Identification of an Anti-A and Anti-B Blood Group Glycosyltransferase Antibody After Incompatible Bone Marrow Transplant

By Marina Mojena and Lisardo Boschá

The occurrence of a potent antibody against plasmatic A and B glycosyltransferase activities has been characterized in a patient (blood group A₁) transplanted with a bone marrow from a blood group O donor. A and B glycosyltransferases were purified to near homogeneity from plasma of A₁ and B blood-group individuals. The half-maximal inhibition of both enzymes was obtained at 1 to 2 μg/mL of the post-transplant IgG fraction, prepared by protein A-sepharose chromatography. A and B glycosyltransferases were also recognized by the post-transplant IgG fraction after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by electrophoretic transfer to nitrocellulose membranes.

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MATERIALS AND METHODS

Overview of the experimental approach and reagents used.

**Materials**: UDP-N-acetyl-D-galactosamine, UDP-D-galactose, and 2'-fucosyllactose were from Sigma (St Louis). Electrophoresis, blotting materials, and other ion-exchange resins were from Bio-Rad laboratories (Richmond, CA). Polyconal anti-A and anti-B sera were purchased from Ortho Diagnostics (Raritan, NJ). UDP-[14C]galactose (155 Ci/mol) was from Amershams International (Amersham, UK). Other chemicals and biochemicals were obtained from Merck (Darmstadt, FRG) and Boehringer (Mannheim, FRG).

Control sera were aliquoted and stored at −40°C. Serum from a patient (MF, blood group A₁) transplanted 26 months previously with a bone marrow from a group O donor was stored in small aliquots at −40°C. The patient suffered from chronic myeloid leukemia and in the postoperative course received doses of 3 mg/kg body weight of cyclosporine A. This serum was the same used in a previous work.

**Purification of A and B glycosyltransferases**: Fresh group A₁ or B serum (450 mL) was filtered through glass-wool and centrifuged at 10,000 g for 15 minutes at 4°C. A₁ glycosyltransferase activity was purified essentially as described by Nagai et al. by sepharose 4B chromatography followed by an affinity chromatography on UDP-hexanolamine-agarose column (0.5 × 3 cm) and eluted with 20 μM UDP-N-acetylgalactosamine. The activity was purified about 160,000-fold. Fractions containing activity were pooled, concentrated, and desalted by means of an Ultracart device (Sartorius, Göttingen, FRG) provided with a cellulose triacetate membrane with a cut-off of 30,000 daltons, and stored at −20°C in the presence of 20% glycerol (vol:vol). A glycosyltransferase was also partially purified from pooled A group sera by DE52 chromatography. In this case two peaks with enzymatic activity were observed after elution of the column by a NaCl gradient (from 50 to 300 mmol/L).

**B glycosyltransferase activity** was purified as described by Nagai et al. by DE52 and hydroxylapatite chromatography. The procedure used was exactly the same except for the elution of the hydroxylapatite column, which was modified as follows: after load-
GLYCOSYLTRANSFERASE ANTIBODY

Fig 1. Purification of A and B glycosyltransferase from human serum. Blood group A, serum (450 ml) was centrifuged at 10,000 g for 15 minutes and applied to a sepharose 4B column. After washing (buffer A: 150 mmol/L NaCl, 0.1 mmol/L EDTA, and 20 mmol/L HEPES, pH 7.2) the activity was eluted with 20 μmol/L UDP (A) followed by dialysis and ultrafiltration through a membrane with a cut-off of 30,000 daltons and the volume reduced to 2 mL. This fraction was applied to a UDP-hexanolamine agarose column and was eluted with 20 μmol/L UDP-N-acetylgalactosamine (B). For the purification of B-glycosyltransferase the serum was diluted 1:3 and applied to a DE52 column. After washing with buffer A diluted 1:3, a gradient of NaCl was applied to elute the enzyme (C). Fractions containing activity were applied to a hydroxylapatite column. After washing with 0.4 mol/L NaCl, the enzyme was eluted with a potassium phosphate gradient (D). For both enzymes, fractions containing activity were ultrafiltrated up to 2 mL and stored at −20°C in the presence of 20% glycerol (vol/vol). Arrows indicate the steps at which the addition of the ligand was produced.

Table 1. Purification of A- and B-Glycosyltransferase Activity From Human Serum

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Step</th>
<th>Specific Activity (U/mg of Protein)</th>
<th>Purification (X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (pooled)</td>
<td>Serum</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sepharose 4B</td>
<td>7,500</td>
<td>75,000</td>
</tr>
<tr>
<td></td>
<td>UDP-hexanolamine agarose</td>
<td>16,000</td>
<td>160,000</td>
</tr>
<tr>
<td></td>
<td>DE52 (100 mmol/L NaCl)</td>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>DE52 (200 mmol/L NaCl)</td>
<td>21</td>
<td>210</td>
</tr>
<tr>
<td>B</td>
<td>Serum</td>
<td>0.2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>DE52</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>Hydroxylapatite</td>
<td>16,800</td>
<td>84,000</td>
</tr>
</tbody>
</table>

The enzyme activity was measured by the agglutination assay. Serum was centrifuged at 10,000 g for 15 minutes prior to use. All purification steps were carried out at 4°C.
elution under mild-acid conditions with 150 mmol/L NaCl, 50 mmol/L acetate, pH 3.5. The eluate was immediately neutralized with 0.1 mol/L HEPES, pH 8.0. This IgG fraction was given to the serum of the patient transplanted with an ABO-incompatible bone marrow, A,- and B-glycosyltransferase activities were purified from human plasma to near homogeneity following Coomassie brilliant blue staining criteria. The purifications were carried out by ion-exchange chromatography and an improved affinity chromatography on a UDP-hexanolamine agarose column. These purifications were essential to characterize the presumed antibody produced by the patient and to rule out the possibility of interfering activities in the enzyme assay (mainly hydrolases and phosphatases). Figure 1 shows the purification profile of both activities through the different steps, and Table 1 summarizes the yield in these enzyme activities. It is interesting to note that if pooled blood-group A is used as a source of the enzyme, two peaks containing activity emerge after DE52 chromatography due to the presence of various isoenzymatic forms of this activity in human populations. For this reason blood-group A, serum was used to purify the enzyme.

To characterize the inhibitory activity present in the post-transplant serum of MF, purified A,- and B-glycosyl-

**RESULTS AND DISCUSSION**

To establish clearly the nature of the inhibitor present in the serum of the patient transplanted with an ABO-incompatible bone marrow, A,- and B-glycosyltransferase activities were purified from human plasma to near homogeneity following Coomassie brilliant blue staining criteria. The purifications were carried out by ion-exchange chromatography and an improved affinity chromatography on a UDP-hexanolamine agarose column. These purifications were essential to characterize the presumed antibody produced by the patient and to rule out the possibility of interfering activities in the enzyme assay (mainly hydrolases and phosphatases). Figure 1 shows the purification profile of both activities through the different steps, and Table 1 summarizes the yield in these enzyme activities. It is interesting to note that if pooled blood-group A is used as a source of the enzyme, two peaks containing activity emerge after DE52 chromatography due to the presence of various isoenzymatic forms of this activity in human populations. For this reason blood-group A, serum was used to purify the enzyme.

To characterize the inhibitory activity present in the post-transplant serum of MF, purified A,- and B-glycosyl-
glycosyltransferases were submitted to SDS-PAGE followed by overnight transfer at 100 mA to a nitrocellulose sheet. Strips of the transferred nitrocellulose membrane were incubated with purified IgG from either A, B, or O blood groups or post-transplant IgG from MF (dilution 1:500). After appropriate washing, the nitrocellulose strips were incubated with 1:3,000 rabbit antihuman IgG peroxidase conjugate. After becoming visible the recognized antigens through the peroxidase activity, a single band was evident in strips incubated in the presence of MF IgG fraction, regardless of the type of glycosyltransferase present (Fig 2). The mol wt of these bands were 50,000 and 44,000 daltons, which are in the range of the reported subunit mol wt of these enzymes.10,11 No staining was evident in strips incubated with IgG from A, B, or O control sera.

The titration of the antglycosyltransferase antibody from MF serum was done by using purified A and B glycosyltransferase. The results are shown in Fig 3. A progressive inhibition of the enzyme activity was observed either through the transformation of O red cells into A or B blood groups or through the incorporation of [14C]galactose into 2'-fucosyllectose for the B-glycosyltransferase activity. The half-maximal inhibition of the purified A and B glycosyltransferase (agglutination assay) was obtained at 1 to 2 μg/mL of IgG for both enzymes and at 1.7 μg/mL of IgG for the B glycosyltransferase assayed by the incorporation test. These results are in the line of previous work on the presence of glycosyltransferase inhibitors in minor incompatible liver transplantation and in minor incompatible BMT and with the reported immunologic homology of the human blood group glycosyltransferases.14 The use of two different methods for the assay of the glycosyltransferase activity as well as the use of purified enzymes guarantee the interaction between the enzymes and the antibody.

From our results on the purification of the glycosyltransferases as well as those from previous work,10,11 the concentration of enzyme in plasma may be estimated to be lower than 100 ng/mL of plasma, although continuously present in this fluid. These circumstances may be considered to explain the rise of the antibody after ABO minor incompatibility BMT.

The use of this antibody against A and B glycosyltransferases as a clinical marker of the evolution of the engrafted bone marrow (or solid organ) in cases of ABO nonisogroup identity between donor and receptor is a subject that requires additional work to establish its value.

ACKNOWLEDGMENT

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REFERENCES

2. Samuelsson BE: Enzymatic synthesis of a blood group A
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