Biochemical Studies on Red Blood Cells From a Patient With the Inab Phenotype (Decay-Accelerating Factor Deficiency)

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A 38-year-old Russian woman (KZ) has been identified as the fourth proposita with the Inab blood group phenotype. Like the first two propositi, she has a chronic intestinal disorder and, as shown for the third proposita, her Inab phenotype is demonstrably inherited. KZ's serum contained anti-IFC, which reacted with a red blood cell (RBC) membrane component with an Mr of 70,000, which is decay accelerating factor (DAF). Her RBCs lacked all Cromer-related blood group antigens and DAF. Her RBCs were no more susceptible than normal control RBCs to lysis in acid lysis or in rabbit or human antibody-initiated complement lysis tests. Northern blots of total RNA isolated from KZ's Epstein-Barr virus-transformed lymphoblasts showed a marked reduction of DAF mRNA when compared with normal. Polymerase chain reaction (PCR) amplification of cDNA confirmed this reduced level of DAF mRNA. Sequencing of the PCR product showed a 44-nucleotide deletion in the mRNA close to the short consensus repeats IIIa/IIIb intron/exon boundary. This deletion results in a change in the reading frame that places a termination codon six amino acids after the deletion. The putative translation product would lack a glycosyl phosphatidyl-inositol linkage site and, therefore, would not be membrane-bound in the RBC.

RED BLOOD CELLS (RBCs) from individuals with the Inab phenotype lack all Cromer-related blood group antigens, of which there are at least eight high incidence (Cr^a, Tc^a, Tc^{ab}, Dr^a, Es^a, WES^b, UMC, and IFC) and three low incidence (Tc^b, Tc^c, and WES^a) antigens.[1] Cromer-related antigens are located on the RBC membrane complement regulatory protein, decay accelerating factor (DAF).[2-4] DAF is an Mr 70,000 glycoprotein that is anchored to the RBC membrane by a glycosyl phosphatidyl-inositol (GPI) linkage.[5] DAF is widely distributed on hematopoietic and nonhematopoietic tissues and has been assigned the white blood cell differentiation antigen cluster number CD55.[6]

DAF regulates the action of C3 convertase on RBC surfaces and its absence has been held to be partly or wholly responsible for the abnormal sensitivity to complement-initiated lysis exhibited by affected RBCs from patients with the acquired condition paroxysmal nocturnal hemoglobinuria (PNH).[7-9] Individuals with the Inab phenotype have a specific deficiency of DAF on their RBCs but have no documented hematologic abnormalities.[1] This is in contrast to the affected RBCs from individuals with PNH that have a deficiency of several GPI-linked proteins (eg, DAF; lymphocyte function associated antigen 3 [LFA-3 or CD58]); acetylcholinesterase (AchE); CD59, synonyms: membrane inhibitor of reactive lysis [MIRL and HRF20]; and homologous restriction factor [HRF], synonyms: membrane attack complex inhibition protein [MIP] and C8bp), and increased susceptibility to hemolysis.[10] Thus, RBCs from individuals with the rare Inab phenotype are valuable in determining the specific role of DAF in these cells. Previous studies on RBCs from other individuals with the Inab phenotype indicated that they do not exhibit the extreme sensitivity to complement-mediated lysis characteristic of PNH RBCs[11] or that they are slightly more sensitive than normal RBCs.[12]

The gene encoding DAF protein is located on chromosome 1, q32.[13,14] This band is also the location of the genes for four other proteins that downregulate the complement system at the level of the C3 convertase (namely, membrane cofactor protein [MCP], complement receptor type 1 [CR1], factor H, and C4 binding protein [C4bp]). The gene for complement receptor type 2 (CR2) is also in this region. This gene cluster constitutes the regulators of complement activation (RCA) gene family.[15]

The amino acid sequence of DAF has been deduced from the nucleotide sequence of cDNA.[16,17] Recent studies on mRNA extracted from Epstein-Barr virus-transformed lymphocytes (EBV-LCL) from the first propositus with the Inab phenotype (Inab) have shown that DAF mRNA is present but in markedly reduced amounts when compared with a normal control.[18]

In this report we describe studies on blood samples from another individual (KZ) with a specific RBC deficiency of DAF who is the fourth proposita with the Inab blood group phenotype. KZ was a 38-year-old Russian woman who was admitted to the hospital for surgery to correct a chronic intestinal disorder of unknown etiology. This fact is of interest because the first propositus with the Inab phenotype had an ileocecal tumor and protein-losing enteropathy[19] and the second propositus had Crohn's disease.[20] In contrast, the third example of the Inab phenotype and her Inab phenotype brother did not exhibit an intestinal disorder.[21] During routine antibody screening, serum from KZ was found to have an antibody that agglutinated all RBCs tested, except her own, and this prompted the investigations described here. Results of serology, immunochemistry, complement regulation, and molecular biology studies are described.

MATERIALS AND METHODS

Standard serologic tests were used throughout. Trypsin and α-chymotrypsin treatment of RBCs was as described previously.[22,23] Human polyclonal RBC typing reagents were from our in-house collection. ABO and antiglobulin reagents were obtained from the

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Bio Products Laboratory, Elstree, UK (anti-IgG, -IgG_{Fc}, -C3d); Ortho Diagnostics, High Wycombe, UK (anti-IgM); and Hoechst, Hounslow, Middlesex, UK (anti-C3c, -C4c, and -C5). Monoclonal antibodies to DAF, NBTS/BRIC 110 (BRIC 110), BRIC 128,[24] and BRIC 230, and BRIC 227, which recognizes an epitope on the LFA-3 glycoprotein, were obtained from Dr D.J. Anstee (IBGRL, Bristol, UK). Rabbit anti-DAF was provided by Dr M. Pangburn (Department of Biochemistry, University of Texas Health Center, Tyler). The acetylcholinesterase activity of whole RBCs was assayed by the method of Ellman and Calloway.[25] Immunoblotting of proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as previously described.[22,26] Human antibodies were purified by adsorption onto, and elution from, antigen-positive RBCs and used for hemagglutination or for immunoblotting as described previously.[26]

Hemolysis tests. The acid lysis, cold antibody lysis, and sucrose lysis tests were performed as published previously.[11] Antibody-initiated human complement lysis tests were performed as follows: A 1/5 dilution of rabbit antihuman RBC serum or undiluted human serum containing anti-I were used to sensitize RBCs. Both were heat-treated (56°C for 30 minutes) to inactivate complement. The RBCs were washed and resuspended to 10^8 RBCs/mL in DGVB++ buffer (2.5 mmol/L sodium veronal, 72.5 mmol/L NaCl, 0.15 mmol/L $CaCl_2$, 0.5 mmol/L $MgCl_2$, 25 g/L glucose, 1 g/L gelatin, pH 7.4). Volumes of 100 μL were added to wells in a flat-bottomed microtiter plate. Fifty microliters of dilutions (1/20 to 1/40) of a fresh normal human serum was added to each well and incubated with shaking for 30 minutes at 37°C. To standardize the complement source, a fresh aliquot of a single-donor serum was thawed from −70°C storage on each occasion that these tests were performed. The plates were centrifuged, and the degree of lysis was calculated by determining the optical densities at 540 nm. Blanks containing serum dilutions but no RBCs were prepared and the percent lysis calculated by comparison with an aliquot of the RBCs lysed with 0.1 mol/L sodium carbonate. Results obtained with KZ's RBCs were compared to results obtained with a panel of up to eight normal RBC controls, matched for anticoagulant and duration and conditions of storage.

Assessment of antibody and C3 binding. The amount of rabbit antibody bound to the patient's washed RBCs was assessed as follows: Duplicate 100 μL aliquots of 10^8 RBC/mL were mixed with 100 μL of phosphate-buffered saline (PBS) containing no antibody, or 1/5, 1/10, or 1/20 dilutions of rabbit antihuman RBC antiserum (made by immunizing rabbits with human RBC ghosts). RBCs were incubated for 30 minutes at 37°C and then washed three times with PBS and resuspended in 200 μL of PBS containing 4×10^6 cpm of ^{125}I-labeled goat antirabbit IgG. The RBCs were left for 16 hours at 4°C, centrifuged (5 minutes at 10,000*g*), and then washed five times with PBS. RBCs were resuspended in 200 μL PBS and transferred to fresh tubes for counting of radioactivity. Goat antirabbit IgG was supplied by Tissue Culture Services (Slough, Berks, UK) and was iodinated with a standard iodogen reaction.[27]

C3 binding was assessed as follows: 100 μL of RBCs (10^8/mL) in DGVB++ buffer were mixed with 100 μL of a 1/5 dilution of heat-treated rabbit antihuman RBC antiserum, plus 200 μL of 1/10 diluted fresh human serum, containing 2×10^6 cpm of ^{125}I-labeled C3. Tubes were taken after 0, 5, 10, or 30 minutes of incubation at 37°C, and centrifuged for 5 minutes at 10,000*g* (sufficient to pellet the ghosts of complement-lysed RBCs). Lysis occurred progressively in test and control RBCs and was incomplete at 30 minutes. RBCs and ghosts were washed five times with PBS, then transferred to fresh tubes to measure radioactivity. C3 was isolated from human plasma[28] and iodinated as described by Fraker and Speck.[27]

Northern blotting of RNA samples. Total RNA was prepared from EBV-LCL as described by Chirgwin et al.[29] Poly(A)$^+$ RNA was isolated from total RNA on oligo (dT) cellulose[30] and blots were performed by the method of Maniatis et al.[31]

Polymerase chain reaction (PCR) amplification. First-strand cDNA was prepared from poly(A)$^+$ RNA using oligo (dT_{16}) as the primer.[31] Avian myeloblastosis virus reverse transcriptase was obtained from Pharmacia (Uppsala, Sweden). First-strand DAF cDNA was amplified by PCR[32] using Taq polymerase on a Perkin-Elmer Cetus DNA thermal cycler (Norwalk, CT) using primers that spanned the predicted coding sequence of DAF cDNA: The sense primers were DAF1 with the sequence GACT-GTGGCCTTCCCCCAGATGTAC corresponding to nucleotides (nts) 168 to 192 of the DAF cDNA isolated by Caras et al[16] and DAF9, CCGGGAGAAATACGAAATG (nts 561 to 579). Antisense primers used were DAFAS2, (CGAATTCG)ATGCA-GATAAGTCTAAGAAACTAGG (nts 1258 to 1282 with the addition of an endonuclease-*Eco*RI site at the 5′ end) and DAFAS4, GCTCTCCAATCATGGTGAATCC (nts 831 to 852). PCR amplified DNA was purified, cloned, and sequenced as described by Avent et al.[33]

RESULTS

Serologic testing. KZ's RBCs typed as group A1; C−c+D−E−e+; M+N+S−s+; P_1++; Lu(a−b+); K−; Le(a−b−); Fy(a+b+); Jk(a−b+) and positive for the following high-incidence antigens: H, P, Kp^b, Js^b, Ku, U, En^a, I, Au^a, Sc1, Yt^a, Vel, Co^a, Di^b, Lan, Kn^a, McC^a, JMH, Ch, Rg, Ge2, Ge3, AnWj, Jr^a, Er^a, LW^a, LW^{ab}, Gy^a, In^b, Rh17, and Yk^a. Her RBCs were not agglutinated by anti-Cr^a, -Tc^a, -Dr^a, -IFC, rabbit anti-DAF, or by the monoclonal anti-DAF antibodies BRIC 110 and BRIC 128. These results indicate that the patient's RBCs are of the Inab phenotype. The direct antiglobulin test (DAT) was negative with anti-IgG, anti-IgGFc, and anti-IgM.

The KZ serum agglutinated all untreated, ficin-, papain-, and trypsin-treated panel RBCs in the low ionic strength solution indirect antiglobulin test using an anti-IgG reagent but did not agglutinate α-chymotrypsin–treated RBCs. KZ serum agglutinated Cr(a−) and agglutinated weakly Dr(a−) RBC samples. It did not agglutinate RBCs with the Inab phenotype and the antibody was identified as anti-IFC.

KZ's siblings were not available for testing; however, RBCs from her mother and father were IFC-positive although the reactivity was weaker than that obtained with control RBC samples (Table 1). Weak reactions were also obtained with the parent's RBCs by hemagglutination tests with the parent's RBCs by hemagglutination tests with anti-Dr^a, -Cr^a, -Tc^a, -IFC (Inab), BRIC 110 and BRIC 128. These results suggest that the parent's RBCs have a heterozygous expression of Cromer-related antigens and that KZ's Inab phenotype was inherited.

Table 1. Results of Hemagglutination Titration Studies

	Patient's Anti-IFC			
	Serum Titer	Score	Eluate Titer	Score
Control	256	64	4	13
KZ	0	0	0	0
Father	32	39	2	6
Mother	NT	NT	2	5

Abbreviation: NT, not tested.

Immunoblotting. BRIC 128 and BRIC 230 reacted in immunoblotting with an Mr 70,000 component from normal RBC membranes, but did not react with KZ or control Inab RBC membranes (Fig 1). Anti-IFC from KZ serum (adsorbed onto normal RBCs and eluted as described in Materials and Methods) reacted with an Mr 70,000 component from normal RBC membranes but did not react with membranes prepared from KZ or Inab RBCs (data not shown). These data are consistent with the interpretation that the KZ RBCs were of the Inab phenotype and lacked or were markedly deficient in DAF glycoprotein. The anti-IFC produced by KZ is probably reactive with an antigenic determinant on DAF.

Tests for other GPI-linked proteins. The reaction pattern by immunoblotting of membranes from KZ RBCs with BRIC 227 (which detects an epitope on the LFA-3 glycoprotein) was indistinguishable from the normal control. The number of copies of CD59 glycoproteins has been determined to be similar on RBCs from KZ, Inab, and a normal control (B. Gardner and D.J. Anstee, personal communication, 1990). Direct assay of AchE activity on the RBCs from KZ and her father gave results of 95% and 85%, respectively, in comparison with three random controls

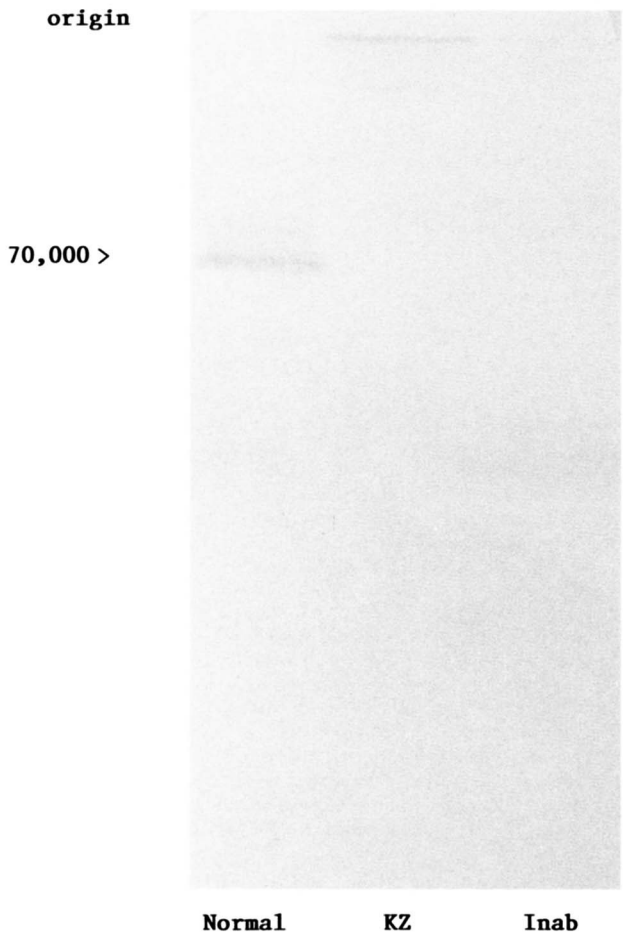


Fig 1. Immunoblot with monoclonal anti-DAF BRIC 128. A component of Mr 70,000 is present in normal but not in KZ or Inab RBC membranes. Similar results were obtained with BRIC 230.

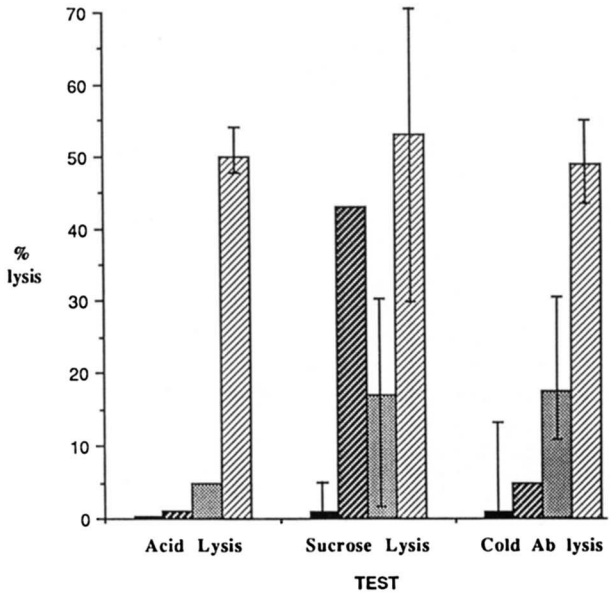


Fig 2. Lysis tests. RBCs from normal individuals (range for 5, ■), KZ (▨), Inab (triplicate tests, □), and PNH patients (three individuals, ▨) were tested in the acid lysis, sucrose lysis, and cold antibody lysis tests as described.[11] The results are compiled from a series of experiments performed on different occasions but tests on normal individuals were always included in each assay.

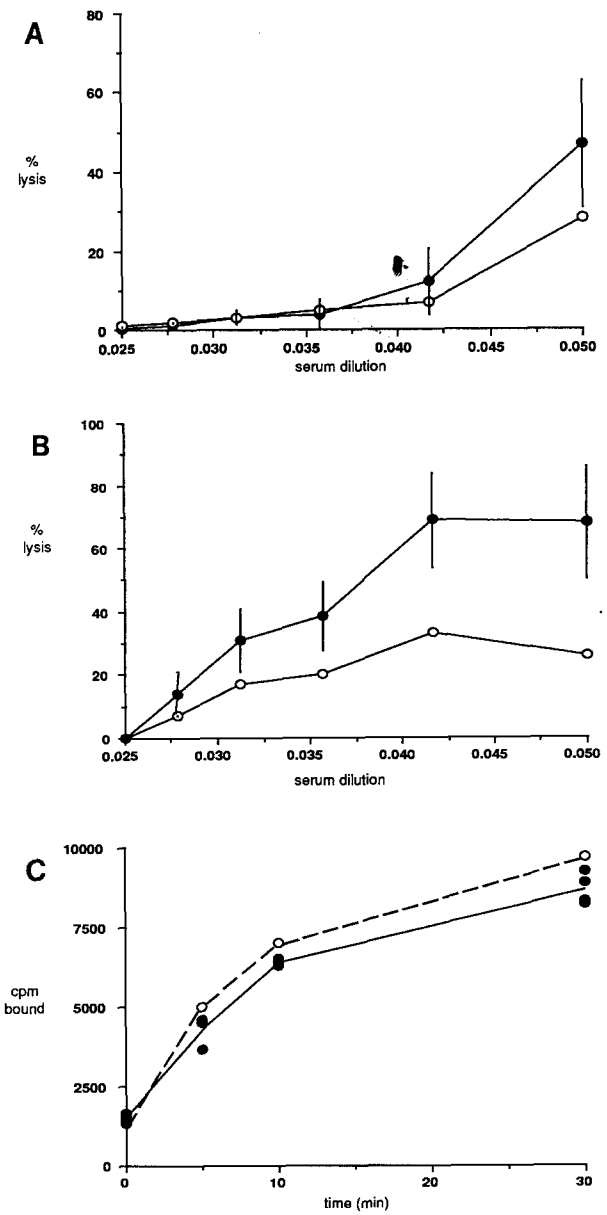
(93% to 104%). By hemagglutination, the KZ RBCs were shown to possess Yt^a, Gy^a, and JMH, and were shown by immunoblotting to possess Gy^a and Hy blood group antigens. These blood group antigens have been reported to reside on GPI-linked proteins.[34,35] These findings suggest that while KZ RBCs lack or are grossly deficient in DAF, other GPI-linked proteins are present.

Susceptibility of RBCs to lysis. In the acid lysis test, the degree of hemolysis obtained with KZ RBCs was within the normal range (Fig 2). RBCs from control Inab gave slightly greater, but not abnormal lysis while an average of 50% of the typical control PNH RBCs hemolyzed (Fig 2). In the cold antibody lysis test, the amount of hemolysis obtained with KZ RBCs was above the average obtained with the normal controls, but much less than the lysis obtained with the PNH control (Fig 2). In the sucrose lysis test, lysis of KZ RBCs was within the range for normal RBCs but less than for PNH RBCs (Fig 2). An osmotic fragility test showed normal results for KZ (data not shown). Because increased hemolysis was obtained in the cold antibody lysis test, a further series of tests was performed in which complement activation was antibody initiated. Such tests should show whether DAF performs a significant C3 convertase regulating function on RBCs.

Antibody-initiated complement lysis tests. RBCs were sensitized with human anti-I and incubated with fresh human serum dilutions. KZ RBCs were no more sensitive to lysis than a panel of eight normal controls (Fig 3A). Another test, performed under the same conditions but with rabbit antihuman RBC serum, showed less lysis in KZ RBCs than in matched controls (Fig 3B). Because KZ RBCs were no more susceptible to lysis than normal in this

antibody-initiated lysis test, an experiment was performed to determine whether they bound less rabbit antihuman RBC antibody. Results of this test showed that the amount of this antibody bound by KZ RBCs (100%) did not differ from four normal control RBC samples (mean 100%).

DATs on KZ's RBCs with anti-C3d, -C3c, -C4c, and -C5 reagents were negative, indicating that complement was not accumulating on KZ's DAF-deficient RBCs in vivo. This result is surprising if RBC DAF plays a major role in regulation of C3 convertase activity at the RBC surface. Because DAF is purported to inhibit the formation and decrease the stability of C3 convertase enzymes,[7] it would be assumed that lack of DAF would result in more C3 being bound per RBC. An experiment was performed to determine whether KZ RBCs fixed more C3 than did control RBCs when complement was deliberately activated on the RBC surface. KZ RBCs bound marginally more C3 than four control samples but the increase was less than 15% (Fig 3C). These results show that KZ's DAF-deficient RBCs are no more susceptible to complement-initiated lysis than normal control cells (Fig 3B), even when complement is deliberately activated on the RBCs in circumstances in which they bind the same amount of sensitizing antibody and C3 as control RBCs (Fig 3C).



DAF mRNA in EBV-LCL. Because EBV-LCL express DAF[18] and because it was difficult to obtain blood samples from KZ, EBV-LCL were prepared from her peripheral blood for analysis of mRNA. A Northern blot of poly(A)$^+$ RNA from the KZ EBV-LCL and a normal EBV-LCL control is shown in Fig 4A. Extended exposure of the autoradiograph shows that DAF mRNA was present in the patient's cell line but in substantially reduced amount. First-strand cDNA produced from poly(A)$^+$ RNA derived from the KZ EBV-LCL was amplified by PCR for 26 cycles using primers DAF1 and DAFAS2. The product from KZ and from Inab was barely detectable; however, after 30 cycles a product of similar size to the control was clearly demonstrable (Fig 4B).

The PCR products from the KZ cDNA were cloned and sequenced. Two independent clones were sequenced from nts 168 to 1282. mRNA from KZ was shown to have a deletion of 44 nts spanning nts 644 to 687 (Fig 5). This deletion was confirmed by sequencing four further PCR clones. PCR amplification of first-strand cDNA using primers DAF9 and DAFAS4 was used to confirm the deletion in the mRNA. The product derived from KZ was 247 bp compared with the 291-bp product obtained from the control (Fig 4).

DISCUSSION

RBCs from KZ are of the Inab phenotype and characteristically lack all of the antigens of the Cromer-related blood group system for which they were tested. KZ RBCs lacked

Fig 3. Antibody-initiated complement lysis tests. (A) Human antibody-initiated human complement lysis tests: RBCs from KZ (○) and from eight normal controls (●) were tested in an assay in which the dilution of sensitizing antibody (human anti-I) was constant and that of human complement rate limiting by dilution ranged from 1/20 to 1/40 (0.025 to 0.050). Dilutions of fresh human serum containing complement were added to sensitized RBCs and incubated as described in Materials and Methods. The means of duplicate assays on KZ and the range for normal RBCs are shown. (B) Rabbit antihuman RBC antibody-initiated human complement lysis tests: RBCs from KZ (○) and from three normal individuals (●) were tested in an assay in which the dilution of sensitizing antibody (rabbit antiserum to human RBCs) was constant and that of human complement rate limiting by dilution ranged from 1/20 to 1/40 (0.025 to 0.050). Dilutions of fresh human serum containing complement were added to sensitized RBCs and incubated as described in Materials and Methods. The means of duplicate assays on KZ and the range for normal RBCs are shown. (C) Classical pathway initiated C3 binding: The amount of C3 bound to RBCs sensitized with rabbit antihuman RBC antibody was measured after incubation of RBCs with a 1/5 dilution of antiserum with 1/10 diluted fresh human serum in the presence of ^{125}I-labeled C3 over a period of 30 minutes at 37°C as described in Materials and Methods. The ranges for four normal control samples (●) and for KZ (○) are shown. Values were corrected for binding of C3 to control RBCs with no complement activation.

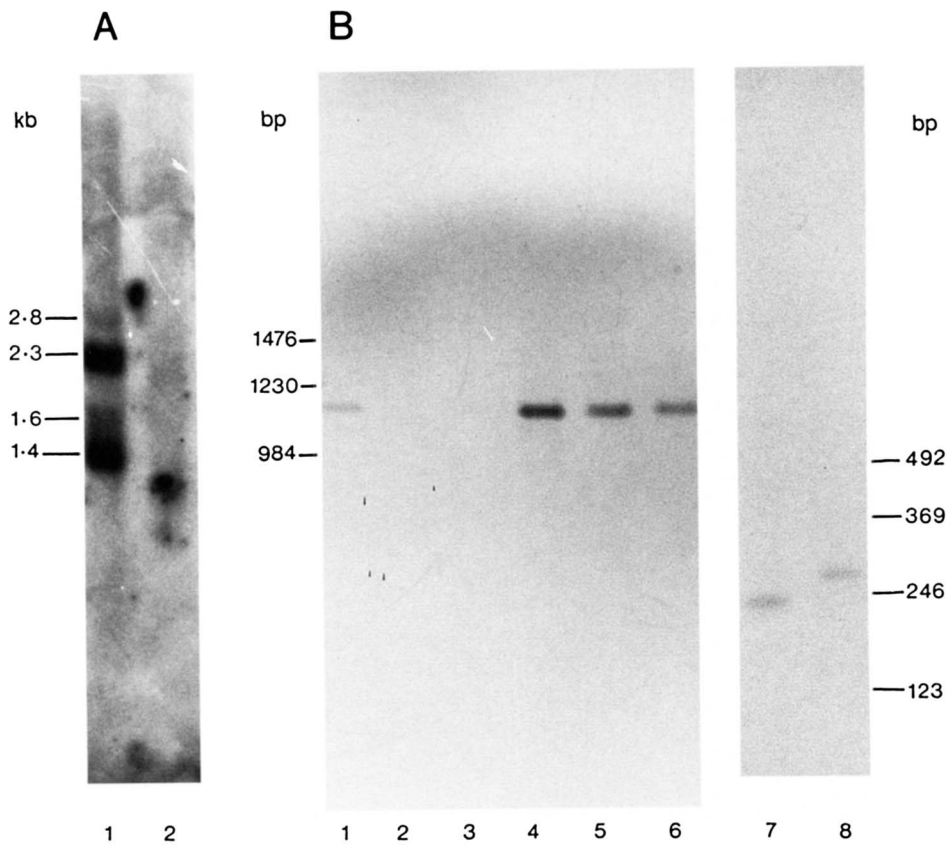


Fig 4. Molecular biology analysis. (A) Northern blot analysis of poly(A)$^+$ RNA and (B) PCR amplification of cDNA derived from cells from KZ. (A) Lane 1 contains control poly(A)$^+$ RNA and lane 2 contains KZ poly(A)$^+$ RNA derived from EBV-LCL. Five micrograms of RNA was loaded onto each lane. The blot was probed with DAFA1 and washed in 0.5X SSC (75 mmol/L NaCl/7.5 mmol/L sodium citrate) containing 1% SDS at 65°C. Exposure of the autoradiograph was for 2 weeks. DAFA1 was a DAF cDNA insert corresponding to nts 1 to 1432 of the DAF cDNA isolated by Caras et al[16] with an additional 20 nts of 5′ noncoding region.[18] (B) Lanes 1, 4, and 8 contain control cDNA; lanes 2, 5, and 7 contain KZ cDNA; and lanes 3 and 6 contain Inab cDNA. The cDNA was produced by PCR amplification using primers DAF1 and DAFAS2 for 26 cycles (lanes 1 through 3), or 30 cycles (lanes 4 through 6), or by using primers DAF9 and DAFAS4 for 30 cycles (lanes 7 and 8).

DAF but possessed other GPI-linked proteins and behaved in a similar manner in sucrose lysis, cold antibody lysis, and acid lysis tests as did Inab RBCs.[11,12] In view of the widely accepted role of DAF in downregulating C3 convertases,[36] it was unexpected that the Inab phenotype RBCs were not more susceptible to lysis than normals in tests where RBCs were sensitized with rabbit antihuman RBC antibody or human anti-I and then exposed to human complement. It was considered possible that KZ RBCs fixed less rabbit antihuman RBC antibody than normals; however, this was not the case. It was also shown that KZ RBCs sensitized with rabbit antibody fixed essentially the same amount of C3b as normal RBCs (Fig 3C). Telen and Green[12] showed that Inab RBCs had a twofold to fourfold increased sensitivity to lysis, compared with a single normal control, when anti-I and human complement were used. These Inab RBCs fixed marginally more C3b via the alternative pathway than a single normal control.

The role of DAF in controlling lysis of PNH RBCs has been re-assessed by Kinoshita,[9] who stated that inhibitors of the membrane attack complex (MAC) are more important than DAF in regulating lysis: the absence or reduction of DAF affects sensitivity to lysis when the MAC inhibitor level is reduced, but not when it is normal. The MAC inhibitors CD59 and HRF/MIP are also absent in PNH RBCs,[37,38] but HRF/MIP is present in Inab.[2] Differences in susceptibility to antibody-dependent complement lysis in Inab phenotype RBCs (KZ and Inab) may depend on variations in MAC inhibitor level. Little is known at present about variation in CD59 or HRF levels, or of the genetic factors controlling the level of expression of these proteins. The decreased lysis of KZ RBCs (Fig 3, A and B) cannot be explained at this time but there was also considerable variation in the extent of lysis of the normal RBCs in this test. The lack of deposition of C3 fragments on circulating KZ RBCs and the failure of KZ RBCs to fix more C3 during deliberate complement activation suggest that the C3 regulatory activity of DAF in vivo, or in this particular form of in vitro classical pathway activation, may be very weak. As noted previously, Telen and Green[12] have reported a possible effect of DAF deficiency on fixation of C3b via the alternative pathway. In in vitro hemolysis assays, or in vivo,

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     C  N  T  G  Y  K  L  F  G  S  T  S  S  F  C  L  I  S  G  S  S  V  Q  W  S  D  P  L
633  TGTAACACAGGGTACAAATTATTTGGCTCGACTTCTAGTTTTTGTCTTATTTCAGGCAGCTCTGTCCAGTGGAGTGACCCGTTG 716  normal DAF cDNA
     ::::::::::::                                                ::::::::::::::::::::::::::::::
633  TGTAACACAGG-------------------------------------------------GCAGCTCTGTCCAGTGGAGTGACCCGTTG 672  KZ DAF cDNA
     C  N  T  G                                                   Q  L  C  P  V  E  *
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Fig 5. Partial sequence and translated amino acid sequence of normal and KZ PCR amplified cDNA. The region shown is the DNA sequence of KZ DAF cDNA adjacent to the 44-nt deletion at the site of the SCRIIIa/SCRIIIb intron/exon boundry and the translated C-terminal amino acid sequence of KZ DAF.

with high RBC density and the constant presence of soluble complement regulatory proteins (eg, factor H and C4bp), the regulatory activity of DAF on RBCs may be negligible compared with that of the soluble proteins and of CR1. However, in other tissues where CR1 is absent and the concentration of plasma proteins is low, DAF may play a more important role.

The abnormal test result noted with KZ RBCs in the sucrose lysis test (Fig 2) was also found in the Inab RBCs studied by Telen and Green.[12] This finding cannot be explained at present but it may be that DAF plays some role in C3 convertase regulation in the unknown mode of activation in this test; it may also be possible that the unusual conditions of this test affect the activity of MAC inhibitors. RBCs from KZ did not have an abnormal osmotic fragility, a test that also exposes RBCs to a low ionic strength environment.

KZ EBV-LCL express a low abundance of DAF mRNA, a result that is similar for Inab.[18] The consequence of the deletion in the KZ mRNA is to change the reading frame downstream of the deletion. This places a termination codon six amino acids after the deletion. Therefore, a translation product would contain 165 amino acids, excluding the leader sequence (Fig 5). Because the potential translation product retains the N-terminal leader sequence and approximately two and a half short consensus repeats (SCR I-IIIa using the nomenclature of Post et al[39]) but lacks a membrane-spanning domain and the GPI-linkage site of normal DAF, any translation product is likely to be secreted from the cell rather than be bound to the membrane. The deletion found in KZ mRNA is very close to the boundary of the intron that Post et al[39] have shown to be present between SCR IIIa and SCR IIIb. Comparison of the 3′ side of the deletion with the corresponding region of normal DAF mRNA shows that the upstream sequence in the normal mRNA is very similar to that of the consensus sequence for 3′ intron splice acceptor sites ($py_{11}xcag$[40]). This finding suggests that the 3′ side of the deletion may be replacing the normal 3′ intron splice acceptor site at the normal SCR IIIa/SCR IIIb splice junction. It is possible that a mutation in KZ has altered the normal splice acceptor site at the SCR IIIa/SCR IIIb junction so that splicing occurs at nucleotide 689. Thus, the deletion present in the mRNA from KZ may not occur in the chromosomal DNA. Similar examples of changes in splice sites have been shown to cause the difference between the glycophorin A and B genes and their products.[41] The low levels of mRNA in KZ RBCs are consistent with a splicing alteration that might alter either the efficiency of splicing, transport out of the nucleus, or the stability of the RNA products. It is interesting to note that the nt substitution (C to T at nt 662[42]) giving rise to the Dr(a−) phenotype is within the region that is deleted in KZ. Dr(a−) RBCs are known to have a weak expression of Cr-related antigens.[1]

Because DAF is present on placental trophoblast epithelium, on those aspects of the tissue that are in direct contact with maternal blood and tissues, it has been suggested that DAF may play a crucial role in protecting the developing conceptus from complement-initiated attack.[43] If this is the case, the question arises as to how individuals with a genetic defect of DAF could survive their development in utero. In normal individuals, Holmes et al[43] have shown by Northern blotting that DAF mRNA can be isolated from placental trophoblasts. It may be that, as in RBCs, other membrane glycoproteins substitute for DAF in this biologically important role. In placental trohoblasts, a probable candidate is membrane cofactor protein (MCP; CD46), which regulates C3 and is expressed on placental tissue.[44] This intriguing question remains unanswered. In contrast, in some cells DAF may perform a role for which there is no substitute. This finding may explain the apparent susceptibility of several Inab phenotype individuals to intestinal disorders. For instance, increased in situ complement activation, resulting from impaired C3 conventase regulation, may lead to cell damage and release of anaphylatoxins and arachidonic acid derivatives, promoting a complex inflammatory mechanism involving a progression of degenerative changes as suggested by Niculescu et al[45] in human atherosclerotic walls.

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