Missing Scott Murphy, a platelet “maestro”

The most recent occasion I had to see Scott before his untimely death was at the 29th BEST meeting, held in Rome in March 2005. Early spring in Rome is unique, as the local environment provides an ideal link between the past—witnessed by its gorgeous monuments—and future dreams and expectations inspired by the Ponentino breeze caressing the seven hills of Rome.

I met Scott as he exited his hotel on the via Veneto. I knew his disease had started a new aggressive phase, but his optimistic smile overcame my concerns when he addressed me with a warm “Buongiorno!” The meeting was scientifically very successful, as usual for all BEST gatherings, but most notable was Scott’s piano performance during dinner, when he played four-hand Diabelli’s Sonatine with Georges Andreu, another musically gifted transfusion medicine professional. This performance was the second in a series begun at an earlier BEST meeting in Paris, where Scott played Mozart’s Twinkle, Twinkle Little Star with the devoted help of his wife Joanie playing the triangle during a dinner at the Musée D’Orsay.

While everyone knows of Scott’s scientific accomplishments, it was his blend of science and art that truly made him a platelet “maestro.” Our scientific interests crossed on several occasions, mainly surrounding the European method to produce platelets for transfusion—the buffy-coat technique—as compared with the “platelet-rich-plasma” procedure used in the United States. In particular, he was instrumental in studies developed by Francesco Bertolini in his laboratory and ours, elucidating the role of acetate in platelet storage.

Finally, remembering Scott without mentioning his deep love and dedication to his wife, their children, and grandchildren would be forgetting one of his most important qualities, his humanity. We, his friends, along with the many patients who have and will continue to benefit from his work, will miss the scientist, the gentleman, and the maestro.

Paolo Rebulla, MD
Director
Center of Transfusion Medicine, Cellular Therapy and Cryobiology
Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena
Via Francesco Sforza 35
20122 Milano MI
Italy
IN MEMORIAM

Katherine M. Beattie, BS, MT(ASCP)SBB 1922–2007

Kay Beattie received her BS in medical technology from Wayne State University in Detroit, Michigan, in 1943 and began her career at Children’s Hospital in Detroit. She became the supervisor of the Blood Bank at Children’s in 1954. In 1957, she received her SBB certification, and in 1961, she became the co-director of the Michigan Community Blood Center in Detroit. In 1975, she joined the Southeastern Michigan region of the American Red Cross (ARC) Blood Services as director of the reference laboratory and education, a position she held until she retired to North Palm Beach, Florida, in 1988.

During her 45-year career, Kay was active at the local, state, and national levels, and was honored often by her peers. She was a member of the Ontario Antibody Club from 1966 to 1988; the Michigan Blood Council, as chairman of the Reference Laboratory Committee from 1977 to 1988; the Invitational Conference of Investigative Immunohematologists from 1976 to 1988; and the ARC Accreditation Reference Committee from 1984 and as chairman from 1987 to 1988. As a member of the Michigan Association of Blood Banks (MABB) since its founding in 1956, Kay had served as chairman of two committees and in all offices, including the presidency. She also served on the AABB Committees on Education, Reference Laboratories, and Quality Control as vice chairman; Scientific Section Coordinating Committee as secretary; as an inspector in the Inspection Program; and on the Committee on Self-Testing and Review.

Among her awards from the AABB, she received the Ivor Dunsford Memorial Award in 1972; the prestigious Emily Cooley Lectureship Award in 1980, which was given for the first time to a medical technologist; and the Chapman-Franzmier Memorial Award in 1998. The MABB has honored Kay four times, with the Merit Award in 1963, the Technical Award in 1971, the Founders Award in 1985, and the Kay Beattie Lecture, a lecture given in her honor each year at the MABB meeting. Kay received the Distinguished Alumni Award in 1982 from Wayne State University.

Kay published 44 scientific papers and was the co-editor of the ARC Immunohematology Methods and Procedures Manual. Her final paper, on resolving ABO discrepancies, was published in 1993 in the Methods Manual.

She was a superb reference technologist and had a phenomenal memory for patients and the antibodies they made. Those of us who knew her will remember her as a mentor who always encouraged us to try harder and do better. For example, she got us on committees and asked us to give talks. She introduced us to famous people at meetings and could always explain something new in blood banking. Kay was always a great friend with a ready joke. When she retired, she did not look back. She took her golf clubs and went at golf like she went at antibodies. When she had it figured out, she was the women’s club champion for many years. Her humor, intelligence, generosity, friendship, honesty, and jokes will be greatly missed.

Delores Mallory
ERRATUM

Vol. 22, No. 4, 2006; page 176

Review: molecular basis of MNS blood group variants

The author has informed the editors of Immunobematology that there is an error on page 176, second column, second paragraph, third sentence. The sentence should read “Some of the classes of Miltenberger did not react with anti-Mi^r but reacted with one or more of the other three specific antisera, e.g., GP.Hil (Mi.V) RBCs did not react with anti-Mi^r but did react with anti-Hil.”

Free Classified Ads and Announcements

*Immunobematology* 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.

Attention: State Blood Bank Meeting Organizers

If you are planning a state meeting and would like copies of *Immunobematology* for distribution, please contact Cindy Flickinger, Managing Editor, 4 months in advance, by fax or e-mail at (215) 451-2538 or flickingerc@usa.redcross.org.
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 -Fya). Some are directly agglutinating (Anti-M,-Wrb 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 Web site at http://www.nybloodcenter.org/research/immunochemistry/current list of monoclonal antibodies available.

Meetings!

**June 6–8 Blood Banks Association of New York State (BBANYS)**
The 56th annual meeting of the Transfusion Medicine Symposium, Blood Banks Association of New York State (BBANYS), cosponsored by the New York State Department of Health, will be held June 6 through 8, 2007, at the Desmond Hotel and Conference Center in Albany, New York. For more information, contact Kevin Pelletier at (518) 485-5341 or meeting06@bbanys.org, or refer to the Web site at http://www.bbanys.org.

**June 7–8 Heart of America Association of Blood Banks (HAABB)**
The spring meeting of the Heart of America Association of Blood Banks (HAABB) will be held June 7 and 8, 2007, at the Embassy Suites in Kansas City, Missouri. For more information, refer to the Web site at http://www.haabb.org.

Notice to Readers: All articles published, including communications and book reviews, reflect the opinions of the authors and do not necessarily reflect the official policy of the American Red Cross.
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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,
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   2. Abstract
   3. Text
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   5. References
   6. Author information
   7. Tables
   8. Figures

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         v. Discussion: Brief review of literature with unique features of this case
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         viii. Tables (see II.B.7.)

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