Glycophorin A-deficient red cells may have a weak expression of C4-bound Ch and Rg antigens

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The blood group antigens Ch and Rg are polymorphisms of C4d. Antigen-positive red blood cells (RBCs) treated with proteases type as Ch-, Rg-. Although RBCs treated with sialidase may type Ch+ Rg+, they cannot be coated with C4 by the 10 percent sucrose method. Since studies of complement binding have shown that glycophorin A (GPA) is an important component for the uptake of C4 by RBCs, we tested all available GPA-deficient RBCs for their Ch and Rg status. Using eluates of human anti-Ch and anti-Rg, and monoclonal anti-Rg, we found that the Ch antigen was only weakly expressed on these RBCs, while Rg expression was variable. Our results imply that in the absence of GPA, C4 binds in vivo to a component or components other than GPA on RBCs. Immunochemistry 1996:12:4-7.

The blood group antigens Ch and Rg are common determinants carried by the fourth component of complement C4 on the C4d fragment. They are plasma/serum antigens that become attached to red blood cells (RBCs). C4 is very polymorphic; two isotypes, C4A and C4B, are encoded by closely linked loci on chromosome 6. Many electrophoretically determined isotypic variants have been reported. Generally, the Rg antigens are carried by the faster-moving acidic C4A band, which binds preferentially to protein; the Ch antigens are usually borne on C4B, an electrophoretically slower, basic band that binds more readily to carbohydrate. Use of C4 coatings and selected anti-Ch and anti-Rg reagents have defined seven Ch and two Rg antigens. Rare examples of Ch on C4A and Rg on C4B are documented.¹

Variation in Ch/Rg antigen strength between individuals has been documented: C4 is present on washed RBCs and the amount of bound C4 varies from person to person.² Further, Giles et al.³ showed that reaction strength with anti-Ch and anti-Rg depended on the number of C4 molecules bound, providing an explanation for the variation in Ch and Rg antigen expression on normal RBCs.

Ch and Rg expression on RBCs is sensitive to protease treatment but unaffected by sialidase treatment.¹⁴ RBCs can be coated with large quantities of C4 in vitro using a 10 percent sucrose solution.⁵ However, it has been shown that sialidase-treated RBCs do not appear to bind to C4 by this method.⁶⁷

Association of glycophorin A (GPA) with complement came from work by Parker and colleagues⁸-¹⁰ and Isenman and Young.¹¹,¹² Investigation of the red cell membrane components involved in the increased binding of C3b on paroxysmal nocturnal hemoglobinuria cells and in pathogenesis of chronic cold agglutinin disease demonstrated involvement of GPA in both of these events.⁸,⁹ Furthermore, Tomita et al.¹⁰ showed that GPA inhibited reactive lysis by preventing the formation or binding of the C5b-7 complex in the complement cascade. They concluded that GPA functions as an inhibitor of the membrane attack complex of complement. Isenman and Young studied the binding of C4 following sensitization of RBCs with various IgM and IgG antibodies; they found that C4 bound to several different surface molecules, including GPA.¹¹,¹² GPA is encoded by a gene GYPB on chromosome 4q28 in close proximity to a homologous gene GYPB, which encodes a related sialoglycoprotein component of the red cell membrane (reviewed in Reid).¹³ RBCs of individuals lacking GYP and/or GYPB lack GPA and/or GPB, and therefore have decreased levels of sialic acid.

These observations led us to study the expression of Ch and Rg antigens on RBCs with low levels of sialic acid. Since RBCs with rare phenotypes En(a−) and M⁴M⁵ lack GPA, the major sialic acid-bearing component of the RBC membrane,¹⁴ they have a reduced level of sialic acid. Therefore, we exploited the serologically detectable RBC antigens Ch and Rg, in order to test En(a−) and M⁴M⁵ RBCs for the presence of C4 acquired in vivo.

Materials and Methods

RBCs were obtained from samples referred to our laboratories or samples from the SCARF exchange pro-
gram. These samples had been collected into anticoagulant to prevent uptake of complement in vitro by the RBCs. The following GPA-deficient RBC samples were tested: En(a−)FIN: EP,15 GW,16,17 TN,18 and SD;19 En(+)-UK: MEP,20 M^kM^k: KM,21 NAS and sib.22 Whenever possible, age-matched controls were used in parallel; Wr(a+b−): MF cells23 were among these controls.

Antisera were obtained from serologic problems that had been investigated or were gifts from colleagues. Two problems arise from using sera: the presence of anti-A and/or anti-B, and the occurrence of HLA antibodies that sometimes react with RBCs.24 To avoid these problems, eluates were used in subsequent testing. Eluates containing anti-Ch (MLE, Chi) and anti-Rg (Pres, Rodg) were made from sensitized complement-coated RBCs using Elu-Kit II (Gamma Biologicals, Inc., Houston, TX). Monoclonal anti-Rg (RGd1) was the gift of Dr. C.M. Giles.

Red cell testing

Ch and Rg phenotypes were detected by using a capillary indirect antiglobulin technique.25 Briefly, RBCs were incubated with a predetermined dilution of anti-Ch or anti-Rg in a tube. The cells were then washed and an equal volume of a 50 percent suspension of sensitized RBCs was run into an anti-IgG reagent in a capillary tube. The tubes were inverted in clay and the reactions were recorded at timed intervals. Only anti-Ch and anti-Rg were used; no attempt was made to determine which of the seven Ch antigens or two Rg antigens were present. Monoclonal anti-Rg was used by a LISS-antiglobulin technique. Briefly, two drops of antibody were mixed with one drop of 2 percent saline-suspended RBCs and two drops of LISS and tested according to manufacturer's instructions (Gamma Biologicals, Inc.).

Hemagglutination inhibition tests

After incubating equal volumes of known Ch+ and Rg+ plasmas and a negative control plasma (all diluted 1 in 2), and anti-Ch or anti-Rg of an appropriate dilution at room temperature for 20 minutes, antigen-positive indicator RBCs were added, and the test was performed as described above.

Results

Red cell testing

Table 1 shows representative results of some initial work with GPA-deficient RBCs. Two M^kM^k samples were from sibs.22 M^kM^k–1 had weak expression of Ch antigens and appeared to lack Rg. His sib lacked both Ch and Rg antigens. On a later occasion, when a fresh sample from M^kM^k–1 was tested, the Ch reaction was much stronger, and weak Rg antigen was also detected. This finding showed that results of tests not done in parallel could not be compared. The En(a−) RBCs shown in Table 1 are from individuals who were not deficient in C4.15,18 The slight variation in strength observed with TN's cells showed that the avidity of the eluate used is also important. The two control RBC samples were from staff members that are used for all Ch and Rg testing because of known variation in antigen expression. Their strength of reaction with different eluates showed little variation over many years of testing, and G was always slightly stronger than T.

After this initial work, as many GPA-deficient samples as possible and age-matched controls were recovered from storage in liquid nitrogen for testing against eluates of anti-Ch and anti-Rg, and monoclonal anti-Rg. Results of these experiments are presented in Table 2. One control sample appeared to be Ch-negative. Another control was weak with anti-Ch (MLE) and with both anti-Rg eluates. Some reagents demonstrated weakness more efficiently than others. The Ch (MLE) eluate was more effective in demonstrating weakness with the En(a−) and M^kM^k samples than anti-Ch (Chi) (Table 2). The Wr(a+b−) RBCs were slightly weaker with the Ch (Chi) eluate than the other positive control RBCs (Table 2). From these results we conclude that RBCs lacking GPA usually have only weak expression of Ch antigen.

Table 1. Ch and Rg phenotyping using a capillary antigulobulin test

<table>
<thead>
<tr>
<th>Year tested</th>
<th>GPA-deficient red cells</th>
<th>Anti-Ch eluate (MLE)</th>
<th>Anti-Rg eluate (Pres)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10'</td>
<td>20'</td>
</tr>
<tr>
<td>1990</td>
<td>M^kM^k–1</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>1990</td>
<td>M^kM^k–2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1991</td>
<td>En(a−)-FIN EP</td>
<td>-</td>
<td>w</td>
</tr>
<tr>
<td>1990</td>
<td>En(a−) FIN TN</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1994</td>
<td>En(a−) FIN TN</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>G</td>
<td>3^+</td>
<td>4^+</td>
</tr>
<tr>
<td>Control</td>
<td>T</td>
<td>2^+</td>
<td>3^+</td>
</tr>
</tbody>
</table>

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Table 2. Comparison of Ch and Rg reactions of GPA-deficient red cells with age-matched GPA-normal Wr(a+) and control red cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Phenotype</th>
<th>Year frozen</th>
<th>Ch (ChA)</th>
<th>Ch (MLE)</th>
<th>Rg (Pesc)</th>
<th>Rg (Rodig)</th>
<th>Monoclonal anti-Rg (EgCell)</th>
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<tr>
<td>SD</td>
<td>En(a-)FIN</td>
<td>1993</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GW</td>
<td>En(a-)FIN</td>
<td>1976</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EP</td>
<td>En(a-)FIN</td>
<td>1985</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>MFB</td>
<td>En(a-)UK</td>
<td>1980</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>KM</td>
<td>MPMB</td>
<td>1992</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>+</td>
</tr>
<tr>
<td>MF</td>
<td>Wr(a+b-)</td>
<td>1983</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5 controls</td>
<td>1976</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1 control</td>
<td>1985</td>
<td>+</td>
<td>w</td>
<td>w</td>
<td>w</td>
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<td>+</td>
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<td>+</td>
</tr>
</tbody>
</table>

*Monoclonal anti-Rg by LISS antiglobulin technique (see Methods)

Weakness was detected in the En(a-)UK sample and slight weakness was detected in the En(a-)FIN (EP) sample; one control sample also expressed weak Rg antigen. The results for En(a-)FIN (EP) illustrate the importance of performing tests on comparable samples in parallel; previous tests had failed to detect Rg antigen on EP's RBCs (Table 1). Variation in condition of RBCs and eluates of different avidity are probable explanations for the differences observed. Titration using Rg eluates were not done because there were insufficient cells for such tests. The monoclonal anti-Rg, which was used by a different technique, gave a slightly different pattern of reactions. Using this reagent, MPM and En(a-)UK RBCs were very weakly positive, and a slight weakening was noticed with the Wr(a+b-) RBCs. But the En(a-)FIN (SD) RBCs appeared to react as strongly as control samples (Table 2).

Ch and Rg antigens in plasma

The plasma from TN, an untransfused En(a-) male donor who has no immune antibodies, inhibited both anti-Ch and anti-Rg. These results showed that his plasma contained the C4-borne antigens Ch and Rg. Thus, an absence or marked decrease in C4 was not the reason for the reduced expression of Ch and Rg antigens. Plasma from other GPA-deficient individuals could not be tested because all had antibodies to high-incidence RBC antigens.

Discussion

GPA-deficient samples are very rare, but most reported examples available were tested. The results showed that Ch and Rg antigens are usually more weakly expressed on GPA-deficient RBCs than on RBCs of normal phenotype. These findings are in agreement with observations of earlier investigators, who found that complement bound to GPA. They also correlate with the findings of Hsu et al. that the amount of IgG bound, and hence the amount of complement bound, was dependent on the surface charge of the RBCs.

Various mechanisms have been postulated to explain the presence of Ch and Rg antigens on RBCs of normal donors. C4 is bound covalently to the surface molecule target. Several laboratories have reported that the isotype C4A carrying Rg antigens showed a preference for amide bond formation, and the C4B isotype carrying Ch antigens showed a preference for ester bond formation. Outcomes could suggest that the more basic C4B bands showed greater dependence on the amount of sialic acid on the RBCs than the more acidic C4A bands, since Ch antigens were more weakly expressed than Rg antigens on GPA-deficient RBCs.

Isenman and Young had shown previously that C4 bound in vitro to several cell surface molecules, including glycoporphin A, and that the two isotypes bound to different components. Since some GPA-deficient RBCs are Ch+Rg+, our results show that C4 binds in vivo to a component or components other than GPA on RBCs. If sialic acid were important for complement binding, as suggested by Hsu et al., there are, of course, several other minor glycoproteins that carry sialic acid, e.g., GPB, GPC, GPD, Lu-glycoproteins, and Xg-protein. Perhaps only a small number of sialic acid sites are required for in vivo binding in normal complement turnover. The function of GPA has not yet been identified. Although lack of GPA retards invasion of the malarial parasite Plasmodium falciparum, this parasite has not apparently conferred any selective advantage, since GPA-deficient phenotypes are rare in all populations tested. Perhaps GPA plays a role in complement binding and assists the immune system in protecting RBCs from complement-mediated lysis. This function cannot be exclusive, since lack of GPA is not deleterious and other
sialoglycoproteins may perform this role in the absence of GPA.

Acknowledgments

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References


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