
EVIDENCE FOR A NOVEL BLOOD GROUP A HEPTAGLYCOSYL CERAMIDE BASED ON A TYPE 3 CARBOHYDRATE CHAIN*

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Jan Holgersson, Henrik Clausen, Sen-itiroh Hakomori, Bo E. Samuelsson, and Michael E. Breimer

From the Department of Medical Biochemistry, University of Göteborg, P. O. Box 33031, S-400 33 Göteborg, the Department of Surgery, Sahlgren's Hospital, University of Göteborg, S-413 45 Göteborg, Sweden, the Royal Dental College, Copenhagen, Denmark, and the Biomembrane Institute and University of Washington, Seattle, Washington 98119

Kidney, ureter, kidney artery, and kidney vein tissue were obtained from a single human transplant specimen. The donors erythrocyte blood group phenotype was Aₜ,Le(a⁻b⁺). Total non-acid glycolipid fractions were isolated and individual glycolipid components were identified by immunostaining thin layer plates with a panel of monoclonal antibodies and by mass spectrometry of the permethylated and permethylated-reduced total glycolipid fractions. The dominating glycolipids in all tissues were mono- to tetraglycosyleramides. In the kidney, ureter, and artery tissue less than 1% of the glycolipids were of blood group type, having more than 4 sugar residues. In contrast, 14% of the vein glycolipids were of blood group type, and the dominating components were type 1 chain blood group A hexaglycosylceramides and A hexaglycosylceramides. Trace amounts of structurally different blood group A glycolipids (type 1 to 4 core saccharide chains) with up to 10 sugar residues were found in the kidney, ureter, and vein tissues, including evidence for a novel blood group A heptaglycosylceramide based on the type 3 chain in the vein. The only detected A glycolipid antigen in the artery tissue was the blood group A difucosyl type 1 chain heptaglycosylceramide (ALe⁶) structure. Blood group Lewis and related antigens (Le⁴, Le⁵, and ALe⁶) were expressed in the kidney, ureter, and artery, but were completely lacking in the vein, indicating that the Le gene-coded α1-4-fucosyltransferase was not expressed in this tissue. The X and Y antigens (type 2 chain isomers of the Le⁴ and Le⁵ antigens) were detected only in the kidney tissue.

In recent years a growing structural complexity of the blood group ABH and related carbohydrate antigens has been documented (1). Several new core saccharide structures carrying the antigenic determinants as well as structures indicating interactions between blood group systems have been found. The expression of these antigens in human tissues is a result of the interaction of several gene systems such as the ABO, H, Se, Le, I, and P blood group systems (1-3). In addition, also other, mostly unknown factors influence the expression of these antigens exemplified by the difference in antigen expression found in various organisms within a single individual (4-6) and also between different cells in a certain organ (7, 8).

An attempt has been made to correlate the tissue-specific expression of different blood group antigens to the embryological origin of the tissue (3). The type 1 chain ABH antigens are thought to be of endodermal origin, regulated by the Se and Le genes, while the type 2 chain antigens, independent of Se and Le genes, are thought to be of ectodermal and mesodermal origin (3). With some exceptions, earlier studies speak in favor of this theory (3). Human erythrocytes (9) and granulocytes (10), being of mesodermal origin, have been shown to contain mainly type 2 chain compounds, whereas the blood group glycolipid antigens in human small intestine are dominated by type 1 chain compounds, which are exclusively present in the epithelial cells of the bowel (7). However, the increasing number of antigens identified have made the situation more complicated, exemplified by the kidney (11) and ureter (8) where the type 4 chain A antigen is the dominating blood group A glycolipid.

The structural difference of the blood group carbohydrate antigens can be recognized by the immune system. This is illustrated by the production of monoclonal antibodies against structurally different blood group A antigens (type 1-4 structures) (4, 12) and in the clinical situation were antibodies specific for a certain A antigen structure have been found in patients receiving ABO incompatible blood transfusions (13) or kidney transplants (14). In many of these ABO incompatible kidney transplantsations, the graft was lost due to kidney artery or kidney vein thrombosis.¹

Therefore, an extended knowledge about the structural polymorphism and the cell- and tissue-specific distribution of the ABH and related antigens within a kidney graft is needed. This paper describes the expression of the non-acid glycolipids in the kidney, ureter, artery, and vein tissues from a single human kidney specimen. The blood group glycolipids were structurally identified by a combination of thin layer chro-

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matography using a panel of specific monoclonal antibodies for detection and by mass spectrometry of the glycolipid mixtures.

**MATERIALS AND METHODS**

**Tissue Specimens**—The kidney tissue specimen discarded for lack of suitable recipient was obtained from the transplantation unit, Sahlgren's Hospital. The donors red blood cells were blood group typed as A, Le(a-b+). As the specimen was aimed for transplantation the shorthand designation for blood group glycolipids, the letter(s) correspond to the blood group A hexaglycosylceramide based on the gangliotetraosylceramide (15). The kidney tissue (Fig. 1A) shows the thin layer chromatogram of the total non-acid glycosphingolipid fractions. All bands seen on the plate, except for these marked +, were colored green indicating below the five-sugar region are seen by chemical staining. The blood group X pentaglycosylceramide (X-5), the Y hexaglycosylceramide (Y-6), and the type 2 chain difucosyl ceramide (A-7-2) were from dog small intestine (26).

**Antigen specificity of the mouse monoclonal antibodies used in the immunostaining experiments**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Fig.</th>
<th>Code no.</th>
<th>Specificity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A all types</td>
<td>Plate B</td>
<td>Dakopatts A561</td>
<td>Terminal A trisaccharide</td>
<td>10</td>
</tr>
<tr>
<td>Anti-A type 1</td>
<td>Plate C</td>
<td>AH-21</td>
<td>A type 1, monofucoyl</td>
<td>20</td>
</tr>
<tr>
<td>Anti-A type 2</td>
<td>Plate D</td>
<td>HH-4</td>
<td>A type 2, monofucoyl</td>
<td>12</td>
</tr>
<tr>
<td>Anti-A type 3</td>
<td>Plate E</td>
<td>TH-1</td>
<td>A type 3</td>
<td>21</td>
</tr>
<tr>
<td>Anti-A type 3 and 4</td>
<td>Plate F</td>
<td>HH-5</td>
<td>A type 3 and 4</td>
<td>12</td>
</tr>
<tr>
<td>Anti-ALeb</td>
<td>Plate G</td>
<td>HH-3</td>
<td>A type 1, difucoyl</td>
<td>22</td>
</tr>
<tr>
<td>Anti-Le*</td>
<td>Plate H</td>
<td>XALA Chembiomed</td>
<td>Le* terminal</td>
<td></td>
</tr>
<tr>
<td>Anti-Leb</td>
<td>Plate I</td>
<td>9ALB Chembiomed</td>
<td>Le*/H type 1 terminal*</td>
<td></td>
</tr>
<tr>
<td>Anti-X</td>
<td>SH-1</td>
<td>X terminals</td>
<td>X terminals</td>
<td>20</td>
</tr>
<tr>
<td>Anti-Y</td>
<td>AH-6</td>
<td>Y terminals</td>
<td>Y terminals</td>
<td></td>
</tr>
<tr>
<td>Anti-A</td>
<td>HH-2</td>
<td>A type 2, difucoyl</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Anti-AGA</td>
<td>MH-3</td>
<td>A on ganglioseries backbone</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This antibody was shown to cross-react with the blood group H active pentasaccharide based on a type 1 chain.


**RESULTS**

Total amount of non-acid glycolipids isolated from the kidney was 78.6 mg, ureter 1.3 mg, vein 2.5 mg, and artery 0.3 mg, corresponding to 3.6, 2.5, 5.4, and 0.7 mg/g dry tissue weight, respectively. Fig. 1A shows the thin layer chromatogram of the total non-acid glycolipid fractions. All bands seen on the plate, except for these marked +, were colored green with the anisaldehyde reagent and thus contained carbohydrate (15). The kidney tissue (Fig. 1A, lane 1 and Table II) contain mono- to tetraglycosylceramides as major components and small amounts of a pentaglycosylceramide. The chemical structures of these compounds have been reported before (7, 33-35) as glucosylceramide, galactosylceramide, lactosylceramide, digalactosylceramide, globotriosylceramide, globotetraosylceramide and 3-galactobiose ceramide (4V'GalGb3Cer) and the blood group X pentaglycosylceramide (III'Fucα1LcCer). No bands migrating below the five-sugar region are seen by chemical staining. The ureter (Fig. 1A, lane 2 and Table II) contain mono- and diglycosylceramides as major components, small amounts of tri- and tetraglycosylceramides and trace amounts of com-
pounds migrating in the five- to seven-sugar region. This glycolipid pattern is similar to the pattern obtained from the analysis of epithelial cells and non-epithelial residue of a ureter from another tissue donor with the same blood group (8). The kidney vein and artery (Fig. 1A, lanes 3 and 4 and Table II) contain mono-, tri-, and tetracylosglycosylceramides and only small amounts of diglycosylceramides. The vein contains also compounds migrating as penta- and hexaglycosylceramides, while the artery tissue lacks these compounds. In addition to the glycolipids identified by the chemical reagent, several minor compounds were identified by mass spectrometry and antibody binding analysis, and the results are described below and summarized in Table III.

**Mass Spectrometry**—Mass spectrometric analysis of the total non-acid glycolipid fractions isolated from the kidney, ureter, kidney artery, and vein tissues revealed the presence of both carbohydrate sequence and molecular ions for Hex-Cer, Hex-Hex-Cer, Hex-Hex-Hex-Cer, and HexNAC-Hex-Hex-Hex-Cer structures in all tissues (Table II). For the more complex glycolipids having more than 4 sugar residues a large variation in the expression of glycolipid species between the different tissues was found. The following presentation will be focused in detail on the blood group type glycolipids from the vein and data from the kidney, ureter, and artery tissue will be shortly described.

Fig. 2 shows selected reconstructed mass chromatograms from the analysis of the permethylated-reduced total non-acid glycosphingolipid mixture of the kidney vein and the mass spectrum recorded at scan number 25 is reproduced in Fig. 3. The curves reproduced in Fig. 2 are the intense ion monomers from the 24:0 fatty acid species of each glycolipid. The carbohydrate sequence shown in the simplified formulae are based on sequence ion information obtained from the analysis of both the permethylated (Fig. 4) and permethylated-reduced (Fig. 3) derivatives. A partial separation of individual glycolipid components, according to molecular size, is obtained by rising the ion source temperature as shown in Fig. 2. The major glycolipid species in the vein are mono- (m/z 659), tri- (m/z 1037), tetra- (m/z 1268) and pentaglycosylceramides (m/z 1472) together with small amounts of di- (m/z 863) and hexaglycosylceramides (m/z 1703) and trace amounts of hepta- (m/z 1847), octa- (m/z 2081), and nonaglycosylceramides (m/z 2313). This is in accordance with the thin layer chromatography data (Table 1, lane 3 and Table II). The major blood group glycolipid is a blood group H pentaglycosylceramide. The number of sugars (3 hexoses, 1 deoxyhexose, and 1 hexosamine) and fatty acid composition (16:0, 20:0, 22:0, 23:0, and 24:0 hydroxy fatty acids) is shown by the intense series of peaks at m/z 1360, 1416, 1444, 1458, and 1472 in Fig. 3. The peak at m/z 1404 formed by a loss of the fatty acid instead of the long chain.

![Thin layer chromatograms of total non-acid glycolipid fractions isolated from the kidney (lane 1), ureter (lane 2), kidney vein (lane 3), and kidney artery (lane 4) of a blood group A₁Le(a-b+) human individual.](image)

**Table II**

<table>
<thead>
<tr>
<th>Glycolipid structure</th>
<th>Kidney</th>
<th>Ureter</th>
<th>Vein</th>
<th>Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex-Cer</td>
<td>0.61 (17)</td>
<td>1.25 (51)</td>
<td>1.21 (22)</td>
<td>0.13 (19)</td>
</tr>
<tr>
<td>Hex-Hex-Cer</td>
<td>0.84 (18)</td>
<td>0.35 (13)</td>
<td>0.24 (6)</td>
<td>0.02 (4)</td>
</tr>
<tr>
<td>Hex-Hex-Hex-Cer</td>
<td>0.92 (26)</td>
<td>0.30 (12)</td>
<td>1.35 (36)</td>
<td>0.22 (32)</td>
</tr>
<tr>
<td>HexNAC-Hex-Hex-Hex-Cer</td>
<td>1.34 (38)</td>
<td>0.58 (24)</td>
<td>1.23 (23)</td>
<td>0.31 (45)</td>
</tr>
<tr>
<td>HexNAC-Hex-Hex-Hex-Galgal3(3Hex-Fuc)</td>
<td>0.05 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fuc2Gal3GlcNAcHex-Hex-Cer</td>
<td>0.44 (8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GalNAc1-Gal2-Fuc1-Gal3-Hex-Hex-Cer</td>
<td>0.33 (6)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Expressed as mg/g dry tissue weight.
* Relative amount expressed as percentage.
The structures were tentatively assigned by a combination of thin layer mobility, immunostaining with monoclonal antibodies, and by mass spectrometry.

<table>
<thead>
<tr>
<th>Tentative structure</th>
<th>Shorthand designation</th>
<th>Kidney</th>
<th>Ureter</th>
<th>Vein</th>
<th>Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>GalNAc1-3(Fucol-2)Gal-Hex-Cer</td>
<td>A-4</td>
<td>(-)</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hex-HexNAc-Hex-Cer</td>
<td>+</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gal BJP(1-3)GlcNAc-Hex-Hex-Cer</td>
<td>X-5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fucol-2GalBl-3GlcNAc-Hex-Cer</td>
<td>H-5</td>
<td>-</td>
<td>(++)</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Gal Bl-2(Fucol-4)GlcNAc-Hex-Cer</td>
<td>Lea-5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fucol-2(Fucol-1-4)GlcNAc-Hex-Cer</td>
<td>Lea-6</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fucol-2GalBl-1(Fucol-3-1)GlcNAc-Hex-Cer</td>
<td>Y-6</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fucol-2GalBl-3GlcNAc-Hex-Cer</td>
<td>Y-6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GalNAc1-3(Fucol-2)GalBl-3GlcNAc-Hex-Hex-Cer</td>
<td>A-6</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>-</td>
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<tr>
<td>GalNAc1-3(Fucol-2)GalBl-3GlcNAc-Hex-Hex-Cer</td>
<td>A-6-2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>GalNAc1-3(Fucol-2)GalBl-3GlcNAc-hex-Hex-Cer</td>
<td>A-7-3</td>
<td>+</td>
<td>+</td>
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<td>GalNAc1-3(Fucol-2)GalBl-3(Fucol-4)GlcNAc-Hex-Cer</td>
<td>A-7-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>GalNAc1-3(Fucol-2)GalBl-3(Fucol-4)GlcNAc-Hex-Cer</td>
<td>A-7-2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>GalNAc1-3(Fucol-2)GalBl-3(Fucol-4)GlcNAc-Hex-Hex-Cer</td>
<td>A-7-4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2dHex,2HexNAc,4Hex,Cer</td>
<td>+</td>
<td>(++)</td>
<td>+</td>
<td>(-)</td>
<td>-</td>
</tr>
<tr>
<td>1dHex,3HexNAc,4Hex,Cer</td>
<td>A-8</td>
<td>+</td>
<td>(+)</td>
<td>(-)</td>
<td>+</td>
</tr>
<tr>
<td>2dHex,3HexNAc,4Hex,Cer</td>
<td>A-9</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>1dHex,4HexNAc,5Hex,Cer</td>
<td>A-10</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>-</td>
</tr>
<tr>
<td>2dHex,4HexNAc,6Hex,Cer</td>
<td>A-11</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+a indicates the presence and - the absence of each compound. No quantification of the relative amount is given. The parentheses indicate that the presence or absence of this compound is not established with certainty.

*Presence or lack of these compounds are based on the antibody staining experiments.

The second major blood group type glycolipid is a blood group A hexaglycosylceramide. The intense fatty acid immunoions are seen at m/z 1591-1703, the ions formed by the loss of the fatty acid at m/z 1635 and the molecular ions are found at m/z 1591-1988 (Fig. 3). Carbohydrate sequence ions are seen at m/z 189, 246, 262, 624, 640, and 855 in Fig. 3 and at m/z 159, 223 (260-32), 260, 606 (636-32), 698, 803, and 1087 in Fig. 4. The evidence for this structure is further strengthened by scan no. 27 of the permethylated derivative (not shown). In this spectrum m/z 1291 (1087 plus a hexose), 1364 (1365+1), a series of peaks at m/z 1733-1789 (whole sugar chain plus h22:0 to h24:0 fatty acids), and molecular ions at m/z 2029 were clearly seen.

In addition to these two major blood group glycolipids several minor components could be identified by the appearance of series of immunoions containing the whole carbohydrate chain and the fatty acid for the permethylated-reduced derivatives. Carbohydrate sequence ions from these components are, however, difficult to identify except for certain fragments which do not coincide with the ions from the major compounds. Evidence for a hexaglycosylceramide (2 deoxyhexoses, 3 hexoses, and 1 hexosamine) was given by ions at m/z 1618 and 1646 (Fig. 2) for the hydroxy 22:0 and 24:0 fatty acid species in the permethylated-reduced spectrum. Carbohydrate sequence ions indicating a Leb or Y determinant were found at m/z 780 (812-32), 812, and 1016 for the permethylated derivative. The presence of a difucosylheptaglycosylceramide is given by the ions at m/z 1819 and 1847 (Figs. 2 and 3) for the non-hydroxy 22:0 and 24:0 fatty acids, and the corresponding molecular ions are seen at m/z 2104 and 2132 (Fig. 3). The carbohydrate sequence ion at m/z 1262 and the peak at m/z 1539 (1538+1) (not shown), corresponding to the whole sugar chain plus part of the ceramide, together with the absence of the sequence ions at m/z 1026 (1058-32) and 1058 in the spectrum of the permethylated derivative, indicate a HexNAc-{(dHex)-}Hex-HexNAc-{(dHex)-}Hex-Hex-Cer structure as shown in Fig. 2 and not a HexNAc-{(dHex)-}Hex-{(dHex)-}HexNAc-Hex-Hex-Cer structure. The presence of this structure was further strengthened by the reactivity in the six-sugar region with the TH1 antibody and the absence of reactivity with the antibodies (HH3 and HH2, respectively) specific for the ALeb and AY structures (see below). In the high mass region, two series of peaks were found indicating the presence of an oktaglycosylceramide (2 deoxyhexoses, 4 hexoses, and 2 hexosamines) and a nonaglycosylceramide (2 deoxyhexoses, 4 hexoses, and 3 hexosamines) given by the immunoion curves at m/z 2081 and 2313, respectively, in Fig. 2. No conclusive sequence ions were identified for these structures. In a second analysis of the permethylated-reduced derivative, a larger amount of sample was analyzed and the magnet was scanned to cover only the high mass region (see "Materials and Methods"). A 10-sugar component (1 deoxyhexose, 5 hexoses, and 4 hexosamines) was identified with immunoions containing the whole sugar chains and the fatty acid present at m/z 2432-2518 for the non-hydroxy 16:0 to hydroxy 24:0 fatty acid species. No peaks were found indicating structures with 11 or more sugar residues. No specific fragments indicating the presence of globoseries-based blood group H (H-6-4) or A (A-7-4) structures were
found in the vein tissue. These structures are present in the kidney (7) and ureter (8) as shown in Table III.

The glycolipid samples from the kidney, ureter, and artery were analyzed as permethylated and permethylated-reduced derivatives in a similar manner and the results are listed in Table III.

Binding of Various Monoclonal Antibodies to Kidney, Ureter, Vein, and Artery Glycolipids Separated on Thin Layer Plates—The immunostaining of the non-acid glycolipid fractions with mouse monoclonal antibodies with different blood group A antigenic specificity together with anti-Le\(^a\) and anti-Le\(^b\) antibodies are shown in Fig. 1, chromatograms B–I. In addition, anti-X, anti-Y, anti-AY, and anti-AGA antibodies were used (not shown). The antigenic specificity of the antibodies used is known in detail (Table I). A very complex pattern of blood group A glycolipids was found, with the kidney and ureter having a similar thin layer immunostaining pattern, the vein being unique in its high expression of type 3 chain A glycolipid antigens and the artery having the ALe\(^b\) glycolipid as its only blood group A glycolipid (Fig. 1, B–G). The six-sugar region of the kidney and ureter (lanes 1 and 2) reacted with the A type 1 and 2 chain antibodies, while this
region of the vein (lane 3) contained at least three different structures reacting with the anti-A type 1 (C), the anti-A type 2 (D), the anti-A type 3 (E), and anti-A type 3 and 4 (F) antibodies. In the seven-sugar region a band comigrating with the A-7-4 reference was stained with the anti-A type 3 and 4 antibody (F) in the kidney and ureter, while this component was absent in the vein and artery. Only a very weak staining was seen in this region with the anti-A type 3 antibody (E), which is known to cross-react weakly with A type 4 structures (21), indicating that this band contain the A type 4 compound. In addition, the type 2 chain A-specific antibody stained the ureter in the seven-sugar region (D, lane 2). The eight/nine-sugar region of the vein was stained strongly by the anti-A type 3 (E) and anti-A type 3 and 4 (F) antibodies, and weakly with the anti-A type 2 (D) antibodies. The other tissues were not stained in the eight/nine-sugar regions except for the kidney, where a faint band with eight sugars reacting with the anti-A type 2 antibody (D, lane 1) and a faint band with nine sugars reacting with the anti-A type 3 and anti-A type 3 and 4 antibodies were present (E and F, lane 1). The 10/12-sugar region stained in the vein with anti-A type 2 (D), anti-A type 3 (E), and anti-A type 3 and 4 (F) antibodies. Neither the anti-A difucosyl type 2 chain antibody (HH-2) nor the anti-AGA, antibody (MH3) showed any reactivity in the tissues analyzed (not shown).

The anti-Le^a (H) and anti-Le^b (I) antibodies reacted with structures migrating as Le^a pentaglycosylceramides and Le^b hexaglycosylceramides, respectively, in the kidney, ureter, and weakly in the artery, while no staining of the vein glycolipids was seen. The strong staining of the anti-Le^b antibody in the
FIG. 4. Mass spectrometric analysis of the permethylated total non-acid glycolipid mixture from the kidney vein of a blood group A,Le(a-b+) human individual. Ten μg of sample was evaporated by a temperature rise of 6 °C/min started at 200 °C. Scan 24 recorded at an ion source temperature of 310 °C is shown. Simplified formulae of the major glycolipid components are given in the figure.

![Mass spectrometric analysis](image)

The five-sugar region of chromatogram I is due to a cross-reaction with the blood group H type 1 pentaglycosylceramide. The anti-ALeb antibody reacted with a seven-sugar compound in the kidney, ureter, and artery migrating as the reference A-7-1 glycolipid (G), while no reaction was seen with the vein glycolipids.

The anti-X antibody strongly stained a band migrating as a pentaglycosylceramide in the kidney, while the other tissues were negative (not shown). A similar staining pattern has been shown for another anti-X antibody (36). The anti-Y antibody stained very weakly a component in the six-sugar region in the kidney, while the other tissues were negative (not shown).

The antibody binding data described above was compared with the results of the mass spectrometric analysis of the other tissues and the blood group glycosphingolipid structures tentatively assigned for the kidney, ureter, vein, and artery are summarized in Table III.

DISCUSSION

The present investigation has used a combination of mass spectrometry and immunostaining of thin layer chromatograms to provide evidence for partial structures of most of the non-acid glycosphingolipids in the kidney, ureter, renal vein, and artery tissue obtained from a single human individual. The analysis was performed on the total non-acid glycolipid mixtures isolated from each tissue. This was necessary due to the small amounts of glycolipids obtained, especially of blood
group type, which made it impossible to purify each component for structural studies. For example, the vein tissue contained 0.7 mg of total glycolipids of which about 14 percent (0.1 mg) were of blood group type and consisted of at least six different molecular species. Due to the individual specific distribution of the blood group type glycolipids (4–6), it is not possible to collect tissue from several individuals in order to increase the amount of each glycolipid. The analytical methods used established the structures of a large number of glycolipid structures present in the tissues. However, there are difficulties in the structural interpretation which can be exemplified by, for example, the four-sugar compound stained by the anti-A antibody reacting with all A determinants (Fig. 1B, lane 3) and the compound strongly stained by the anti-A type 3 antibody migrating in the six-sugar region (Fig. 1E, lane 3) in the vein tissue. The four-sugar compound may be a blood group A tetraglycosylceramide based on the lactosyl core, previously identified in rat small (37) and large (38) intestine. This compound has not been found in human tissues before although its precursor, the H trisaccharide, has been found in human small intestinal epithelial cells (7). In the mass spectrometric analysis, fragments originating from such a blood group A tetraglycosylceramide all coincide with either the ions from the more abundant blood group A hexaglycosylceramide or globotetraosylceramide. Therefore, no unambiguous structural information concerning this compound is obtained. However, the use of the newly developed four sector instruments, where mass spectrometry-mass spectrometry analysis is performed (39), may overcome this problem in mixture analysis (40). The only carbohydrate sequence having six sugars and a terminal A trisaccharide identified by mass spectrometry was HexNAC-(dHex)-HexHexNAC-Hex-Hex-Cer which originates from the type 1 and 2 chain A hexaglycosylceramides. However, these structures do not bind the TH-1 antibody. The binding could be explained by a seven-sugar repetitive A determinant based on lactosylceramide as suggested by the known binding epitope of the TH-1 antibody (21), GalNAco1-3(Fucu1-2)Galβ1-3GalNAc-R. The ions seen at m/z 1819 and 1847 in the permethylated-reduced spectrum (Fig. 3) could derive from such a structure, but could also originate in the ALeb and AY structures. However, the absence in the permethylated spectrum of the sequence ions at m/z 1058 and 1026 (1058–32), which are specific for the HexNAC-(dHex)-HexHexNAC-terminal (41), together with the lack of reactivity with the anti-ALeb and anti-AY antibodies exclude these structures as candidates for explaining the ions at m/z 1819 and 1847 (Fig. 3). Despite such problems in the interpretation of the results obtained by the mass spectrometric and thin layer chromatographic analysis, no other analytical methods are available at present to analyze glycolipid samples obtained in small amounts from unique and small tissue specimens.

The dominating glycolipid present in all tissues were mono- to tetraglycosylceramides except for the vein and artery which only contained small amounts of diglycosylceramides (Table II). The mono- and diglycosylceramides present in the ureter consist of mainly one slow-moving band (Fig. 1A, lane 2) which is due to a unique ceramide composition of C16:0 and C20:0 bases in combination with hydroxy 22:0 and 24:0 fatty acids (42). These molecular species originate from the epithelial cells of the ureter while the non-epithelial glycolipids contained d18:1 fatty acids, which are mainly short-chain non-hydroxy 16:0 to 24:0 fatty acids (8). The tri- and tetraglycosylceramides of the ureter have a less hydroxylated ceramide, and these components are present in the non-epithelial part of the tissue. The high degree of hydroxylation in epithelial tissues like the transitional epithelium of the urinary tract (8) and the epithelial cells of the small intestine (7) has been suggested to be due to the high physicochemical demands to which these cell membranes are exposed. The hydroxylated ceramide type has been suggested to stabilize the membrane and decrease its water permeability by forming lateral hydrogen bonds (42, 43).

A large difference in the expression of the blood group type glycolipids in the different tissues was found. The kidney vein contained large amounts of type 1 chain-based H penta- and A hexaglycosylceramides and small amounts of several additional blood group A compounds based on type 1, 2, and 3 core saccharides. The vein, however, was unique in its high expression of both short- and long-chain blood group A glycolipid antigens, and especially in its relatively high content of type 3 chain-based antigens (Fig. 1E, lane 3). A prerequisite for expression of the latter chain type is an active β1-3galactosyltransferase adding a galactose residue to the terminal N-acetylgalactosamine of the A trisaccharide (44). The expression of blood group antigens in the kidney and ureter was very similar to each other, having type 1 and 4 chain structures as the dominating species (8, 11). The kidney artery contained trace amounts of the ALeb heptaglycosylceramide but lacked completely the monofucosyl type 1 and type 2, the type 3, and the type 4 chain A structures. The arterial endothelium especially is known to be very sensitive to ischemia, which might explain the low abundance of blood group type glycolipids in the artery. However, the presence of the ALeb hexaglycosylceramide contradicts this explanation. For the Lewis antigens another expression pattern was found. The Le<sup>a</sup> penta- and Le<sup>b</sup> hexaglycosylceramides together with the ALeb heptaglycosylceramide were present in the kidney, ureter, and in small amounts in the artery, while the vein completely lacked these antigens. This indicates that the Le gene code α1-4 fucosyltransferase is not expressed in the vein. Further support for this is the accumulation in the vein of the H-5 and A-6 glycolipids, which are the precursors for the Le gene enzyme forming the Le<sup>a</sup>- and A-7 glycolipids, respectively. The reason for, and the regulation of, this tissue-specific expression of blood group glycolipids is not known. However, it has been suggested that the glycosyltransferase expression is regulated at the level of transcription. In support of this suggestion is the finding that the level of Ga12,6-sialyltransferase mRNA varies up to 100-fold in different rat tissues, corresponding to the activity of the enzyme (45). Also Ga12,6-sialyltransferase mRNA of varying sizes have been found in different rat tissues, which in part has been explained by an alternative splicing from a common gene sequence (46).

Immunohistochemical studies of the expression of blood group antigens in human kidneys have shown that the ABH antigens present in the endothelial cells of the blood vessels were expressed independent of the secretor status of the individuals, while the ABH antigens in the distal tubulus and collecting ducts were only present in secretor individuals (47–49). The blood group Le<sup>a</sup> and Le<sup>b</sup> antigens were only present in the distal and collecting tubules and were absent in the blood vessels (48, 49). As expected Le<sup>b</sup> antigens are absent in non-secretor individuals (49). According to Oriol and coworkers (3), mesodermal-derived tissues have ABH antigens with type 2 chain, which are independent of the Se and Le genes, while ABH antigens derived from tissues of endodermal origin have type 1 chain core and are synthesized by the Se and Le gene-derived enzymes. In the kidney, the vessels are of mesodermal origin and the collecting tubules of endodermal origin. The A type 1 chain antigens and the Lewis antigens

* R. Oriol and J. Wallwork, personal communication.
may therefore be located in the collecting tubules as well as the A type 4 structure, for which the expression in different kidneys is Se gene-dependent (19). In the ureter, the ABH antigens are located in the transitional epithelium and blood vessels (50), but the secretor status of the donors was not located in the epithelial cells of the ureter (8).

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Blood group A glycolipid antigen expression in kidney, ureter, kidney artery, and kidney vein from a blood group A1Le(a-b+) human individual. Evidence for a novel blood group A heptaglycosylceramide based on a type 3 carbohydrate chain.

J Holgersson, H Clausen, S Hakomori, B E Samuelsson and M E Breimer


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