En(a-) phenotype in a Japanese blood donor

Y. OKUBO, T. SENO, H. YAMAGUCHI, Y. MIYATA, C.A. GREEN, AND G.L. DANIELS

The first Japanese En(a-) individual (T.N.) was found by screening red cells from 250,000 Japanese blood donors with monoclonal anti-En. His serum contained no atypical antibodies and his partial red cell phenotype was M–N–S+s–, although a trypsin-resistant N antigen was detected. His red cells were En(a−) and Wr(b−), as determined by various human and mouse monoclonal antibodies. The absence of glycophorin A (GPA) and the presence of apparently normal glycophorin B (GPB) were demonstrated by immunoblotting with antibodies to the extracellular and cytoplasmic domain of GPA and to epitopes common to GPA and GPB. Sialic acid levels of T.N.'s intact red cells were substantially lower than those of control MN cells. Serologic tests suggested that both of T.N.'s parents were heterozygous for a recessive GPA deficiency gene. Immunochemistry 1993;9:105.

Antigens of the MNS blood group system are located on two sialic-acid-rich glycoproteins, glycophorin A (GPA) and glycophorin B (GPB) (for review, see Cartron and Rahuel). GPA expresses a trypsin-sensitive M or N antigen, whereas GPB expresses a trypsin-resistant N antigen and S or s.

The typical En(a−) phenotype, En(a−)Fin, results from complete absence of GPA and presence of normal GPB. Consequently, trypsin-sensitive M or N antigens are not expressed, but a trypsin-resistant N is present.

En(a−)Fin was found in an extended Finnish family in Finland and in the United States by Furuhjelm et al. and Walker et al., and in a French-Canadian family by Taliano et al. The three independently ascertained propositi from the Finnish family and the Canadian propositus were all found through the presence of antibodies to nonpolymorphic determinants on GPA, collectively known as anti-En. Red cells of the original En(a−) proposita, an English woman with anti-En, described by Darnborough et al. as lacking most of the GPA molecule, did not have normal GPB, so she probably had a GP(A–B) hybrid molecule. Thus, the English and Finnish types of En(a−) have completely different genetic backgrounds.

We report here a third family with the En(a−)Fin phenotype. The propositus was found as a result of screening Japanese blood donors with monoclonal anti-En.

Materials and Methods

IgG1 monoclonal anti-En4FR (OSK4) used for screening blood donors, monoclonal anti-En4FS (OSK4-1), and monoclonal anti-En4TS (OSK4-2) were produced from mice immunized with human red cells. Other monoclonal antibodies to epitopes of GPA and anti-Wr were provided by Dr. D.J. Anstee, International Blood Group Reference Laboratory, Bristol, United Kingdom; Dr. R. Fraser, Glasgow and West of Scotland Transfusion Centre, Carluke, United Kingdom; and Dr. L. Messeter, MonoCarb AB, Lund, Sweden.

Standard serologic techniques were used. Blood donors were screened by testing bromelain-treated red cells with OSK4 in 120-well microtiter plates. Eluates were prepared by the digitonin-acid method. Red cells were trypsin-treated as described previously.

Immunoblotting was carried out as described by Daniels et al.. Briefly, red cell membrane proteins were separated by SDS 10 percent polyacrylamide gel electrophoresis, electroblotted onto filters, and immunostained using murine monoclonal antibodies, horseradish peroxidase conjugated anti-mouse globulin, and 4-chloro-1-naphthol as substrate.

Sialic acid levels on intact red cell membranes were determined by thiobarbituric acid assay as reported by Warren, after treatment with sialidase (RDE: 0.05 units) at 37°C for 60 minutes.

Cells of the propositus were analyzed by flow cytometry using a monoclonal anti-N reactive with GPA and GPB.

Results

Red cells of 250,000 Japanese blood donors were tested with anti-En4 OSK4. All red cells reacted except those of T.N. The serum of T.N. contained no atypical antibodies, and his red cells were M–N–S+s– as determined by selected MNS phenotyping reagents. However, they did express a weak, trypsin-resistant N antigen, characteristic of the N antigen of GPB. They
were also confirmed as En(a−) by several sera from previously described En(a−) propositi (M.E.P., VB., G.W., E.R.P., and R.L.) and by monoclonal antibodies to epitopes along the extracellular domain of GPA. No Ena determinant was detected on the T.N. red cells by adsorption and elution tests with OSK4, OSK4-1, or OSK4-2. The T.N. red cells were Wr(b−) as determined by the original alloanti-Wrb (M. Fr), four sera from En(a−) individuals known to contain anti-Wrb, and two monoclonal anti-Wrb. Further testing showed that T.N.’s red cells did not express any of the following low-frequency antigens associated with the MNS system: He, Vw, Mur, Mg, Vr, Mr, St, Ria, Cla, Ny, Hut, Hil, Ms, Far, sD, Mit, Dan, Hop, Nob, Or, SAT, or M1.

Flow cytometry with anti-N gave the following value of fluorescence per channel for the red cells of T.N.: 36.7. Control values were NN, 202.5; MN, 124.9; MM, 15.9; and Minkk, 8.2.

T.N.’s D+ red cells were directly agglutinated by incomplete anti-D reagents, which do not usually directly agglutinate untreated D+ red cells, and by Glycine soja lectin. Both results suggested that T.N.’s red cells have reduced sialic acid levels. The sialic acid level of T.N.’s intact red cells was about 39 percent (55.1 µg/mL packed cells) of that of control M+N+ red cells (average of 5 samples: 140.4 µg/mL packed cells).

Results of serologic tests on the red cells of the family of T.N. are shown in Figure 1 and Table 1. Red cells of his father, mother, and sister were En(a+) and Wr(b+). The D+ red cells of all three were directly agglutinated by incomplete anti-D, suggesting that they are heterozygous for the rare gene producing no GPA. The parents of the propositus were not consanguineous.

Immunoblotting demonstrated that the T.N. red cell membranes contained no GPA. Immunostaining with monoclonal antibodies to epitopes on various regions of the extracellular domain of GPA (anti-M, R10, and R18) and with an antibody that detects an epitope on the cytoplasmic domain of GPA (BRIC 163) revealed no bands with the T.N. cells; GPA and its dimers GPA2 and GPAB were immunostained when M+ control cells were used (Fig. 2). Anti-N and R1.3,

Table 1. Blood typings of the T.N. family

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monoclonal antibodies to epitopes common to the N-terminal regions of the extracellular domains of GPA and GPB, revealed that the T.N. red cells had GPB and GPB2, but no GPA, GPA2, or GPAB. All bands representing GPA, GPB, and their dimers were apparent with N+ control cells (Fig. 2).

SDS polyacrylamide gel electrophoresis followed by protein staining with Coomassie blue demonstrated reduced mobility of Band 3 (anion exchange protein) in T.N.'s cells and in M^k cells, which also lack GPA, compared with that of control cells (Fig. 3).

**Discussion**

In several centers of the Japanese Red Cross Society, red cells of donors have been screened for rare blood group phenotypes by the use of monoclonal antibodies. This procedure has proved very beneficial for increasing our rare blood registry.

Red cells of only one of 250,000 Japanese donors, T.N., failed to react with an antibody to GPA. Further investigation demonstrated that the T.N. red cells lack GPA and have apparently normal GPB, giving rise to the following characteristics: they are M-N as determined by selected antibodies, but have a trypsin-resistant N antigen on GPB; they have reduced sialic acid levels; and their Band 3 (anion exchange protein) has increased apparent molecular weight, presumably caused by increased glycosylation. Consequently, the red cell phenotype of the T.N. RBCs is indistinguishable from the typical En(a-) phenotype, En(a-)Fin. T.N. is the first En(a-) individual found in Japan and the first En(a-) propositus with no anti-En^a in his serum.

Recently, another Japanese M-N-S+s-, En(a-) donor whose serum did not contain anti-En^a was found at the Chiba Red Cross Blood Center. This propositus (H.S.) is not related to T.N.

T.N. is probably homozygous for a rare recessive gene at the GP7A locus that produces no GPA. His parents are M+N− and M−N+, and both are En(a+), but serologic tests indicated that their red cells have reduced sialic acid content, suggesting that both parents are heterozygous for the GPA deficiency gene.

Southern blot analysis of the red cells from two of the En(a-) propositi of the extended Finnish family suggested that they had a deletion of most of the GPA gene, including all of the region encoding the mature protein. Clearly, a number of different genetic backgrounds, apart from a deletion of all or most of the gene, could result in red cells lacking GPA. An example would be loss of a vital exon or a single base deletion resulting in a reading frame shift.

Molecular analysis of T.N.'s rare phenotype is currently under way, and it will be interesting to see if his RBC GPA deficiency arose in the same way as it did in the En(a-) individual in Finland.

**References**

12. Murata S. Personal communication.

Yasuto Okubo, MD, Vice-Director, Osaka Red Cross Blood Center, 4–13 Morinomiya 2-chome, Jotoku, Osaka, Japan; Taiko Seno, MT, and Hideo Yamaguchi, MD, Osaka Red Cross Blood Center, Osaka, Japan; Yoshihisa Miyata, PhDr B, Shonan Red Cross Blood Center, Atsugi, Japan; Carole A. Green, FIMLS, and Geoffrey L. Daniels, PhD, MRC Blood Group Unit, London, UK.

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