Glycosphingolipid expression in spontaneously aborted fetuses and placenta from blood group p women. Evidence for placenta being the primary target for anti-Tjα-antibodies

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A 12-week-old fetus and one 17-week-old fetus + placenta were obtained after spontaneous abortions from two women of blood group p. The 17-week-old fetus was dissected into intestine, liver, brain and residual tissue. Nonacid glycosphingolipid fractions were prepared from the tissues. Glycolipid characterization was carried out using thin layer chromatography immunostained with monoclonal antibodies and bacteria and by 1H NMR spectroscopy and mass spectrometry. In the placental fraction substantial amounts of globotetraosylceramide (P-antigen) and globotriaosylceramide (Pk-antigen) were identified. In contrast, the fetuses contained only trace amounts of these structures, as revealed by immunostaining. These results indicate that the primary target for the antibodies of the anti-Tjα serum is the placenta tissue, resulting in termination of the pregnancy.

Keywords: Blood group p, glycosphingolipids, spontaneous abortions, mass spectrometry, NMR spectroscopy

The human blood group P-system contains three antigens, Pα, P and P1 (Table 1) which have been identified as glycosphingolipids [1, 2]. Individuals belonging to the rare blood group p phenotype lack all these antigens on their red cells and have preformed or natural antibodies against them, defined as anti-Tjα serum, which is believed to be a mixture of anti-P, anti-Pα and anti-P1 antibodies [3]. Women belonging to the p blood group have a high incidence of early spontaneous abortions believed to be caused by anti-Tjα antibodies [4, 5] since the father (statistically) always belongs to another group within the P blood group system (P1, P2). The mechanisms for these abortions, however, are not fully understood. IgG antibodies produced by Rh immunizations are known to pass the placental barrier and bind to the red blood cells of the fetus.

Mature placentas have been shown to contain the P- and Pk-antigens [4, 6]. Spontaneously aborted fetuses of p women are usually macroscopically intact, in contrast to fetuses aborted due to Rh incompatibility (B. Cedergren, unpublished observations). In order to answer the question whether the fetus or the placenta or both is/are a target for the anti-Tjα antibodies we have analysed two cases of spontaneously aborted fetuses from p women.

Materials and methods

Glycosphingolipid preparation

A 17-week-old fetus and placenta were obtained after spontaneous abortion from a woman of blood group A1 p Le(a−b+) and stored at −80 °C. The father belonged to blood group P2 (having the Pk- and P-antigens but lacking

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Table 1. Structures of antigens mentioned in the text.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pk-Antigen</td>
<td>Galz1-4Galβ1-4Glcβ1-1-Ceramide</td>
</tr>
<tr>
<td>P-Antigen</td>
<td>GalNAcβ1-3Galz1-4Galβ1-4Glcβ1-1-Ceramide</td>
</tr>
<tr>
<td>P-Antigen</td>
<td>GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1-Ceramide</td>
</tr>
<tr>
<td>A-6-1</td>
<td>GalNAcβ1-3(Fucz1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-1-Ceramide</td>
</tr>
<tr>
<td>A-7-1</td>
<td>GalNAcβ1-3(Fucz1-2)Galβ1-3(Fucz1-4)GlcNAcβ1-3Galβ1-4Glcβ1-1-Ceramide</td>
</tr>
<tr>
<td>A-6-2</td>
<td>GalNAcβ1-3(Fucz1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1-Ceramide</td>
</tr>
<tr>
<td>A type 3</td>
<td>GalNAcβ1-3(Fucz1-2)Galβ1-3GalNAcβ1-3(Fucz1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1-Ceramide</td>
</tr>
<tr>
<td>A type 4</td>
<td>GalNAcβ1-3(Fucz1-2)Galβ1-3GalNAcβ1-3(Fucz1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1-Ceramide</td>
</tr>
</tbody>
</table>

a In the short-hand designation for blood group glycolipids, the letter indicates blood group determinant, the first numeral is the number of sugar residues, and the second numeral the type of carbohydrate chain. Thus A-6-1 means a hexaglycosylceramide with a blood group A determinant based on a type 1 chain.

the P1-antigen). After thawing, the placenta was separated from the fetus which was further macroscopically dissected into intestine, liver, brain and residual tissue. An additional 12-week-old fetus spontaneously aborted from a p woman was obtained. After lyophilization, total nonacid glycosphingolipid fractions were prepared from the different tissues as described [7].

Thin layer chromatography with immunostaining and bacterial overlay

This layer chromatograms were run on glass plates (Merck, Darmstadt, Germany) for chemical detection and on glass plates (Whatman International Ltd, Maidstone, UK) or aluminium sheets (Merck, Darmstadt, Germany) for immunostaining and bacteria overlay. The solvent was chloroform–methanol–water (60:35:8, by vol.). Chemical detection was achieved with anisaldehyde reagent [7]. The immunostaining and bacterial overlay were performed as described [8–10]. Antibodies used were anti-P [4], anti-Pk [11] and the following blood group A monoclonal antibodies: Dakopatts A581 reactive with terminal A trisaccharide irrespective of core chain type [12], AH-21, reactive with monofucosyl A type 1 determinant [13], HH-3, reactive with difucosyl A type 1 determinant [14], TH-1, reactive with A type 3 determinant [14] and HH-5, reactive with A type 3 and 4 determinants [14]. Serum from the p woman who aborted the 17-week-old fetus and placenta was tested against the prepared glycosphingolipid fractions and the detection of bound antibodies was accomplished using anti-IgG3 antibodies (BIO-Zac) [15]. 125I labelled antimouse immunoglobulins (Dakopatts, Denmark) were used as sandwich antibodies. 35S labelled P-fimbriated Escherichia coli bacteria (HB101/pPIL2GI-15) recognizing the Galz1-4Gal sequence were kindly provided by Dr T. Korhonen.

Proton NMR spectroscopy

400 MHz proton NMR spectroscopy of the native glycolipids in 0.5 ml [2H6]dimethylsulfoxide containing 2% 2H2O at a probe temperature of 30 °C was performed on a Varian XL400 apparatus (Varian, USA). Chemical shifts are given relative to tetramethylsilane.

Mass spectrometry

Mass spectrometry of the permethylated-reduced glycolipids [16–18] was performed on a high-field ZAB-2F mass spectrometer (VG Analytical Ltd, Manchester, UK) equipped with a PDP 11/250 data system. The sample was analysed with electron ionization (EI) using the ‘in beam’ technique [19].

Results

Thin layer chromatography

The results from analysis with thin layer chromatography are shown in Fig. 1. As can be seen in Fig. 1, plate II, the Galz1-4Gal specific E. Coli strain binds the reference globoside (lane 9) and two compounds in the placenta fraction (lane 3), one in the four sugar region and one in the three sugar region. Faint staining with the bacteria is also seen of compounds in the fractions from the 12-week-old fetus (plate II, lane 7) and the residual tissue of the 17-week-old fetus (lane 8). The P-antibody (Fig. 1, plate III) has affinity for one compound in the placenta fraction (lane 3) and for one in the fraction from the 17-week-old fetus (lane 8). One band in the placenta fraction is also stained by pk-antibody (Fig. 1, plate IV, lane 3) and weak staining can be seen in the fraction from the residual fetus tissue (lane 8). Note that no binding can be detected to the fraction from A1 p Le(a–b+) plasma (lane 1) while the fraction from A1 p, Le(a–b+) plasma (lane 2) is positive with the reagents used.

The results from the analyses with the different blood group A antibodies are not shown. Earlier studies have shown that placenta tissue probably does not express blood group A and B antigens [20, 21], and the positive results we obtained with the anti-A antibodies are most likely due to remaining maternal red blood cells, as the staining of the placenta fraction was very similar to the staining of red blood cells (not shown). Both the intestine and the residual tissue fractions were stained with all A-antibodies used, while the liver and the brain were negative.