The Dombrock blood group system: a review

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The Dombrock blood group system (Do) consists of two antithetical antigens (Do^a and Do^b) and five antigens of high prevalence (Gy^a, Hy, Jo^a, DOYA, and DOMR). Do antigens are carried on the Dombrock glycoprotein, which is attached to the RBC membrane via a glycosylphosphatidylinositol linkage. The gene (*DO, ART4*) encoding the Do glycoprotein, located on the short arm of chromosome 12, has been cloned and sequenced, allowing the molecular basis of the various Do phenotypes to be determined. Do^a and Do^b have a prevalence that makes them useful as genetic markers; however, the paucity of reliable anti-Do^a and anti-Do^b has prevented this potential from being realized. The ease with which these antigens can be predicted by analysis of DNA opens the door for such studies to be carried out. Anti-Do^a and anti-Do^b are rarely found as a single specificity, but they have been implicated in causing hemolytic transfusion reactions. This review is a synthesis of our current knowledge of the Dombrock blood group system. ***Immunohematology* 2010;26:71–78.**

Key Words: ART, blood group system, Dombrock blood group system, mono-ADP-ribosyltransferase, ADP-ribosyltransferase

The Dombrock (Do) blood group system illustrates the value of different methods for the advancement of knowledge. Classical hemagglutination showed certain characteristics of the antigens and antibodies, a relationship of Hy to Gy^a, and the fact that the Gy(a–) phenotype was the null of the Dombrock blood group system. Immunoblotting provided a tool to allow further characterization of the Dombrock glycoprotein, including the fact that it is linked to the RBC membrane by a glycosylphosphatidylinositol (GPI) anchor. In silico analysis aided in cloning the *DO* gene and led to PCR-based assays not only to identify the nucleotide changes associated with the antigens but also to screen for antigen-negative donors and identify new alleles. Transfection and hybridoma technology has been used for the production of monoclonal antibodies to Do, and DNA array technology provides a means to do high-throughput testing of donor blood. This review summarizes the understanding of the Dombrock blood group system that has evolved as methods were developed and applied.

History

Anti-Do^a was identified in 1965,[1] and anti-Do^b, which recognizes the antithetical antigen, was described 8 years later.[2] These two antibodies define three phenotypes, the prevalence of which differs in various ethnic groups (Table 1). For populations other than Whites,[2,4] studies have been restricted to testing with anti-Do^a, and thus the numbers given for Do(a–b+) in Table 1 are calculated from the prevalence given for Do^a.[4–6] Do^a and Do^b were placed in the Dombrock blood group system (DO; 014) by the ISBT Working Party on Terminology for Red Cell Surface Antigens in 1985.[7]

Table 1. The Dombrock blood group system

RBC phenotype	Reactivity with anti-					Occurrence (%) in				
	Do^a	Do^b	Gy^a	Hy	Jo^a	Whites	Blacks	Japanese	Thai	Chinese
Do(a+b–)	+	0	+	+	+	18	11	1.5	0.5	
Do(a+b+)	+	+	+	+	+	49	44	22	13	
Do(a–b+)	0	+	+	+	+	33	45	76.5	86.5	
Gy(a–)	0	0	0	0	0	Rare	Rare*	Rare	Not found	Rare†
Hy–	0	wk	wk	0	0/wk	Not found	Rare	Not found	Not found	Not found
Jo(a–)	wk	0/wk‡	+	wk	0	Not found	Rare	Not found	Not found	Not found

*One Gy(a–) Black proband has been reported.[3]
†One Gy(a–) Chinese proband has been found (unpublished data).
‡RBCs will most often be Do(b–) when the Do glycoprotein is encoded by *JO/JO* but Do(b+^{W}) when encoded by *HY/JO*.

The high-prevalence antigens Gregory (Gy^a) and Holley (Hy), described independently in 1967[8,9] were shown to be phenotypically related. RBCs from Caucasians with the Gy(a–) phenotype are Hy–, and RBCs from Black people of African descent with the Hy– phenotype are Gy(a+^{w}).[10] On the basis of this observation, Gy^a and Hy were upgraded from the ISBT Series of High Incidence Antigens to the Gregory Collection (206) (Table 2).[11] Gy^a and Hy were shown to be located on the same glycoprotein by immunoblotting in 1991.[12]

Table 2. ISBT terminology for the Dombrock (014) blood group system antigens

Traditional name	ISBT name	ISBT number	Previous ISBT number
Do^a	DO1	014001	
Do^b	DO2	014002	
Gy^a	DO3	014003	206001; 900005
Hy	DO4	014004	206002; 900011
Jo^a	DO5	014005	901004; 900010
DOYA	DO6	014006	
DOMR	DO7	014007	

The high-prevalence antigen Jo^a was first described in 1972[13] and later shown to have a phenotypic association with Gy^a and Hy because RBCs with either the Gy(a–) phenotype or the Hy– phenotype are also Jo(a–).[14,15] Jo^a was assigned a number in the ISBT Series of High Incidence Antigens before its association with Gy^a and Hy was realized.[7] When Jo^a was shown to reside on the Gregory glycoprotein by immunoblotting,[16] it was not placed in the Gregory collection but it was promoted directly to the Dombrock blood group system.[17,18] Another high-prevalence antigen, Jc^a, was shown to be associated with Gy^a and Hy,[19] and although it was reported to be the same as Jo^a,[20] there remained some doubt and an ISBT number was not assigned to Jc^a. On the basis of subsequent work, this doubt was justified (see later discussion).[21]

In 1992, Banks and coworkers[22] revealed that in addition to being Hy– and Jo(a–), Gy(a–) RBCs were Do(a–b–). Thus, it was shown that RBCs with the Gy(a–) phenotype are the null phenotype of the Dombrock blood group system.[23] After this discovery, Gy^a, Hy, and Jo^a were assigned ISBT numbers in the Dombrock blood group system (Table 2).[17,18]

Dombrock Glycoprotein

Antigens in the Dombrock blood group system are carried on a GPI-linked glycoprotein.[12,16,24] The Dombrock glycoprotein has an apparent M_r of approximately 47,000 to 58,000 in SDS-PAGE under nonreducing conditions.[12,23] In the membrane-bound form, the Do glycoprotein has five potential *N*-linked glycosylation sites and four or five cysteine residues.[25] The susceptibility of some Do antigens to sulfhydryl compounds suggests that the tertiary conformation of the glycoprotein is dependent on disulfide bonds.

The Do glycoprotein is expressed primarily on erythroid cells in adult bone marrow and in fetal liver. Expression may also occur in the lymph nodes (on lymphocytes), testes, spleen, and fetal heart. Although the Do glycoprotein is a member of the mono-ADP-ribosyltransferase family, no enzyme activity has been demonstrated on the RBC. ADP-ribosyltransferases catalyze the transfer of ADP-ribose from NAD^+ to a specific amino acid in a target protein that modulates protein function. ADP-ribosylation can be reversed by ADP-ribosyl hydrolases, which remove the ADP-ribose and restore protein function.[26,27] Thus, Do may be involved in the regulation of cellular protein function.

Dombrock Antigens

The characteristics of antigens in this system are summarized in Table 3. The susceptibility and resistance of antigens in the Dombrock system to treatment of RBCs with various proteolytic enzymes and DTT, and their absence from paroxysmal nocturnal hemoglobinuria (PNH) type III RBCs,[24] can be used to aid the identification of antibodies to Dombrock antigens.

Table 3. Characteristics of antigens in the Dombrock blood group system

Resistant to papain or ficin treatment of antigen-positive RBCs. Often the reactivity is enhanced.
Sensitive to trypsin treatment of antigen-positive RBCs.
Do^a, Do^b, Gy^a, Hy, DOYA, and DOMR are sensitive to DTT (200 mM) treatment of antigen-positive RBCs. Jo^a is variably affected by such treatment. All antigens are resistant to treatment of antigen-positive RBCs with 50 mM DTT.
Weakened by α-chymotrypsin or AET treatment of antigen-positive RBCs.
Expressed on cord RBCs; although Gy^a, Hy, and Jo^a may be weaker than on RBCs from adults.
Absent from PNH III RBCs.
Some variation in expression on different RBCs and on RBCs from different people.
Carried on a mono-ADP-ribosyltransferase (ART-4), a GPI-linked protein.
Do^a, Do^b, Hy, and Jo^a are not highly immunogenic.

AET = 2-aminoethylisothiouronium bromide; GPI = glycosylphosphatidylinositol; PNH = paroxysmal nocturnal hemoglobinuria.

Dombrock Antibodies

Studies involving the Dombrock blood group system have been hampered by the paucity of reliable monospecific antisera. Antibodies in the Dombrock blood group system can be difficult to identify. This is especially true for the differentiation of anti-Hy from anti-Jo^a. As will be described later, determination of the molecular basis associated with Hy and Jo^a has provided an explanation for this

particular difficulty. Common characteristics of antibodies to Dombrock blood group system antigens are summarized in Table 4.

Table 4. Characteristics of antibodies to Dombrock antigens

Usually IgG.
React optimally by column agglutination technology or by the IAT using papain- or ficin-treated RBCs.
Usually weakly reactive.
Do not bind complement.
Stimulated by pregnancy and by transfusion.
Usually present in sera containing other alloantibodies. The exception is anti-Gy^a, which often occurs as a single specificity.
Often deteriorate in vitro and fall below detectable levels in vivo.
Have not caused clinical HDFN (positive DAT only) but have caused transfusion reactions.

HDFN = hemolytic disease of the fetus and newborn.

Clinical Importance

Although transfusion reactions caused by anti-Do^a or anti-Do^b have been reported,[28–37] they may be underreported. One reason is that events usually associated with transfusion reactions may not be observed. For example, the DAT is often negative, no antibody is eluted from the patient's RBC samples after transfusion, there is no lag phase in the antibody reactivity, and no increase in titer of the antibody is observed. However, in our experience, the provision of Do(a–) or Do(b–) blood as predicted by DNA analysis has improved RBC survival in patients with the corresponding antibody who receive chronic transfusions. At least one anti-Hy has caused biphasic destruction of Hy+ RBCs[38]; other examples of anti-Hy, and anti-Gy^a and anti-Jo^a, have caused moderate transfusion reactions. In the absence of Gy(a–) blood, Hy– blood has been a suitable substitute (personal observations). Some anti-Gy^a appear to be benign: 10 Gy(a+) units were transfused to a man with anti-Gya without adverse consequences.[39] Antibodies in the Dombrock blood group system typically do not cause clinical HDN, although RBCs of some antigen-positive babies were positive in the DAT. One baby of a mother with anti-DOMR was born icteric and required phototherapy.[40]

The *DO* Gene

For some time, it has been known that *DO* is located on the short arm of chromosome 12 (Fig. 1).[41] In silico analysis aided in identification of a candidate *DO* gene, which has been cloned and sequenced (GenBank accession number; AF290204).[25] *DO* is the first blood group gene to be cloned by an in silico approach.[42]

The *DO* gene is identical to *ART4*, described in 1997 (GenBank accession number X95826),[43] and has been renamed *DO* (GenBank accession number NM_021071, AF290204). The data sets for the chromosomal arms of *ART3* and *ART4* were inadvertently switched,[43] so *ART4* was incorrectly reported to reside on the long arm of chromosome 12. Had it not been for this switch, it is likely that *ART4* would have been recognized as the *DO* gene in 1997! Although the original publication for *ART4* did not include exon 1,[43] the entire sequence of *ART4* is identical to *DO* (F. Koch-Nolte, personal communication). Cloning and sequencing of *DO* allowed the determination of the molecular basis associated with various Do phenotypes (see later discussion) and led to PCR-based assays to screen for antigen-negative donors and identify new alleles.

The *DO* gene consists of three exons distributed over 14 kilobase pairs (kbp) of DNA (Fig. 1). The messenger RNA, which consists of 1.1 kbp, is predicted to encode a protein of 314 amino acids that has both a signal peptide and a GPI-anchor motif (Fig. 1).[25] Both these are cleaved and not present in the membrane-bound form of Do. The *DO*B* allele encodes an Arg-Gly-Asp (263-RGD-265) motif. RGD motifs within adhesive ligands are commonly involved in cell-to-cell interactions involving integrin binding.[44] However, because the *DO*A* allele, and the chimpanzee *DO* (*pDO*) homolog, encode asparagine (N) instead of aspartic acid (D),[25,45] it is unlikely that the RGD motif encoded by the *DO*B* allele is a critical one.[46]

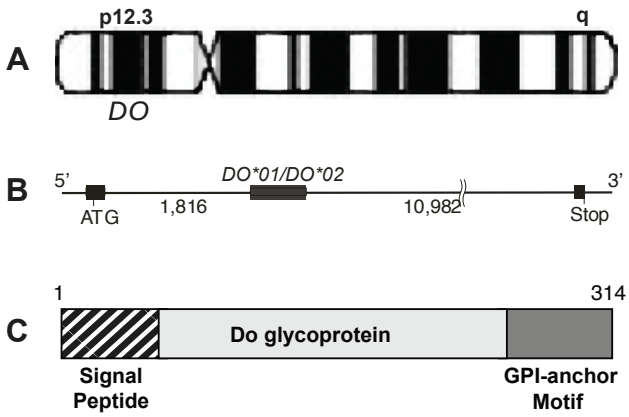

Fig. 1 Diagram of *DO*, its organization, and the Do glycoprotein. (**A**) Location (12p12.3) of *DO* on the short arm of chromosome 12. (**B**) The organization of the three *DO* exons. (**C**) The Do protein with signal peptide at the amino terminus and glycosylphosphatidylinositol (GPI)-anchor motif at the carboxyl terminus.

Molecular Basis of Antigens and Phenotypes

The Polymorphic Do^a (DO1) and Do^b (DO2) Antigens

The common forms of *DO*A* and *DO*B* alleles differ in three nucleotide positions in exon 2. Two are silent nucleotide changes (378C>T, Tyr126Tyr; 624T>C, Leu208Leu); the third is a missense change (793A>G, Asn265Asp), which encodes, respectively, Do^a and Do^b (Table 5).[25] These three nucleotide changes can be readily differentiated by PCR-RFLP, using *Dra*III for 378C>T,[47] *Mnl*I for 624T>C,[47] and BSeRI for 793A>G.[49] Allele-specific PCR also can be used to differentiate *DO*A* from *DO*B*.[50] The ability to distinguish *DO*A* from *DO*B* makes it feasible to predict the Do type of patients and blood donors. This is a tremendous advantage

because, owing to the paucity of reliable reagents, screening for large numbers of Do(a–) or Do(b–) blood donors using classical hemagglutination methods has not been feasible.

Table 5. *DO* alleles defining phenotypes†

Phenotype	ISBT allele name	Nucleotide change	Exon	Amino acid change
DO:1 or Do(a+)	*DO*01* or *DO*A*			
DO:2 or Do(b+)	*DO*02* or *DO*B*	793A>G	2	Asn265Asp[25,47]
DO:–4 or Hy–	*DO*02.–04*‡	323G>T	2	Gly108Val[21]
DO:–5 or Jo(a–)	*DO*01.–05*‡	350C>T	2	Thr117Ile[21]
DO:–6 or DOYA–	*DO*01.–06*‡	547T>G	2	Tyr183Asp[48]
DO:–7 or DOMR–	*DO*02.–07*‡	431C>T; 432C>A	2	Ala144Glu[40]

†Reference allele *DO*01* (AF290204; shaded) encodes Do^a, Gy^a, Hy, Jo^a, DOYA, and DOMR antigens.See Table 6 for alleles that result in the Gy(a–) phenotype.
‡ISBT proposed allele name pending ratification June 2010.

The High-Prevalence Gy^a (DO3) Antigen

An absence of Gy^a, in addition to an absence of all antigens in the Dombrock blood group system, defines the Do$_{null}$ [Gy(a–)] phenotype. To date, *DO* has been described to be silenced by five molecular bases (Table 6). Two of them, a mutation in the donor splice site[52] and a mutation in the acceptor splice site,[51] lead to outsplicing of exon 2. The third mechanism is a nonsense change in a *DO*A-HA* allele [350C; 378T (*DO*B*); 624T (*DO*A*); 793A (*DO*A*), see later section].[52] A fourth proband has a deletion of eight nucleotides within exon 2 that leads to a frameshift and a premature stop codon,[53] and the fifth is attributable to an amino acid substitution of Phe62 to Ser.[54]

Table 6. Molecular basis for Gy(a–) phenotype†

Phenotype	Allele name	Nucleotide change	Exon	Amino acid change
DO:–3 or Gy(a–)	*DO*02N.01*§	IVS1–2 a>g	2	Skips exon 2[51]
DO:–3 or Gy(a–)	*DO*02N.02*§	IVS1 +2 t>c	2	Skips exon 2[52]
DO:–3 or Gy(a–)	*DO*01N.03*‡§	442C>T	2	Gln148Stop[52]
DO:–3 or Gy(a–)	*DO*01N.04*§	343de1343–350	2	Frame-shift; premature; stop codon[53]
DO:–3 or Gy(a–)	*DO*01N.05*§	185T>C	2	Phe62Ser[54]

†Changes from the reference allele (GenBank accession number AF290204) are given.
‡The background for this allele is actually *DO*A-HA* (378T, 524T, 793A).[55]
§ISBT proposed allele name pending ratification June 2010.

The High-Prevalence Hy (DO4) Antigen

The nucleotide change associated with Hy+/Hy– phenotypes is 323G>T in exon 2, which is predicted to encode Gly108Val. The change is associated with the absence of Hy and is on an allele carrying 378C (*DO*A*), 624C (*DO*B*), and 793G (*DO*B*) (Table 5). Its association with 793G (265Asp) explains why RBCs with the Hy– phenotype are invariably Do(a–b+). There are two forms of the allele giving rise to the Hy– phenotype, one with 898G (300Val) and the other with 898C (300Leu). Nucleotide 898C (300Leu) is present on the allele encoding the wild-type Hy+. As the 898G allele was present in the sister of the original Hy– proband it was named *HY1*, and the *HY* allele with the wild-type nucleotide 898C, *HY2*.[21] Testing with one potent example of anti-Jo^a showed that some Hy– RBCs express Jo^a very weakly.[56]

The High-Prevalence Jo^a (DO5) Antigen

A single nucleotide change of 350C>T in exon 2 is predicted to encode isoleucine at amino acid residue 117. Nucleotide 350T is associated with the absence of the Jo^a antigen and is predominantly on an allele carrying 378T (*DO*B*), 624T (*DO*A*), and 793A (*DO*A*) (Table 5). The genotype of people whose RBCs have the Jo(a–) phenotype can be *DO*JO/JO* or *DO*HY/JO*.[21] Its association with 793A (265Asn) explains why most RBCs with the Jo(a–) phenotype are Do(a+b–) (Fig. 2). RBCs from individuals with the *DO*HY/JO* genotype will type Do(a+b+^{w}).

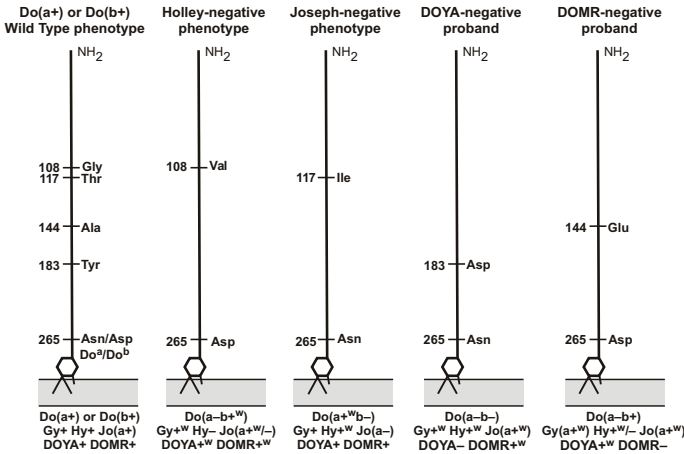

Fig. 2 Diagram showing amino acids associated with the various Do phenotypes. The effect of the amino acid changes associated with Hy–, Jo(a–), DOYA–, and DOMR– phenotypes on the other Do antigens is given under each stick figure. The figures for the DOYA– and DOMR– phenotypes are each based on the only known proband.

The Jc^a Antigen

DNA analysis on four samples that had been originally typed as Jc(a–) revealed a combination of *DO*HY* and *DO*JO* alleles (*DO*HY1/HY1; DO*HY1/HY2; DO*HY1/JO; DO*JO/JO*). These results show that Jc^a is not a discrete antigen.[21]

The High-Prevalence DOYA (DO6) Antigen

The nucleotide change associated with DOYA+/DOYA– phenotypes is *DO*A.547T>G* in exon 2, which is predicted to change Tyr183 to Asp (Table 5). In the DOYA– Turkish Kurd proband, this change silenced the expected Do[a], so her RBCs typed Do(a–b–). They also have a weakened expression of Gy[a], Hy, and Jo[a] (Fig. 2).[57]

The High-Prevalence DOMR (DO7) Antigen

The novel nucleotide changes associated with DOMR+/DOMR– phenotypes are 431C>A and 432C>A, which are predicted to encode Ala(GCC)144Glu(GAA) (Table 5). The change is associated with the absence of the DOMR antigen and is on a DO*B-WL allele (see later section). Its association with 793G (265Asp) explains why RBCs with the DOMR– phenotype are Do(b+), albeit weakly. RBCs from the DOMR– Brazilian Black proband have a weakened expression of Gy[a], Hy, and Jo[a] (Fig. 2).[40]

Other *DO* Alleles

Testing of DNA from Blacks from Brazil or the Congo led to the recognition of additional *DO* alleles. These are *DO*A-HA*,[55] *DO*A-SH*,[58] *DO*A-WL*,[59] *DO*B-SH*,[55] *DO*B-WL*,[58] *DO*B-SH-Q149K*,[60] and *DO*B-I175N* (it is possible that this last allele could be on a *DO*B-WL* background) (Table 7).[60] The initials used in these allele names are derived from the initials of investigators who reported them. Information is lacking about the nature of the Do glycoprotein or antigenic expression, if any, encoded by these alleles. These alleles are not known to encode novel antigens.

Chapel-Fernandes and coworkers[60] measured the expression of various Do proteins on transduced K562 cells by flow cytometry using monoclonal anti-Do. They report that the protein encoded by *DO*B-SH, DO*B-WL*, and *DO*B-SH-Q149K* alleles was expressed in lower copy number than the protein encoded by *DO*B* or *DO*B-I175N* alleles. This finding may provide insights into the variable expression of Do[b] on RBCs.

DO and Anthropology

The chimpanzee *DO* allele has the human wild-type nucleotides at positions 323, 350, and 898 and has 378T (*DO*B*), 624C (*DO*B*), and 793A (*DO*A*).[21] This sequence has not been found in humans and does not provide an insight as to the primordial *DO* gene. The sequence of the allele from three unrelated chimpanzees was identical (GenBank accession numbers AF373016, AF373017, and AF374727).

Transfection of *DO* cDNA

Cells transfected with *DO* cDNA have been used to study expression levels of Do.[25,60] Cells transfected with *DO* cDNA have also been used as an immunogen to produce several monoclonal antibodies (MoAbs). Two MoAbs, MIMA-52 that recognizes a DTT-sensitive epitope and MIMA-53 that recognizes a DTT-resistant epitope, strongly agglutinate RBCs from humans [except Gy(a–)] and other great apes but not from lesser apes, old world monkeys, new world monkeys, prosimians, rabbits, dogs, sheep, or mice.[45] Three other MoAbs (MIMA-64, MIMA-73, and MIMA-98) agglutinated RBCs from humans, chimpanzees, greater apes, and

Table 7. *DO* alleles, including some that were described only at the DNA level

Allele name		nt (aa)	nt (aa)	Nt	nt	nt (aa)	nt (aa)	nt (aa)
ISBT	Other	323 (108)	350 (117)	378	624	793 (265)	898 (300)	Other
*DO*01*	*DO*A*	G (Gly)	C (Thr)	C	T	A (Asn)	C (Leu)	
*DO*01.–05*	*DO*JO*	G (Gly)	T (Ile)	T	T	A (Asn)	C (Leu)	
*DO*01.–06*	*DOYA*	G (Gly)	C (Thr)	C	T	A (Asn)	C (Leu)	547T>G (Tyr183Asp)
	*DO*A-HA*†	G (Gly)	C (Thr)	T	T	A (Asn)	C (Leu)	
	*DO*A-SH*†	G (Gly)	C (Thr)	C	C	A (Asn)	C (Leu)	
	*DO*A-WL*†	G (Gly)	C (Thr)	C	T	A (Asn)	G (Val)	
*DO*02*	*DO*B*	G (Gly)	C (Thr)	T	C	G (Asp)	C (Leu)	
*DO*02.–04*	*DO*HY1*	T (Val)	C (Thr)	C	C	G (Asp)	G (Val)	
*DO*02.–04*	*DO*HY2*	T (Val)	C (Thr)	C	C	G (Asp)	C (Leu)	
*DO*02.–07*	*DOMR*	G (Gly)	C (Thr)	T	C	G (Asp)	G (Val)	431C>A and 432C>A (Ala144Glu)
	*DO*B-SH*†	G (Gly)	C (Thr)	C	C	G (Asp)	C (Leu)	
	*DO*B-SH-Q149K*†	G (Gly)	C (Thr)	C	C	G (Asp)	C (Leu)	445C>A (Gln149Lys)
	*DO*B-WL*†	G (Gly)	C (Thr)	T	C	G (Asp)	G (Val)	
	*DO*B-I175N*†	G (Gly)	C (Thr)	T	C	G (Asp)	C (Leu)	524T>A (Ile175Asn)

†Alleles defined only through DNA-based assay; not investigated serologically.
aa = amino acid; nt = nucleotide.

lesser apes but not RBCs from the other animals (unpublished observations). A homolog of the DO has been found in mouse,[43] and thus the lack of reaction with mouse RBCs indicates that the specific epitope recognized by the MIMA anti-Do is restricted to apes.[61]

Expression of Dombrock Antigens

The molecular basis associated with Hy– and Jo(a–) phenotypes was determined only after numerous samples were analyzed. During this analysis, it became clear that RBC samples had been misidentified. The most significant was that of SJ after whom the Joseph phenotype and Jo^a antigen were named. Surprisingly, DNA from SJ typed *DO*HY1/HY2* and DNA from her Hy+ brother was *DOHY2/DO*B*.[21] Thus, any "anti-Jo^a" that was identified by using SJ RBCs may actually be anti-Hy. The use of such RBCs and antibodies as reagents has led to the inadvertent incorrect labeling of reagents. To correctly identify anti-Hy and anti-Jo^a, RBCs whose type has been predicted by DNA analysis should be used. The various possible combinations and expected antigen expression are given in Table 7. RBCs from people with *DO*JO/JO* or *DO*JO/HY* genotypes will have the same phenotype, although the latter may have a slightly weaker expression of Hy.

The close proximity of amino acids associated with Hy (residue 108) and Jo^a (residue 117) antigen expression may explain why Hy– RBCs lack or have an extremely weak expression of Jo^a and why Jo(a–) RBCs have a weak expression of Hy (Fig. 2). The two critical residues are separated by only eight amino acids, which is within the range of an antigenic determinant.[62,63] The reason for the weak expression of Gy^a on Hy– RBCs, the weak expression of Do^b on RBCs with the Hy– phenotype, and the weak expression of Do^a on RBCs with the Jo(a–) phenotype is still not understood but likely involves conformation or the effect of steric hindrance or charge on the conformation. Interestingly, the amino acid changes involved with an absence of DOYA or DOMR also weaken other Do antigens (Fig. 2).

It is apparent that the immune response in different people with the same Dombrock phenotype varies. One possibility is that Hy– patients may produce anti-Do^a in addition to anti-Hy, and Jo(a–) patients may produce anti-Do^b in addition to anti-Jo^a. Furthermore, a patient with a Jo(a–) phenotype and a *DO*HY/JO* genotype should have RBCs that type Do(a+^{w}b+^{w}) Gy(a+) Hy(a+^{w}) Jo(a–) and thus would be expected to make only anti-Jo^a. The results of DNA analysis of serologically identified samples with unusual Do phenotypes provide an explanation for the diversity of typing results in antibody producers and for the diversity of the reactivity of their plasma and serum of their immune response.

Conclusions

Determination of the molecular basis underlying the antigens in the Dombrock blood group system has several advantages and has changed practice in transfusion medicine. The first, as outlined earlier, is the possibility of using RBCs with a bona fide antigen profile in antibody identification studies. The second advantage is to predict the phenotype of patient RBCs to aid in the antibody identification process; the third is the ability to type donors for *DO*A* and *DO*B* and thereby select Do(b–) RBCs for transfusion to a patient who has or has had anti-Do^b. Yet another value is to be able to type donors whose RBCs are used in antibody identification panels.[64] This is, perhaps, the first instance in which DNA analysis is more reliable than hemagglutination. This is because the antibodies (especially anti-Do^a and anti-Do^b) are rarely available as a single specificity with strength and volume to make accurate typing possible.

There is no known disease entity associated with the Do^a form of the Dombrock glycoprotein (RGD→RGN) nor, indeed, with an absence of the entire glycoprotein [Gy(a–)]. The presence of RGN in the chimpanzee homolog[21] suggests that this, and not RGD, may be the primordial sequence. Expression of Dombrock and other ectoenzymes (Kell and Yt) on RBCs may provide a readily transportable steady-state level of these enzymes for tissues in the vascular space.

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