The first case of the p phenotype in a Gurkha Nepalese

C.K. LIN, K.H. MAK, C.K. CHENG, AND C.P. YANG

A serum sample from a Gurkha Nepalese soldier, residing in Hong Kong, was found to cause hemolysis of reagent ABO red cells (RBCs) in the reverse blood grouping test. Subsequent follow-up studies revealed that he was of the p phenotype, with potent anti-PP1Pk that was strongly hemolytic both at room temperature and 37°C. The anti-PP1Pk was composed of IgG and IgM, and its various components were separable. Immunohematology 1998;14:30–32.

Key Words: p phenotype, anti-PP1Pk, red cell hemolysin

Red blood cells (RBCs) of the rare p phenotype lack all the P blood group–related antigens. People of such phenotype form anti-PP1Pk (anti-Tja), which reacts with RBCs of almost all random individuals. Therefore, the p phenotype can be termed as Tj(a−). The P antigen, first reported as Tja, was named after a woman, Mrs. Jay, who was suffering from gastric carcinoma (the “T” stands for tumor and “j” for the patient). The P1 antigen is encoded by the PI gene, whereas a different locus is involved in the production of P and Pk antigens. P is a high-frequency antigen whereas the Pk phenotype occurs in only 0.01 percent of the population.

Anti-PP1Pk is primarily naturally occurring, though the antibody is not present at the time of birth. The various antibody components in anti-PP1Pk, anti-P, -P1, and -Pk may or may not be separable. Anti-PP1Pk, usually composed of a mixture of IgG and IgM molecules, is often hemolytic. Infusion of as little as 25 mL of ABO-matched random blood units to individuals with anti-PP1Pk has been reported to cause severe transfusion reactions. Anti-PP1Pk is implicated in repeated spontaneous abortions (46% of females with the antibody). Anti-PP1Pk or anti-P, implicated in abortions, is often predominantly of the IgG3 subclass. The purpose of this article is to give an account of the serologic findings on the anti-PP1Pk detected in a Gurkha Nepalese.

Case Report

A serum sample from a 21-year-old male Gurkha Nepalese soldier was referred to us for investigation. It was observed that his serum caused strong hemolysis of reagent RBCs in the reverse blood grouping test and in antibody identification tests both at room temperature (RT) and by a 37°C saline indirect antiglobulin test (SIAT).

Materials and Methods

Commercial reagents were purchased from Ortho Diagnostics Systems Inc. (Raritan, NJ), Gamma Biologicals (Houston, TX), and Biotest Diagnostics Corp. (Denville, NJ). Rare RBCs and sera were obtained from the International Immunological Exchange Group of Serum, Cells, and Rare Fluids (Houston, TX).

Standard techniques, as described in the American Association of Blood Banks Technical Manual, were used. In order to determine whether the various components of the anti-PP1Pk were separable, the antibody was adsorbed with P1− RBCs at 4°C for 2 hours. The adsorbed serum was tested against P1+, P1−, and P2k RBCs. Elution by the xylene technique was performed on the P1− RBCs after adsorption, and the eluate also was tested against each of these RBCs at RT and with 37°C SIAT.

Differentiation between IgG and IgM was carried out by dithiotreitol (DTT) treatment of the serum. The DTT-treated serum was incubated at RT in parallel with a phosphate-buffered saline (PBS) blank and spun after 30 minutes and examined for agglutination. Thereafter, the DTT-treated serum and the PBS blank were incubated at 37°C for 45 minutes, and an SIAT was performed using both anti-IgG and a polyspecific anti-human globulin reagent.

Results

The patient’s RBC phenotype was group O, D+C+E−c−e+; M+N+S−s+; P1−; Le(a+b+); Fy(a+b−); K−k+; Jk(a+b−); he was also Tj(a−), P−, and Vel+. His serum completely hemolyzed a panel of random RBCs at RT and at 37°C. The serum also agglutinated P2k RBCs (2+) at RT and partially hemolyzed the cells at 37°C. Negative reactions were obtained with Tj(a−) RBCs at both temperatures. Therefore, he is of the p (Tj[a−]) phenotype, and anti-PP1Pk (anti-Tj3) was detected in his serum.
The antibodies that were removed or were present in the eluate are given in Table 1. As shown in Table 1, P1− cells may contain traces of Pk antigen, requiring the use of enzyme-treated cells for its demonstration. The results of adsorption and elution are given in Table 2. The adsorbed serum strongly agglutinated three examples of P1− RBCs at 16°C. Weaker reactions were obtained at 37°C. At 37°C SIAT, the eluate gave a 4+ reaction with P1+ RBCs and papain-treated P2k RBCs, a 2+ to 3+ reaction with P1− RBCs and a negative reaction against untreated P2k RBCs. These results indicate that the anti-PP1Pk contained separable anti-P1, potent anti-P, and weak anti-Pk.

The presence of anti-P1 was further confirmed by neutralization of the P1 antibody with P1 substance. The titration results of the anti-PP1Pk against P1− RBCs are given in Table 3. At RT and 37°C, the hemolysin titer was 8; the agglutination titer was 32 at RT and 8 at 37°C. The SIAT titer was 64. In the DTT test, the agglutination of RBCs at RT was completely abolished (the PBS agglutination titer was 32), whereas by the SIAT, both the DTT-treated serum and the PBS control gave a titer of 32. The anti-PP1Pk, therefore, was composed of a mixture of IgM and IgG.

### Discussion

Similar to other rare phenotypes, there is a high incidence of consanguinity in people with the p phenotype and studies conducted among siblings have confirmed the recessive nature of the inheritance. The p phenotype is usually identified through laboratory investigation of the serologic phenomena mediated by the naturally occurring anti-PP1Pk or in females with a history of spontaneous abortions in successive pregnancies. The p phenotype has an estimated incidence of less than 1 in 100,000 in the global population. A higher incidence of the phenotype is found in Sweden (14 in 100,000) and in Japan (1 in 10,000 to 1 in 30,000). Isolated cases have been reported in Ecuador, Tunisia, Algeria, North America, and Peru. We have screened over a million blood donors and have not encountered another p phenotype in the Hong Kong Chinese population. The Nepalese soldiers of Hong Kong are Gurkhas, members of a people originating in the mountains of Nepal. It is estimated that around 10,000 of these soldiers have been tested by our laboratory in Hong Kong through blood donations. We were unable to conduct a family study in this case because of a language barrier, and we cannot determine the incidence of the p phenotype in the Nepal population.

### Table 1. Antibodies removed after adsorption with P1− RBCs and antibodies present in the RBC eluate

<table>
<thead>
<tr>
<th>Antigens in the P1− RBCs</th>
<th>Antibodies removed</th>
<th>Antibodies that remained</th>
<th>Antibodies present in the eluate</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>P, Pk</td>
<td>Anti-P plus traces of anti-Pk†</td>
<td>Anti-P plus most of the anti-Pk†</td>
<td>Anti-P plus traces of anti-Pk for its demonstration</td>
<td>Presence of anti-P1 was confirmed by neutralization with P1 substance</td>
</tr>
</tbody>
</table>

* Red blood cells
† Most of the anti-Pk remained, as the P1− RBCs were not enzyme-treated

### Table 2. Characterization of the anti-PP1Pk by an adsorption–elution procedure using P1− RBCs

<table>
<thead>
<tr>
<th>Tests vs.</th>
<th>Adsorbed serum tested at 16°C</th>
<th>37°C</th>
<th>Eluate tested at 16°C</th>
<th>37°C</th>
<th>Control supernatant 16°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 examples of P1+ RBCs:</td>
<td>P1+</td>
<td>4+</td>
<td>2+</td>
<td>–</td>
<td>4+</td>
<td>–</td>
</tr>
<tr>
<td>P1+</td>
<td>4+</td>
<td>1+</td>
<td>1+</td>
<td>–</td>
<td>4+</td>
<td>–</td>
</tr>
<tr>
<td>P1+</td>
<td>4+</td>
<td>1+</td>
<td>1+</td>
<td>–</td>
<td>4+</td>
<td>–</td>
</tr>
<tr>
<td>3 examples of P1− RBCs:</td>
<td>P1−</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2+</td>
<td>–</td>
</tr>
<tr>
<td>P1−</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>P1−</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1 example of untreated P2k RBCs:</td>
<td>P2k</td>
<td>1+</td>
<td>1+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Papain-treated: P2k</td>
<td>4+</td>
<td>2+</td>
<td>–</td>
<td>2+</td>
<td>4+</td>
<td>–</td>
</tr>
</tbody>
</table>

### Table 3. Titration of the anti-PP1Pk against P1− RBCs

<table>
<thead>
<tr>
<th>Serologic investigation</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titer of serum at RT*</td>
<td>2</td>
</tr>
<tr>
<td>Titer of serum at 37°C</td>
<td>8</td>
</tr>
<tr>
<td>Titer of serum by SIAT†</td>
<td>NT‡</td>
</tr>
</tbody>
</table>

*Room temperature
†Saline indirect antiglobulin test
‡Not tested
References

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