REVIEW

The Diego blood group system: a review

D. Figueroa

The Diego blood group system (DI) currently encompasses 22 antigens. Three of the antigens are of high prevalence and the other 19 are of low prevalence. The antigens of the Diego blood group system are carried on the erythroid band 3 protein anion exchanger 1 (AE1), the product of a single gene, *SLC4A1* (solute carrier family 4, anion exchanger, member 1). AE1 is a member of a family of three anion exchangers or transporters expressed in a variety of tissues. This protein is involved in carbon dioxide transport from tissues to lungs. It is also found in the kidney, where it is involved in acid secretion. Antibodies to Diego system antigens with the exception of anti-Di^a, -Di^b, -Wr^a, -ELO and -DISK do not seem to be of clinical significance for transfusion or of importance in hemolytic disease of the fetus and newborn. ***Immunohematology* 2013;29:73–81.**

Key Words: Diego, AE1 protein, band 3, *SLC4A1*

History

Currently there are a total of 22 antigens in the Diego blood group system. Three of the antigens are of high prevalence, Di^b, Wr^b, and DISK. The other 19 antigens are of low prevalence. Within the 22 antigens there are only three sets of antithetical antigens: Di^a/Di^b, Wr^a/Wr^b, and Wu/DISK. None of the other 17 low-prevalence antigens has an antithetical high-prevalence antigen yet described. Figure 1 illustrates the molecule that carries the Diego system antigens.

The discoveries of these antigens exemplify the tenacity and curiosity of many immunohematologists and researchers. These individuals investigated many serum samples and in some cases came out with more questions (findings) than answers. On many occasions the new antibody discovered was present in sera that were under investigation for some other reason and contained antibody(ies) to other low-prevalence antigens. In other instances the antibody was discovered because it caused hemolytic disease of the fetus and newborn (HDFN) or an incompatible crossmatch in the presence of a negative antibody detection test.

The first antigen assigned to the Diego system, Di^a, was reported by Layrisse et al. in 1955. They reported an antibody to a low-prevalence antigen that caused a fatal HDFN.[1]

Diego Blood Group Antigens Carried on Band 3 Protein

The antigens of the Diego blood group are carried on the erythroid band 3 protein anion exchanger 1 (AE1), the product of a single gene, *SLC4A1* (solute carrier family 4, anion exchanger, member 1). The gene is located on chromosome 17q21.31, and the cluster differentiation assignment is CD233. *SLC4A1* consists of 20 exons that span more than 18 kbp of DNA.[2]

The erythroid band 3 protein is a member of a family of three anion exchangers or transporters—AE1, AE2, and AE3—and is expressed in a variety of tissues. AE1 is the major integral membrane protein of the erythrocyte. This protein is a chloride-bicarbonate exchanger involved in carbon dioxide transport from tissues to lungs. AE1 is expressed in erythrocytes and the kidney, with the kidney having a different isoform of the protein. AE1 is not expressed by nonerythroid blood cells.[3]

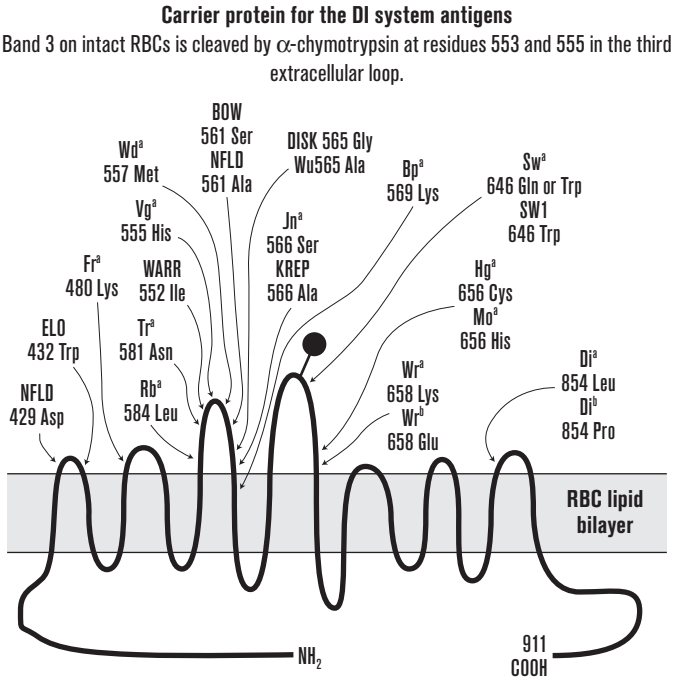

Fig. 1 Diagram of band 3 and the locations of the Diego system antigens. Reprinted from *The Blood Group Antigen FactsBook,* 3rd ed. Reid ME, Lomas-Francis C, Olsson ML. Diego Blood Group System, page 386, Copyright 2012, with permission from Elsevier.

Mutations of the band 3 gene have been implicated in Southeast Asian ovalocytosis (SAO), hereditary spherocytosis, congenital acanthocytosis and distal tubular acidosis. Band 3 SAO has the Memphis I variant and is not functional as anion transporter.[4] Another variant is known as band 3 Memphis II. Spring et al. reported that the Di^a antigen is carried on the Memphis II variant of band 3.[5]

It has been reported that naturally occurring antibodies to low-prevalence antigens in the Diego system are common in the plasma of patients with hyperactive immune systems, e.g., those with autoantibodies. This may be related to the exposure of the senescent cell antigen, which resides on protein residues in band 3.[6]

Band 3 and Glycophorin A

Band 3 and glycophorin A (GPA) are the two most abundant integral proteins of the red blood cell (RBC) membrane (Figure 2). The extracellular domains of both proteins are highly polymorphic. Band 3 carries the antigens of the Diego blood group system, and GPA and glycophorin B (GPB) carry the antigens of the MNS system. There is evidence that band 3 and GPA associate in the RBC membrane, and the Wr^b antigen, although a product of the band 3 gene, requires a complex of GPA and band 3 for normal expression. The discovery of a novel GPA mutation (Ala84Pro) giving rise to aberrant Wr^b expression provided some of the initial information with regard to the site of interaction of the two proteins.[7]

In contrast to GPA-related antigens (which are the result of events between two closely linked genes and different genetic mechanisms), the antigens on band 3 are exclusively caused by single-nucleotide polymorphisms (SNPs) in the band 3 gene, *SLC4A1*.[8] All low-prevalence antigens in the DI system, with the exception of Bp^a, occur from extracellular amino acid substitutions. Some amino acid residues have been associated with more than one substitution, each giving rise to separate antigens, for example NFLD/BOW, Jn^a/KREP, and Hg^a/Mo^a. Table 1 lists the 22 phenotypes encoded by the alleles in the DI system and summarizes the alleles and amino acid substitutions.

Effect of Enzymes on the Diego System Antigens

The effect of enzymes on the Diego blood group antigens is directly related to their location. Band 3 has two α-chymotrypsin cleavage sites in the third extracellular loop, at Tyr553 and Tyr555. Therefore the antigens in the third loop are sensitive to α-chymotrypsin, and those in the fourth and

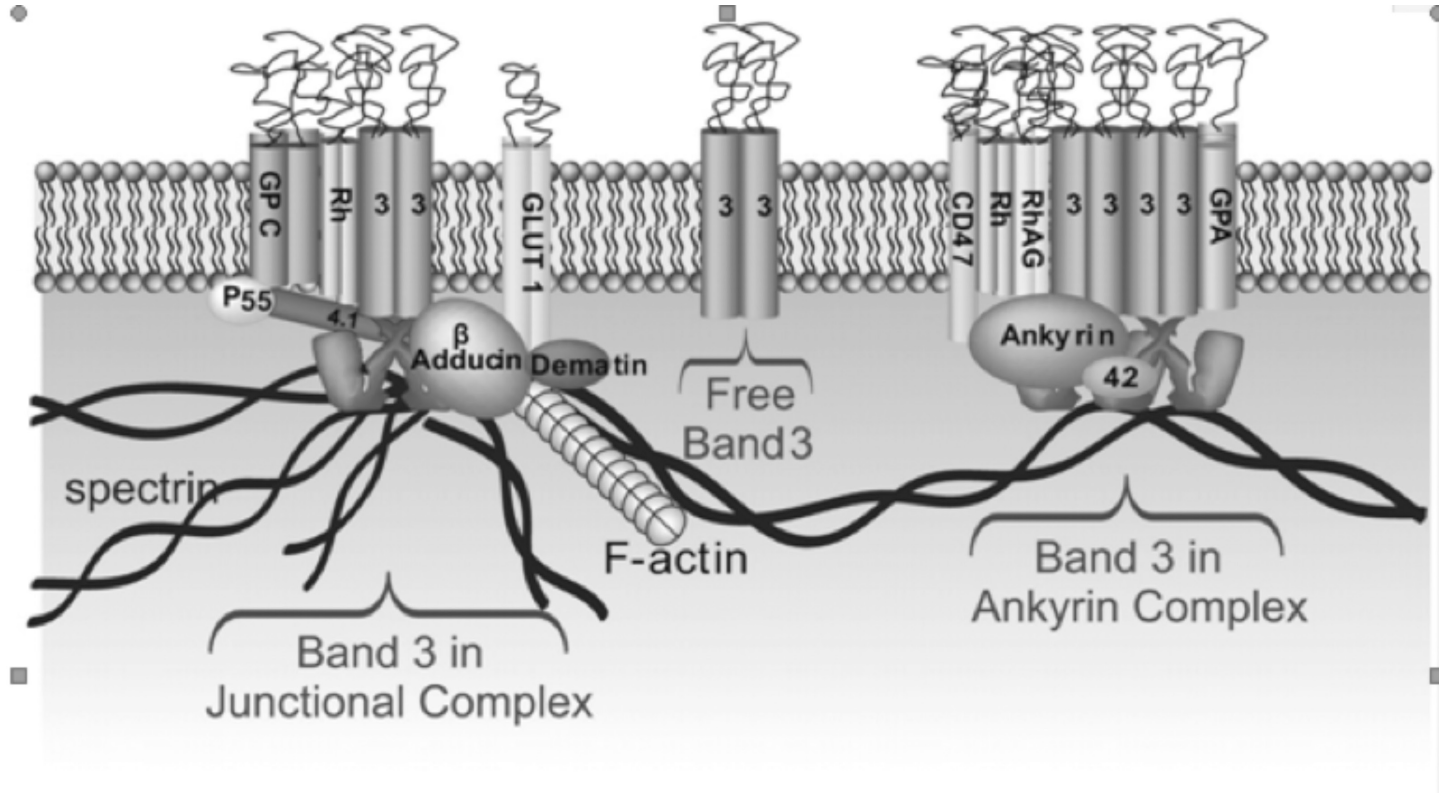

Fig. 2 Schematic diagram of the erythrocyte membrane (lateral view) showing major membrane-spanning proteins. Source: Kodippili GC, et al. Blood 2009;113:6237–45.

Table 1. Diego blood group system phenotypes. NOTE: Reference allele *DI*2 (DI*B)* encodes Di^b (DI2), DI4, and DI22.

Phenotype encoded by allele	ISBT No.	Allele name	Nucleotide change	Exon	Amino acid change
Di(a+b–)/DI:1,–2	10.1	*DI*01*	2561C>T	19	Pro854Leu
Wr(a+b–)/DI:3,–4	10.3	*DI*02.03*	1972G>A	16	Glu658Lys
DI:5 or Wd(a+)	10.5	*DI*02.05*	1669G>A	14	Va1557Met
DI:6 or Rb(a+)	10.6	*DI*02.06*	1643C>T	14	Pro548Leu
DI:7 or WARR+	10.7	*DI*02.07*	1654C>T	14	Thr552Ile
DI:8 or ELO+	10.8	*DI*02.08*	1294C>T	12	Arg432Trp
Wu+, DISK– /DI:9,–22	10.9	*DI*02.09*	1694G>C	14	Gly565Ala
DI:10 or Bp(a+)	10.10	*DI*02.10*	1707C>A	14	Asn569Lys
DI:11 or Mo(a+)	10.11	*DI*02.11*	1967G>A	16	Arg656His
DI:12 or Hg(a+)	10.12	*DI*02.12*	1966G>T	16	Arg656Cys
DI:13 or Vg(a+)	10.13	*DI*02.13*	1663T>C	14	Tyr555His
DI:14 or Sw(a+)	1014	*DI*02.14.01; DI*02.14.02*	1937G>A; 1936C>T	16; 16	Arg646Gln; Arg646Trp
DI:15 or BOW+	10.15	*DI*02.15*	1681C>T	14	Pro561Ser
DI:16 or NFLD+	10.16	*DI*02.16*	1287A>T; 1681C>G	12; 14	Glu429Asp; Pro561Ala
DI:17 or Jn(a+)	10.17	*DI*02.17*	1696C>T	14	Pro566Ser
DI:18 or KREP+	10.18	*DI*02.18*	1696C>G	14	Pro566Ala
DI:19 or Tr(a+)	10.19	*DI*02.19*	1653C>G	14	Lys551Asn
DI:20 or Fr(a+)	10.20	*DI*02.20*	1438G>A	13	Glu480Lys
DI:21 or SW1+	10.21	*DI*02.21*	1936C>T	16	Arg646Trp

ISBT = International Society of Blood Transfusion.

seventh loops are resistant. Data available for some of the DI system antigens indicate that they are resistant to the effects of dithiothreitol, sialidase, and acid. Refer to Table 2 for details on the effect of enzymes and other chemicals on the DI system antigens.

Antigens and Antibodies in the Diego System

Like most antigens, the low-prevalence antigens in the Diego system were discovered by first encountering the antibodies that define them. Most of the low-prevalence antigens in the Diego system, DI5 to DI21, are of very low prevalence. Some antigens have only been found in one family; therefore, information on their characteristics and clinical significance is very limited. It is important to mention that although the antigens are rare, the antibodies to them are very common. Table 3 summarizes the antibodies, their immunoglobulin types, and the limited information regarding their clinical significance.

Di^a (DI1)

In 1955, Layrisse et al. reported an antibody to a low-prevalence antigen that caused a fatal HDFN.[1] The Di^a antigen is the result of a 2561C>T transition in exon 19, Pro854Leu, in the seventh ectoplasmic loop of band 3. The antibody was named Di^a after the antibody producer, Mrs. Diego. Further family studies of the proposita and investigations using the maternal serum helped to unveil the interesting history of the Di^a antigen and the genetic, anthropologic, and clinical relevance of the antigen.

The Di^a antigen is characteristic of individuals of Mongoloid descent, and the *DI*A* alleles have never been found in individuals of unmixed European descent. The Di^a antigen has been used as an anthropologic population migration marker. An example of this migration marker is the presence of the Di^a antigen in Poles, which can be explained by the invasion of southern Poland by Tartars in the 13th to 17th centuries.[9] Gene frequency in eastern Asians has been reported as 1 to 5 percent. In Korea and Tibet the frequency ranges from 7 to 8 percent. In some South American Indian tribes, the gene frequency can be as high as 40 percent; it is nonexistent in others. In Central American Indians, the frequency can also be high. In North American Indians, the frequency ranges from 2 to 12 percent in some tribes. The gene is absent in Eskimos.[6]

Anti-Di^a is most often RBC stimulated and mostly immunoglobulin (Ig) G1 and IgG3. A few examples of

Table 2. Effect of enzymes and other chemical treatments on the Diego system antigens

Antigen	Ficin/ papain	Trypsin	α-chymotrypsin	Pronase	Sialidase	DTT (200 mM)	Acid
BOW	R	R	S	S	PR	R	PR
Bp[a]	S	S	S	S	PR	PR	PR
Di[a]	R	R	R	R	R	R	R
Di[b]	R	R	R	R	R	R	R
DISK	R	R	S	S	PR	R	PR
ELO	R	R	V	V	PR	R	PR
Fr[a]	R	PR	PR	PR	PR	PR	PR
Hg[a]	R	R	R	R	PR	PR	PR
Jn[a]	R	R	S	PS	PR	R	PS
KREP	R	R	S	PS	PR	R	PR
Mo[a]	R	R	R	R	PR	R	PR
NFLD	R	R	S	S	PR	R	PR
Rb[a]	V	R	V	V	PR	PR	PR
Sw[a]	R	R	R	PR	PR	PR	PR
SW1	R	R	R	PR	PR	PR	PR
Tr[a]	R	R	S	PS	PR	PR	PR
Vg[a]	R	R	S	S	PR	PR	PR
WARR	R	R	S	S	PR	R	PR
Wd[a]	R	R	S	PS	PS	R	R
Wr[a]	R	R	R	R	R	R	R
Wr[b]	R	R	R	R	R	R	R
Wu	R	R	S	S	PR	R	PR

DTT = dithiothreitol; R = resistant; S = sensitive; PR = presumed resistant; V = variable; PS = presumed sensitive.

NOTE: The effect of the listed chemicals on the DI antigens was collected from many of the references listed at the end of this review.

Table 3. Immunoglobulin type and clinical significance of Diego system antibodies

Antibody	Immunoglobulin class	HDFN	HTR
BOW	IgG, some IgM	NI	NI
Bp[a]	IgM	No	NI
Di[a]	IgG	Mild–severe	None–severe
Di[b]	IgG	Mild	No–moderate
DISK	IgM, IgG	Possible	Possible
ELO	IgG	Mild–severe	NI
Fr[a]	IgG, IgM	Pos DAT only	NI
Hg[a]	IgM, IgG	NI	NI
Jn[a]	IgM	NI	NI
KREP	IgM	NI	NI
Mo[a]	IgM, IgG	NI	NI
NFLD	IgM, IgG	NI	NI
Rb[a]	IgM	NI	NI
Sw[a]	IgM, IgG	NI	NI
SW1	IgM, IgG	NI	NI
Tr[a]	IgM, IgG	NI	NI
Vg[a]	IgM	NI	NI
WARR	IgG	Mild	NI
Wd[a]	IgM	NI	NI
Wr[a]	IgM, IgG	Mild–severe	None, severe, immediate, delayed
Wr[b]	IgM, IgG	NI	NI
Wu	IgM, few IgG	NI	NI

HDFN = hemolytic disease of the fetus and newborn; HTR = hemolytic transfusion reaction; DAT = direct antiglobulin test; Ig = immunoglobulin; NI = no information.

NOTE: The HDFN and HTR information presented was collected from many of the references listed at the end of this review.

agglutinins with anti-Di[a] specificity have been reported in individuals with no known RBC exposure. A few examples of anti-Di[a] have been shown to activate complement, and they have demonstrated the capability of causing in vitro hemolysis as well as causing severe immediate or delayed transfusion reactions.[10] As previously mentioned, anti-Di[a] has caused serious HDFN. It has been recommended that in populations at risk a Di(a+) RBC be added to the routine antibody detection cells.

Di[b] (DI2)

The Di[b] antigen differs from Di[a] by a single amino acid change, Leu854Pro, on band 3. The antigen is found in all populations. Di[b] is weakly expressed in individuals with SAO, and it has also been reported as weakened in several Mexican American individuals.

Anti-Di[b] was first reported by Thompson et al. in 1967.[11] The antibody may cause mild cases of HDFN, yield a positive direct antiglobulin test (DAT), or exhibit no clinical manifestation. Anti-Di[b] has caused moderate or delayed transfusion reactions. Anti-Di[b] may show dosage in serologic testing. A few examples of autoanti-Di[b] have been reported.[12]

Wr[a] (DI3)

The Wr[a] antigen was first reported by Holman in 1953 and assigned to the DI system in 1995.[13] Wr[a] differs from the Wr[b] antigen by a single nucleotide change, 1972G>A, at exon 16, producing the amino acid change Glu658Lys.

Anti-Wr[a] is a relatively common antibody and has a wide range of reactivity. The antibody can be an IgM agglutinin reacting at temperatures below 37°C or an IgG agglutinin reacting only at the antiglobulin phase of testing (AGT). Anti-

Wr^a has caused severe HDFN and hemolytic transfusion reactions (HTRs). It has been speculated that there may be some examples of anti-Wr^a that are benign, as routine antibody detection reagent RBCs do not contain the Wr^a antigen and HTR caused by anti-Wr^a has not been reported since the widespread use of immediate spin (IS) and electronic compatibility testing.[14]

Wr^b (DI4)

The Wr^b antigen was first described in 1971 by Adams et al., but not assigned to the DI system until 1995.[15] The Wr^b antigen expression is dependent on the presence of GPA and band 3.

Wr^b is absent from En(a–) erythrocytes, which lack GPA. However, GPA from En(a+), Wr(b–) RBCs has an amino acid sequence identical to that of GPA from En(a+), Wr(b+) erythrocytes. Wr^b requires the interaction of GPA with erythrocyte integral membrane protein, band 3.[4,7,16]

The Wr(b–) phenotype with normal GPA may be explained by the change from a negatively to a positively charged residue at position 658 of band 3. Erythrocytes that are Hill-positive and Dantu-positive carry a hybrid sialoglycoprotein containing part of the GPA and GPB amino acid sequence and are also Wr(b–).[17]

Owing to the rarity of the Wr(b–) phenotype, data on the clinical significance of anti-Wr^b are scanty. One alloanti-Wr^b, in a Wr(a–b–), GPA-variant individual, was evaluated by chemiluminescence assay, and the findings suggested the antibody was likely to cause destruction of incompatible RBCs.[18] Many examples of autoanti-Wr^b have been reported. Some examples of the autoantibody have been clinically significant and others benign.

Wd^a (DI5)

The Wd^a antigen was reported in two large nuclear Hutterite families. In 1981, Lewis and Kaita first reported the antibody defining the Wd^a antigen.[19] Wd^a is destroyed by α-chymotrypsin.[20] Wd^a is the result of a single point mutation that causes the amino acid change Val557Met.

Anti-Wd^a can be found in sera containing other low-prevalence antigens. The antibody often reacts as a saline agglutinin. Only one IgG example has been reported. No cases of HTR or HDFN have been reported.

Rb^a (DI6)

The Rb^a antigen was described by Contreras in 1978.[21] Rb^a is destroyed by α-chymotrypsin.[22] Rb^a is the result of a single point mutation that causes the amino acid change Pro548Leu.

The antibody can be found in sera containing antibodies to other low-prevalence antigens and often reacts as a saline agglutinin. Only one IgG example of anti-Rb^a has been reported. No cases of HTR or HDFN have been reported.

WARR (DI7)

Coghlan et al. reported a case in 1991 of mild HDFN caused by an antibody to a low-prevalence antigen that they named WARR.[23] The antigen is very rare and has been found in two related kindreds. The antigen was detected in the oldest member of the kindred, an absentee Shawnee. It is believed that the antigen may be unique to Native American individuals. Issitt and Anstee reported that even though the antigen is rare, the antibody is not.[22] WARR is the result of a single point mutation that causes the amino acid change Thr552Ile.

ELO (DI8)

The low-prevalence RBC antigen ELO was detected in 1979 (unpublished observations cited by Tippett). The findings were published by Coghlan et al. in 1993.[24] ELO was assigned to the DI system in 1998, when Zelinski et al. published their findings that established the association between ELO and the gene controlling Diego blood group polymorphisms.[25] The ELO antigen (Arg432Trp) is carried in the first ectoplasmic loop of band 3.

HDFNs caused by anti-ELO have been reported. In experiments with enzyme-treated erythrocytes, Jarolim et al. reported that in the two anti-ELO samples they studied, one reacted with α-chymotrypsin and pronase-treated RBCs, which was an unexpected finding.[26]

Wu (DI9)

The Wu antigen was also called Hov and Haakestad by different investigators. The antigen was reported by three different individuals and given three different names. In 1972, an abstract describing Mo^a[27] was published that mentioned a new low-prevalence antigen called Haakestad. Then in 1973, Szaloky et al.[28] described an antibody they named anti-Hov, and in 1976, Kornstad described the Wu antigen.[29] In 1987, Kornstad[30] published data that showed that Haakestad and Hov were the same antigen, and in 1992, Moulds et al.[31] showed that Hov and Wu were the same antigen. Wu was selected as the official name. This antigen is well developed at birth, and it is destroyed by α-chymotrypsin and pronase.

The antigen Wu (Wulfsberg), Gly565Ala, is carried in the third ectoplasmic loop of band 3. There is a serologic relationship between Wu and two other low-prevalence antigens in the DI system, NFLD and Bow, which are described

later.[32] Adsorption studies demonstrated that RBCs positive for either antigen will remove all three antibodies. This is likely to happen as these antigens are the result of amino acid substitutions at the same position, and cross-reactivity surely occurs.[17] The Wu antigen is antithetical to the DISK antigen (DI22).

Bp^a (DI10)

In 1964, Cleghorn (unpublished observations 1962–1967) found a serum that contained multiple antibodies to multiple known low-prevalence antigens and also contained a new specificity that he named anti-Bp^a.[33]

The Bp^a antigen is associated with the amino acid substitution Asn569Lys in the transmembrane domain close to the third extracellular loop. This area is sensitive to trypsin, α-chymotrypsin, pronase, and papain.[4]

Mo^a (DI11)

Kornstad and Brocteur, in 1972, described the first example of anti-Mo^a in a serum being used to screen random donors for the low-prevalence antigen Jn^a.[27] Mo^a is the result of a mutation at the fourth ectoplasmic loop that changed the amino acid at position 656 (Arg656His). Another mutation at the same amino acid position gives rise to the Hg^a antigen (see next section). Mo^a antigens are not destroyed by trypsin, α-chymotrypsin, ficin, or pronase.

Hg^a (DI12)

Anti-Hg^a was reported by Rowe and Hammond in a serum that contained a warm reactive autoantibody along with anti-Pt^a, -Wd^a, and -BOW.[34] The fourth ectoplasmic loop carries the Hg^a (Hughes) antigen and is associated with the amino acid residue substitution Arg656Cys. Hg^a is not destroyed by trypsin, α-chymotrypsin, ficin, or pronase.

Vg^a (DI13)

Young, in 1981, reported the presence of anti-Vg^a in one serum that also contained anti-Wu.[35] Anti-Vg^a is not uncommon in sera containing multiple antibodies to low-prevalence antigens. Vg^a (Van Vugt), Tyr555His, is carried in the third ectoplasmic loop of band 3 and is destroyed by α-chymotrypsin and pronase.

Sw^a (DI14)

Anti-Sw^a was described by Cleghorn in 1959.[36] In 1987, Contreras et al. reported heterogeneity among anti-Sw^a and reported the existence of a second antigen related to Sw^a.[37] It was found that some Sw(a+) RBCs reacted with all examples of anti-Sw^a, but others only reacted with some of the Sw^a antisera. The difference was shown to be qualitative, not quantitative. The RBCs that reacted with all anti-Sw^a were said to contain two antigens, Sw^a and SW1. The RBCs that only reacted with some of the anti-Sw^a were said to be Sw(a+) but negative for SW1. Family studies confirmed the theory that the difference between the two antigens is a qualitative one.[38] In addition, a serologic relationship between Sw^a, SW1, and the low-prevalence antigen Fr^a has also been reported.[37]

Two mutations in exon 16 have been associated with the Sw^a antigen, 1937G>A, which changes Arg646Gln, and 1936C>T, which causes the Arg646Trp change.

BOW (DI15)

Anti-BOW was first described by Chaves et al. in 1988.[39] The antibody caused an unexplained incompatible crossmatch. BOW is the result of a single point mutation that causes the amino acid change Pro561Ser, and it is carried in the third ectoplasmic loop of band 3. The antigen is destroyed by pronase and α-chymotrypsin. The BOW antigen is antithetical to the NFLD antigen.

NFLD (DI16)

Lewis et al., in 1984, described a new low-prevalence antigen, NFLD, in a white family of French extraction.[40] In 1988, Okubo et al. described the presence of the NFLD antigen in the Japanese population.[41] It is interesting to mention that tests with NFLD+ RBCs in Japan detected 67 examples of anti-NFLD, and most of these examples were single antibodies.

NFLD is associated with the amino acid residue substitution Pro561Ala as the result of the single nucleotide change 1681C>G. As expression of the BOW antigen is also the result of a substitution at amino acid residue 561 (Pro561Ser), NFLD and BOW can be considered to be antithetical antigens. Expression of NFLD is also associated with a second mutation, 1287A>T, which causes the amino acid change, Glu429Asp. This suggests that the NFLD epitope may be formed through an association or interaction between the first (residue 429) and third (residue 561) extracellular loops of band 3.

Jn^a (DI17)

Kornstad et al. reported a new antibody, anti-Jn^a, in a serum containing anti-Wr^a and anti-Bp^a.[41] They presented studies of three unrelated individuals with Jn(a+) RBCs. Two of them presented with nucleotide substitutions of Pro556Ser. The third individual showed a change that predicted Pro556Ala and was later shown to define the KREP antigen (see next section). These changes were recognized at the serologic level

before the polymorphisms were established by Poole et al. in 1997.[43]

KREP (DI18)

Found in 1997 during investigation of the second Jn(a+) proband, KREP was named after the antigen-positive donor and assigned to the Diego blood group system in 1998. This antigen is the result of an SNP at 1696C>G, which causes the amino acid change Pro556Ala. As expression of both Jn^a and KREP antigens is the result of substitutions of Pro556, they can be considered antithetical antigens.[43]

Tr^a (DI19)

Cleghorn found anti-Tr^a in 12 of 18 sera that also contained anti-Wr^a.[33] This antigen has been called Lanthois in some publications. Tr^a is the result of a single point mutation that causes the amino acid change Lys551Asn.

Fr^a (DI20)

The Fr^a antigen has been reported in three Mennonite kindreds in Manitoba, Canada. A point mutation in exon 13 of *SLC4A1* accounts for a Glu480Lys substitution in band 3, which controls Fr^a antigen expression.[44] Based on these results Fr^a was assigned to the Diego blood group system. Fr^a seems to be related to Sw^a. Both Sw(a+) SW:1 and Sw(a+) SW:–1 will adsorb anti-Sw^a and anti-Fr^a, but Fr(a+) RBCs will adsorb anti-Fr^a but not anti-Sw^a.[45]

SW1 (DI21)

SW1 was documented in 1987, after being revealed by heterogeneity among sera containing anti-Sw^a. It was assigned to the Diego blood group system in 2000.[46] The SW1 antigen is linked to a mutation in exon 16 (1936C>T), which causes the Arg646Trp amino acid change. Refer to the Sw^a review in a previous section.

DISK (DI22)

A high-prevalence antigen antithetical to DI9 (Wu) has been identified.[47] The new antigen, DI22 (DISK), was characterized by an apparently naturally occurring, strongly agglutinating antibody reactive at both 18°C and 37°C and by the indirect antiglobulin test. DISK was shown to be sensitive to α-chymotrypsin treatment, but resistant to other commonly used proteases. Targeted sequence analysis of *SLC4A1* exon 14 revealed homozygosity in the proband and heterozygosity in a sample from her brother for the mutation 1694G>C that encodes Gly565Ala. The RBCs of her brother reacted more weakly with her antibody, suggesting that the anti-DISK exhibits dosage in DI:9, 22 individuals.

Poole et al. suggest that anti-DISK may be of clinical significance based on its serologic characteristics even though it could not be confirmed at the time.[47]

The Diego Null Phenotype

In 1983, Biro et al.[48] described a healthy Mexican blood donor initially thought to be Di(a–b–), but further testing showed the donor's RBCs were Di(a–b+^{w}). The donor's RBCs were tested with eight samples of anti-Di^b and failed to react with two of them, but it was shown that the donor's RBCs will adsorb and elute the anti-Di^b present in the two antisera that were macroscopically nonreactive. Other reports have mentioned the weakened reactivity of the Di^b antigens of some individuals.[48–50]

In 2000, Ribeiro and colleagues reported the case of a child with severe hereditary spherocytosis caused by the absence of band 3.[51] Defects in *SLC4A1* are the cause of spherocytosis type 4. Spherocytosis is a hematologic disorder characterized by numerous abnormally shaped erythrocytes, which are spheroidal and may cause chronic hemolytic anemia. The absence of band 3 suggests that the patient represents the Di(a–b–) phenotype.

Contrary to initial belief, homozygosity for mutations in *SLC4A1* (lack of band 3) is not incompatible with life. That the lack of band 3 is not incompatible with life has been demonstrated in experimental evidence using engineered knockout mice and naturally occurring knockouts in Japanese black cattle, and it also has been reported in humans.[3,52,53]

In the article, Ribeiro et al.[51] indicated that the child probably survived because the family history was known, including the underlying mutation that caused band 3 and protein 4.2 to be absent. At the time, the prognosis of the child was uncertain; she was transfusion dependent and also sustained by oral doses of bicarbonate to counteract the renal acidosis that resulted from the absence of band 3 in her kidneys.

Summary

For 40 years (1955–1995), Di^a and Di^b were the Diego system. In 1995, Wr^a and Wr^b were assigned to the system. Since then, a combination of serologic knowledge with discoveries from biochemical and molecular research has allowed another 18 antigens to be assigned to the system; all but one are of low prevalence.

There is scanty or no data on the clinical significance of most of the antibodies in the Diego system. Anti-Di^a, -Wr^a, -ELO, -BOW, -Sw^a, -Fr^a, and -SW1 have been associated with HDFN, and anti-Wr^a has also been associated with HTR. Antibodies against the high-prevalence antigen Di^b have been associated with mild HTR.

The antibodies directed against low-prevalence antigens in the Diego system are produced with no obvious immunizing stimulus. Antibodies to all low-prevalence antigens are almost invariably found in antisera that contain multiple antibodies to low-prevalence antigens. In contrast, anti-Di^a is usually found as a single specificity and only occasionally does it occur in plasma containing multiple antibodies to low-prevalence antigens.

Mutations in the *SLC4A1* gene that encodes the Di system antigens can result in various defects, some of which are critical to the organism, such as hereditary spherocytosis or renal tubule acidosis. Other mutations seem to be benign, producing changes that result in new antigens.

Acknowledgments

I thank Marie Holub for lending her time and expertise to the review of this manuscript.

References

1. Layrisse M, Arends T, Dominguez SR. Nuevo grupo sanguíneo encontrado en descendientes de Indios. Acta Med Venez 1955;3:132–8.
2. Schofield, AE, Martin, PG, Spillett, D, Tanner, MJ. The structure of the human red blood cell anion exchanger (EPB3, AE1, band 3) gene. Blood 1994;84:2000–12.
3. Telen MJ. Erythrocyte blood group antigens: not so simple after all. Blood 1995;85:299–306.
4. Daniels G. Human blood groups. 2nd ed. Oxford, UK: Blackwell Science, 1995:363.
5. Spring FA, Bruce LJ, Anstee DJ, Tanner MJ. A red cell band 3 variant with altered stilbene disulphonate binding is associated with the Diego (Dia) blood group antigen. Biochem J. 1992 Dec 15;288(Pt 3):713–716.
6. Poole J. The Diego blood group system—an update. Immunohematology 1999;15:135–43.
7. Poole J. Red cell antigens on band 3 and glycophorin A. Blood Rev 2000;14:31–43.
8. Tanner MJ. The structure and function of band 3 (AE1): recent developments (review). Mol Membr Biol 1997;14:155–65.
9. Daniels G. Human blood groups. 2nd ed. Oxford, UK: Blackwell Science, 1995:354.
10. Issitt PD, Anstee DJ. Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific Publications, 1998:584.
11. Thompson PR, Childers DM, Hatcher DE. Anti-Di^b—first and second examples. Vox Sang 1967;13:314–18.
12. Issitt PD, Anstee DJ. Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific Publications, 1998:590.
13. Holman CA. A new rare human blood group antigen (Wr^l). Lancet 1953;2:119–20.
14. Issitt PD, Anstee DJ. Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific Publications, 1998:586.
15. Adams J, Brovisac M, Brooks W, Johnson NR, Issitt PD. An antibody, in the serum of a Wr(a+) individual, reacting with an antigen of very high frequency. Transfusion 1971;11:290–1.
16. Telen MJ, Chasis JA. Relationship of the human erythrocyte Wr^b antigen to an interaction between glycophorin A and band 3. Blood 1990;76:842–8.
17. Bruce LJ, Ring SM, Anstee DJ, Reid ME, Wilkinson S, Tanner MJ. Changes in the blood group Wright antigens are associated with a mutation at amino acid 658 in human erythrocyte band 3: a site of interaction between band 3 and glycophorin A under certain conditions. Blood 1995;85:541–7.
18. Poole J, Banks J, Kjeldsen-Kragh J, et al. Second example of MiV/MiV phenotype with anti-Wr^b. A case study (abstract). Transfus Med 1997; 7(Suppl 1):24.
19. Lewis M, Kaita H. A "new" low incidence "Hutterite" blood group antigen Waldner (Wda). Am J Hum Genet 1981;33: 418–20.
20. Daniels G. Human blood groups. 2nd ed. Oxford, UK: Blackwell Science, 1995:359.
21. Contreras M, Stebbing B, Mallory D, et al. The Redelberger antigen Rba. Vox Sang 1978;35:397-400.
22. Issitt PD, Anstee DJ. Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific Publications, 1998:591.
23. Coghlan G, Crow M, Spruell P, Moulds M, et al. A 'new' low-incidence red cell antigen, WARR: unique to Native Americans? Vox Sang 1995;68(3):187–90.
24. Coghlan G, Green C, Lubenko A, Tippet P, Zelinski T. Low-incidence red cell antigen ELO (700.51): evidence for exclusion from thirteen blood group systems. Vox Sang 1993;64: 240–43.
25. Zelinski T, Punter F, McManus K, Coghlan G. The ELO blood group polymorphism is located in the putative first extracellular loop of human erythrocyte band 3. Vox Sang 1998;75:63–5.
26. Jarolim P, Rubin HL, Zakova D, Storry J, Reid ME. Characterization of seven low incidence blood group antigens carried by erythrocyte band 3 protein. Blood 1998;92: 4836–43.
27. Kornstad L, Brocteur J. (abstract). A new, rare blood group antigen, Moa (MOEN). Book of Abstracts Joint Congress ISBT/ AABB 1972:p 58.
28. Szaloky A, Sijpesteijn NK, Hart M vander. A New Blood Group Antigen, Hov. Vox Sang 1973;24(6):535-41.
29. Kornstad L, Howell P, Jorgensen J, Larsen AM, Wadsworth LD. The rare blood group antigen, Wu. Vox Sang 1976;31: 337–43.
30. Kornstad L, Jerne D, Tippett, P. The Haakestad antigen is identical with the Hov antigen. Vox Sang 1987;52(1-2):120–2.
31. Moulds M, Kaita H, Kornstad L, Lubenko A. Evidence that the low-incidence antigens termed Wu(700.12) and HOV(700.38) are identical. Vox Sang 1992;62(1):53–4.
32. Kaita H, Lubenko A, Moulds M, Lewis M. A serologic relationship among the NFLD, BOW, and Wu red cell antigens. Transfusion 1992;32:845–7.

33. Cleghorn TE. Cited by Race RR, Sanger R. Blood groups in man. 6th ed. Oxford, UK: Blackwell Scientific, 1975.
34. Rowe GP, Hammond W. A new low-frequency antigen, Hga (Hughes). Vox Sang 1983;45:316–19.
35. Young S. Vga: a new low incidence red cell antigen. Vox Sang 1981;41:48–9.
36. Cleghorn TE. A 'new' human blood group antigen, Swa. Nature 1959;184:1324–5.
37. Contreras M, Teesdale P, Moulds M, et al. Sw^a: a subdivision. Vox Sang 1987;52:115–19.
38. Issitt PD, Anstee DJ. Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific Publications, 1998:596.
39. Chaves MA, Leak MR, Poole J, Giles CM. A new low-frequency antigen BOW (Bowyer). Vox Sang 1988;55:241–3.
40. Lewis M, Kaita H, Allderdice PW, Bergren M, McAlpine PJ. A "new" low incidence red cell antigen, NFLD. Hum Genet 1984;67:270–1.
41. Okubo Y, Yamaguchi H, Seno T, Egashira S, Green CA, Kaita H. The NFLD antigen in Japan. Hum Hered 1988;38:122–4.
42. Kornstad L, Kout M, Larsen AM, Orjasaeter H. A rare blood group antigen, Jna. Vox Sang 1967;13:165–70.
43. Poole J, Bruce LJ, Hallewell H, et al. Erythrocyte band 3 mutation Pro561→Ser gives rise to the BOW antigen and Pro 566→Ala to a novel antigen KREP (abstract). Transfus Med 1997;(Suppl 1):17.
44. McManus K, Lupe K, Coghlan G, Zelinski, T. An amino acid substitution in the putative second extracellular loop of RBC band 3 accounts for the Froese blood group polymorphism. Transfusion 2000;40:1246–9.
45. Issitt PD, Anstee DJ. Applied blood group serology. 4th ed. Durham, NC: Montgomery Scientific Publications, 1998:597.
46. Reid M, Lomas-Francis C. The blood group antigen factsbook 2nd ed. New York: Elsevier, 2004:330.
47. Poole J, Thornton NM, Tilley L, et al. Novel high incidence antigen in the Diego blood group system (DISK) and clinical significance of anti-DISK. Vox Sang 2010;99(Suppl 1):54–5.
48. Biro V, Garratty G, Johnson CL, Marsh WL. Depressed blood group antigens on red cells from a Mexican donor. Transfusion 1983;23:65–6.
49. Edwards-Moulds JM, Alperin JB. Studies of the Diego blood group among Mexican-Americans. Transfusion 1986;26: 234–6.
50. Issitt PD, Wren MR, Rueda E, Maltz M. Red cell antigens in Hispanic blood donors (letter). Transfusion 1987;27:117.
51. Ribeiro ML, Alloisio N, Almeida H, et al. Severe hereditary spherocytosis and distal renal tubular acidosis associated with the total absence of band 3. Blood 2000;96:1602–4.
52. Inaba M, Yawata A, Koshino I, et al. Defective anion transport and marked spherocytosis with membrane instability caused by hereditary total deficiency of red cell band 3 in cattle due to a nonsense mutation. J Clin Invest 1996;97:1804–17.
53. Southgate CD, Chishti AH, Mitchell B, Yi SJ, Palek J. Targeted disruption of the murine erythroid band 3 gene results in spherocytosis and severe haemolytic anaemia despite a normal membrane skeleton. Nat Genet 1996;14:227–30.

Dolores Figueroa, MT(ASCP)SBB, IRL System Specialist, Blood Systems Laboratories, 2424 W. Erie Dr., Tempe, AZ 85282.