CONCISE REPORT

Immunochemical Studies on Tn Erythrocyte Glycoprotein

By L. T. Lee, S. Frank, D. S. de Jongh, and C. Howe

Glycoproteins were extracted from membranes of erythrocytes that displayed Tn polyagglutination and were compared chemically and immunologically with glycoproteins of group O, MN cells. Tn glycoprotein had lower than normal NANA: protein and sugar: protein ratios, as revealed by direct analysis and polyacrylamide gel electrophoresis, and displayed slower immunoelectrophoretic mobility than glycoprotein from group O, MN cells. Agglutination of Tn cells by Salvia sclarea lectin was inhibited by Tn glycoprotein but not by O, MN glycoprotein. Tn and MN glycoproteins were equally potent inhibitors of influenza virus HA. Our findings indicate that Tn-specific determinants are part of the glycoprotein molecule.

ON THE SURFACE of normal human erythrocytes, the MN blood group antigenic determinants are borne on the principal integral transmembranal protein, glycophorin.\(^1,2\) Desialylation of erythrocytes eliminates the MN specificity and exposes the T antigen (Thomsen-Friedenreich), the antigenic determinant of which is a beta-linked galactosyl residue.\(^3\) Subsequent treatment with beta-galactosidase exposes still other determinants (Tn) that specifically bind the lectin, Salvia sclarea.\(^4\) Erythrocytes with Tn specificity are unusual, but may come to light when the cells of a particular individual are found to be agglutinated by most randomly selected adult human sera. Such erythrocytes therefore may present difficulties in ABO blood group typing or crossmatching.\(^5\) We encountered a donor (L.M.) in 1974 whose erythrocytes were reportedly of blood group A, but were found to be polyagglutinable and reactive with S. sclarea lectin.\(^*\) This signified that they had acquired Tn specificity. We have isolated glycoproteins in soluble form from this donor’s erythrocytes, and have compared these glycoproteins immunologically and chemically with glycoproteins from normal erythrocytes of group O, MN, which are unreactive with S. sclarea lectin.

MATERIALS AND METHODS

Single donor units of whole blood (group O, MN), which had been deemed unacceptable for transfusion, were generously supplied by the Blood Center for Southeast Louisiana. A sample of 50 ml of whole blood (group A) in EDTA was obtained from donor L.M. Hemoglobin-free membranes were prepared from washed erythrocytes according to the method of Dodge et al.\(^6\) as modified by Howe and Lee.\(^7\) Glycoprotein was extracted from the membranes with chloroform: methanol (2:1) according to the methods of Hamaguchi and Cleve\(^8\) and Lee et al.\(^9\) The glycoproteins extracted from Tn and MN erythrocytes are designated, respectively, Tn gp and MN gp. The nomenclature of Fairbanks et al.\(^10\) is used in referring to major and minor glycoprotein bands in polyacrylamide gel electrophoresis. Antiserum to the major erythrocyte glycoprotein (PAS-1) was prepared in rabbits as previously described.\(^8\) Saline extracts were made from S. sclarea seeds obtained commercially (G.W. Park Seed Co., Greenville, S.C.) and from Glycine soja (soybeans) obtained locally. S. sclarea seeds (2 g) were soaked in 40 ml 0.15M NaCl for 10–12 hr at room temperature and blended for 1 min at high speed in a Sunbeam bar blender. The material was allowed to stand for 12–14 hr at 4°C. Heavy sediment was removed by centrifugation at 900 g in an International Centrifuge for 15 min at 26°C, followed by further clarification at 110,000 g for 1 hr at 4°C in a Beckman L-350 centrifuge. The supernatant was dialyzed against deionized water for 12–14 hr at 4°C and lyophilized. The yield of soluble material was approximately 35 mg/g S. sclarea seeds. An antiserum to the soluble extract of S. sclarea was made in rabbits. Saline extracts of S. sclarea, S. horminum, and Glycine soja were also prepared according to the method of Bird.\(^11\) These lectins define, respectively, specificities of polyagglutinable cells as (Tn), (Tn, Cad) and (T, Tn, and Cad).\(^12\) Agglutination and inhibition of agglutination assays were carried out using phosphate-buffered saline (PBS), pH 7.2, as diluent and erythrocytes (MN or Tn) in 1% suspension in PBS. Reducing sugar was performed according to the procedure of Lewis et al.\(^13\) Hexosamine was done by the method of Elson and Morgan,\(^14\) after hydrolysis in 2N HCl, 2 hr at 100°C and neutralization. N-acetylneuraminic acid (NANA) was determined according to the method of Warren\(^15\) following hydrolysis in 0.1N HSO₄, 30 min at 80°C. Protein was measured by the method of Lowry et al.\(^16\) Polyacrylamide gel electrophoresis (PAGE) was performed according to the method of Maizel\(^17\) in 0.1% SDS in 0.05M Tris/glycine buffer, pH 8.6, in which samples were preheated for 5 min at 100°C. Gels were stained with Coomassie brilliant blue (CBB) or periodic acid Schiff (PAS) according to the method previously described.\(^8\) Immunoelectrophoretic analysis\(^18\) and virus hemagglutination inhibition\(^19\) were done as previously reported.

RESULTS

In order to determine what proportion of donor L.M.’s erythrocytes possessed Tn specificity, cells were mixed with varying volumes and dilutions of...
In ERYTHROCYTE GLYCOPROTEIN 1229

Table 1. Composition and Activity of Erythrocyte Glycoproteins

<table>
<thead>
<tr>
<th>Glycoprotein Preparation</th>
<th>Percent Protein*</th>
<th>Percent NANAt</th>
<th>Percent Reducing Sugar†</th>
<th>Percent Hexosaminet</th>
<th>μg Inhibiting 5 HAU Influenza A Virus (FM 1)</th>
<th>μg Inhibiting 4+ Tn Cells With 20 μg S. sc/area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn</td>
<td>80</td>
<td>4.6</td>
<td>10</td>
<td>8</td>
<td>0.5</td>
<td>50</td>
</tr>
<tr>
<td>MN</td>
<td>60</td>
<td>12.7</td>
<td>15</td>
<td>10</td>
<td>0.2</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

*Lowry method.18 BSA as standard.
†After hydrolysis in 0.1 N H₂SO₄ for 30 min at 80°C.19 Crystalline NANA as standard.
‡After hydrolysis in 2 N HCl for 2 hr at 100°C and neutralization.13
§μg protein.
∥Hemagglutinating units.

In an attempt to amplify immunologically the agglutination of Tn erythrocytes by S. sclarea lectin, rabbit antiserum to the lectin was absorbed with A₁B erythrocytes and used in the manner of antiglobulin serum in the indirect Coombs test. Replicate serial dilution agglutination titrations of Tn cells with S. sclarea lectin were set up. The direct titer was consis-

Glycine soja, S. sclarea, and S. horminum lectins and kept at 26°C long enough for agglutinated cells to sediment. Unsedimented cells were then drawn off and retested with each lectin.20 Repeated attempts to recover inagglutinable (non-Tn) erythrocytes failed, regardless of the proportions, dilutions, and specificities of the lectins used. These results indicate that over 90% of donor L.M.’s erythrocytes had Tn specificity. No further attempts were made to separate Tn from non-Tn cells prior to preparation of hemoglobin-free membranes. L.M. (Tn) erythrocytes gave 4+ agglutination with a minimum of 23 μg S. sclarea protein, as well as with lectins from S. horminum and Glycine soja.

Results of chemical analyses and inhibition assays on chloroform-methanol-extracted glycoproteins of Tn and MN erythrocytes are shown in Table 1. The protein content of Tn gp was 20% higher than that of MN gp, while reducing and amino sugar values were lower in Tn than in MN gp. NANA in Tn gp was less than half the normal value. Tn gp was more than twice as potent as MN gp in inhibiting agglutination of Tn cells with S. sclarea lectin. Immunoelectrophoretic analysis with monospecific antiserum to the major erythrocyte glycoprotein9 revealed that Tn gp was distinctly slower in mobility than MN gp (Fig. 1). In SDS-PAGE, stained with CBB, Tn gp and MN gp gave essentially similar patterns, except for the heavier protein band in Tn gp in the region above PAS-2 (Fig. 2A). Gels stained with PAS reaction, however, showed striking differences between MN and Tn gp. In the latter, PAS-1 and 2 were greatly reduced and PAS-3 and 4 were not detectable, as compared with the normal (MN) pattern (Fig. 2B).

Tn cells were agglutinable by several strains of influenza virus, type A (FM1, PR301) and one strain of type B (Lee) virus and gave titers comparable to those with group O (MN) erythrocytes. Tn and MN cells were equally well agglutinated by parainfluenza type 1 (Sendai) virus, but neither was agglutinated by measles virus (results not shown). Tn gp was slightly less potent than MN gp in inhibiting hemagglutination by group A influenza virus (Table 1).
negative results leave unidentified the agglutinating factor in *S. sclarea* extract, which comprised several other distinct protein components demonstrable on PAGE (results not shown), none of which were reactive with Tn cells by any of the methods used.

**DISCUSSION**

We have attempted to determine the immunologic specificity of the Tn gp antigen by examining glycoproteins extracted by the chloroform-methanol method from Tn erythrocytes and comparing the soluble products with glycoproteins similarly prepared from normal (MN) cells. The most striking difference between Tn gp and MN gp was in the content of NANA. The lower NANA in Tn gp accounts for the faint PAS staining in SDS-PAGE and the slower immunoelectrophoretic mobility of this antigen compared with MN gp. These results were consistent with previously reported reduction in immunoelectrophoretic mobility of erythrocyte glycoprotein caused by desialylation with influenza viral neuraminidase. Reduced electrophoretic mobility has also been demonstrated in subjects with Tn cells,22 as well as in cases showing acquired abnormalities of erythrocytes characterized by deficiency in NANA. The double arcs seen in the immunoelectrophoretic pattern of Tn gp were previously seen in analyses of normal erythrocyte glycoprotein, and are attributed to random aggregation of antigenically identical macromolecules.

Hemagglutination by myxoviruses and paramyxoviruses is dependent on the presence of NANA in terminal glycosidic linkage on the major membrane glycoprotein (glycophorin). Tn erythrocytes were as readily agglutinated by these viruses as were MN cells containing normal amounts of NANA. Evidently the markedly reduced NANA content of Tn cells in itself did not affect reactivity with viral HA, either directly or in competitive inhibition of hemagglutination. Polyagglutinability occasioned by the uncovering of T or Tn antigens appears not to be related simply to reduced surface NANA, but rather to more subtle structural aberrations caused by enzymatic treatment.25 The specific reactivity of Tn erythrocytes with *S. sclarea* lectin, however, appears to be at least partially dependent on reduced NANA content, because agglutination of Tn cells by the lectin was inhibited by Tn gp but not by MN gp. The Tn-specific determinants are therefore part of the glycophorin molecule. The component in *S. sclarea* extracts that is specifically reactive with Tn antigen(s) remains unidentified.
REFERENCES

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