Case report and literature review: transient Inab phenotype and an agglutinating anti-IFC in a patient with a gastrointestinal problem

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BACKGROUND: The Inab phenotype is a rare deficiency of all Cromer antigens. These antigens are carried on the decay-accelerating factor (DAF, CD55) molecule that is attached to the red blood cell (RBC) membrane by a glycosylphosphatidylinositol (GPI) anchor. Although typically inherited, an acquired and transient form of the Inab phenotype also exists. A patient with the triad of transient Inab phenotype, a direct-agglutinating anti-IFC, and gastrointestinal (GI) abnormalities is reported.

CASE REPORT: An 18-month-old boy with gastroesophageal reflux disease requiring a feeding tube, milk and soy intolerance, and severe growth retardation, as well as vision and hearing deficits from cytomegalovirus infection, was identified when pretransfusion testing revealed a potent panagglutinin (titer > 2000 at 4°C). This antibody did not react with Dr(a–) and IFC RBCs, and the autocontrol was negative. The patient's RBCs lacked CD55 by flow cytometric techniques but had normal levels of CD59 and antigens such as Ytª and Emm, carried on GPI-linked proteins, thus excluding paroxysmal nocturnal hemoglobinuria. Several months after initial detection, the anti-IFC was virtually undetectable and his cells reacted weakly with anti-IFC, anti-Drª, and anti-CD55. RBCs from the propositus' parents and brother demonstrated normal CD55 and CD59 expression.

CONCLUSION: This is the first example of a direct-agglutinating anti-IFC. The cause of the transient depression in CD55 protein (and thus Cromer system antigens) and appearance of anti-IFC remains unknown, as does the relationship between the patient's GI system abnormalities and these serologic findings.

There are 14 antigens in the Cromer blood group system, 11 of high incidence and three of low incidence (Table 1).[1-6] All of these antigens are located on the complement regulating protein decay-accelerating factor (DAF, CD55), which is tethered to the red blood cell (RBC) membrane by a glycosylphosphatidylinositol (GPI) anchor.[7] There are three molecular backgrounds that give rise to weakened or absent expression of Cromer antigens. First, somatic mutations in the X-linked phosphatidylinositol-glycan-complementation class A (*PIG-A*) gene result in a biosynthetic defect in GPI anchor synthesis and hence the absence of all GPI-linked proteins on the RBC surface.[8] The absence or reduction of these proteins on RBCs occurs in paroxysmal nocturnal hemoglobinuria (PNH). In its most extreme form, when the two main regulators of complement, CD55 and membrane inhibitor of reactive lysis (CD59) are completely lacking on the RBC surface; intravascular hemolysis occurs and has been associated with thrombosis (reviewed in Rosse and Nishimura[9]).

Second, reduced Cromer system antigen expression occurs in the case of the rare Dr(a–) RBCs.[10] The molecular basis for this phenotype is a single-nucleotide polymor-

phism (SNP) in exon 5 of *DAF*, 596C>T, which results in alternative splicing of the *DAF* mRNA such that the resulting major transcript lacks 44 nucleotides.[10-12] In addition, this alternative splicing shifts the reading frame and introduces a premature stop codon; the translated protein cannot attach to the membrane and is not present on the membrane of the RBCs.[12] A small minority of the *DAF* mRNA transcripts, however, contain this SNP but do not utilize the cryptic splice acceptor site.[13] These minor transcripts are translated normally with the result that there is a reduced copy number of CD55 and weakened expression of Cromer system antigens on Dr(a–) cells.[13]

Third, Inab phenotype RBCs, named after the original Japanese propositus, lack all Cromer system antigens.[14] This rare phenotype has been previously reported in nine people and is typically recognized by the presence of an alloantibody (anti-IFC) in their serum to a high-incidence antigen of the Cromer system absent on these cells (Table 2).[14-20] Several SNPs have been identified underlying this phenotype. The original Japanese propositus[14] and another unrelated Japanese individual[18] demonstrated a nonsense 261G>A polymorphism in exon 2 of the *DAF* gene rendering the protein unable to be tethered to the GPI anchor. Two Japanese individuals with the Inab phenotype were reported to have a different SNP, 263C>A, which causes a 26-bp deletion in the *DAF* mRNA secondary to the activation of a cryptic splice site.[17,19] Recently, another Japanese proband with the Inab phenotype and a novel nonsense SNP (508C>T, Arg136Stop) was identified.[20] The molecular basis for the Inab phenotype in several patients of Jewish,[15] Italian,[16] and African American[18] descent has not been elucidated. Curiously, some of the first patients with the Inab phenotype were noted to have a variety of gastrointestinal (GI) system abnormalities, whereas others with the Inab phenotype lacked these problems (Table 2).

In addition, there are two reports of transient IFC-negative individuals. One was a previously healthy Kenyan national with HbS trait, unexplained splenic infarcts, and transient Inab phenotype.[21] The patient had no GI abnormalities and had never received a transfusion, yet an anti-IFC–reactive at indirect antiglobulin test (IAT) was present in his serum sample, and his cells were clearly lacking CD55 by flow cytometric analysis.[21] More recently, an elderly female patient with chronic lymphocytic leukemia and transient Inab phenotype with anti-IFC was reported.[22] We report another example of a patient with transient Inab phenotype, who unlike the previous transient Inab phenotype propositi, had GI abnormalities and a direct agglutinating anti-IFC.

CASE REPORT

The propositus is a Caucasian male born at 38 weeks' gestation with congenital cytomegalovirus disease causing blindness and deafness. The patient had severe growth retardation (height, first percentile; weight, 0 percentile) and suffered significant gastroesophageal reflux disease as well as milk and soy

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TABLE 1. The molecular basis of Cromer system antigens*

High-incidence antigen	ISBT number	Antithetical antigen	ISBT number	Amino acid polymorphism
Cr^a	CROM1			Ala193Pro
Tc^a	CROM2	Tc^b	CROM3	Arg18Leu
Tc^c	CROM4			Arg18Pro
Dr^a	CROM4			Ser165Leu
Es^a	CROM6			Ile46Asn
IFC	CROM7			See text for details
WES^b	CROM9	Wes^a	CROM8	Leu48Arg
UMC	CROM10			Thr216Met
GUTI	CROM11			Arg206His
SERF	CROM12			Pro182Leu
ZENA	CROM13			His208Gln
CROV	CROM14			Glu122Lys

* Adapted from Reid et al.,[2] Storry et al.,[3] Banks et al.,[4] Storry and Reid,[5] Ivankovic et al.[6]

TABLE 2. Comparison of all reported individuals with Inab phenotype

Author	Ethnicity	Anti-IFC	GI abnormalities*	Transient	SNP
This study	Caucasian	Yes	GERD, food intolerances	Yes	None
Hue-Roye et al.[20]	Japanese	Yes	None	No	508C>T
Banks et al.[22]	Swedish	Yes	None	Yes	Unknown
Uchikawa et al.[19]	Japanese	Yes	Unknown	No	263C>A
Matthes et al.[21]	Kenyan	Yes	None	Yes	None
Daniels et al.[18]	Japanese	Yes	Capillary angioma	No	261G>A
	African American	Yes	PLE	No	Unknown
Wang et al.[17]	Japanese	Unknown	None	No	263C>A
Lin et al.[16]	Italian American	Yes	None	No	Unknown
	Italian American	No	None	No	Unknown
Walthers et al.[15]	American	Yes	Crohn's disease	No	Unknown
Daniels et al.[14]	Japanese	Yes	PLE	No	261G>A

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

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intolerances such that a feeding tube was required. At age 1 year (April 2004), he came to the attention of the blood bank when routine pretransfusion testing, performed in advance of cochlear implant surgery, revealed an ABO typing discrepancy due to a potent panagglutinin. Additional samples were obtained when the patient returned for a follow-up visit at age 16 months (August 2004). At that time his complete blood count was within normal limits. Additional samples were also obtained after surgery at age 18 months (October 2004).

MATERIALS AND METHODS

RBC serology

Standard serologic techniques were performed throughout this case study investigation.[23] Reagent RBCs and antibodies to blood group antigens were from either ImmucorGamma (Norcross, GA) or Ortho Clinical Diagnostics (Raritan, NJ), or from the private collections of our institutions. Gel column technology was from Ortho Clinical Diagnostics.

Immunoglobulin G (IgG) monoclonal antibodies to CD55 and CD59 were a gift from G. Halverson of the Lindsay F. Kimball Research Institute at the New York Blood Center and were tested against RBCs in gel columns containing mouse anti-IgG (MicroTyping Systems, Pompano Beach, FL). *Escherichia coli* (*E. coli* 12F), containing the 075X adhesin (Dr^a adhesin),[24] was a gift from C.F. Marrs (School of Public Health, University of Michigan). Bacterial hemagglutination tests were performed on buffered gel cards (Ortho Clinical Diagnostics) as described previously.[25]

Flow cytometry

Washed RBCs (50 μL, 3% suspension) were incubated with IgG monoclonal anti-CD55 and CD59 (50 μL) for 30 minutes at room temperature. After being washed, the RBCs were incubated with fluorescein isothiocyanate (FITC)-labeled mouse anti-IgG, washed, and then resuspended in 500 μL of phosphate-buffered saline-1 percent paraformaldehyde. After fixation, cells were analyzed by one-color flow cytometry (FACScan, Becton Dickinson, Hialeah, FL) with the accompanying software (Cell-Quest, Becton Dickinson). Cells were gated on forward and orthogonal scatter, and the percentage FITC-positive cells was determined. All samples were stained in duplicate and recorded as the mean percent positive cells and mean channel fluorescence.

Sequencing of DNA

Genomic DNA was isolated from whole blood (QIAamp blood kit, Qiagen, Inc., Valencia, CA). The regions of *DAF* that include and flank exon 2, exon 3, exon 4, exon 5, and exon 6 (which encode SCRs 1-4) were individually amplified with sets of primers derived from Banks and colleagues[4] and Telen and coworkers[26] with modified primers DAFSCR2R (GAGTTCTAGCATGAATGAAGGAAGGG) and DAFSCR4F (GCATCTCTTGTTGGTAATGCTGG). The primers were synthesized by Life Technologies, Inc. (Gaithersburg, MD). Two microliters of DNA per reaction was amplified by 5 U *Taq* DNA polymerase (HotStarTaq, Qiagen) in a 50-μL reaction mixture containing 2.0 mmol per L MgCl$_2$ (1.5 mmol/L MgCl$_2$ for exon 2 primers), 1× PCR buffer, 0.2 mmol per L 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 in the Laboratory of Microchemistry at the New York Blood Center with an automated DNA sequencer (ABI 373XL, Applied Biosystems, Inc., Foster City, CA), and reagents (ABI Big Dye reagents with BD Half-Term, GenPak, Stony Brook, NY). The PCR products were sequenced in both directions.

RESULTS

Serology

April through August 2004. The following description combines the serologic findings from the propositus' April and August 2004 blood samples. Unexpected reactivity with A$_1$ cells on the serum ABO typing of this apparent group A patient was noted. Additional studies revealed a strongly reactive panagglutinin (2-3+^{s}) in immediate-spin tests with all panel cells tested, including a sample from cord blood. Much weaker (microscopic 1+ to 1+^{s}) reactivity was noted with all panel cells tested at albumin 37°C, at albumin IAT, and by papain IAT. The autologous control was negative in all phases of testing. The antibody failed to agglutinate cells when saline was then added in place of AHG reagent in the IAT.

The maximum titer of this panagglutinin was 2000 at 4°C. It agglutinated RBCs of the following rare phenotypes: Emm–, Gy(a–), M^{k}M^k, p, and P$_2$^{k} and did not agglutinate Dr(a–), IFC–, and type III PNH. The antigen recognized by this panagglutinin was ficin-, papain-, and trypsin-resistant but α-chymotrypsin-sensitive. The failure of this antibody to agglutinate Dr(a–), IFC–, and type III PNH RBCs, along with its reaction pattern with enzyme-treated RBCs, was suggestive of an antibody to a high-prevalence antigen of the Cromer blood group system.

The patient's RBCs were negative in the DAT. The extended phenotype of his RBCs revealed that he was positive for the following antigens: A, D, C, E, c, e, M, S, s, Fy^a, Fy^b, Jk^a, Jk^b, Wr^b, Yt^a, I, P, Vel, and Emm. His RBCs were negative for the following antigens: N, P1, K, Dr^a, and IFC. His RBCs did not react with anti-CD55 when tested on mouse anti-IgG gel cards nor did they demonstrate agglutination in gel cards containing *E. coli* 12F with the 075X

adhesin.[25] Overall, in view of the IFC-negative phenotype of the propositus, this antibody is considered to have anti-IFC specificity, although typically anti-IFC would be expected to react weakly with Dr(a–) RBCs.

October 2004. The antibody detecting a high-prevalence antigen of the Cromer blood group system was virtually undetectable in his serum sample; it reacted only microscopically by albumin IAT with one of six panel cells tested and also with a cord RBC sample. The propositus' plasma also reacted weakly at room temperature with four of seven RBC samples suspended in saline. As before, the patient's RBCs were negative in the DAT, but his RBC phenotype had changed such that his cells were now weakly reactive with anti-Dr[a] and anti-IFC. His RBCs still failed to be agglutinated by *E. coli* 12F. Weak reactivity was now observed when his cells were tested with monoclonal anti-CD55 by mouse anti-IgG gel cards. At this time the RBCs of his mother, father, and brother were also obtained and evaluated for the expression of CD55; these family members demonstrated normal expression of CD55.

Flow cytometry

CD55 was virtually absent from the RBCs of the propositus in August (gated events, 1.6 percent of control; Fig. 1). However, in October the number of CD55-positive cells (gated events) had increased to 28 percent of control. CD59 was expressed normally in specimens from both dates. This finding, taken together with the presence of the GPI-anchored Yt[a] and Emm antigens on his RBCs, excludes PNH as the cause of reduced CD55 expression. The levels of CD55 and CD59 were also evaluated on RBCs from his parents and his brother in October: as in agglutination-based studies, RBCs of these family members demonstrated normal CD55 and CD59 expression.

Sequencing of DNA

Exons 2 through 6 of *DAF* and their flanking intronic sequences were sequenced and found to be identical to the consensus sequence.

DISCUSSION

This is the first report of a patient with the triad of GI abnormalities, transient Inab phenotype, and a direct aggluti-

nating antibody directed at a high-prevalence Cromer antigen. The specificity is considered to be anti-IFC based on the findings that the patient's RBCs were IFC– and that the antibody did not agglutinate IFC– or Dr(a–) RBCs. The failure to agglutinate Dr(a–) RBCs is most likely due to the very low levels of Cromer antigens expressed on RBCs with this phenotype. Unlike a previous case report of a patient with transient Inab phenotype with anti-IFC,[21] our patient did not manifest thrombotic complications, although in both cases a generalized absence of GPI-linked proteins was excluded by the presence of normal amounts of CD59 on the RBC surface. Exons 2 through 6 of *DAF* were consensus in both propositi. Unfortunately, we have been unable to obtain further blood samples from the current propositus and his family, nor can we be certain of the length of time the current propositus had the transient Inab phenotype with the anti-IFC. It is interesting to note that the anti-IFC reported here was a direct agglutinin that
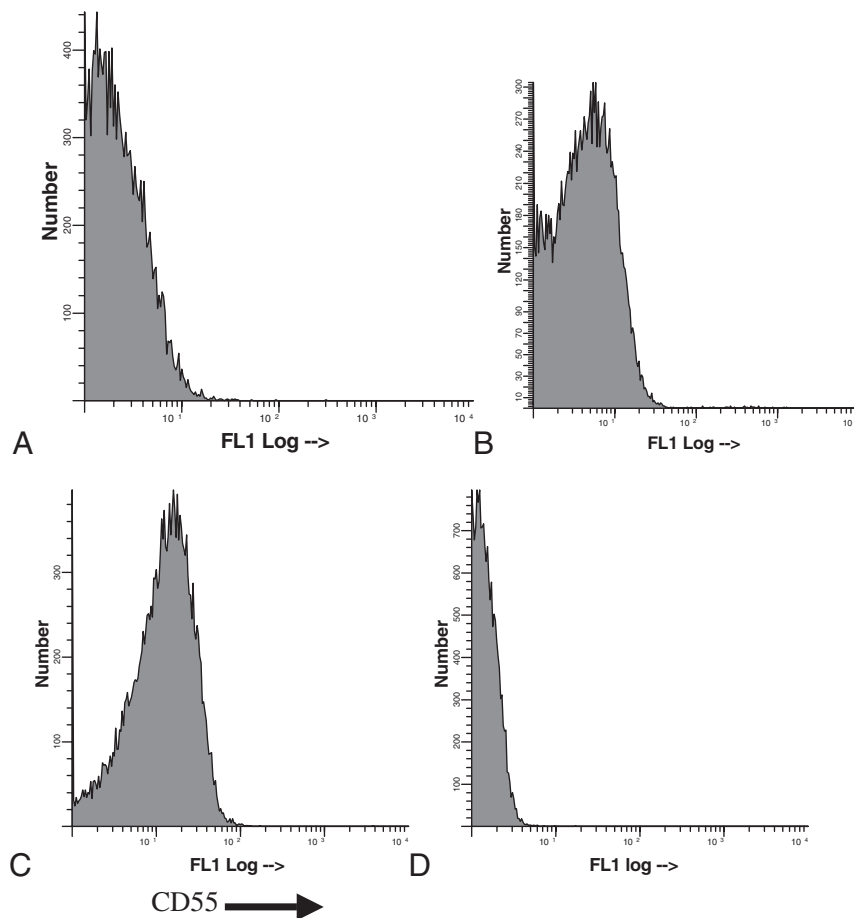
Fig. 1. Comparison of the expression of CD55 on the propositus' RBCs in August 2004 (A) and again in October 2004 (B). In August, most of his cells (98.4%) had a complete absence of CD55. In October a significant proportion of his RBCs demonstrated increasing levels of CD55, and his circulating anti-IFC was virtually undetectable. (C) Positive control (RBCs donated by a healthy laboratory volunteer); (D) isotype control.

reacted strongly at 4°C but there was also reactivity, albeit weaker, at IAT; however, reactivity was absent when saline was added in place of the AHG reagent. There is precedent for changes in the GI flora stimulating transient anti-blood group antibodies: a 20-day old, K– neonate with sepsis caused by the uncommon *E. coli* variant O125:B15 produced anti-A and anti-K. Both antibodies were IgM and not of maternal origin. Cell-free isolates of these bacteria could inhibit the agglutination caused by these antibodies and thus bacteria were presumed to have been the sensitizing stimulus. When the infection resolved, the antibodies disappeared.[27]

Still, the stimulus for developing the current anti-IFC, as in the two previous reports of patients with transient Inab with anti-IFC remains unclear.[21,22] The patient in the earlier report had been a blood donor for many years before developing this antibody, and no unexpected antibodies had been detected in his serum at the time of his donations.[21] The other patient with transient Inab phenotype had not been previously transfused, but had been pregnant (J. Poole, personal communication, June 2005).[22] The current propositus was a very young male who had also never been transfused. Although we do not have a blood sample from before April 2004, the appearance and subsequent disappearance of the anti-IFC most likely correlates to the presence (and absence) of the corresponding antigen on the patient's RBCs. Anti-IFC is not invariably present in people with the Inab phenotype: a 70-year-old Italian-American individual who, along with his sister, inherited the Inab phenotype did not demonstrate anti-IFC; given that both he and his sister demonstrated this rare phenotype, it is most likely inherited. Thus anti-IFC is not necessarily present in Inab individuals, even those with a genetic etiology to their phenotype.[16] The appearance of anti-IFC was also noted in the original Inab propositus who presented with an anti-IFC; he had received transfusion of fresh-frozen plasma but apparently not RBCs.[14]

A common thread linking the first few reports of patients with the Inab phenotype was that these propositi had GI system abnormalities; the original propositus (Inab) had a protein-losing enteropathy,[14] as did a 12-year-old African American boy who almost certainly had the Inab phenotype.[18] Walthers and coworkers[15] reported a third Inab phenotype patient who had Crohn's disease, whereas Daniels reported a Japanese woman with the Inab phenotype who suffered from blood capillary angioma of the small intestine.[18] Although CD55 is known to be expressed on numerous epithelial surfaces including the gut,[28] a causal association between its deficiency and anatomical lesions of the gut has not been established. DAF knock-out mice had heightened susceptibility to dextran sulfate sodium–induced colitis, suggesting its role in protecting the gut from insult.[29] These similarities have to be interpreted in light of the six other Inab phenotype

patients, including the two transient Inab patients,[21,22] who did not demonstrate nor have a history of GI abnormalities.[16,17,20]

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