GUTI: a new antigen in the Cromer blood group system

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BACKGROUND: The Cromer blood group system consists of seven high-incidence and three low-incidence antigens carried on decay-accelerating factor (DAF). This report describes the identification and characterization of a new Cromer high-incidence antigen, named GUTI.

STUDY DESIGN AND METHODS: RT-PCR and sequence analysis were performed on cDNA prepared from a Chilean donor whose serum contained the alloantibody (anti-GUTI). Based on the observed point mutation, a PCR-RFLP assay using *Mae*I was developed. To map the epitope, DAF-deletion mutants were tested by immunoblotting with anti-GUTI.

RESULTS: Sequence analysis revealed a substitution of 719G>A in *DAF* in the proband. The proband's parents and two daughters were heterozygotes for 719G>A, one sister whose RBCs typed GUTI– was homozygous for 719A, and one sister had the wild-type *DAF* (719G). Seven additional heterozygote samples were identified among 214 Chileans. No heterozygotes were found in 197 New York donors. Analysis using DAF-deletion mutants showed the antigenic determinant to be within short consensus repeat (SCR) 4.

CONCLUSION: This study describes a novel high-incidence antigen (GUTI) in the Cromer blood group system characterized by the amino acid arginine at position 206 in SCR4 of DAF. The GUTI-negative proband has a substitution mutation that predicts for histidine at this position.

The antigens of the Cromer blood group system are carried on decay-accelerating factor (DAF; CD55), which is a member of the regulators of complement activation family of proteins.[1] DAF has four homologous short consensus repeat (SCR) domains followed by an *O*-glycosylated serine- and threonine-rich region attached to a glycosylphosphatidylinositol (GPI) membrane anchor.[2] The Cromer blood group system consists of seven high-incidence antigens and three low-incidence antigens,[3] each of which, with the exception of IFC, is associated with a single amino acid change in DAF. The identification of the locations of the various Cromer antigens on DAF was accomplished by testing the corresponding antibodies against stable transfectants expressing full-length and deletion mutants of DAF.[4] $Tc^a/Tc^b/Tc^c$, Es^a, and WES^a/WES^b are located in SCR 1; Dr^a is within SCR 3; and Cr^a and UMC are within SCR 4.[4-6] The Dr(a–) phenotype is characterized by an amino acid substitution of Ser165Leu of DAF. The underlying molecular basis of Dr(a–) has been shown to have a profound effect on total DAF expression on RBCs, and

ABBREVIATIONS: CHO = Chinese hamster ovary stable transfectants; DAF = decay-accelerating factor; SCR = short consensus repeat.

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thus Cromer antigens are very weakly expressed on Dr(a–) RBCs.[7] PNH III RBCs lack all GPI-linked proteins and therefore lack DAF protein.[8]

In this report we describe a new high-incidence antigen (GUTI) in the Cromer blood group system identified by an alloantibody (anti-GUTI) made by a transfusion recipient. The GUTI antigen has been numbered by the ISBT Committee on terminology for RBC surface antigens as 021.011. GUTI is associated with a nucleotide 719G in exon 6 of *DAF* that is predicted to encode arginine at position 206 of DAF. This association was determined by studying the proband whose RBCs lack the GUTI antigen. His DAF genes have nucleotide 719A, which predicts histidine at position 206 of DAF. We also show that the 719G>A mutation has an allele incidence of 5.3 percent in Mapuche Indians in Chile.

CASE STUDY

A 41-year-old Chilean male blood donor has donated 22 whole-blood units since 1995 at blood-donor clinics held by the Hamilton Center of the Canadian Blood Services. In 1986, he was in a motor vehicle accident (in Chile) after which a steel plate was inserted to immobilize an unstable fracture of the femur. Because he had significant bleeding during the surgery, he was transfused with three units of RBCs.

Each time the proband donated blood, an antibody to a high-incidence antigen was detected in his plasma, in addition to the expected ABO alloantibodies. The donor's RBCs are group O, D+C+E–c+e+, M–N+ S+s+, P_1+, Lu(a–b+), K–k+, Kp(b+), Le(a–b–), Fy(a–b+), Jk(a+b+), Cr(a+), Tc(a+), Dr(a+), Es(a+), WES(b+), and In(b+).

The proband's plasma was reactive with all RBCs tested, except his own and one sister, by an IAT, either in saline or PEG, using anti-human IgG. The titer of the alloantibody was 16. His plasma was also reactive with papain-, ficin-, trypsin-, AET-, or DTT-treated RBCs and with RBCs of the following phenotypes: En(a–), U–, Rh_{null}, D––, Lu:–3, Au(a–), K_0, Fy:–3, Jk:–3, Di(b–), Wr(b–), Yt(a–), Xg(a–), Sc:–1, Do(a–b+), Do(a+b–), Gy(a–), Hy–, Jo(a–), Co(a–), LW(a–), Ch–, Rg–, Ge–, Cr(a–), Tc(a–), Es(a–), UMC–, Kn(a–), McC(a–), Yk(a–), JMH–, Cs(a–), Er(a–), p, Vel–, Lan–, At(a–), Jr(a–), Emm–, and Sd(a–). His plasma was did not react with α-chymotrypsin-treated RBCs and with two samples each of Dr(a–) RBCs and PNH III RBCs. These observations suggested that the proband's alloantibody is directed to a "new" high-incidence antigen in the Cromer system.

This new antigen has been named GUTI (derived from the donor's family name and with his permission) and the alloantibody is anti-GUTI. To date, we have tested over 1000 different blood-donor RBC samples with the proband's plasma and all have been reactive. We have also tested serum from three recipients of the proband's RBCs, as well as serum from his wife, but could not detect any reactivity in these sera with his GUTI– RBCs.

MATERIALS AND METHODS

Samples

Peripheral blood samples were obtained from the proband, his wife, two daughters, parents, two sisters, a cohort of Chileans comprised of 114 Mapuche Indians, 100 medical students at the Universidad Católica de Chile, and 197 New York blood donors that included 98 samples from donors who identified themselves as being Black. All samples were obtained following Institutional Review Board (IRB) approved protocols.

RT-PCR and sequence analysis

Total RNA was isolated from 10 mL of blood by a modified method[9] (TRIzol, Gibco BRL, Grand Island, NY) and reverse-transcribed according to a commercially available kit protocol (SuperScript II, Gibco BRL) using oligo d(T) as a primer. Amplification of the coding sequences of *DAF* was performed with two sets of overlapping oligonucleotide primers (synthesized by Life Technologies, Gaithersburg, MD), CD55 5′F/CD55 5′R and CD55 3′F/CD55 3′R (Table 1). cDNA (2 μL/reaction) was amplified by 5 U *Taq* DNA polymerase (HotStarTaq, QIAGEN, Valencia, CA) in a 50-μL reaction mixture containing 1.5 m*M* $MgCl_2$, 1 × PCR buffer, 0.2 m*M* dNTPs, and 100 ng of forward and reverse primer. Amplification was achieved over 35 cycles with a final extension time of 10 minutes. The PCR products were sequenced directly and were also cloned into the pCR 2.1 cloning vector using a kit (TOPO TA Cloning Kit, Invitrogen, Carlsbad, CA). DNA sequences were obtained using a sequencer (ABI 373XL, Applied Biosystems, Foster City, CA), and ABI Big Dye reagents with BD Half-Term (GenPak, Stony Brook, NY). We used the numbering system of Lublin et al.,[2,5] that is, nucleotide number 1 is the "A" of the initiation codon (AUG of *DAF*), whereas amino acid number 1 is the Asp that starts the mature protein (minus the signal peptide of 34 amino acids).

TABLE 1. Oligonucleotide primers used in the analysis of *DAF*[4]

Name	Primer sequence (5′ to 3′)	Annealing temperature	Expected size (bp)
CD55 5′F	cgtccttgttctaacccggc	55°C	690
CD55 5′R	tctctgcactctggcaacgg		
CD55 3′F	cagctctgtccagtggagtg	60°C	575
CD55 3′R	actaggaacagtctgtatacttg		
DAFSCR4F	gcatctcttgttggtaatgctg	60°C	350
DAFSCR4R	caacccacatatagacctgaggg		

PCR-RFLP analysis

A point mutation was observed in the cDNA sequence of the proband (719G>A) that ablated a *Mae*II restriction enzyme site. Consequently, genomic DNA was isolated from fresh whole blood (QIAamp Blood Kit, QIAGEN), and a 350-bp region of *DAF* that included exon 6 was amplified from the proband, his wife, and two daughters using the DAFSCR4F and DAFSCR4R set of primers (Table 1). PCR was performed under the conditions given above. The PCR products were subjected to *Mae*II digestion and separated on an 8-percent polyacrylamide gel and stained with ethidium bromide. To confirm the mutation, the PCR products from the donor, his family members, and selected Chileans were sequenced.

Localization of the epitope using deletion mutants

Chinese hamster ovary stable transfectants (CHO) expressing either DAF or DAF-deletion mutants, from which individual DAF SCR domains have been removed, were established as described.[10] For immunoblot analysis, lysates were prepared from these cell lines in 1-percent Triton X-100, Tris-buffered saline at pH 7.4, 10 μg per mL of aprotinin, 10 μg per mL of leupeptin, and 1 m*M* PMSF, separated by SDS-PAGE on 9-percent acrylamide gels and electroblotted onto nitrocellulose membranes. Membranes were blocked with 3-percent milk powder in Tris-buffered saline at pH 7.4 with 0.05-percent Tween 20, and then incubated with either an eluate containing anti-GUTI (Elu-*Kit*II, Gamma Biologicals, Houston, TX) or a pool of two murine MoAbs to human DAF (1H4 and 8D11) that recognize, respectively, epitopes in SCR3 and SCR4.[10] Immunoblots were developed with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (Pierce, Rockford, IL).

RESULTS

Sequence analysis

Direct sequencing of the RT-PCR products from the proband's DNA had a point mutation corresponding to 719G>A. The single-point mutation was confirmed by sequencing TA clones in both directions from both fragments of *DAF*. Nucleotide 719 lies in exon 6, which encodes the fourth SCR of DAF protein. Nucleotide 719G>A predicts an amino acid change of Arg206His and ablates one of two *Mae*II restriction enzyme sites in this exon.

PCR-RFLP analysis

RFLP analysis of the 350-bp product amplified from the proband and one of his sisters indicated homozygosity for the point mutation, with bands of 206 bp and 144 bp. The PCR amplicon from his wife and other sister showed two bands of 175 bp and 144 bp, consistent with the expected digestion pattern of the wild-type sequence. A smaller band of 31 bp was not visible in the gel. Samples from his mother, father, and both daughters were heterozygous for the mutation and the wild-type sequence and demonstrated bands of 206, 175, and 144 bp. PCR-RFLP analysis on the proband, his wife, and the daughters is shown in Fig. 1. Sequence analysis of the PCR products confirmed the RFLP findings.

Analysis of DNA from the Chileans revealed 6 heterozygotes among the 114 Mapuche Indians (5.3%) and 1 among the 100 medical students. No additional homozygotes were identified. The *DAF* 719G>A mutation was not found in any of the 197 blood donors from New York. Exon 6 from the seven heterozygote samples and seven Mapuche Indians with the wild-type *DAF* were sequenced. In each case, the sequence agreed with the result obtained by PCR-RFLP. The mother and only sibling of the heterozygous Chilean medical student were both heterozygous in the PCR-RFLP assay. The mother's ancestors are from the southern part of Chile.

Localization of the epitope using deletion mutants

In immunoblot analysis of recombinant DAF expressed in CHO, anti-GUTI recognized a band of 70 kDa that was present in the CHO stable transfectant expressing DAF but absent in the parent CHO cell line (Fig. 2). In testing a panel of DAF-deletion mutants that contain individual deletions of the four SCR domains, anti-GUTI reacted with each of the deletion mutants except DAF ΔSCR4 (Fig. 2), and thus the epitope recognized by the GUTI antibody mapped to the SCR4 region of DAF.

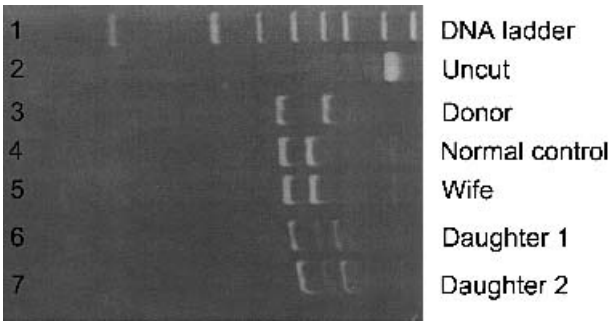

Fig. 1. *Mae*II RFLP analysis of *DAF* exon 6. Lane 1: 50-bp DNA ladder. Lane 2: 350-bp uncut PCR amplicon. Lane 3: Digestion of the proband's PCR product shows homozygosity for the point mutation, with bands of 206 bp and 144 bp. Lane 4: The PCR amplicon from a normal control DNA, which showed two bands of 175 bp and 144 bp, respectively, consistent with the expected digestion pattern of the wild-type sequence. Lane 5: The PCR amplicon from the DNA of the donor's wife, which is comparable with the normal control. The expected 31-bp fragment was not visible in the gel. Lanes 6 and 7: Samples from both daughters were heterozygous for the mutation and the wild-type sequence and demonstrated bands of 206, 175, and 144 bp.

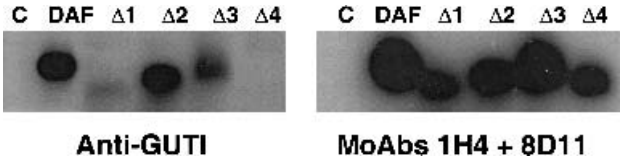

Fig. 2. GUTI antibody binds to DAF SCR4. Cell lysates of a panel of CHO stable transfectants expressing DAF or one of the DAF-deletion mutants that lack a single SCR domain were screened by immunoblot analysis. The immunoblot was probed with anti-GUTI or with a pool of the DAF MoAbs 1H4 and 8D11; a pool was used so that at least one MoAb would recognize each deletion mutant. The CHO control cell lysate (not transfected with DAF) is designated C. Each of the transfectants expresses DAF protein, as shown by the immunoblot with the DAF MoAbs, but GUTI serum loses reactivity with the DAF ΔSCR4 mutant.

DISCUSSION

We describe a novel high-incidence antigen in the Cromer blood group system that has been numbered by the ISBT as 021.011. The antigen, GUTI, is associated with an arginine residue at position 206 in DAF. The lack of GUTI antigen on the proband's RBCs is associated with a single nucleotide mutation (719G>A) in exon 6, predicting an amino acid change (Arg206His) in the fourth SCR of DAF. We demonstrated, through the use of recombinant deletion mutants, that anti-GUTI reacted specifically with an epitope in SCR4, which is consistent with our molecular findings. Thus, the GUTI antigen lies between the Cr^a and UMC antigens (Fig. 3). A search for an antibody to the corresponding antithetical low-incidence antigen has been unfruitful thus far. In hemagglutination tests, no weakening of GUTI antigen was noted on Cr(a–) or UMC-negative RBCs and no weakening of the Cr^a antigen was noted on GUTI-negative RBCs.

The proband was born approximately 1000 km south of Santiago, and his ancestors, both maternal and paternal, have apparently lived in Chile continuously for over 300 years. His genetic background is of particular interest in that his first male ancestors came to Chile from Spain and probably chose their mates from among the indigenous population of Chile. Subsequent generations probably also occasionally intermarried, resulting in a progeny with a Western European indigenous Chilean gene admixture.

The absence of a high-incidence antigen in a restricted ethnic group is demonstrated among other Cromer blood group antigens and is remarkable. The Cr(a–) and Tc(a–b+) phenotypes are found predominantly in the Black population. Similarly, the Dr(a–) phenotype is restricted to Jews originating from the Bukharan area of Uzbekistan[7,11-13] and Japanese,[14,15] and the single example of the UMC– phenotype was identified in a Japanese blood donor.[16] Similarly, our results show that the GUTI– polymorphism (*DAF* 719G>A) is found in Chileans.

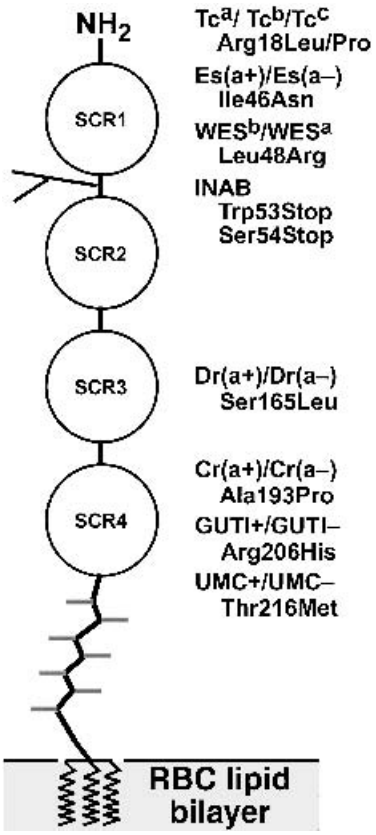

Fig. 3. Location of amino acid substitutions associated with Cromer blood group antigens on DAF. The GUTI antigen lies between Cr^a and UMC antigens.

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