The Cromer blood group system: a review

J.R. Storry, M.E. Reid, and M.H. Yazer

The antigens of the Cromer blood group system reside on decay-accelerating factor (DAF), a protein belonging to the regulators of complement activation family. The blood group system consists of 12 high-prevalence and three low-prevalence antigens. The molecular basis for the antigens is known, and with the exception of IFC, each antigen is the product of a single nucleotide change in the *DAF* gene and has been localized to one of the four complement control protein (CCP) domains on the DAF protein. The RBCs of people with the Cromer null phenotype, Inab, lack DAF but do not appear to demonstrate increased susceptibility to hemolysis. Antibodies to Cromer antigens are rarely encountered, although there is evidence that the antibodies may cause accelerated destruction of transfused RBCs. There is no risk of HDN associated with Cromer system antibodies because the placenta is a rich source of fetally derived DAF, which is thought to adsorb the antibodies
***Immunohematology* 2010;26:109–117.**

Table 1. Summary of the Cromer antigens and the associated amino acid residues*

High-prevalence antigen	ISBT No.	Critical amino acid	Amino acid specifying negative phenotype	Antithetical low-prevalence antigen	ISBT No.	Critical amino acid
Cr^a	CROM1	Ala227	Pro227			
Tc^a	CROM2	Arg52		Tc^b	CROM3	Leu52
				Tc^c	CROM4	Pro52
Dr^a	CROM5	Ser199	Leu199			
Es^a	CROM6	Ile80	Asn80			
IFC	CROM7					
WES^b	CROM9	Leu82		WES^a	CROM8	Arg82
UMC	CROM10	Thr250	Met250			
GUTI	CROM11	Arg240	His240			
SERF	CROM12	Pro216	Leu216			
ZENA	CROM13	His242	Gln242			
CROV	CROM14	Glu156	Lys156			
CRAM	CROM15	Gln247	Arg247			

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Key Words: Cromer, DAF, decay-accelerating factor, blood group, Inab, RBC

The Cromer blood group antigens are carried on decay-accelerating factor (DAF, CD55), a member of a family of proteins known as the regulators of complement activation. In 1965, an antibody in the serum of a Black prenatal patient, Mrs. Cromer, which reacted with all RBCs except her own and those of two siblings, was reported.[1] RBCs from Mrs. Cromer were Go(a+), and initially her antibody was named anti-Go^b because it was thought to detect the high-prevalence antigen antithetical to Go^a (Go^a was only years later discovered to result from a partial D rearrangement categorized as D^{IVa}). In 1975, Stroup and McCreary[2] reported four additional examples of the antibody and renamed it anti-Cr^a after the propositae. In 1982, Daniels et al.[3] described an antibody in the serum of a Japanese man (Inab) that was reactive with all RBCs, including those of his mother, father, and brother. Inab RBCs were shown to be the null phenotype of the Cromer system. Subsequently, other examples of antibodies to high-prevalence antigens were linked to Cromer by failure of the antibody to react with Inab RBCs, sensitivity of the antigen to α-chymotrypsin treatment of RBCs, and specific inhibition of the antibody by concentrated plasma or urine from an antigen-positive person. To date, 12 high-prevalence antigens and 3 low-prevalence antigens have been described in the Cromer system (Table 1).[4] Transfusion data regarding the clinical significance of antibodies to Cromer antigens are mixed; however, no cases of hemolytic disease of the fetus and newborn (HDFN) have been described. These clinical details are discussed later in more detail.

Cromer Blood Group Antigens Are Carried on DAF

In 1987, a human cell-surface glycoprotein with an approximate M_r of 70 kDa, found on RBCs, WBCs, and platelets, was characterized and shown to carry the Cromer antigens.[5] Telen et al.[6] and Parsons et al.[7] showed independently that this glycoprotein was DAF and that people with the Inab phenotype lacked DAF on their RBCs. DAF is linked to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor and is expressed on all hematopoietic cells and on the vascular endothelium where it protects against complement-mediated attack.[8] It is also widely expressed on epithelium in the gastrointestinal (GI), genitourinary, and central nervous systems. A soluble form is present in plasma and body fluids.[9]

The gene encoding DAF was mapped to chromosome 1 in 1987.[10] It was cloned and sequenced in the same year: DAF encodes a protein of 381 amino acids that includes a 34-residue signal peptide.[11,12] The mature protein is arranged into four complement control protein (CCP) domains, each of approximately 60 amino acids, followed by a 70-amino acid region that is rich in serine and threonine residues.[8] In this review, for the numbering of nucleotides and amino acids in DAF, nucleotide A of the initiation methionine codon is designated as nucleotide 1 and the first amino acid (methionine) of the mature protein is designated as residue 1 (Fig. 1). These numbers may differ from those in the original reports.

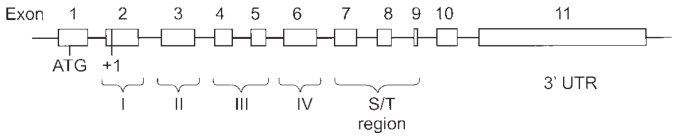

Fig. 1. The organization of the *DAF* gene. ATG in exon 1 is the initiation codon, the A of which is nucleotide 1.[8] Roman numerals indicate the region of the gene that encodes the complement control protein (CCP) domains of the protein. The S/T region refers to the exons that encode a serine/threonine-rich region. 3′ UTR is the 3′ untranslated region. Reprinted with permission from the *Journal of Immunology*.[13] Copyright 1990. The American Association of Immunologists, Inc.

Before DAF was identified as the carrier protein for Cromer blood group antigens, traditional methods of establishing exclusion of new Cromer antigens from existing blood groups were used. These methods include comprehensive family studies and serologic characterization of the antibodies. Consanguinity has been shown in four families in which the propositus lacks a high-prevalence Cromer blood group antigen.[14–17] DNA sequencing now provides a rapid method of identifying the genetic basis of the antigens. Furthermore, two elegant sets of experiments, one using the monoclonal antibody immobilization of erythrocytes assay (MAIEA)[18] and the other using a series of DAF deletion mutant proteins expressed in Chinese hamster ovary (CHO) cell lines, permitted epitope mapping of the antigens to one of the four CCPs on DAF (Fig. 2).[20–22] These experiments are important because although DNA sequencing can be used to identify a missense nucleotide change in *DAF*, proving that the change is responsible for the presence or absence of an antigen is difficult when there are only one or two examples of the phenotype to test. The MAIEA confirmed that Cromer antigens are on DAF, and the use of well-characterized monoclonal anti-DAF in combination with human polyclonal antibodies to different Cromer antigens permitted spatial mapping. Western blotting experiments using CHO cell lines that expressed all or part of the DAF protein were tested with specific antibodies to Cromer antigens and the DAF protein. The results of these assays correlated with the DNA sequence predictions.

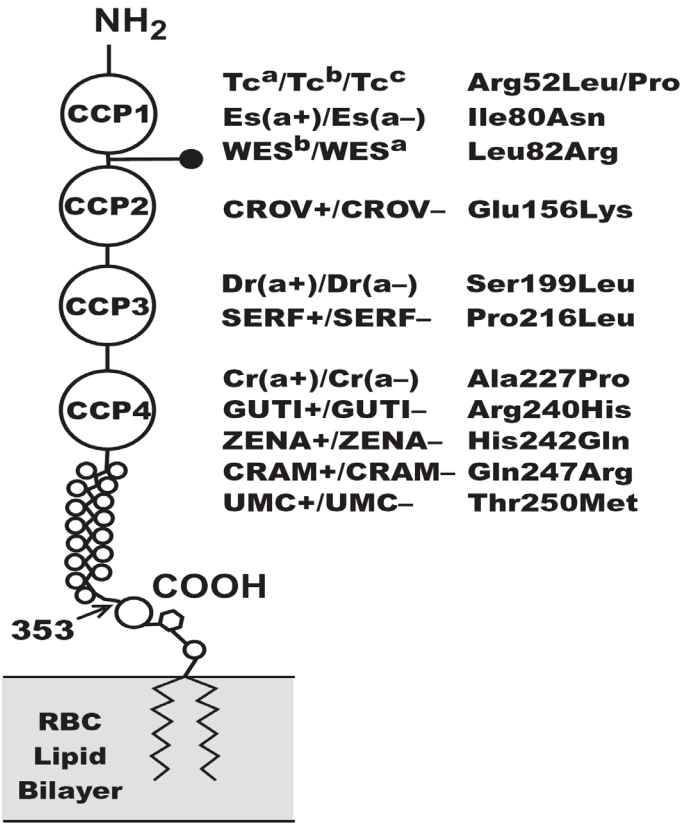

Fig. 2. Schematic diagram of the glycosylphosphatidylinositol-linked DAF protein demonstrating its four complement control protein (CCP) regions and the location of the Cromer antigens along with their amino acid mutations. Reprinted with permission from John Wiley and Sons.[19]

Characteristics of the Antigens and Antibodies

Cr^a

The first example of anti-Cr^a was described in 1965 in the serum of a Black woman.[1] The antibody reacted with the RBCs of all random donors and with those of more than 4000 Black donors but not with the proposita's own RBCs or those of two of her siblings. With the exception of one example found in the serum of a Spanish American woman,[23] all examples of anti-Cr^a have been found in Black individuals.[24] Although anti-Cr^a may be stimulated by pregnancy,[25] there have been no cases of HDFN attributable to the antibody (see later discussion). The molecular basis of the Cr(a–) phenotype is a nucleotide change, 679G>C in exon 6, which is predicted to encode an amino acid substitution of Ala-227Pro in the CCP4 of DAF.[22] No low-prevalence antigen has been identified as being associated with this change.

Tc^a, Tc^b, Tc^c

Tc^a, a high-prevalence antigen, was first reported in 1980.[26] The antibody was found in the serum of two Black women (GT and DLC), and the new antigen defined by the G*T* and DL*C* sera was named Tc^a after the two propositae.

Neither patient had been transfused. Presumably, their antibodies had been stimulated by pregnancy, although there was no evidence of HDFN. The DLC antibody was distinct from all other antibodies to known high-prevalence antigens. Tests with a panel of RBCs that lacked an unidentified high-prevalence antigen revealed compatibility with the RBCs from GT. Cross-testing confirmed that the antibodies were mutually compatible. Studies on 28 members of DLC's family revealed two additional Tc(a–) people, neither of whom had produced anti-Tc^a.[14] Previously, Stroup and McCreary[2] had found that the RBCs from one of their Cr(a–) patients, AJ, reacted very weakly with the GT serum, and a possible relationship between the two antigens was proposed. The RBCs of Inab were compatible with the "new" antibody; however, the Inab serum was reactive with RBCs from both AJ and DLC. The low-prevalence antigen Tc^b was identified when the Tc(a–) RBCs of WM reacted with one of four sera containing anti-Go^a.[27] After adsorption of the anti-Go^a, the serum reacted with 6 of 103 RBC samples from randomly selected Black individuals. Additionally, the RBCs of 11 Tc(a–) individuals were agglutinated by the absorbed serum. Thus, it was concluded that the new antibody detected an antigen, Tc^b, which was antithetical to Tc^a. The occurence of Tc^b in a random Black population was calculated to be 5 percent.[28] No Tc(b+) Caucasians have been found. The Tc(a–) phenotype also has been described in Caucasians.[29] An antibody was detected in the serum of an 18-year-old woman (DWL) who had been transfused with four units of RBCs during her second pregnancy. Her RBCs typed as Tc(a–b–), and the antibody was compatible with Tc(a–b+) RBCs. A family study showed that the proposita's parents and three of four siblings were incompatible, but the RBCs of DWL's serologically compatible sister also typed Tc(a–b–) and were nonreactive with the antibody. The low-prevalence antigen was named Tc^c and is the antithetical antigen of Tc^a. Six months later, DWL's serum was reactive with Tc(a+b–) and Tc(a–b+) RBCs but not with her own RBCs or those from her Tc(a–b–c+) sister. Subsequent testing showed that the antibody was compatible with RBCs of the Inab phenotype. It was proposed that the antibody was an inseparable anti-Tc^{a}Tc^b.[29] The molecular basis of the Tc^a/Tc^b antigens was defined by direct sequence analysis as a single nucleotide change, 155G>T, that is predicted to encode Arg52Leu in the CCP1 of DAF.[22] Another nucleotide change at the same position (155G>C) was found in a Tc(a–b–c+) sample, which is predicted to encode a change of Arg52Pro.[20]

Dr^a

Another antibody to an apparent Cromer-related high-prevalence antigen was reported in 1984.[15] Before surgery, an antibody to a high-prevalence antigen was identified in the serum of MD, an Israeli woman of Bukharan origin. In family studies, MD's serum was compatible with the RBCs from her sister, whose serum also contained an antibody that demonstrated identical specificity. Both women were multiparous and had been transfused. The RBCs of both MD and her sister had weakened expression of Cr^a and Tc^a antigens and were only weakly incompatible with the Inab serum and that of Owens (see IFC).[15] The antibody was shown to be distinct from anti-Cr^a and anti-Tc^a and was named anti-Dr^a, after MD. Two other examples of anti-Dr^a in unrelated individuals of Uzbekistani Jewish descent were described.[30,31] Family studies performed on the second reported example revealed three Dr(a–) individuals, two sons and a daughter, none of whom had anti-Dr^a in their serum. Weakened expression of Cr^a and Tc^a antigens (and subsequently of other high-prevalence Cromer antigens) was also observed in these individuals. A 38-year-old Russian woman (KZ), who presented with a chronic intestinal disorder, was originally reported as an example of the Inab phenotype, but subsequent studies showed that her Dr(a–) RBCs were weakly reactive with anti-Tc^a and anti-IFC.[32,33] Although KZ's siblings were not available for testing, samples from her parents were tested. RBCs from both parents were positive for IFC; however, the reactivity was weaker than that of control RBCs, consistent with an underlying heterozygous genotype which included the silencing/variant allele. The Dr(a–) phenotype has been also described in the Japanese population. Daniels et al.[34] reported anti-Dr^a in the serum of a female blood donor who had two children but no history of transfusion. The donor's RBCs reacted weakly with anti-Cr^a, anti-Tc^a, anti-WES^b, anti-UMC, and six of nine monoclonal anti-DAFs but did not react with anti-Dr^a. Another Japanese Dr(a–) proband, a blood donor, was reported by Uchikawa et al.[35] The donor's RBCs were only weakly agglutinated by five monoclonal anti-DAFs and with the original anti-IFC, but not by anti-Dr^a. The molecular basis is the same for Israelis and Japanese. A single nucleotide change of 596C>T produces Ser199Leu.[33,34,36] The nucleotide change creates an alternative splice site that is used preferentially but not exclusively. However, the missplicing results in a 44–base pair (bp) deletion in the coding sequence that alters the open reading frame and results in a premature stop codon. The small amount of full-length DAF that is translated carries the 199Leu residue, and RBCs type as Dr(a–). The Cromer antigens on Dr(a–) RBCs are very weak and may not be detected by hemagglutination. In tests with monoclonal antibodies, Dr(a–) RBCs express only 40 percent DAF when compared with normal RBCs.[36] The Dr^a antigen is the receptor for fimbriae from 075X-positive *Escherichia coli*, an organism that is associated with urinary tract infection, cystitis, and protracted diarrhea.[37]

Es^a

The original antibody found in the serum of a woman of Mexican descent was detected in routine compatibility testing and was reactive with all random RBCs tested.[16] The RBCs of two of three siblings, as well as RBCs of the Inab phenotype, were compatible with the antibody. The woman's parents were first cousins. The second example was identified in an African American man who was being

treated for Alzheimer's disease, diabetes, and anemia. He had no history of transfusion and no siblings were available.[38] The molecular basis of the Es(a–) phenotype was determined to be a single nucleotide change of 239T>A, which predicts a change of Ile80Asn in CCP1 of DAF.[20]

IFC (The Inab or Cr_{null} Phenotype)

The Inab phenotype is the null phenotype of the Cromer blood group system, and the RBCs of people with this rare phenotype are DAF-deficient.[3] The sera of several of these individuals with the Inab phenotype contained an antibody that was reactive with all random RBCs tested, including RBCs lacking the high-prevalence Cromer antigens. The antibody was named anti-IFC, and RBCs of the Inab phenotype are IFC negative.[17]

To date, this rare phenotype has been identified in nine people: five Japanese,[3,34,39–41] one Jewish American,[42] an Italian American woman and her brother,[43] and an African American[34] (Table 2). It is likely that another African American male was also of the Inab phenotype; however, his RBCs were never tested.[44] The first two probands with the Inab phenotype, a Japanese man and a Jewish American man, were both diagnosed with disorders of their GI tracts, and it was unclear whether the "null" status was an acquired phenomenon associated with their disease or an inherited characteristic.[3,42] However, the parents of the propositus were first cousins, suggesting that the Inab phenotype arose from the inheritance of a rare recessive gene. The Japanese patient, Osad-II,[34] was diagnosed with a capillary angioma of the small intestine.[34] Similarly, the aforementioned African American boy had a diagnosis of protein-losing enteropathy,[44] as did the African American patient.[34] However, no history of intestinal disease was reported in the 86-year-old Italian American,[43] who had been admitted to the hospital with a fractured hip, or for one of the remaining two Japanese individuals.[41]

Table 2. Description of reported patients with the Inab phenotype*

Reference	Ethnicity	anti-IFC	GI abnormalities	Transient	Nucleotide change
48	Caucasian	Yes	GERD, food intolerances	Yes	None
38	Japanese	Yes	None	No	508C>T
47	Swedish	Yes	None	Yes	Unknown
37	Japanese	Yes	Unknown	No	263C>A
46	Kenyan	Yes	None	Yes	None
31	Japanese	Yes	Capillary angioma	No	261G>A
	African-American	Yes	PLE	No	Unknown
36	Japanese	Unknown	None	No	263C>A
40	Italian-American	Yes	None	No	Unknown
	Italian-American	No	None	No	Unknown
39	American	Yes	Crohn's disease	No	Unknown
3	Japanese	Yes	PLE	No	261G>A

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GERD = gastroesophageal reflux disease; GI = gastrointestinal; PLE = protein-losing enteropathy.

DAF is important in protecting cells from complement attack; thus it was surprising to find that IFC-negative and Dr(a–) RBCs that either lack DAF completely or have reduced DAF do not show a marked susceptibility to lysis in vitro.[45,46] The relative stability of the RBCs in these people contrasts sharply with the state of RBCs in persons experiencing paroxysmal nocturnal hemoglobinuria (PNH). PNH III RBCs have a gross deficiency of all GPI-linked glycoproteins, of which DAF is just one.[47] As DAF is expressed on GI tissue,[48] it is enticing to speculate that a link exists between these patients with the Inab phenotype and their assorted GI disorders, although the association of DAF deficiency and chronic disease of the intestine remains unproved.

The molecular basis of the Inab phenotype has been determined in the five Japanese probands (Table 2). In two people, a nucleotide change of 261G>A is predicted to change Trp87 to a stop codon.[33,34] In two other Japanese Inab people, a nucleotide change of 263C>A introduces a cryptic splice site in exon 2.[39,40] Use of this splice site results in a 26-bp deletion and alters the open reading frame. As a consequence, Ser88 is changed to a stop codon. In all cases, there is no DAF protein in the membrane. A third single-nucleotide polymorphism (SNP) found in another Japanese individual with the Inab phenotype, 508C>T, is predicted to introduce a stop codon in the third CCP domain (Arg170Stop) and is likely another silencing change, but in the lone report of this single nucleotide change, the level of DAF on the propositus's RBCs was not assayed.[41]

There have been several reports of individuals with a transient Inab phenotype (Table 2). The first instance was in a 54-year-old man from Kenya with a history of HbS trait and α-thalassemia.[49] He was admitted to the hospital with abdominal pain from an enlarged spleen with infarcts that eventually required its removal. On admission, the patient had an IgG antibody against a high-prevalence antigen that behaved like an antibody in the Cromer system, namely anti-IFC. His RBCs lacked CD55 and, thus, all the Cromer antigens for which he was tested (Cr^a, Tc^a, Dr^a, WES^b, UMC, IFC), although expression of CD59 was normal, indicating that he did not have PNH. Interestingly, this patient had been a frequent blood donor up until the time of his illness, and no unexpected antibodies had ever been detected in his serum. He also did not give a history of any other GI problems, other than his acute splenomegaly, nor had he been transfused. He had a consensus *DAF* gene. By 17 months after splenomegaly, the anti-IFC was extremely weak and the high-prevalence Cromer antigens had begun to reappear on his RBCs, along with CD55. The authors point out that a causal link between the patient's disease and the transient Inab phenotype is speculative.

The second example of a patient with a transient Inab phenotype was a 78-year-old woman with a history of three pregnancies and chronic lymphoblastic leukemia (CLL) that appeared to be transforming into the acute variety.[50] She too initially had anti-IFC in her serum, and her RBCs did not express Cr^a, Dr^a, Tc^a, or IFC. During the next 3 months, her antibody disappeared and her RBCs began to express Cromer RBCs. It is enticing to speculate that the disappearance of this patient's anti-IFC was related to chemotherapy that she might have received for her acute leukemia, but the authors did not detail the extent and nature of her treatment, nor did they mention whether she had any GI abnormalities. Also, *DAF* gene sequencing was not reported.

The third patient with a transient Inab phenotype was a very young boy with multiple medical problems including congenital cytomegalovirus (CMV), which led to blindness and deafness.[4] He was also at the 0 percentile for both height and weight, and had severe GI reflux disease and some food intolerances. When he was 1 year old, an ABO discrepancy was noted and he had an antibody to a high-prevalence antigen that reacted at the immediate spin phase as well as at IAT. This antibody also behaved like an antibody in the Cromer system in terms of its reactivity with chemically treated RBCs and its failure to agglutinate Dr(a–), IFC–, and PNH III RBCs. Furthermore his RBCs did not express Dr^a, IFC, or CD55; Yt^a and Emm were present on his RBCs, thus excluding PNH. Although this antibody did not weakly agglutinate the Dr(a–) RBCs, it was considered to be anti-IFC. Exons 2 through 6 of *DAF* were consensus. He had not been transfused before these serologic investigations. Approximately 6 months later the anti-IFC was virtually undetectable, and Dr^a, IFC, and CD55 were beginning to be expressed on his RBCs.

WES^a, WES^b

In 1987, a low-prevalence antigen, WES, was described in the Finnish population, with an occurrence of 0.6 percent.[51] The antibody was first detected during routine compatibility testing using serum from a 77-year-old woman. Subsequently, many more examples of the antibody were identified when WES+ RBCs were used to screen donor plasma samples. Family studies showed WES to be inherited as an autosomal dominant characteristic. Two further examples of WES+ RBCs were found in 392 samples from Black American blood donors, and five WES+ donors were found in a population of 245 Black North London donors. In a study of 3072 American donors drawn in eight different blood centers across the United States, 7 of 1460 Black donors and 2 of 1612 White donors were WES+, giving an occurrence of 0.48 percent and 0.12 percent, respectively.[52]

Daniels et al.[53] described an antibody to a high-prevalence antigen produced by a Black WES+ prenatal patient (Wash). At delivery, the infant's RBCs had a weakly positive DAT result and the antibody could be eluted, but there was no evidence of clinical HDFN. The antibody failed to react with a presumed WES+ homozygote of Finnish origin (Hel). Both the proposita's RBCs and those of Hel were positive for Cr^a, Tc^a, Dr^a, and other Cromer-related antigens. The Wash serum reacted with RBCs lacking high-prevalence Cromer-related antigens but showed weak reactivity only with Dr(a–) RBCs and was nonreactive with RBCs of the Inab phenotype. Additionally, the serum failed to react with α-chymotrypsin–treated or pronase-treated RBCs. It was proposed that the antigen recognized by the Wash serum was antithetical to WES. The low-prevalence antigen WES was renamed WES^a and the high-prevalence antigen was named WES^b. Molecular characterization of WES^a and WES^b showed that the antigens are encoded by a single nucleotide difference in codon 48. A change of 245T>G of the wild-type *DAF* results in the loss of the WES^b antigen and expression of WES^a. At the protein level, this is predicted to encode a change of Leu82Arg on CCP1 of DAF. Tests with anti-WES^b showed that Es(a–) RBCs react less strongly than Es(a+) RBCs; additionally, WES(a+b–) RBCs react very weakly with anti-Es^a, reactivity only being demonstrable by adsorption or elution tests.[31] Because the amino acid responsible for the Es^a antigen is Ile^{46}, just two residues away, this finding is not surprising.[20]

UMC

UMC, another high-prevalence antigen of the Cromer blood group system, was reported in 1989.[54] The only example of anti-UMC described was found in the serum of a Japanese blood donor. Although untransfused, the proposita had three children. The antibody reacted with all panel RBCs tested, including RBCs lacking known high-prevalence Cromer antigens, although the RBCs of one sibling were compatible, as were the RBCs of a patient with PNH and those of the Inab phenotype. Testing of 45,610 Japanese blood donors did not reveal another UMC– individual. The molecular basis of the UMC– phenotype was shown to be another single nucleotide change of 749C>T, which is predicted to encode Thr250Met on CCP4 of DAF.[20]

GUTI

An antibody (anti-GUTI) in the serum of a Canadian blood donor of Chilean descent was strongly reactive with a panel of RBCs of common phenotype.[55] Tests after different protease treatments showed the antibody reactivity was ablated after treatment of test RBCs with α-chymotrypsin or pronase. Treatment with papain, trypsin, or 200 mM DTT did not affect reactivity. Subsequent tests with RBCs known to lack high-prevalence antigens in the Cromer system and with PNH III RBCs showed that the plasma contained an antibody to a novel antigen in the Cromer blood group system. Molecular analysis of the donor's blood identified a nucleotide change, 719G>A, in *DAF* that corresponded to an amino acid change of Arg240His in CCP4 of DAF.[55] Immunoblotting analysis using anti-GUTI with DAF deletion mutants confirmed the location of the antigen on CCP4.

The donor's parents and two daughters were shown by PCR-RFLP analysis to be heterozygous for the nucleotide change. One sister was homozygous for normal *DAF*. Another sister was homozygous for the 719A change and her RBCs were compatible with anti-GUTI. Screening of more than 1000 North American blood donors did not reveal any additional GUTI-negative RBCs. PCR-RFLP analysis of DNA samples from 114 Native Chileans showed that the polymorphism was present with an occurrence of 5.3 percent.[55]

SERF

The first example of anti-SERF was detected in a pregnant Thai patient.[56] Her IgG antibody was suspected to be against a high-prevalence antigen in the Cromer system because it demonstrated the typical reactivity pattern with chemically treated RBCs, did not react with IFC– or Dr(a–) RBCs, and only reacted weakly against RBCs in which the Cromer antigens were transiently suppressed. The antibody was confirmed to target DAF using monoclonal antibodies in the MAIEA. A single nucleotide change in exon 5 of *DAF*, 647C>T, causes a Pro216Leu amino acid substitution in the third CCP. These authors[56] found the frequency of the SERF-negative allele to be 1 percent in a sample of 100 Thai individuals, and this frequency was subsequently confirmed in a large screening study of 1041 Thai blood donors.[57]

ZENA

The first and only example of anti-ZENA was discovered in a pregnant Syrian-Turkish woman whose IgG antibody to a high-prevalence antigen did not react with IFC– and Dr(a–) RBCs.[19] The reaction of this antibody with chemically treated RBCs was consistent with an antibody to a Cromer system antigen, although the patient's Cromer phenotype was unremarkable. A novel nucleotide change in exon 6 of the *DAF* gene, 726T>G (His242Gln, in the fourth CCP of the DAF protein), was discovered when the gene was analyzed. The propositus's serum reacted with the RBCs from a variety of family members, all of whom were shown to be heterozygous for this SNP. Consistent with other pregnancies complicated by anti-Cromer antibodies, her baby was born without evidence of HDFN, and the baby's DAT was negative. No ZENA– individuals were found among the 150 Israeli blood donors that were screened.[19]

CROV

The antibody to this creatively named antigen (from *CRO*mer and *CRO*atia + *V*inkovci, the city from which the proposita hailed) was found in the serum of a woman with multiple medical and surgical problems.[19] She had been pregnant three times but had not been transfused. The antibody was an IgG antibody to a high-prevalence antigen that failed to react with IFC– RBCs and produced only weak agglutination with Dr(a–) RBCs. The reactivity pattern of anti-CROV with protease-modified RBCs was also suggestive of an antibody in the Cromer system, and her Cromer RBC phenotype was unremarkable. The genetic investigation of this proposita revealed a nucleotide change in exon 3, 466G>A (Glu156Lys, in the second CCP of the DAF protein), and similar to the ZENA proposita, her serum agglutinated RBCs from some first-degree relatives who were shown to be heterozygous for this nucleotide change. As with the population screening for ZENA, no further CROV-negative alleles were found when 100 Croatian DNA samples were tested.[19]

CRAM

The first example of anti-CRAM was identified in a Somali woman who was in her eighth week of gestation of her third pregnancy.[19] She had not been transfused. Her IgG antibody to a high-prevalence antigen demonstrated the typical Cromer pattern of reactivity to chemically treated RBCs, did not react with IFC– RBCs, and reacted only weakly with Dr(a–) RBCs and those with a transient suppression of Cromer system antigens. Her Cromer phenotype was pedestrian. Monoclonal antibodies specific to the first CCP of the DAF protein caused RBC agglutination, although another monoclonal antibody to CCP 3 or 4 did not cause agglutination, thereby revealing the location of the amino acid change, Gln247Arg in the fourth CCP, caused by a 740A>G nucleotide change in exon 6 of *DAF*. The authors indicted that it was not possible for them to perform screening of Somali individuals—thus, the frequency of this allele in this population is not known—and cautioned that although some Cromer alleles are prevalent in certain populations, it is premature to link an allele to the ethnicity of a proband.[19]

Characteristics of Cromer Antigens

Cromer blood group antigens are sensitive to treatment of RBCs with α-chymotrypsin or pronase, but they are not affected by treatment with trypsin, papain, or ficin.[31,58] Reducing agents such as 2-aminoethylisothiouronium bromide (AET), 2-ME, or DTT weaken the antigens but generally do not disrupt them completely.[31] This is somewhat surprising, as DAF is known to have a number of intrachain disulfide bonds and the protein is detectable by immunoblotting techniques only under nonreducing conditions.[4] Although DAF is a cell membrane–bound glycoprotein, small amounts are present in the plasma and other body fluids of normal individuals.[5,48] Hemagglutination inhibition studies have shown that antibodies to Cromer antigens may be inhibited by concentrated plasma and urine from an antigen-positive person.[31] In one study by Judd et al.,[59] a commercial preparation of human platelet concentrates for immunohematologic use was shown to adsorb anti-Cr^a from four different sera. The authors warned of the potential hazards of using such preparations, designed for the removal of HLA antibodies, in the adsorption of potentially clinically significant antibodies.

Clinical Significance of Cromer Antibodies

Because persons lacking the various high-prevalence antigens of the Cromer blood group system are rare, there are few suitable donors for patients who are sensitized. In vivo and in vitro survival studies have been used to determine the consequences of transfusing antigen-positive RBCs to sensitized patients. In vitro studies, such as the mononuclear phagocyte assay (MPA) or the monocyte monolayer assay (MMA), suggested that these antibodies could be clinically significant.[14,59–63] A number of in vivo chromium 51 (^{51}Cr) -labeled RBC survival studies with Cr(a+) RBCs have been reported.[23,44,59,62,64–66] With the exception of one case,[66] increased destruction of incompatible RBCs, albeit variable, was observed. However, Smith et al.[23] reported the uneventful transfusion of a patient with anti-Cr^a with two units of Cr(a+) RBCs. Based on the results of a ^{51}Cr survival study in one patient, Ross and McCall[64] proposed that incompatible blood could be transfused "without significant untoward effect." In a case described by Whitsett and Oxendine,[66] normal survival of Cr(a+) RBCs was observed in a patient with anti-Cr^a. The antibody was reactive in an MMA and was IgG. Two incompatible units were transfused successfully, as determined by the absence of clinical signs of hemolysis. Kowalski et al.[67] reported a hemolytic transfusion reaction in a woman who was transfused with three units of crossmatch-compatible RBCs. Seven days after transfusion, the patient's hemoglobin had dropped to 5.5 g/dL with no overt signs of bleeding. Anti-Tc^a was identified in the posttransfusion serum, and an MMA was strongly reactive. Anderson et al.[65] observed decreased RBC survival of incompatible RBCs in a patient with anti-Tc^a, and there was no increase in antibody titer. RBC survival at 16 days was normal.

Although the antigens of the Cromer blood group system are well developed on fetal RBCs near term, there have been no cases of fetal demise directly attributed to these antibodies. This might appear somewhat anomalous, as Cromer antibodies are mostly IgG, predominantly of the IgG1 subclass, and would be expected to cross the placenta.[51,62,68] Elegant studies on human trophoblast cells by Holmes et al.[69] showed that there was an elevated concentration of DAF on cells at the immediate fetomaternal interface, and proposed that the elevated concentration of DAF in the trophoblast is a protection mechanism of the fetus against complement-mediated maternal attack. Support for this hypothesis was demonstrated in a case of a postpartum woman whose anti-Cr^a was elutable from the placenta itself even though the antibody was not present in her plasma or that of her newborn.[70]

That the placenta can become a "sink" for Cromer antibodies is supported by six different case reports: four women whose serum contained anti-Cr^a with titers that were at least 64 in the first trimester and the patient with anti-CRAM whose antibody titered to 128 in her second trimester[19] demonstrated loss of reactivity of the antibody as the pregnancy progressed, in at least two cases to undetectable levels at delivery. (Anti-CRAM was not detectable 1 month before parturition; thus, it was probably not detectable at the time the baby was born.[19]) Another woman, whose serum contained anti-Dr^a with a titer of 512 at 22 weeks, had no detectable antibody at delivery. The DAT performed on the cord RBCs of five of these babies was negative (in the lone anti-CRAM case the neonatal DAT was not reported[19]), and there was no evidence of HDFN in any of these cases.[25,71,72] In three of the cases the antibody, including anti-CRAM, was detected again in the serum of these women after delivery, again suggesting an adsorption mechanism rather than the cessation of antibody production.[19,71] Although no cases of fetal demise have been directly attributed to Cromer antibodies, it is worthwhile to point out that in at least one case, anti-Cr^a was detectable at a titer of 128 by week 15 and had declined to a titer of 8 by week 20, which was 2 weeks before fetal demise. The role of the antibody in the demise is not known, and this patient's next pregnancy was successfully carried to term despite the presence of anti-Cra at a maximum titer of 64 at week 7.[70] Interestingly, a patient with an anti-Dr^a titer that ranged between 256 and 512 gave birth to two babies who were not affected by HDFN despite one baby's having a positive DAT with anti-Dr^a recoverable in the eluate.[73] The titer of the anti-Dr^a in this woman did not change appreciably during either pregnancy.

Conclusions

The Cromer blood group system consists of 2 pairs of antithetical antigens (Tc^a/Tc^b/Tc^c and WES^a/WES^b) and 10 high-prevalence antigens on DAF that are characterized serologically by their sensitivity to α-chymotrypsin and pronase. With the exception of IFC, molecular analysis has shown that each antigen is the result of a single nucleotide change in *DAF* that affects a single amino acid in the resulting protein. In general, the different phenotypes demonstrate an ethnic bias that suggests a spontaneous mutation in that population. The recent identification of four new Cromer system antigens suggests that there are probably other rare and exciting polymorphisms of *DAF* that await discovery.

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Jill R. Storry, PhD, FIBMS, Coordinator, Special Serology, Department of Clinical Immunology and Transfusion Medicine, University and Regional Laboratories, Division of Haematology and Transfusion Medicine, Department of Laboratory Medicine, Lund University, Lund, Sweden; Marion E. Reid, PhD, Director, Immunochemistry Laboratory, New York Blood Center, New York, NY; and Mark H. Yazer, MD (corresponding author), Department of Pathology, University of Pittsburgh, Pittsburgh, PA, and The Institute for Transfusion Medicine, 3636 Boulevard of the Allies, Pittsburgh, PA 15213.