Cromer-related blood group antigens and the glycosyl phosphatidylinositol-linked protein, decay-accelerating factor DAF (CD55)

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Abstract: Cromer-related blood group antigens are located on the complement regulatory glycoprotein, decay-accelerating factor (DAF). DAF is not detectable on red cells from individuals with a Cromernull phenotype (termed Inab, which is probably an inherited condition. DAF is also absent from a subpopulation of red cells (PNH III) from patients with paroxysmal nocturnal hemoglobinuria (PNH), an acquired hematological defect. PNH III red cells, like Inab cells, lack all the Cromer-related antigens described to date.

Abbreviations used:
CD55 cluster differentiation antigen 55
DAF decay-accelerating factor
PI phosphatidylinositol
PNH paroxysmal nocturnal hemoglobinuria
RBC red blood cell

Eleven Cromer-related blood group antigens have been identified on red blood cells (RBCs). Eight (Cr\textsuperscript{a}, Te\textsuperscript{a}, Te\textsuperscript{b}, Dr\textsuperscript{a}, Es\textsuperscript{a}, WES\textsuperscript{a}, UMC, and IFC) are high-frequency antigens while three (Te\textsuperscript{c}, WES\textsuperscript{b}, and WES\textsuperscript{c}) are low-incidence antigens.\textsuperscript{1} These inherited antigens are located on the RBC membrane-bound complement regulatory protein called decay-accelerating factor (DAF).\textsuperscript{2,3} The gene controlling the expression of DAF is located within the cluster of complement regulatory genes on the long arm of chromosome 1 (p32).\textsuperscript{4} The red cells of individuals of the rare Cromernull phenotype (Inab) lack DAF and all Cromer-related antigens.

Nomenclature
The International Society of Blood Transfusion (ISBT) working party on terminology for red cell surface antigens has recently published a new category of blood group specificities: The Collections.\textsuperscript{5} The Collections (series 201 to 210) are blood group specificities that have not been assigned to established blood group systems but have a serological, biochemical, or genetic connection.

The Cromer-related antigens have been assigned the 202 series (Table 1).

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Serological Characteristics of Cromer-Related Antigens
Cromer-related antigens are denatured by \textalpha-chymotrypsin but not by ficin, papain, trypsin, or sialidase treatment of RBCs. Promase is reported to have a variable effect in that WES\textsuperscript{a} and WES\textsuperscript{b} antigens are more readily destroyed than other Cromer-related antigens. RBCs treated with dithiothreitol (DTT) and 2-aminoethylisothiouronium bromide (AET) express Cromer-related antigens more weakly than do untreated RBCs.\textsuperscript{1} Dr(a-) RBCs express weakly all other Cromer-related antigens that are present on these RBCs.
**Cromer-Related Antibodies**

Cromer-related antibodies are usually IgG1 and have not been implicated in hemolytic transfusion reactions or hemolytic disease of the newborn. Some monoclonal antibodies have been described that detect epitopes on DAF (BRIC 110, BRIC 128, 143-50, F2B-7.2).

**Individuals With the Inab Phenotype**

Individuals with the Inab phenotype (Cromer_null) lack DAF on their RBCs but possess other phosphatidylinositol-linked (PI-linked) proteins. This is in contrast to a subpopulation of RBCs from patients with PNH, which have an acquired deficiency of all PI-linked proteins and an increased susceptibility to complement-mediated lysis. Individuals with the Inab phenotype can make anti-IFC, which agglutinates all RBCs except those with the Inab phenotype and those red cells classed as PNH III.

The Inab phenotype has been reported in four propositi. The first was a Japanese male with an ileoceleal tumor and protein-losing enteropathy. His intestinal disease was corrected by hemicolectomy, but his red cells still lacked Cromer-related antigens. The parents of this patient were cousins, but no family was available for testing. The second individual was a Jewish American male with Crohn's disease, a form of protein-losing enteropathy. The third was an American female of Italian descent. One of her four siblings also had the Inab phenotype, providing some evidence that the phenotype was inherited. Neither the proposita nor her brother had a history of an intestinal disorder. The fourth was a woman with a chronic intestinal disorder of unknown etiology that required surgery; the family study suggested that the Inab phenotype was inherited.

**Molecular Biology Studies on Cells From an Inab Individual**

Molecular biology studies on lymphocytes from the first propositus with the Inab phenotype have been described. No expressed differences in the DAF gene of normal control cells and of those from the Inab individual were detected by Southern blotting studies. The authors suggested that in this individual, the genetic information contains a mutation that affects transcription of processing of DAF mRNA, because Epstein-Barr virus (EBV)-lymphoblastoid cell lines derived from his peripheral blood lymphocytes had a gross reduction in the level of DAF mRNA compared to normal controls.

**Decay-Accelerating Factor**

DAF is a glycoprotein that is anchored to the RBC membrane by a glycosyl phosphatidylinositol (PI) linkage. PI-linked proteins possess the following features: phosphatidylinositol is attached to a non-acetylated glucosamine residue which, in turn, is attached via at least three other sugar residues to ethanolamine. Ethanolamine is attached to the carboxy terminal amino acid of the polypeptide chain. Thus, the protein is attached to a lipid via sugar residues, and this lipid resides in the outer leaflet of the lipid bilayer (Fig. 1).

Fig. 1. Decay-accelerating factor (DAF) glycoprotein.

The amino acid sequence of DAF has been deduced from nucleotide sequencing of cDNA. The amino acid terminus of DAF contains four repeating homologous regions of 60 amino acids, then a region of approximately 70 amino acids that is rich in serine and threonine residues. There is an N-linked oligosaccharide attached to one of the four amino acid repeats and many O-linked oligosaccharides in the serine/threonine-rich region. DAF was recently designated as CD55 at the 4th International Workshop and Conference on Human Leukocyte Differentiation Antigens.

DAF is a membrane glycoprotein of many types of cells, including RBCs, neutrophils, lymphocytes, monocytes, platelets, endothelial cells, and epithelial cells. DAF is also present in a soluble form in plasma,
tears, saliva, urine, synovial fluid, and cerebrospinal fluid.\textsuperscript{21}

DAF inhibits the action of C3 convertase on cell surfaces. Its absence is responsible for the abnormal sensitivity to complement-mediated lysis exhibited by a proportion of RBCs from patients with PNH.\textsuperscript{22} These RBCs (termed PNH III) have an acquired deficiency of all PI-linked proteins [e.g., DAF, lymphocyte function associated antigen 3 (LFA-3 or CD58), acetylcholinesterase (AchE)] as well as other complement-regulatory proteins [namely, MIRL, membrane attack complex inhibition protein/homologous restriction factor (MIP/HRF), complement receptor type 1 (CR1)].\textsuperscript{10,11} Individuals with the Inab phenotype have no known hematological abnormality, although their RBCs lack DAF. Thus, RBCs with the Inab phenotype are valuable in determining the role of DAF.

RBCs with the Inab phenotype are susceptible to lysis in the sucrose lysis test but not in the acidified serum test. Variable results of antibody-mediated complement lysis tests may be a reflection of how the tests were performed.\textsuperscript{7-9} Nevertheless, RBCs from individuals with the Inab phenotype do not have accumulations of C3c, C3d, C4d, or C5, and there is no evidence that they have in vivo hemolysis.\textsuperscript{7-9} It is possible that the presence of other PI-linked proteins (which are absent from PNH III RBCs but present in Inab RBCs) protects the Inab RBCs from antibody-mediated complement-lysis. This would suggest that DAF is one of many complement-regulatory proteins with overlapping functions.\textsuperscript{1}

References
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15. Tate CG, Uchikawa M, Tanner MA, et al. Studies on the defect which causes absence of decay accelerating factor (DAF) from the peripheral blood cells of an individual with the Inab phenotype. Biochim J 1989;261:489-93.

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