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Ophelia by John William Waterhouse

Ophelia is a favorite subject of the pre-Raphaelites and, in particular, John William Waterhouse. The painting, completed in 1910, is one of his more famous. It depicts the doomed daughter of Polonius picking flowers near the river in the moments before she drowns. The red and white blossoms represent both the vitality and the transience of life, as well the curse and fate of lost innocence. The scene is pastoral, but the darker hues and the intensity in her gaze and in how tightly she clutches the flowers and her dress betray her true state of mind. Ophelia mirrors Hamlet in her brooding over the moral collapse around her but is unable to resist the descent into madness.

Like sweet bells jangled, out of tune and harsh;
That unmatch'd form and feature of blown youth
Blasted with ecstasy: O, woe is me,
To have seen what I have seen, see what I see!

The XG blood group, reviewed in this issue, has been investigated in genetic marker studies suggesting possible linkage with manic-depressive illness.
The Scianna system was named in 1974 when it was appreciated that two antibodies described in 1962 in fact identified antithetical antigens. However, it was not until 2003 that the protein on which antigens of this system are found and the first molecular variants were described. Scianna was the last previously serologically defined, protein-based blood group system to be characterized at the molecular level, marking the end of an era in immunohematology. This story highlights the critical role that availability of laboratory reagents for serologic testing has played in the initial characterization of a blood group and sets the stage for the development of new reagents, such as recombinant proteins, to assist in this process. The central role that genetics has played, both by classical pedigree analysis and by molecular techniques, in the discovery and characterization of this blood group is reviewed. *Immunohematology* 2011;27:41–57.

The high- and low-prevalence antigens that constitute the Scianna (SC) blood group system are caused by variants in the erythroid membrane–associated protein (ERMAP).1 SC was initially identified by serologic methods; the clinical significance of antibodies specific to SC is uncertain, although case reports demonstrating rare cases of hemolytic disease attributed to SC variants exist. Genetic analyses in both the classical and molecular approaches, have been central to the discovery and elaboration of the SC system. This article reviews the story of the SC blood group from a genetic point of view, emphasizing the way it has been brought into focus thanks to genetic tools ranging from pedigree analysis to physical mapping.

**History**

**Nomenclature: Sc1, Sc2, Sc3, and Sc4**

The story of the 13th International Society of Blood Transfusion (ISBT) blood group system began in 1962, when a new high-prevalence antigen was reported alongside a coexisting anti-D in a 25-year-old, multiparous woman of Italian descent in Miami, Florida, who experienced several fetal deaths as a result of hemolytic disease of the fetus and newborn (HDFN).2 She came to clinical attention because of difficulty obtaining compatible blood. Her ABO and Rh typings were O ccddee, and her husband’s were O CCDee. After an unremarkable first pregnancy and birth, she experienced three subsequent and progressively earlier fetal demises—at term, at 7, and at 6 months’ gestation—in the late 1950s. After her second fetal death, her anti-D titer was demonstrated at 256, and the new antibody to a high-prevalence antigen, originally named anti-Sm, was demonstrated at a titer of 16. An informative family study revealed three antigen-negative siblings with a likely autosomal dominant mode of antigen inheritance, and no unrelated antigen-negative specimens were identified in a population survey of 600 D− random individuals. A clue to the genetic position of the responsible locus was present even in this defining family: based on the pedigree, it could not be determined whether the new antigen was part of the Rh system as it was in linkage disequilibrium with cc in that kindred.

In spite of this very dramatic introduction, the clinical importance of the new antigen was uncertain, as the concurrent anti-D clearly could account for the proband’s unfortunate obstetric history. While the work of the Miami group was in the pipeline for publication, the Winnipeg Rh Laboratory, in Manitoba, reported an antibody to a new low-prevalence antigen arising in a 50-year-old man with stomach cancer.3 In this patient, the antibody originally named anti-Buα, found in serum Char., was identified during a routine pretransfusion crossmatch. As the patient had been transfused with three units of blood 14 days earlier, this delayed serologic transfusion reaction was investigated, which revealed that although his serum was crossmatch-compatible with all three donor samples before transfusion, it reacted with one of the three samples after transfusion. A follow-up survey of 18 panel red blood cells (RBCs) demonstrated one reactive cell, suggesting a relatively high prevalence for this new antigen; however, this proved not to be the case, as only one of the next 1,000 donors was positive. The families of all three of these probands took part in pedigree analysis, one of which was extremely informative, with a kindred of both parents and nine offspring. These studies in classical genetics demonstrated that the new locus segregated independently from ABO, MNSs, P, Rh, Kell, Kidd, Duffy, and X-chromosome.

**Genetics and Inheritance**

It did not take long for the relationship between the Sm and Buα to be postulated, tested, and proven. In 1964, the anti-Buα serum was used to type the available members of the index Sm family (Fig. 1). The importance of using this...
serum as a typing reagent is underscored by the fact that it was required to demonstrate that the parent generation consists of a mating of two Sm/Bu
a heterozygotes (parents PM Sr. and RM): the F1 generation consists of four Sm– homozygotes, one Sm/Bu
a heterozygote (individual AM), and one Bu
a/Bu
a homozygote (individual CS). Without it, the zygosities of AM and CS could not be determined. This is the only outbred family in the Sm/Bu
a literature in which both parents are Sm/Bu
a heterozygotes.

Concurrent with their suggestion that Sm and Bu
a were the result of a biallelic polymorphism, the Winnipeg Rh Laboratory also reported an extensive study of a Mennonite population in whom the Bu
a antigen had a considerably higher frequency of 5 in 348 samples than the 1 in 1,000 prevalence observed in other Caucasian populations.4,5 Although they tested 145 Caucasian families for Bu
a and rigorously examined 19 based on the presence of Bu
a, it was not until additional reagent serum was available in 1966 that definitive proof of this relationship was found.

Further examples of anti-Bu
a were discovered in a group of individuals from Poland and the United Kingdom (particularly the strongest serum Soch.) who had produced anti-Bu
a after having been artificially immunized with D+ cells to stimulate the production of Rh antibodies.6 Through a careful study of the donors used for these stimulations, three additional subjects who were also stimulated with these cells were found to have created an anti-Bu
a, although it was much weaker than that in the Soch. serum. The prevalence of the Bu
a allele was determined as 0.88 percent in a Warsaw and 0.67 percent in a London cohort.

Using this reagent after adsorption to remove the iatrogenically generated anti-D also present in the serum, the Winnipeg Rh Laboratory identified a large, six-generation Mennonite kindred that included two cousin matings of Sm/Bu
a heterozygotes, which produced 20 offspring.7 Through a methodical review of the serologic data from samples from the kindred, which showed the effects of antibody dosage on antigen expression, this report confirmed and expanded the definition of the new system to exclude all blood groups reported before 1962 (except Diego, Yt, and Auberger) and Do
a and Cs
a. Independence from Yt was established in two British families.8,9 Finally, the currently accepted nomenclature (Table 1) was proposed in 1974 to reflect the surname of the index family in the

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Table 1. Alleles, encoded antigens, and ISBT terminology of the Scianna blood group system

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Trivial name</th>
<th>Allele</th>
<th>Genotype</th>
<th>Wild-type High-prevalence antigen</th>
<th>Variant Low-prevalence antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sc1</td>
<td>Sc+ (Sm)</td>
<td>SC*01</td>
<td>169g</td>
<td>57Gly Sc1</td>
<td>SC*02</td>
</tr>
</tbody>
</table>
| Sc2     | Sc+ (Bu
a)    | SC*02  |          |                               | SC*03N.01†                 |
| Sc3     | Sc null      | SC*03  | 307Δ2    | Frameshift: null              | 332Stop: null              |
| Sc4     | Rd           | SC*04  | 178c     | 60Pro Rd−                      | 60Ala Rd+                 |
| Sc5     | STAR         | SC*05  | 139g     | 47Glu STAR+                    | 47Lys STAR−                |
| Sc6     | SCER         | SC*06  | 242g     | 81Arg SCER+                    | 81Gln SCER−               |
| Sc7     | SCAN         | SC*07  | 103g     | 35Gly SCAN+                    | 35Ser SCAN−               |

† Provisional name suggested by the International Society of Blood Transfusion (ISBT) Working Party on Red Cell Immunogenetics and Blood Group Terminology.
The discovery of anti-Sm, Scianna, such that Sm was renamed Sc1 and Bu* was renamed Sc2.

**The SC Gene Lies on Chromosome 1**

The focus then shifted from definition of the system to characterization of the locus responsible for the antigen at a chromosomal level. Much of this seminal work was performed in the Winnipeg Rh Laboratory. So it is not surprising that their detailed investigations of the genetic mapping of chromosome 1 contributed significantly to the progress on SC. Their unique access to informative kindreds certainly hastened this process, and in a series of reports from 1976 to 1978, linkage between RH and SC was established, showing a logarithm of the odds ratio (LOD score) of 5.34 at a recombination fraction, \( \theta = 0.10 \) if paternally segregated, and the relative position of SC was determined and refined.

**A Third Antigen in the System: Sc3 or Scianna Null Alleles**

The appearance of “minus-minus” phenotypes, i.e., individuals whose RBCs tested negative for both Sc1 and the antithetical Sc2 antigen (Sc:–1,–2), was first documented in 1973 and demonstrated the existence of apparent SC null alleles. However, the term Sc3 was not coined until 1980 when an antibody from an Sc:–1,–2 individual demonstrated no evidence of a separable anti-Sc1 or anti-Sc2. The index patient examined in 1973 was a female surgical patient from the Likiep Atoll in the Marshall Islands, who had been transfused 7 months earlier without crossmatching difficulty. Her cells phenotyped as Sc:–1,–2 as did those from one cousin, but both women’s RBCs could still adsorb anti-Sc2. Despite four pregnancies with an Sc:1,–2 husband, the proband’s cousin had a negative antibody screen. Because these RBCs reduced the titer of anti-Sc2, but not anti-Sc1, they may have had some weak Sc2 expression, and a new antigen was not definitively characterized at that time.

However, in 1980, a 67-year-old man in New York being treated with chemoradiotherapy for metastasis to the throat of a carcinoma of unknown origin required a preoperative transfusion, and demonstrated a positive antibody screen. He had been transfused 4 years earlier without event. Unlike the Likiepian Sc:–1,–2 erythrocytes, RBCs from this patient did not reduce the titer of either anti-Sc1 or anti-Sc2. When tested against the Likiepian Sc:–1,–2 cells, the New York serum did not agglutinate the RBCs. No other Sc:–1,–2 cells were found in a family study. No separable anti-Sc1 was present in this patient’s serum, which is somewhat unusual because patients who completely lack all known antigens for a blood group and have been transfused typically make a polyclonal mixture of antibodies directed at the common epitopes of that system.

The next report of a null phenotype for SC was described in 1986, in another Pacific Islander. This patient was a 4-year-old girl from Papua New Guinea with thalassemia major who had been transfused many times. Anti-Sc3 was demonstrated. Erythrocytes from the patient’s mother were compatible, and both mother and daughter were found to have the Sc:–1,–2 phenotype. In this community, the Sc:–1,–2 phenotype was surprisingly common: A survey of 29 family members and apparently unrelated villagers revealed 6 others (2 of whom had no obvious relationship to the patient) who were also Sc:–1,–2. This very high phenotype prevalence (20.6%) makes heterozygosity for a putative recessive Sc3 allele in the patient’s father quite likely (although his RBC phenotype is not reported in the short abstract), as well as explaining the homozygosity for the same allele observed in her mother.

Additional Sc:–1,–2 individuals have been reported (including a patient who experienced an apparent delayed hemolytic transfusion reaction), some of whom have also lacked several other high-prevalence antigens. Evidence that each patient in their series produced antibodies with different specificities is discussed in Antibodies section.

**Radin: A “New” Low-Prevalence Antigen Is Linked to Scianna (via RH)**

When Radin was first described, in 1967, the relationship between Radin and Scianna was not appreciated. Although the clinical significance of antibodies generated to antigens within the SC system in general is controversial, the analysis of the antibodies that led to the discovery of the Radin antigen is based on its clinical importance, with the initial description encompassing five cases of mild to moderate hemolytic disease of the newborn, of which one required exchange transfusion and one appeared in the first pregnancy. Additional cases and confirmation of the low general frequency (1 in 205) appeared shortly thereafter.

Once again, it was the unique analysis performed by the Winnipeg Rh Laboratory that elucidated the putative connection between Radin and SC. Linkage analysis of eight propositi in ten nuclear families demonstrated linkage between Rd and RH. Similar to the Sc1/Sc2 linkage analysis in the present dataset, the Winnipeg analysis demonstrated heterochiasmy, with the paternal LOD score exceeding 3 at a recombination fraction, \( \theta = 0.10 \), whereas the corresponding maternal LOD neither suggests nor refutes linkage (LOD = 0.41 at \( \theta = 0.10 \)). This finding was only sufficient to propose the connection between SC and Rd, as heterozygotes at both of these (i.e.,...
double recombinants) had not been found. Incorporation of the gene encoding the Rd antigen into the bigger picture of chromosome 1 mapping placed it so close to SC that it was proposed even at that time that the gene encoding Rd “is either very closely linked to or identical with SC.”

There were few additional reports on the SC blood group system for more than 20 years until its molecular basis was finally resolved in 2003. Since then, molecular analysis has identified the three additional alleles underpinning the three antibodies described by Devine and coworkers in 1988.

**Molecular Basis**

The ERMAP Expresses the SC Antigens

The molecular basis of the SC blood group system was identified in 2003 by merging the genetic mapping data with protein chemistry showing that the SC antigens were on a single glycoprotein of approximately 60 to 68 kDa that must be expressed by RBCs. The SC gene had been mapped to chromosome 1, and based on its linkage to the RH genes, the chromosomal location had been further refined to 1p34 to 1p36. This led to the identification of a strong candidate gene.

The ERMAP is a 475-amino acid, type 1 single-pass membrane glycoprotein that is a member of the butyrophilin (BTN) family and is encoded by the ERMAP gene. It consists of a predicted signal sequence of 29 amino acids at the NH₂ terminus, an extracellular immunoglobulin V domain (amino acids 50–126), a short transmembrane domain spanning amino acids 157 through 176, and an intracellular carboxyl terminus encompassing a B30.2 domain from amino acids 238 through 395.

The nomenclature for the transcripts of ERMAP is complicated and has been revised many times. At least two transcript variants have been described, which result from use of an alternative upstream promoter and alternative splicing. The longer variant is 3,423 bp and is designated as transcript 1, or as transcript b by GenBank curators, as it was the second transcript variant identified, and it includes an additional upstream exon (GenBank NM_001017922.1). The shorter variant is designated as transcript 2 or as transcript a, is 3,369 bp, excludes this upstream exon (GenBank NM_018528.3), begins transcription 45 bp upstream from the start of exon 2, and was the first variant discovered; thus, early reports describe this gene as consisting of 11 exons. However, the revised current nomenclature for this gene is based on the presence of 12 exons spanning approximately 28 kb. The relative production of these two mRNAs is not known, nor has it been determined whether the use of either promoter is favored in some tissue types or physiologic conditions. Both transcripts share the common ATG start codon in exon 3; thus, there is no predicted difference in the protein product of the two transcripts. They differ in both the transcript initiation site and the DNA stretch of exon 2 that becomes part of the final transcript; consequently, there are two alternative exon 2s, named exon 2a and exon 2b. The longer transcript 1 comprises exon 1, exon 2b, and exons 3 through 12, whereas the shorter transcript 2 starts with exon 2a and also includes exons 3 through 12 (Fig. 2).

The Ensembl genome browser curators have delineated five transcripts in ERMAP, three of which are protein coding and two of which generate a processed transcript only. Two of the Ensembl transcripts correspond to GenBank’s transcripts 1 and 2; however, unique to the Ensembl database is a 3,949-bp transcript (named ERMAP-002), which is reported as protein coding and generates a short protein encoding 385 amino acids rather than the usual 475. However, only the two transcripts that encode the 475–amino acid protein are included in the Consensus Coding Sequence Project (CCDS; both with identification number CCDS475), so the biological significance of the 3,949-bp transcript is unclear.

In addition to explaining the Sc₁/Sc₂ biallelic single nucleotide polymorphism (SNP) at Gly57Arg, a binucleotide GA deletion was identified at nt307 (now known as SC*03N.01, provisional name suggested by the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology). This deletion causes a frameshift and premature stop codon, which explain the SC null phenotype. The Rd antigen (Sc₄) was defined as the Pro60Ala variation, and two other variants in the presumed leader signal peptide (54C>T and 76C>T) were described in the original study elucidating the molecular basis by Wagner and colleagues (Table 2).

Understanding the Variants Discovered in the Post-ERMAP Era

The knowledge of the gene responsible for the antigens of this blood group protein opened the floodgates through which variant descriptions of unknown, unresolved serologic cases promptly flow. This was clearly the case for SC, for which three new variants were discovered within just a few years after the characterization of ERMAP as the SC gene. Careful molecular follow-up of the three patients described by Devine and coworkers in 1988 revealed three distinct ERMAP/SC variants, demonstrating the importance of molecular testing in the resolution of serologic SC mysteries. The success of these investigations depended not only on the cooperation among an international collaborative, but also on the supportive participation of two patients and...
a family who kindly released two autologous RBC units. It was appreciated in the initial case reports in 1988 that the SC protein carried multiple high-prevalence antigens other than Sc1/2. Sera or eluates from these three Sc1,–2 patients who had developed high-prevalence antibodies did not react with the Sc:–1,–2 null RBCs, but the samples were not mutually compatible.

Sc5 (STAR)

In 1982, a 65-year-old man with a history of transfusion of three units of crossmatch-compatible whole blood before presentation underwent routine preoperative blood bank testing, demonstrating anti-C and anti-e. He was transfused with another three units of C–e– RBCs, and 1 week later, a new anti-Jkβ and an antibody against a high-prevalence antigen were detected. Because his serum reacted with all cells except his own (phenotyped as Sc:1,–2), those of a sibling, and Sc:–1,–2 RBCs, it was suspected that he had developed an antibody to another antigen on the SC protein. Blood needs for the patient were met with autologous units. Blood samples from the proband and 16 family members had been frozen. Sequencing of the exons encoding the extracellular and transmembrane domains demonstrated homozygosity at a new nonsynonymous polymorphism at amino acid 47 (glutamic acid to lysine), which is in the N-terminal domain. This variation is catalogued as rs56047316 in the SNP database (dbSNP), and it results from the guanine–adenine transition at nucleotide 139 in the complementary DNA (cDNA). Frequencies of this allele in population studies have not been reported, nor have additional cases of this antibody.

Sc6 (SCER) and Sc7 (SCAN)

The remaining two antibody cases described by Devine and colleagues were concomitantly resolved using the same approach as for the Sc5 antigen. These investigators sequenced all 11 exons of ERMAP known at the time, which encompasses all cDNA. They also solicited others to submit suspected SC variants and the eight other orphan low-prevalence antigens By, To, Ptα, Jeα, Liα, SARA, and Skα. The proband for case 1 reported by Devine and colleagues demonstrated homozygosity for a guanine–adenine transition at cDNA nucleotide 242, predicted to cause an amino acid change at position 81 (from arginine to glutamine), which is in the immunoglobulin V loop. The case 2 proband demonstrated a new homozygous,
nonsynonymous variant at cDNA nucleotide position 103 (again a guanine–adenine transition), corresponding to a glycine to serine change at amino acid 35 in the NH₃ terminus. This specimen also demonstrated two other SNPs (at cDNA nucleotides 54C>T and 76C>T), which had been previously reported, are common in Caucasian populations, and are in tight linkage disequilibrium (LD). Because these variants are both in the predicted leader sequence (and the 54C>T is a silent mutation), they are not predicted to directly impact on ERMAP structure. This report also excluded the eight orphan low-prevalence antigens from the SC system because the only variants found were in the leader sequence or in introns.

Molecular Basis of SC Null Phenotypes

Central to the set of specimens originally reported with the discovery of ERMAP as SC was an Sc:−1,−2 sample from a Saudi Arabian pedigree, which demonstrated homozygosity at three SNPs: the common 54C>T and 76C>T (described in an earlier section) as well as a 2-bp deletion starting at cDNA nucleotide 307 (307Δga) that is predicted to cause a frameshift mutation and early termination codon after 113 amino acids. The nt54 and nt76 SNPs were genotyped in an additional 111 European blood donors and were found in tight LD. The frameshift would account for a complete lack of an intact ERMAP protein in the cell membrane.

Two Sc:−1,−2 individuals from northern and southern California were then sequenced in a follow-up study, and both shared a new SC null allele formed by a nonsense mutation at codon 332. This is the first report of a variant in the B30.2 intracellular domain in a patient immunized to SC. It is not, however, the only variant in the B30.2 domain (Table 2), because three others have been described

Table 2. Erythroid membrane–associated protein polymorphisms in the coding region and global population frequencies

<table>
<thead>
<tr>
<th>Sc name</th>
<th>Descriptive name</th>
<th>dbSNP ID:</th>
<th>cDNA nt</th>
<th>mRNA*</th>
<th>Wild-type (WT)</th>
<th>Variant</th>
<th>Protein effect</th>
<th>Amino acid (aa) codon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sc1 vs. Sc2</td>
<td></td>
<td>rs56025238</td>
<td>169</td>
<td>439</td>
<td>g (Sc1)</td>
<td>nonsynonymous substitution</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Sc4</td>
<td>Rd</td>
<td>rs56136737</td>
<td>178</td>
<td>448</td>
<td>c</td>
<td>nonsynonymous substitution</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Sc6</td>
<td>SCER</td>
<td>rs53954154</td>
<td>219</td>
<td>489</td>
<td>c</td>
<td>nonsynonymous substitution</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Sc null (Δga together)</td>
<td></td>
<td>rs56695242</td>
<td>307</td>
<td>577</td>
<td>g</td>
<td>del −1</td>
<td>frameshift</td>
<td>103</td>
</tr>
<tr>
<td>Sc null (Δga together)</td>
<td></td>
<td>rs56151267</td>
<td>308</td>
<td>578</td>
<td>a</td>
<td>del −1</td>
<td>frameshift</td>
<td>103</td>
</tr>
<tr>
<td>rs35147822</td>
<td>775</td>
<td>1045</td>
<td>t</td>
<td>c</td>
<td>nonsynonymous substitution</td>
<td>259</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs34441268</td>
<td>788</td>
<td>1058</td>
<td>g</td>
<td>a</td>
<td>nonsynonymous substitution</td>
<td>263</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs35972628</td>
<td>888</td>
<td>1158</td>
<td>g</td>
<td>a</td>
<td>silent</td>
<td>296</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sc null</td>
<td></td>
<td>rs55773259</td>
<td>994</td>
<td>1264</td>
<td>c</td>
<td>nonsense (STOP)</td>
<td>332</td>
<td></td>
</tr>
<tr>
<td>rs55872827</td>
<td>1324</td>
<td>1594</td>
<td>t</td>
<td>c</td>
<td>nonsynonymous substitution</td>
<td>442</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs56405033</td>
<td>1355</td>
<td>1625</td>
<td>t</td>
<td>c</td>
<td>nonsynonymous substitution</td>
<td>452</td>
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<tr>
<td>rs55677363</td>
<td>1356</td>
<td>1626</td>
<td>g</td>
<td>t</td>
<td>silent</td>
<td>452</td>
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</tbody>
</table>

*NCBI Reference Sequence Position: NM_001017922.1

Minor allele frequencies for some populations are determined by reports of serologic phenotype and assuming individuals positive for a rare antigen are heterozygotes.

cDNA = complementary DNA; dbSNP = single nucleotide polymorphism database; IgV = immunoglobulin V; mRNA = messenger RNA; nt = nucleotide.
Table 2. Minor allele frequencies for some populations are determined by reports of serologic phenotype and assuming individuals positive for a rare antigen are

<table>
<thead>
<tr>
<th>WT aa</th>
<th>Variant aa</th>
<th>Exon</th>
<th>Protein domain</th>
<th>Minor allele frequency in blood donors*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>Val</td>
<td>3</td>
<td>Leader</td>
<td>0.054 (dbSNP)</td>
</tr>
<tr>
<td>Leu</td>
<td>Leu</td>
<td>3</td>
<td>Leader</td>
<td>0.28 (German†), 0.1 (dbSNP)</td>
</tr>
<tr>
<td>His</td>
<td>Tyr</td>
<td>3</td>
<td>Leader</td>
<td>0.33 (German†), 0.25 (Caucasian North American), 0.1 (dbSNP), 0.05 (African-American)</td>
</tr>
<tr>
<td>Gly</td>
<td>Ser</td>
<td>4</td>
<td>NH₃-terminus</td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>Lys</td>
<td>4</td>
<td>NH₃-terminus</td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>Arg</td>
<td>4</td>
<td>IgV loop</td>
<td>0.01 (Caucasian North American, Brazilian), 0.008 (German†), 0.005 (Hispanic American), 0.002 (African-American), 0 (Asian American)</td>
</tr>
<tr>
<td>Pro</td>
<td>Ala</td>
<td>4</td>
<td>IgV loop</td>
<td>0.003 (Slavs), 0.002 (Caucasian North American, Danish, German, New York Jewish)</td>
</tr>
<tr>
<td>Arg</td>
<td>Arg</td>
<td>4</td>
<td>IgV loop</td>
<td>0.017 (dbSNP)</td>
</tr>
<tr>
<td>Arg</td>
<td>Glu</td>
<td>4</td>
<td>IgV loop</td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>frameshift</td>
<td>4</td>
<td>IgV loop</td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>frameshift</td>
<td>4</td>
<td>IgV loop</td>
<td></td>
</tr>
<tr>
<td>Cys</td>
<td>Arg</td>
<td>12</td>
<td>B30.2 domain</td>
<td>0.022 (dbSNP)</td>
</tr>
<tr>
<td>Gly</td>
<td>Glu</td>
<td>12</td>
<td>B30.2 domain</td>
<td>0.011 (dbSNP)</td>
</tr>
<tr>
<td>Glu</td>
<td>Glu</td>
<td>12</td>
<td>B30.2 domain</td>
<td>0.011 (dbSNP)</td>
</tr>
<tr>
<td>Arg</td>
<td>STOP</td>
<td>12</td>
<td>B30.2 domain</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Leu</td>
<td>12</td>
<td>C-terminus</td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>Pro</td>
<td>12</td>
<td>C-terminus</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Pro</td>
<td>12</td>
<td>C-terminus at a 3' dileucine (LL) phosphorylation motif</td>
<td>0.054 (dbSNP)</td>
</tr>
<tr>
<td>Leu</td>
<td>Leu</td>
<td>12</td>
<td>C-terminus at a 3' dileucine (LL) phosphorylation motif</td>
<td>0.054 (dbSNP)</td>
</tr>
</tbody>
</table>

in dbSNP, two of which are nonsynonymous amino acid changes (C259R and G263E) and one of which is silent (E296E). This result suggests that an early translation termination, even as late as at codon 332 of the expected 475, sufficiently interferes with correct protein trafficking, membrane insertion, or stability so as to render the individual susceptible to alloimmunization at the Sc1/2 site (codon 57).

**Other Variants in ERMAP: Using dbSNP and HapMap**

Variants in the coding region, particularly those in the extracellular domain of transmembrane proteins, are of particular clinical interest in transfusion medicine, as their location provides an obvious mechanism for putative clinical significance by alloimmunization. However, much variation in the human genome is found outside of coding regions, and ERMAP is no exception. Studies of this variation are a critical tool in the investigation of human disease and basic sciences. Several collaborative projects in human genomics focus on gathering, sharing, and interpreting human genetic variation, and although a full cataloguing of these is beyond the scope of this review, we will present variants in ERMAP that are part of two such projects: dbSNP and the International HapMap Project. Table 2 integrates some of these variants (identified by their rs numbers) with those described in the transfusion medicine literature. These data allow informative LD studies of ERMAP (Fig. 3). This LD map shows two distinct LD blocks that correspond to the exons encoding the extracellular immunoglobulin-like domains and the intracellular B30.2 domains of ERMAP. The importance of this pattern of LD is that it reveals a possible evolutionary vestige of the modular nature of the butyrophilin-like (BTN1) protein family and is even more pronounced in the Nigerian population. Long stretches of
LD such as that illustrated in the extracellular domain of ERMAP, where all of the SC antigens are encoded, raise the possibility that a selective advantage by genetic hitchhiking may be operating at ERMAP.

Global Variation
Some ethnographic trends have already been historically appreciated with SC variants during the early years of their investigation (Table 2). For instance, Sc4 has been identified most often in Ashkenazi Jews and Slavic populations, and the few Sc−1,−2 phenotypes have been reported particularly in populations from Oceania. Sc2 appears to be more common in a consanguineous Canadian Mennonite population (derived from a small region in Eastern Europe), but it remains very rare in or absent from populations of African or Native American descent. However, it is not sufficient to rely on case reports to propose generalizations regarding population frequencies. Fortunately, recent technologies are available to rapidly genotype ERMAP variants using an automated genotype-calling platform, and additional population studies will continue to be reported.

Biochemistry and Physiology

ERMAP is a Member of the Butyrophilin-like Family of the Immunoglobulin Superfamily
By virtue of its extracellular immunoglobulin V and the intracellular B30.2 domains, ERMAP is a member of the BTNL protein family, which is a subset of the immunoglobulin superfamily. The compact, globular 110–amino acid immunoglobulin domain defines this superfamily and is found in three operational domain subclasses: variable (immunoglobulin V), intermediate (immunoglobulin I), or constant (immunoglobulin C). These proteins are central to many immunologic processes, especially cell adhesion, costimulation, and signalling. The immunoglobulin superfamily is one of the largest in all eukaryotic organisms. Other members of this family well known to the transfusion medicine community...
include the Lutheran, LW, OK, JMH, and Indian blood groups, which are found on the B-CAM, ICAM4, CD147, CD108, and CD44 molecules, respectively. BTN proteins are within the B7-CD28-like branch of this superfamily and have been extensively studied in cows. The prototype of the human BTN family of proteins is BTN1A1, which is primarily expressed in the lactating breast, where it makes up 20 percent of the protein in the membrane of milk fat globules. The name derives from the Greek butyros and philos, meaning “having an affinity for butterfat.” Although ERMAP is carried on chromosome 1, the genes for many members of this family are located in the major histocompatibility complex (MHC) on chromosome 6. The lactational and immunologic functions of BTNs as a part of the secretory granule-to-plasma membrane zipper complex and as an inhibitor of T-cell activation are only recently described, and even less is known about the BTNL proteins. There are six known human BTNL proteins, which are defined by their homology to BTN proteins (Fig. 4). The most extensively studied of these is BTNL2, the gene for which is located in the MHC class II cluster at 6p21.3. A truncated splice site mutation in BTNL2 has been associated with sarcoidosis, and although this SNP has been investigated in many other infectious and autoimmune diseases, some of these associations have been attributable to LD with the MHC. BTNL2 inhibits T-cell activation by inhibiting proliferation in response to the stimulatory T-cell receptor signal. This inability for BTNL2 to propagate a cell signal has led some investigators to propose its role as “decoy receptor” because it lacks the B30.2 intracellular domain present in most other BTN and BTNL proteins.

The B30.2 Cytoplasmic Domain: A Role in Erythropoiesis?

The B30.2 (or PRYSPRY) domain was described in 1993 with the discovery of an exon in the MHC class I region showing similarity to other mammalian and amphibian proteins, which have since been termed the tripartite motif family. Such a domain was demonstrated in bovine BTN and in human BTN proteins. The B30.2 domain is proposed to be a recent evolutionary adaptation in the immune system of mammals as a fusion of two ancient domains (PRY and SPRY), which are found in all eukaryotes. The genes for proteins in this family have a modular structure, suggesting that they arose by duplication. Mutations in the B30.2 domain in the tripartite motif branch of the B30.2 proteins have been associated with Opitz syndrome (MID1 protein) and familial Mediterranean fever (pyrin protein). However, human syndromes have not been associated with mutations in the B30.2 domain of the BTN or BTNL families.
The crystal structure of the B30.2 domain of pyrin was recently elucidated. A β-barrel consisting of two antiparallel β-sheets has been described, forming a central cavity. The ligand(s) that interact with the B30.2 domain have yet to be described, although binding analyses suggest that it is the site of protein–protein interactions. The crystal structure of one other B30.2 domain interacting with a peptide has shown that there is a conformationally rigid peptide binding pocket (consisting of a core β-sandwich around variable loops) binding to a short-sequence motif, which may allow multiple intracellular targets to bind. Recently, BTN1A1 has been shown to bind xanthine oxidoreductase via B30.2, apparently stabilizing the milk fat globule membrane in mammary tissue, but it is also hypothesized to function as a novel signaling pathway in nonmammary tissues or perhaps in the innate immune system via generation of reactive oxygen species.

A role for B30.2 domains in erythropoiesis has been revealed through study of an unlikely model organism: Antarctic icefish. These animals are the only vertebrate taxon that fails to produce RBCs (Fig. 5), and as such have been studied in a hunt for genes important in erythropoiesis and cardiovascular biology. Using this approach, a new B30.2-containing protein, called bloodthirsty (bty), was identified in the pronephric kidney of a red-blooded Antarctic rockcod (Notothenia coriiceps), which was present at levels tenfold higher than those in an icefish (Chaenocephalus aceratus). Interestingly, disruption of bty synthesis in zebrafish suppressed both erythrocyte production and hemoglobin synthesis, and although interactions between bty and ERMAP have been hypothesized, they have not been empirically demonstrated (H. William Detrich, personal communication, January 20, 2010).

**Functional Studies of ERMAP**

The function of ERMAP itself, however, is not well understood. The murine homolog, Ermap, was described first and named as such because it was found to be produced exclusively in erythroid cells. The human homolog was characterized shortly thereafter, and Northern blots demonstrated high expression in hematopoietic tissues, such as fetal liver and bone marrow, with weaker expression in peripheral blood leukocytes, thymus, lymph node, and spleen. Set has been demonstrated on phagocytic leukocytes using an antibody absorption technique. Recent RNA array expression analysis supports this finding, having detected low levels of ERMAP transcripts in leukocytes, especially monocytes; however, quantitative protein expression remains to be assessed. An in silico analysis of nucleotide database searches of human expressed-sequence tags using the ERMAP transcript 1 (GenBank NM_001017922.1) as the probe detected the transcript in cDNA libraries from hematogenous tissues, such as bone marrow, a chronic myeloid leukemia cell line, peripheral blood pool, thymus, spleen, and fetal liver, as well as in neural tissues. However, it is difficult to definitively ascribe transcript production to the nonerythroid cells in the neural tissues, as contamination from the vasculature cannot be completely eliminated. ERMAP mRNA reaches peak levels in fetal liver in the 18th through 20th weeks, and it is present from the 15th through 32nd weeks in fetal bone marrow, suggesting that ERMAP may be related to the migration of erythroid cells to these sites during hematopoietic development.

Additional evidence that ERMAP may be involved in erythroid differentiation has been put forward, although only abstracts of these reports are available in the English literature. Using fluorescent quantitative polymerase chain reaction, ERMAP expression has been found in the K562 cell line, which is derived from chronic myeloid leukemia and is of undifferentiated granulocytic lineage. To test the degree of erythroid-specificity of ERMAP in hematopoiesis, this group used cytarabine (Ara-C) to induce these cells toward erythroid differentiation and 12-O-tetradecanoylphorbol-13-acetate to induce development toward the macrophage lineage, but found an increase in ERMAP mRNA after an Ara-C stimulation only. This finding suggests that although ERMAP may be present on cells of the monocyte/macrophage lineage, its functional importance may be limited in these cell types. RNA silencing experiments using an ERMAP shRNA/K562 cell line also reported by this group showed decreased ERMAP expression and morphologic features, including the relative amounts of surface erythrocyte maturation markers, leading the authors to conclude that ERMAP shRNA inhibited Ara-C–induced erythroid differentiation.

A second model of erythroid differentiation using umbilical cord blood with two naturally occurring hormones (namely stem cell factor and IL-3) and erythropoietin to provoke differentiation also showed a concurrent rise in ERMAP mRNA with erythrocyte maturation. Many structural features of ERMAP point toward a putative role in immunity, perhaps by adhering to other cells or pathogens via its extracellular immunoglobulin V domain, or by immunoregulatory mechanisms such as the modulation of cellular activation signals via B30.2 as is observed in its BTN family members. Much work is needed in this area to better characterize the proteins that interact with ERMAP, both extracellularly and intracellularly, and to determine whether ERMAP has a central role in erythropoiesis itself.
Antibodies in the System

The discovery of the SC blood group system began with and has relied on the detection and investigation of alloantibodies. The effects of enzymes and chemicals and the in vitro characteristics of SC antibodies were recently reported elsewhere. In general, SC antigens are resistant to ficin plus papain, trypsin, α-chymotrypsin, sialidase, and 50 mM dithiothreitol (DTT), and they are sensitive to pronase and 200 mM DTT. The exception to this trend is the variable sensitivity of Sc4 to trypsin and α-chymotrypsin. Enzymatic properties of anti-SCER and anti-SCAN are only described in the initial case report as showing no change in the strength of antibody activity when tested with RBCs that had been treated with ficin, papain, trypsin, ZZAP (mixture of 0.1 M DTT plus 0.1% cysteine-activated papain), or chloroquine diphosphate. Anti-STAR demonstrated enhanced reactivity with enzyme-treated RBCs. Patients or donors in whom an antibody to an SC antigen has been reported (Table 3) have been thoroughly reviewed recently.

Thirteen of the 19 reports of alloantibodies to SC system antigens listed in Table 3 have occurred in cases in which they do not appear to manifest a major clinical significance. All six of the reports with clinical significance occurred in cases of HDFN. All six of the reported autoantibodies to SC system antigens in patients were in the context of clinically significant autoimmune hemolytic anemia, although five of these cases were presented as an abstract only, without the full clinical details. Alleles Sc4–7 were not tested in any of these cases. Such an investigation is warranted because heterozygosity for alleles with weak expression in the Rh blood group system has been associated with D autoantibodies.

Clinically important reactions to an erythrocyte antibody typically result from significant RBC destruction and usually manifest in the patient as a hemolytic anemia or HDFN. In the SC system, significant HDFN defined the blood group in a patient named Ms. Scianna; however, she also had anti-D to account for her severe obstetric complications in addition to the anti-Sc1. Anti-Sc2 has been reported in a case of HDFN requiring simple transfusion in a 20-day-old infant. Importantly, the search for antibodies to low-prevalence antigens in this patient was only performed because the neonate and the mother were ABO-compatible: had they been ABO-incompatible, the HDFN would likely have been attributed to this, and the anti-Sc2 antibody may not have been detected.

The recent availability of recombinant reagents to assist in the detection of SC antibodies may bring this ability into the wider immunohematology arena. This novel technique will allow one to appraise the relevance of SC antibodies in conjunction with other antibodies and tease out their relative clinical importance, which may have been previously underestimated. The ERMAP protein has been expressed in its native form in a eukaryotic system, and so although the initial report describes the utility of this reagent for detecting the high-prevalence SC antigens, a screening protein for low-prevalence antigens Sc2 and Sc4 would also be feasible.

Some SC alloantibodies (Table 3) resulted in delayed serologic transfusion reactions without associated hemolysis, including a so-called naturally occurring anti-Sc4 described in a 55-year-old man without a transfusion history. Many of these patients were transfused with crossmatch-compatible blood without incident. However, some surgeries were delayed owing to lack of availability of crossmatch-compatible units, and other patients were transfused with autologous products in nonurgent scenarios.

The remaining reports of important reactions involving SC antibodies are in cases of autoimmune hemolytic anemia. Auto-anti-Sc1 and -Sc3 have been eluted from direct antiglobulin test–positive RBCs of these patients. In many cases, these antibodies were transient and associated with decreased expression of SC antigens. Several patients underwent splenectomy in addition to treatment with many types of immunosuppressant medications. One patient with erythroid hypoplasia and diagnosed with an Evans-like syndrome even underwent a total of 20 plasma exchanges over the course of 11 weeks. After the ninth exchange, the antibody was no longer detectable, although it reappeared 1 week later. He was finally transfused after 16 exchanges, despite the continued presence of the antibody, with a resultant rise in his hematocrit from 14% to 30%.

Clinical Significance

Is It Worth Phenotype or Genotype Matching?

Of the 23 cases of SC alloantibodies catalogued here (Table 3), one case of HDFN and one delayed hemolytic transfusion reaction (case 2) relate sufficient information in their reports to convincingly attribute clinical relevance to SC antibodies. The HDFN cases described in the initial report of the Radin antigen also speak to the potential importance of antibodies to Sc4 (especially the case that required exchange transfusion); however, these cases are not reported in sufficient detail to definitively implicate anti-Sc4 to the exclusion of all other causes. Even the very dramatic presentation of Ms. Scianna’s antibody does not incriminate anti-Sc1 as the cause of her poor obstetric outcomes, as an anti-D of a higher titer was also found. The fundamental question of when and whether antibodies
Table 3. Published reports of antibodies to antigens in the Scianna blood group system

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Allo or auto?</th>
<th>Authors</th>
<th>Year</th>
<th>Case identifier</th>
<th>Scianna phenotype</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Ethnicity</th>
<th>Important reactions?</th>
<th>Other antibodies</th>
<th>Transfusion/pregnancy history</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-Sc1</td>
<td>Allo</td>
<td>Schmidt et al.</td>
<td>1962</td>
<td>Mrs. N.S.</td>
<td>Sc:–1,2</td>
<td>25</td>
<td>F</td>
<td>Caucasian</td>
<td>HDFN</td>
<td>anti-D</td>
<td>Multiparous, after 1st baby with HDFN.</td>
</tr>
<tr>
<td></td>
<td>Auto</td>
<td>Kaye et al.</td>
<td>1990</td>
<td>Sc:–1,2</td>
<td>28</td>
<td>F</td>
<td>Indian</td>
<td>No HDFN</td>
<td>Excluded all except E and Lu(a)</td>
<td></td>
<td>G2, 1st was uncomplicated with negative Ab screen.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tregellas et al.</td>
<td>1979</td>
<td>Sc:1,–2</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>McDowell et al.</td>
<td>1986</td>
<td>Case 1</td>
<td>Sc:1,–2</td>
<td></td>
<td></td>
<td></td>
<td>Hemolytic anemia</td>
<td></td>
<td>4 units RBC transfused earlier.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>McDowell et al.</td>
<td>1986</td>
<td>Case 2</td>
<td>Sc:1,–2</td>
<td>(1+, but 3+ 2 years later)</td>
<td></td>
<td></td>
<td>Hemolytic anemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Owen et al.</td>
<td>1992</td>
<td>Sc:1,–2</td>
<td>10 months</td>
<td>F</td>
<td>West Indies</td>
<td>Hemolytic anemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Auto</td>
<td>Ramsey and Williams</td>
<td>2010</td>
<td>Sc:1</td>
<td>20</td>
<td>F</td>
<td>Caucasian</td>
<td>Hemolytic anemia, acute hemolysis</td>
<td>15 units RBC 3 years earlier during chemotherapy, previously negative Ab screen.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Auto vs. Allo</td>
<td>Steane et al.</td>
<td>1982</td>
<td>Case 2: pt C.D.</td>
<td>22</td>
<td>M</td>
<td>Caucasian</td>
<td></td>
<td>Not reported.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anderson et al.</td>
<td>1963</td>
<td>Mr. Char.</td>
<td>Sc:1,–2</td>
<td>50</td>
<td>M</td>
<td>Caucasian</td>
<td>DSTR vs. DHTR</td>
<td>3 units RBC transfused 2 weeks earlier.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seyfried et al.</td>
<td>1986</td>
<td>Four donors</td>
<td>Sc:1,–2</td>
<td>4</td>
<td>F</td>
<td>Papua New Guinea</td>
<td>DSTR vs. DHTR</td>
<td>Many transfusions.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Auto</td>
<td>DeMarco et al.</td>
<td>1995</td>
<td>Sc:1,–2</td>
<td>29</td>
<td>F</td>
<td>Caucasian</td>
<td>HDFN</td>
<td>Negative maternal Ab screen</td>
<td>60, no transfusion history.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>McCreary et al.</td>
<td>1973</td>
<td>Sc:1,–2</td>
<td>22</td>
<td>F</td>
<td>Likiep Atoll, Marshall Islands</td>
<td>Transfused 7 months earlier without difficulty.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nason et al.</td>
<td>1980</td>
<td>Sc:1,–2</td>
<td>67</td>
<td>M</td>
<td>Caucasian</td>
<td>DSTR</td>
<td>Transfused 4 years earlier without incident.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Woodfield et al.</td>
<td>1986</td>
<td>Sc:1,–2</td>
<td>4</td>
<td>F</td>
<td>Fijian Islands</td>
<td>DSTR vs. DHTR</td>
<td>3 units RBC transfused 2 weeks earlier.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Auto</td>
<td>Peloquin et al.</td>
<td>1989</td>
<td>Case 1</td>
<td>weak Sc1 and Sc3</td>
<td>64</td>
<td>F</td>
<td>Hemolytic anemia</td>
<td>Hb 10.0 g/dL, DAT + (IgG and C3d).</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peloquin et al.</td>
<td>1989</td>
<td>Case 2</td>
<td>weak Sc1 and Sc3</td>
<td>54</td>
<td>F</td>
<td>Hemolytic anemia</td>
<td>4 units RBCs transfused earlier.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rausen et al.</td>
<td>1967</td>
<td>Family 3–Ha</td>
<td>Sc:–4 (Rd+)</td>
<td>F</td>
<td>Northern European</td>
<td>HDFN</td>
<td>“Newborn with hemolytic disease” in 4th child.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rausen et al.</td>
<td>1967</td>
<td>Family 4–We</td>
<td>Sc:–4 (Rd+)</td>
<td>F</td>
<td>Native American</td>
<td>HDFN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rausen et al.</td>
<td>1967</td>
<td>Family 5–Gr</td>
<td>Sc:–4 (Rd+)</td>
<td>F</td>
<td>German Jewish or Scotch-Irish</td>
<td>HDFN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lundsgaard and Jensen</td>
<td>1968</td>
<td>Mrs. J.P.</td>
<td>Sc:–4 (Rd+)</td>
<td>47</td>
<td>F</td>
<td>DSTR: No clinical evidence of hemolysis</td>
<td>Had a 9-year-old child.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lundsgaard and Jensen</td>
<td>1968</td>
<td>Mr. L.C.</td>
<td>Sc:–4 (Rd+)</td>
<td>55</td>
<td>M</td>
<td>DSTR: No clinical evidence of hemolysis</td>
<td>Anti-Vw</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Winn et al.</td>
<td>1976</td>
<td></td>
<td>19</td>
<td>M</td>
<td>Caucasian</td>
<td>Negative DAT, negative Ab screen before transfusion.</td>
<td>92, no transfusion.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>anti-Sc5</td>
<td>Devine et al.</td>
<td>1988</td>
<td>Case 3</td>
<td>Sc:1,–2</td>
<td>65</td>
<td>M</td>
<td>Irish and English</td>
<td>DSTR</td>
<td>anti-C anti-e anti-Jk4</td>
<td>3 units RBC 3 years earlier.</td>
</tr>
<tr>
<td>(anti-STAR)</td>
<td></td>
<td>Devine et al.</td>
<td>1988</td>
<td>Case 3</td>
<td>Sc:1,–2</td>
<td>65</td>
<td>M</td>
<td>Irish and English</td>
<td>DSTR</td>
<td>anti-C anti-e anti-Jk4</td>
<td>3 units RBC 3 years earlier.</td>
</tr>
<tr>
<td></td>
<td>anti-Sc6</td>
<td>Devine et al.</td>
<td>1988</td>
<td>Case 1</td>
<td>Sc:1,–2</td>
<td>76</td>
<td>M</td>
<td>German</td>
<td>DSTR</td>
<td></td>
<td>3 units RBC 6 years earlier.</td>
</tr>
<tr>
<td>(anti-SCER)</td>
<td></td>
<td>Devine et al.</td>
<td>1988</td>
<td>Case 1</td>
<td>Sc:1,–2</td>
<td>76</td>
<td>M</td>
<td>German</td>
<td>DSTR</td>
<td></td>
<td>3 units RBC 8 years earlier.</td>
</tr>
<tr>
<td></td>
<td>anti-Sc7</td>
<td>Devine et al.</td>
<td>1988</td>
<td>Case 2</td>
<td>Sc:1,–2</td>
<td>50</td>
<td>M</td>
<td>German, English, and Native American</td>
<td>DHTR</td>
<td>anti-D</td>
<td>3 units RBC 3 years earlier.</td>
</tr>
<tr>
<td>(anti-SCAM)</td>
<td></td>
<td>Devine et al.</td>
<td>1988</td>
<td>Case 2</td>
<td>Sc:1,–2</td>
<td>50</td>
<td>M</td>
<td>German, English, and Native American</td>
<td>DHTR</td>
<td>anti-D</td>
<td>3 units RBC 3 years earlier.</td>
</tr>
</tbody>
</table>

Ab = antibody; ADCC = antibody-dependent cell-mediated cytotoxicity; AHG = antihuman globulin; AIHA = autoimmune hemolytic anemia; CHF = congestive heart failure; DAT = direct antiglobulin test; DHTR = delayed hemolytic transfusion reaction; DOL = day of life; DSTR = delayed serologic transfusion reaction;
### Table 3

<table>
<thead>
<tr>
<th>Clinical synopsis</th>
<th>Other laboratory data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neonate with 3+ DAT, eluted anti-Sc1, but Hgb = 13.5 g/dL and no HDFN. Mother B, Rt; infant B, rr; husband Sc:1–2.</strong></td>
<td>IgG3 subclass; steady rise in ADCC despite constant titer throughout pregnancy; 9 weeks postpartum ADCC increased ×4 and titer doubled (16 to 32).</td>
</tr>
<tr>
<td>Healthy blood donor.</td>
<td>Antibody demonstrated in serum, not in plasma, suggesting autoantibody is only seen when it reacts with a coagulation factor; IgG3; very weak DAT (both IgG and C3b), weak anti-Sc1 eluted.</td>
</tr>
<tr>
<td><strong>Sudden-onset jaundice, pallor, fever with hepatomegaly, Hgb = 4.1 g/dL. AHA diagnosed, treated with steroids and IVIG, transfusion, urgent splenectomy. Transfusion-dependent for 4 weeks, then steroids stopped 7 months after splenectomy.</strong></td>
<td>3+ DAT and anti-Sc1 in IAT. Eluates by heat, ether, and glycine acid were nonreactive, but xylene eluate showed anti-Sc1.</td>
</tr>
<tr>
<td><strong>Hodgkin disease in remission, presented with 3 weeks of fatigue, 2-day Hgb decrease (9.1 to 5.7 g/dL). Gross hematuria and Tbil = 5.5 mg/dL during 3 units RBC transfusion.</strong></td>
<td>Transient Sc antigen weakening; serum from initial presentation reacted 3+ with patient’s RBCs 2 years later.</td>
</tr>
<tr>
<td><strong>Evan’s-like syndrome with anemia during a lung infection, 2 years of hospitalizations, steroids, splenomegaly, stem cell transplant, transfused safely after 16 plasma exchanges.</strong></td>
<td><strong>Carcinoma of the stomach, pretransfusion antibody screen positive. Hemolysis could be the reason for the 2nd blood request, but this is not stated.</strong></td>
</tr>
<tr>
<td>Healthy donors iatrogenically immunized for anti-D production.</td>
<td><strong>Jaundice in infant (Tbil = 14.3 mg/dL) on DOL2, postphlebotomy discharged Hct = 45%, DOL20 Tbil = 0.5 mg/dL, Hct 17.3% with pallor, tachypnea, tachycardia; transfused 45 ml; Hct 4 months later = 38.3% Mother B+; infant B-</strong>.</td>
</tr>
<tr>
<td>Preoperative testing.</td>
<td><strong>Reported DAT+, but “presence of an antibody in his plasma which reacted with all erythrocytes tested except his own.” Antibody undetectable after the 9th exchange, then disappeared after 4 more exchanges.</strong></td>
</tr>
<tr>
<td>Preoperative positive antibody screen for metastatic carcinoma surgery. Not transfused owing to deteriorating clinical condition and lack of compatible blood.</td>
<td><strong>Jaundice in infant (Tbil = 14.3 mg/dL) on DOL2, postphlebotomy discharged Hct = 45%, DOL20 Tbil = 0.5 mg/dL, Hct 17.3% with pallor, tachypnea, tachycardia; transfused 45 ml; Hct 4 months later = 38.3% Mother B+; infant B-</strong>.</td>
</tr>
<tr>
<td><strong>2 children Rd+, both had HDFN; 1 child Rd-, did not have HDFN.</strong></td>
<td>Antibody dropped to below detectable levels shortly after discovery.</td>
</tr>
<tr>
<td><strong>Preoperative positive antibody screen for atonic goiter surgery. Considered as a “naturally occurring antibody.”</strong></td>
<td><strong>Preoperative Ab screen for revision of right hip arthroplasty, procedure delayed until autologous units could be collected.</strong></td>
</tr>
<tr>
<td><strong>11 units RBC on 3 dates for gunshot wound surgery (7/5, 7/17, and 8/9), 1 unit found incompatible on crossmatch (RT, 37°C, and AHG) but screening RBCs still negative.</strong></td>
<td><strong>Antibody was undetectable after splenectomy and not stimulated by use of XM-compatible blood.</strong></td>
</tr>
<tr>
<td><strong>1 week after transfusion of 3 units RBC, Ab screen showed new anti-JK and a high-frequency antigen. DAT–, patient eventually diagnosed with lymphoma and transfused with autologous units.</strong></td>
<td><strong>DAT weak with IgG and C3d, eluates negative. Antibody disappeared within 70 days.</strong></td>
</tr>
<tr>
<td><strong>Preoperative positive Ab screen for revision of right hip arthroplasty, procedure delayed until autologous units could be collected.</strong></td>
<td><strong>DAT 3+ with C3d, negative with IgG, eluate negative. Serum antibody weaker after transfusion; additional follow-up not possible.</strong></td>
</tr>
<tr>
<td><strong>Moderately strong DAT (polyspecific and IgG-eluade showed anti-Rd), weakened with time. Negative Ab screen preoperatively.</strong></td>
<td><strong>2 children Rd+, both had HDFN; 1 child Rd-, did not have HDFN.</strong></td>
</tr>
<tr>
<td><strong>10 children in pedigree: 5 Rd– with no HDFN; 1st 3 Rd+ without HDFN, last 2 Rd+ with HDFN (1st of these requiring exchange transfusion).</strong></td>
<td><strong>4 children in family: 1 Rd– with no HDFN; 1st 2 Rd+ without HDFN, last 1 Rd+ with HDFN.</strong></td>
</tr>
<tr>
<td><strong>4 children in family: 1 Rd– with no HDFN; 1st 2 Rd+ without HDFN, last 1 Rd+ with HDFN.</strong></td>
<td><strong>5 children in family: 3 Rd– with no HDFN; 1 stillborn (3rd in birth order) followed by 1 Rd+ with HDFN.</strong></td>
</tr>
<tr>
<td><strong>5 children in family: 3 Rd– with no HDFN; 1 stillborn (3rd in birth order) followed by 1 Rd+ with HDFN.</strong></td>
<td><strong>Moderately strong DAT (polyspecific and IgG-eluade showed anti-Rd), weakened with time. Negative Ab screen preoperatively.</strong></td>
</tr>
<tr>
<td><strong>Preoperative positive antibody screen for atonic goiter surgery. Considered as a “naturally occurring antibody.”</strong></td>
<td><strong>11 units RBC on 3 dates for gunshot wound surgery (7/5, 7/17, and 8/9), 1 unit found incompatible on crossmatch (RT, 37°C, and AHG) but screening RBCs still negative.</strong></td>
</tr>
<tr>
<td>1 week after transfusion of 3 units RBC, Ab screen showed new anti-JK and a high-frequency antigen. DAT–, patient eventually diagnosed with lymphoma and transfused with autologous units.</td>
<td>Serum reacted with all cells except Sc:1–2, autologous, and the patient’s sibling (phenotype Sc:1–2), Identity of antigen discovered with molecular testing years afterward.</td>
</tr>
<tr>
<td>Preoperative positive Ab screen for revision of right hip arthroplasty, procedure delayed until autologous units could be collected.</td>
<td>Identity of antigen discovered with molecular testing years afterward.</td>
</tr>
<tr>
<td>12 days after transfusion of 2 units RBC after orthopedic surgery, patient had defect in hematocrit, pretransfusion Ab screen positive, weakly DAT+ (IgG and C3).</td>
<td>Eluates reacted with all cells except Sc:1–2. Antibody no longer detected within 9 months. Identity of antigen discovered with molecular testing years afterward.</td>
</tr>
</tbody>
</table>

Hct = hematocrit; HDFN = hemolytic disease of the fetus and newborn; Hgb = hemoglobin; IAT = indirect antiglobulin test; IgG = immunoglobulin G; IVIG = intravenous immunoglobulin; RBCs = red blood cells; RT = room temperature; Tbil = total bilirubin; XM = crossmatch.
to SC system antigens are of clinical importance remains unresolved because these antibodies are not routinely characterized, especially if they are detected along with other, better-understood alloantibodies that can account for a clinical presentation.

On the Detection of SC System Antibodies in Routine Pretransfusion Testing

The most important obstacle to understanding the clinical consequences of transfusing across SC antigens is that serologic reagents to simply define these antigens in both patients and test RBCs have not been widely available. As they do for the Dombrock blood group system, molecular approaches help resolve these situations. It has been estimated that approximately 13 percent of the transfused population are immunologic responders capable of creating alloantibodies, but unless RBC reagents with SC antigens are included in the routine laboratory workup, these antibodies may go unnoticed. It is also problematic if an antibody to one of the high-prevalence SC antigens is in fact detected because the mere presence of the antibody does not necessarily correspond to clinical importance. Such a qualitative assay is insufficient. The quantitative characterization of the antibody, such as by titer, has only been performed in a few SC antibody cases, and we recommend that this parameter be more routinely evaluated in cases of possible hemolysis caused by antibodies in the SC system and in most other blood group systems. Especially because HDFN has been reported, establishing a better understanding of antibody potency as detected by the titer would help inform practice guidelines.

An economic and rational approach would be the routine use of recombinant SC protein during pretransfusion testing before titration to effectively screen only for antibodies present above a threshold titer. In this way, nuisance antibodies (akin to the low-titer cold autoantibodies detected before routine testing at higher temperatures) would fall under the radar as desired and precious laboratory resources would not be spent working up these most likely incidental findings. Only higher titer antibodies would be detected, focusing the laboratory investigation on cases more likely to be of consequence to the patient. If these laboratory techniques do lead to the increased identification of SC system alloantibodies, the medical importance of respecting them in a particular patient’s transfusion recommendation remains debatable.

Should We Genotype for SC Alleles?

The integration of molecular diagnostics with transfusion medicine has been a slow, but steady, process, with applications ranging from individual patient prenatal diagnosis to routine high-throughput donor testing. Although the rate of implementation of these technologies varies among nations and local transfusion services, it is expanding. The transfusion community should move these procedures from potentially inaccessible research laboratories to standard clinical practice. For optimal performance of such an approach toward personalized medicine, ideally all donors and all recipients should be evaluated at a molecular level. Economic barriers to this strategy are falling as high-throughput and multiplexed assays are achieved, because the incremental cost of adding a handful of additional SNPs when designing a DNA microarray is negligible. Consequently, molecular testing strategies are shifting from decisions about which variants to include in a genotyping system that force the prioritization of well-studied variants already known to have medical importance, to finding effective platforms to analyze data derived from genotyping as many known variants as possible, regardless of their allele frequencies or uncertain clinical effects.

The most compelling reason to genotype SC variants in greater depth, both by increasing the number of donors and patients and also by including more variants regardless of their prevalence, is that such data are necessary to clarify the presently ambiguous role of SC in clinical transfusion practice. At the same time, valuable data for research into ERMAP biology are accrued without added costs. Inclusion of the SC*01 to SC*07 alleles in high-throughput assays would be a move in the right direction, and in cases of unresolved serology, a referral to the molecular laboratory for an in-depth investigation may be desirable. By using a high-throughput genetic test to determine the potential for alloimmunization by a variant homozygote, we can collect such genetic data almost for free. The pursuit of alloantibodies in such variant homozygous patients is a feasible strategy to achieve complete resolution of all alloantibodies in a posttransfusion sample submitted as a transfusion reaction investigation. Consequently, knowing these genetic data gives us the opportunity to better define which antibodies are of clinical importance.

Conclusion

The SC story is an excellent example of the essential roles that classical genetics played in the original physical mapping of a blood group system and is an exceptional illustration of transfusion laboratories that were in the right place at the right time to finally identify the responsible locus in the era of whole genome analysis. Parsing out the meaning of global polymorphism frequency distributions in light of founder effects or selection pressures would be a useful adjunct to in vitro studies of transcript utilization.
and protein expression and function. The continued study of ERMAP homologues, such as the BTLN family of proteins in general, and their physiologic interactions in other species, such as the B30.2-containing bloodthirsty gene in Antarctic icefishes, is a promising avenue to explore fundamental insights into erythropoiesis. Investigators in transfusion medicine are uniquely poised not only to carry out this work but also to lead the way toward a more comprehensive understanding of the “lucky 13th” blood group, SC, making us the lucky ones indeed.

Acknowledgment

We gratefully acknowledge Professor Guillaume Lecointre for kindly providing the photograph of blood from the Antarctic icefish (Fig. 5).

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Possible suppression of fetal erythropoiesis by the Kell blood group antibody anti-Kp


Antibodies to antigens in the Kell blood group system are usually immunoglobulin G, and, notoriously, anti-K, anti-k, and anti-Kp can cause severe hemolytic transfusion reactions, as well as severe hemolytic disease of the fetus and newborn (HDFN). It has been shown that the titer of anti-K does not correlate with the severity of HDFN because, in addition to immune destruction of red blood cells (RBCs), anti-K causes suppression of erythropoiesis in the fetus, which can result in severe anemia. We report a case involving anti-Kp in which one twin was anemic and the other was not. Standard hemagglutination and polymerase chain reaction (PCR)-based tests were used. At delivery, anti-Kp was identified in serum from the mother and twin A, and in the eluate prepared from the baby’s RBCs. PCR-based assays showed twin A (boy) was KEL*84T/C (KEL*03/KEL*04), which is predicted to encode Kp(a+b+). Twin B (girl) was KEL*84T/C (KEL*04/KEL*04), which is predicted to encode Kp(a–b+). We describe the first reported case of probable suppression of erythropoiesis attributable to anti-Kp. One twin born to a woman whose serum contained anti-Kp experienced HDFN while the other did not. Based on DNA analysis, the predicted blood type of the affected twin was Kp(a+b+) and that of the unaffected twin was Kp(a–b+). The laboratory findings and clinical course of the affected twin were consistent with suppression of erythropoiesis in addition to immune RBC destruction. Immunohematology 2011;27:58–60.

Key words: erythropoiesis—suppression, Kell blood group system, hemolytic disease of the newborn, Kp

The first antigen in the Kell blood group system, K, was discovered in 1946 as the result of hemolytic disease of the newborn. The system now includes 31 antigens. Antibodies to K, k, Kp, Kp, Kp, Js, and Js are the most clinically important. Next to anti-A, anti-B, and anti-D, anti-K is the most common immune RBC antibody. Antibodies to antigens in the Kell blood group system are usually IgG, and, notoriously, anti-K, anti-k, and anti-Kp cause severe hemolytic transfusion reactions, as well as severe hemolytic disease of the fetus and newborn (HDFN). It is well known that the titer of anti-K does not correlate with the severity of HDFN; indeed, severe HDFN caused by anti-K has been associated with lower antibody titers, bilirubin levels, and reticulocytosis than has hemolytic disease of the newborn caused by anti-D. The anemia seen in these infants is caused by a combination of immune destruction and suppression of erythropoiesis in the fetus. Vaughan et al. demonstrated experimentally that proliferation of K+ erythroid progenitors was inhibited by anti-K, and suggested that the K glycoprotein plays a role in erythropoiesis.

Cases of HDFN involving antibodies to other antigens in the Kell system of antibodies are rare. We retrospectively report a case of HDFN in twins of a mother whose serum contained anti-Kp. The Kp(a+) twin was anemic, and the Kp(a–) twin was not.

Case History

A 31-year-old gravida 3, para 2 woman with a history of a negative antibody screen delivered ABO-identical, Rh-compatible fraternal twins at full term (37 weeks’ gestation). The mother had two previous pregnancies delivered by cesarean section; no problems were noted in the medical record. At delivery, the cord hemoglobin (Hb) for twin A (male) was 10.2 g/dL and for twin B (female) was 17.0 g/dL. Unfortunately, there were no cord bilirubin or reticulocyte counts done for either twin.

Because of anemia, shortly after birth twin A received a single-aliquot RBC transfusion that was compatible with the maternal serum. On day 1, he received an additional single-aliquot transfusion with RBCs and was discharged on day 5 with a Hb of 15.1 g/dL and an absolute reticulocyte count of 0.16 × 10^9/L (normal range of 0.125–0.325 × 10^9/L). Transfusion was again required on day 25 when the Hb reached a nadir of 7.5 g/dL with a decreased absolute reticulocyte count of 0.007 m/μL. Additional transfusions were given on days 30 and 39 to maintain a Hb level above 9.0 g/dL. After transfusion of two aliquots on day 39, the Hb of twin A was 9.6 g/dL and on day 43 and 52 it was 9.3 g/dL and 9.5 g/dL, respectively. Two weeks later the Hb was 10.3 g/dL, and 6 months later it was 12.6 g/dL. The infant’s bilirubin peaked at 5 mg/dL on day 1 and fell steadily during the monitoring period. White blood cell and platelet counts remained normal.

Materials and Methods

Hemagglutination

Standard hemagglutination tests were used throughout. The cord blood samples were tested by tube method for
ABO, Rh, and direct antiglobulin test (DAT). The maternal and paternal samples were tested by MTS gel cards (Ortho Clinical Diagnostics, Raritan, NJ).

Polymerase Chain Reaction–Restriction Fragment Length Polymorphism Analysis for KEL*03/KEL*04

Genomic DNA was isolated from epithelial cells captured by the buccal swabs obtained from the twins using a DNA kit (QIAamp DNA Blood Mini Kit, QIAGEN, Inc., Valencia, CA). Exon 8 of KEL was amplified using a forward oligonucleotide primer (Life Technologies, Inc., Gaithersburg, MD) designed within exon 8 (TACCTGACTTACCTGAATCAGCTGGGAACC) and a reverse oligonucleotide primer designed at the 3′ end of exon 8 and into intron 8 (tcttctggcccccagttccaggcacCATGA). Five microliters of DNA per reaction was amplified by 5 units of Taq DNA polymerase (HotStarTaq, QIAGEN Inc., Valencia, CA) in a 50-μL reaction mixture containing 2.5 mM MgCl₂, 1 × PCR buffer, 0.2 mM dNTPs, and 100 ng of forward and reverse primer. The PCR amplification was achieved in 35 cycles with a final extension time of 10 minutes, using 62°C as the annealing temperature. The PCR 202-bp products were digested using the restriction enzyme NlaIII. A restriction enzyme site for this restriction enzyme is present in the KEL*03 variant (841T) but not in the wild-type (841C).

Results

Hemagglutination

At the time of delivery, anti-Kp\(^{a}\) was identified in the maternal serum. No other unexpected antibodies were present in the maternal serum. Tests using the serum from twin A and an eluate prepared from his RBCs also contained anti-Kp\(^{a}\) (Table 1). Antibody testing on days 25, 30, and 39 demonstrated the persistence of anti-Kp\(^{a}\) in the baby’s serum.

Polymerase Chain Reaction–Restriction Fragment Length Polymorphism Analysis for KEL*03/KEL*04

Twin A (boy) was KEL*841T/C (KEL*03/KEL*04), which is predicted to encode Kp(a+b+). Twin B (girl) was KEL*841C/C (KEL*04/KEL*04), which is predicted to encode Kp(a–b+).

Conclusions

We describe the first reported case of possible suppression of erythropoiesis attributable to anti-Kp\(^{a}\). One twin born to a woman whose serum contained anti-Kp\(^{a}\) experienced HDFN and anemia, while the other did not.

Based on DNA analysis, the predicted blood type of the affected twin was Kp(a+b+) and that of the unaffected twin was Kp(a–b+). The laboratory findings and clinical course of the affected twin were consistent with suppression of erythropoiesis in addition to immune RBC destruction. The steadily declining bilirubin levels concurrent with a declining hemoglobin level and decreased absolute reticulocyte count provide supportive evidence for this interpretation. It is possible that the change in the reticulocyte count was induced, or exacerbated, by transfusion. Although this was a retrospective study and not all data were available to us, it is worth documenting, especially because one twin was an antigen-negative twin and served as a control.

Antigens in the Kell blood group system appear on erythroid progenitor cells early in erythropoiesis.\(^{7,8}\) Because erythroid progenitor cells do not contain hemoglobin, immune destruction of these cells by anti-Kp\(^{a}\) could explain the absence of hyperbilirubinemia in our patient. The restriction of Kell messenger RNA and protein to erythroid precursors explains why white blood cell and platelet counts remained normal in the affected twin.\(^{9,10}\) The anemia of the affected twin resolved after 3 months, and his Hb has remained within the normal range. Twin B had no postdelivery complications and provides a biologic control for factors other than maternal alloantibody as a cause of the anemia. Although anti-K is most commonly associated with erythropoietic suppression, twin A shows that antibodies to other antigens in the Kell blood group system can also have this capability.

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References


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The use of massive transfusion protocols (MTPs) in trauma patients presenting with near-exsanguinating injuries has increased in the past several years. This is largely a result of the increasing evidence suggesting that transfusion of blood components in ratios more closely approximating the composition of whole blood may reduce mortality.1–4 Inherent in the design of these protocols is the necessity for the timely transfusion of plasma. Unfortunately, the need for plasma often precedes the determination of blood group.

Group AB plasma is commonly used in MTP when the patient’s blood type has not yet been determined. The foundation for this practice is rooted in the theory that group AB plasma is “universal” because of the absence of anti-A and anti-B hemagglutinins. Likewise, it is analogous to the belief that group O red blood cells (RBCs) are universal and may be transfused to all patients, regardless of blood type. This universal blood component practice continues to this day, based on concerns that infusion of incompatible plasma may produce hemolysis, a potentially fatal complication. Unfortunately, in the United States the availability of group AB plasma is limited because only 4 percent of all blood donors in North America are group AB.5 Therefore, plasma from group AB donors may not be available in sufficient quantities to satisfy the requirements for an MTP in all trauma settings.

Historically, this risk of hemolysis has outweighed concerns about the potential risks associated with compatible but nonidentical plasma. Although experience in populations receiving apheresis platelets has proved that the concern about intravascular hemolysis is warranted,5–18 the belief that group O red blood cells is universal plasma has been repeatedly challenged in several patient populations, including those undergoing cardiac surgery,19 receiving treatment for hematologic malignancies,20 or receiving stem cell transplants.21 For patients in whom a hemolytic reaction may occur, there are three important observations to consider. First, minor incompatibilities appear to be rare events, with one study suggesting an incidence of 1 in 9000.22 Second, in these anecdotal case reports in which hemolytic reactions occurred, the suspected components contained plasma from group O donors.6,23,24 After careful review of these case reports, no patient belonging to group B or group AB was found to have experienced a hemolytic event after transfusion with group A plasma. Third, and most interestingly, it appears that correlations between these hemolytic episodes and agglutinin titers exist.17,25–27

If the level of anti-A and anti-B agglutinin titers could be quantified in compatible but nonidentical plasma units, perhaps a novel approach to a plasma substitution could be made for MTPs. For example, if group AB plasma is not available or is in short supply from local blood bank sources, it may be feasible and preferable to use group A plasma. Because approximately 15 percent of the North American population are group B or group AB, the use of group A plasma is already acceptable for approximately 85 percent of the general population. Published experience from different institutions indicates that transfusion of 300 to 500 mL of incompatible plasma in 1 day and transfusion of 1 L of incompatible plasma in 1 week have been practiced without significant clinical sequelae in adult patients. In addition, many institutions use a critical titer of 64 or higher as the cutoff when saline solution is used for labeling a unit as “high-titer.”24 Therefore, it would be logical to conclude that it may be safe to transfuse 1 L of group A plasma to a group B or group AB patient as long as the anti-B agglutinin titer is less than 16.

The goal of this study is to identify acceptable group A plasma units that could theoretically be safely transfused as universal plasma to any patient during MTP. To determine concept feasibility, we propose classifying group A plasma donors into those with low titers (LT) of anti-B agglutinins (≤ 64 but > 16) and those with very low titers (VLT) of anti-B agglutinins (≤ 16). In the trauma setting, while a blood group determination is being performed, it may be possible to transfuse more than 1 unit of group A plasma during the initial MTP shipments if group AB plasma is unavailable. Group A plasma meeting these specifications could be used to supplement the group AB plasma inventory in the treatment of trauma patients requiring MTP. The solution...
proposed in this pilot study assumes established criteria supported by the currently documented literature.

**Materials and Methods**

Fresh plasma (less than 48 hours old) was obtained from the pilot tubes of 100 consecutive group A blood donors during December 2008. All donors consented to research-related activities as part of the general consent procedure at the Community Blood Center/Community Tissue Services (CBC/CTS), Dayton, Ohio, and all were eligible donors based on an approved donor health questionnaire. These documents were reviewed by the Food and Drug Administration and are on file at the Center for Biologics Evaluation and Research as required by the Code of Federal Regulations. Demographic data including sex, age, and history of previous pregnancy were recorded from the information on the questionnaire.

The plasma from each of these donors was diluted with 0.85 percent saline solution (Blood Bank Saline, Fisher Scientific USA, Pittsburgh, PA) to prepare dilutions of 1:16 (1 part plasma and 15 parts saline) and 1:64 (1 part plasma and 63 parts saline) in sufficient quantities for testing. A freshly collected random group B donor unit was selected as the testing RBCs. The group B RBCs were washed and prepared as a 4% concentration in saline. One drop of the 4% group B RBCs was added to 0.2 mL of each of the 1:16 dilutions of the group A plasmas and mixed well at room temperature (27°C). The mixtures were centrifuged and read macroscopically for agglutination; reactions were recorded as either negative (no agglutination observed) or positive (any macroscopic agglutination observed, regardless of strength). All tubes were then transferred to a 37°C dry heat incubator and were allowed to incubate for 30 minutes. The tubes were again centrifuged and read macroscopically for agglutination with the same criteria used for recording as negative or positive.

Similarly, one drop of the 4% group B RBCs was added to 0.2 mL of each of the 1:64 dilutions of the group A plasmas and mixed well at room temperature. These mixtures were also centrifuged and read macroscopically for agglutination, and recorded as either negative or positive using the criteria previously established. All tubes were then transferred to a 37°C dry heat incubator and were allowed to incubate for 30 minutes. The tubes were centrifuged and read macroscopically for agglutination, and recorded as either negative or positive using the criteria previously established.

All dilutions and testing were performed by one of two experienced CBC/CTS immunohematology reference laboratory technologists. The resulting patterns of reactivity were analyzed for correlation with age, sex, and history of pregnancy. Statistical analysis with the Pearson chi-square test was then used to determine any correlation between the patterns and age. Significance was determined at α = 0.05.

**Results**

Among the 100 plasma samples, seven different patterns of reactivity were noted. Sixty three percent of donor samples were negative at a titer of 64 both at room temperature and at 37°C. These donor samples can be classified as LT. Fifteen percent of donor samples were negative at titers of 64 and 16 both at room temperature and at 37°C and can be classified as VLT (Table 1).

**Table 1.** Donor sample demographic data and agglutinin titer

<table>
<thead>
<tr>
<th>N</th>
<th>Mean age, y (range)</th>
<th>Male: female</th>
<th>Pregnancies</th>
<th>RT(VLT)</th>
<th>RT(LT)</th>
<th>37°C(VLT)</th>
<th>37°C(LT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>39 (17–75)</td>
<td>4:19</td>
<td>13/19</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>47 (19–71)</td>
<td>7:4</td>
<td>4/4</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>39</td>
<td>51 (20–72)</td>
<td>19:20</td>
<td>15/20</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>48 (43–51)</td>
<td>2:3</td>
<td>3/3</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>53 (50–56)</td>
<td>0:3</td>
<td>3/3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>53 (46–59)</td>
<td>4:0</td>
<td>0/0</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>54 (38–77)</td>
<td>8:7</td>
<td>7/7</td>
<td>-</td>
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</table>

LT = low titer ≤ 64; RT = room temperature 27°C; VLT = very low titer ≤ 16.

Although low titers and very low titers occur in both sexes and across all age ranges, some general patterns do emerge. The three most commonly observed patterns of reactivity are (1) positivity at titers of 16 and 64 at room temperature and 37°C (pattern A), (2) positivity at a titer of 16 at room temperature and 37°C but negativity at a titer of 64 at both temperatures (pattern B), and (3) negativity at both titers at both temperatures (pattern C).

The ratio of men to women within pattern A is 4:19; the ratio is more evenly balanced within patterns B and C. The relationship of these three patterns of reactivity with regard to age is noted in Table 2. There is a statistically significant difference (p < 0.029) in the prevalence of patterns A and C in donors younger than 50 years of age compared with donors who are older than 50 years. Pattern A is more common in younger donors, whereas pattern C is more common in older donors.
The results of this laboratory study demonstrate that evaluation of anti-B agglutinin titers in group A plasma as VLT may serve to identify acceptable group A plasma units that may be substituted in MTP when blood type has not yet been determined.

As the demand for plasma resuscitation increases, transfusion services are developing algorithms for maintaining an inventory of thawed plasma. Because of the relative scarcity of group AB plasma, in general, it is simply not practical to maintain thawed group AB plasma. This translates into a delay in plasma distribution because the process of obtaining a blood bank specimen, determining a blood type, and issuing the plasma can be challenging. In addition, group AB plasma may involve additional risks because of the formation of circulating immune complexes, especially when transfused to group O patients. In fact, in one large study mortality was increased whenever type-compatible but not type-specific plasma was transfused. However, statistical significance was reached only when group AB plasma was given to group O patients, and in this case when more than five units of group AB plasma were transfused to a group O patient, the relative risk of increased mortality was 1.26 (p = 0.004). Thus, even if group AB plasma were available in sufficient amounts, it might not be ideal. A possible solution to this dilemma is to identify a type of plasma that is present in abundance and can be safely transfused to any trauma patient, at least up to a certain volume, thus allowing sufficient time for a blood group determination to be made. Group A plasma is relatively abundant, and because nearly 85 percent of trauma patients are group A or group O, they can safely receive group A plasma. For the 15 percent of patients for whom the transfusion of group A plasma creates a minor incompatibility, published experience has demonstrated that reactions caused by minor incompatibilities are extremely rare.

Multiple reviews in the published literature have provided details about the anecdotal cases in which a minor incompatibility has resulted in a serious adverse outcome. The overwhelming majority of these cases involve group O plasma transfused to a group A patient, although there are sporadic instances in which group O plasma was transfused to a group B patient. The reports warn that the issue may be underreported and that physicians should be vigilant when transfusing incompatible plasma. Nonetheless, the preponderance of the published clinical experience seems to indicate that the transfusion of incompatible group A plasma appears to be safe.

Despite the lack of a reported problem with the transfusion of group A plasma to either a group B or a group AB patient, additional factors support using group A plasma. There appears to be a correlation between adverse clinical consequences and high ABO agglutinin titers, which provides an avenue for interventions to deal with the issue of the rare but potentially significant minor incompatibilities. Some transfusion services are identifying agglutinin titers before issuing platelet components containing incompatible plasma. Evaluation of anti-B agglutinin titers in group A plasma may serve to identify acceptable group A plasma units that could theoretically be safely transfused to any patient. In determining these titers, the laboratory method is important, and various laboratories have used tube agglutination, gel technology, or microtiter plates in these determinations. In our pilot study, agglutination titers were determined using tube agglutination, and the titers we used as thresholds were based on relatively conservative values from reports in the platelet literature in which tube agglutination was used. A recent review containing an extensive summary of hemolytic reactions associated with minor incompatibility in patients transfused with platelets indicated that the range of titers fell between 128 and 32,000 using antiglobulin reagent. The rationale for the use of titers in our study was to base our proposed solution on conservative assumptions with the use of available platelet transfusion data. It may turn out that these assumptions are overly conservative. Another element that is important in the laboratory method is the choice of RBCs used to perform the titer. We used RBCs from a random group B whole blood donor. Because of variation in antigen sites in group A RBCs, titers are best performed using blended pools of group A RBCs; it is not clear that the same issue exists in group B RBCs, although further investigation of this open question may be warranted.

In the preceding paragraph, the conservative assumptions are as follows: (1) group A plasma will be statistically compatible with 85 percent of patients, (2)
minor incompatibilities are extremely rare, (3) there has been no reported case of a minor reaction with group A plasma in either a group B or group AB patient, (4) serious adverse reactions can be associated with certain threshold agglutinin levels, and (5) conservative values from evidence-based publications were used to set an acceptable agglutinin titer threshold level. Based on these assumptions, our study showed that 63 percent of our donors could safely donate one unit of incompatible plasma and that 15 percent could safely donate up to 1 L of incompatible plasma to any patient. Furthermore, we found that the VLT donors are more common in donors older than 50 years of age. Therefore, a possible recruitment strategy in our donor population would involve screening donors older than 50 years of age to establish a dedicated group of VLT donors for MTP.

With regard to the limitations of this pilot study, there are several. First, the donor sample size is only 100. This number was selected because the purpose of the study was merely to test for feasibility of the proposed approach. Second, the donor population is a midwest population, and therefore these results may not extrapolate to other donor populations. The determinants of agglutinin levels appear to be largely determined by geography and environmental factors, and one study of populations in Asia found higher agglutinin levels in donors older than 50 years of age. Although it is well known that immune function deteriorates with increasing age, this population had increased agglutinin titers. Our results of a midwest North American population demonstrated the opposite. Surprisingly, our population showed titer levels that appear to be independent of history of pregnancy. The significance of these findings remains to be determined.

Because populations of blood donors will have different patterns of agglutinin titers based on ethnicity and age, select blood centers could develop specific recruitment strategies to provide these VLT universal plasma products to meet local and regional needs. Finally, and most importantly, this new concept of VLT group A plasma as universal plasma in MTP has not been studied in trauma patients requiring MTP and will require thoughtful study design to test the hypothesis.

Conclusions

The results of this study suggest a fresh perspective on resuscitation in trauma patients. When a massively injured trauma patient arrives at the trauma center, it is theoretically possible to initiate MTP with thawed VLT group A plasma instead of thawed group AB plasma. Because MTP suggests a ratio of one RBC unit to one plasma unit during acute hemorrhage, the time required to transfuse the initial MTP shipment of four RBC units and four plasma units (essentially equivalent to 1 L of plasma) would provide transfusion services with ample time to determine blood type and then issue the appropriate type-specific blood components. When handled in this fashion, group AB plasma may remain frozen and only be thawed for use in those patients with group AB blood.

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Group A as universal plasma donors in massive transfusion protocols


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Manuscripts

The editorial staff of Immunohematology welcomes manuscripts pertaining to blood group serology and education for consideration for publication. We are especially interested in case reports, papers on platelet and white cell serology, scientific articles covering original investigations, and papers on new methods for use in the blood bank. Deadlines for receipt of manuscripts for consideration for the March, June, September, and December issues are the first weeks in November, February, May, and August, respectively. For instructions for scientific articles, case reports, and review articles, see Instructions for Authors in every issue of Immunohematology or on the Web at www.redcross.org/immunohematology. Include fax and phone numbers and e-mail address with all manuscripts and correspondence. E-mail all manuscripts to immuno@usa.redcross.org
Prevalence of *RHD*DOL and *RHCE*ce(818T) in two populations

C. Halter Hipsky, D.C. da Costa, R. Omoto, A. Zanette, L. Castilho, and M.E. Reid

The alleles *RHCE*ceBI (RHCE*ce 48C, 712G, 818T, 1132G) and *RHCE*ceSM (RHCE*ce 48C, 712G, 818T) encode the low-prevalence Rh antigen STEM. These alleles frequently travel in cis with *RHD*DOL. To estimate the frequency of these alleles, we tested a total of more than 700 samples in two populations. Blood samples were obtained from patients with sickle cell disease and from blood donors of African descent. DNA extractions and analyses were performed by standard methods. In the United States, none of 70 patient samples had the *RHCE*818nucleotide change. Two of 220 donors (frequency of 0.009) were heterozygous for *RHCE*818C/T (*RHCE*ceBI). One of these samples had RHD/RHD*DOL and the other had RHD/RHD*DOL-2. In these 290 samples, no other RHD*DOL alleles were found. In Brazil, 1 of 244 patients with sickle cell disease (frequency of 0.004) and 1 of 171 donors (frequency of 0.006) were heterozygous for *RHCE*818C/T (*RHCE*ceBI). Testing of more than 500 additional samples from people of African descent, selected because they had a diverse range of common and variant RHCE alleles, did not reveal a sample with RHD*DOL or RHD/RHD*DOL-2 (Fig. 1B). We tested more than 700 samples in New York and in Brazil to estimate the frequency of RHCE*ce818C>T and to investigate RHD in these samples.

Key words: blood groups, Rh alleles, low-prevalence antigens, population study

The low-prevalence Rh antigen STEM (RH49) was reported in 1993. The antigen was serologically shown to be associated with an altered e phenotype because approximately 65 percent of hr<sup>–</sup> and 30 percent of hr<sup>+</sup> red blood cells (RBCs) from South African donors were reported to be STEM+. STEM has a variable expression, which is an inherited characteristic. Anti-STEM has induced mild hemolytic disease of the fetus and newborn. Owing to the paucity of anti-STEM and typed RBCs, little further work has been done and no other serologic reports have appeared in the literature.

The STEM antigen is encoded by two RHCE alleles: RHCE*ceBI (RHCE*ce 48C, 712G, 818T, 1132G) and RHCE*ceSM (RHCE*ce 48C, 712G, 818T) (Fig. 1A). The nucleotide (nt) 818C>T change is believed to be associated with expression of STEM, because the other nucleotide changes occur in other alleles. In analyzing DNA from samples known to be STEM+ or DAK+, it was revealed that these variant RHCE are usually in cis to *RHD*DOL or *RHD*DOL-2 (Fig. 1B). We tested more than 700 samples in New York and in Brazil to estimate the frequency of RHCE*ce818C>T and to investigate RHD in these samples.

**Figure 1.** Diagram of selected RHCE (A) and RHD (B) alleles. The 10 exons are numbered. Nucleotide changes are indicated in italics and amino acids are given in parentheses below the exon in which they are found. Changes from the wild-type nucleotides and amino acids are written in bold.
Materials and Methods

Blood samples were obtained from patients with sickle cell disease and from blood donors who self-identified as being of African descent. Genomic DNA was isolated from the whole blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, Inc., Valencia, CA). Initial screening of samples was carried out by sequencing RHCE- and RHD-reverse transcriptase–polymerase chain reaction (PCR) products, as described previously, or analyzing RHCE*818 and RHCE*1132 by standard PCR products generated with genomic DNA using RHCE-specific primers, followed by restriction fragment length polymorphism (RFLP) using, respectively, MwoI and Tsp45I restriction enzymes. Genomic DNA was amplified using RHD-specific primers flanking exon 4 and exon 8, and products were submitted for sequencing to determine the presence of RHD*DOL (nt 509T>C in exon 4) and RHD*DOL-2 (nt 509T>C in exon 4 and nt 1132C>G in exon 8). PCR products were sequenced in both directions.

Results

In the United States, none of 70 patient samples had the RHCE*818 nucleotide change. Two of 220 donors (frequency of 0.009) were heterozygous for RHCE*818C/T. Both samples had the change at RHCE*ce1132C>G and, thus, are RHCE*ceBI. One sample had RHD/RHD*DOL and the other had RHD/RHD*DOL-2. No homozygous RHCE*818T sample was found. In these 290 samples, no other RHD*DOL alleles were found.

In Brazil, 1 of 244 patients with sickle cell disease (frequency of 0.004) and 1 of 171 donors (frequency of 0.006) were heterozygous for RHCE*818C/T and for RHCE*ce1132C/G and, thus, are also RHCE*ceBI. The samples were not analyzed for RHD.

Taking the combined data, 4 samples in a total of 705 tested had RHCE*818C/T (all 4 were RHCE*ceBI), which gives an allele frequency of 0.006.

Conclusions

Although the numbers are small, our study shows that in the United States, the frequency of RHCE*818T (RHCE*ceBI) is 0.007 (2 in 290 samples; 580 alleles), and in Brazil it is 0.004 (2 in 415 samples; 830 alleles). Our findings show that RHCE*818T, which encodes the STEM antigen, is not as rare as previously thought. This raises the question as to whether anti-STEM is also more prevalent but not identified. In the cohort of samples tested, the RHD*DOL or RHD*DOL-2 alleles were in cis with the RHCE*ce(818T) allele; no RHD*DOL or RHD*DOL-2 was found in the absence of RHCE*ce(818T).

Acknowledgments

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Notice to Readers

Immunohematology is printed on acid-free paper.
The XG blood group system is best known for its contributions to the fields of genetics and chromosome mapping. This system comprises two antigens, Xg\textsuperscript{a} and CD99, that are not antithetical but that demonstrate a unique phenotypic relationship. XG is located on the tip of the short arm of the X chromosome with exons 1 to 3 present in the pseudoautosomal region of the X (and Y) chromosome(s) and exons 4 to 10 located only on the X chromosome. Xg\textsuperscript{a} demonstrates a clear X-linked pattern of inheritance. MIC2, the gene encoding the CD99 antigen, is found in the pseudoautosomal region of both the X and Y chromosomes. Anti-Xg\textsuperscript{a} is comparatively rare, and only two examples of anti-CD99 have ever been identified. Alloanti-Xg\textsuperscript{a} is considered clinically insignificant; only one example of autoanti-Xg\textsuperscript{a} has been reported, but it resulted in severe hemolytic anemia. Insufficient data exist to determine the clinical significance of anti-CD99. Linkage of XG to several X-borne genes encoding inherited disorders has been demonstrated. CD99 is an adhesion molecule,\textsuperscript{5,8} and high levels are associated with some types of cancer. Xg\textsuperscript{a} and CD99 enjoy a unique phenotypic relationship, which will be discussed in further detail in the Genetics and Inheritance section. Fourteen years later, in 1995, Uchikawa et al. reported the only examples of alloanti-CD99, detected in two unrelated, healthy Japanese blood donors.\textsuperscript{2} CD99, became part of the XG blood group system officially in 2000 when it was confirmed that MIC2 and XG are adjacent, homologous genes.\textsuperscript{4}

Because of its location in the pseudoautosomal boundary region of the sex chromosome, study of the inheritance of Xg\textsuperscript{a} has helped to define the mechanisms responsible for Turner, Klinefelter, and other syndromes resulting from an abnormal number of sex chromosomes. Sex chromosome aneuploidies are commonly associated with infertility.

### Nomenclature

The XG blood group system has been assigned the ISBT symbol and number XG and 012, respectively. The ISBT terminology for the two antigens in the system, Xg\textsuperscript{a} and CD99, is listed in Table 1. No antithetical counterpart to the Xg\textsuperscript{a} antigen has been discovered, so the failure of RBCs to react with anti-Xg\textsuperscript{a} defines the silent allele, Xg, or an absence of Xg\textsuperscript{a}.

### Table 1. Nomenclature

<table>
<thead>
<tr>
<th>ISBT symbol</th>
<th>Xg\textsuperscript{a}</th>
<th>CD99</th>
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<tbody>
<tr>
<td>ISBT number</td>
<td>012001</td>
<td>012002</td>
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</table>

### Genetics and Inheritance

Xg\textsuperscript{a} is inherited as an X-linked, dominant trait, and the pattern of inheritance is as expected for an X-borne characteristic.\textsuperscript{1,8} If Xg\textsuperscript{a} is present, it is expressed on RBCs.
The Xg(a+) phenotype is found in 88.7 percent of females and 65.6 percent of males. (Table 2.) Females can have a single (Xg+/Xg) or a double dose (Xg+/Xg•) of Xg•. Having only one X chromosome, males can be hemizygous for Xg• (Xg•/Y) or be Xg/Y and not express the antigen at all.6

**Table 2. Phenotype prevalence and genotype frequencies**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Male (%)</th>
<th>Female (%)</th>
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<tbody>
<tr>
<td>Xg(a+)</td>
<td>65.6</td>
<td>88.7</td>
</tr>
<tr>
<td>Xg(a–)</td>
<td>34.4</td>
<td>11.3</td>
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</table>

Lyonization is a process by which one X chromosome is inactivated early in somatic cell development in XX females.9 The process is random in each cell, ensuring that all genes on the two X chromosomes are found at the phenotypic level. By studying RBCs of Xg•/Xg women it can be shown that all cells carry Xg•, proving that Xg• is not subject to inactivation or Lyonization. XG was the first X chromosome locus proven to escape this process.10 It was later shown that MIC2 is not subject to Lyonization either.11

New Guineans, Australian aborigines, Navajo Indians, and Sardinians are reported to have the highest prevalence of Xg•. On the other hand, native Taiwanese; Chinese in Singapore, Hong Kong, Taiwan, and Hakka; and Malays in Singapore are observed to have the lowest incidence.3,12–14 Interestingly, Asian populations with a higher prevalence of Xg(a–) phenotypes do not have a higher incidence of anti-Xg•.5

Using monoclonal antibodies, it has been shown that CD99 is present on all tissue cells tested; however, the expression of the adhesion molecule on RBCs is unique. CD99 is expressed at different levels on RBCs, and this expression is directly related to the presence or absence of Xg•.10 In females, the Xg(a–) phenotype is always associated with low expression of CD99. (Table 3.) Curiously, among Xg(a–) males, 74 percent are high expressors of CD99.17 To explain this quantitative polymorphism, Goodfellow and Tippett proposed the existence of a locus on the Y chromosome, YG, analogous to XG on the X chromosome. Like XG, YG would have two alleles, YG• and Yg. In Xg(a–) males, the presence of YG• would result in high expression of CD99, and the absence of YG• (Yg) would result in low expression of CD99.7,16

The presence of YG has been substantiated by family studies,17 but to date, there has been no Yg• antigen identified. It is important to note that MIC2 is responsible for expression of CD99 on all tissue cells, but XG and YG create the CD99 quantitative polymorphism observed on RBCs.8

**Molecular Basis**

Sex chromosomes have two genetically distinct regions: sex chromosome–specific sequences and pseudoautosomal sequences. Pseudoautosomal refers to a structurally homologous region on sex chromosomes, the function of which is to facilitate pairing during male meiosis.8 XG spans the pseudoautosomal boundary between the two regions of the X chromosome at Xp22.3; exons 1 to 3 are located in the pseudoautosomal region (PAR) and exons 4 to 10 are found in the sex chromosome–specific region.4–6 MIC2, the closest neighbor to XG, is located in the PAR at position Xp22.2. An identical copy of MIC2 is found in the PAR region of the Y chromosome at Yp11.2.4 Almost half (48%) of the predicted amino acid sequences of XG and MIC2 are identical.16

**Biochemistry**

Immunoblotting of immunoprecipitates confirmed that CD99 and Xg• antigens are located on different structures, later confirmed to be sialoglycoproteins.8,19 CD99 and Xg• are associated in the membrane, possibly in the form of a heterodimer.7,18

The RBC membrane molecule expressing Xg• was first identified in 1989 by Herron and Smith, by immunoblotting and electrophoresis.19 Several years later, Petty and Tippett used immunoblotting and immunoprecipitation to show that Xg• is carried on a broad band of apparent molecular weight 24.5 to 29.5 kDa, consisting of a darkly stained component at 24.5 kDa and a more diffusely stained component between 26.5 and 29.5 kDa.18 No bands are observed with protease-treated cells, and the apparent molecular weight of Xg• is decreased after treatment with neuraminidase, suggesting that Xg• resides on a sialoglycoprotein.
Xg\(^a\) is sensitive to treatment with ficin, papain, trypsin, bromelin, \(\alpha\)-chymotrypsin, and pronase, and is resistant to sialidase, 0.2 \(\text{M} \) dithiothreitol (DTT), neuraminidase, and 2-aminoethylisothiouronium bromide.\(^4,13,20\) The effects of acid are unknown. The expression of the antigen decreases as the RBC ages and it has been shown by indirect radioimmunoassay to have an in vivo half-life of 47 days.\(^21\)

Early experiments using a microcomplement fixation method and indirect radioimmunoassay suggested that Xg\(^a\) might be present on other cell lines and tissues,\(^22\) but subsequent testing was unable to duplicate these results. XG is expressed on hematopoietic tissues\(^8\) and is strongly expressed as mRNA in fibroblasts.\(^20\)

Apart from humans, the only animals found to have Xg\(^a\) on their RBCs are one species of gibbons. Of 52 gibbons tested, 30 percent of males and 53 percent of females were Xg(a+). Chimpanzees (67); gorillas (2); orangutans (20); another species of gibbons (5); various monkeys, including 60 baboons; and a few nonprimates, including mice and dogs, were all Xg(a−).\(^3,4\)

Monoclonal anti-CD99 identify a protein of apparent molecular weight of 32 kDa.\(^5,8\) Immunoblotting and immunoprecipitate studies suggest the CD99 protein is composed of two polypeptides, a 32-kDa surface component, and a 30-kDa intracellular polypeptide.\(^28\) CD99 is sensitive to treatment with trypsin, \(\alpha\)-chymotrypsin, ficin, papain, and pronase\(^5\) and, like Xg\(^a\), is carried on a sialoglycoprotein. CD99 is resistant to 0.2 M DTT and is usually resistant to neuraminidase treatment; results vary with sialidase treatment.

CD99 is present on all human cell types and tissues tested.\(^38\) High levels of CD99 are a tumor marker in Ewing sarcoma, some neuroectodermal tumors, lymphoblastic lymphoma, and acute lymphoblastic leukemia.\(^8\) CD99 was not detected on the RBCs or peripheral blood lymphocytes of 10 gibbons, regardless of their Xg\(^a\) phenotype. CD99 was detected on RBCs and fibroblasts of chimpanzees and gorillas, but not of orangutans or any of the other mammals tested.\(^23\)

### Antibodies in the System

Anti-Xg\(^a\) is comparatively rare; most immunohematologists will never see anti-Xg\(^a\) in their careers. When identified, it is usually found as a lone antibody specificity. Some examples of anti-Xg\(^a\) are naturally occurring, yet are more frequently IgG than IgM.\(^4\) Optimal methods for identification include room temperature incubation (even when they are IgG in composition\(^3\)), indirect antiglobulin test (IAT), and capillary testing.\(^4\) One IAT-reactive anti-Xg\(^a\) was shown to have IgG1 and IgG2 subclasses.\(^24\) Some examples of anti-Xg\(^a\) have been shown to fix complement.\(^1\)

The only reported example of autoanti-Xg\(^a\) was found in a pregnant woman. The antibody appears to have caused significant hemolytic anemia, but the possibility that the hemolytic anemia was exacerbated by the pregnancy was not ruled out.\(^25\)

As a result of the sex-related frequency differences of Xg\(^a\), it is expected that men would make more examples of anti-Xg\(^a\). Although this is true, it is striking that greater than 85 percent of antibody makers are men when the incidence of the Xg(a−) phenotype is only 23 percent higher in men than in women. It has been postulated, although not studied, that this higher than expected prevalence of anti-Xg\(^a\) in men may be associated with the expression of CD99.\(^6\)

The first example of anti-CD99 ever made was a mouse monoclonal antibody called 12E7, which was generated to screen for antigen differences in human acute lymphocytic leukemia.\(^26\) The only two examples of anti-CD99 ever detected were found in two unrelated, healthy Japanese blood donors. One antibody was reported to react with variable strength with different RBC samples. The second blood donor had a history of pregnancy. One of the two siblings of the second blood donor was determined to be CD99 negative also.\(^2\) The antibodies were determined to be IgG in nature and reacted optimally by the IAT.\(^5\) It is not known whether the antibodies were capable of binding complement.

### Clinical Significance

Alloanti-Xg\(^a\) has never been reported to cause hemolytic disease of the fetus or newborn or a hemolytic transfusion reaction.\(^4\) One patient received six units of antigen-positive blood with no signs of a reaction.\(^27\) A chromium survival study on another patient showed normal survival of Xg(a+) RBCs and no posttransfusion increase in antibody strength.\(^28\) One male Japanese patient had a slight temperature elevation and exhibited urticaria when transfused with Xg(a+) blood. Subsequent transfusions with Xg(a−) blood were tolerated with no symptoms.\(^29\)

The one example of autoanti-Xg\(^a\) reported was identified in a pregnant female. The antibody was IgG in nature, activated complement, and caused severe hemolytic anemia. The infant was Xg(a+) and demonstrated a strongly positive direct antiglobulin test at birth, and anti-Xg\(^a\) was eluted from the infant’s cells. The clinical course was remarkable only for a mild rise in bilirubin after birth.\(^25\)

Because the only two examples of anti-CD99 were found in healthy blood donors, information on the clinical significance of this antibody is not known.
XG is linked to genes responsible for several genetic disorders. Ichthyosis is a disorder in which the skin resembles scales on a fish. Ocular albinism is unique in that only the eyes lack pigment. Retinoschisis is a sex-linked form of macular degeneration affecting only men. High levels of CD99 have been reported in Ewing sarcoma, some neuroectodermal tumors, lymphoblastic lymphoma, and acute lymphoblastic leukemia.

Summary and Future Perspectives

The XG blood group system is most important for the insight it has provided into the study and understanding of meiotic errors that lead to sex chromosome aneuploidies. As the roles of Xg and CD99 become more clearly delineated, knowledge of the forgotten blood group system may aid in the development of cures for certain genetic disorders and types of cancers.

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Erratum
Vol. 27, No. 1, 2011, pp. 14, 16, and 18
Red blood cell phenotype matching for various ethnic groups

The author has informed the editors of Immunohematology that there is an error on pages 14, 16, and 18. The running head should appear as K.S.W. Badjie et al.

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E-mail or fax information to immuno@usa.redcross.org or phone (215) 451-2538
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-A\textsubscript{1}, -M, -s, -U, -D, -Rh\textsubscript{17}, -K, -k, -Kp\textsuperscript{a}, -Js\textsuperscript{b}, -Fy\textsuperscript{a}, -Fy\textsuperscript{3}, -Fy\textsuperscript{6}, -Wr\textsuperscript{b}, -Xg\textsuperscript{a}, -CD\textsubscript{99}, -Do\textsuperscript{b}, -H, -Ge\textsubscript{2}, -Ge\textsubscript{3}, -CD\textsubscript{55} (both SCR\textsubscript{2/3} and SCR\textsubscript{4}), -Ok\textsuperscript{a}, -I, and anti-CD\textsubscript{59}. Most of the antibodies are murine IgG and require the use of anti-mouse IgG for detection (Anti-K, -k, and -Kp\textsuperscript{a}). Some are directly agglutinating (Anti-A\textsubscript{1}, -M, -Wr\textsuperscript{b}, and -Rh\textsubscript{17}) and a few have been humanized into the IgM isoform (Anti-Js\textsuperscript{b}). The antibodies are available at no charge to anyone who requests them. Please visit our Web site for a complete list of available monoclonal antibodies and the procedure for obtaining them.

For additional information, **contact**: Gregory Halverson, New York Blood Center, 310 East 67th Street, New York, NY 10021/e-mail: ghalverson@nybloodcenter.org, phone: (212) 570-3026, FAX: (212) 737-4935, or visit the Web site at www.nybloodcenter.org >research >laboratories >immunochemistry >current list of monoclonal antibodies available.

Specialist in Blood Bank (SBB) Program
The Department of Transfusion Medicine, National Institutes of Health, is accepting applications for its 1-year Specialist in Blood Bank Technology Program. Students are federal employees who work 32 hours/week. This program introduces students to all areas of transfusion medicine including reference serology, cell processing, HLA, and infectious disease testing. Students also design and conduct a research project. NIH is an Equal Opportunity Organization. Application deadline is December 31, 2011, for the July 2012 class. See www.cc.nih.gov/dtm > education for brochure and application. For further information **contact** Karen M. Byrne at (301) 495-8645 or KByrne@mail.cc.nih.gov
Announcements, cont.

Online Specialist in Blood Bank (SBB)
Certificate and Masters in Clinical Laboratory Management Program
Rush University
College of Health Sciences

Continue to work and earn graduate credit while the Rush University SBB/MS program prepares you for the SBB exam and the Diplomat in Laboratory Management (DLM) exam given by ASCP Board of Certification! (Please note acceptable clinical experience is required for these exams).

Rush University offers online graduate level courses to help you achieve your career goals. Several curricular options are available. The SBB/MS program at Rush University is currently accepting applications for Fall 2011. For additional information and requirements, please visit our website at: www.rushu.rush.edu/cls/

Rush University is fully accredited by the Higher Learning Commission [HLC] of the North Central Association of Colleges and Schools and the SBB Certificate Program is accredited by the Commission on Accreditation of Allied Health Education Programs (CAAHEP).

Applications for the SBB/MS Program can be submitted online at the following website: http://www.rushu.rush.edu/admiss/hlthadm.html

Contact: Yolanda Sanchez, MT(ASCP)SBB, Director, by email at Yolanda_Sanchez@rush.edu or by phone at 312-942-2402 or Denise Harmening, PhD, MT(ASCP), Director of Curriculum by e-mail at Denise_Harmening@rush.edu
Masters (MSc) in Transfusion and Transplantation Sciences at The University of Bristol, England

Applications are invited from medical or science graduates for the Master of Science (MSc) degree in Transfusion and Transplantation Sciences at the University of Bristol. The course starts in October 2011 and will last for 1 year. A part-time option lasting 2 or 3 years is also available. There may also be opportunities to continue studies for PhD or MD following the MSc. The syllabus is organized jointly by The Bristol Institute for Transfusion Sciences and the University of Bristol, Department of Pathology and Microbiology. It includes:

- Scientific principles of transfusion and transplantation
- Clinical applications of these principles
- Practical techniques in transfusion and transplantation
- Principles of study design and biostatistics
- An original research project

Application can also be made for Diploma in Transfusion and Transplantation Science or a Certificate in Transfusion and Transplantation Science.

The course is accredited by the Institute of Biomedical Sciences.

Further information can be obtained from the Web site: http://ibgrl.blood.co.uk/MSc/MscHome.htm

For further details and application forms please contact:

Dr Patricia Denning-Kendall
University of Bristol
Paul O’Gorman Lifeline Centre
Department of Pathology and Microbiology
Southmead Hospital
Westbury-on-Trym, Bristol BS10 5NB, England
Fax +44 1179 595 342, Telephone +44 1779 595 455, e-mail: p.a.denning-kendall@bristol.ac.uk.
Advertisements, cont.

**National Platelet Serology Reference Laboratory**

**Diagnostic testing for:**
- Neonatal alloimmune thrombocytopenia (NAIT)
- Posttransfusion purpura (PTP)
- Refractoriness to platelet transfusion
- Heparin-induced thrombocytopenia (HIT)
- Alloimmune idiopathic thrombocytopenia purpura (AITP)

**Medical consultation available**

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

For further information, contact:

**Platelet Serology Laboratory** (215) 451-4205
Janet Demcoe (215) 451-4914
demcoej@usa.redcross.org
Sandra Nance (215) 451-4362
snance@usa.redcross.org

American Red Cross Biomedical Services
Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594

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**National Neutrophil Serology Reference Laboratory**

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

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

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

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

For further information, contact:

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

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

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Reference and Consultation Services

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

For information, contact:
Mehdizadeh Kashi
at (503) 280-0210
or write to:
Tissue Typing Laboratory
American Red Cross Biomedical Services
Pacific Northwest Region
3131 North Vancouver
Portland, OR 97227

CLIA licensed, ASHI accredited

IgA/Anti-IgA Testing

IgA and anti-IgA testing are available to do the following:
- Identify IgA-deficient patients
- Investigate anaphylactic reactions
- Confirm IgA-deficient donors

Our ELISA for IgA detects protein to 0.05 mg/dL.

For additional information contact:
Janet Demcoe at (215) 451-4914,
or e-mail:
demcoej@usa.redcross.org,
or write to:
American Red Cross Biomedical Services
Musser Blood Center
700 Spring Garden Street
Philadelphia, PA 19123-3594
ATTN: Janet Demcoe

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National Reference Laboratory for Blood Group Serology

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

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

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

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Phone, business hours:
(215) 451-4903
Fax:
(215) 451-2538

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

For additional information:
Kathy Kaherl
at (860) 678-2764
e-mail:
kaherlk@usa.redcross.org
or write to:
Reference Laboratory
American Red Cross Biomedical Services
Connecticut Region
209 Farmington Ave.
Farmington, CT 06032

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What is a certified Specialist in Blood Banking (SBB)?
• Someone with educational and work experience qualifications that successfully passes the American Society for Clinical Pathology (ASCP) board of certification (BOC) examination for the Specialist in Blood Banking.
• This person will have advanced knowledge, skills, and abilities in the field of transfusion medicine and blood banking.

Individuals who have an SBB certification serve in many areas of transfusion medicine:
• Serve as regulatory, technical, procedural, and research advisors
• Perform and direct administrative functions
• Develop, validate, implement, and perform laboratory procedures
• Analyze quality issues preparing and implementing corrective actions to prevent and document nonconformances
• Design and present educational programs
• Provide technical and scientific training in transfusion medicine
• Conduct research in transfusion medicine

Who are SBBs?
Supervisors of Transfusion Services  Executives and Managers of Blood Centers  LIS Coordinators  Educators
Supervisors of Reference Laboratories  Research Scientists  Consumer Safety Officers
Quality Assurance Officers  Technical Representatives  Reference Lab Specialists

Why become an SBB?
Professional growth  Job placement  Job satisfaction  Career advancement

How does one become an SBB?
CAAHEP-accredited SBB Technology program or grandfather the exam based on ASCP education and experience criteria.

Fact: In recent years, a greater percentage of individuals who graduate from CAAHEP-accredited programs pass the SBB exam compared to individuals who grandfather the exam. The BEST route for obtaining an SBB certification is to attend a CAAHEP-accredited Specialist in Blood Bank Technology Program.

Which approach are you more compatible with?

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

<table>
<thead>
<tr>
<th>Program</th>
<th>Contact Name</th>
<th>Phone Contact</th>
<th>Email Contact</th>
<th>Website</th>
<th>Onsite or Online Program</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walter Reed Army Medical Center</td>
<td>William Turkcan</td>
<td>202-782-6210</td>
<td><a href="mailto:William.Turcan@amedd.army.mil">William.Turcan@amedd.army.mil</a></td>
<td><a href="http://www.militaryblood.dod.mil/fellow">www.militaryblood.dod.mil/fellow</a></td>
<td>Onsite</td>
</tr>
<tr>
<td>American Red Cross, Southern California Region</td>
<td>Michael Coover</td>
<td>909-659-2746</td>
<td><a href="mailto:CooverM@usa.redcross.org">CooverM@usa.redcross.org</a></td>
<td>none</td>
<td>Onsite</td>
</tr>
<tr>
<td>ARC-Central OH Region</td>
<td>Joanne Kosanek</td>
<td>614-253-2740 x 2270</td>
<td><a href="mailto:kosanekJ@usa.redcross.org">kosanekJ@usa.redcross.org</a></td>
<td>none</td>
<td>Onsite</td>
</tr>
<tr>
<td>Blood Center of Wisconsin</td>
<td>Lynne LeMense</td>
<td>414-937-6403</td>
<td><a href="mailto:Lynne.Lemense@bcw.edu">Lynne.Lemense@bcw.edu</a></td>
<td><a href="http://www.bcw.edu">www.bcw.edu</a></td>
<td>Onsite</td>
</tr>
<tr>
<td>Community Blood Center/CTS Dayton, Ohio</td>
<td>Nancy Lang</td>
<td>937-461-3293</td>
<td><a href="mailto:nlang@cbicts.org">nlang@cbicts.org</a></td>
<td><a href="http://www.cbicts.org/education/sbb.html">www.cbicts.org/education/sbb.html</a></td>
<td>Online</td>
</tr>
<tr>
<td>Gulf Coast School of Blood Bank Technology</td>
<td>Clare Wong</td>
<td>713-791-6201</td>
<td><a href="mailto:cwwong@giveblood.org">cwwong@giveblood.org</a></td>
<td><a href="http://giveblood.org/index.php?page=sbb-distance-program">http://giveblood.org/index.php?page=sbb-distance-program</a></td>
<td>Online</td>
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<tr>
<td>Hoxworth Blood Center/University of Cincinnati</td>
<td>Susan Wilkinson</td>
<td>513-558-1275</td>
<td><a href="mailto:Pamela.hurt@usu.edu">Pamela.hurt@usu.edu</a></td>
<td><a href="http://www.huxworth.org">www.huxworth.org</a></td>
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<td>Indiana Blood Center</td>
<td>Jayanna Slayten</td>
<td>317-815-5186</td>
<td><a href="mailto:jslayten@indianablood.org">jslayten@indianablood.org</a></td>
<td><a href="http://www.indianablood.org">www.indianablood.org</a></td>
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<tr>
<td>Johns Hopkins Hospital</td>
<td>Lorraine Blagg</td>
<td>410-502-9684</td>
<td><a href="mailto:Lblagg@jhmi.edu">Lblagg@jhmi.edu</a></td>
<td><a href="http://pathology.jhu.edu/department/divisions/transfusion/sbb2.cfm">http://pathology.jhu.edu/department/divisions/transfusion/sbb2.cfm</a></td>
<td>Online</td>
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<tr>
<td>Medical Center of Louisiana</td>
<td>Karen Kinkle</td>
<td>504-803-3054</td>
<td><a href="mailto:kkirk@luhs.ac.edu">kkirk@luhs.ac.edu</a></td>
<td>none</td>
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<tr>
<td>NIH Clinical Center Department of Transfusion Medicine</td>
<td>Karen Byrne</td>
<td>301-496-8335</td>
<td><a href="mailto:Kbyrne@mail.cc.nih.gov">Kbyrne@mail.cc.nih.gov</a></td>
<td><a href="http://www.cc.nih.gov/dtm/research/sbb.html">www.cc.nih.gov/dtm/research/sbb.html</a></td>
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<td>Online</td>
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<tr>
<td>Transfusion Medicine Academic Center at Florida Blood Services</td>
<td>Marjorie Doty</td>
<td>727-568-5433 x 1514</td>
<td><a href="mailto:mdoty@ftoblood.org">mdoty@ftoblood.org</a></td>
<td><a href="http://www.ftoblood.org">www.ftoblood.org</a></td>
<td>Online</td>
</tr>
<tr>
<td>University Health System and Affiliates, San Antonio</td>
<td>Linda Myers</td>
<td>210-731-5329</td>
<td><a href="mailto:lmyers@bloodntissue.org">lmyers@bloodntissue.org</a></td>
<td><a href="http://www.sbbefsa.org">www.sbbefsa.org</a></td>
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<tr>
<td>University of Texas Medical Branch at Galveston</td>
<td>Janet Vincent</td>
<td>409-772-3055</td>
<td><a href="mailto:jvincent@utmb.edu">jvincent@utmb.edu</a></td>
<td><a href="http://www.utmb.edu/abb">www.utmb.edu/abb</a></td>
<td>Online</td>
</tr>
<tr>
<td>University of Texas SW Medical Center</td>
<td>LeAnne Houston</td>
<td>214-684-1780</td>
<td><a href="mailto:mls.sshp@utsouthwestern.edu">mls.sshp@utsouthwestern.edu</a></td>
<td><a href="http://utsouthwestern.edu/mls">http://utsouthwestern.edu/mls</a></td>
<td>Online</td>
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</table>
Immunohematology
Journal of Blood Group Serology and Education

Instructions for Authors

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

II. SCIENTIFIC ARTICLE, REVIEW, OR CASE REPORT WITH LITERATURE REVIEW
A. Each component of the manuscript must start on a new page in the following order:
1. Title page
2. Abstract
3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables
8. Figures

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

III. EDUCATIONAL FORUM
A. All submitted manuscripts should be approximately 2000 to 2500 words with pertinent references. Submissions may include:
   1. An immunohematologic case that illustrates a sound investigative approach with clinical correlation, reflecting appropriate collaboration to sharpen problem solving skills
   2. Annotated conference proceedings

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

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

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A

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Featuring—
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**Blood Group Antigens & Antibodies**

*A guide to clinical relevance & technical tips*

by Marion E. Reid & Christine Lomas-Francis

This compact “pocketbook” from the authors of the *Blood Group Antigen FactsBook* is a must for anyone who is involved in the laboratory or bedside care of patients with blood group alloantibodies.

The book contains clinical and technical information about the nearly 300 ISBT recognized blood group antigens and their corresponding antibodies. The information is listed in alphabetical order for ease of finding—even in the middle of the night. Included in the book is information relating to:

- Clinical significance of antibodies in transfusion and HDN.
- Number of compatible donors that would be expected to be found in testing 100 donors. Variations in different ethnic groups are given.
- Characteristics of the antibodies and optimal technique(s) for their detection.
- Technical tips to aid their identification.
- Whether the antibody has been found as an autoantibody.

**Pocketbook Education Fund**

The authors are using royalties generated from the sale of this pocketbook for educational purposes to mentor people in the joys of immunohematology as a career. They will accomplish this in the following ways:

- Sponsor workshops, seminars, and lectures
- Sponsor students to attend a meeting
- Provide copies of the pocketbook

(See www.sbbpocketbook.com for details to apply for funds)

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**Ordering Information**

The book, which costs $25, can be ordered in two ways:

- Order online from the publisher at: www.sbbpocketbook.com
- Order from the authors, who will sign the book. Send a check, made payable to “New York Blood Center” and indicate “Pocketbook” on the memo line, to:
  
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  New York Blood Center  
  310 East 67th Street  
  New York, NY 10065

Please include the recipient’s complete mailing address.