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Review: Biochemistry of carbohydrate blood group antigens

L. G. GILLIVER AND S. M. HENRY

This review presents the basics of the structural chemistry of blood group glycoconjugates, with special reference to red cell serology. Its aim is to create an appreciation of the inherent subtleties of the carbohydrate blood group antigens, which are currently poorly understood within the field of blood transfusion. It is hoped that a better understanding of the intricacies of the carbohydrate blood group systems will lead to further contributions to the body of knowledge within this growing field.

Introduction

In 1900, Karl Landsteiner discovered the carbohydrate blood group system of ABO after mixing the cells and serum of his colleagues. Over the next 100 years many other blood group systems were discovered and later resolved at both the molecular and the genetic level. It is now clearly recognized that most blood group systems are either carbohydrate, protein, or protein-carbohydrate in nature, although they may associate with other components and complexes to be recognized as specific blood group determinants. Essentially, a blood group antigen is any polymorphic three-dimensional conformation expressed on the outer membrane of a RBC against which an antibody is available. The concept of the three-dimensional shape is very important because this is what antibodies recognize, and the number of potential shapes that can be found in carbohydrate chains is enormous. The variability in characteristics such as sugar type, linkage type, branch positioning, shape, charge, ring size, and epimeric (pairs of monosaccharides which differ only in the configuration about one carbon atom are epimers of each other e.g., D-glucose and D-mannose are epimers with respect to the C2 carbon atom) and anomeric configuration results in more than $1.05 \times 10^{12}$ possible structural combinations from a six-sugar glycan (the largest size expected for a carbohydrate epitope). This is compared with only 46,656 combinations possible with a chain made up of six amino acids, more than seven orders of magnitude lower than the diversity possible in glycans.\(^1\)

Fortunately, despite the vast potential for complexity in carbohydrate chains, there is generally only a limited range of structures representing the carbohydrate blood group epitopes (glycotopes), although many of the structural differences are subtle and some epitope surfaces may be common. These subtleties may represent different blood group antigens and in some cases, specific antibodies may recognize the common surfaces. For example, anti-A,B recognizes the similarities between A and B antigens, not the subtleties which distinguish them.\(^2\) Unfortunately, this potential variation makes structural determination of carbohydrate antigens complex, requiring combinations of powerful techniques such as nuclear magnetic resonance spectroscopy and mass spectrometry to elucidate the structures of carbohydrate antigens without ambiguity.\(^3,4\) Progress in determining the structures of carbohydrate blood group antigens has been slow, hindered by a combination of factors, such as the fact that carbohydrate blood group antigens are not the primary product of a gene—being rather secondary biosynthetic products—and that the isolation and purification of carbohydrate blood group antigens in requisite quantities is difficult. As a result, we know only the basic structures of the major glycotopes and antigens expressed in the carbohydrate blood group systems. We have not yet characterized many of the phenotypic subtleties, such as those that determine ABO subgroups.

Because carbohydrate blood group antigens are important to transfusion medicine and are becoming more recognized in transplantation (especially xeno-
transplantation) and studies of microorganism binding and interactions, it is becoming more important for serologists to understand the biochemistry of carbohydrate blood group antigens.

We presently recognize several blood group systems and collections where the blood group glycotopes are comprised entirely of carbohydrate, such as ABO, Lewis, H, II, P, Sd, Cad, etc.; or others, such as MN, where carbohydrate moieties are essential to the structure of the epitope; or even others, where carbohydrates are associated with the character of the antigen—e.g., GPI-linked membrane proteins such as Cromer, Dombrock, Cartwright, and John Milton Hagen, etc.

This review is not intended to cover the carbohydrate blood group systems, rather it is intended to provide a basic understanding of carbohydrate blood group chemistry, and in this regard we will use ABO antigens as examples.

**Monosaccharides**

The common blood group-related sugars, namely β-D-glucose (Glc), N-acetyl-β-D-glucosamine (GlcNAc), β-D-galactose (Gal), N-acetyl-β-D-galactosamine (GalNAc), α-L-fucose (Fuc), and D-mannose (Man) are all six-carbon sugars (hexoses). The carbonyl group (C=O) of each residue is an aldehyde (HC=O), and thus they are all classified as aldohexoses. Another special monosaccharide which contains nine carbons is also common in blood group antigens, with the most common blood group variation being N-acetyl-α-D-neuraminic acid (also referred to as NeuAc or sialic acid). There are various forms of sialic acid, but these will not be reviewed here.

Sugar molecules can have configurational isomers which differ in their arrangement in space even though they have identical composition. The variation in the configuration of individual carbohydrates was first characterized in relation to the smallest chiral carbohydrate, glyceraldehyde, by German chemist Emil Fischer in the late 19th century. In extremely simple terms, chirality is “handedness”—that is, the existence of left/right apposition. For example, your left hand and right hand are mirror images and therefore “chiral.”

The enantiomeric (mirror image) configurations Fischer described were designated D and L by Rosanoff in 1906. Today, all monosaccharides are classified according to this principle, which depends on the orientation of the hydroxyl group attached to the highest numbered chiral carbon (Cₙ₋₁), where n is the total number of carbons in the structure. If this hydroxyl group points to the right when the carbonyl group is at the top, the monosaccharide is said to be in the D configuration. The mirror image of this structure, with the hydroxyl group pointing to the left, is the L configuration. The D and L configurations of glucose are shown as open chain Fischer projection formulas (Fig. 1). In blood group glycoconjugates, sugars exist almost exclusively in the D configuration, except fucose, which is found in the L configuration. The chiral centers of saccharides can also be designated as R or S under the Cahn-Ingold-Prelog system. This is a more modern notation; however, it is not often used in carbohydrate chemistry because it causes confusion in the classification of some monosaccharides.

The seven common blood group sugars almost exclusively form pyran rings—six atoms forming the ring, compared with furan, which is a five-atom ring (Fig. 2).

Numbering of the carbons in the pyranose ring formation of hexose and nonose sugars is shown in Figure 3.

The pyran ring comes about through the formation of an oxygen bridge between a carbon (carbon 1 in the common hexose blood group sugars, or carbon 2 in sialic acid) and the oxygen of the hydroxyl group five carbons away. In hexoses, carbon 5 rotates so that its hydroxyl group apposes carbon 1. The double-bonded oxygen on carbon 1 is reduced—taking the hydrogen
from the hydroxyl group of carbon 5, in the process oxidizing the hydroxyl group to an oxygen radical with only one bond. This leaves carbon 1 as a radical also, with only three bonds. This is one less than the preferred complement of bonds in the case of both atoms, enabling the formation of a bond between them (see Fig. 4 for the scheme of this reaction, using D-galactose as an example). The scheme is basically the same for sialic acid, except that the bridge is formed between carbon 2 and the oxygen on carbon 6.

The two ring formations possible for each sugar are either the α or β anomeric forms. These are two isomeric forms that differ only in the configuration at the carbon involved in the formation of the oxygen bridge, which is carbon 1 in the common blood group hexose sugars and carbon 2 in sialic acid. This carbon is termed the anomeric carbon, and the configurational difference occurs in the orientation of its hydroxyl group in relation to the oxygen on the anomic reference atom, which is carbon 5 in most hexoses.

Fig. 2. Furan and pyran rings of d-galactose. The open chain formation of d-galactose is shown in the middle of the figure. The furanose (lower left) and pyranose (lower right) forms of d-galactose (β is used in this example) are formed from the open chain structure. The generic furan (upper left) and pyran (upper right) ring formations are also shown.

Fig. 3. Carbon numbering in pyranose rings. Most of the common blood group sugars are 6-carbon (hexoses), except for sialic acid, which has 9 carbons (nonose). Carbons are numbered in a clockwise direction from the oxygen bridge.

Fig. 4. Scheme showing the formation of the pyran ring. Step 1—Carbon 5 (square) of the open chain form of d-galactose rotates so that its hydroxyl group apposes carbon 1 (circle). Step 2—The double-bonded oxygen on carbon 1 is reduced and the hydroxyl group of carbon 5 is oxidized, leaving carbon 1 and the oxygen on carbon 5 each with one less than their preferred number of bonds. Step 3—A bond forms between carbon 1 and the oxygen of carbon 5 to yield β-D-galactopyranose.

Fig. 5. Fischer linear ring projection showing the configurational difference at the anomeric carbon hydroxyl group (top arrows) of α-D-galactose (left) and β-D-galactose (right) in relation to the anomeric reference oxygen (bottom arrows). The anomeric carbon hydroxyl group and the anomeric reference oxygen are on the same side of the carbon chain in the α-form, and on opposite sides of the carbon chain in the β-form.
When in the Fischer linear representation of the ring (Fig. 5), the α form occurs when the anomeric carbon hydroxyl is on the same side of the carbon chain as the oxygen on carbon 5. Accordingly, in the β form, the anomeric carbon hydroxyl and the oxygen on carbon 5 are on opposite sides. Due to the rotation of carbon 5 in hexoses (carbon 6 in sialic acid) and hence its hydroxyl group (Fig. 4), this rule cannot be applied to the Haworth projection of the ring. However, the anomeric form can be deduced in the Haworth projection—it is α when the anomeric hydroxyl group is down, and β when the anomeric hydroxyl is up.

The Haworth projections of the α and β forms of mannose and galactose are shown in Figure 6. The two cyclic α and β anomeric forms exist in equilibrium with the open chain structure, however, the open chain configuration is present only in minute amounts. Because these anomers are interchangeable, the anomericity of the sugar is really only of interest once it is made permanent by bonding with another saccharide.

![Haworth projection formulae of the sugar residues commonly found in blood group–related glycoconjugates.](image)

Figure 6 also shows the other saccharides commonly found in blood group antigens. D-glucose and D-galactose are most often found in the β form, while L-fucose, which is almost identical in structure to galactose (except that fucose does not have a hydroxyl group on its sixth carbon, thus it is also known as 6-deoxygalactose) is found in the α form. D-mannose is a sugar residue commonly found in glycoproteins (exclusively N-linked oligosaccharides) and exists in both the α and β configuration.

N-acetyl-D-glucosamine and N-acetyl-D-galactosamine are examples of amino sugars (Fig. 6). The carboxyl group (COOH) of carbon 1 gives sialic acid its acid characteristics, and because of this, glycoconjugates incorporating it are known as gangliosides, which are a subset of acidic glycosphingolipids. The acidic glycosphingolipid group also encompasses uronoglycosphingolipids (containing uronic acid residues), sulfoglycosphingolipids (containing sulfate ester groups), phosphoglycosphingolipids (containing phosphate mono- or diester groups), and phosphonoglycosphingolipids (containing [2-aminoethyl]hydro-xyrophosphoryl groups).

![The acetamido group of N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and sialic acid.](image)

N-acetyl-D-glucosamine most commonly adopts the β form in blood group glycoconjugates, while N-acetyl-D-galactosamine most commonly adopts the α configuration—although the β form is also seen. Sialic acid is unlike the other sugar residues discussed here in that it has nine carbons and the anomeric carbon is carbon 2 (Fig. 6). It is substituted at carbon 5 with an acetamido group as described for carbon 2 of N-acetyl-D-glucosamine and N-acetyl-D-galactosamine.
Up to this point, the pyranose-forming monosaccharides have all been represented as having planar rings with functional groups projecting above or below. In reality this is not the case—the electron orbitals of the carbons and the oxygen in the ring and the large functional groups attached to the carbons prevent the rings from existing in a planar form. The result is that the ring contorts into one of three three-dimensional shapes, two of which are suggestive of a chair and one of which looks like a boat (Fig. 8). The functional groups attached to the ring carbons are able to adopt either an axial position—perpendicular to the general plane of the ring—or an equatorial position—in line with the general plane of the ring. In this way a single saccharide can potentially assume three different three-dimensional shapes, although in practice the lowest energy conformation is preferred, i.e., the one which minimizes the proximity of large functional groups. The functional groups in the equatorial orientation are less crowded together than those in the axial position, and so the lowest energy conformation occurs when as many as possible of the large hydroxyl groups, rather than the relatively small hydrogens, are situated in the equatorial orientation. Only saccharides with alternating chirality, such as β-D-glucose (Fig. 1), can position all the ring hydroxyl groups in the equatorial position, because crowding in the axial orientation is reduced due to the ring hydrogens being able to alternate above and below the plane of the ring (Fig. 9).

**Bonds and Linkages**

Sugar residues can be joined into chains by the synthesis of glycosidic bonds. As previously mentioned, the two monosaccharide cyclic forms—α and β—are in equilibrium with the open chain formation when in aqueous solution. However, when joined together by glycosidic linkages, the residues become fixed in ring formation except for the last sugar in the chain.12

Glycosidic bonds are the result of a condensation reaction between the hydroxyl group of carbons 2, 3, 4, 6, or 8 of one residue and the anomic carbon hydroxyl group of a second sugar. The bond is considered to belong to the sugar whose anomic carbon is involved, which for hexoses is carbon 1, and is expressed as 1-2, 1-3, 1-4, or 1-6 depending on the point of attachment. Sialic acid, whose anomic carbon is carbon 2, forms 2-3, 2-6, or 2-8 bonds.

Two types of glycosidic bond are possible; this depends on the configuration of the anomic carbon hydroxyl group involved in the bond. The bond formed when the anomic hydroxyl group is in the α configuration is designated an α bond. Likewise, a β bond will result when the anomic hydroxyl is in the β configuration. The A type 1 structure shown in Figure 10 has two α bonds and three β bonds.

**Fig. 8.** Chair and boat conformations of pyranose rings. There are two variants of the chair conformation, which differ mainly in the axial versus equatorial orientations of the ring functional groups (X and Y). In the boat conformation, the functional groups labeled A are in an axial position, while those labeled E are equatorial.

**Fig. 9.** β-D-glucopyranose in its preferred chair conformation. All the hydroxyl groups are in the equatorial orientation. Note that the hydrogens alternate above and below the plane of the ring in the axial positions.

**Fig. 10.** Example of α and β linkages in the A type 1 molecule. This diagram shows A type 1 in two schematic forms; one structural and the other alphanumeric—both a simple form (lower right) and an expanded form associated with the structural diagram to identify the appropriate sugars and bonds. R = the remainder of the molecule.
terminal (and sometimes also a sub-terminal) position. A terminal sialic acid residue prevents further elongation of the chain except for the addition of another sialic acid residue through an \(\alpha\)2-8 linkage (Fig. 11).

Different glycoconjugate precursor chain structures have been identified and categorized into six groups based on the identity of the terminal disaccharide sugar residues and the type of glycosidic linkage that joins them together. These terminal disaccharide structures of the six groups are shown in Table 1.

### Table 1. Six terminal disaccharide chain types.

<table>
<thead>
<tr>
<th>Terminal Disaccharide Structure</th>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
<th>Type 4</th>
<th>Type 5</th>
<th>Type 6</th>
</tr>
</thead>
</table>
| Gal\(\beta\)1-3GlcNAc\(\beta\)1-R | \n| Gal\(\beta\)1-4GlcNAc\(\beta\)1-R | \n| Gal\(\beta\)1-4GalNAc\(\alpha\)1-R | \n| Gal\(\beta\)1-3GalNAc\(\beta\)1-R | \n| Gal\(\beta\)1-4Gal\(\beta\)1-R | \n| Gal\(\beta\)1-4Glc\(\beta\)1-R | \n
### Biosynthesis of Saccharide Chains

According to carbohydrate terminology, an oligosaccharide consists of 3 to 10 sugar residues and a polysaccharide contains more than 10 residues. The blood group structures can exist in both linear and branched forms and range from a few saccharide units up to more than 60 sugar residues. Irrespective of the size of any given polysaccharide, it is generally accepted that the actual surface recognized by antibody (glycotope) is usually no larger than 2–3 sugars. However, sometimes glycotopes may look bigger (up to six sugars) because some extra sugars are needed to stabilize the three-dimensional configuration of the sugars actually recognized by the antibody (R. Oriol, personal communication). The actual structure and size of the polysaccharide is determined by interactions of various polymorphic blood group glycosyltransferases and other monomorphimic glycosyltransferases. Glycosyltransferases are gene-encoded proteins (enzymes), and are responsible for the stepwise addition of substrate residues to acceptor structures. It is therefore clear that the biosynthesis of carbohydrate blood group antigens is not a straightforward gene equals antigen process. Instead, a gene must encode a functional protein (enzyme), which must be retained in the Golgi apparatus, and in the presence of appropriate precursors and substrates a carbohydrate blood group antigen can be synthesized (Fig. 12). Even this is overly simplistic, because the formation of a carbohydrate blood group antigen requires the interaction of several glycosyltransferases unrelated to blood groups before the blood group determinant(s) can be added. Furthermore, competition and sometimes cooperation occur between the various glycosyltransferases, some of which show redundancy and degeneration. Redundancy is said to occur when the same carbohydrate structure can be made by two separate enzymes. For example, Se is capable of transferring a fucose to the terminal \(\beta\)-o-galactose of both type 1 and 2 chains, while H can only utilize type 2 chains. Thus, H type 2 can be made by two genetically different enzymes. The presence of Se-formed H type 2 antigens on Bombay RBCs is the basis of some para-Bombay phenotypes. Degeneration refers to the situation...
where one enzyme can make two different structures. Using the same example, Se shows degeneration in that it can make type 1 and type 2 H structures. Thus, it can be seen that the occurrence of specific glycoconjugates is indirectly controlled by genes through the occurrence and subsequent synthetic actions of enzymes. After glycoconjugates are synthesized, they are transported to various destinations in and out of the cell, as well as being incorporated in the cell membrane where they can be detected by various antibodies. A more comprehensive review of the pathways leading to the expression of the blood group-related glycosphingolipids can be found elsewhere.13

Glycolipids vs. Glycoproteins
Glycans can be conjugated to protein or lipid molecules in biological systems; such molecules are called glycoproteins or glycolipids, respectively. The glycosylation pathways followed by glycoproteins and glycolipids are not always identical. This possibly reflects the different biological functions of membrane-bound glycoconjugates (glycolipids and glycoproteins) versus soluble structures (usually glycoproteins).15,16

Glycolipids
Glycosphingolipids are a class of glycolipids which are composed of a carbohydrate chain and a sphingolipid tail, the trivial name of which is ceramide. Sphingolipids are common components of cell membrane lipid bilayers and are composed of a long-chain base (LCB) amino linked to a fatty acid (FA). Due to the long hydrophobic hydrocarbon chains present in LCBs and the FAs, ceramides are able to insert into the lipid bilayer of RBCs. The hydroxyl group on carbon 1 of the sphingolipid is oxidized to form the $\beta$-linkage with the anomeric carbon of $\beta$-D-glucose to form a monoglycosyl sphingolipid (Fig. 13). This linkage is designated $\beta$1-1 because the two hydroxyl groups involved belong to carbon 1 of their respective structures.

There is considerable heterogeneity in ceramides, mainly due to variations in hydrocarbon chain length, level of saturation (presence of double bonds), and the number of hydroxyl groups—in fact, there are over 60 different variations of LCBs, each of which can be combined with one of 20 common variants of FAs. It is speculated that some of the variants may be important for presentation of the antigen.

Glycoproteins
Glycans can be attached to proteins via nitrogen or oxygen—N- or O-glycosidically linked, respectively. N-glycosidically linked glycoproteins are found primarily in serum and cell membranes, while O-glycosidically linked glycoproteins are located in exocrine gland secretions and mucins, as well as in cell membranes, although this is less frequent.7

O-linked glycoproteins
An O-linkage occurs between the hydroxyl groups of serine or threonine amino acid residues and the anomeric carbon hydroxyl group of N-acetyl-$\alpha$-D-galactosamine, with the linkage type being $\alpha$1 (Fig. 14). Glycolipids are also O-linked, but the sugar

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**Fig. 13.** Structure of a sphingolipid (ceramide) and a monoglycosyl sphingolipid. Top: the sphingolipid is composed of a FA (nervonic acid C24:115—upper chain) and a LCB (4-sphingenine d18:1—lower chain). Below: $\beta$-D-glucose is joined to the sphingolipid via a $\beta$-linkage between carbon 1 of the saccharide and ceramide.

**Fig. 14.** Structure of glycoprotein O- and N-linkages. Left and center: O-linked glycoproteins where N-acetyl-D-galactosamine is linked through an $\alpha$ bond to the oxygen of the amino acids serine or threonine. Right: N-linked glycoprotein where N-acetyl-$\alpha$-D-glucosamine is linked through a $\beta$ bond to the nitrogen of the amino acid asparagine. $R_1$ and $R_2$ represent the remainder of the polypeptide chain.
involved is β-D-glucose. Unlike the N-linked glycoproteins, there is no single common oligosaccharide core structure for O-linked glycoproteins. Instead there are six distinct di- and trisaccharide core structure types and a variety of terminal structures. O-linked glycoproteins range from the very simple mono-glycosylated structures to highly complex molecules containing large amounts of galactose and N-acetylgalcosamine, along with lesser amounts of N-acetylgalactosamine, fucose, and sialic acid. O-linked glycoproteins do not contain mannose residues.

**N-linked glycoproteins**

An N-linkage occurs between the nitrogen of the amino acid asparagine and the hydroxyl group of N-acetyl-β-D-glucosamine, with the linkage type being β1 (Fig. 14).

N-linked glycoproteins have a common pentasaccharide structure consisting of Man3GlcNAc2. There are three main groups into which N-linked glycoproteins can be divided: high mannose type, complex type, and hybrid type. A comprehensive description of these types can be found elsewhere.

**Red Cell Glycosylation and Serology**

The outside of the human RBC is highly glycosylated, incorporating both glycoproteins and glycolipids. The major function of the glycoconjugate coat of the RBC is probably to make them more hydrophilic and to provide a "hard" but flexible protective coating. However, if this was the only function of red cell glycosylation, then it could be achieved by the expression of large amounts of simple carbohydrate chains, which does not account for the complex pattern of glycosylation that actually exists. Some of the glycoconjugates are very complex and bear genetically defined polymorphisms, for example ABH, Lewis, and P. Why these polymorphisms exist is unknown, but there is emerging evidence of evolutionary and biological function.

The intention of this review is to present basic concepts of carbohydrate chemistry in order to develop an understanding of the complexities that exist within carbohydrate blood group systems. Traditionally, the antigens of these systems have been viewed generically, without appreciation of the subtle differences between them or the impact this may have on phenotyping or biological function. Not only is there a variety of glycotopes within an antigen designation, but there also is extensive variation in the carbohydrate molecules which carry the glycotopes (Fig. 15).

This range of variants can be further expanded by the polymorphic proteins and lipids which then support them. The presentation of the carbohydrate chain at the membrane surface may differ significantly among the different types, as shown by molecular modeling. The membrane orientation of saccharide chains may vary from almost perpendicular (type 1), to nearly parallel (types 2, 3, and 4). These different conformations of the carbohydrate chains are important for antibody binding and for the immunogenic and microbial-interaction characteristics. Some may actually determine phenotype characteristics, although this has not yet been determined. In addition, the carrier molecule may influence the positioning of the carbohydrate chain on the RBC.
the cell membrane in relation to other membrane components. From the perspective of blood transfusion, the current lack of depth of understanding of carbohydrate blood group antigens has been accepted as adequate. However, as can be seen from other blood group systems (e.g., Rh and HLA), there is value to be found in unraveling their intricacies. It is hoped that by investigating the subtleties of the carbohydrate blood group systems, important new biological information, in particular microbial interactions, will be discovered.

References
Manuscripts: The editorial staff of *Immunohematology* welcomes manuscripts pertaining to blood group serology and education for consideration for publication. We are especially interested in case reports, papers on platelet and white cell serology, scientific articles covering original investigations, and papers on the use of computers in the blood bank. Deadlines for receipt of manuscripts for the March, June, September, and December issues are the first weeks in November, February, May, and August, respectively. Instructions for scientific articles and case reports can be obtained by phoning or faxing a request to Mary H. McGinniss, Managing Editor, *Immunohematology*, at (301) 299-7443, or see “Instructions for Authors” in every issue of *Immunohematology* or on the Web. Include fax and phone numbers and e-mail address with your manuscript.

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Neonatal alloimmune thrombocytopenia due to anti-HPA-2b (anti-Ko^a)

M. Goldman, E. Trudel, L. Richard, S. Khalife, and G. M. Spurlin

Most severe cases of neonatal alloimmune thrombocytopenia (NAIT) are due to anti-HPA-1a (anti-Pl^a) antibodies. We report a case of NAIT due to anti-HPA-2b that resulted in intracranial hemorrhage. A 33-year-old G2P1A0 Caucasian woman had a routine ultrasound at 34 weeks. The fetus appeared to have a left hemispheric hematoma. IVIG, 1g/kg, was started immediately and administered weekly until delivery. One day after receiving the first dose of IVIG, fetal platelet count was 18 × 10^9/L, and Hb was 116 g/L. Eleven mL of matched platelets compatible by monoclonal antibody immobilization of platelet antigens (MAIPA) assay were transfused in utero, raising the platelet count to 62 × 10^9/L. Repeat transfusions were done later that week and 1 week later, with pretransfusion counts of 19 × 10^9/L and 16 × 10^9/L, respectively. Delivery by C-section was done at 35.5 weeks, after the third platelet transfusion. Platelet count at birth was 77 × 10^9/L. Drainage of the hematoma was performed after transfusion. Testing with a solid phase ELISA revealed reactivity against GPIb/IX. MAIPA testing after platelet treatment with the protease inhibitor leupeptin demonstrated the presence of anti-HPA-2b. On PCR-SSP the mother was HPA-2a homozygous, the father was HPA-2a/2b. Antibodies against the HPA-2b antigen located on the GPIb/IX complex have been reported in rare cases of NAIT. Testing is complicated by proteolytic degradation of the antigen-bearing fragment. Compatible platelets are easily found since approximately 85 percent of donors are HPA-2a/2a.


Key Words: neonatal thrombocytopenia purpura, NAIT, anti-HPA-2b, anti-Ko^a

Neonatal alloimmune thrombocytopenia (NAIT) is caused by maternal antibodies directed against an antigen on paternal and fetal platelets. NAIT can result in intracranial hemorrhage and death or severe neurologic sequelae. Unlike its RBC counterpart, erythroblastosis fetalis, NAIT may affect the firstborn infant. In the Caucasian population, most severe cases of NAIT are due to anti-HPA-1a (anti-Pl^a). Anti-HPA-5b (anti-Br) antibodies account for approximately 20 percent of serologically proven NAIT. They usually are associated with milder thrombocytopenia and therefore lead to less severe clinical manifestations. HPA-2 (Ko, Sib) platelet alloantigens are located on GPIbα. Anti-HPA-2b antibodies have been found in multitransfused patients and in rare cases of NAIT. We report an additional case of severe NAIT due to anti-HPA-2b. The case illustrates the importance of performing sufficient laboratory investigations to detect antibodies other than anti-HPA-1a in a clinical setting suggestive of NAIT, in order to supply appropriate phenotyped platelets for transfusion support.

Case Report

The patient, a 33-year-old Caucasian woman of Greek origin, G2P1A0, had an unremarkable first pregnancy. During her second pregnancy, a routine ultrasound of the fetus at 34 weeks’ gestation showed a left hemispheric hematoma. She was taking no medication and had no other medical problems. IVIG (1g/kg/week) was started. Percutaneous umbilical cord blood sampling (PUBS) done 1 day post IVIG administration showed a fetal Hb of 116 g/L and a fetal platelet count of 18 × 10^9/L. Microplate ELISA testing demonstrated strong reactivity against GPIb/IX in the
maternal serum as well as against HLA Class I antigens. Three intrauterine transfusions of 11 mL each were given using platelets that were crossmatch compatible in the monoclonal antibody immobilization of platelet antigens (MAIPA) assay. Perinatal transfusions and the fetal platelet count are illustrated in Figure 1. After Cesarean delivery, a fourth crossmatch-compatible transfusion was given and the hematoma, reconfirmed on ultrasound, was evacuated. No petechiae or other signs of bleeding were noted at delivery. The baby was discharged home and is developmentally normal to date, although a seizure disorder is present.

Material and Methods

**GTI PAK™ 12 assay**

In the ELISA assay, patient’s serum was added to the microwells of the GTI PAK™ 12 platelet antibody screening kit (GTI, Brookfield, WI) coated with platelet glycoproteins and HLA class I antigens, allowing for antibody to bind when present. After washing away unbound immunoglobulins, an alkaline phosphatase–labeled anti-human IgG was added. Unbound anti-IgG was washed away and the enzyme substrate P-nitrophenyl phosphate was added. After incubation, the reaction was stopped by a sodium hydroxide solution and the optical density was measured in a spectrophotometer at 405 nm.

**MAIPA**

The MAIPA assay was slightly modified from the method described by Kiefel et al. Platelets (20 × 10^6) isolated from EDTA-anticoagulated blood from known donors were washed, suspended in 50 µL of PBS-BSA 2%, and incubated with 50 µL of human serum at 37°C for 30 minutes. Platelets were then washed in PBS-BSA 2% and incubated with 0.2 µg of the glycoprotein-specific monoclonal antibody (anti-GP1b/IX, Immunotech, Marseille, France) at 37°C for another 30 minutes. Platelets were washed in 100 µL of isotonic saline and centrifuged × 3 at 12,000-14,000 g for 2 minutes. After the last centrifugation, the platelets were suspended in 100 µL solubilization buffer (containing 1% leupeptin) and allowed to lyse at 4°C for 30 minutes. The lysates were then centrifuged at 12,000–14,000 g for 30 minutes at 4°C and 50 µL of the supernatant was diluted in 200 µL of Tris buffer saline (TBS). One hundred µL of the dilution was distributed into wells of a microtiter plate coated with goat antihuman IgG (1 in 500). After overnight incubation at 4°C the microplate was washed × 5 with TBS and incubated 120 minutes at 4°C with 100 µL of goat anti-human IgG labeled with peroxidase (1 in 500). The wells were then washed × 5 and filled with 100 µL of orthophenylenediamine (OPD) substrate solution. After a 15-minute incubation at room temperature in the dark, the enzyme reaction was measured in a spectrophotometer at 490 nm.

**Allele-specific PCR (PCR-SSP)**

Amplification was run using 25 µM each of the specific primers, as described by Skogen et al. The primers are designed as follows:

- HPA-2a specific primer: 5′-CCCCCAGGGCTCCTGAC-3′
- HPA-2b specific primer: 5′-CCCCCAGGGCTCCTGAT-3′
- HPA-2c common primer: 5′-GCAGCCAGCGACGAAAATA-3′

The cycling protocol used includes one cycle of 94°C for 5 minutes; 30 cycles of 94°C for 1 minute, 65°C for 2 minutes, and 72°C for 1 minute; and one cycle of 72°C for 10 minutes. The mixture is made using 1.5 mM MgCl2 10 X reaction buffer, 25 µM dNTPs mix, 5 U/µL Taq polymerase (2.5 U/amplification), and the human growth hormone gene as an internal control.

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<td>Control serum</td>
<td>0.121</td>
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<td>Maternal serum</td>
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<td>0.269</td>
<td>0.298</td>
<td>0.319</td>
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Results

On GTI PAK™ 12 testing, strong reactivity was demonstrated against GPIb/IX in the maternal serum (OD = 0.919 vs. OD = 0.036 for negative control), as well as against HLA Class I (OD = 1.741 vs. OD = 0.056 for negative control).

MAIPA testing using leupeptin-treated platelets and monoclonal antibody anti-GPIb/IX demonstrated reactivity against paternal and known HPA-2b(+) platelets, as demonstrated in Table 1.

In PCR-SSP, the mother was found to be HPA-2a/2a, while the father was HPA-2a/2b.

Discussion

The HPA-2 (Ko) alloantigen system was first described by van der Weerdt et al. in 1961. The gene frequency of HPA-2b is 0.09 in Caucasians in the United States.
States, 0.18 in African Americans, and 0.13 in Koreans.9

The GPIb/IX/V complex is the platelet receptor for von Willebrand Factor (vWF). The HPA-2 alloantigens are located in the N-terminal globular portion of the molecule, as is the vWF binding site. In vitro, anti-HPA-2b antibodies have been shown to inhibit vWF binding.7 It is therefore possible that the antibody may affect platelet function as well as platelet survival.

The N-terminal globular portion of the GPIb molecule is sensitive to proteases.7 Therefore, it is preferable to use a protease inhibitor, such as leupeptin, during MAIPA testing to avoid degradation of the antigen. In our protocols, 1% leupeptin is added to platelet suspensions before testing, and also at the step of solubilization in the lysing buffer.

The GTI PAK™ 12 microplate testing system permits relatively rapid identification of reactivity against a given platelet glycoprotein. However, we and others have found that alloantibodies may be undetectable in PAK™ 12 testing or may give nonspecific reactions.10,11 Therefore, although the GTI kit is useful as a screening test, it must be complemented by other laboratory investigations. The MAIPA assay, first described by Kiefel et al.,6 is used in many platelet serology laboratories. At the 10th ISBT International Platelet Genotyping and Serology Workshop, in 2000, the MAIPA assay was reported to be used alone, or in combination with other methods, by 28 of 38 laboratories.12 Although this assay may be more sensitive and specific than the GTI kit, it also requires a minimum of 10 hours of technical time and an overnight incubation period. In addition, a monoclonal antibody against the glycoprotein complex of interest must be added. MAIPA testing using monoclonal antibodies solely against GPIIb/IIIa and GPla/IIa in the expectation of finding an anti-HPA-1a or anti-HPA-5b antibody, respectively, would have given a negative result in this case. In addition, the use of a frozen platelet panel or of platelets that have been stored without protection from proteases may lead to antigen degradation and a negative result. Finally, allele-specific PCR confirms the alloantigen incompatibility between the two parents. However, it does not prove that an alloantibody is actually present. Because alloantisera are extremely rare, genotyping permits the laboratory to develop a panel of platelets of known HPA-2 specificities and to establish a bank of HPA-2a/2a donors.

There have been several other reported cases of NAIT due to anti-HPA-2b. Kroll et al.13 reported two cases in Germany. In the first case, the third child of healthy parents was found to have a postpartum platelet count of $53 \times 10^9/L$ at delivery, which decreased to $11 \times 10^9/L$ on day 4. Three maternal platelet transfusions were necessary to prevent bleeding. In the second case, a second pregnancy was complicated by erythroblastosis fetalis due to anti-CD treated by intrauterine transfusion. Fetal death occurred, and a platelet count of $3 \times 10^9/L$ was found. Because of the small numbers of cases reported and the laboratory diagnostic difficulties discussed, it is difficult to know if these cases represent the true range of severity of NAIT due to anti-HPA-2b.

The frequency of NAIT due to anti-HPA-2b is difficult to determine. In a large prospective study by de Moerloose et al.,14 of 8388 newborns in Switzerland, 40 newborns had NAIT; anti-HPA-1a and anti-HPA-5b antibodies were detected in three and two mothers, respectively, and no anti-HPA-2 antibodies were present. In a study by Hohlfeld et al.,15 of 5194 neonates in France undergoing fetal blood sampling for cytogenetic analysis and various infections and hematologic disorders, anti-HPA-1a and anti-HPA-5b were detected in 23 and 2 mothers, respectively, but no anti-HPA-2 antibodies were present. In a series of 975 cases of NAIT reported by McFarland et al.16 spanning 11 years of testing by a tertiary referral center, only three cases of anti-HPA-2b were detected. In all three cases, anti-HPA-2b was found in the presence of other alloantibodies (anti-HPA-3a in one, anti-HPA-3b in another, and anti-HPA-1a and anti-HPA-3a in the third).

Although anti-HPA-2b antibodies are a rare cause of NAIT, they should be considered in a clinical setting that is suggestive of NAIT, when more commonly seen antibodies have not been found. The detection of these antibodies in the MAIPA assay requires fresh platelets, treatment with leupeptin, and the use of an anti-GPIb/IX monoclonal antibody. The GTI PAK™ 12 assay may be useful in screening for these antibodies.

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References


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Quantitation of red cell-bound IgG, IgA, and IgM in patients with autoimmune hemolytic anemia and blood donors by enzyme-linked immunosorbent assay

A. A. Bencomo, M. Diaz, Y. Alfonso, O. Valdés, and M. E. Alfonso

This paper describes an enzyme immunoassay for the quantitative determination of IgG, IgA, and IgM immunoglobulins on RBCs. Ether eluates made from RBCs were followed by an enzyme-linked immunosorbent assay of immunoglobulin concentration. Calibration curves were derived from immunoglobulin standards and the number of molecules of each isotype per RBC was calculated. The assay was carried out in 200 healthy blood donors and 62 patients with warm autoimmune hemolytic anemia (AIHA), two of them with a negative DAT. For healthy blood donors, mean values were 58 IgG, 16 IgA, and 3 IgM molecules per RBC. For patients with a positive DAT, the mean values were 3435 IgG, 157 IgA, and 69 IgM molecules per RBC. An increased level of IgA was found in 12 patients without IgA autoantibodies demonstrable in RBC eluates. Increased IgG levels were also observed in patients with a negative DAT and, in one case, an increased level of IgA was also found. The enzyme-linked immunosorbent assay using ether eluates is a sensitive method for quantitating RBC autoantibodies in patients with AIHA as well as immunoglobulins bound to RBCs in healthy individuals. Immunohematology 2003;19:47–53.

Key Words: enzyme-linked immunosorbent assay, autoimmune hemolytic anemia, IgG, IgA, and IgM immunoglobulins, RBCs, quantitative method

Autoimmune hemolytic anemia (AIHA) is characterized by immune-mediated hemolysis associated with the presence of IgG, IgA, or IgM immunoglobulins on the RBC membrane. The most common type of AIHA is associated with warm-reactive antibodies and in most cases they are detectable by the DAT. The presence of immunoglobulins can also be revealed on the surface of RBCs of DAT-negative healthy individuals by more sensitive methods. Several methods to quantitate RBC-bound immunoglobulins have been reported, including an immunoradiometric assay, autoanalyzer assays, antiglobulin consumption assays, and enzyme-linked immunosorbent assay (ELISA). Quantitation of immunoglobulins in a patient with AIHA would allow a more precise method of monitoring the disease or defining the synergistic effect of the different immunoproteins in causing RBC destruction.

We have developed an enzyme immunoassay for the quantitative determination of immunoglobulin isotypes in ether eluates made from RBCs. This method can be used to study RBC-associated immunoglobulins in patients with AIHA and in healthy individuals.

Materials and Methods

Blood samples and ether elution

After informed consent had been obtained from patients and blood donors, blood samples were collected from venous blood in EDTA (Merck). The samples were centrifuged at 270 × g for 8 minutes at room temperature, and the plasma and buffy coat were removed.

Ether eluates were prepared after washing 1 mL of packed RBCs × 10 using 10 volumes of saline. Elution was performed by adding 1 mL of PBS, pH 7.2, containing 0.4% BSA (Sigma Chemical Co.) and 1 mL of diethyl ether (Merck) to 1 mL of RBCs (an average of 1.1 × 10^10 RBCs). The mixture was incubated in a 37°C water bath for 10 minutes, with frequent mixing. After centrifugation, the upper layer of ether was discarded and the hemoglobin-stained eluate was transferred into a test tube. The residual ether was evaporated at 37°C for 15 minutes. An average of 1 mL of eluate was recovered. Eluates were kept frozen at −30°C until used.
ELISA for the quantitation of IgG, IgA, and IgM on RBCs

IgG, IgA, and IgM standard curves were prepared from reference sera (Nor-Partigen, Behring) in concentration ranges from 0.0025 to 0.1 µg/mL in PBS, 0.05% Tween (Tw) 20 (Merck), and 0.4% BSA (PBS/Tw/BSA). Flat-bottomed 96-well plates (Maxisorp, Nunce Immunoplates) were precoated with 100 µL per well of 5 µg/mL goat anti-human IgG, IgA, or IgM (Sigma) in 0.05M carbonate buffer, pH 9.6, at 4°C temperature overnight. After washing × 4 in PBS/Tw, 0.2%, 100 µL per well of standards or diluted eluates in PBS/Tw/BSA were incubated at 37°C for 60 minutes. The plates were washed × 4 in PBS/Tw, 0.05%, before addition of 100 µL per well of peroxidase conjugated (Sigma) goat anti-human IgG or IgM diluted 1 in 5000 or goat anti-human IgA diluted 1 in 1000. The plates were incubated with the conjugate at 37°C for 60 minutes and then washed as above. A substrate solution (100 µL per well) of 0.24 mg/mL of O-phenylenediamine (Merck) in 0.05M phosphate citrate buffer, pH 5.0, with 0.024% of hydrogen peroxide (Merck) was added. Color development was stopped after 15 minutes by adding 100 µL per well of 3M H2SO4 (BDH) and absorbance was read at 492 nm on the Titertek Multiscan MC plate reader. Absorbances of duplicate determinations were plotted against the concentration of the standard points (linear scale). The amount of IgG, IgA, and IgM was calculated and the results were expressed as the approximate number of molecules of immunoglobulin per RBC, using Avogadro’s constant and the molecular weights of IgG or IgA (160,000 daltons) and IgM (970,000 daltons). According to Avogadro’s constant, 1 µg contains 376 × 10¹⁰ molecules of IgG or IgA and 62 × 10¹⁰ molecules of IgM. The number of molecules of immunoglobulins on RBCs is equal to the concentration in µg/mL of Igs in eluates, multiplied by the molecules of Igs in 1µg, and divided among the number of RBCs in 1 mL.

Effect of test sample milieu on IgG, IgA, and IgM quantitation by ELISA

We obtained free hemoglobin from human RBCs as follows: To 6 mL × 10 washed RBCs an equal volume of hypotonic lytic solution (5M sodium phosphate buffer, pH 7.4) was added. We placed the mixture in a 37°C water bath for 10 minutes. The hemoglobin solution was collected by centrifugation at 370 × g for 30 minutes at room temperature and dialyzed overnight against 20 volumes of PBS. IgG, IgA, and IgM standard curves from reference serum (Nor-Partigen, Behring) were prepared in the hemoglobin solution and one sample without immunoglobulins was used as a blank. To 1 volume of each point of standard curves prepared in the hemoglobin solution and the blank, an equal volume of diethyl ether was added and mixed. The mixture was incubated at 37°C for 10 minutes. The upper layer of ether was carefully discarded and the residual evaporated at 37°C for 15 minutes. BSA and Tw 20 were added to each sample to obtain a concentration of 0.4% and 0.05%, respectively. The ELISA was carried out with standard curves with this treatment and with standard curves prepared in PBS/Tw/BSA. The slopes of the curves were compared with the t test.

Analytical evaluation of the ELISA

The limit of detection was calculated as the concentration of the dilution of the first point of the curves that gave an absorbance more than the mean absorbance of the blank, plus 3 standard deviations (SDs). The undiluted and diluted 1 in 5, 1 in 10, 1 in 100, and 1 in 500 in PBS/Tw/BSA of hemoglobin solution treated with ether and the PBS/Tw/BSA were used as blanks and also as a control for nonspecific binding by the assay. The mean and SD were calculated on 20 repeat estimation assays.

The working range of the assay was determined by testing in replicates of 10 at each point of the standard curves of IgG, IgA, and IgM and the coefficient of variation (CV) was calculated. The working range was accepted as that over which CV was no greater than 10%.

Parallelism was investigated after serial dilution in PBS/Tw/BSA of seven eluates made from RBCs with reactions of 4+ (IgG, IgA) or 3+ (IgM), 1+, and negative in the DAT with anti-IgG (BPL; Elstree, UK), IgA, and IgM (CLB; Amsterdam).

The eluate of a pool of ten DAT-negative RBCs as well as two eluates with IgG, IgA, and IgM autoantibodies, and a standard human serum with known concentration of immunoglobulins (Immunotrol, Bio-Merieux, France) adjusted to a concentration of 0.052 µg/mL of IgG, IgA, and IgM, were used to determine the precision of the ELISA. The intra-assay and the inter-assay coefficient of variation on separate days were calculated on 20 determinations.

Test samples

The ELISA was carried out in duplicate in ether eluates from 200 healthy blood donors with a negative
DAT, 60 DAT-positive patients with warm AIHA, and two patients with AIHA and a negative DAT. Hemolytic anemia was documented by a low hemoglobin concentration, increased reticulocytes, increased unconjugated bilirubin concentration, the presence of marrow erythroid hyperplasia, and the exclusion of other hemolytic disorders. Thirty-nine patients had idiopathic AIHA; the hemolytic anemia in the remainder was associated with other conditions: four patients had systemic lupus erythematosus, four had unspecified respiratory infections, four had chronic lymphocytic leukemia, three were on methyldopa, three had chronic active hepatitis, two had multiple myeloma, two had acute lymphocytic leukemia, and one had non-Hodgkin's lymphoma. Patients' ages ranged from 1 to 84 years (median 45 years) and 41 were female and 21 were male. The eluates from blood donors were tested undiluted and diluted 1 in 5; those for patients were tested after serial dilution in PBS/Tw/BSA to ensure that the tests were carried out within the working ranges for the assay.

Immunohematologic studies

The immunoglobulin classes of the autoantibodies were determined by the DAT using monospecific anti-IgG (BPL), -IgM, and -IgA reagents (CLB, Amsterdam) and in the eluates by a microplate test.

The microplate test was performed using microtiter plates with V-shaped bottom wells (Greiner) as follows: 10 µL of the mixture of three kinds of packed group O RBCs (R₁R₁, R₂R₂, and rr), were incubated with 100 µL of the eluate at 37°C for 1 hour in a tube. The RBCs were washed × 4 and 0.5% of RBC suspensions in saline were made. Anti-IgG, -IgA, and -IgM were diluted 1 in 2, 1 in 5, 1 in 10, and 1 in 20 in PBS with 5% of fetal calf serum (Gibco, UK). Then 25 µL of the antiserum dilutions and the diluent (as negative control) were transferred into appropriate microtiter wells and an equal volume of the sensitized RBC suspension was added. After overnight incubation at 4°C, the microtiter plate was placed at a 60° angle for 10 minutes, then inverted for 5–10 minutes and read. When the result was positive, the cells remained together in a tight button, when negative they ran down in a smear.

All the patients with a positive DAT had IgG autoantibodies. In 11 and six patients, IgA and IgM autoantibodies, respectively, were also present. Similar patterns of immunoglobulin classes were found in the eluates and with the DAT. Antibodies were not detected in eluates from patients with AIHA and a negative DAT.

Results

Curves of IgG, IgA, and IgM represent the mean of ten consecutive standard curves, each performed in duplicate. Immunoglobulin standards made up in hemoglobin and treated with ether did not significantly affect the standard curves (p > 0.49) (Fig. 1). The hemoglobin solution treated with ether used as a blank gave values of optical density from 0.053 to 0.061 similar to the PBS/Tw/BSA (0.051). The working ranges for the ELISA were found to be 0.0025 to 0.07 µg/mL for IgG and IgM and 0.0025 to 0.06 µg/mL for IgA. The limit of detection for IgG corresponded to 0.00062

Fig. 1. Curves for quantitating IgG (Fig. 1A) and IgA (Fig. 1B) with immunoglobulin standards prepared in PBS, Tween 20 (0.05%), and BSA (0.4%) (●) and in hemoglobin treated with ether (●). The curve for IgM is similar to that for IgG.
µg/mL, for IgA to 0.0011 µg/ml, and for IgM to 0.0068 µg/ml (approximately 1 molecule of Igs per RBC). The ELISA showed excellent parallelism for all the samples diluted in the measuring ranges with interdilutional coefficient of variation from 5.9% to 16% for IgG samples, from 2.9% to 13% for IgA samples, and from 2.4% to 8.6% for IgM samples (Fig. 2). The intra-assay variation ranged from 3.6% to 6.7% for IgG samples, from 3.1% to 6.5% for IgA samples, and from 5.5% to 6.7% for IgM samples. The inter-assay variation ranged from 9.2% to 10% for IgG samples, from 7.5% to 9.5% for IgA samples, and from 8.2% to 9.3% for IgM samples.

It was established that the immunoglobulins present in the eluates were not contaminated by serum immunoglobulins after ten washes of the RBCs (as described in Materials and Methods). The supernatant of the last wash gave values of optical density similar to those of the blank in the ELISA.

The ELISA on healthy blood donors gave mean and standard deviation values of 58 ± 35 molecules of IgG per RBC (range from 2 to 146), 16 ± 11 molecules of IgA per RBC (range from 1 to 81), and 3 ± 2 molecules of IgM per RBC (range from 1 to 11).

In AIHA patients with a positive DAT, the range of results for IgG was from 206 to 20,000 molecules per RBC, with a mean of 3435 molecules. In the group of patients with IgA autoantibodies the range of results was from 90 to 385 molecules per RBC, with a mean of 157 molecules. An increased level of IgA, with a range from 94 to 385 molecules per RBC, was found in 12 patients with a negative DAT and in the test of eluates by microplate with anti-IgA. In the remainder of cases, the values of molecules of IgA per RBC were within the range found in blood donors. A mean value of 69 with a range from 26 to 109 molecules per RBC was obtained in patients with IgM autoantibodies and results were within the normal range in the rest of the patients. The results are shown in Figure 3.

Discussion

The sandwich ELISA described provides a method for the quantitation of RBC-bound IgG, IgA, and IgM immunoglobulins. The assay was carried out in ether eluates because our laboratory routinely performs elution in the investigation of autoantibodies in AIHA and because the testing of eluates is a more sensitive method to detect immunoglobulins than direct testing of the RBCs.14

Differences in the milieu in test samples and immunoglobulin standards can produce nonspecific effects, which modify the kinetics of antigen-antibody reactions and could affect the estimation of Ig concentration from the standard curve.11 However, the presence of hemoglobin and the ether treatment of immunoglobulins did not affect standard curves. Therefore, all the test samples were investigated with immunoglobulin standards prepared in PBS/Tw/BSA. Hemoglobin has a peroxidase activity and obscures the antibody assay if a peroxidase conjugate is used.15 In this ELISA the hemoglobin in eluates was removed by washing and did not interfere with the peroxidase

![Fig. 2.](image-url)
Quantitation of red cell immunoglobulins
c
conjugate. The unaffected immunoglobulin detection after ether treatment was not surprising in view of the known use of this elution method for recovering antibody attached to RBCs, although Dumaswala et al. found decreased detectability of IgG after treatment with another organic solvent such as xylene, using an immunoblotting technique with peroxidase-labeled anti-IgG.

The ELISA showed a wide measuring range; the lower limit corresponded approximately to one molecule of immunoglobulin per RBC. Accordingly, this assay is suitable for the quantitation of immunoglobulins on RBC eluates from DAT-positive patients and from normal subjects. Although the working range had an upper limit of 0.07 µg/mL for IgG and IgM, and 0.06 µg/mL for IgA, it could be extended by dilution of high concentration samples with PBS/Tw/BSA. This was in agreement with the excellent dilution parallelism over the measuring range showed by assay with all sample types tested.

We found the mean amount of 58 IgG, 16 IgA, and 3 IgM molecules per RBC by using the eluates from healthy donors. Other methods gave similar results for IgG and IgM. There has been only one previous report of the number of RBC-associated IgA molecules on normal human RBCs. That study reported a median of < 29 molecules per RBC, which is comparable to the amount quantitated by our assay.

There was considerable variation in the number of IgG, IgA, and IgM molecules per RBC in patients with AIHA, demonstrating the ability of the assay to measure widely different degrees of sensitization in clinical samples. The results are comparable with previous studies where the reported ranges in patients with AIHA were from 230 to about 30,000 IgG molecules, from 20 to 168 IgM molecules, and from < 29 to 4500 IgA molecules per RBC.

We encountered IgA autoantibodies in 18 percent of DAT-positive patients, which is similar to the 14 percent reported previously by Sokol et al. An increase of RBC-bound IgA by ELISA was also found in 12 patients without IgA autoantibodies demonstrable on RBCs by the DAT and in the eluates by a microplate test. The clinical significance of antibodies only detected by ELISA should be further elucidated because an increased amount of cell-bound immunoglobulins may also be due to immune complexes and nonspecific adsorption of Igs onto RBCs rather than as a result of autoantibodies.

Similar considerations are applicable to the results found in two patients with AIHA and a negative DAT without antibodies demonstrated in eluates. However, in these cases there were findings to suggest that IgG and IgA (in one case) antibodies detected by ELISA were responsible for the hemolysis. The hemolytic anemia in these patients was associated with chronic lymphocytic leukemia and non-Hodgkin's lymphoma, respectively. It is known that the incidence of AIHA is higher in patients with these hematological malignancies than in the general population. Previous investigators have demonstrated IgG and IgA autoantibodies on RBCs with more sensitive methods.
than the DAT in patients with a DAT-negative AIHA.\textsuperscript{21-25} The patients were diagnosed by clinical manifestations including the exclusion of other hemolytic disorders and a positive clinical course following administration of corticosteroid.

These results showed that the quantitative determination of immunoglobulins on eluates by ELISA appears to be useful for the serologic diagnosis of DAT-negative AIHA, although the assay should be evaluated with a larger number of cases.

Previous investigation identified RBC autoantibodies of the IgM class in approximately 30 percent of patients with warm AIHA by enzyme-linked DAT.\textsuperscript{26} We found a frequency (around 10\%) of warm IgM autoantibodies that is similar to those found in earlier studies\textsuperscript{20} in both the DAT and the ELISA. This difference may be attributable to multiple washing required by our assay, which carries the possibility for loss of small quantities of IgM autoantibodies prior to elution. In addition, it can be difficult to obtain IgM autoantibodies in eluates. An investigation showed that heat elution was the better method compared to acid stromal, chloroform-trichlorethylene, and freeze-thaw methods.\textsuperscript{27} We detected IgM antibodies in ether eluates of only three of the six patients with IgM autoantibodies, when a conventional IAT with anti-IgM was used (data not shown). However, all the IgM autoantibodies revealed by the DAT were detected in the eluates by the microplate test and ELISA. As described previously, ELISA also quantitated a very small quantity of IgM on RBCs of healthy blood donors. We have no explanation for our different findings from a previous report.\textsuperscript{26}

In essence, the ELISA, using ether eluates, is a sensitive method to follow patients with RBC autoantibodies as well as immunoglobulins bound to RBCs in healthy individuals. The assay can be also extended to measure IgG subclasses or C3 by using appropriate specific antiserum.

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Attention: State Blood Bank Meeting Organizers
If you are planning a state meeting and would like copies of *Immunohematology* for distribution, please contact Mary McGinniss, Managing Editor, 4 months in advance, by phone or fax at (301) 299-7443.
ABO and Rh(D) blood typing on the PK 7200 with ready-to-use kits

P. Moncharmont, A. Plantier, V. Chirat, and D. Rigal

The performance of ready-to-use kits was evaluated on the PK 7200 blood grouping system. The Olymp Group (kit 1) and Olymp Group II (kit 2) containing anti-A, -B, -AB, and -D reagents were tested for first and second determinations of A, B, and D antigens. More than 500 RBC samples, including several variant ABO and D phenotypes, were evaluated for specificity, repeatability, reproducibility, and sensitivity. Specificity was tested with well-characterized reagent RBCs. Repeatability was established by at least 12 assays per run with three reagent RBCs, and reproducibility was established on one run per day for 5 days. No discrepancy was observed in ABO and D determinations with either kit. In repeatability, three discrepancies were found with group A and B RBCs with kit 1. In reproducibility, no discrepancies were observed. The kit 1 anti-A reagent detected A, but not A, RBCs and anti-AB detected both. A B RBC was detected by both kits. Among eight weak D phenotypes, six were positive with kit 1. With kit 2, only one of five weak D phenotypes was detected.

Key Words: ABO, Rh(D), PK 7200 automated blood grouping system

Improvements in ABO and D typing may be related to the introduction of new technologies1 or to changes in reagents.2 New technologies include microplate testing, i.e., the gel test,3 and solid phase systems.4 Blood typing reagents manufactured from human or animal plasma or serum have been replaced with new monoclonal reagents. With new reagents and technologies, automation of blood typing using computerized systems has been tested and introduced into the laboratories. Recently, ready-to-use reagents linked to one automated system were offered by the manufacturer. We tested their ready-to-use kits for ABO and D blood typing on a fully automated blood grouping system, the PK 7200, and detected certain problems regarding identification of weak D phenotypes.

Materials and Methods

Methods

Two ready-to-use kits, the Olymp Group (kit 1) and Olymp Group II (kit 2) (Diagast Laboratories, Loos, France) were evaluated on the PK 7200 (Olympus Biology, Rungis, France). Monoclonal anti-A, -B, -AB, and -D reagents were used in these kits. Clones for each kit were different (see Table 1). Specific microplates are used on the PK 7200. In each well, hemagglutination reading is enhanced by a charge-coupled device (CCD)
ABO and D typing on the PK 7200 camera with a high-resolution image. Results are reported as positive, negative, or indeterminate. Control samples: group A, B, AB, O, D+, and D– were tested before and after each run.

Four parameters were evaluated: specificity, repeatability, reproducibility, and sensitivity. For each antibody specificity, at least 100 RBC samples known to be positive for the antigen and 100 RBC samples known to be negative for the antigen were tested. Repeatability was established on at least 12 assays per run, with three well-characterized RBC samples per reagent. Reproducibility was determined in one run per day for 5 successive days with the same RBC samples, which were stored at 4°C. For sensitivity, the following weak phenotypes were tested: A3, A*, and B3 for the ABO system and weak D RBCs. When a falsely negative test was obtained with a reagent, the variant RBCs were retested.

Results

Three hundred samples of A, B, AB, and O RBCs and 240 D+ and D– RBCs (122 D+, 118 D–) were tested versus kit 1. Two hundred sixty-nine samples of A, B, AB, and O RBCs and 329 samples of D+ and D– RBCs (226 D+ and 103 D–) were tested versus kit 2. No discrepancies were observed in A, B, or D determinations. However, in repeatability testing, three discrepancies were detected with group A and B RBCs with kit 1. Two were group A and one was group B. No discrepancy was found with kit 2. In reproducibility testing, no discrepancy was observed with kit 1 or kit 2 with RBCs tested for 5 successive days (Table 2).

Table 2. ABO discrepancies (repeatability and reproducibility)

<table>
<thead>
<tr>
<th>RBCs</th>
<th>Repeatability*</th>
<th>Reproducibility†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kit 1</td>
<td>Kit 2</td>
</tr>
<tr>
<td>Group A</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Group B</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Group AB</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Established on ≥ 12 assays per run vs. three well-characterized RBCs per reagent  
†Determined by one run per day for 5 successive days with the same RBCs

With kit 1 anti-A reagent detected A3 RBCs but not the A* RBCs, and the anti-B reagent gave a positive result with the B3 RBCs. These three variant RBCs were detected also by the anti-AB reagent. Kit 2 detected the A3 and B3 RBCs. Unfortunately, no fresh A* RBCs were available at the time we tested kit 2.

With kit 1, eight known weak D RBC samples were tested. Four were positive, two gave indeterminate results, and two gave a falsely negative result. With kit 2, of the five weak D RBC samples tested, only one was positive, none gave indeterminate results, but four gave falsely negative reactions (Table 3).

Table 3. Detection of weak D phenotypes

<table>
<thead>
<tr>
<th>Kit</th>
<th>(8)*</th>
<th>Kit 2</th>
<th>(5)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive</td>
<td>indeterminate</td>
<td>negative</td>
<td>positive</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

*Number of samples tested

Discussion

Our findings indicate that using the PK 7200 and ready-to-use kits, difficulties remain, particularly in typing blood donors with variant RBCs (i.e., A3 and weak D). With the first-generation Olympus blood grouping system, the PK 7100, sensitive monoclonal anti-D reagents did not guarantee detection of weak D phenotypes. Confirmation with another sensitive technology (e.g., a gel test) was needed. The detection system for the PK 7200 includes a CCD camera to enhance the detection of hemagglutination in the reaction wells and, therefore, increase the effectiveness of the tests. Nevertheless, to avoid falsely positive or falsely negative reactions, reagents must be adapted to the automated system in use. Recently, some manufacturers have offered ready-to-use reagents.

Our study shows that two ready-to-use kits failed to detect weak D phenotypes. Despite improvements in the PK 7200, kit 2 failed to detect four of five weak D phenotypes. The anti-D reagents in kits 1 and 2 are different. Kit 1 anti-D reagent contains a mix of four monoclonal antibodies, while kit 2 has only one. The anti-D reagents in both kits include an IgM monoclonal antibody (P3x61 clone for kit 1, and HM 10 for kit 2). In fact, Jones et al. recommend the use of an IgM monoclonal anti-D with high avidity in saline for detection of weak D phenotypes. Williams observed good detection of weak D phenotypes with both the IgM monoclonal antibody clone P3x61 (84%) and clone HM 10 (74%). In our short study, kit 1 anti-D (P3x61) detected 75 percent (6 of 8) of the weak D phenotypes, whereas with kit 2 (HM 10) anti-D reagent, which contains one monoclonal antibody, only 20 percent (1 of 5) were detected. Furthermore, Scott and Voak state that, for detecting all weak D phenotypes, a single monoclonal anti-D is not adequate. To increase the efficiency in blood donor typing, particularly for weak D phenotypes, kit 2 must be used with another kit for the same automated system or with another technology (e.g., a gel test).
Improvement in kit 2, i.e., addition of one or more anti-D monoclonal antibodies, is needed. Lastly, detection of A\textsubscript{x} RBCs is increased by using an anti-AB reagent.

Several hypotheses could explain the discrepancies observed with kit 1 anti-A and anti-B reagents with the repeatability assay. First, a lower volume of RBCs and/or reagent could have been dispensed by the PK 7200 in the wells of the microplate. Nevertheless, at the time of the assay, no other grouping difficulty was observed. Secondly, a wrong reading was possible, but no problem was noted with the CCD camera. Even though the assay was performed according to the manufacturer’s directions, these discrepancies are not clearly explained. Nevertheless, discrepancies with kit 1 anti-A and anti-B reagents were only observed with the repeatability assay. The other tests for ABO blood grouping gave good results with kit 1.

**Conclusion**

The ready-to-use kits for ABO and D blood typing on the PK 7200 blood grouping system are efficient in specificity, repeatability, and reproducibility for routine testing of RBCs with normal ABO and D antigen expression. However, numerous discrepancies, namely falsely negative reactions, remain with kit 2 for weak D phenotypes. We recommend the use of kit 2 in association with kit 1 or with another ready-to-use reagent or technology for weak D typing.

**References**


Pierre Moncharmont, MD, PhD, Chief of office, Department of Immunology, E.F.S. Rhône-Alpes Site de Lyon, 1-3 rue du Vercors, 69364 Lyon cedex 07 France; Annick Plantier, Technician, Véronique Chirat, Technician, and Dominique Rigal, MD, PhD, Director, E.F.S. Rhône-Alpes Site de Lyon, Lyon, France.
One hundred voluntary blood donors in Hanoi were typed for antigens in the MNS, Rh, Kell, Duffy, and Kidd blood group systems. They were also tested for the presence of the Mi.III (GP.Mur) phenotype and Lewis system antigens. The Jk phenotype frequencies were markedly different from those previously reported. The frequency of the Mi.III phenotype was similar to that reported in Chinese and Taiwanese.

**Key Words:** blood groups, phenotype frequencies, Vietnam, Jk, Miltenberger

There is minimal information on blood group frequencies in Vietnam in English-language scientific journals. The few reports have not included the Miltenberger (Mi) phenotypes, of which Mi.III (GP.Mur) is present with a relatively high frequency in Chinese, Thai, and Taiwanese populations. There is also a likelihood of erroneous information on Jk phenotypes in previous publications.\(^1,2\)

### Materials and Methods

To investigate the frequency of the Mi.III (GP.Mur) phenotype and to reevaluate Jk phenotype frequencies, 100 volunteer blood donors in Hanoi were typed for antigens of the MNS, Rh, Kell, Duffy, and Kidd blood group systems and antigens in the Lewis system. ABO groups were not performed, as previous reports from different areas of Vietnam were in general agreement. A total of 160 donors were tested for Mi.III, using anti-Mur and anti-Mur/Hop sera.

Blood group antisera were donated by CSL Biosciences (Australia) and Gamma Biologicals (USA). Anti-Jk\(^a\) and anti-Jk\(^b\) were IgM monoclonal reagents. Human anti-Mur and anti-Mur/Hop sera were kindly supplied by Mr M.K. Mak of the Hong Kong Red Cross Blood Transfusion Service. All blood typings were performed manually by the tube method according to manufacturers’ instructions. Blood samples were collected randomly from volunteer blood donors at the blood bank at Thanh Nhan Hospital, Hanoi.

### Results

Most of the blood group phenotype frequencies were similar to those in other Asian populations (Table 1). However, the Jk phenotype frequencies were markedly different from those previously reported in Vietnam (Table 2). The Mi.III phenotype was present at a level similar to that reported in the Chinese and the Taiwanese (6%), but testing of an additional 60 donors for Mi.III raised the frequency to 6.5 percent (Table 1).

### Discussion

The phenotype frequencies reported here should be considered to be approximate and some amendments may be expected when larger numbers of Vietnamese are tested.

For the majority of the phenotypes, the results corresponded closely to those obtained on blood donors in Ho Chi Minh City (South Vietnam) by Tran Van Bé,\(^1\) but significant differences were noted in the Jk system.

The discrepancies between our Jk typings and those reported in earlier studies of the Vietnamese population are striking.\(^1,2\) The earlier studies reported a high frequency of the Jk(a−b−) phenotype, while the

| Table 1. Approximate phenotype frequencies of Hanoi donors based on testing 100 donors |
|-----------------|------|-----|-----|-----|-----|-----|
| MNSs Rh Fy Jk Kell Le |
| Ms 58% CDe 57% Fy(a+b−) 88% Jk(a+b−) 25% K−k+ 100% Le(a+b−) 0% |
| Ms 7% CcDe 13% Fy(a+b+) 11% Jk(a+b+) 53% K+k+ 0% Le(a−b+) 62% |
| Ms 18% cDDe 6% Fy(a−b+) 1% Jk(a-b+) 22% K+k− 0% Le(a+b+) 8% |
| Ms 17% cDE 3% |
| CcDDe 19% |
| cDDe 1% |
| cDDe 1% |

Mi.III (GP.Mur) 6.0%*  
*Testing of an additional 60 donors raised the incidence to 6.5%
Jk(a+b+) phenotype was completely absent in one study and present at only 5.26 percent in the other. Our results show an absence of the Jk(a–b–) phenotype, with frequencies much closer to those reported in other Asian populations. It is possible that the Jk typings in the earlier Vietnamese studies were erroneous due to the use of low-potency antisera that failed to detect the Jk(a+b+) phenotype.

The presence of the Mi.III phenotype in the Vietnamese population had not been reported previously, but its presence was expected as it had been reported in Thailand, Hong Kong, China, Taiwan, and in Chinese in Australia. Mi.III was present in 6.5 percent of 160 donors. Variation in reactions with two reagents labeled as anti-Mi.III suggests that Mi.VI (Bun) may also be present in North Vietnam as it is in Thailand. Only those samples that reacted with both reagents were classified as Mi.III in the tables. King et al. have shown the value of immunoblotting in determination of Mi phenotypes, but molecular biological and immunoblotting techniques could not be performed in this study. It is hoped that such tests will be performed in the future to more accurately identify the Mi phenotypes present.

Antibodies to the antigens represented in the Mi.III phenotype have previously been reported as causing both hemolytic transfusion reactions and hemolytic disease of the newborn in Asians. Given a 6.5 percent frequency of the Mi.III phenotype in the Vietnamese, it is possible that its presence has resulted in some of the cases of transfusion reactions and hemolytic disease of the newborn that have been reported to be due to unidentified antibodies. It becomes important that screening panels used in Vietnam include RBCs with the Mi.III phenotype, as do those used in Hong Kong.

Further studies to determine whether the differences in JK phenotype frequencies between tests on Hanoi (North Vietnam) donors and on donors from Ho Chi Minh City (South Vietnam) represent a true variation in the population are warranted. Testing of ethnic minorities found in the mountain regions of Northwest Vietnam would also be of anthropological interest.

Table 2. Different phenotype frequencies in the Jk system, according to three studies

<table>
<thead>
<tr>
<th>References</th>
<th>Jk(a+b–)</th>
<th>Jk(a+b+)</th>
<th>Jk(a–b+)</th>
<th>Jk(a–b–)</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study 1</td>
<td>25%</td>
<td>53%</td>
<td>22%</td>
<td>0%</td>
</tr>
<tr>
<td>Tran Van Bé 2</td>
<td>25.85%</td>
<td>0%</td>
<td>21.10%</td>
<td>55.05%</td>
</tr>
<tr>
<td>Do Trung Phan et al. 2</td>
<td>13.68%</td>
<td>5.26%</td>
<td>62.12%</td>
<td>18.94%</td>
</tr>
</tbody>
</table>

References
10. Lin M, Broadberry RE. An intravascular haemolytic transfusion reaction due to anti-Mi(a) in Taiwan. Vox Sang 1994;67:320.
Letter to the Editors

**Vel– donors in Yugoslavia**

The Vel antigen belongs to the high-frequency antigen series (901), ISBT number 901.001.\(^1\) It was described for the first time in 1952 when it was discovered in the serum of a previously transfused patient,\(^2\) and the second example was described in 1955.\(^3\) The Vel- phenotype appears to be inherited as a recessive character.\(^4\) Since the frequency of the antigen is > 99 percent and the antibody usually causes hemolysis,\(^4\) Vel– donors have found a place on many rare blood group donor lists. Anti-Vel is sometimes difficult to identify. In 1994, a misidentified anti-Vel, which caused a fatal hemolytic transfusion reaction due to inappropriate use of serologic techniques, was described.\(^5\)

Anti-Vel was detected in the serum of a group A patient during routine selection of blood for transfusion. She had a history of two pregnancies and had been transfused with four units of blood 4 years before.

Serum prepared from the patient’s plasma, obtained by manual plasmapheresis, was used to type donors’ blood for the Vel antigen. We typed 3108 group A donors and 3633 group O donors.

The serum demonstrated strongly positive reactions at room temperature, by IAT, and versus enzyme-treated RBCs. Antibody specificity, anti-Vel, was determined by the IBGRL in Bristol. Further investigation of the patient’s RBCs confirmed the following phenotype: group A, CcDee, NNss, Lu(a–b+), kk, Le (a–b+), Fy(a–b+), Jk(a–b+), P1 +, and Vel–. The antibody not only reacted in saline and by IAT, but also demonstrated hemolytic activity (IBGRL). The titer of anti-Vel was 256 at room temperature and by IAT. The IAT was also performed using monospecific reagents. Anti-IgG and -IgA reacted 2+ by IAT. Anti-IgM, -C3, and -C4 reacted 4+ by IAT.

Out of 6741 donors typed for Vel, two Vel– donors (0.02966%) were identified. The calculated frequency of Vel was 0.9997, which is in accordance with the results obtained in other Caucasian populations.

This case points out the necessity of establishing a list of rare donors in our country and listing them with an international blood exchange.

References


Platelets in Thrombotic and Non-thrombotic Disorders is an informative and extensively referenced textbook that is highly recommended to biomedical scientists and clinical hematologists with strong interests in platelet physiology and disease states. The editors have assembled an outstanding group of contributors to provide overviews of platelet physiology, pathologic platelet disorders, and the pharmacology of platelet-dependent disease. This textbook will be most useful to scientists studying the pathophysiology of platelet disorders and the basic science of platelet function; many of the chapters, especially the 29 physiology chapters, include the relevant historical context and hard-to-find seminal references that are essential for methodology, manuscript preparation, and general background. The textbook was published in 2002; therefore, the most up-to-date references are from 2000–2001, including several from 2001 that are listed as in press.

The scope of this textbook encompasses a large number of separate chapters, necessitating relatively concise presentation of data and discussions; this enables the reader to quickly focus on a particular topic, although more cross-referencing between chapters would be helpful for future editions. The chapters on platelet physiology are highly focused to provide the reader with state-of-the-art knowledge on distinct aspects of platelet function. For example, platelet signaling is discussed over four separate chapters, including the roles of cAMP and cGMP, tyrosine kinases, protein kinase C, and calcium. These briefs include important methodological detail that is well referenced for benchwork. There is a strong group of platelet pathology chapters that discuss thrombocytopenia; a more comprehensive background in earlier chapters on platelet circulation kinetics might better serve these chapters. One novel aspect of this textbook is the number of chapters that explore the interactions of platelets with heterotypic cells in both normal physiology and disease. These include vascular control of platelet function, leukocyte-platelet conjugate formation in vascular disease, the role of platelets in tumor invasiveness and metastatic potential, and the interaction of platelets with various pathogens, both in vivo and during storage. Several other chapters are highlighted below to provide a snapshot of some of the specific strengths of this text.

A concise, highly relevant overview of the most critical issues and controversies in platelet storage and transfusion is provided by Scott Murphy. This chapter is valuable for hematology and transfusion medicine fellows and includes an excellent discussion of platelet preparation and storage methodologies and comprehensive tables for quick reference. The clinical focus also encompasses the relevant indications for platelet transfusion and includes a succinct outline of alloimmunization and strategies for finding compatible platelets. Thomas Kunicki authors a readable overview of gene regulation of platelet function. This chapter includes details on the regulation of megakaryocyte-specific genes found in hematopoietic-derived cell lines and of transcription factors, including the GATA and Ets families. Recent studies that have examined platelet polymorphisms as risk factors for clinically relevant atherothrombosis are also discussed, including clearly presented tables on $\alpha_{2}\beta_{1}$ density and polymorphisms of GPIb$\alpha$, VNTR, and $\alpha_{IIb}\beta_{3}$.

The chapter on platelet procoagulant function by Hemker is broadly written to give a concise overview of the interactions between platelets and soluble/plasma coagulation factors. There has been an explosion of new information in this field since this chapter was prepared, but the general principles related to platelet-associated thrombin generation are clearly written and serve as a firm background for further reading and research. Several chapters are included on the evaluation of trials of antiplatelet therapy for cardiovascular, cerebrovascular, and peripheral vascular disease. All of these include comprehensive tables and detailed analyses of antiplatelet efficacy and outcomes, as well as summary recommendations on current therapeutic regimens.

Future scenarios for antiplatelet therapy are discussed in the chapter on pharmacogenetics. This chapter highlights some of the possible targets of
pharmacogenomic therapy, including those that are applicable to general risk, e.g., high C-reactive protein population subsets, and platelet-derived risk factors, including polymorphisms of GPIIIa (PLA1/2), differential thienopyridine metabolism, and the novel P2Y12 receptor; the latter two are relevant to platelet ADP-receptor antagonist therapy. There is also an interesting discussion of the possibilities for future screening of atherothrombotic risk factors, and the subsequent use of targeted antiplatelet therapy.

Platelets in Thrombotic and Non-thrombotic Disorders emphasizes strongly focused chapters and comprehensive referencing on platelet physiology and function; this extensive framework for up-to-date platelet knowledge makes this text essential for hematologists and blood bankers in training, and for researchers and veteran clinicians with an interest in hemostasis.

Henry M. Rinder, MD
Associate Professor of Laboratory Medicine and Internal Medicine (Hematology)
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BOOK REVIEW


This reviewer has a research interest in adoptive immunotherapy, and gladly agreed to review Cellular Therapy: New Frontiers in Transfusion Medicine. I was not disappointed. Although the book is relatively short, the chapters are detailed and well-referenced and present an appropriate spectrum of currently practiced cellular therapy techniques and more esoteric applications.

The first chapter is a concise yet thorough historical perspective on cellular therapy written by Harvey Klein, MD, and originally delivered as the 2001 Emily Cooley Memorial Lecture. Covering topics including the development of the blood cell separator, initial observations supporting the clinical feasibility of adoptive immunotherapy, somatic cell gene therapy, and the cellular vaccines, this chapter nicely covers the continuing evolution of transfusion medicine in the area of cellular therapy.

There are comprehensive chapters on established cellular therapy methods that many blood banks are already performing. Mobilization and collection of peripheral blood progenitor cells, increasingly used for hematopoietic cell transplantation, is discussed by David Stronccek, MD, and Susan Leitman, MD. Included are mobilization protocols, expected results, and donor issues. As a companion chapter, Elizabeth Read, MD, and Charles Carter describe methods for T-cell depletion of hematopoietic progenitor cell products prior to allogeneic transplantation, and the NIH clinical experience with this procedure. While not quite “how-to” guides, these chapters would nonetheless serve as excellent introductions for practitioners whose clinicians may request these services.

An evolving methodology with particularly exciting potential is pancreatic islet cell transplantation for type I diabetes, as discussed by Elizabeth Read, MD, Janet Lee, MT(ASCP), and David Harlan, MD. The authors cover pancreas procurement, processing to isolate islets, and the current status of clinical trials. After reading this chapter, it seems increasingly likely that islet cell processing will become a technique largely performed and overseen by transfusion medicine specialists, and this chapter is a good primer on the subject.

The remaining three chapters are more forward-looking, covering areas that very few physicians and technologists currently have experience with. For example, David Williams, MD, discusses genetic modification of stem cells, with an emphasis on retroviral and other vectors currently in use. Ena Wang, MD, and Francesco Marincola, MD, discuss immunotherapies for solid tumors. Finally, Catherine Verfaillie, MD, and colleagues give an overview on tissue-resident stem cells, including mesenchymal stem cells.

In summary, I recommend this well-written book for anyone who wants an introduction to where transfusion medicine may be heading, and what components we may be asked to prepare in the future.

John D. Roback, MD PhD
Emory University School of Medicine
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**Blood group antigens**


**Blood group antibodies**


Blood group genetics


phenotype caused by a new mutation at the ABO locus. Transfusion 2002;42:294-301.

Blood group biochemistry

Monoclonal antibodies

Red cell serology/methods

White cell/platelet serology/methods

Hemolytic anemias
1. Frohn C, Jabs WJ, Fricke L, Goer G. Hemolytic anemia after kidney transplantation: case report...


Hemolytic disease of the newborn


Transfusion/transplantation


Disease associations


Miscellaneous


Monoclonal antibodies available. The New York Blood Center has developed murine monoclonal antibodies that are useful for donor screening and for typing red cells with a positive DAT. Anti-Rh17 is a direct agglutinating monoclonal antibody. Anti-Fy\textsuperscript{a}, anti-K, anti-Js\textsuperscript{b}, and anti-Kp\textsuperscript{a} are indirect agglutinating antibodies that require anti-mouse IgG for detection. These antibodies are available in limited quantities at no charge to anyone who requests them. Contact: Marion Reid, New York Blood Center, 310 E. 67th Street, New York, NY 10021; e-mail: mreid@nybc.org

Workshop on Blood Group Genotyping. The ISBT/ICSH Expert Panel in Molecular Biology has recommended that a workshop be held on blood group genotyping by molecular techniques. The results of the meeting would culminate in a report at the ISBT Congress in 2004 in Edinburgh. It was decided that only laboratories that provide a reference service in blood group genotyping would be included in the workshop. One of the aims of the workshop would be to establish an external quality assurance plan. If you have any suggestions as to how the workshop should be organized, we would be grateful for your opinions. If you are interested in taking part in such a workshop, please contact Geoff Daniels (geoff.daniels@nbs.nhs.uk). Offer presented by Geoff Daniels, Martin L. Olsson, and Ellen van der Schoot.

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Classification AD

SBB Program. The Department of Transfusion Medicine, National Institutes of Health, is accepting applications for its 1-year Specialist in Blood Bank Technology Program. Students are federal employees who work 32 hours per week. This program introduces students to all areas of transfusion medicine, including reference serology, cell processing, and HLA and infectious disease testing. Students also design and conduct a research project. NIH is an Equal Opportunity Employer. Application deadline is June 30, 2003, for the January 2004 class. Contact: Karen M. Byrne, NIH/CC/DTM, Bldg. 10/Rm. 1C711, 10 Center Drive, MSC 1184, Bethesda, MD 20892; e-mail: kbyrne@mail.cc.nih.gov or visit our Web site: www.cc.nih.gov/dtm.

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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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   - B. Initials and last name of each author (no degrees; all CAPS), e.g., M.T. JONES
   - C. Running title of ≤ 40 characters, including spaces
   - D. 3 to 10 key words

2. Abstract
   - A. 1 paragraph, no longer than 200 words
   - B. Purpose, methods, findings, and conclusions of study
   - C. Abstracts not required for reviews

3. Text (serial pages)
   - Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Results of surveys and review papers are examples that may need individualized sections.
   - A. Introduction
      - Purpose and rationale for study, including pertinent background references.
   - B. Case Report (if study calls for one)
      - Clinical and/or hematologic data and background serology.
   - C. Materials and Methods
      - Selection and number of subjects, samples, items, etc. studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer's name, city, and state. Do not use patients' names or hospital numbers.
   - D. Results
      - Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable.
   - E. Discussion
      - Implications and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction.

4. Acknowledgments
   - Acknowledge those who have made substantial contributions to the study, including secretarial assistance.

5. References
   - A. In text, use superscript, arabic numbers.
   - B. Number references consecutively in the order they occur in the text.
   - C. Use inclusive pages of cited references, e.g., 1431–7.
   - D. Refer to current issues of *Immunohematology* for style.

6. Tables
   - A. Number consecutively, head each with a brief title, capitalize first letter of first word (e.g., Table 1. Results of ...), and use no punctuation at the end.
   - B. Use short headings for each column, and capitalize first letter of first word.
   - C. Place explanations in footnotes (sequence: *, †, ‡, §, ¶, **, ††).

7. Figures
   - A. Figures can be submitted either drawn or photographed (5" × 7" glossy).
   - B. Place caption for a figure on a separate page (e.g., Fig. 1. Results of ...), ending with a period. If figure is submitted as a glossy, put title of paper and figure number on back of each glossy submitted.
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8. Author information
   - A. List first name, middle initial, last name, highest academic degree, position held, institution and department, and complete address (including zip code) for all authors. List country when applicable.

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