The $P_1H$ antigen and antibody

P. P. Moors

$P_1H$, a newly discovered compound antigen associated with both the ABO and P systems, occurs in approximately 7 percent of Natal (South African) blacks. The compound antigen is evident only when the red cells have exceptionally strong expression of both $P_1$ and $H$ antigens, and it is apparently a dominant character. The antigen is thought to originate by steric rearrangement in the molecule, or to be the product of competition between $P^1$ and $H$ gene transferases for the available paragloboside. The corresponding antibody, anti-$P_1H$, has been made by both $P_1$ and $P_2$ people and is a weak cold agglutinin. It is not adsorbed by red cells carrying either strongly expressed $P_1$ or $H$ antigens, but is adsorbed by and eluted from $P_1H+$ red cells sensitized by anti-$P_1H$. The antibody is inhibited by $P_1$ but not by $H$, Lewis, or $Sd^a$ substances. *Immunohematology* 1993;9:9-7.

The structures in the red cell membrane that carry the ABO system (ISBT (International Society of Blood Transfusion) No. 001) and I collection (ISBT No. 207) determinants also bear those of the P system (ISBT No. 003). The precursor substance of both $H_2$ and $P_1$ glycolipid is lacto-$N$-neotetraosyl-ceramide (type 2 chain), otherwise known as paragloboside. The structure of paragloboside is $\beta$Gal(1→4)$\beta$GlcNAc (1→3)$\beta$Gal(1→4)Glc-Cer. Blood group antibodies with the combined ABO and I specificities anti-IA, -IB, -IH, -iH, -iBH, and -iAB and those with the combined I and P specificities anti-IP$_1$, -IP$_2$, -iP$_1$, and -iP have all been recorded. This article details a further antibody, not previously described, having the combined ABO and P specificity anti-$P_1H$.

Materials and Methods

The red cells were phenotyped and antibodies were tested using standard serologic methods. The parallel titrations were first conducted "blind" and then repeated, with the indicator red cells positioned in a different order. The titers were confirmed with further examples of polyclonal anti-$P_1$ and, if ABO-compatible, with anti-$H$ from a Bombay person and with a potent anti-$H$ from a group B person. The eluates were recovered in saline by the 56°C technique of Landsteiner and Miller. The substances used in the inhibition tests were three examples of hydatid cyst fluid (buffered to pH 7.0 to avoid nonspecific results), pooled treated saliva from several secretors of $H$, pooled treated saliva from several nonsecretors of $H$, and pooled urine from several guinea pigs. The scoring of agglutination is a modification of the method proposed by Marsh.

Results

Identification of antibody

The unknown antibody was a weak cold agglutinin. It was identified in each of three 10-donation pools of group B human sera being standardized as polyclonal anti-A blood-grouping reagents. When the pools were subsequently tested against a local red cell panel to eliminate unwanted antibodies, all three were found to agglutinate one panel sample microscopically. Because the agglutination failed to correspond with any known common antibody specificity, the stored unpooled aliquots of sera were thawed and retested individually. The agglutination was traced to an Indian donor, from whom one donation had been included in each pool.

In the studies made in Durban, the Indian donor's antibody reacted 1+ (just visible to the naked eye) by saline technique at 20°C with an occasional group O or group B black donor's red cells. The agglutination was not enhanced in one- or two-stage bromelin tests, and the indirect antiglobulin tests were negative. The agglutination had a "mixed-field" appearance, i.e., some of the red cells were agglutinated but the majority were not. The antibody was inactivated by 2-mercaptoethanol, suggesting that it was type IgM. The antibody was successfully adsorbed by and recovered in eluates from presensitized antigen-positive red cells. Neither $O_hP_1$ (H−) nor OP$_2$ (H+) red cells adsorbed the antibody, and the eluates from these cells gave negative results. Negative results were also obtained when the red cells had exceptionally strong $H$ but weaker than normal $P_1$ antigens, and when they had exceptionally strong $P_1$ but weaker than normal $H$ antigens. In the inhibition tests, the antibody was inhibited by hydatid cyst fluid but not by secretor or nonsecretor saliva or by guinea pig urine. This finding showed that the antibody was inhibited by $P$ but not by $H$, Lewis, or $Sd^a$ substances. Two of the antibody donors were group B and the remainder were group O. Group A donors were excluded because their sera frequently contained anti-$H$. The red cells of the anti-
body donors were type P₁ or P₂ and I⁺, I⁻ and had normal H antigen status in titrations with Ulex europaeus lectin (anti-H). All the autoantibody tests gave negative results; however, autoadsorption and elution studies were not done. No other atypical antibodies were detected in the donors' sera.

Seventeen further examples of the antibody have since been identified, mainly in black donors. The Blood Group Reference Laboratory in London, UK, where the first example was sent for study, suggested that the antibody was a type of anti-P₁.

Investigations with antigen-positive red cells

In titrations with human anti-H and Ulex europaeus lectin and with anti-P₁, the red cells agglutinated by the unknown antibody were found to react exceptionally strongly (see Table 1). In titrations with anti-I, the I antigen of the cells was not found to be enhanced. Negative results were obtained when the cells were tested with group AB serum and with Arachis hypogea and Glycine soja lectins.

Exclusion of other specificities

Although the inhibition results suggested that the unknown antibody belonged in the P system, the following alternative specificities were excluded: anti-C, -Cw, -enhanced D, -Di, -E, -Fy, -He, -Hil, -In, -Jk, -Jk, -Js, -K, -Kp, -Le, -Le, -Lu, -M, -McC, -M₁, -Mo, -Radin, -S, -sD, -Sk, -St, -VS, -Wb, and -Xg.

Family inheritance studies

Three family studies were made (see Figure 1). One family (Family 2) showed that the unknown antigen was inherited as a dominant character. Increased H antigen was evidently encoded by one but not both O genes in the propositi. As no family member was type P₂, the genetic background for the increased P₁ antigen could not be determined.

Antigen frequency studies

Table 2 shows the results of random frequency studies with the unknown antibody in group O black, Caucasian (white plus Indian), and Coloured (mixed race) blood donors of Natal and the Eastern Cape Province. The highest frequencies, 7 percent and 6.9 percent, occurred in Natal and Eastern Cape black donors, respectively. The corresponding antigen has been identified so far in a total of 192 persons (see Table 2).

Discussion

The Indian donor's antibody was named anti-P₁H because it apparently reacted exclusively with red cells that carried exceptionally strong P₁ and H antigens simultaneously. As with the anti-IH of Rosenfield et al., the letter H was not meant to suggest that the antibody was inhibited by H substance.

In 1968, Brain noted that the red cells of group O and A₁ Bantu (Zulu blacks) in Natal frequently had stronger H antigen than those of Natal whites of the same ABO group. The red cells of group B Bantu and whites, however, had similar quantities of H antigen. This author's studies with anti-P₁H have since shown that the occasional group B black has exceptionally strong H antigen. Moreover, blacks with little or no H antigen on their red cells occasionally have high titer anti-H in their sera.

According to Watkins, provided that the glycosyltransferases in the cells synthesizing glycolipids can compete freely, the pool of lactosyl ceramides in the cells is converted, either to ceramide trihexoside (PK) and hence to globoside (P) or, through the sequential

Table 1. Titration results: two P₁H+ red cell samples and a P₁H− control versus anti-P₁ and anti-H

<table>
<thead>
<tr>
<th>Red cells</th>
<th>ABO group</th>
<th>Reaction with anti-P₁H</th>
<th>Score</th>
<th>Dilutions of anti-P₁</th>
<th>Score</th>
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<td></td>
<td>1 2 4 8 16 32 64 128 256</td>
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<tr>
<td>P₁H+ No. 1</td>
<td>0</td>
<td>±</td>
<td>3</td>
<td>4+ 4+ 3+ 2+ 1+ w</td>
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<tr>
<td>P₁H+ No. 2</td>
<td>0</td>
<td>1+</td>
<td>5</td>
<td>4+ 4+ 3+ 3+ 1+ w</td>
<td>0 0 0</td>
</tr>
<tr>
<td>P₁H− control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1+ 1+ w</td>
<td>w 0 0 0</td>
</tr>
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<tr>
<th>Red cells</th>
<th>ABO group</th>
<th>Reaction with anti-P₁H</th>
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<td>1 2 4 8 16 32 64 128 256</td>
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<td>P₁H+ No. 1</td>
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<td>±</td>
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<td>4+ 4+ 4+ 4+ 4+ 3+ 2+ 1+ w</td>
<td>0 0 0</td>
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<tr>
<td>P₁H+ No. 2</td>
<td>0</td>
<td>1+</td>
<td>5</td>
<td>4+ 4+ 4+ 4+ 4+ 3+ 3+ 1+ w</td>
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</tr>
<tr>
<td>P₁H− control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4+ 4+ 4+ 2+ ±</td>
<td>0 0 0 0 0</td>
</tr>
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addition of N-acetylglucosamine and galactose, to paragloboside (lacto-N-neotetraosyl ceramide). The paragloboside is then converted to a Type 2 H structure and hence to A and B structures, to a P1 determinant by the addition of an alpha-galactosyl residue, or to sialoparagloboside by the addition of sialic acid. From the red cell membrane to the nonreducing sugars at the molecular terminus, the glycosphingolipid sequence is evidently P, then I, i, etc. and then H, A, and B.\(^1\) However, the molecule is not a rigid, straight structure, but is three-dimensional, and carbohydrate moieties situated some distance away from each other may therefore actually be very close together. The existence of anti-IP1, -iP1, -IA, -IH, and other compound antibodies to which anti-P1H has now been added confirms the steric arrangement suggested.

The P\(_1\) antigen is known to vary considerably in strength on the red cells of different people. It is also frequently expressed more strongly in blacks than in Caucasoids.\(^1\) Moharram\(^9\) suggested that the variations were inherited, but this suggestion has not been confirmed. P\(_1\)H antigen may be the result of competition between P\(_1\) and H gene transferases for the available paragloboside.

Alternatively, this author speculates whether P\(_1\)H antigen is in some way associated with the globoseries H antigen GL-6. The structure of GL-6 is Fuco(1-->2)Galβ(1-->3)GalαCer.\(^10\)

In attempting to understand how anti-P1H may be made by persons whose own red cells are P1+ and H+, this author suggests that P1H is neither P1 nor H but a separate antigen. The antigen is formed only when the red cells express both P1 and H exceptionally strongly; alternatively, it is formed when the membranes of the red cells carry exceptionally large numbers of P1 and H epitopes simultaneously. As a result, steric rearrangement takes place in the molecule, and P1H antigen appears. Naturally, the presence of strong P1 and strong H means that the red cells are also agglutinated strongly by both anti-P1 and anti-H. The people whose red cells bear normal-strength P1 and H antigens, those whose red cells bear either strong P1 or strong H but not both, those with red cells bearing either P1 or H but not both, and those with red cells bearing neither P1 nor H antigens may make anti-P1H in response to stimulation by P1H+ red cells.

**Table 2.** P1H antigen frequency studies

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total tested</th>
<th>Number P1H+</th>
<th>% P1H+</th>
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<tr>
<td>Natal blacks</td>
<td>1973</td>
<td>138</td>
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<tr>
<td>Eastern Cape blacks</td>
<td>290</td>
<td>20</td>
<td>6.9</td>
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<tr>
<td>Natal Coloureds</td>
<td>288</td>
<td>9</td>
<td>3.1</td>
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<tr>
<td>Eastern Cape Coloureds</td>
<td>240</td>
<td>8</td>
<td>3.3</td>
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<tr>
<td>Natal Caucasoids (whites &amp; Indians)</td>
<td>2198</td>
<td>17</td>
<td>1.7</td>
</tr>
</tbody>
</table>
Acknowledgments

This author thanks Mrs. Elizabeth Smart and Mr. Ravi Reddy, Natal Blood Transfusion Service, and Mrs. Ulla Vaaja, Eastern Province Blood Transfusion Service, for expert technical assistance.

References


Phyllis P. Moores, DSc, Head, Immunohematology Research Laboratory, Natal Institute of Immunology, P.O. Box 2356, Durban, 4000 Natal, South Africa.

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