

Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

VOLUME 20, NUMBER 4, 2004



American Red Cross

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Immunohematology

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LETTER TO THE READERS

Introduction to the review articles

In this, the last of four special issues celebrating *Immunohematology's* 20th anniversary, we offer a potpourri of review articles exploring the clinicopathologic correlations of a number of conditions of interest to immunohematologists and blood bankers. The management of patients with antibodies to blood group antigens or antigen-antibody incompatibilities after hematopoietic progenitor cell (HPC) or solid organ transplantation is complemented by knowledge of the pathophysiology of RBC membrane components and how RBC antigen compatibility is likely to change throughout the course of HPC or organ transplantation. Patients with IgA-mediated anaphylactic transfusion reactions also challenge the transfusion service on a number of levels: providing timely laboratory diagnoses as well as additional compatible blood components. Blood banks challenged by the appearance of blood group antibodies or anti-IgA antibodies in patients they support may also need to turn to an organization with access to rare units. A working knowledge of how rare units are obtained facilitates high-quality patient care. The reviews in this special issue provide a starting point and a host of references for further directed exploration of three complex topics: the function of blood group-specific RBC membrane components, immunohematologic aspects of transplantation, and IgA anaphylactic transfusion reactions. A detailed description of the American Rare Donor Program complements and completes the thoughtful discussions in our other reviews.

Allogeneic blood transfusion remains the most commonly performed tissue transplant in the United States. This procedure is occasionally complicated by the formation of alloantibodies directed against glycoprotein and glycolipid determinants on the RBC surface. Jill Storry reminds us that RBC-specific antibodies characterize phenotypic variants of surface molecules that perform a myriad of functions. These include regulation of RBC ontogeny, metabolism, and structural integrity; complement and chemokine regulation; and even the disease resistance of individuals expressing certain variants.

Drs. Vincent LaRoche, D. Ted Eastlund, and Jeffrey McCullough tackle the peri-transplantation transfusion management of hematopoietic progenitor cell and

solid organ recipients mismatched with their allografts for ABO, Rh, and other RBC antigen polymorphisms. A number of immunohematologic complications occur in the setting of isoagglutinin or isoantigen incompatibility as planned or unplanned donor immune system engraftment occurs or as the recipient's immune system recognizes incompatibilities in the allograft. The timing of these changes may be a source of confusion as transfusion needs change during the course of patients' care.

Dr. Ralph Vassallo, and Drs. Nicole Zantek and S. Gerald Sandler, address the issues surrounding the initial care and evaluation of patients experiencing severe allergic reactions to transfused blood products. The investigation of anti-IgA as the cause for anaphylaxis is time-consuming, and thus a source of frustration for physicians faced with immediate transfusion decisions. An appreciation of how the immunology laboratory approaches the diagnosis may be as helpful to clinicians as an appreciation of caregivers' clinical imperatives is to the immunohematologist.

Cynthia Flickinger, Tammy Petrone, and Ann Church then provide an overview of the American Rare Donor Program (ARDP), a collaborative effort between the American Red Cross and AABB. A database of approximately 55,000 high-frequency-antigen-negative, multiple-common-antigen-negative, or IgA-deficient donors is maintained and continually augmented through donor or family screening. It can be accessed through its 80 member institutions. The ARDP's ability to provide rare blood products to alloimmunized individuals in a timely fashion is an essential service linking clinicians and blood bankers seeking to help patients in need.

Finally, in addition to these important reviews, two original articles and several noteworthy communications are presented in this, the last issue of the year.

*Ralph R. Vassallo, MD
Associate Medical Editor and
Guest Editor of this issue
Senior Medical Director
American Red Cross Blood Services
Penn-Jersey Region
700 Spring Garden Street
Philadelphia, PA 19123*

Review: the function of blood group-specific RBC membrane components

J.R. STORRY

Billions of RBCs course through the heart and arteries per second. Blood flow through capillaries and veins is somewhat slower but a RBC circulates throughout the body many thousands of times during its 120-day lifespan. Thus, the contact that a RBC has with the vessel walls and with leukocytes and platelets is extremely intimate and unique. RBCs must be inert enough to perform their function of gaseous exchange without compromise but, as is becoming clearer, they must be active enough to respond to the microenvironment in which they find themselves.

The extracellular surface of the RBC membrane consists of glycoproteins and glycolipids. Blood group antigens have been found on most of these components and have provided a useful “handle” by which to isolate and study them. There are few proteins on the RBC membrane on which blood groups have not been identified, such as the glucose transporter and MRL (CD59). This review will describe the blood group-active RBC membrane components only and their relevance in a normal physiological state. Instead of being listed according to ISBT order, the blood groups have been grouped according to known or likely function (see Table 1). Blood group antigens will be discussed where they are relevant to the function of the carrier component. For more details regarding the blood group antigens themselves, two excellent reference texts (among many others) are recommended: *The Blood Group Antigen FactsBook* and *Human Blood Groups*.^{1,2} The reference list is by no means exhaustive, since the functional groups are the subjects of detailed reviews themselves. In fact, these reviews have been cited wherever possible and original papers cited only where appropriate. The reader is also referred to another excellent and recent review that addresses both structure and function of RBC blood group antigens.³

Figure 1 shows the assignment of the blood group-active molecules to a primary functional group. This is somewhat artificial and it is important to keep in mind that many of these molecules may have more than one function. There are two large groups: the transport proteins and the adhesion molecules. Aside from these two groups, blood group-carrying proteins include those involved in complement regulation and proteins that are known to be enzymes. There are two families of glycoporphins, the glycoporphin A (GPA) family, whose function is not clearly defined, and the glycoporphin C (GPC) family, whose structural role, at least, has been identified. Finally, most of the integral membrane proteins or membrane-associated proteins of the RBC are glycosylated. These polysaccharides carry blood group antigen determinants. A brief discussion of their function is included. The role of blood group antigens as receptors for various pathogens is not discussed in this review. The interested reader is referred to the excellent reviews in the reference list.^{4,5}

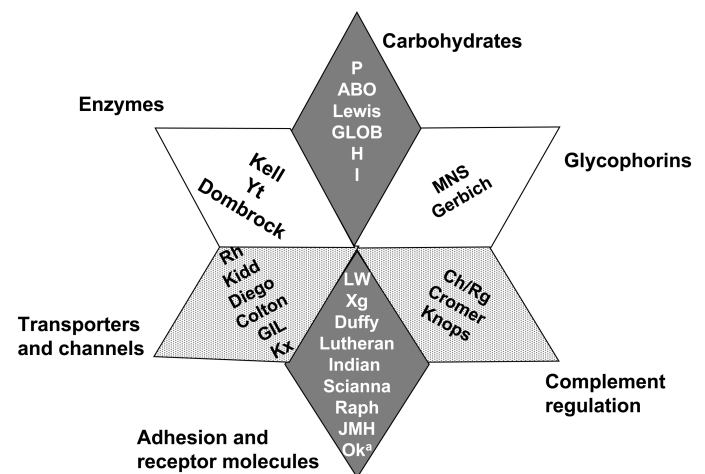


Fig. 1. Blood group systems grouped according to functional molecule.

Table 1. Blood group-carrying RBC membrane components

Carrier type	Blood group system (ISBT)	Carrier molecule	Known function of carrier molecule	Secondary/probable function of the carrier molecule	Clinical manifestation of the absence of the carrier molecule
	Rh	RhD, RhCE, (RhAG)*	NH ₄ ⁺ gas exchange?	Structural	Stomatocytosis; mild compensated anemia
	Kidd	HUT/11	Urea transporter		Reduced ability to concentrate urine
Transporter or channel	Diego	AE-1	Anion exchanger; structural		Spherocytosis; severe anemia, failure to thrive
	Colton	AQP1	Water channel		Reduced osmotic water permeability
	Kx	Xk glycoprotein	Not known	Structural	Acanthocytosis; McLeod syndrome
	GIL	AQP3	Water/glycerol transporter		None
	Lutheran	Lutheran glycoprotein (B-CAM)	Adhesion	Structural?	None
	Duffy	DARC	Chemokine receptor		None
	Xg	Xg glycoprotein, CD99	Adhesion		None
	Scianna	ERMAP	Adhesion		None
	Landsteiner-Wiener	LW glycoprotein (ICAM-4)	Adhesion		None
Adhesion or signalling	Indian	Hermes antigen (CD44)	Adhesion		Not known
	OK	Neurothelin (CD147)	Adhesion		Not known
	RAPH	MER2 (CD151)	Adhesion		Multiple system disorder
	JMH	Sema7A (CDw108)	Adhesion		None
	MNS	GPA/GPB	Not known	Structural?	None
Glycophorin	Gerbich	GPC/GPD	Structural		Elliptocytosis; mild anemia
	Chido/Rodgers	C4	C' regulation		None
	Gromer	DAF (CD55)	C' regulation		Association with intestinal disorder?
Complement regulation	Knops	CR1 (CD35)	C' receptor/C' regulation		Not known
	Kell	Kell glycoprotein	Enzyme	Structural	None
Enzyme	Yt	AcHE	Enzyme		Not known
	Dombrock	ART4	Enzyme		None
	ABO	CHO [†]	Unknown	Innate defense?	None
	P	CHO	Unknown	Innate defense?	None
	Lewis	CHO	Unknown	Innate defense?	None
Carbohydrate	H	CHO	Unknown	Innate defense?	None
	I	CHO	Unknown	Innate defense?	None
	GLOB	CHO	Unknown	Innate defense?	Recurrent spontaneous abortion

*RhAG does not carry blood group antigens but it is an integral protein of the functional complex

†Carbohydrate

Transporters and Channels

The primary function of the RBC is to deliver oxygen to the tissues in exchange for metabolic wastes such as carbon dioxide and urea. The exchange of oxygen for carbon dioxide occurs passively and is based on oxygen dissociation from Hb, which in turn is based on the oxygen affinity of the Hb molecules. However, there are many transporters and channels on or through the membrane that move bioactive molecules to and from the extracellular domain and cytosol. Transporter and channel proteins are multiple-membrane-spanning proteins that either actively transport compounds across the membrane or provide a hydrophilic channel through the lipid bilayer.⁶ In addition to their transmembrane functions, some of these proteins are connected to the cytoskeleton and therefore play a role in the maintenance of RBC shape. Transport proteins with and without a structural role will be discussed in separate sections.

Transport proteins with structural functions

Structural function is not included in Figure 1; however, as Table 1 shows, there are RBC membrane proteins that play a crucial role in maintenance of cell shape. It is becoming clear that our current perception of the RBC skeleton and its interactions with the integral membrane proteins is oversimplified, and there is increasing evidence to demonstrate the association of the Rh complex with the band 3 complex: interaction of the Kell/Kx complex with GPC, Lu/B-CAM with spectrin, and so on.⁷⁻⁹ Furthermore, phosphatidylserine in the lipid bilayer has been shown to interact directly with spectrin subunits.^{10,11} This information creates a picture in which the underlying spectrin-actin web is in close and constant contact with the lipid bilayer as well as with an extracellular matrix of functional protein complexes which, in turn, interact with themselves and other complexes.^{7,12,13}

Band 3 (AE-1, anion transporter) carries the antigens of the Diego blood group system.¹ It is the most abundant protein in the RBC membrane, with an estimated 1×10^6 copies per cell.¹⁴ This fascinating protein has two distinct domains: an intracellular N-terminal domain of 359 amino acids that forms structurally important associations with the cytoskeleton and interacts with several glycolytic enzymes and a transmembrane, channel-forming domain that occurs as homodimers and homotetramers to form the anion transporter.¹⁴ Its contribution to the maintenance of RBC structure is demonstrated in people with

hereditary spherocytosis (HS), where it has been shown that 20 percent of HS cases are due to the heterozygous inheritance of one of numerous mutations identified in the *SLC4A1* gene encoding band 3, and in Southeast Asian ovalocytosis, which is due to the inheritance of one gene with a 27-base pair deletion.¹⁵ It was commonly thought that homozygosity for mutations in *SLC4A1* would be incompatible with life; however, there are now living examples of natural "knockouts" in Japanese black cattle and in humans, as well as experimental evidence from engineered "knockouts" in laboratory mice.¹⁶⁻¹⁸ Studies of RBCs taken from the cord blood of a severely anemic, hydropic baby girl showed the complete absence of band 3 and protein 4.2 as well as a significant reduction in spectrin, ankyrin, and GPA.¹⁷ At the time of the report, she was 3 years old and was sustained by regular RBC transfusions as well as oral doses of bicarbonate to counteract the renal acidosis that resulted from the absence of band 3 in her kidneys. Analysis of RBCs from Japanese black cattle that had homozygous inheritance of a nonfunctional band 3 gene gave similar results: absence of band 3 and protein 4.2, 44% actin, and 26% spectrin.¹⁶ These examples show the importance of band 3 in the stable assembly of the spectrin-actin web that makes up the RBC cytoskeleton. Surprisingly, they also showed that while band 3 is not essential for life, it is critically important for prolonged survival. The role of GPA in the band 3 complex is not clear, although there is evidence to suggest that GPA acts as a chaperone in getting band 3 to the RBC membrane.¹⁹ However, it is not essential in this role, since persons who lack GPA have normal levels of band 3, although glycosylation of the protein is altered.^{19,20} More recently, the presence or absence of GPA has been shown to affect the efficacy of anion transport.²¹

The transport function of band 3 is the exchange of anions: specifically, it exchanges equimolar concentrations of Cl^- ions for HCO_3^- ions. This function is not only important in the removal of CO_2 from tissues but also important in maintaining pH in the kidney, where band 3 is located on the basolateral membrane of the collecting tubule alpha-intercalated cells and abnormalities of band 3 cause distal renal acidosis.^{15,22,23}

The extracellular loops of band 3 carry the Diego blood group antigens. Many low-frequency antigens of the Diego blood group system reside on the third extracellular loop, which also carries sequences thought to be important in RBC senescence and in the adhesion of sickle RBCs.^{3,24} Polymorphisms on the

intracellular domain or transmembrane domains are not as forgiving, and it is these mutations that lead to misfolding of the protein and have been shown to result in hereditary spherocytosis.²⁵

The Rh complex consists of a core tetramer of two RhAG molecules and two molecules of either RhD or RhCE.^{26,27} In addition, the LW, CD47, glycophorin B (GPB), and Duffy glycoproteins interact with the complex, as evidenced in individuals with the Rh_{null} phenotype, whose RBCs not only lack the RhD, RhCE, and RhAG proteins but also have reduced levels of expression of the accessory proteins.²⁸ Rh_{null} RBCs are stomatocytic and are associated with a mild compensated hemolytic anemia. Although a role for the complex in the maintenance of RBC structure was evident from the abnormal cell shape in the Rh_{null} syndrome, interaction with the cytoskeleton has been shown only recently. Beckmann et al.^{29,30} demonstrated that in K562 cells the expression levels of Rh were increased if co-transfected with band 3, and Nicolas et al.¹³ demonstrated that the cytoplasmic tails of Rh and RhAG polypeptides interacted with ankyrin-R. In addition to the direct attachment of the Rh proteins, CD47, another important member of the Rh complex, has been shown to associate with protein 4.2.^{12,31-33}

The transport function of the Rh complex has still not been defined. However, RhAG shares approximately 40 percent evolutionary homology with genes encoding ammonium transport proteins (Amt proteins) and there is growing experimental evidence of its role as an ammonium transporter.³⁴⁻³⁷ A role in gas transport, specifically CO₂, has been suggested, possibly for a macromolecular complex involving band 3 and the Rh complex.^{34,38}

Another transmembrane protein that demonstrates evidential proof of structural and transport functions (although neither has been proved experimentally) is the Xk protein. In RBCs, the Xk protein is covalently linked to the Kell glycoprotein through a disulfide bond. The K₀ phenotype, in which the Kell glycoprotein is absent from the RBC membrane, is not associated with morphological change or with any pathology; however, the amount of Xk protein present is reduced.^{39,40} In contrast, the absence of Xk protein is associated with a markedly reduced amount of Kell glycoprotein. The RBCs are acanthocytic, with variable amounts of poikilocytes and other abnormal cells.⁴¹ Abnormal RBC shape and reduced expression of Kell blood group antigens are symptoms of a late-onset multi-system disorder known as the McLeod syndrome,

which is characterized by neurological abnormalities that develop, typically, in the fifth decade of life.^{42,43} Xk is a multipass membrane-spanning protein with homology to neurotransmitter transporters.⁴⁴ However, its substrate in RBCs has not been identified and the pattern of Xk expression in other tissues is different from that of Kell.

Other transport proteins

The specific and controlled passage of water molecules occurs through one of two water channels in the RBC membrane. These proteins, named aquaporins, were first identified by the discovery of aquaporin 1 (AQP1, channel-forming integral protein, CHIP) in RBCs through the association of the Colton blood group system.⁴⁵ While AQP1 is the primary water transporter in RBCs, it is also found in the kidney, lung, vascular epithelium, brain, and eye. The absence of AQP1, first demonstrated in rare individuals with the Co(a-b-) phenotype, showed that AQP1 was not the only water transporter, because these individuals were seemingly healthy. However, the RBCs from Co(a-b-) persons demonstrate a markedly reduced osmotic permeability. In addition, these individuals exhibit an inability to concentrate urine and decreased pulmonary vascular permeability.⁴⁶

The second aquaporin to be identified in the RBC membrane through its blood group "handle," the Gil antigen, was AQP3.^{47,48} AQP3 transports water, but its primary function is the transport of glycerol. It also transports urea with a low permeability. Apart from RBCs, AQP3 is also found in the kidney, skin, lung, eye, and colon. AQP1 and AQP3 are two members of a growing family of similar transmembrane proteins involved in the transport of water, glycerol, urea, and arsenite. Currently there are 11 members distributed across many different tissues.⁴⁶

While AQP3 has a moderate affinity for urea, the protein that is primarily responsible for urea transport in the RBC is the urea transporter, or HUT11. This protein carries the antigens of the Kidd blood group system.⁴⁹ The relationship of the Kidd blood group system with a protein involved in urea regulation was first demonstrated when Jk(a-b-) RBCs were shown to be resistant to lysis with 2M urea.⁵⁰ Further studies showed that urea transport in Jk(a-b-) cells was greatly reduced when compared with that in RBCs of normal Jk phenotype and was consistent with simple diffusion across the membrane.⁵¹ Rapid transport of urea across the RBC membrane is necessary in the kidney, where

high urea concentrations make the RBCs susceptible to dehydration.⁵²

Adhesion and Receptor Molecules

The view of the RBC as an inert bag of Hb has changed considerably over the past decade, with the identification of many blood group-bearing glycoproteins as adhesion molecules and receptors (Table 1). The Duffy protein was identified as the exclusive RBC receptor for *Plasmodium vivax* in 1976, when it was shown that the Fy(a-b-) RBCs of Africans were resistant to invasion by the parasite.⁵³ Nearly 20 years later, the physiological role of the Duffy glycoprotein as the chemokine receptor was established when it was shown that the chemokines IL-8, MGSA, RANTES, and MCP-1 bound with high affinity.⁵⁴⁻⁵⁶ Duffy, which has homology with G-coupled signalling proteins, has been proposed to act as a sink for excess circulating chemokines, thus sequestering these highly bioactive molecules.

The high-incidence antigen JMH is carried on a glycosylphosphatidylinositol (GPI)-linked protein, Sema7A (H-Sema-L, CD108). While Sema7A is expressed on RBCs, it is preferentially expressed on activated T lymphocytes and thymocytes.⁵⁷ Sema7A is one of a large family of semaphorins, molecules whose primary function was considered to be cell-signalling in neural axonal development. More recently, however, it has been shown that several semaphorins are important cell-signalling molecules in the immune system.⁵⁸ Sema7A binds specifically to plexin-C1, a virus-encoded semaphorin protein receptor, and stimulates the production of proinflammatory cytokines such as IL-6 and IL-8, as well as being a potent monocyte activator.⁵⁹

The antigens of the Indian blood group system (and, most likely, the AnWj antigen) are markers for CD44, another adhesion molecule on RBCs. CD44 is found on lymphocytes and monocytes and is a marker for some tumor cells.¹ Although CD44 is found in soluble forms, its role is not fully understood.⁶⁰ Regulation of the *CD44* gene is complex, and alternative isoforms of the protein are produced in different cell lineages and at different stages of development or activation.⁶¹ CD44 is a receptor for hyaluronan but interacts with many extracellular matrix proteins, such as fibronectin, collagens, growth factors, chemokines, and metalloproteinases. It is an important signalling molecule for leukocytes and is involved in leukocyte rolling and attachment to epithelial cells, homing of lymphocytes to lymphoid

organs, and recruitment to sites of inflammation.⁶² Additionally, CD44 plays a role in tumor metastasis, possibly via hyaluronan attachment to basement membranes.⁶¹ There is also clear evidence that CD44 interacts with proteins that bind to actin in the cytoskeleton. The role of CD44 on RBCs is not clear, but it may play a role in anchoring erythroid precursors. Furthermore, monoclonal antibodies to CD44 have been shown to stimulate erythropoiesis.⁶³

Adhesion molecules of the immunoglobulin superfamily on RBCs

The glycoproteins that carry the Lutheran, LW, Scianna, and Ok^a blood groups are all adhesion molecules that belong to the immunoglobulin superfamily (IgSF) of transmembrane proteins.^{63,64} IgSF proteins are defined as having one or more immunoglobulin-like domains: discrete regions of the protein that bear similarity to the V-set or C-set domains of immunoglobulin molecules. The similarity is structural and they contain relatively few highly conserved residues; however, the majority of the proteins play a role in cell-cell recognition.⁶⁵ On RBCs, the Lutheran antigens are found on two isoforms of the Lu glycoprotein derived from alternate splicing of the *LU* gene. The shorter isoform (also known as B-CAM) lacks a cytoplasmic domain but the extracellular region is identical to the Lu glycoprotein and has 5 IgSF domains.^{62,63} Lu/B-CAM binds specifically to laminin 10/11 and is expressed relatively late in erythropoiesis. Thus, it has been suggested that the protein plays a role in the migration of mature RBCs from the bone marrow to the circulation. Similarly, it may play a role in the trafficking of hemopoietic cells from fetal liver (where it is upregulated) to the bone marrow during fetal development.^{24,66} A pathophysiological role for Lu/B-CAM has been explored in sickle cell disease, where it has been shown that expression of the proteins is increased and might contribute to vaso-occlusive events in these patients.^{67,68}

The LW glycoprotein has 2 IgSF domains and it belongs to the intercellular adhesion molecule (ICAM) family designated ICAM-4. Unlike most of the adhesion molecules on the RBC, which have a wide tissue distribution, LW is restricted to RBCs and is weakly expressed on the placenta.⁶³ Like all ICAM family members, LW is a ligand for LFA-1 but it is also a specific ligand for VLA-4 on hemopoietic cells and α_v integrins on nonhemopoietic cells.^{24,66} LW is expressed early in erythropoiesis and it has been proposed that its

function is to tether erythroid progenitors to the erythroblastic islands for their subsequent maturation.⁶⁶ There is recent evidence that ICAM-4 exists as a secreted isoform that may play a regulatory role in cell attachments, particularly in erythroblast attachment.⁶⁹ Furthermore, ICAM-4 has been shown to be a ligand for the α IIb/ β 3 integrin on platelets, which suggests an active role for RBCs in thrombosis and hemostasis.⁷⁰

Enzymes

There are two GPI-linked proteins and one integral membrane protein on the RBC surface that carry blood group antigens and are known to have enzymatic activity, although their role on erythrocytes is not clear.

The Kell blood group protein, discussed briefly in context with the Xk glycoprotein, is a type II integral membrane protein that belongs to a family of neutral endopeptidases, specifically the neprilysin (M13) family of zinc metalloproteinases.⁴⁰ It acts as a highly specific endothelin-3-converting enzyme although it converts both endothelin-1 and endothelin-2, albeit less efficiently.⁷¹ Although the role of the Kell glycoprotein on the RBC has not been elucidated, endothelins are potent vasoconstrictors and it is possible that Kell may be involved in local vascular tone. Maternal antibodies to Kell blood group antigens on fetal RBCs have been shown not only to cause immune-mediated destruction of these RBCs but also to inhibit proliferation and maturation of erythrocyte precursors.⁷² The early appearance of the Kell glycoprotein in erythropoiesis suggests that Kell might participate in lineage determination.^{40,73,74}

In contrast to the Xk protein, to which it is covalently linked in the RBC membrane, the absence of the Kell glycoprotein is not associated with any morphological changes or with any clinical pathology. Interestingly, however, the Kell glycoprotein has been shown to associate with protein 4.1 and thus, indirectly, with both the cytoskeleton and GPC.⁸ The significance of this is not understood yet, although both GPC and Kell appear early in erythropoiesis.^{75,74} Twenty-four antigens have been assigned to the Kell glycoprotein, each of which is associated with a single amino acid change. None of these polymorphisms has been shown to affect function; however, the amino acid substitution responsible for the Kp^a antigen has been shown to affect intracellular trafficking of Kell to the RBC membrane and consequently results in or causes a reduction in the expression of Kell.⁷⁵

Acetylcholinesterase, a GPI-linked protein on RBCs, carries the antigens of the Yt blood group system.^{76,77} While its function is well explored with regard to regulation of acetylcholine at neuromuscular junctions,⁷⁸ its role on RBCs is not known. One example of the Yt(a-b-) phenotype has been described and no obvious pathology was observed; however, this was a transient reduction of the protein.⁷⁹

Another GPI-linked protein with enzyme activity is the Dombrock glycoprotein (ART4), an ADP-ribosyltransferase that carries the antigens of the Dombrock blood group system.⁸⁰ As yet, no RBC enzyme activity has been identified for this protein. The absence of the glycoprotein is characterized by the Gy(a-) phenotype and individuals of this phenotype do not appear clinically affected in any way.

Complement-Related Proteins

The activation and regulation of the complement cascade is tightly monitored by both cell-bound and plasma proteins. The primary erythrocyte receptor is complement receptor 1 (CR1, CD35), which carries the antigens of the Knops blood group system.⁸¹⁻⁸³ CR1 is a member of a large family of complement control proteins (CCP). It is a large protein that consists of 30 homologous domains known as CCP domains or short consensus repeat domains. These are arranged into 4 long homologous repeat domains.⁸⁴ CR1 not only has a high binding affinity for C3b and C4b, and is therefore important in the removal of immune complexes, but also acts a cofactor in their breakdown, thus protecting the RBC from autohemolysis. The Sl(a-) phenotype, found only on the RBCs of persons of African origin, has been shown in vitro to confer some protection against invasion of erythrocytes by *Plasmodium falciparum*. Rosetting of uninfected RBCs around an infected cell has been proposed as an important mechanism for transmission. Sl(a-) RBCs or those with low CR1 copy number show reduced rosetting.^{4,85}

The antigens of the increasingly polymorphic Cromer blood group system are carried on decay accelerating factor (DAF, CD55).^{86,87} This GPI-linked protein is present not only on RBCs but also on the vascular endothelium and gastrointestinal and urinary epithelia. It is also a soluble protein in plasma. DAF is an important regulatory protein in both the classic and the alternative pathways of complement activation, serving to accelerate the decay of C3 and C5 convertases.³ Interestingly, DAF is also found in high levels on the apical surface of trophoblast cells in the

placenta, where it is thought to protect the fetus from maternal complement-mediated attack.⁸⁸ While there is no definitive pathology associated with the null phenotype (Inab phenotype, IFC-), a gastrointestinal disorder has been reported for the majority of the IFC-probands.² Interestingly, DAF has been shown to be a receptor for Dr-fimbriated strains of *Escherichia coli*, specifically the Dr^a polymorphism.⁸⁹

Although not strictly a RBC membrane component, the Chido and Rodgers antigens are polymorphisms of C4, one of the complement proteins found in plasma. C4 is adsorbed onto the RBC surface and thus the antigens are detected on its surface.⁹⁰ Two isoforms of C4 are found, C4A and C4B, of which C4A is more efficient in solubilizing immune complexes and preventing immune precipitation. The absence of C4 has been associated with a number of autoimmune diseases.⁹¹ Additionally, an association of C4 and GPA was demonstrated by GPA-deficient RBCs that were shown to have reduced amounts of Ch antigens, although Rg antigen expression was variable.⁹²

Glycophorins

As mentioned in the introduction, there are two families of glycophorins on the RBC membrane. The GPA family consists of GPA and GPB, which are single-pass membrane proteins and carry the antigens of the MNS blood group system. Although GPA and GPB have been shown to be components of larger protein complexes, respectively, band 3 and the Rh complex, their functions have not been defined clearly. Both proteins are heavily glycosylated by sialic-acid-containing oligosaccharides, and it has been suggested that their function on the mature RBC is to provide a highly negatively charged barrier that limits interaction with other cells and prevents cell aggregation.²⁴

Alternative splicing of the unrelated *GYPC* gene generates two glycophorin molecules: glycophorin C and D (GPC, GPD), both of which carry the antigens of the Gerbich system. Both proteins are also glycosylated by sialic acid-containing oligosaccharides and contribute to the negatively charged glycocalyx.⁹³ However, GPC has also been shown to be another important site of attachment of the RBC membrane to the cytoskeleton, mediated through the cytoplasmic tail, which forms a complex with protein 4.1 and p55.⁹³ This complex is important in maintaining cell shape and mechanical stability. Absence of GPC, i.e., the Leach phenotype (Gerbich null) results in a mild elliptocytosis: the RBCs are more rigid and

mechanically unstable. Absence of protein 4.1 results in a more pronounced elliptocytosis and these RBCs have ~70 percent of normal levels of GPC.³

Carbohydrate Antigens

The carbohydrate antigens include those antigens in the ABO, P, Lewis, H, I, and GLOB blood group systems. With the exception of I antigen, which is defined by a tri-mannose structure at the branch points of complex carbohydrates, these antigens are defined by terminal or subterminal sugar residues on both simple and complex oligosaccharides N-linked to membrane proteins or attached to extracellular lipids. Carbohydrate antigens are not restricted to RBCs but are widely distributed on many tissues and in body fluids. For instance, ABH antigens are found on RBCs; platelets; lymphocytes; respiratory, digestive, and urinary epithelia; vascular endothelium; sensory receptors; and epidermis; and in saliva, plasma, tears, and semen.¹

While the function of the carbohydrate antigens has not been definitively assigned, it is thought that they may have a role as adhesins in embryogenesis and malignancy.^{24,94} It is also likely that they provide a first line of defense against invading pathogens. The naturally occurring antibodies produced by individuals who lack these carbohydrate antigens are not present at birth, and are produced in the first 6 months of life as the infant's immune system becomes active.⁹⁵ It is thought that they are made in response to the constant bacterial, viral, and other pathogenic challenges of everyday life.⁹⁶ No pathology has been associated with the null phenotypes of each of these blood group systems, with the exception of women with the P₁^K, P₂^K, and p phenotypes, who are prone to spontaneous abortion. However, the absence of an expected antigen can serve as an indicator of disease.⁹⁷

Conclusion

Initially, the identification and study of erythrocyte blood group antigens taught us much about cell membrane structure and immunology. I hope that this review makes clear that the continuing study of these molecules is teaching us about the complex interactions between the RBC and its environment in health. Although they have not been discussed in this review, the roles of blood group antigens as receptors for pathogens and as susceptibility/resistance markers for disease has also taught us much about the physiological role of the RBC.

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Jill R. Storry, PhD, FIBMS, Post Doctoral Fellow, Department of Transfusion Medicine, Institute of Laboratory Medicine, Lund University, Lund SE-22185, Sweden.

Review: immunohematologic aspects of allogeneic hematopoietic progenitor cell transplantation

V. LAROCHE, D.T. EASTLUND, AND J. MCCULLOUGH

Since its beginning, allogeneic hematopoietic progenitor cell transplantation (HPCT) has brought new and interesting challenges to the field of transfusion medicine. The blood bank and transfusion medicine programs often play essential roles in the many steps required to achieve a successful transplantation, from donor recruitment; collection, processing, and infusion of the progenitor cell product; to ensuring adequate blood component therapy is available until engraftment occurs.

This latter role is of particular interest to the field of immunohematology, as recipients of allogeneic HPCT undergo a gradual change of hematopoietic and immune systems, from recipient to donor type,^{1,2} and many become alloimmunized to transfused components.^{3,4} For a time, a mixed chimerism state exists: RBCs that originate from the donor's hematopoietic progenitor cells appear in the recipient's circulation while the recipient's older RBCs are still present. In parallel, lymphocytes from both individuals can be found in the recipient, either early in the posttransplant period because of passive infusion through the graft product or later, after engraftment of the donor's progenitor cells.

ABO antigen disparity between donor and recipient is common. In 30 to 40 percent of transplants, recipient and donor harbor different ABO antigens on their RBCs.⁵ Even more commonly, recipients and donors have different non-ABO RBC antigens. In this setting, ensuring an adequate and compatible blood supply requires careful attention. Moreover, the combination of two different RBC phenotypes and lymphocytes from two individuals capable of forming alloantibodies against these RBC

phenotypes is the grounds upon which posttransplantation hemolytic reactions can occur. In addition to the presence of blood group incompatibilities, other factors that influence the responsiveness and activity of the recipient and the donor immune system and the rate of engraftment also have an impact upon the risk of posttransplant hemolysis.

There are many causes of hemolysis following HPCT. ABO incompatibility remains a frequent cause. In major ABO incompatibility, where the recipient is able to form alloantibodies against the donor's RBCs (e.g., O recipient and A donor), and minor ABO-incompatibility, where the donor lymphocytes are able to form antibodies against the recipient's RBCs (e.g., A recipient and O donor), episodes of hemolysis can occur. Both forms of incompatibility can coexist at the same time (e.g., A recipient and B donor). This is referred to as "bidirectional" incompatibility. Although usually less severe, and less frequently reported, other blood groups can also be associated with posttransplant hemolysis. In addition to the potential for alloimmunization, RBC autoantibodies and autoimmune hemolytic anemia (AIHA) are known complications of HPCT. However, one must be cautious in using the terms *autoimmune* and *alloimmune* in the HPCT setting. As suggested by Petz and Garraty,⁶ the term autoimmune should be restricted to donor:anti-donor antibodies or recipient:anti-recipient antibodies.

In addition to immune hemolysis following HPCT, this review will look at the role of blood group incompatibility in other complications of HPCT: GVHD, engraftment, and graft rejection.

Immune Hemolytic Reactions Secondary to Blood Group Incompatibilities

Major ABO incompatibility

A hemolytic reaction can occur when infusing a hematopoietic progenitor cell (HPC) product containing ABO-incompatible RBCs into the recipient. HPCs harvested from marrow⁷ and, to a lesser degree, peripheral blood and cord blood, can have sufficient numbers of RBCs to cause a severe acute hemolytic reaction. This complication is easily prevented by removing RBCs from the progenitor cell product. However, two other complications related to major ABO incompatibility must be mentioned.

Major ABO incompatibility can lead to delayed erythropoiesis mediated by the recipient's isohemagglutinins.^{8,9} This complication can also lead to pure RBC aplasia of the donor's engrafted marrow. The incidence inferred from case-control studies is up to 30 percent of major ABO-incompatible transplants. This is usually a transient phenomenon that resolves with the disappearance of the recipient's isohemagglutinins,^{9,10} but RBC aplasia has lasted up to 8 months in one case.¹¹

Delayed hemolysis has been occasionally reported in this setting.¹² Persistence of recipient-derived antibodies has been described,¹³ and in some cases IgG antibodies were detectable for longer than 100 days posttransplant. In these infrequent cases in which recipient antibodies persist, a delayed hemolytic reaction can occur when the donor's RBCs produced from the transplanted marrow emerge into the patient's circulation. The onset of delayed hemolysis due to major ABO incompatibility is usually between 30 and 100 days. The exact incidence is unknown. Factors modulating the risk of this hemolytic reaction are not well known, but case-control studies suggest that the use of cyclosporine without methotrexate as GVHD prophylaxis could entail a higher risk of hemolysis.¹²

Minor ABO incompatibility

This form of incompatibility can lead to two different causes of hemolysis: delayed hemolysis due to passenger lymphocyte syndrome (PLS) and acute hemolysis during stem cell infusion, due to passive transfer of donor ABO antibodies in the graft that are incompatible with the recipient's RBCs. Acute hemolysis due to passive transfer of donor antibodies that bind with the recipient's RBCs can cause hemolysis immediately after infusion, particularly in

donors with extremely high titers of anti-A or -B. Prevention of this potential cause for hemolysis is achieved by removing plasma from the HPC product, as discussed below. In this section, we will focus on the PLS. Passive transfer of antibodies through transfusion of minor incompatible platelet products, although not necessarily related to the donor-recipient incompatibility, is a related issue that we will also review in this section.

1. PLS

PLS is defined as hemolysis caused by antibodies produced by donor lymphocytes present in the graft at the time of infusion.⁶ It is believed, based upon studies performed mostly in solid organ transplantation, that mature lymphocytes transfused in the graft proliferate and begin producing antibody against the recipient's incompatible ABO blood group antigens,¹⁴ a form of blood group graft-versus-host reaction.

The onset of PLS due to ABO incompatibility has been reported in different series to be somewhere between posttransplant day 5 and day 20.¹⁵⁻¹⁷ Because of its early onset in the transplant period, it is uncertain whether this syndrome is caused by passenger lymphocytes that do not engraft or progenitor cells that have engrafted, differentiated, and matured into educated B lymphocytes.

Hemolysis in PLS, with an increased bilirubin and LDH, and a low haptoglobin, can be severe. It usually lasts until all the recipient's RBCs have been hemolyzed or until the passenger lymphocytes undergo apoptosis. One inconsistency regarding PLS is that, in some cases, the calculated RBC loss exceeds the estimated mass of incompatible recipient RBCs at the time of transplant. This has raised the possibility of a "bystander hemolysis,"^{18,19} wherein transfused RBCs are hemolyzed along with the recipient's RBCs, even though they do not express incompatible A or B antigens.

Serologic findings in these circumstances have revealed that, except in severe cases, significant hemolysis may become clinically apparent before the DAT becomes positive and/or the eluate demonstrates the involved anti-A or -B.¹⁵ In a retrospective study of seven minor ABO-incompatible transplants complicated by severe hemolysis and renal failure in four patients, Gajewski et al.¹⁷ suggested that a positive DAT is usually observed at the time hemolysis becomes apparent. As discussed below, PLS and hemolysis have been observed with minor Rh incompatibility and other blood group antigens as well. Finally, there has

also been a report of hemolysis in an A₁ recipient with an A₂ donor whose anti-A₁ was the causative antibody.²⁰

The true incidence of hemolysis in minor incompatible HPCT is unknown, but case series show a frequency varying from 2 to 70 percent.^{15,16,21} In the largest series of minor ABO-incompatible HPCT, the frequency of hemolysis was 2 percent.²¹ Hemolysis secondary to PLS seems more frequent with group O HPCs infused into group A recipients.¹⁵

2. Minor ABO-incompatible platelet transfusion

Large volumes of ABO-incompatible plasma given during platelet transfusions (e.g., O donor and A recipient) can lead to hemolysis or increase the risk of developing hemolysis in minor ABO-incompatible transplant recipients. Transfused antibodies, upon formation of immune complexes with the recipient's soluble A or B substance, may also reduce the survival of the transfused platelets. To avoid these problems, it is now common to limit the volume of ABO-incompatible plasma that a patient can receive. This limit can be a percentage of the patient's estimated blood volume or an absolute volume limit. For instance, we limit adults to no more than one liter of ABO-incompatible plasma per week. In addition to this, we test incompatible plasma and do not transfuse the platelet component unless the titer is < 1:256.

Influence of the progenitor cell product type, conditioning regimen, and GVHD prophylaxis regimen

Other factors affect the risk of hemolysis by having the potential to tip the balance in favor of the recipient's or the donor's immune system. Currently, HPCs can be collected directly from the bone marrow (BM) or from a donor's peripheral blood after stimulation with an appropriate growth factor (e.g., granulocyte-colony stimulating factor) to boost peripheral blood progenitor counts. HPCs can also be harvested from fresh umbilical cord blood at the time of delivery. Each source has different characteristics. Peripheral blood stem cells (PBSC) are known to contain higher numbers of donor lymphocytes along with progenitor cells.²² Akin to solid organ transplantation, where PLS is correlated with the number of lymphocytes in the graft,²³ in transplants using PBSC as a source of HPCs, PLS should be more frequent. At the present time, the exact incidence of hemolysis due to minor ABO incompatibility in PBSC compared to BM is unknown. Nevertheless, case

reports^{24,25} suggest hemolysis might be more severe with minor ABO-incompatible PBSC transplants. Few data regarding hemolytic complications exist for cord blood transplantation. Yomtovian et al.²⁶ reviewed 14 myeloablative minor ABO-incompatible cord blood transplants and found no donor isohemagglutinins, compared with 15 instances of detectable donor isohemagglutinins in the course of 24 PBSC transplants performed at the same center during the same period.

The intensity of the conditioning regimen has also been examined as potentially modulating the risk of hemolysis. Although conditioning regimens are referred to as myeloablative or nonmyeloablative, the reality is that a number of transplant protocols are situated in between the truly myeloablative and nonmyeloablative. This allows a better tailoring of the treatment to balance the toxicity of the conditioning regimen with the risks of graft failure, relapse, and GVHD.²⁷ These types of lower intensity conditioning regimens increase the time to complete engraftment and the process of engraftment is more gradual, creating a state of mixed chimerism in the recipient during which both hematopoietic and immune systems temporarily coexist. This kind of engraftment kinetic is usually not seen in standard myeloablative transplants. In the patients with severe hemolysis described by Bolan et al.,²⁵ two out of three had undergone a nonmyeloablative transplant. Worel et al.²⁸ also published a series of four patients with severe hemolysis after minor ABO-incompatible transplants, three of whom had received a nonmyeloablative conditioning regimen. It is difficult to draw any conclusions, however, due to confounding factors. Most notably, all five cases had received only cyclosporine as GVHD prophylaxis, which appears to be a risk factor for hemolysis. In fact, Mielcarek et al.²⁹ could not find such a high frequency of hemolysis in 40 nonmyeloablative transplants with minor ABO incompatibility. They attributed this difference to their GVHD prophylaxis regimen, which consisted of cyclosporine and mycophenolate mofetil.

The most important factor modulating the risk of hemolysis, in fact, seems to be the medications used for GVHD prophylaxis. There are many reports supporting the contention that patients on cyclosporine alone for GVHD prophylaxis, as opposed to cyclosporine and methotrexate or mycophenolate mofetil, have an increased risk of hemolysis.^{15,17,25} There is also one report of severe hemolysis in a minor ABO-incompatible transplant patient receiving tacrolimus (FK506) as the sole prophylaxis for GVHD.³⁰

Long-term complications of ABO incompatibility

Various studies have looked at the potential impact of ABO incompatibility on different long-term outcomes of HCPT, such as survival, the occurrence of GVHD, and relapse rates. Although mortality is influenced by all the other outcome measures and relapse is affected by the occurrence of GVHD, we will present them in distinct categories for the sake of clarity.

1. Mortality

Unlike in solid-organ transplantation, ABO incompatibility does not appear to be an unfavorable prognostic factor for survival in HPCT.³¹⁻³³ Transfusing ABO-mismatched platelets³⁴ or large amounts of incompatible plasma could, however, have a detrimental effect on survival.³⁵ A recent retrospective study comparing survival among 153 ABO-identical and -mismatched bone marrow or PBSC transplants³⁶ did not find any significant differences, except in five patients who had bidirectional ABO incompatibility. These patients had a significantly lower survival. However, four of these were matched-unrelated transplants, which are known to have lower survival. Another recent study, by Stussi et al.,³⁷ looked at 562 consecutive transplants, of which 361 were ABO-identical, 98 were minor ABO-incompatible, 86 were major ABO-incompatible, and 17 had bidirectional incompatibility. They also found that only patients with bidirectional incompatibility had lower survival compared with major, minor, and no ABO incompatibility. On the contrary, a study by Mehta et al.³⁸ with 119 matched-related bone marrow transplants also showed no impact on overall survival or treatment-related mortality between ABO-identical and -mismatched patients, but they had only six patients with bidirectional ABO incompatibility, who they analyzed along with the minor ABO-incompatible patients.

2. Relapse

Mehta et al.³⁸ showed a better event-free survival and lower relapse rate among those who were ABO mismatched. The strength of this study is the uniformity of the conditioning regimen used and the underlying diagnosis (acute myelogenous leukemia in first remission), which made for easier comparison between groups. Worel et al.²⁸ looked at the impact of ABO incompatibility on relapse rate in 40 nonmyeloablative transplants and found that the probability of relapse was significantly higher for

ABO-mismatched patients compared with ABO-identical. However, Stussi et al.³⁷ did not find any difference in relapse rates between ABO-compatible and ABO-incompatible transplants. In this study, the majority of transplants used bone marrow as a stem cell source and there were no nonmyeloablative conditioning regimens.

3. GVHD

The association of minor ABO incompatibility and GVHD has been debated in the medical literature for a long time.³⁹ Studies examining the incidence of GVHD among minor ABO-incompatible transplants have produced contradictory results,^{40,41} with the two more recent studies^{37,42} showing an increased incidence of GVHD in the minor ABO-incompatible groups. Stussi et al.,³⁷ however, only found a significant difference if they included milder forms of GVHD. In the more severe cases, the difference was not statistically significant.

Rh and other blood group incompatibility

Both acute and delayed alloimmune hemolytic reactions caused by antibodies directed against Rh antigens have been reported^{43,44}; they are probably the most frequent causes, after ABO incompatibility. Antibodies to the C, c, E, and other non-Rh blood groups (Kidd and Lewis) have been implicated in hemolytic reactions, but anti-D remains the most common antibody involved in that setting.⁴⁵ One notable difference with ABO incompatibility is that antibodies against Rh antigens do not occur naturally, limiting potential acute hemolytic reactions to already sensitized donors (PLS) or recipients. The frequency of non-ABO alloimmunization after transplantation is low in most studies,⁴⁴ ranging from 1 to 6 percent. Moreover, Hows et al.¹⁵ reported a rate of hemolysis of 25 percent among Rh-alloimmunized transplant recipients. However, Hows et al.¹⁵ and Young et al.⁴⁶ described delayed hemolytic reactions arising in the setting of apparently nonsensitized donors and recipients.

Prevention of hemolysis in Rh-incompatible donor-recipient pairs depends upon the direction of the incompatibility and whether or not the donor or recipient is already alloimmunized. With major Rh incompatibility in a previously sensitized recipient, RBC depletion of the graft should be performed using a validated protocol at the cellular therapy laboratory. An alternative would be to perform plasmapheresis on the recipient to lower the titer of the antibody,

although this is much more cumbersome than, and probably not as effective as, removing incompatible RBCs from the graft. With minor Rh incompatibility in a previously sensitized donor, plasma depletion of the graft, with or without washing, should be sufficient to prevent acute hemolysis.

Prevention Strategies and Treatment of Hemolytic Reactions

For prevention of hemolytic reactions due to major ABO incompatibility, RBCs are removed from the graft product. Different methods exist to deplete RBCs. For example, a sedimenting agent, hydroxyethyl starch, is commonly used at our center.⁴⁷ At the present time, it remains unclear what is the maximal safe number or volume of incompatible RBCs left in the HPC product after depletion, but a residual volume of 20 mL or less seems safe. Other strategies have been proposed to prevent hemolysis in major ABO incompatibility. Recipient plasma exchange to reduce the antibody titer has been attempted, with the goal of a post-plasma exchange titer of 1:16 or less. Although this can be an effective approach, the logistics of scheduling a recipient for this procedure along with the conditioning regimen the recipient needs to receive (chemotherapy, radiotherapy, or both) can be problematic, especially for patients with higher titers, who need more than one exchange. Also, the impact of plasma exchange on chemotherapeutic drugs used for conditioning is not well known. For all of these reasons and others not detailed here, this option is less attractive than removing RBCs from the graft.

In minor ABO-mismatched HPCT, it is common practice to reduce the plasma volume of the progenitor cell product to decrease the amount of the potentially offending antibody. Not all cases of minor ABO incompatibilities need to have plasma reduction of their progenitor cell products, however, as the incidence of clinically significant hemolysis is probably low overall and the benefits to be expected from that intervention are offset by the loss of hematopoietic progenitor cells due to graft manipulation. There is no clear guideline as to when to proceed with plasma reduction. Petz and Garraty⁴⁸ propose a threshold of 1:256 for IgM or IgG anti-A or anti-B antibodies for consideration of plasma reduction of the graft. It is interesting to note that this practice of plasma reduction bears some inconsistencies with current knowledge about hemolysis in minor ABO incompatibility HPCT. First, it has been shown that

antibody titers are not predictive of hemolysis in minor ABO or other blood group incompatibility.¹² Even though it makes sense to use some criterion to avoid plasma reduction in all HPC products, the use of a specific antibody titer such as 1:256 is arbitrary. Moreover, in theory, plasma reduction should only be truly effective in preventing hemolysis due to the passive transfer of antibodies. Mature lymphocytes are not significantly removed by this process.

Other interventions to prevent or treat hemolysis in minor ABO incompatibility include recipient RBC exchange to replace the recipient's RBCs with group O cells. Prophylactic RBC exchange is not usually recommended, however, as the recipient's remaining RBC volume will be between 20 and 30 percent and can be as high as 40 percent in some cases,²⁵ a level insufficient to prevent significant hemolysis. Nevertheless, experts agree that RBC exchange should be considered for higher risk patients, such as those receiving unrelated peripheral blood stem cells and cyclosporine alone as GVHD prophylaxis.³⁰

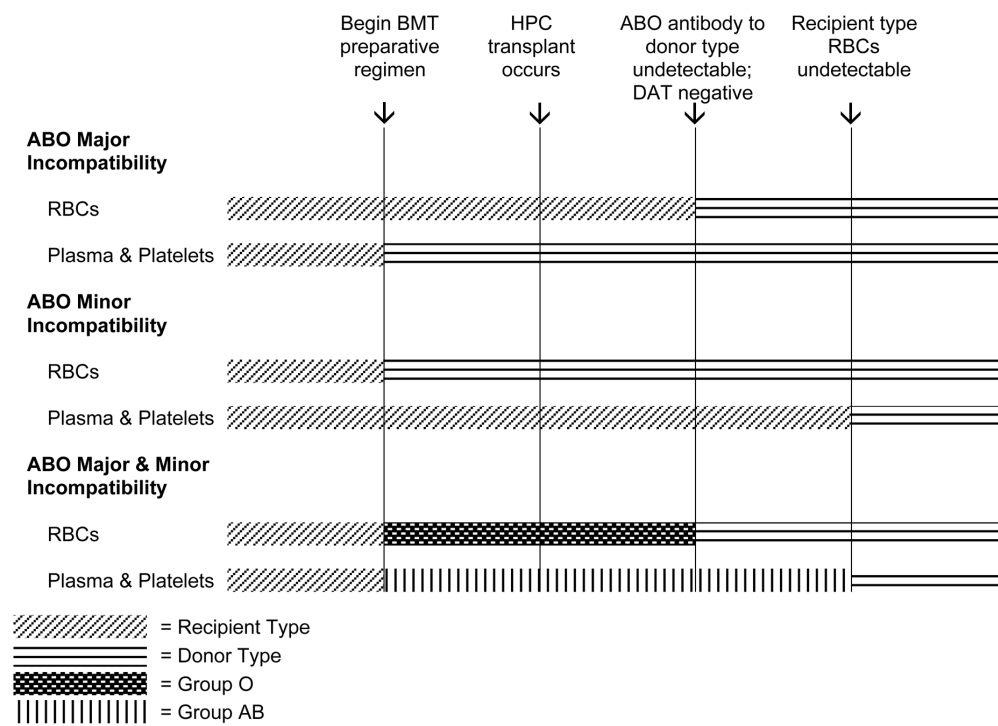
Treatment of immune hemolysis consists of supportive measures such as transfusion and hydration to maintain adequate renal perfusion. At our center, the selection of blood products is guided by a protocol approved by our transfusion committee, which addresses each form of ABO incompatibility⁴⁰ (see Fig. 1). In most cases, using group O RBCs and AB plasma or platelets is easiest, but recipient- or donor-compatible products can also be used in the early phase after transplantation for major and minor ABO incompatibility, respectively.

For delayed erythropoiesis and pure red cell aplasia (PRCA) following major ABO-incompatible HPCT, there is no standard treatment. Tapering immunosuppression, erythropoietin, steroids, and plasma exchange to reduce isohemagglutinin titers have all been tried.

Autoimmune Hemolysis Following HPC Transplantation

Depending upon one's point of view, autoimmunity in the context of HPCT can refer to two different situations: first, donor lymphocytes within the recipient may produce antibodies against the donor's RBCs; second, recipient lymphocytes may produce antibodies against the donor's RBCs. The second situation is, in fact, not usually considered autoimmunity as much as rejection. However, both situations may present with signs of hemolysis and a positive DAT, and

Recommended blood groups of various components to be transfused to recipients of ABO-incompatible cell or marrow transplants at various intervals in the transplantation process:



Notes:

Granulocytes, when required, should be the same ABO group as is recommended for RBCs because of the large volume of RBCs present in these concentrates.

When a patient requires transfusion of granulocytes and platelets, the platelets which are transfused may be of the same ABO group as the granulocytes, so that a single apheresis donor per day may provide both components.

Fig. 1. Blood group guidelines for blood component transfusion in ABO-incompatible HPC transplant recipients.

differentiating between true autoimmunity and rejection can prove difficult.

Post-HPCT AIHA (donor:anti-donor antibodies) is a well known complication, but few large series have examined its incidence and outcome. From Chen et al.⁴⁹ and Drobyski et al.,⁵⁰ the incidence is thought to be from 3 to 5 percent. The onset of warm AIHA ranged from 7 to 25 months in these two series. Cold-antibody AIHA appeared earlier than warm-antibody AIHA in the series published by Chen et al. Outcome was poor in both studies. Positive DATs without hemolysis are not infrequent following HPCT. Little is known about the significance of this finding.

We recently reviewed⁵¹ pediatric cases of positive DATs with clinical signs of hemolysis in HPCT recipients at the University of Minnesota. Of 439 HPCTs performed between 1995 and 2001, there were 34 cases of positive DATs and 19 of 34 also had

significant hemolysis warranting increased transfusion frequency. The overall cumulative incidence was 4 percent at 1 year (95% CI: 2%-6%) and 5 percent at 3 years after HPCT (95% CI: 3%-7%). The median time to develop AIHA was 4 months from HPCT (range, 2 to 32 months). Looking at DAT results, 14/19 had panreactive warm IgG only, two had IgG plus complement, and three had complement only. Elution and adsorption studies revealed an antibody specificity in 4 of the 16 cases with warm IgG-positive DATs: one had both an anti-LW^{ab} and an anti-Jk^a, one had an anti-LW^{ab} alone, one had an anti-E, and one had an anti-e. All cases occurred in patients receiving unrelated-donor HPCT. Patients transplanted for the treatment of an underlying metabolic disease and patients younger than 10 years had a higher risk of developing AIHA. Occurrence of AIHA was not associated with acute or chronic GVHD. The conditioning regimen, source of progenitor cells, and GVHD prophylaxis were also not associated with higher risk of AIHA. Three of 19 died of complications of hemolysis. In four cases, the onset of AIHA coincided with graft failure, raising the question of a true diagnosis of AIHA versus rejection by the recipient's immune system.

Conclusion

Transfusion medicine physicians have an important role in HPCT. These patients place substantial demands upon the blood bank and create interesting and challenging immunohematology issues. RBC incompatibility, alloimmunization, and autoimmunization are significant factors involved with the success of HPCT. Hemolysis can be acute or delayed. A close follow-up of these patients is necessary, as indirect

signs of RBC destruction can, in some cases, precede the detection of antibodies by current serologic methods. Although still debated, blood group incompatibility can potentially play a role in the outcome of HPCT. Further studies are needed to increase our knowledge of alloimmune and autoimmune hemolysis following HPCT, with the hope of improving the management of these serious complications.

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Vincent Laroche, MD, Blood Banking/Transfusion Medicine Fellow, Department of Laboratory Medicine & Pathology—MMC 609; D. Ted Eastlund, MD, Assistant Professor, Department of Laboratory Medicine & Pathology—MMC 609; and Jeffrey McCullough, MD, Professor, Department of Laboratory Medicine & Pathology—MMC 609, University of Minnesota, 420 Delaware Street SE, Minneapolis, MN 55455.

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Review: IgA anaphylactic transfusion reactions.

Part I. Laboratory diagnosis, incidence, and supply of IgA-deficient products

R.R. VASSALLO

Despite yielding a definitive diagnosis in fewer than 20 percent of anaphylactic transfusion reactions, investigation for IgA deficiency and the presence of presumably pathogenic IgG anti-IgA is useful in patient management. Individuals with demonstrated anti-IgA are thereafter committed to receiving IgA-depleted cellular products or IgA-deficient plasma and derivatives to prevent recurrent severe reactions. Unfortunately, in populations of IgA-deficient individuals screened for anti-IgA, the predictive value of the test in the absence of a prior reaction is quite low. Anti-IgA testing is complex and limited to a few reference laboratories, many of which still employ a labor-intensive hemagglutination assay developed in the late 1960s. Timely decisions regarding further transfusion management of patients experiencing anaphylaxis often rely upon more rapidly obtained assays of the IgA concentration as an indicator of the likelihood of subsequent demonstration of anti-IgA. The scarcity of IgA-deficient banked plasma products and dedicated plateletpheresis donors has led to the development of American Rare Donor Program policies designed to appropriately allocate these precious resources. The test methods used to establish the diagnosis of IgA deficiency and identify the approximately one-third of these individuals with anti-IgA are discussed, along with the incidence of abnormal tests in various populations. Also presented are testing recommendations for the identification of an IgA-mediated mechanism for transfusion-associated anaphylaxis and qualification of patients to receive rare IgA-deficient plasma-containing products. *Immunohematology* 2005;21:226–233.

Key Words: IgA, anti-IgA, IgA deficiency, transfusion reactions, anaphylaxis, immunodiffusion, hemagglutination, ELISA

The diagnosis of an IgA-related anaphylactic reaction requires a clinical diagnosis that is confirmed by specialized assays for IgA concentration and anti-IgA. This review will address these laboratory assays, their application to defining the incidence of IgA-related transfusion reactions, and the provision of IgA-deficient blood products. In the accompanying

review, Sandler and Zantek address the clinical diagnosis of IgA anaphylactic reactions and bedside management.

The Role of IgA in Major Transfusion Reactions

Numerous well-documented mechanisms exist by which blood products can trigger major allergic reactions (i.e., anaphylaxis or severe anaphylactoid reactions).¹ Besides unrecognized coincidental events unrelated to the transfusion, these mechanisms include: administration of blood containing antigens to which the recipient is presensitized (e.g., medications, foods, or chemicals like latex or plasticizers), infusion of biological mediators produced during product storage, transfer of donor IgE antibodies against substances in the recipient's blood, transfusion of presensitized individuals with polymorphic forms of common serum proteins (e.g., haptoglobin [especially in Japanese populations], transferrin, α 1-antitrypsin, albumin, etc.), and, central to this review, exposure of recipients with preformed antibodies to transfused IgA. These preformed antibodies may be class-specific (anti-IgA), subclass-specific (anti-IgA1 or -IgA2), or allotype-specific (anti-IgA2m[1] or -IgA2m[2]). Subclass- and allotype-specific antibodies are frequently referred to as being of "limited specificity." The most severe reported reactions tend to result from class-specific antibodies in severely IgA-deficient patients.² Limited-specificity antibodies have been reported to be associated with less severe reactions,

often in patients with low or even normal concentrations of IgA.

Sandler et al.³ reported that only 18.1 percent of 359 sera from patients with anaphylactic or anaphylactoid transfusion reactions contained anti-IgA of any specificity by passive hemagglutination assay. Clearly, analogous to the old medical dictum, “not all that wheezes is asthma,” not all that anaphylaxes during transfusion is IgA-related. A robust risk estimate of the incidence of transfusion-associated major allergic reactions comes from the Québec Hemovigilance system.⁴ For RBC transfusions, the reported risk was 4.3 per 100,000 units, and for platelet transfusions, 62.6 per 100,000 platelet pools. Domen and Hoeltge found 10.4 severe reactions per 100,000 platelet, 3.5 per 100,000 plasma, and 1.7 per 100,000 RBC transfusions in a retrospective review at the Cleveland Clinic.⁵ The reported rate of IgA-related major allergic reactions to RBCs from single institutions is 1.7 per 100,000⁶ and 2.1 per 100,000.⁷ These rates are not inconsistent with Sandler et al.’s finding, as one of the four cases used to generate the higher of the two institutional rates occurred in the setting of an urticarial reaction (estimated frequency 400 per 100,000 RBC transfusions),⁴ which no doubt was reported because of a coincidental myocardial infarction. Furthermore, and more importantly, the true risk estimates from Québec (and Ohio) are likely somewhat higher due to underrecognition and underreporting of transfusion reactions.

The exact cause for most major allergic reactions often goes undetermined.¹ Nevertheless, the proven ability of washed cellular or IgA-deficient cellular or plasma products to prevent recurrent reactions in recipients with previous reactions mandates a prompt, carefully planned investigation for the presence of pathogenic antibodies. Many advocate the screening of patients at high risk for development of anti-IgA before blood products or derivatives are administered, to prevent anaphylaxis.⁸ This latter approach would obviously be important when, not uncommonly, one considers prophylactic use of IgA-containing IVIG for frequent infections or therapeutic use in coexisting autoimmune diseases. Others note that detection of anti-IgA by passive hemagglutination does not necessarily predict risk for anaphylactic reactions.² This screening approach may then unnecessarily commit patients with positive tests to a lifelong policy of IgA-deficient plasma transfusion and could result in harmful delays. The development of anti-IgA assays

that more reliably distinguish individuals at risk for major allergic reactions is therefore of paramount importance.

Epidemiology of IgA Deficiency

Selective IgA deficiency (i.e., IgA only) is the most common primary immunodeficiency in the Western World.⁹ In up to 20 percent of individuals, IgA deficiency may be accompanied by an IgG subclass deficiency, most commonly affecting IgG2 ± IgG4,¹⁰ or may be part of a broader condition, common variable immunoglobulin deficiency, characterized by total absence of IgG, IgA, and, sometimes, IgM. The clinical definition of selective IgA deficiency has been recently revised to a somewhat higher serum IgA concentration (< 7 mg/dL in individuals > 4 years old)¹¹ than that previously advocated by the World Health Organization (< 5 mg/dL). Up to 80 percent of these individuals are at risk of developing sinopulmonary infections or allergies of variable clinical significance, as well as autoimmune disorders and malabsorption syndromes, within 20 years of diagnosis.¹² “Severe IgA deficiency,” however, has been variously defined as < 0.5 mg/dL,¹³ < 0.16 mg/dL,¹⁴ or < 0.05 mg/dL.¹⁵ The American rare donor program uses the < 0.05 mg/dL level to classify its donors as IgA-deficient (A. Church, personal communication). This assures that products provided to patients sensitized to IgA contain far lower concentrations of IgA than those implicated in the development of major allergic reactions.¹⁶ Class-specific anti-IgA develop in individuals with the very lowest concentrations of IgA. As stated above, individuals who have relatively low or even normal concentrations but who lack an IgA subclass or allotype may develop limited-specificity antibodies, reported to be capable of inducing allergic reactions upon IgA challenge, almost all of which are minor reactions. The incidence of IgA deficiency reported in the literature varies not only with the population studied, but also with the detection limits of the screening test. In the United States, 1 in 328 blood donors had an IgA concentration below 5 mg/dL by immunodiffusion assay.¹⁷ International estimates using similarly sensitive assays for IgA concentration include 1 in 168 unselected Spanish children,¹⁸ 1 in 406 Czech blood donors,¹⁹ and 1 in 442 Australian blood donors,²⁰ but only 1 in 4100 Chinese²¹ and 1 in 18,500 Japanese blood donors.²² Identification of individuals whose plasma contains such low concentrations of IgA (< 0.05 mg/dL) that they may donate products for

sensitized patients requires assays not routinely available outside of specialized laboratories. Using more sensitive screening assays, incidence estimates in the same or similar populations reveal that roughly half of those found to be IgA deficient have plasma concentrations low enough to qualify them as IgA-deficient donors. Among blood donors, 1 in 507 Finns,²³ 1 in 875 English,²⁴ and 1 in 886 Americans²⁵ qualify, but as only 1 in 93,000 Japanese manifested concentrations this low,²⁶ establishment of an IgA-deficient donor registry in that country has been quite challenging.

Principles of Testing for IgA-Mediated Transfusion Reactions

The definitive diagnosis of an IgA-mediated allergic reaction requires the demonstration of anti-IgA. However, because the most severe allergic reactions usually occur in the setting of class-specific antibodies in severely IgA-deficient individuals, and quantitative IgA assays are faster and more widely available than anti-IgA assays, many algorithms for the evaluation of anaphylaxis begin with a determination of pretransfusion serum IgA concentrations. (A pretransfusion specimen is necessary because transfused plasma would be expected to contain normal concentrations of IgA, artificially elevating posttransfusion measurements). The identification of severe IgA deficiency in a patient who suffered a major allergic reaction vastly increases the likelihood of a role for anti-IgA in the reaction. Sandler et al.³ found that among 359 individuals experiencing a major allergic transfusion reaction, 76.3 percent with serum IgA < 0.05 mg/dL had class-specific IgA antibodies. A low or normal IgA concentration cannot entirely rule out a role for anti-IgA in an allergic reaction, since antibody assays may demonstrate a limited-specificity antibody in up to 0.7 percent of patients. These antibodies are of uncertain clinical significance, since the majority of reported reactions with limited-specificity antibodies are urticarial, not anaphylactic.³ Table 1 lists some of the available methods to determine serum IgA concentrations, their sensitivity, and the length of time required to perform them.

Quantitation of IgA

Three basic principles underlie assays of plasma IgA concentrations. The first involves quantitation through analysis of precipitates or light-scattering characteristics of IgA:anti-IgA immune complexes. The

Table 1. Characteristics of IgA quantitation assays

Assays	Sensitivity	Assay time
Immunoelectrophoresis	20–25 mg/dL	≤ 24 hrs.
Double diffusion	5–25 mg/dL	≤ 24 hrs.
Radial immunodiffusion (RID)	0.5–5 mg/dL	14–48 hrs.
Nephelometry or turbidometry assays	0.2–50 mg/dL	< 1 hr.
Solid phase red cell adherence (SPRCA)	1 mg/dL	< 1 hr.
Passive hemagglutination inhibition (PHAI)	0.05–0.5 mg/dL	3–5 hrs.
Radioimmunoassay (RIA)	0.0002–0.002 mg/dL	≤ 24 hrs.
Enzyme-linked immunosorbent assay (ELISA)	0.005–0.05 mg/dL	5–6 hrs.

second utilizes hemagglutination as an end point of the antigen:antibody reaction, the third uses specifically labeled antibodies to measure IgA concentrations. In the immune complex-based test category, immunodiffusion, immunoelectrophoretic, and nephelometric/turbidometric assays are available. Ouchterlony double diffusion employs an agar plate whose central well is filled with anti-human-IgA. Serum or plasma samples are placed in surrounding wells and absence of a precipitin arc signifies sample concentrations below the cutoff established by the antibody and sample dilutions used. This test and immunoelectrophoresis are the least sensitive quantitation techniques, best suited to screening individuals for further testing. (Immunoelectrophoresis is usually used in searching for high concentrations of paraproteins, but it can identify levels in the lower ranges.) Low-level radial immunodiffusion kits are available commercially; they can also identify patients requiring further testing to distinguish those with the very lowest concentrations of IgA who may have formed class-specific IgA antibodies. All three technologies require extended plate incubation before test results are available. Nephelometers and turbidometers provide faster results but are not uniformly available in the hospital setting. Both project light through a sample cuvette in which IgA standards or patient test samples are reacted with a known concentration of anti-human-IgA. Nephelometers measure the light scattered through the sample at an angle from the incident beam, while less sensitive turbidometers measure direct light attenuation as antigen-antibody complexes form in solution. Both compare these to the light-exiting dilutions of IgA standards to derive a measured concentration. Rate nephelometers improve upon the sensitivity of the technique by determining the rate of

change of light scattering rather than static values. All of these techniques, if rapidly available at the point of patient care, may increase the probability of detecting an anti-IgA mechanism for major allergic transfusion reactions by defining a population of individuals more likely to have extremely low concentrations of IgA and, thus, class-specific anti-IgA.

In an effort to devise a rapid, more sensitive screening technique for IgA deficiency, Schulenburg et al.²⁷ developed a solid phase RBC adherence assay comparable in sensitivity to but faster than radial immunodiffusion and more widely available than rate nephelometry. Others have found the assay technically difficult to establish,¹⁵ and the test is not commercially available.

The use of highly sensitive immunoassays to quantitate plasma IgA is limited to specialized reference laboratories that frequently cannot provide rapid results when immediate postanaphylaxis transfusion decisions must be made. They are able to distinguish individuals with plasma IgA concentrations below 0.05 mg/dL (i.e., severely IgA-deficient individuals). Highly sensitive quantitation of IgA in patients experiencing anaphylactic transfusion reactions is most useful if anti-IgA assays would introduce intolerable delays in patient care and faster, less sensitive screening studies are unavailable. In nonemergent situations, however, use of rare IgA-deficient products is unwarranted without first demonstrating circulating anti-IgA. High sensitivity studies are also an absolute requirement to establish a panel of IgA-deficient blood donors. One of the first assays developed for high-sensitivity quantitation of IgA was the passive hemagglutination inhibition assay.²⁸ Still in use today, this method is adaptable to automated high volume blood donor screening on Olympus PK or Groupamatic platforms.^{29,30} In this assay, group O negative RBCs are coated with purified IgA using tannic acid or chromic chloride. Test sera are incubated with a diluted anti-human-IgA and then added to a suspension of RBCs in microplate wells. After centrifugation, wells with visible agglutination of the test cells indicate sera with very low concentrations of IgA insufficient to neutralize the anti-human-IgA. Because the assay uses RBC agglutination as an end point, anti-RBC antibodies in test sera invalidate the assay. More and more, highly sensitive enzyme-linked immunoassays are supplanting this technically demanding test. The most sensitive technique, radioimmunoassay, is costly, involves use of radionuclides,

and has been largely replaced by ELISA. Several ELISA techniques have been described, but as yet no FDA-approved commercial kits are available, and these tests must be locally developed and validated.^{31,32}

Anti-IgA and Its Demonstration

Reported rates of what percentage of at-risk IgA-deficient individuals actually develop IgG or IgM anti-IgA depend upon the primary immunodeficiency state studied and, in some cases, concurrent disease states. For otherwise healthy, selective-IgA-deficient individuals, antibodies have been identified in 36.8 percent of those with IgA concentrations < 5 mg/dL³³ and 28 percent,¹⁴ 28.8 percent,³ and 29 percent³⁴ of those with concentrations < 0.05 mg/dL. Interestingly, in individuals with combined IgA and IgG2 deficiency, 60 percent of tested subjects had antibody,³⁴ as did 50 percent of selective-IgA-deficient patients whose clinical course was complicated by rheumatoid arthritis (RA), 77 percent of those with juvenile RA, and 100 percent of those with systemic lupus erythematosus.³³ Seventy-three percent of IgA-deficient individuals with ataxia-telangiectasia were demonstrated to have anti-IgA.³⁵ These data collectively indicate that a clinically recognizable state of immune dysregulation superimposed upon selective IgA deficiency is associated with the formation of anti-IgA and that the application of anti-IgA assays to individuals with progressively higher concentrations of IgA probably picks up additional, limited-specificity antibodies in those with IgA subclass, or allotype deficiencies, or IgA autoantibodies. A frequently stated estimate of the detection of anti-IgA in approximately one-third of IgA-deficient individuals is dependent upon both the composition of the IgA-deficient population as well as the sensitivity of the various methods used to demonstrate antibodies.³⁶

The commonly misquoted rate of 1 in 20,000 to 1 in 47,000 for IgA-related major transfusion reactions comes from the limited data reported by Bjerrum and Jersild (3 reactions, only 1 of which was actually anti-IgA-related in ~60,000 transfusion events)⁶ and Pineda and Taswell (4 anti-IgA-related cases in ~188,000 transfusions).⁷ The true rates are therefore 1 in 47,000 to 1 in 60,000, or a combined rate of ~1 in 50,000. All five patients reacted to whole blood or packed cells and, while this is not explicitly stated,³⁵ are presumably compared to a RBC transfusion denominator. Therefore, while 1 in 1200 Americans has IgA deficiency with co-existing anti-IgA,³ the rate of anti-IgA-mediated

reactions to RBC transfusions is almost 42 times less frequent, i.e., a major allergic reaction may occur in only 2.4 percent of those with identified antibodies! Clearly, we are currently unable to identify what feature of these antibodies truly defines their pathogenicity. The importance of the isotype of anti-IgA also remains unresolved. A 1986 Duke University study detected IgE anti-IgA by ELISA in 9 of 18 patients with allergic transfusion reactions, two of whom had suffered unmistakable anaphylactic events.⁸ However, Mayo Clinic researchers using an immunoradiometric assay (IRMA) were unable to detect IgE anti-IgA in any of 101 sera from patients with urticarial reactions, at least three of which contained demonstrable IgG anti-IgA.³⁵ They also found no IgE antibody in 18 other sera with IgG class-specific anti-IgA by IRMA and passive hemagglutination. IgE antibodies were similarly unreported in a series of patients with anaphylactic transfusion reactions in the setting of selective IgA deficiency.³⁷ Methods to reliably distinguish clinically relevant anti-IgA have yet to be identified. As a result, recommendations cannot be made regarding which individuals with anti-IgA are able to safely receive IgA-containing plasma products or how often patients with previously identified severe IgA deficiency should be screened for antibody formation.

Despite the shortcomings of anti-IgA studies in predicting who is likely to experience a major allergic reaction, a positive result in any of the several available assays is a requirement before elective release of rare IgA-deficient plasma. Available testing methods are listed in Table 2. The first test developed was the passive hemagglutination assay (PHA). It remains a sensitive and reliable, but time-consuming and technically demanding, method available in a small number of reference labs. The assay involves coating group O, D- RBCs with purified myeloma proteins using tannic acid or chromic chloride. Tannic acid-treated RBCs are stable for longer periods (14 days versus 2 for chromic chloride-treated cells) but may manifest nonspecific serum reactivity to "tanned cells." Separate cells are coated with IgA1, IgA2m(1), and IgA2m(2), and two different sources of each protein are

Table 2. Available anti-IgA assays

Passive hemagglutination assay (PHA)
Immunoradiometric assay (IRMA)
Flow cytometry microbead immunoassay (MIA)
Enzyme-linked immunosorbent assay (ELISA)
Particle gel immunoassay (PaGIA)

included in the panel. Diluted test sera are added to V-bottom microplates and observed for hemagglutination following centrifugation. Class-specific antibody is present when all six examples of IgA-coated RBCs agglutinate. Antibody subclass specificity to IgA1 or IgA2 can be determined by reaction with only IgA1- or IgA2-coated cells, while allotype-specific antibodies react only with the two examples of IgA2m(1) or IgA2m(2). Specificity is confirmed by neutralization with purified total IgA or IgA of the appropriate subclass or allotype. Antibody reactivity may also be titrated using serial sample dilutions. Major allergic reactions are often associated with class-specific antibodies of high titer (> 1:1,000); these antibody levels have been reported to remain quite stable over a median follow-up period of 19 years.¹³ Sandler et al.³ reported that the correlation of clinical severity of reactions and antibody titers was not good, possibly because in the wake of an acute reaction, some of the antibody may be removed in complexes with infused IgA. The assay detects hemagglutinating antibodies (IgM and IgG), but cannot identify IgE antibodies. Invalid runs may also occur when reactivity is seen without clear class, subclass, or allotype distinction, due either to nonspecific antibody reactions with storage-induced IgA neo-epitopes or to variable specific reactivity to different IgA myeloma proteins of the same subclass or allotype. The test cannot be interpreted in the presence of RBC antibodies, recognized by their reactivity with uncoated control RBCs. Selection of antigen-negative RBCs for IgA coating can prevent this false reactivity.

A flow cytometry-based microbead immunoassay has been described, using IgA-coated polystyrene beads which are incubated with serum samples.³⁸ A FITC-conjugated anti-human IgG causes fluorescence of beads incubated with test sera containing anti-IgA. Fluorescence is detected and anti-IgA is quantitated using a standard flow cytometer. The requirement for a dedicated flow cytometry facility has limited the applicability of this assay.

Solid phase immunoradiometric assays utilizing IgA coupled to microcrystalline cellulose are available.³⁵ Test sera are incubated overnight with the cellulose-IgA reagent and probed for anti-IgA binding with radiolabeled anti-human IgG using a gamma counter. A number of reference laboratories offer this test, which reliably detects class-specific and, somewhat less reliably so, limited-specificity antibodies. Enzyme immunoassays, which incubate test samples in

IgA-coated microplates, are available.³⁹ Anti-IgA is detected using enzyme-conjugated anti-human IgG and binding specificity is confirmed by neutralization with the IgA preparation used to coat the plates. The ease of this method over the other previously described methods offers advantages in speed and efficiency in reference labs offering anti-IgA studies. ELISA techniques may not be as sensitive in detecting limited-specificity antibodies as a passive hemagglutination assay (PHA), possibly due to the lower concentrations of IgA2 in polyclonal IgA preparations (personal observation).

A new detection technology based on a standard particle-gel immunoassay technique (PaGIA) is under development. Diluted test samples are incubated with red polystyrene beads coated with IgA in the reaction chamber on an ID-Micro Typing System card just above an anti-human IgG gel-containing microwell. Sera containing anti-IgA result in agglutination of the beads, which become trapped in or above the gel during centrifugation. Unagglutinated beads (i.e., those in wells containing test sera without anti-IgA) travel through the gel particles and form a visible pellet at the bottom of the microtube. Results are read macroscopically. Very little is available in the literature about this technology, which, if found to be sufficiently sensitive, is rapid and uses equipment available in many blood banks.^{40,41} One study found the technique sensitive to high-titer (> 1:1,000) anti-IgA demonstrated by passive hemagglutination techniques, but less so for lower titer antibodies.⁴¹ No false positives were seen in either study.

Testing Algorithms and Qualification for Rare Donor Plasma Products

Because of the known risks of re-exposing individuals who have experienced IgA-related anaphylactic transfusion reactions to IgA-containing blood products, cellular blood products are washed to remove plasma. Plasma products, however, must be provided from IgA-deficient donors. The American Rare Donor Program (ARDP), described in this issue of *Immunohematology*, will identify locations of IgA-deficient frozen plasma and request shipment to authorized requesting U.S. blood centers and, rarely, those in other countries. All donors in their database are tested twice, using high-sensitivity IgA assays to ensure severe deficiency (i.e., < 0.05 mg/dL). Because of the limited supply of plasma, ARDP technical and medical staff carefully assess each request.

For elective and most nonemergent requests, ARDP policies generally require the demonstration of class-specific or limited-specificity anti-IgA in a patient's serum, regardless of whether the patient has previously experienced an allergic transfusion reaction. Since the likelihood of non-IgA-related causes of anaphylactic transfusion reactions far outweighs the likelihood of relation to anti-IgA (only 18% in Sandler et al.'s study),³ prior to the release of IgA-deficient plasma, a serum IgA concentration must be determined by the most expedient method. In an emergency, when IgA-antibody testing has not been completed, individuals without detectable IgA by either high-sensitivity or more rapidly available low-sensitivity testing (see test sensitivities in Table 1) will be provided with IgA-deficient plasma until antibody testing is found to be negative. Patients with low but detectable concentrations of IgA rarely have limited-specificity antibodies identified upon appropriate testing. In addition, since most, if not all, of the reactions associated with limited-specificity antibodies are minor ones, IgA-deficient plasma cannot be provided until antibody studies demonstrate subclass- or allotype-specific antibodies. In unusual circumstances, short-term distribution of IgA-deficient plasma may be required after direct communication between the patient's physician and ARDP medical staff.

Since platelets are suspended in large volumes of plasma and platelet washing is technically demanding, the ARDP also maintains a list of blood centers that have IgA-deficient donors in their apheresis programs. ARDP staff can facilitate the recruitment of these donors when platelet washing is locally unavailable or patients continue to experience reactions with washed platelet units. Generally, IgA-deficient cryoprecipitate is not available through the ARDP.

Summary

The laboratory investigation of an anaphylactic transfusion reaction includes testing for anti-IgA. Washed or frozen, deglycerolized RBC transfusions need not be delayed during a laboratory investigation to determine the need for special IgA-deficient blood products. Due to the involved nature of antibody testing, surrogate assays for the absence of IgA may be completed within a time frame commensurate with making important clinical decisions regarding plasma or platelet transfusion. The testing requirements of the American Rare Donor Program, a valuable resource for rare plasma units, have been discussed.

Prophylactic testing for anti-IgA in patients with known IgA-deficiency who are anticipated to require blood transfusion or IVIG treatment is reasonable, but identifies many individuals who are not at risk for major allergic reactions, due to the poor predictive value of the assay. In addition, how often one should test individuals who have not formed antibody is unknown, though one study found that 11.8 percent of individuals with IgA concentrations < 0.05 mg/dL developed antibodies over a follow-up period of 16 to 21 years.¹³ The need for ongoing investigation to determine a better marker for allergic risk is clear and urgent.

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Ralph R. Vassallo, Jr., MD, Senior Medical Director, American Red Cross Blood Services—Penn-Jersey Region, 700 Spring Garden Street, Philadelphia, PA 19123.

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Review: IgA anaphylactic transfusion reactions.

Part II. Clinical diagnosis and bedside management

S.G. SANDLER AND N.D. ZANTEK

In Part I, Dr. R.R. Vassallo addressed laboratory assays for IgA and anti-IgA, their application to defining the incidence of IgA-related transfusion reactions, and the provision of IgA-deficient blood products. Part II will address the clinical diagnosis of IgA anaphylactic transfusion reactions and bedside management. The viewpoint of this review is that IgA anaphylactic transfusion reactions are serious but uncommon events that must be distinguished from other acute generalized reactions that occur more commonly, for example, transfusion-related acute lung injury (TRALI). Subsequent transfusions in persons with a history of an IgA anaphylactic transfusion reaction should be safe and uneventful using either washed RBCs or platelets or plasma from IgA-deficient donors. Clinical diagnosis of IgA anaphylactic transfusion reactions must be confirmed by laboratory testing for anti-IgA before unnecessarily committing a patient to a lifelong requirement for these specialized blood components if blood transfusion is needed in the future.

Definitions

Conventionally, IgA anaphylactic transfusion reactions are defined as anaphylaxis occurring within 1 hour of transfusing a plasma-containing blood product in a patient who is IgA deficient (< 0.05 mg/dL) and whose plasma contains anti-IgA.¹ In addition, there are case reports of persons with normal or low concentrations of IgA whose transfusion-related anaphylactoid reactions have been attributed to IgA antibodies of limited serologic specificity (i.e., IgA subclass antibodies). Published case reports of IgA anaphylactic transfusion reactions use the terms *anaphylaxis* and *anaphylactoid*, which require additional definitions, as follows.

Anaphylactic reactions

Anaphylaxis is a generalized, immediate, type I hypersensitivity reaction caused by IgE-mediated activation and degranulation of mast cells and basophils.² Typical reactions are unexpected, explosive, and catastrophic, and they may be fatal. Usually, signs and symptoms are cutaneous (hives), pulmonary (wheezing), cardiovascular (hypotension), and/or gastrointestinal (diarrhea). However, many different presentations have been reported (Table 1).

Table 1. Signs and symptoms of IgA anaphylactic reactions

Cutaneous:	Urticaria, flushing, hives, rash, angioedema
Respiratory:	Wheezing, dyspnea, bronchospasm, stridor
Cardiovascular:	Hypotension, shock, loss of consciousness, cardiac arrhythmia, syncope, cardiac arrest
Gastrointestinal:	Abdominal cramps, diarrhea, vomiting

Anaphylactoid reactions

An anaphylactoid reaction is clinically similar, but not mediated by IgE.² Since nearly all published case reports of IgA anaphylactic transfusion reactions have been confirmed by laboratory tests that do not specifically identify *IgE* anti-IgA, most reported cases are, technically, anaphylactoid reactions. In the first published report, Vyas and colleagues classified IgA transfusion reactions into two groups: (1) anaphylactic reactions associated with severe clinical symptoms, class-specific anti-IgA, and absence of plasma IgA and (2) anaphylactoid reactions associated with milder clinical reactions, anti-IgA of limited serologic reactivity, and normal plasma concentrations of IgA.³ Of the 40 case reports of IgA-related transfusion reactions that have been published in journal articles,

only eight are classified by their authors as anaphylactoid.¹ Authors of case reports for IgA transfusion reactions generally use the terms anaphylactic (versus anaphylactoid) to distinguish severe (versus milder) reactions or class-specific (IgA) (versus limited-specificity anti-IgA1 or -IgA2) antibodies. The conventional distinction between IgE and IgG or IgM anti-IgA is not apparent when reading published case reports of IgA anaphylaxis. Given this lack of precise definitions in published case reports, we will use the designation *IgA anaphylactic transfusion reactions* in this review to include all acute generalized reactions attributed to anti-IgA.

Differential Diagnosis

The first report of an IgA anaphylactic reaction was published in 1968, prior to recognition of several other acute generalized transfusion reactions (Table 2). TRALI, the most pertinent acute transfusion reaction to be distinguished from IgA anaphylaxis, was first recognized in 1985,⁴ i.e., after more than 75 percent (31/40) of the case reports of IgA anaphylaxis had already been published.¹ Other transfusion-related anaphylactic reactions that were recognized after most cases of IgA anaphylaxis were published include anti-Rga (1984),⁵ anti-Cha (1992),⁶ anaphylactic reactions to HLA-incompatible platelets (1993),⁷ and IgE-mediated anti-haptoglobin (2000).⁸ Passively transfused drugs (penicillin, aspirin)^{9,10} or drug antibodies (anti-cephalosporin)¹¹ have been reported to cause anaphylactic reactions. As the number of other potential causes for severe generalized transfusion reactions increases, it becomes apparent that some clinical diagnoses of IgA anaphylactic reactions, including early published case reports confirmed by weakly reacting IgA antibodies of limited specificity,

Table 2. Other transfusion-related acute generalized reactions that may be pertinent in the differential diagnosis of IgA anaphylaxis

ABO hemolytic reactions
TRALI ^{4,15}
Bacterial contamination/endotoxemia
Angiotensin-converting enzyme inhibitors ¹⁷
Circulatory overload ⁸
Anti-Rg ⁵
Anti-Ch ⁶
HLA-incompatible platelets ⁷
Anti-haptoglobin ⁸
Passively transfused allergens ⁹⁻¹¹
Coincidental exposure to allergens (food, medication, latex, others)

may have been misdiagnoses. This personal impression is supported by our experience testing blood samples from patients with suspected IgA anaphylactic reactions that were referred to the American Red Cross National Reference Laboratory. Of 359 samples only 65 (18.1%) contained an IgA antibody of any specificity.¹² Thus, 81.9 percent of 359 anaphylactic transfusion reactions that were suspected, clinically, to be anti-IgA anaphylactic transfusion reactions were, in fact, caused by other factors.

ABO hemolytic transfusion reactions

The differential diagnosis of an immediate systemic reaction in a person receiving a RBC transfusion should begin by excluding a possible ABO-related acute hemolytic transfusion reaction. Prompt diagnosis and aggressive management can reduce formerly high fatality rates of ABO hemolytic reactions to (only) 10 percent.¹³ A clerical check to verify positive identification of the blood sample used for compatibility testing, the patient, and the blood component should quickly resolve this issue. The absence of a pink or red color (reflecting hemoglobin monomers or dimers) in samples of the patient's posttransfusion urine or plasma excludes a diagnosis of an acute hemolytic transfusion reaction.

TRALI

TRALI is recognized with increasing frequency and is now the most frequent cause of fatal transfusion reactions reported to the U.S. Food and Drug Administration.¹⁴ Like IgA anaphylactic transfusion reactions, TRALI presents with predominantly pulmonary findings, e.g., shortness of breath, cyanosis, and hypoxia.^{14,15} In contrast to the predominance of upper airway signs (stridor, bronchospasm) in anaphylactic reactions, the clinical findings in TRALI are more typical of noncardiogenic pulmonary edema. Fever is present in most cases of TRALI, whereas fever is uncommon in IgA anaphylactic transfusion reactions.

Bacterial contamination and endotoxemia

The sudden onset of hypotension and shock during the first few minutes of a blood transfusion may raise the question of an IgA anaphylactic transfusion reaction. The presence of rigors and fever, sometimes over 40°C, should direct diagnostic efforts toward bacterial contamination and sepsis.¹⁶

Angiotensin-converting enzyme (ACE) inhibitors

Patients who are being treated with ACE inhibitors for hypertension or heart failure may develop "anaphylactoid" reactions (flushing and hypotension) during albumin infusions.¹⁷ The reaction is believed to result from the presence of prekallikrein activator in albumin products, which converts prekallikrein to bradykinin, a vasoactive peptide. ACE inhibitors cause the accumulation of bradykinin. The temporal association of an infusion of albumin, together with the absence of hypoxia or other pulmonary symptoms, points away from an IgA anaphylactic reaction.

Circulatory overload

An infant or elderly patient who develops acute hypoxia during a blood transfusion should be evaluated first for possible circulatory overload due to hypervolemia.¹⁸ Rales and other signs of pulmonary congestion exclude IgA anaphylaxis.

Coincident exposure to allergens

The visual image of a patient in anaphylaxis who is receiving a blood transfusion logically directs the differential diagnosis to the blood product. Nevertheless, that patient may be experiencing a coincidental acute IgE- (or non-IgE-) mediated reaction to medication, food, or exercise.² Latex gloves and certain other latex-containing hospital appliances are recognized causes of anaphylactic reactions.² However, awareness of the risk that latex presents to patients and hospital personnel has greatly reduced the presence of latex-containing products in patient care areas.

Bedside Management

There are two principal aspects of the management of patients with IgA anaphylactic reactions: (1) treatment of the acute clinical event and (2) selection of blood products for subsequent transfusions.

Treating IgA anaphylactic transfusion reactions

Effective management of an IgA anaphylactic transfusion reaction begins, proactively, by establishing a hospital policy that ensures all transfusions will be administered in clinical areas where appropriate medications, equipment ("crash cart"), and trained personnel are readily available. A detailed list of medications and equipment may be found in standard textbooks.² Minimum equipment includes an oxygen source and masks, needles and tubing for intravenous fluids, defibrillator, suction apparatus, laryngoscope,

endotracheal tubes, nonlatex gloves, and an Ambu-bag. The medications should include epinephrine (aqueous, 1:1000), injectable diphenhydramine, injectable corticosteroids, dopamine, and atropine. Specific treatment depends on the prominence of individual clinical findings. First, the blood transfusion must be discontinued and intravenous access maintained with 0.9% sodium chloride for administration of parenteral medications. For shortness of breath, oxygen via a face mask should be started and a decision made whether or not intubation (endotracheal tube) is needed. Epinephrine is the most effective medication for relieving the acute symptoms of anaphylaxis. The exact dose and route depend on the patient's circulatory status. Often anaphylaxis has a biphasic course, i.e., after the initial episode has resolved following treatment there is a recurrence of acute symptoms requiring re-treatment within the next 2 hours. Depending on the severity of the initial reaction, patients should be observed in an intensive care setting for 2 to 24 hours after resolution of all symptoms.

Subsequent transfusions: IgA anaphylaxis suspected, but not confirmed

The above scenario describes an acute generalized reaction which occurred during a blood transfusion that may (or may not) have been an IgA anaphylactic transfusion reaction. If additional transfusions are required in such a situation, they may be needed before laboratory testing for anti-IgA can be completed. A similar situation occurs when a patient needing an urgent blood transfusion gives a medical history of a prior transfusion-related anaphylactic reaction, but testing for anti-IgA was not performed or the results are not available. In this situation, the safest blood components are washed red blood cells¹⁹ and platelets or plasma from known IgA-deficient donors.²⁰ If a platelet transfusion is required before an IgA-deficient component can be supplied, platelets may be washed, although considerable skill and experience is required to avoid platelet clumping, activation, and loss of platelets.¹⁹ If FFP is urgently required before IgA-deficient components can be supplied, withholding transfusions may create a greater risk to the patient than initiating a slow transfusion of standard FFP in a carefully monitored nursing unit. Since anti-IgA was detected in only 18.1 percent of cases of suspected IgA anaphylaxis (see above), there is a reasonable possibility that anti-IgA is not present and subsequent

transfusions will be uneventful. While such an approach is not without risk, we are unaware of any published case report describing a fatality that was directly caused by an IgA anaphylactic transfusion reaction.^{1,21,22} The only published report of a fatal IgA anaphylactic transfusion reaction that we have been able to locate is that of a 57-year-old man with a normal concentration of plasma IgA, a low titer anti-IgA of limited serologic specificity (titer = 64), and an autopsy report of hemorrhagic pulmonary edema, focal severe coronary artery disease of the left anterior descending artery, and focal early ischemic change in the anterior wall of the left ventricle.¹⁰

Subsequent transfusions: prior IgA anaphylactic reaction confirmed by anti-IgA

The management of a patient whose history of one or more anaphylactic transfusion reactions has been confirmed by a test for plasma anti-IgA is straightforward in principle and difficult in practice. In principle, subsequent transfusions of blood products that are deficient in IgA should be safe and uneventful. In practice, such blood products are not readily available in hospital transfusion services or in most blood centers. If plasma or platelets are needed, and if time allows, the first and safest strategy should be an effort to locate IgA-deficient components via the community blood center. Certain community blood centers have lists of donors who have been tested and confirmed to be IgA-deficient and, using emergency release procedures, the centers may collect and supply such components in an urgent situation. In addition, lists of IgA-deficient blood donors have been established in the United States, Canada, Australia, and certain countries in Europe and Asia.^{20,23} In the United States, IgA-deficient blood components may be requested via the American Rare Donor Program, Philadelphia, Pennsylvania (215-451-4900; 24 hours/7days). A low-content IgA IVIG product is available in the United States (Gammagard S/D IGIV 5%; Baxter Healthcare Corporation, Glendale, CA). Standard RBCs may be transfused after automated washing to remove residual IgA-containing plasma.¹⁹ Of course, any RBCs that become available if whole blood is collected from IgA-deficient donors for plasma or platelets may be transfused without washing.

Lastly, if severe bleeding with a coagulopathy occurs in a patient with IgA deficiency and anti-IgA, and platelets or FFP from an IgA-deficient donor is not readily available, an “off-label” infusion of recombinant

activated coagulation factor VII (rFVIIa; NovoSeven, Novo Nordisk, Bagsvaerd, Denmark) may be a reasonable alternative. Case reports describe efficacy of infusions of rFVIIa in certain systemic bleeding disorders where platelets²⁴ or FFP²⁵ would be indicated. While none of these case reports relate to IgA-deficient patients specifically, rFVIIa does not contain IgA and, therefore, should not precipitate a recurrent anaphylactic reaction.

Summary

Numerous published case reports of IgA-related anaphylactic transfusion reactions (1968–present) alert us that certain IgA-deficient persons are at risk for acute generalized transfusion reactions, presenting with severe shortness of breath. However, such IgA anaphylactic transfusion reactions are uncommon, and the majority of clinical diagnoses are not confirmed by detection of anti-IgA in the patient’s plasma. The recognition of other transfusion-related acute generalized reactions in recent years requires expanding the differential diagnosis for such events to include other possible diagnoses, most importantly TRALI. Clinical diagnoses of IgA anaphylactic transfusion reactions should be followed up promptly by testing for anti-IgA. Failure to follow up a clinical diagnosis by testing for anti-IgA will complicate management of any future blood transfusions and potentially commit the patient to a lifelong requirement for highly specialized IgA-deficient blood products.

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S. Gerald Sandler, MD, Director, Transfusion Medicine (corresponding author); and Nicole Dodge Zantek, MD, PhD, Departments of Pathology and Laboratory Medicine, Georgetown University Hospital, 3800 Reservoir Road, NW, Washington, DC, 20007.

Review: American Rare Donor Program

C. FLICKINGER, T. PETRONE, AND A. CHURCH

In the early 1960s, both the American Red Cross (ARC) and the AABB realized the need for organizing rare donor information in an effort to meet the needs of patients with antibodies to high-frequency antigens or with multiple common antibodies. Both organizations began rare donor databases: the ARC Rare Donor Registry and the AABB Rare Donor File. In the mid 1990s, a task force was formed to combine efforts and merge the two programs. On November 1, 1998, the American Rare Donor Program (ARDP) was formed. The associated computer database is maintained by the ARC and is accessible by the ARDP staff at the Philadelphia site. Both the AABB and the ARC provide funding for the program. Currently, there are 80 ARDP members and more than 34,000 active rare donors in the database.

Membership Requirements

Membership in the ARDP is associated with obtaining and maintaining immunohematology reference laboratory (IRL) accreditation by the AABB or meeting IRL accreditation criteria defined by the ARC. In addition, facilities that have been evaluated and approved for pending AABB IRL accreditation status are also granted ARDP membership during the 2-year accreditation process.

Once ARDP membership is attained, members may submit donors for entry in the database as well as request searches for rare products for their customers or patients. In addition, members are responsible for supporting ARDP activities by meeting one of the defined criteria on an annual basis. These criteria include registering at least ten new rare donors, screening at least 1000 donors for high-frequency antigens, performing a potentially productive family study, providing antisera to another ARDP facility for use in screening for high- or low-frequency antigens, and shipping at least 15 rare products to other facilities in response to ARDP patient requests. Other member responsibilities include implementing a process to

ensure that the facility can respond to ARDP requests on a 24 hour/7 day per week basis as well as serving as a "portal" to the ARDP for nonmember facilities that request rare blood products. This enables nonmember facilities to access ARDP resources to procure rare blood products for their patients, thus making the ARDP accessible to everyone in need. It is the responsibility of the ARDP member, acting as the portal, to confirm the specificity of the alloantibody(ies) reported by the nonmember facility, either by performing testing on a submitted sample or by reviewing worksheets from the investigation that identified the antibody(ies).

Rare Donor Submission and Maintenance

Rare donor submission to the ARDP requires that the donors meet rare criteria, defined as being high-frequency-antigen-negative, multiple-common-antigen-negative, or IgA deficient. Donors submitted as high-frequency-antigen-negative may be of any ABO blood group and negative for a high-frequency antigen occurring in fewer than 1 in 1000 random donors. Donors entered as multiple-common-antigen-negative must be group O or group A and must meet one of the following sets of defined antigen-negative criteria:

Set 1: R_1, R_2, R_0 or rr , AND $K:-1$, AND $S-$ or $s-$, AND $Fy(a-)$ or $Fy(b-)$, AND $Jk(a-)$ or $Jk(b-)$

Set 2: R_1, R_2 or rr , AND $K:-1$, AND $Fy(a-b-)$

All antigens submitted as negative must be confirmed with at least two sources of antisera. Donors submitted as IgA deficient must have documentation of IgA concentrations < 0.05 mg/dL determined on two separate samples submitted to the ARDP for IgA testing. Copies of the ARDP IgA test results must accompany the donor submission form. Upon request, the ARDP will perform confirmatory testing for high-frequency-antigen testing and perform IgA donor testing at no charge for ARDP member facilities.

Members mail submission forms with donor antigen testing, along with either complete or partial donor demographics, to the ARDP office staff for verification of rare status and entry into the database. Once the data have been entered, a donor packet is generated. For member facilities submitting complete donor demographics, this packet consists of a phenotype card, a letter explaining the entry of the donor into the ARDP and outlining the importance of this donor's blood products to patients in need, as well as an ARDP informational pamphlet. For member facilities submitting partial demographics, only the ARDP informational pamphlet is provided. These materials are forwarded to the submitting member facility for distribution to the donor.

Once the donor has been registered, it is important that current donor demographics be maintained so that effective donor recruitment can be conducted. Donor demographic information is updated semiannually, either through an address-update card sent by the ARDP office staff or by a facility-specific practice. Information from this address-update card or from a member facility may serve to deactivate or delete a donor from the database. Monthly donor activity reports with details of donor record changes are issued to all member facilities.

Requests for Products

Requests for both nonrare and rare RBC products, as well as for IgA-deficient plasma products, may be made by member facilities, although each type of request is handled differently. A request for nonrare blood products may be submitted after a facility's contacts with other institutions have been non-productive. This request is faxed to the ARDP office staff, who broadcast the fax to ARDP member facilities. No further action is taken by the ARDP staff. Facilities holding the desired blood products contact the requesting member facility directly.

A request may be made for blood products that are considered rare due to the product's lack of a high-frequency antigen and/or lack of multiple common antigens. Although these two types of requests are handled in the same manner, determining the *in vivo* significance of an antibody to a high-frequency antigen may greatly facilitate the search for available products. A patient's sample can be tested by the monocyte monolayer assay (MMA) to predict the antibody's ability to cause an overt transfusion reaction. If the MMA test result indicates that the antibody is currently

not stimulating monocyte activity, there is a high likelihood that antigen-positive RBC products can be transfused safely. This result can modify the phenotype requested and simplify the product search, at least for the current transfusion episode. Although it is advantageous to have this information prior to requesting products, MMA testing is often not undertaken until a product search is under way. Regardless, it provides valuable information that can expedite the search process.

A request for rare blood products cannot be faxed but must be verbally conveyed to the ARDP staff. Once patient and facility contact information is recorded, the ARDP staff initiates a donor search in the database according to the ABO, Rh, and phenotype desired. The computer program produces a list of member facilities with donors who match the ABO, Rh, and phenotype desired. ARDP staff then place phone calls to these facilities to determine product availability. If products are found, ARDP staff convey the information regarding the patient and the requesting facility to the shipping facility. Shipping and billing arrangements are coordinated between the shipping and receiving facilities. If products are not found through the initial phone process or if no donors of the desired phenotype are registered in the database, a broadcast fax is sent to all member facilities. The intent of this fax is to identify available products from donors not registered in the ARDP database or products that have been imported but not used for the designated patient. Facilities holding desired products are instructed to call the ARDP staff to obtain shipping information and to ensure proper documentation of the transaction. Communication between ARDP staff and the requesting facility is paramount in keeping the requesting facility updated about the ARDP search. If the broadcast fax elicits no response, a follow-up process is initiated by the ARDP manager or associate. This involves a phone call to the requesting facility, notifying them of the fax response and ascertaining the patient's transfusion status. Should the transfusion need still exist, a discussion of alternative immediate steps begins: assessing the availability of autologous donations; searching for and testing patient siblings; submitting a patient sample for MMA testing, if not already performed; or investigating alternative methods of treatment, such as unlicensed, research hemoglobin solutions for compassionate use. If these steps are not possible or prove unproductive, the ARDP manager or associate can request the recruitment of member

facilities' donors, if any can be identified. When this is unsuccessful, an international search can be performed.

The request for IgA-deficient-plasma products requires confirmation that the patient has anti-IgA in his or her serum. This confirmation may be documented by anti-IgA assay results or by submitting a sample to the ARDP for testing. However, if a clinician strongly suspects that a patient has anti-IgA and an urgent patient need for plasma products disallows testing completion, IgA-deficient plasma products can be released with the authorization of the medical directors who oversee the ARDP.

International Search

Several criteria mandated by the FDA affect the initiation of an international search, the first of which is assurance that there are no available blood products of the desired phenotype in the United States. Typically, this criterion is met through taking the above-stated steps as well as contacting non-ARDP blood centers. The second criterion centers around the fact that imported blood products are considered "unlicensed" by the FDA, since they may not meet current FDA testing criteria. It is important that both the physician and the patient understand the nature of these "unlicensed" products. A physician-signed letter attesting to the intent to transfuse and to the understanding of the "unlicensed" nature of the imported products is required. Another criterion is that the receiving facility must have a procedure in place to ensure that the imported products are only transfused to the designated patient. They must not be placed into the general inventory for routine use. It is the responsibility of the ARDP manager or associate to ensure that all of these criteria are met before moving forward with the international search. At that point, the ARDP contacts international locations for available products. If products are found, the ARDP coordinates the shipment of the products and provides appropriate follow-up. Again, communication is critical in ensuring that this process is expedient and effective in obtaining the desired blood products for the patient.

Considerations in the Fulfillment of ARDP Requests

There are several considerations in the fulfillment of requests made to the ARDP for rare blood products. The most obvious is that these products are by definition rare, meaning that only 1 in 1000 donated

units of blood will match the phenotype needed. Theoretically, this means 4000 units would need to be screened to find four units of blood to fill a request. More difficult yet are ARDP requests for very rare products: those requests in which only 1 in 10,000 units of donated blood will fill the request.

In addition to a request for RBC products requiring a rare antigen type, some of the patients for whom these requests are made may have developed antibodies to one or more of the more common antigens found on RBCs. These requests further restrict the ARDP search to those RBC products that also lack the more common antigen(s) for the corresponding antibody(ies) present. It is easy to see how this situation may cause complexity and limit the number of donor products able to fulfill the request.

Effectiveness of the Process

The activity of the ARDP is reflected in Tables 1 and 2. As seen in Table 1, submission of new rare donors is on the decline. If 2004 activity continues at the current rate, only about 2400 new donors will be entered into the database this year. However, since donor submission is a requirement of membership, this number may actually increase as the year closes, reflecting members' efforts to meet their membership obligations. More importantly, however, the number of active donors is increasing, indicating a greater potential to fulfill patients' needs. Interestingly, while the number of requests is growing steadily, the number of shipped products is declining, indicating that the increase in active donors may not necessarily translate into more available products or into products of the desired phenotype.

Table 1. General ARDP activity

ARDP activity	2004	2003	2004 8 mos/12 mos*
New donors registered	3164	3024	1588/2382*
Total active donors	31,983	33,002	34,496
Requests made to ARDP	578	670	441/662*
Phone calls placed to fill orders	3086	3172	2092
Units shipped	1739	1536	995/1492*

*Projected values based upon the 8-month data

The ability of the process to meet a patient's needs is reflected in Table 2. The initial phone search results in fulfillment of approximately 72 percent of patient requests. This figure has remained fairly constant.

Table 2. Patient request fill rate

Total requests	2002 580	2003 670	2004 (8 mos) 441
● Requests completely filled by phone search	415 (71%)	491 (73%)	317 (72%)
● Requests not completely or partially filled by phone search (fax sent)	165 (29%)	179 (27%)	124 (28%)
● Requests completely filled by fax	95	83	52
• Percent of total requests	16%	12%	12%
• Percent of fax requests	29%	46%	42%
Requests completely filled by phone/fax	510 (88%)	574 (86%)	369 (84%)
Requests completely or partially unfilled	70 (12%)	96 (14%)	72 (16%)
● Requests partially filled by fax	24	27	12
• Percent of total requests	4%	4%	3%
• Percent of fax requests	15%	15%	10%
● Requests unfilled by fax	46	69	60
• Percent of total requests	8%	10%	14%
• Percent of fax requests	28%	39%	48%

However, a decline is apparent in the number of requests filled through the extended or fax search for products. Although the percentage of faxed orders has remained constant, the percentage of partially filled or unfilled requests is slowly increasing, indicating the unavailability of rare RBC products.

In addition, certain phenotypes are constantly in demand and frequently unavailable, as indicated in Table 3. Typically, those phenotypes characteristic of the African American population are the most sought after and the hardest to find. The chronic need for RBC products for patients with sickle cell anemia and the relatively lower donor rate amongst the African American community exposes these patients to high-frequency antigens from Caucasian populations; namely, U, e determinants (hr^b and hr^s), and Js^b, as well as to many of the common antigens.¹ This exposure

Table 3. Completely or partially unfilled requests by phenotype

Requests	2004	2003	2004 (8 mos)
Total requests completely or partially unfilled	70	96	72
U-*	14 (20%)	20 (21%)	12 (17%)
hr ^s -*/hr ^b -*	7 (10%)	13 (14%)	8 (11%)
Di(b-)*	3 (4%)	13 (14%)	5 (7%)
Vel-*	5 (7%)	3 (3%)	3 (4%)
Kp(b-)*	4 (6%)	2 (2%)	2 (3%)
Js(b-)*	0	3 (3%)	5 (7%)
Yt(a-)*	4 (6%)	1 (1%)	2 (3%)

*Antigen alone or in combination with other common antigens

results in this patient population often making multiple clinically significant alloantibodies. The search for products for these patients is always difficult and demonstrates the need for more African American donors and for better methods of screening.

In addition to submitting donors to fill rare RBC requests, it is also vital to have an abundant resource of IgA-deficient donors for patients requiring plasma products. Currently, there are just over 100 registered IgA-deficient donors in the database and only one member facility performing screening for these donors. With these small numbers, it is apparent why these products are so closely monitored by the ARDP staff to ensure that only patients truly requiring them receive these resources.

Searching for Rare Donors

Facilities attempt to identify rare donors by performing mass antigen screening of donated RBC products. Mass screening allows new donors with rare phenotypes to be identified. Antigen screening on a mass scale typically means that one or two specific antigens are tested in large batches. These batches sometimes test for antigens that, if absent from the RBC surface, qualify the product as rare. Other times, the lack of a single antigen is not rare, but the lack of a combination of antigens qualifies the product as rare. In these instances, the initial batched test result may prompt the facility to test the donated unit for lack of additional antigens that may constitute a rare combination. If testing reveals that a product lacks a high-frequency antigen or a rare combination, the facility has identified a new rare donor.

The most important resource in the screening process is personnel; therefore, restrictions in antigen screening tend to be related to staffing. A testing facility needs an adequate number of staff to devote time to the performance of mass antigen screening. The most productive screening tends also to be proactive rather than "just in time" to fill urgent requests. Staff shortages in laboratories limit the amount of time that can be dedicated to mass antigen screening activities. Unfortunately, proactive screening can be one of the first tasks omitted during times of high work volume or staffing shortages.

The Indiana/Ohio Region of the American Red Cross has a unique and fortunate opportunity in that a retired medical technologist volunteers at its facility. This volunteer has been trained to perform antigen screening. By providing a few hours of his time twice

a week, he is able to screen almost 2½ days' worth of blood donations for two common antigens. Utilizing this volunteer's services has enabled the facility to support the blood needs of its customers' sickle cell programs. Procuring enough antigen-negative RBC products to meet the needs of the sickle cell patient population in this facility is an enormous achievement, since this facility has comparatively few African American donors relative to the number of patients requiring RBC products. To assist in the screening process, staff members sequester samples from the blood units collected from self-identified African American donors. In addition to the antigen testing performed by the volunteer, staff also perform antigen screening on these sequestered samples as workload permits. Through these continued efforts, the facility was able to submit 12 new donors to the ARDP database in the month of February 2004 alone, while simultaneously supporting its customers' needs for antigen-negative RBC products for their sickle cell programs.

"Proactive antigen screening of red blood cells can really pay off," according to Letitia Price, Assistant Director of the Reference Laboratory at the Community Blood Centers (CBC) of South Florida, Inc. This laboratory screens at least 100 new donors per week. Recognition of the rare donor is a critical factor in the ongoing fulfillment of requests for rare blood products. Ms. Price further states that "we send a mailing to our rare donors on every holiday and they get a rare donor T-shirt when they are first identified." "In addition," she continues, "we will actually call our donors who lack a high-incidence antigen and wish them a good holiday." The CBC of South Florida exported 435 rare units to other members of the ARDP while only importing two rare units during 2003. This was attributed to their aggressive screening process and donor retention efforts.

The newest endeavor within the Indiana/Ohio region of the ARC is to utilize staff from another department to perform antigen screening during downtime. A staff member is being trained to perform capillary testing which will allow screening for high-frequency antigens. This strategy was again dictated by the needs of the facility's customers. Because the

facility tests patients who have developed antibodies to high-frequency antigens, the laboratory supervisor, Peggy Ball, states "I know we have people out there who are negative for these high-frequency antigens, we just need to find them."

Automated methods are available for mass screening. Gel technology, for example, uses significantly fewer donor RBCs and less reagents per test. Automated testing can also reduce technologists' workload and, in theory, reduce error rates as well. The unfortunate dilemma in the use of automated methods for antigen screening is the cost of equipment, supplies, and maintenance, and the validation of such equipment. These costs may prohibit a facility from using this resource, even though it is available.

Although the ARDP specifically deals with rare donors and rare patient requests as defined in the criteria, the rarest blood product is truly that product that is unavailable when needed. This fact stresses the need for continual and aggressive donor recruitment throughout the United States. It is through the dedicated efforts of donors, technologists, volunteers, office staff, doctors, nurses, international contacts, couriers, and even airport customs staff, that the blood supply is maintained and the ARDP can be effective. All of these individuals work toward one common goal—to provide safe blood products to patients in need, since together, we can save a life!

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Cynthia Flickinger, MT(ASCP)SBB, Tammy Petrone, MT(ASCP), and Ann Church, MT(ASCP)SBB, American Red Cross Blood Services, Penn-Jersey Region, 700 Spring Garden Street, Philadelphia, PA 19123-3594.

A review of the ISBT rare blood donor program

G. WOODFIELD, J. POOLE, S.T. NANCE, AND G. DANIELS

One of the challenges in transfusion medicine is to provide compatible blood for patients who are negative for a high-frequency blood group antigen and who have an alloantibody against the antigen. Another category of patients for whom compatible blood is often difficult to find is those with a combination of alloantibodies, as donors negative for the various antigens can be difficult to locate.

Sooner or later, most blood transfusion services come up against these situations and require rare blood units. Numerous procedures are commonly used to define the situation more clearly. Additional phenotyping of the patient is performed, use is made of special RBC identification panels, elution studies are often required, and random or selected units of blood may be tested to find compatible blood. Family members may be typed and matched, and autologous transfusion may be considered, as well as the use of Hb substitutes and hematinics. It can be a very challenging time for the immunohematology laboratory, particularly if a delay in transfusion is likely to be life threatening.

On these rare occasions, the availability of a well-developed national or regional blood group reference laboratory is helpful. Even then, additional international help may be required, as not all national laboratories have available the extensive range of techniques, reference RBCs, and antisera to aid in final diagnosis and confirmation of antibody identity. When identification is made, it may not be possible to find donors with the appropriate blood type from the resources of that particular country.

To assist in this scenario, the ISBT established an international program aimed at providing a system that can find compatible rare blood donors worldwide.¹ This system has evolved and been developed since the 1960s and has been used extensively in many urgent situations.

Main Aspects of the Rare Blood Program

(1) The international panel of donors of rare blood type

The need for a rare donor registry was noted at an early stage of blood transfusion development. By 1959, the AABB had developed the rare donor file, and the American Red Cross, the rare donor registry.² About the same time, a similar panel was formed in France to meet local needs.³

There was a clear requirement for a more international panel of donors, and in 1965 the ISBT established an international donor panel (IDP) of 300 donors from 10 centers.¹ The first lists of donors were circulated in 1968⁴ to all World Health Organization (WHO) national blood group reference laboratories as well as to other contributing centers. By 1985, the IDP had grown to 1500 donors and paper lists were sent to 110 centers throughout the world. At present, the IDP contains details on 4000 donors derived from 24 countries⁵ and may be accessed by authorized users via the Web site <http://www.bloodnet.nbs.nhs.uk/ibgrl/>. The lists continue to be compiled and maintained by the IBGRL in Bristol, UK.

(2) The IBGRL

The UK blood group reference laboratory was formed in 1946 for the National Blood Transfusion Service. In 1953, it gained WHO recognition for its RBC reference work and became known as the IBGRL. It was initially established in London, but since 1990 it has been in Bristol as part of the Bristol Institute for Transfusion Sciences within the National Blood Service. Since 1987, it has been under the direction of Professor David Anstee. Ms. Joyce Poole is the reference manager and has responsibility for compiling and maintaining the IDP. In 2001, Dr. Geoff Daniels became head of IBGRL Molecular Diagnostics. It

provides reference services not only to the UK but also, under the auspices of WHO, to blood services worldwide, and works closely with other blood group reference laboratories.

(3) The ISBT Working Party on Rare Blood Donors

A further development was the formation by the ISBT of a Working Party on Rare Blood Donors (WPRBD) in 1986. This was first chaired by Ms. Delores Mallory from the United States, and from 1996 by Dr. Graeme Woodfield from New Zealand. The objectives of the WPRBD included developing guidelines on all matters related to the use, labelling, testing, and transport of rare blood, providing a resource for ongoing information on this topic, communicating with the WHO IBGRL, and upgrading international information on rare blood programs.^{6,7}

This group now has a membership of 19 individuals drawn from all the major continents. It meets at ISBT congresses as well as having frequent contact by electronic media. It has been active in drawing up definitive guidelines for the handling of rare blood,⁸ and the latest version of these is in preparation.

In addition, it has arranged many lectures and seminars on various aspects of rare blood at both national and International meetings, particularly those of the ISBT. This has increased the knowledge regarding the problems of rare blood supply for many countries. The result has been increased contributions to the IDP and more clinical requests for serologic investigations. There has been renewed interest in the subject, with countries such as China now embarking on a major program centered on rare blood matters.⁹

The WPRBD has also carried out two international surveys of rare blood usage, the last being in 1999.¹⁰ Useful information has been obtained. It became apparent that numerous countries do not have blood transfusion systems that are able to utilize the benefits of the rare blood program. Many others were unaware of the program. A wide range of problems was uncovered, including matters related to transportation, customs clearance, and reimbursement. Each of these matters has been or is being considered by the WPRBD and incorporated into a reviewed guideline document.

At present, a number of other matters are also under active discussion, including the possible requirement for an international inventory of frozen blood units, the need for an international donor card, the types of available boxes for blood transport, and legal matters.

(4) National Rare Blood Centers

Over the years, numerous countries in Europe, America, and Asia developed extensive national programs for the supply of rare blood. Notable among these were the programs of France, The Netherlands, Germany, Australia, Japan, and South Africa. Such programs are often supported by the development of frozen rare RBC banks. Within the UK there is a National Frozen Blood Bank which is housed at the NBS Birmingham Center.

In the United States, the American Red Cross National Reference Laboratory for Blood Group Serology in Philadelphia, Pennsylvania, manages the American Rare Donor Program (ARDP), in joint partnership with the AABB. The ARDP coordinates the provision of blood negative for high-frequency antigens as well as rare multiple-antigen-negative blood for the 70 ARDP member facilities across the USA. This program has more than 35,000 active donors. There is excellent cooperation with the IBGRL.

Korea, Singapore, and Malaysia have rare donor registries. Japan developed an innovative rare blood-screening program that resulted in the discovery of many rare donors of considerable international value.¹¹ These donors have recently become available on the IDP.

In Australia and New Zealand there are good facilities. South Africa has made significant contributions to international programs for rare blood. In South America a recent review indicated that only a few countries had adequate facilities for the provision of rare blood.¹²

(5) Frozen blood

There is no international system that coordinates the stocks of nationally stored frozen blood. Most frozen blood is retained for national use, as international transport between countries more than a few hours apart is difficult. This is because the donor unit usually needs to be thawed in the donor blood center, imposing a limited time within which the units of blood must be transfused. There are also difficulties in transporting low-glycerol, liquid nitrogen-stored units, although there is less problem with high-glycerol-stored units, as these can be sent using solid carbon dioxide as the cooling medium. If the receiving center has the capacity to store and thaw frozen RBC units, this can make the use of frozen blood efficient.

New methods of sterile thawing of frozen RBC units are becoming available and these may prove

useful in extending the shelf life of frozen/thawed RBCs.¹³ If these methods come into common use, the role of frozen blood in managing patients internationally may increase. There is no doubt that if frozen blood can be rapidly available for clinical use, this would be very helpful, as there is invariably a time lag before blood from a distant donor can be collected, transported, and transfused to a patient.

What is the Way in the Future for the Rare Donor Blood Program?

It is clear that there will be a continued need for the provision of this type of specialized service. As more countries develop their blood service technology, identification of increased numbers of clinical problems will occur, requiring advanced serologic assistance. This will result in the need for rare blood units to be supplied on an international basis. The present migrations of people throughout the world and mingling of different populations may well result in difficult serologic problems arising. This phenomenon is at present seen in many western countries that need to deal with the complex transfusion management of diseases that were once seen only in tropical countries. Good examples of this type of activity are seen with the serologic problems of transfusion in sickle cell disease and thalassemia.

Although many serologic problems can be investigated and determined at the local or national level, it is useful to have available the resources of an advanced serologic laboratory for final elucidation and confirmation. The IBGRL can carry out such advanced serologic work, as it has the necessary staff and experience as well as the local support of other specialized laboratories. There is also close contact and cooperation with scientists working in other countries. With the use of the IDP, the IBGRL can advise enquirers where rare blood of the appropriate types can be found.

It is possible that more careful use of transfusion will result in a decrease in the rate of RBC antigen immunization in certain selected patient groups. It has been demonstrated, for instance, that the use of blood donations from similar racial groups for patients with sickle cell disease has an appreciable effect on the need for rare blood donations.¹⁴ In the United States, requests for rare products for sickle cell patients make up at least 20 percent of the requests for rare blood to the ARDP.¹⁵ This has prompted establishment of donor collection programs targeted to peoples descended from Africa. Preliminary reports show decreased

alloimmunization of patients if blood from African blacks, matched for C, E, and K, is used for all transfusions. More stringent criteria for the clinical use of blood may also result in less immunizations taking place and reduce need for rare blood units. New therapeutic modalities such as hydroxyurea may eliminate the need for future blood transfusions in children with beta-thalassemia major.¹⁶

New developments possibly mean that the use of rare blood may not be required in some clinical situations. If Hb solutions are eventually made available, these could prove useful in some lifesaving situations where the only alternative therapy would have been rare blood units. Another potential therapy may be the transfusion of PEG-treated cells that seem able to largely escape the immune reaction caused by blood group antibodies.¹⁷ However, the suitability of this technology has been put in doubt by the recent identification of antibodies to RBCs suspended in PEG in up to 25 percent of donors.^{18,19}

Molecular genetics will facilitate the screening for suitable blood donors and make the determination of blood group phenotypes even more reliable.²⁰ The genes for all 29 blood group systems have now been cloned and the molecular bases for all the clinically significant polymorphisms are known. Currently, there are many research teams working on the development of high-throughput methods for determining multiple blood group genotypes from genomic DNA. Such methods might involve ELISA detection of PCR amplified products in microtiter plates,²¹ though more advanced techniques could exploit DNA microarray technology, providing the opportunity to produce vast quantities of data. These technologies, when fully automated, will make it possible to screen large numbers of donors for all clinically significant polymorphisms, so that a vast database of fully genotyped donors will become available. This would be invaluable for treatment of transfusion-dependent patients, either to provide compatible blood for those who have blood group antibodies or to provide matched blood to prevent antibody production.

Development of molecular techniques to screen for donors lacking antigens of very high frequency presents a greater challenge. The genes for some antigens of high frequency, such as Vel, Lan, At^a, and Jr^a, are still to be cloned. In addition, null phenotypes, such as Rh_{null} and K₀ (Kell-null), have a plethora of genetic backgrounds, with a different mutation found for almost every case examined.²²

Looking much further into the future, it might become possible to culture therapeutic quantities of erythroid cells from CD34⁺ progenitors. Consequently, for patients requiring regular transfusion, a continuous culture could be maintained, either of their own RBCs or of those from a closely matched donor.

It is clear that the IDP needs to be retained and expanded. Attrition through illness, death, and retirement means that there is always a need for new rare donors. Although some of these may be found from studies of specific families with rare groups, there is still a requirement for well-developed blood centers to set up screening programs for certain categories of donors and particularly those blood groups that are internationally in short supply. Countries with national rare donor lists should try to place their donors wherever possible on the IDP, as this does not preclude local or national use of the donation. An interesting study from Germany²³ demonstrated that despite progress in this area of rare blood supply, about one-third of hospitalized patients with antibodies to high-frequency antigens had unsatisfactory transfusion support and that increasing the supply of just four types of blood would solve many of the problems. Even in the United States, similar shortages of rare donor products have been noted,^{24,25} again emphasizing the need for expanded searches for rare donor phenotypes.

In the scientific advances being made, the blood donor with a rare phenotype should not be overlooked. A recent meeting in Israel to honor and show appreciation to rare blood donors of that country²⁶ was well received and is a model that could well be copied in other countries. Such events generate good publicity for the donor service but also act as an encouragement to individual blood donors. It is anticipated that a future World Blood Donor Day may be at least partially focused on rare blood donors and the contribution they make to the saving of life.

In conclusion, the rare blood donor program of the ISBT has been able to meet many of the needs for rare blood throughout the world for the last 40 years. However, it will still require continued support in the future to enable it to expand and develop the good work already done. This will require international cooperation, particularly in the screening for rare blood types and the selection and care of rare blood donors. Although new advances may in the future make the need for rare blood less urgent, these are not yet available, and so the need for the present rare donor program will remain for the foreseeable future.

Addendum

Current members of the ISBT Working Party on Rare Blood Donors are:

David Anstee (United Kingdom), Willie Flegel (Germany), Sanmukh Joshi (Sultanate of Oman), Piere-Yves Le Pennec (France), Cyril Levene (Israel), Marie Lin (Taiwan), Sandra Nance (United States), Marcia Novaretti (Brazil), Marijke Overbeeke (Holland), Joyce Poole (United Kingdom), Marion Reid (United States), Elizabeth Smart (South Africa), Silvano Wendel (Brazil), Graeme Woodfield (New Zealand) (Chairman), Vered Yaholm (Israel), Tani Yoshihiko (Japan), Ziyang Zhu (China), Christine Lomas-Francis (United States), and Francine Noizat-Pirenne (France). The IDP can be reached at www.bloodnet.nhs.uk/ibgrl.

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Graeme Woodfield, MD, PhD, Chairman, ISBT Working Party on Rare Blood Donors, Department of Molecular Medicine and Pathology, Private Bag 92019, University of Auckland, New Zealand; Joyce Poole, FIBMS, Reference Manager, IBGRL, National Blood Service, Southmead Road, Bristol BS10 5ND, UK; Sandra Taddie Nance, MS, MT(ASCP)SBB, Director, National Reference Laboratory for Blood Group Serology, ARC, Penn-Jersey Region, 700 Spring Garden Street, Philadelphia, PA 19123, and Geoff Daniels, PhD, Head of Molecular Diagnostics, IBGRL, Bristol, UK.

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The gene encoding the I blood group antigen: review of an I for an eye

M.E. REID

Unlike most blood group antigen pairs, the I and i antigens are not antithetical (produced by allelic pairs) but, rather, they are reciprocal. The I antigen is formed by the action of an enzyme (a glycosyltransferase), which adds branches onto the i antigen. Thus, branched I antigen is formed at the expense of its precursor, the linear i antigen. The antigens are present on all blood cells and have a wide tissue distribution. Soluble I antigen is found in milk, saliva, and amniotic fluid, and a small amount is in plasma. The function of these antigens is unknown but the I antigen has a decreased expression and the i antigen has a concomitant increased expression in conditions that result in increased hematopoiesis. The gene encoding the branching transferase has been cloned and sequenced, and the mechanism underlying the i adult phenotype with and without association with cataracts has been elucidated. *Immunohematology* 2004;20:249–252.

Key Words: I blood group, blood groups, human blood groups, carbohydrate antigen, cataracts

Low-titer cold agglutinins are present in the plasma of all adults. The most common of these autoantibodies is a heterogeneous group of specificities known as anti-I, which agglutinate RBCs from the vast majority of adults, but do not react (or react only weakly) with RBCs from newborn infants. Rare individuals who inherit the so-called adult i (or I-) phenotype express very little or no I antigen on their RBCs, and generally have alloanti-I in their plasma. I and i antigens are carbohydrates on membrane glycoproteins and glycolipids. They are, respectively, branched and linear chains on internal structures of ABH-active carbohydrate chains.

The reciprocal relationship between I and i has been of interest for many years and the two antigens have resided in Collection 207 of the ISBT.¹ With the cloning of the gene that encodes the branching enzyme (β -1,6-N-acetylglucosaminyltransferase) that forms the I antigen, the ISBT Committee for Terminology for Red Cell Surface Antigens placed the I antigen in its own blood group system

(027), leaving the i antigen by itself in Collection 207.² The purpose of this review is to summarize the findings regarding the gene that encodes the branching enzyme (β -1,6-N-acetylglucosaminyltransferase) and its association with congenital cataracts. Comprehensive reviews about serologic aspects can be found in current immunohematology textbooks.

Brief History

The I antigen was named by Wiener et al.³ The letter “I” was used to denote the high degree of “individuality” shown by a patient whose RBCs lacked the antigen. The anti-I in the patient’s serum was of high titer and showed that the I antigen was expressed to variable strengths on RBCs from different donors. Although RBCs from the majority of adults express the I antigen, their plasma contains anti-I, albeit of low titer. These cold reacting autoanti-I are present in plasma/serum from most people. While RBCs from adults express I, RBCs from newborn infants do not.^{4,5} The i antigen was named after two cold-reacting antibodies were found that behaved in the manner opposite to that of anti-I. These i antibodies strongly agglutinated cord RBCs and adult i RBCs but only weakly agglutinated RBCs from adults.^{6,7} The relationship of I and i is given in Table 1.

Table 1. The relationship of I to i

RBCs	Occurrence	Antigen Expression		Characteristics of anti-I
		I	i	
Adult	Common	Strong	Weak	Weak; reacts at 4°C
Cord	All	Weak	Strong	
Adult i	Rare	Trace or none	Strong	Moderate; reacts at 4°C and RT*
Disease association				
CHAD	Uncommon	Strong	Weak	Strong; reacts at 4°C, RT, 37°C
	After <i>Mycoplasma pneumoniae</i> infection	Strong	Weak	Strong; reacts at 4°C, RT

*Room temperature

It has been believed for some time that the *I* gene encodes a transferase which adds a branch to the *i* antigen to produce *I* antigen.

Disease Association

A decreased expression of *I* antigen and concomitant increased expression of the reciprocal *i* antigen are associated with leukemia, Tk poly-agglutination, thalassemia, sickle cell disease, HEMPAS, Diamond Blackfan anemia, myeloblastic erythropoiesis, sideroblastic erythropoiesis, and any condition that results in stress hematopoiesis.⁸

Anti-*I* is associated with cold hemagglutinin disease (CHAD) and develops following some infections of mycoplasma pneumonia.⁹ Anti-*i* has been found in a variety of conditions, including autoimmune hemolytic anemia (AIHA), myeloid leukemia, and CHAD, which appear to have in common reticulosis. Anti-*i* has been reported to be present in the serum of from 8 percent to 90 percent of patients with infectious mononucleosis.^{10,11}

***I* Blood Group System**

The gene encoding the *I* antigen, called *I* or *IGnT* (*GCNT2*), consists of three exons spanning approximately 100 kbp of gDNA on chromosome 6p24.¹²⁻¹⁵ Three forms of exon 1 are differentially spliced to give one of three transcripts: *IGnTA*, *IGnTB*, or *IGnTC*.¹⁶⁻¹⁸ This gene encodes *N*-acetylglucosaminyltransferase (β 6GlcNAc-transferase), which is the branching enzyme for the *I* antigen. The transferase needed for expression of *I* antigen on RBCs is encoded by *IGnTC* and consists of 402 amino acids. The transferase needed for expression of *I* antigen in lens epithelium and other tissues is encoded by *IGnTB*.^{17,18} (Fig. 1) The simplest *I* antigen on RBCs is represented by a 6-linked type 2 branch on a type 2 inner core (Gal β 1-4GlcNAc β):

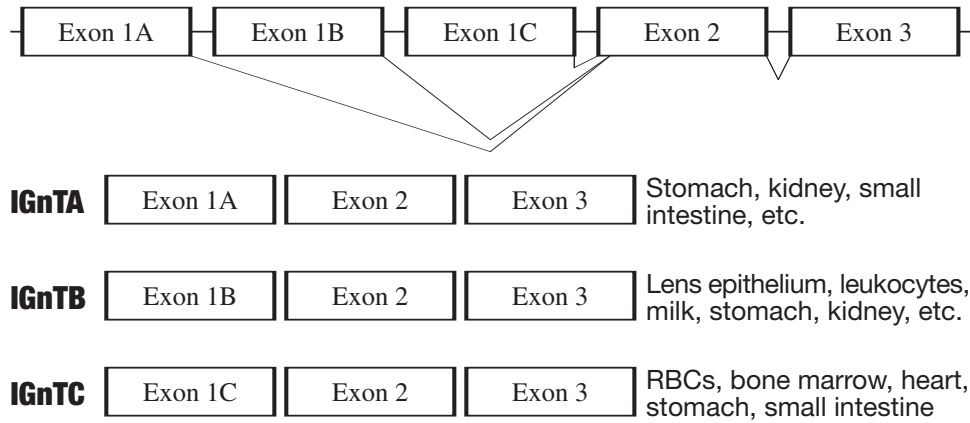
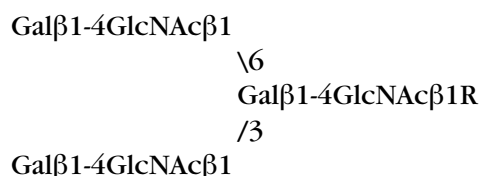
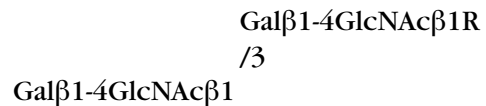


Fig. 1. The *IGnT* gene. Transcripts are formed by use of one of the three alternative forms of exon 1.

While the *i* antigen is a simple linear type 2 chain:



The expression of *I* antigen is enhanced on RBCs treated with proteolytic enzymes. The antigen on RBCs is resistant to disulphide compounds and acid.

Anti-*I* is usually IgM, but rarely it can be IgG. It reacts optimally at ambient temperatures or 4°C and can bind complement, causing hemolysis. It is usually an autoantibody, and it can occur as an alloantibody in rare people with the adult *i* phenotype. In CHAD, anti-*I* is a common specificity with a high thermal amplitude. So-called compound specificities (anti-*IA*, -*IB*, -*IAB*, -*IH*, -*IPI*, -*ILe^{bH}*) have been described.

Molecular basis associated with *I*- (adult *i*) phenotype

In Taiwanese¹⁷ and in Japanese,¹⁸ three mutant alleles have been described. In Caucasians,¹⁶ two alleles have been identified to give rise to the *I*-phenotype (Table 2).

Table 2. Molecular basis associated with *I*- (adult *i*) phenotype

Taiwanese	
Family S	<i>IGnTC</i> exon 3 1043G>A Gly348Glu
Family W	<i>IGnTC</i> exon 3 1043G>A Gly348Glu 1148G>A, Arg383His
Family C	Deletion of <i>IGnT</i> exons 1B, 1C, 2 and 3
Japanese	
	<i>IGnTC</i> 1006G>A in exon 2 Gly334Arg; 1043G>A in exon 3 Gly348Glu
Caucasians	
4 probands	<i>IGnTC</i> exon 1C 505G>A Ala169Thr
1 proband	<i>IGnTC</i> exon 1C 505G>A Ala169Thr; 683G>A; Arg228Gln

Association Between Adult i Phenotype and Congenital Cataracts in Asians

In Japanese, the adult i phenotype has been associated with the occurrence of congenital cataracts.^{19,20} In Caucasians, the association is less strong or absent.²¹ The determination of the molecular basis associated with the adult i phenotype provides an explanation for this phenomenon. Mutations in exon 2 and/or exon 3 of *IGnT*, as in Taiwanese and Japanese, result in no β 6GlcNAc-transferase activity and no I antigen in lens epithelium or in RBCs. Thus, people with these mutated alleles have the I-phenotype and congenital cataracts. In contrast, mutations in exon 1C of *IGnT* lead to markedly reduced β 6GlcNAc-transferase activity, normal I antigen expression in lens epithelium, and very low levels of I antigen in RBCs.¹⁶⁻¹⁸

Conclusion

The analysis of the human I locus (*IGnT* gene) has provided insight into the formation and expression of the I antigen, the adult i phenotype, and the relationship to congenital cataracts. From these studies, it appears that the human *IGnT* gene plays an essential role in maintaining lens transparency. However, there is currently no direct evidence for this. This provides another example of the association of a blood group with an observed effect and the value of documentation of serologic phenomena.

Acknowledgments

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Marion E. Reid, PhD, Immunochemistry Laboratory, New York Blood Center, 310 East 67th Street, New York, NY 10021.

Attention: State Blood Bank Meeting Organizers

If you are planning a state meeting and would like copies of *Immunohematology* for distribution, please **contact** Cindy Flickinger, Managing Editor, 4 months in advance, by fax or e-mail at (215) 451-2538 or flickingerc@usa.redcross.org.

COMMUNICATIONS

Letter to the Editor-in-Chief

My reminiscence of the early days of the American Red Cross Rare Donor Registry

Only I know how the idea for the Rare Donor Registry germinated. I arrived in Washington, DC, from the Milwaukee Blood Center to assume the Directorship of the American National Red Cross Blood Services on January 1, 1967. General Jimmy Collins, then President of the American Red Cross, closed off the entrance of the E Street Marble Palace making it possible for me to have a laboratory for my research right across from my office. The laboratories of the Research Director and the reference laboratories were in the basement. One of my first actions was to have the liquid oxygen generator in the basement removed. I had expert advice that agreed with my fear that the device was a potential time bomb.

In 1959 I had founded the Rare Donor File of the AABB. It was moved to the Minneapolis War Memorial Blood Center when I left for Washington, DC. (Memory was faulty when the AABB awarded a special plaque to the people in Minneapolis for this achievement). As the National Director of the huge ANRC Blood Services I felt it incumbent to start a similar project for the Red Cross. What to call it? I decided on the American Red Cross Rare Donor Registry. Mrs. Elizabeth Dole, then President of the American Red Cross, honored me for this achievement a few years ago. At that time the registry contained 25,000 rare donors and had been involved in solving many related problems and saving many lives. The AABB Rare Donor File and the Red Cross Rare Donor Registry have now been combined and their great work is continuing.

How did we start? We set up the best AutoAnalyzer available. If you can believe it, the results of whether a red cell sample was agglutinated or not was read out on a filter paper strip. It worked. As I recall, Karen Anderson set up the system and got it to work. Leon La Touche was trained to run the unit. Early on, he identified a few rare donors, one of whom was Colton(a-). Of course that sample had been sent from our St. Paul Center. Leon quickly got the nickname, "Green Thumb" because he ran the machine

proficiently, and he was very lucky. I will now reveal a prejudice that did not make me popular with the blood centers of that time. We set up a centralized system because I did not believe that more than a few of our 57 blood centers had the expertise to do a good job and also, if they did find rare donors, whether they would share the information with us. Thus all the work was done by Leon. We assigned geographically selected centers to send us blood samples in ACD tubes that we furnished. That is how we found the Colton(a-) sample, because we knew there was a greater likelihood of finding one among the Scandinavians living in Minnesota. I confess I did not pay much attention to the details of the operation after I had it going. The problems of operating the Blood Services required my full-time attention.

John Peoples relates that when he arrived in 1973 the old AutoAnalyzer had been retired and testing was done in tubes. He developed a microplate technique to conserve reagents. Then, in 1974, we utilized our first computer for the rare donor program. John has related some of the outstanding performances of those days. We made the front page of the Washington Post by releasing for shipment two units of very rare LW-negative units from our donor who lived in the DC area. Our policy was to keep inventories of the rarest units frozen in our laboratory. This was to avoid the problem of coordinating shipments from several locations. Thus, we shipped ten Bombay-negative units to Cook County Hospital in Chicago. They were deglycerolized but the surgeon changed his mind. Unfortunately, they were not recovered for refreezing which is possible by the high glycerol technique devised by Dr. Harold Meryman who was at that time in charge of the cryobiology unit. This was a loss of almost all of the national Bombay negative blood supply. Dr. Bhatia of Bombay kindly sent us a few units to help the situation.

We needed reagents not commercially available. Therefore, we solicited antibody-containing plasmas

COMMUNICATIONS CONT'D

from our centers. Dr. Edwin Steane with the help of Roger Collins started the reagent production program for producing the needed products. We also coordinated our resources with the British program which, in those days, was located at the Lister Institute in London. I do not know where it is now but surely it still exists.

Happily, the AABB and ARC programs have now been merged into the American Rare Donor Program

(ARDP) under the able direction of Sandra Nance and Ann Church (215-451-4351 or 4900).

Tibor J. Greenwalt, MD
Hoxworth Blood Center
PO Box 670055
3130 Highland Avenue
Cincinnati, OH 45267-0055

COMMUNICATIONS CONT'D

Letter from the Editor-in-Chief

The end and the beginning

This is the final issue in the yearlong celebration of the 20 years of publication of *Immunohematology*. Dr. Ralph Vassallo is the guest editor of this issue and it is another exceptional collection of reviews and original publications. Dr. Ralph Vassallo is a Medical Director at the Penn-Jersey American Red Cross and an Associate Medical Editor for *Immunohematology*.

We thank the four guest editors, Christine Lomas-Francis, MSc; Scott Murphy, MD; Sandra Nance, MS, MT (ASCP) SBB; and Ralph Vassallo, MD; and the authors of the reviews and original articles of the four issues of the 20th anniversary year. Each issue was superb and will be used as reference material by many. Christine Lomas-Francis gave us an issue devoted to an up-to-date review of the blood groups. Dr. Murphy's issue covered antibodies, primarily to platelets, in conditions related to NAIT, PTP, TRALI, and ITP. The issue edited by Sandy Nance is another wonderfully useful issue for serologists and clinicians, covering causes of drug-induced anemia; what to do when all units are incompatible (crying is not acceptable); and evaluating a patient with hemolytic anemia. The final issue, that of Dr. Vassallo, finishes with four excellent reviews covering a mixture of articles about the function of blood group antigens, managing patients with anti-IgA, and those who have received incompatible hematopoietic progenitor cell transplantation. An

important review to everyone who orders and receives blood is the one that discusses the function of the American Rare Donor Program (ARDP). Two original articles and a letter from Dr. Tibor Greenwalt, who started the two rare donor programs that make up the ARDP and who is this year's recipient of the AABB Karl Landsteiner Memorial Award, finish off the year.

All of this was planned by the 20th Anniversary Committee—Mary McGinniss, Delores Mallory, Scott Murphy, Sandy Nance, Jerry Sandler, and Christine Lomas-Francis—and was executed by the guest editors and managed by Mary McGinniss, managing editor. The final issue was co-managed by Mary and Cindy Flickinger, and produced by many others who worked hard to make this journal look so good and read so correctly.

We should not forget all of the authors who have contributed articles over the last 20 years, nor the readers who subscribe each year and tell us how much they enjoy *Immunohematology*. It is a unique journal that fills a need of our readers. We hope it will continue to fill that need for 20 more years.

The next 20 years will be even more exciting as great news awaits our readers in 2005!

Delores Mallory
Editor-in-Chief

COMMUNICATIONS CONT'D

Letter From the Editor-in-Chief

To Contributors to the 2004 Issues

The journal depends on readers, authors, editorial board, peer reviewers, and our Penn-Jersey staff. We wish we could thank all of you personally, but doing so is not practical. Instead, we thank each of you as members of an honored group.

First and foremost, we thank the authors for their reviews, scientific articles, case reports, book reviews, and letters to the editors that come not only from the United States but from many countries of the world. This has given the journal an international flavor.

Our editorial board is a prestigious one and we depend on them, not only for peer reviews, but for guidance in policy and suggestions for improvements. Special thanks go to our medical editors, who review every article for medical content, and to Christine Lomas-Francis, MSc, our technical editor, who reads every article for technical content. The current board is listed by name in the front of each issue of the journal.

Our peer reviewers did a wonderful job in 2004. In each December issue we list them by name with thanks to each.

Patricia Arndt, MT(ASCP)SBB	A.J. Hibbard, MD	Marilyn K. Moulds, MT(ASCP)SBB
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We also want to thank the staff at Penn-Jersey, Linda Berenato and Marge Manigly, who do everything else to get the journal ready for press. They word-process all articles, keep up with subscriptions, and handle all e-mail, to name a few tasks. We also thank Lucy Oppenheim, our copy editor; George Aydinian, our proofreader; and Paul Duquette, our electronic publisher.

Finally, thanks go to our readers, whose enthusiasm and interest in the journal make it all worthwhile.

Delores Mallory
Editor-in-Chief

SPECIAL COMMUNICATION

Letter From the Editor-in-Chief

Mary H. McGinniss, AB(ASCP)SBB, Managing Editor of *Immunohematology*, Retires

Mary McGinniss began her career as Managing Editor at *Immunohematology* in 1986, when the journal was only 2 years old. Mary, a research biologist, had just retired from the National Institutes of Health's Department of Transfusion Medicine after an exceptional career that spanned 30 years. She published many papers during her career and is probably best known for her original research with Dr. Louis H. Miller and the discovery of the association of Duffy blood groups with malaria.

Mary's work with *Immunohematology* has been the foundation on which the journal rested for the past 18 years. She edited each issue meticulously, with love and skill. Her sharp eye was the reason each issue was virtually error-free and so consistently true to the style of *Immunohematology*. At a time when many other journals seem to have more and more errors, *Immunohematology* had Mary, who handled each issue with the care and precision that she applied when performing a compatibility test.

She styled all of the manuscripts for the past 18 years. For many authors whose first language was not English, Mary rewrote entire paragraphs of their manuscripts, for which they were most grateful. One of Mary's joys in life has been to mentor new authors, guiding them in writing effective scientific articles. She has a rare editorial talent that many persons who have published in *Immunohematology* will attest to.

She also had an amazing ability to organize four issues each year: 25-30 manuscripts and authors, 50-60

reviewers, proofs, index, "ads," corrections, returns to the authors, and on and on, until each issue was published. *Immunohematology* is published only quarterly, so hers was a part-time job—she took it to give her something to do for a few hours a day! And she could always retrieve back information, even a year later.

Mary is a joy to work with. She never forgets deadlines and gets things done on time. She is happy and can always find a way through a crisis—hurricane, snowstorm, blood shortage, sick kids or cats, no matter the problem. Maybe retiring helps lend an air of calm. Authors and coworkers enjoy the time they have conversing with Mary. She is always helpful, knowledgeable, and available. It's not often that a managing editor has the talent and background that ours did.

Mary McGinniss will be missed by the authors whose papers are better for her talent and attention, by the staff with whom she worked over these 18 years, and, to a degree that can not be expressed, by me. It was one of the best days of my life and for *Immunohematology* when Mary called and said she was ready to work! We dedicate this issue to her, knowing well that every issue is an individual tribute to her excellence, dedication, and love.

Delores Mallory
Editor-in-Chief

BOOK REVIEW

Immune Hemolytic Anemias. Lawrence D. Petz and George Garratty. Churchill Livingstone, 2003. ISBN: 0-443-08559-5. Hardcover, 591 pages. List price: \$115. To order call 1-800-545-2522.

Twenty-four years after the first edition of *Acquired Hemolytic Anemias* was published, Drs. Petz and Garratty have completed the second edition, a thoroughly updated version that has been renamed *Immune Hemolytic Anemias* to reflect its expanded scope. This new edition is a monumental work, both in the broad range of subjects that it encompasses and in the technical detail of individual chapters. The authors cite and comment on a remarkably large number of published journal articles—for example, 774 references in the chapter on the clinical characteristics of autoimmune hemolytic anemia and 494 references in the chapter on its treatment. Nevertheless, the text retains the readable style and expert insights that characterized the first edition.

This reviewer is particularly interested in three subjects that have been added since the first edition: hemolytic disease of the fetus and newborn (HDFN), immune hemolysis associated with transplantation, and hemolytic transfusion reactions. These subjects are reviewed here with particular emphasis.

The chapter on HDFN is organized in a format that is used for most other chapters. First, there is a review of the serologic basis of the subject (antibody specificity and quantification), followed by a discourse on clinical issues (Rh immunoprophylaxis, diagnosis of HDFN, and treatment). The presentation of former and current therapies for HDFN is comprehensive, beginning with a description of the first neonatal exchange transfusion in 1950, followed by commentaries on phototherapy (“Some authors have suggested that the technique is over-used”), preterm delivery, plasma exchange, IVIG, specific immunoadsorption, promethazine hydrochloride, and oral erythrocyte membranes. Table 1 lists 91 antibodies other than ABO that have been reported to cause HDFN. To my surprise, references to reports of specific blood group antibodies causing HDFN are not individually cited in Table 1. For example, if a reader would like to know what published literature supports the authors’ classification of anti-f as a “severe,” or anti-Vel as a

“mild,” cause of HDFN, the reader will need to look elsewhere. Tables with individual references to reports of specific antibodies causing HDFN are included in Issitt and Anstee’s *Applied Blood Group Serology* (1998), which this reviewer uses and finds useful. Given the extensive citations and references in Drs. Petz and Garratty’s text, it seems that this subject is sufficiently important to have individual references in the table. Also, this list seems to be incomplete. This reviewer is currently concerned with a case of HDFN caused by anti-Rh17 (-Hr₀). That designation does not appear in the table, although cases of HDFN, some severe, have been published and are cited in certain other textbooks.

The subject of immune hemolysis associated with transplantation is divided into two subtopics, hematopoietic cell transplantation and solid organ transplantation. Illustrations describe the clinical courses of 18 actual cases of hemolysis associated with ABO mismatched transplants, drug treatment, transfusion (“passenger lymphocyte syndrome”), and others. The references are as up to date as can be expected for a textbook; 62/365 (17%) of the articles referenced in this chapter were published during the last 5 years (1998–2003).

The chapter on hemolytic transfusion reactions is based on the authors’ extensive personal experiences. Recognizing the many important contributions that Drs. Petz and Garratty have made to our understanding of hemolytic anemia in sickle cell disease, it is not unexpected that nine pages of this chapter are dedicated to this topic. In 1997, Petz and colleagues coined the term “sickle cell hemolytic transfusion reaction syndrome” for the syndrome characterized by an acute or delayed hemolytic transfusion reaction, symptoms suggestive of a sickle cell pain crisis, reticulocytopenia, and persistent anemia. In the same year, Garratty reported that in 83 percent of hemolytic transfusion reactions in sickle cell disease, the anemia was more severe *after* transfusion than before. These and other aspects of hemolytic transfusion reactions in sickle cell disease are well illustrated in the authors’ seven case reports. Also, there is a section on hospital-based systems for reducing the incidence of hemolytic reactions due to transfusion of blood to the wrong

BOOK REVIEW CONT'D

patient. Human error is an important cause of hemolytic transfusion reactions, and several studies document the resultant morbidity and mortality. The authors describe devices that are intended to reduce the risk of human error, including a coded locking system and a barcoded wristband and blood bag system. Although the authors note that a commercial variation of the barcode system has "subsequently become available," the manufacturer withdrew that system from the market 4 years ago. Other manufacturers are currently developing similar systems to market and fill the void. I mention this minor inaccuracy only because it illustrates the risk of publishing a textbook that attempts to encompass a broad range of highly technical and rapidly evolving subjects. Surely, the authors will not be able to wait another 24 years to publish the third edition, because a timely update *will* be needed to keep the book's information up to date.

Lastly, it is important that readers know that the retail price for this volume is only \$115. I assume that the compromise necessary to keep the price so affordable was to omit color illustrations. The volume's black-and-white illustrations are clear and communicate the information adequately. A color photograph to illustrate the Donath-Landsteiner test and a few

other color illustrations of subtle hemolysis would have been nice. However, the trade-off, which makes the volume accessible to a wider readership, is clearly the better choice.

In summary, this second edition represents a major revision, as well as an expansion into new and pertinent subjects, including HDFN, transplantation, and hemolytic transfusion reactions. The expert presentations of laboratory and clinical aspects of acquired hemolytic anemias, which made the first edition a world-renowned classic, are retained. I intend to use, and will recommend, this book as the first resource to consult for authoritative information on immune hemolytic anemias. I highly recommend it to immunologists, blood bank specialists (technologists, physicians, and other scientists), and practicing clinicians who seek an expert and affordable reference textbook for their bookshelves.

*S. Gerald Sandler, MD
Professor of Medicine and Pathology
Director, Transfusion Medicine
Georgetown University Hospital
3800 Reservoir Road, NW
Washington, DC 20007*

IMPORTANT NOTICE ABOUT MANUSCRIPTS

FOR

IMMUNOHEMATOLOGY

Please **e-mail** all manuscripts for consideration to Marge Manigly at mmanigly@usa.redcross.org

SPECIAL SECTION

Blood-Banking: Past, Present and Future

Many years ago...
The crossmatch at RT,
And no QC.
Plenty of discovery:
ABO, MN and P.
Albumin and anti-Rh(D).
No mention of QC.
Things were very elementary!

Then came...
Enzymes and IAT,
But no QC.
Minor crossmatch, DAT.
Rh neg. meant cde.
We sought incompatibility
Without QC.
We walked a tight-rope then, maybe.

And then,
FDA and BOB!
At last: "QC".
C3 in AHG,
Let's find that alloantibody.
JCAH and CAP,
More and more QC.
We sought to avoid calamity.

And now...
Forget RT incompatibility
Except for anti-A and -B.
Ignore MN, P₁, Le,
Donor iso/alloantibody.
But anti-Vel? Really?
And keep on with QC.
It's in print for us to see.^{3,4}

And in the future...
No C3 in AHG,
It's not necessary (except for DAT).²
And no more crossmatch IAT???¹
Just the screen, and crossmatch RT.
How much simpler things will be.
And lots, lots more QC.
Will this become reality??

So then we'll have...
The crossmatch at RT,
But with QC.
Bilirubin? Bloody-p?
Renal failure? DIC?
And perhaps a workshop at AABB
On how to QC our QC!
Some say we'll cure somebody???

But wait...
Enough jocularity
Concerning this controversy
In immunohematology.
Let us the data see.
Facts, not someone's fancy,
Beyond dispute are necessary,
And undoubtedly some QC.

So let's find out...
About RT incompatibility,
C3 in AHG, e-t-c.
But do it scientifically!
Then hopefully
We will agree
On what's important, clinically,
And rationalize QC.

W. John Judd

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Reprinted as published in the *Red Cell Free Press* 1978; 3(No.1):6.

SPECIAL SECTION CONT'D

A Word of Caution About Interpreting Panels

Recently a hospital blood bank submitted samples to our reference laboratory for antibody identification. They were perplexed because they identified anti-Fy^a but found the sample to be compatible with a Fy(a+) unit.

We tested the serum with reagent red cell panel Spectra 90577 by our routine methods of 15 minute room temperature incubation, 30 minutes at 37°C with 30% bovine albumin, and with antiglobulin serum. We also tested the serum with a ficin-treated panel. The serum agglutinated three (3) of four (4) Kell positive cells (weak) at room temperature, and all four (4) Kell positive cells (1+) with antiglobulin serum. The reactions were the same with the ficin panel. We did not test the patient's cells for Kell because he had a weakly positive direct antiglobulin test.

The above results are straightforward and ordinarily would be acceptable evidence that this patient has anti-Kell. However, considering that the referring hospital had thought the antibody was anti-Fy^a, we tested the serum with another reagent red cell panel Pfizer 1435. The serum agglutinated both Kell positive and Kell negative cells at room temperature and with antiglobulin serum, with no apparent specificity.

The serum was retested with reagent red cell panel Spectra 90577 at 18°C ± 2° and reacted with all P₁ positive cells (weak-1+). It did not agglutinate any of

the P₁ negative cells or three additional P₁ negative Kell positive cells. The patient cell specimen was P₁ negative.

This antibody was in fact anti-P₁ and it was simply an unlucky coincidence that the reactions of the first panel fit a Kell pattern. A calculation of the p factor of an antibody identification based on four cells positive and six cells negative is $p = .004$, which is statistically significant and well below the requirements of BPD 6.38 which requires a confidence factor of $p .01$. It was only because of the discrepancy between our results and those from the hospital that we questioned the anti-K (Kell) and tested further.

We are not suggesting that this incident indicated the need for routine testing below room temperature. It does, however, remind us that interpretation of panel results is a responsibility we accept for others. Each one deserves careful evaluation of the patient history and information supplied by the hospital, because even serological results with a high statistical probability of accuracy may be in error.

Bev Pohl

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Phone, Fax, and Internet Information: If you have any questions concerning *Immunohematology, Journal of Blood Group Serology and Education*, or the *Immunohematology Methods and Procedures* manual, **contact** us by e-mail at immuno@usa.redcross.org. For information concerning the National Reference Laboratory for Blood Group Serology, including the American Rare Donor Program, please contact Sandra Nance, by phone at (215) 451-4362, by fax at (215) 451-2538, or by e-mail at snance@usa.redcross.org

SPECIAL SECTION CONT'D

Reagent Production

Progress has been made on antiserum reagent production in amongst the sound of electricians, plumbers, and carpenters making a valiant attempt to finish their work before a unit of antibody plasma was placed in their way. Products available as of this date are:

Anti-Kell	8 ml	Indirect antiglobulin test
Anti-M	5 ml	Saline room temperature
Anti-S	5 ml	Indirect antiglobulin test

Products coming up in the near future will be anti-P₁, anti-Cellano and anti-Duffy.

Products will be available to all Centers at no charge. We will have to limit the volume of the anti-M and anti-S to 25 ml per order until we can establish the volume requirements and demands of the Centers for antiserum reagents of these rarer types and coordinate our production volumes accordingly.

We would like to offer a full range of antiserum screening reagents without any cost or restriction to all Centers but the supply of finished reagents is completely dependent on antibody plasma units coming from the Centers.

So please help us to help you.

If you have antibody plasmas that you are currently diverting to cold storage or plasma pooling for fractionation, please divert them to National Headquarters Reagent Production. We require urgently antibodies to all systems. The strength of reaction is not totally important; if it is strong enough to identify its specificity it will be strong enough to use in reagent production.

For any information or inquiries on the antiserum production program or reagents contact Roger Collins at National Headquarters, Washington, D.C., 202-857-2046.

Roger Collins
Reagent Production
American National Red Cross
Washington, DC

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ANNOUNCEMENTS

Monoclonal antibodies available at no cost. The Laboratory of Immunochemistry at the New York Blood Center has developed a wide range of monoclonal antibodies (both murine and humanized) that are useful for screening for antigen-negative donors and for typing patients' RBCs with a positive DAT. Monoclonal antibodies available include anti-M, -Fy^a, -Fy^b, -K, -k, -Kp^a, -Js^b, -Do^b, -Wr^b, and -Rh17. For a complete list of available monoclonal antibodies, please see our Web site at www.nybloodcenter.org/framesets/FS-4C7.htm. Most of those antibodies are murine IgG and, thus, require the use of anti-mouse IgG for detection, i.e, anti-K, -k, and -Kp^a. Some are directly agglutinating (anti-M, -Wr^b, and -Rh17), and a few have been humanized into the IgM isoform and are directly agglutinating (anti-Js^b and -Fy^a). The monoclonal antibodies are available at no charge to anyone who requests them. **Contact:** Marion Reid (mreid@nybloodcenter.org) or Gregory Halverson (ghalverson@nybloodcenter.org), New York Blood Center, 310 East 67th Street, New York, NY 10021.

HEMATOLOGÍA HABANA' 2005— First Announcement.

The 5th National Congress and the 7th Latin American Meeting in Hematology, Immunology, and Transfusion Medicine will present a scientific program at the International Conference Center, Havana, Cuba, May 16-20, 2005. A preliminary program lists malignant hemopathies, disorders of RBC membranes, immunotherapy, histocompatibility, immunohematology, hemolytic disease of the newborn, regenerative medicine, and blood components as some of the topics. For more information **contact:** Prof. José M. Ballester, President, Organizing Committee, Hematology Habana' 2005, Apartado 8070, Ciudad de la Habana, CP 10800, Cuba, e-mail: ihidir@hemato.sld.cu; Web site: www.loseventos.cu/hematologia2005.

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Further information can be obtained from the Web site:

<http://www.blood.co.uk/ibgrl/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

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2. Abstract
3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables—see 6 under Preparation
8. Figures—see 7 under Preparation

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Clinical and/or hematologic data and background serology.
 - C. Materials and Methods

Selection and number of subjects, samples, items, etc. studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer's name, city, and state. Do not use patients' names or hospital numbers.
 - D. Results

Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable.
 - E. Discussion

Implications and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction.

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Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.

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6. One table and/or figure allowed.

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Becoming a Specialist in Blood Banking (SBB)

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

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

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

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

Who are SBBs?

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

Why be an SBB?

Professional growth Job placement Job satisfaction Career advancement

How does one become an SBB?

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

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

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

Conclusion:

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

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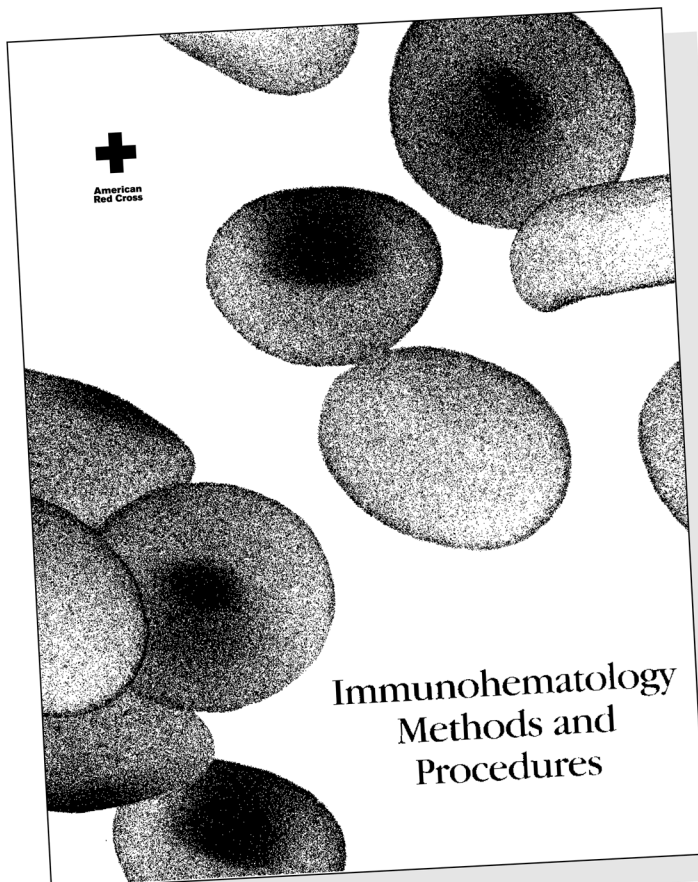
PROGRAM	CONTACT NAME	CONTACT INFORMATION
Walter Reed Army Medical Center	William Turcan	202-782-6210; William.Turcan@NA.AMEDD.ARMY.MIL
Transfusion Medicine Center at Florida Blood Services	Marjorie Doty	727-568-5433 x 1514; mdoty@fbsblood.org
Univ. of Illinois at Chicago	Veronica Lewis	312-996-6721; veronica@uic.edu
Medical Center of Louisiana	Karen Kirkley	504-903-2466; kkirk1@lsuhsc.edu
NIH Clinical Center Department of Transfusion Medicine	Karen Byrne	301-496-8335; Kbyrne@mail.cc.nih.gov
Johns Hopkins Hospital	Christine Beritela	410-955-6580; cberite1@jhmi.edu
ARC-Central OH Region, OSU Medical Center	Joanne Kosanke	614-253-2740 x 2270; kosankej@usa.redcross.org
Hoxworth Blood Center/Univ. of Cincinnati Medical Center	Catherine Beiting	513-558-1275; Catherine.Beiting@uc.edu
Gulf Coast School of Blood Bank Technology	Clare Wong	713-791-6201; cwong@giveblood.org
Univ. of Texas SW Medical Center	Barbara Laird-Fryer	214-648-1785; Barbara.Fryer@UTSouthwestern.edu
Univ. of Texas Medical Branch at Galveston	Janet Vincent	409-772-4866; jvincent@utmb.edu
Univ. of Texas Health Science Center at San Antonio	Bonnie Fodermaier Linda Smith	SBB Program: 210-358-2807, bfodermaier@university-health-sys.com MS Program: 210-567-8869; smithla@uthscsa.edu
Blood Center of Southeastern Wisconsin	Lynne LeMense	414-937-6403; Irlmense@bcsew.edu

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

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