En(a–)FIN phenotype in a Pakistani

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An antibody to a high-incidence antigen in the serum from a Pakistani female (SD) was identified as anti-Ena. Her red cells have the En(a–)FIN phenotype and lack glycoporphin A. This is the first En(a–) to be described in a Pakistani individual and represents the fourth family to be reported. *Immunohematology* 1995;11:51-53.

Eight individuals whose red blood cells (RBCs) express the En(a–)FIN phenotype have been described. Only two of these individuals are alive and available for study.1 RBCs with the En(a–)FIN phenotype lack glycoporphin A (GPA), which is a sialoglycoprotein that traverses the RBC membrane lipid bilayer once. GPA carries M and/or N blood group antigens at its amino-terminus. En(a–)FIN RBCs possess glycoporphin B (GPB) and therefore carry trypsin-resistant, α-chymotrypsin-sensitive N, S, and s antigens, and the U antigen.

The En(a–)FIN phenotype has been described in five members of a Finnish kindred,2-4 in one member of a French-Canadian family,5 and in two Japanese blood donors.1 We describe the first Pakistani individual with this phenotype and the third person with this phenotype still living.

Materials and Methods

RBCs and antisera were available from our current collections and had been obtained from multiple sources. Monoclonal antibodies (mAbs) with specificities to different epitopes on GPA (R1.3, BRIC 119, R18, BRIC 165) were supplied by Dr. D.J. Anstee, International Blood Group Reference Laboratory, Bristol, UK. MAb 148 (clone: F84, 3E8.E2) was obtained from Immucor, Inc., Norcross, GA. E3 was obtained from Dr. Rubinstein at the New York Blood Center, New York, NY, and anti-band 3 (B9277) was purchased from Sigma Chemical Co., St. Louis, MO.

RBC membranes were prepared and electrophoretically separated under reducing conditions on 10 percent acrylamide gels with a 3 percent acrylamide stacking gel.6 Major proteins were visualized with Coomassie brilliant blue stain,7 and sialoglycoproteins were visualized with periodic acid Schiff's Base stain (PAS).8 Immunoblotting was performed as described by Mallinson et al.,9 except that 5 percent bovine milk powder in phosphate-buffered saline at pH 7.3 was used as the blocking agent.

Standard serologic tube tests were used for phenotyping, direct antiglobulin tests (DAT's), antibody identification, adsorption, and elution.

Case History

SD is a healthy 35-year-old female of Pakistani ancestry with no history of having been transfused. She has no siblings and her parents, who live in Pakistan, were not available for testing. SD was admitted to the hospital for a spontaneous abortion. During antibody screening tests and initial panel studies, her serum was found to have an antibody to a high-incidence antigen.

Results

RBCs from SD were group B, D+c+c+E-e+ (most probable genotype R1+r, M-N+w+S-s+, P1-, K-k+, Fy (a+b-), Jk(a+b-). The autologous control and DATs were negative, and no antibody was eluted from SD's RBCs.

Initially, serum from SD agglutinated all RBCs tested except her own at room temperature (1+), at 37°C (2+), and by the indirect antiglobulin technique (IAT) (3+). Further testing showed that her serum was nonreactive with M, M, En(a–)UK, and En(a–)FIN RBCs, but was reactive with Wr(a+b-) RBCs (3+), as well as papain-treated RBCs (2+) of common phenotype. SD's serum was adsorbed twice with papain-treated RBCs. The adsorbed serum was nonreactive with papain-treated RBCs, but was reactive with the same RBCs untreated. These tests suggested that both anti-Ena FR and anti-EnaFS were present. An eluate prepared from RBCs sensitized with SD's serum agglutinated staididase-treated RBCs, indicating that her antibody is not dependent on sialic acid for its activity.

SD's RBCs were nonreactive with human anti-Ena (EP, GW, BJ), anti-Wr(b) (MF), and mAbs BRIC 119 and R18. BRIC 119 detects an epitope on GPA between amino acid residues 31 and 40, and R18 reacts with an epitope on GPA between amino acid residues 46 and 67.10 SD's RBCs were agglutinated by *Vicia graminea* and *Glycine soja*, indicating the presence of 'N' antigen on GPB and a reduced sialic acid level, respectively.

Immunohemochromatographic procedures were used to confirm the En(a–)FIN phenotype of SD's RBCs by demonstrat-
ing that they lacked GPA. Coomassie blue stain showed the expected decreased mobility of band 3 (Fig. 1, first 2 tracks), while PAS staining demonstrated the presence of GPB and GPC and the absence of GPA (Fig. 1, 3rd and 4th tracks). PAS staining also revealed the more intense staining of band 3 as compared to normal membranes.

The more sensitive immunoblotting technique was used to confirm these observations. R1.3 (an mAb recognizing an epitope at the amino-terminus of GPA and GPB) showed the presence of GPB and the absence of GPA from SD’s RBCs (Fig. 1, tracks 5 and 6). E3 (anti-M, BRIC 119, R18, and BRIC 163 (which reacts with an epitope on the C-terminus of GPA) did not react with SD membranes (data not shown). Immunoblotting with antiband 3 clearly showed the higher molecular mass of this component in SD’s RBC membranes (Fig. 1, last 2 tracks).

**Discussion**

SD is a healthy Pakistani female whose RBCs lack GPA and whose serum contains anti-EnA with both anti-EnA PS and anti-EnA FR components. While her RBCs lack all blood group antigens located on GPA, they have ‘N’, which is carried on GPB. The absence of GPA from SD’s RBCs has resulted in a characteristic increased glycosylation of band 3. SD is probably homozygous for a rare deletion of the GYPH locus that produces no GPA in the RBC membrane. If a fresh sample of blood can be obtained from SD, molecular studies will be performed to determine if her RBC GPA deficiency arose by the same mechanism as it did in an En(a−) individual from Finland.

The clinical significance of SD’s anti-EnA was not determined; however, previous examples of anti-EnA, produced by En(a−)FIN individuals, have been clinically significant. A hemolytic transfusion reaction was reported by Furuhjelm et al. after an En(a−)FIN patient (GW) received a transfusion of En(a+) RBCs. Anti-EnA was described in an untransfused woman (EP) who had been pregnant twice. When EP’s antibody was detected in a preoperative compatibility test, the IgG antibody was detectable only in undiluted serum by the IAT; however, following stimulation by only 5 mL of En(a+) blood during a 51Cr survival study, the patient’s antibody titer rose to 16. The results of the survival study showed a significant destruction of the 51Cr-labeled cells with recoveries of 100 percent at 1 hour, 83.4 percent at 2 hours, 48.8 percent at 4 hours, and 9.5 percent at 8 hours. Since SD was brought to our attention at the time of her spontaneous abortion and was lost to follow-up, we have no in vivo data regarding the clinical significance of her antibody.

**References**

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