Serological and immunochemical characteristics of Ge-negative red cells and anti-Ge

M. E. Reid

Abstract: Gerbich-negative red cells lack β and γ sialoglycoproteins (SGPs), which are now known to carry the Gerbich (Ge) antigens. Gerbich and Yus-type Ge-negative red cells possess distinct diffuse SGPs that migrate on sodium dodecyl sulphate polyacrylamide gel electrophoresis in a position between those normally occupied by β and γ SGPs. Both these SGPs lack Ge2 antigens and possess epitopes recognized by monoclonal anti-β. These SGPs differ from each other in at least two ways. The SGP associated with the Gerbich type also lacks the Ge3 antigen and is resistant to trypsin treatment. The SGP associated with the Yus type possesses the antigenic determinant recognized by anti-Ge3 and is sensitive to trypsin digestion.

Gerbich (Ge)-positive red cells and Ge-negative red cells of the Gerbich, Yus, and Leach types have distinct immunochemical profiles. Ge-negative red cells lack β and γ SGPs, which carry the antigenic determinants recognized by anti-Ge. This paper summarizes a compilation of serological and immunochemical data, and the interested reader is encouraged to read the referenced articles for more information and experimental details.

Red Cells

Ge-positive

Ge-positive red cells carry four distinct SGPs (Fig. 1). These are, listed in descending order of their monomeric relative molecular weights: α SGP (synonyms: glycophorin A, PAS 1, PAS 2, MN glycoprotein); β SGP (synonyms: glycophorin C, component E, PAS 2', glycoconnectin); γ SGP (synonyms: glycophorin C, glycophorin D, component E); and δ SGP (synonyms: glycophorin B, PAS 3, Ss glycoprotein).

All four SGPs carry blood group antigenic determinants. The M, N, and Ena antigens are located on α SGP molecules, and ‘N’, S, s, and U antigens are located on δ SGP molecules.1 Gerbich antigens are located on β and γ SGPs. Thus, Ge-positive red cells possess the antigenic determinants that are recognized by anti-Ge2, anti-Ge3, and anti-β.

Ge-negative

Of the three types of Gerbich-negative red cells described in this paper, the Gerbich type (Ge:-2,-3) is the most common. It was identified nearly 30 years ago by Rosenfield et al.2 The Yus type (Ge:-2,3) is the next most common and was identified a year after the Gerbich type by Barnes and Lewis.3 The Leach type (also Ge:-2,3) is the least common and was not detected until 1984, when a Ge-negative red cell sample was tested against an apparent panagglutinating monoclonal antibody.4 This red cell sample, later identified as the Ge:-2,3 Leach type, was not agglutinated by this antibody. Since then, five other Leach type individuals have been reported.4-6 A fourth type of Ge-negative was identified by Booth et al.;7 however, because red cells and serum from this Melanesian type (Ge:-1,2,3) are unavailable and have not been tested immunochemically, this type and its corresponding antibody, anti-Ge 1, will not be described in this report.

The sialoglycoprotein patterns of the three types of Ge-negative red cell membranes differ from Ge-positive red cell membranes and from each other (Fig. 1).4,5,8-11 Red cells from individuals with the Leach-type of Ge-negative lack β and γ SGP molecules and possess a normal complement of α and δ SGPs. Therefore, red cells with the Leach phenotype lack all Gerbich antigens but possess the antigenic determinants that are carried on α and δ SGPs. Red cells from individuals with the Gerbich and Yus types also lack β and γ SGPs and have a normal complement of α and δ SGPs. However, these Ge-negative red cells differ from the Leach type in that each
possesses a distinctive abnormal SGP that migrates between the positions normally occupied by $\beta$ and $\gamma$ SGPs. The abnormal SGP associated with the Gerbich type migrates on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with a relative molecular weight of 30,500–34,500 and will be referred to as $\beta^{\text{Ge}}$ in this communication. The abnormal SGP associated with the Yus type migrates on SDS-PAGE with a relative molecular weight of 32,500–36,500 and will be referred to as $\beta^{\text{Yus}}$ (Table 1).

Both $\beta^{\text{Ge}}$ SGP and $\beta^{\text{Yus}}$ SGP migrate in a much wider band on SDS-PAGE than do $\alpha$, $\beta$, $\gamma$, or $\delta$ SGPs. The reason for this is thought to be that $\beta^{\text{Ge}}$ and $\beta^{\text{Yus}}$ SGPs may be heterogeneously glycosylated. Unlike normal $\beta$ SGP, the $N$-glycosidically-linked oligosaccharide structure on both $\beta^{\text{Ge}}$ SGP and $\beta^{\text{Yus}}$ SGP contains repeating lactosaminyl units (Gal $\beta$ 1-3 GlcNAc).

It is heterogeneity in the number of these lactosaminyl units that gives rise to populations of SGP molecules with different relative molecular weights. This, in turn, results in the broad diffuse bands on SDS-PAGE.\(^\text{11}\)

Gerbich type Ge-negative red cells lack Ge2 and Ge3 antigenic determinants but possess the epitopes recognized by monoclonal anti-$\beta$ reagents. Yus-type Ge-negative red cells lack Ge2 antigens but possess Ge3 antigens and the epitopes recognized by the monoclonal anti-$\beta$ reagents.

The absence of $\beta$ and $\gamma$ SGPs from Ge-negative red cell membranes and the genetic relationship of the abnormal SGPs to $\beta$ SGP have been confirmed by recent molecular biological studies.\(^\text{12,13}\) These studies have shown that $\beta^{\text{Ge}}$ and $\beta^{\text{Yus}}$ SGPs are products of altered forms of the $\beta$ SGP gene, located on chromosome 2. The products of normal and altered $\beta$ SGP genes are inherited in an autosomal codominant manner.\(^\text{12-15}\)

### Antibodies

**Alloanti-Ge**

Anti-Ge can be produced in response to transfusion or pregnancy, or it can occur without known stimulus. Anti-Ge is most commonly of the IgG1 subclass.\(^\text{16}\)

Anti-Ge has not caused clinically severe hemolytic disease of the newborn, even though the Gerbich antigens are expressed on cord red cells and anti-Ge crosses the placental barrier. Some examples of anti-Ge have caused transfusion reactions.\(^\text{17,18}\)

Excluding anti-Gel, anti-Ge antibodies have one of three serological specificities: anti-Ge2, anti-Ge3, and anti-$\beta$. Anti-Ge2 agglutinates Ge-positive but not Ge-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>$\beta$, $\gamma$, and $\beta$-related sialoglycoprotein content of Ge-positive and Ge-negative red cells</th>
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</thead>
<tbody>
<tr>
<td>Red cells</td>
<td>Sialoglycoproteins present</td>
</tr>
<tr>
<td>Ge-positive</td>
<td>$\beta$</td>
</tr>
<tr>
<td></td>
<td>$\gamma$</td>
</tr>
<tr>
<td>Ge-negative</td>
<td>None</td>
</tr>
<tr>
<td>Leach type</td>
<td>$\beta^{\text{Ge}}$</td>
</tr>
<tr>
<td>Gerbich type</td>
<td>$\beta^{\text{Yus}}$</td>
</tr>
<tr>
<td>Yus type</td>
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</tbody>
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**Fig. 1.** Schematic representation of PAS-stained gels after SDS-PAGE of membranes prepared from Ge-positive red cells and three types of Ge-negative red cells.

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negative red cells of any type. Anti-Ge3 agglutinates Ge-positive and Yus-type red cells but not Gerbich or Leach-type red cells. Anti-β agglutinates all red cells except those from Leach-type individuals (Table 2). Ge antibodies do not agglutinate Ge-positive red cells that have been treated with papain, ficin, or pronase, indicating that these antigens are located on protease-sensitive red cell membrane components.

Anti-Ge2 can have one of three immunochemical specificities: anti-γ, anti-β, and anti-γ + β (Table 3). Anti-γ is by far the most common specificity, and its antigenic determinant is on the extracellular trypsin-sensitive domain of γ SGP between amino acid residues 1 and 20 (Fig. 2). Anti-Ge2 does not react with sialidase-treated red cells, indicating that sialic acid is part of its antigenic determinant.

Anti-Ge3 reacts with an antigenic determinant that is present on β, γ, and βYus SGPs (Table 3). This antigenic determinant is located on the extracellular trypsin-sensitive domains of these SGPs. This antigen is probably located in the proximity of the trypsin cleavage site, which, on normal β SGP, is located between amino acid residues 47 and 48. Anti-Ge3 is thought to recognize an antigenic determinant on β SGP that includes amino acid residues 41 to 49 and the O-glycosidically linked tetrasaccharide attached to serine at residue 42 (Fig. 2). Anti-Ge3 usually reacts with sialidase-treated red cells, although the reactivity may be weaker than with untreated red cells. This indicates that sialic acid is not a major structural part of its antigenic determinant. Anti-β does not react with β, βYus, and βGe SGPs but does react with βGe SGP. Anti-β does not react with sialidase-treated red cells, indicating that sialic acid is a structural part of its antigenic determinant. Some monoclonal anti-β are directed against an epitope which includes amino acid residues 1 through 4 on β, βYus, and βGe SGPs (Fig. 2).

**Autoanti-Ge**

Autoanti-Ge from two patients with autoimmune hemolytic anemia and one patient with aplastic anemia have been studied. One autoanti-Ge was an anti-Ge2, which reacted only with normal β SGP. Another, anti-Ge3, reacted with β, γ, and βYus SGPs. The other had a serologic and immunochemical specificity of anti-Ge

### Table 2
Serological specificity of anti-Ge

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Reactive red cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Ge2</td>
<td>Ge-positive</td>
</tr>
<tr>
<td>Anti-Ge3</td>
<td>Ge-negative — Yus type</td>
</tr>
<tr>
<td>Anti-β</td>
<td>Ge-positive</td>
</tr>
<tr>
<td></td>
<td>Ge-negative — Yus type</td>
</tr>
<tr>
<td></td>
<td>Ge-negative — Gerbich type</td>
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<table>
<thead>
<tr>
<th>Ge-positive red cells:</th>
<th>anti-β</th>
<th>anti-Ge2</th>
<th>anti-Ge3</th>
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<tbody>
<tr>
<td>β SGP</td>
<td>41</td>
<td>49</td>
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</tr>
<tr>
<td>γ SGP</td>
<td>1</td>
<td>20</td>
<td>28</td>
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</table>

<table>
<thead>
<tr>
<th>Ge-negative red cells:</th>
<th>anti-β</th>
<th>anti-Ge3</th>
</tr>
</thead>
<tbody>
<tr>
<td>βYus SGP</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>βGe SGP</td>
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</tr>
</tbody>
</table>

Arrow indicates the trypsin cleavage sites

**Fig. 2.** Probable SGP regions recognized by various Anti-Ge.
specificity that was indistinguishable from monoclonal anti-β and reacted with β, β^{Ge}, and β^{Yus} SGPs.

Discussion

The existence of distinct high-incidence Gerbich antigens on human red cells has been known for many years. Recently, immunochemical studies have identified those red cell membrane components that possess these antigens, and the structural relationships between the common and variant membrane components. More recently, molecular biological studies have identified, at the c-DNA level, the origin of these variant forms. Investigation of genetic variants, identified by serological techniques, helps to determine how these variant and normal genes function.

References

16. Vengelen-Tyler V, Morel PA. Serologic and IgG subclass characterization of Cartwright (Yt) and Gerbich (Ge) antibodies. Transfusion 1983;23:114–6.