A Major Echinococcus multilocularis Antigen Is a Mucin-type Glycoprotein*

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The metacestode of Echinococcus multilocularis is surrounded by a carbohydrate-rich laminated layer, which plays a key role in the establishment of the infection in the mammalian host. A major component of the laminated layer is an antigen referred to as Em2(G11). This highly species-specific antigen has been used for serodiagnoses of alveolar echinococcosis and is suggested to contain carbohydrates as major constituents. The results of this work have shown that immunoaffinity-purified Em2(G11) subjected to size-exclusion chromatography eluted mainly in the void volume, indicating a high molecular weight structure of this antigen. Amino acid analysis revealed a large proportion of threonine and proline residues in Em2(G11). The carbohydrate moiety of the antigen was found to be composed of galactose, N-acetylgalactosamine, and N-acetylgalactosamine with a ratio of 2:4:1.0:0.5 as determined by gas-chromatography/mass spectrometry. An epitope tag was introduced to the beta-eliminated glycans, and an integrated mass spectrometric O-glycan profiling and sequencing approach was employed to obtain detailed sequence and linkage information of the unseparated glycoform pool. Novel glycoforms containing mucin-type core Gal1-3GalNAc and branched core structures attached to both serine and threonine residues are described. The data presented reveal that the Em2(G11) antigen is a mucin-type glycosylated protein.

Alveolar echinococcosis caused by the metacestode stage of Echinococcus multilocularis is a severe hepatic disorder that resulted in up to 100% lethality in untreated patients (1, 2). The metacestode of this tapeworm species is a multivesicular structure composed of an inner syncytial layer supported externally by an acellular laminated layer. This surrounding laminated layer is a heavily glycosylated structure that is suggested to play an important role in protecting the parasite from physiological and immunological host reactions (3). A structural component associated with the laminated layer is an antigen referred to as Em2(G11) as shown by the reaction of a monoclonal antibody (MABG11) species-specific for this antigen (4). Em2(G11) was shown to be the major antigenic component of the antigen fraction Em2 as described earlier, and both antigen preparations were found to have identical diagnostic properties for specific antibody detection in patients with alveolar echinococcosis (4, 5). The metacestode antigen purified with solid-phase MABG11 was shown to be largely composed of carbohydrates by its resistance to proteinase K digestion and lectin binding activity (5). A strong binding affinity was displayed by this antigen with lectins having specificities for galactose (Gal), N-acetylgalactosamine (GalNAc), N-acetylgalactosamine (GlNAC), and N-acetylsarminic acid (NANA). Recent analyses of the antigenicity of Em2(G11) demonstrated that the antigen stimulates antibody production in the absence of MHC class II-restricted T-cell help (6). Such T-cell-independent TI-2 antigens have previously been characterized as high molecular weight polysaccharides with multiple identical antigenic epitopes, low in vivo degradability, and the inability to be cognitively recognized by T-cell receptors (7, 8). Because of these properties, TI-2 antigens are unconventional immunogenic molecules for T-cell activation and for inducing effective high affinity IgG synthesis. Em2(G11) as a TI-2 antigen does not stimulate T-cell proliferation in vitro and induces an IgG response lacking subsequent avidity maturation and is therefore claimed to be a relevant factor contributing to the lack of protection against the proliferating metacestode in vivo (6).

This work was undertaken to elucidate the structural nature of the immunoaffinity-purified Em2(G11) antigen using a mass spectrometric O-glycan-profiling method. Evidence is presented that this antigen is a mucin-type glycosylated protein, and to our knowledge, this is the first report on such carbohydrate structures as constituents of a cestode antigen.

EXPERIMENTAL PROCEDURES

Size-exclusion Chromatography—The E. multilocularis Em2(G11) antigen was immunoaffinity-purified according to the method described by Deplazes and Gottstein (4) using the monoclonal antibody MABG11 coupled to CNBr-activated-Sepharose. The combined eluates of three immunoaffinity runs (11 ml in total) were lyophilized, redissolved in 300 μl of phosphate-buffered saline containing 0.1% N-acetylglucosamine and 0.02% sodium azide, and loaded onto an Amersham Biosciences Superdex 200 HR 10/30 column equilibrated with the aforementioned buffer at 0.1 ml min⁻¹. One-milliliter fractions were collected and screened by sandwich-enzyme-linked immunosorbent assay using the monoclonal antibody MABG11 (4). The elution positions of ω-globulin

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§ The abbreviations used are: MAb, monoclonal antibody; ESI-MS, matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry; MS-MS, tandem mass spectrometry; MALDI-TOF-MS, matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry; MS-MS, tandem mass spectrometry; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylgalactosamine; Me₆SO, dimethylsulfoxide; NANA, N-acetylsarminic acid; PMAA, partially methylated alditol acetate.
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(150 kda) and aprotinin (6.5 kda) were determined in a separate run under identical conditions. The immunoreactive fractions 3–5 (void volume) were pooled, dialysed against distilled water, and adjusted to 10 ml with water. A blank sample (buffer only) was passed through the same purification procedure starting with the immunoaffinity column. 

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS)—Methylated 2-deoxy-2-fluoro-2-hydroxybenzoic acid in ethanol. 0.3 µl of the matrix solution was spotted onto the target to form a thin matrix layer. Subsequently, 0.5 µl of the permethylation products was dried from a 2,5-dihydroxybenzoic acid matrix by redissolving the material in 20 µl of acetonitrile. The chloroform phase was vacuum-dried and redissolved in 50% water. The chloroform layer was removed by adding 2 ml of 1% acetic acid in methanol three times and drying under a stream of nitrogen. The sample was acetylated by transferring 400 µl of acetic anhydride, 400 µl of pyridine, and incubating under argon at room temperature overnight. The partially methylated alditol acetates (PMAAs) were dried under a stream of nitrogen and recovered in 4 ml of dichloromethane. The organic phase was washed four times with 2 ml of water, dried under a stream of nitrogen, and redissolved in 50 µl of dichloromethane. The sample was subjected to GC-MS with electron impact ionization (70 eV). The PMAAs were identified by means of their retention time and their characteristic mass spectra. Helium with a pressure of a 2.5 bar was used as carrier gas. The sample was applied via an all-gas-moving needle injector. Separation was achieved through a 0.25-mm × 60-m RTX-5-bonded phase column (Restek, Bad Homburg, Germany) with the temperature gradient of 130–200 °C at 1.5 °C/min and 200–290 °C at 4 °C/min. The analyses were performed using a MAT900 instrument (Finnigan-MAT, Bremen, Germany).

RESULTS

Purification of the Em2(G11) antigen was carried out by immunoaffinity and size-exclusion chromatography. As shown in Fig. 1, most of the immunoreactive material eluted from the Superdex column as a broad peak in the void volume (1300 kDa) down to a hydrodynamic volume corresponding to the elution position of γ-globulin (150 kDa). A minor peak eluting in the void volume (150 kDa) and aprotinin (6.5 kDa) were determined in a separate run under identical conditions. The immunoreactive fractions 3–5 (void volume) were pooled, dialysed against distilled water, and adjusted to 10 ml with water. A blank sample (buffer only) was passed through the same purification procedure starting with the immunoaffinity column. 

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Fig. 1. Size-exclusion chromatography of the Em2(G11) antigen. One-milliliter fractions were collected and screened by sandwich-enzyme-linked immunosorbent assay using the monoclonal antibody MAbG11. The elution positions of γ-globulin (150 kDa) and aprotinin (6.5 kDa) are indicated by arrows. Chromatography was carried out using an Amersham Biosciences Superdex 200 HR 10/30 column.
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Table I
Results of the amino acid and monosaccharide analyses of Em2(G11) antigen

<table>
<thead>
<tr>
<th>Component</th>
<th>Before β-elimination</th>
<th>After β-elimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asx</td>
<td>1.9 ± 0.24</td>
<td>2.0 ± 0.16</td>
</tr>
<tr>
<td>Glx</td>
<td>1.5 ± 0.20</td>
<td>1.3 ± 0.17</td>
</tr>
<tr>
<td>Ser</td>
<td>2.0 ± 0.32</td>
<td>0.7 ± 0.10</td>
</tr>
<tr>
<td>His</td>
<td>0.1 ± 0.14</td>
<td>0.3 ± 0.15</td>
</tr>
<tr>
<td>Gly</td>
<td>0.9 ± 0.13</td>
<td>1.6 ± 0.37</td>
</tr>
<tr>
<td>Thr</td>
<td>13.6 ± 1.67</td>
<td>7.2 ± 0.21</td>
</tr>
<tr>
<td>Ala</td>
<td>2.7 ± 0.31</td>
<td>2.3 ± 0.15</td>
</tr>
<tr>
<td>Arg</td>
<td>2.0 ± 0.35</td>
<td>1.8 ± 0.13</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.2 ± 0.05</td>
<td>0.2 ± 0.03</td>
</tr>
<tr>
<td>Val</td>
<td>1.9 ± 0.15</td>
<td>1.8 ± 0.08</td>
</tr>
<tr>
<td>Met</td>
<td>0.9 ± 0.14</td>
<td>1.2 ± 0.11</td>
</tr>
<tr>
<td>Phe</td>
<td>0.3 ± 0.04</td>
<td>0.3 ± 0.03</td>
</tr>
<tr>
<td>Ile</td>
<td>0.8 ± 0.11</td>
<td>0.6 ± 0.11</td>
</tr>
<tr>
<td>Leu</td>
<td>1.5 ± 0.24</td>
<td>1.4 ± 0.08</td>
</tr>
<tr>
<td>Lys</td>
<td>2.2 ± 0.27</td>
<td>1.7 ± 0.40</td>
</tr>
<tr>
<td>Pro</td>
<td>6.4 ± 0.67</td>
<td>5.3 ± 0.21</td>
</tr>
<tr>
<td>Gal</td>
<td>37.5 ± 4.83</td>
<td></td>
</tr>
<tr>
<td>GalNac</td>
<td>15.7 ± 3.58</td>
<td></td>
</tr>
<tr>
<td>GlcNac</td>
<td>8.1 ± 2.00</td>
<td></td>
</tr>
</tbody>
</table>

* The data are background-corrected. Values are the means ± S.D., n = 3.

at approximately 6.5 kDa (aprotinin) could be detected consistently in varying amounts and might be attributed to a degradation product of the Em2(G11) antigen. The immunoreactive material of apparent high molecular weight was used for all subsequent analyses.

**Amino Acid Analyses**—To address the question whether Em2(G11) does contain protein aliquots of the antigen was examined by amino acid analysis (Table I). Unusually high contents of both threonine and proline residues along with substantial amounts of serine suggest that a mucin-type polypeptide core may be a part of the antigen structure. Nonreductive β-elimination of the material resulted in a 65 and 47% decrease of serine and threonine, respectively, in the amino acid composition, indicating the presence of O-linked glycans attached to both hydroxyamino acids.

**Carbohydrate Compositional Analysis**—The immunoreactive material obtained after size-exclusion chromatography was subjected to acid methanolysis and trimethylsilane derivatization, and the reaction products were analyzed by GC-MS. The major monosaccharide component in the sample was found to be Gal together with significant amounts of GalNac and GlcNac (Table I). The ratio of Gal:GalNac:GlcNac was calculated to be 2.4:1:0.5, and the ratio of carbohydrate to protein ~ 3:1 (w/w).

**Sequence Analysis of the Em2(G11) O-Glycans**—To obtain more detailed information about the glycosylation of the Em2(G11) antigen, O-glycans were released by reductive β-elimination using NaBH₄/NaBD₄ (0.2/0.8 M) as a reducing agent. By this treatment, a tag was introduced to the saccharide aldolts, resulting in a characteristically altered isotopic pattern that can be clearly visualized by MALDI-TOF-MS (Fig. 2A). This pattern allowed the unambiguous distinction between signals from saccharides and those from contaminants and facilitated the interpretation of the MS-MS spectra. Purification and desalting of the β-elimination reaction products were accomplished by passage through a combined column consisting of a reverse-phase cartridge over cation exchange resin. This simple purification procedure yielded an O-glycoform mixture sufficiently pure for structural analyses by mass spectrometric means. The sample was subjected to permethylation, and five different saccharide aldol composition could be assigned by MALDI-TOF-MS, i.e., HexHexNac-ol, Hex₂HexNac-ol, HexHexNac[Hex]HexNac-ol, Hex₂HexNac-ol, HexHexNac-ol, and Hex₂HexNacHexNac-ol (Table II). The ions were detected as their Na⁺ adducts accompanied by less intense K⁺ adducts. The electrospray mass spectrum revealed a prominent abundance of HexNac-ol in addition to the compounds deduced from the MALDI-TOF mass spectrum including a major signal at m/z 535.4 corresponding to the Na⁺ adduct of the disaccharide HexHexNac-ol (Fig. 2B). An addition of sodium acetate to the sample solution led to the appearance of mainly singly charged sodium adducts in the electrospray ionization mass spectra. Ions corresponding to the assigned saccharide compositions were selected and analyzed by collision-induced dissociation. The results of these experiments are summarized in Table III. The branched structure D could be deduced from the product ions (m/z 259, 282, 285, 521, and 544) derived from the precursor m/z 780.4 (Fig. 3A, and Table III, structure D). For the precursor ion m/z 739.4, the sequence Hex-HexHexNac-ol was deduced from the product ions m/z 259 and 521 and the abundant ion m/z 463 together with the complementary ion m/z 299 (Fig. 3B and Table III, structure C). From the collision spectrum of the precursor ion at m/z 984, two branched structures (Table III, structures E1 and E2) were deduced. The product ion at m/z 285 is diagnostic for the branched structures, and together with the ions at m/z 766, 748 and 259, 504, and 530, the more abundant sequence HexHexNac-[Hex]-HexNac-ol was derived. Additional ions at m/z 282, 725, 463, and 544 suggest a further sequence, HexNac-[Hex]-HexNac-ol. The oligosaccharide aldol HexHexNac-ol was determined by the dominant product ion m/z 952 together with the less intense ions at m/z 259, 521, and 690, m/z 766 and 463, and the diagnostic...
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TABLE II

Monosaccharide compositions derived from a MALDI-TOF mass spectrum of the mixture of saccharides released from Em2(G11)

The m/z values refer to the monoisotopic peaks of the NaBD₄-reduced, permethylated saccharitols. Because of the low molecular mass cut-off setting (550 Da), only the K⁺ adduct signal was observed for the disaccharide HexGlcNAc-ol. n.d., not determined.

<table>
<thead>
<tr>
<th>M + Na⁺</th>
<th>m/z measured</th>
<th>m/z calculated</th>
<th>Saccharide composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>n.d.</td>
<td>551.35</td>
<td>551.26</td>
<td>HexGlcNAc-ol</td>
</tr>
<tr>
<td>739.41</td>
<td>755.30</td>
<td>755.36</td>
<td>HexGlcNAc-ol</td>
</tr>
<tr>
<td>780.4</td>
<td>796.38</td>
<td>796.39</td>
<td>HexGlcNAcHexGlcNAc-ol</td>
</tr>
<tr>
<td>984.53</td>
<td>1000.43</td>
<td>1000.49</td>
<td>HexGlcNAcHexGlcNAc-ol</td>
</tr>
<tr>
<td>1188.49</td>
<td>1204.47</td>
<td>1204.59</td>
<td>HexHexGlcNAcHexGlcNAc-ol</td>
</tr>
</tbody>
</table>

TABLE III

Sequence analysis of the Em2(G11) O-glycans by ESI-MS

The sequences of the permethylated saccharitols were deduced from the collision-induced dissociation product ions together with their corresponding precursor ions. The m/z values refer to the NaBD₄-reduced permethylated saccharitols.

<table>
<thead>
<tr>
<th>M + Na⁺</th>
<th>Fragment ions</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>331.2</td>
<td>169, 211, 299</td>
</tr>
<tr>
<td>B</td>
<td>535.4</td>
<td>259, 299, 347</td>
</tr>
<tr>
<td>C</td>
<td>739.4</td>
<td>259, 299, 317, 445, 463, 503, 521</td>
</tr>
<tr>
<td>D</td>
<td>780.4</td>
<td>228, 259, 285, 303, 521, 544, 562</td>
</tr>
<tr>
<td>E1</td>
<td>984.4</td>
<td>259, 285, 486, 504, 521, 530, 748, 766</td>
</tr>
<tr>
<td>E2</td>
<td>1188.4</td>
<td>259, 285, 486, 504, 521, 530, 748, 766</td>
</tr>
<tr>
<td>F</td>
<td>1188.4</td>
<td>259, 285, 303, 445, 463, 521, 530, 690, 708, 725, 748, 766, 952, 970</td>
</tr>
</tbody>
</table>

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The sequences of the permethylated saccharitols were deduced from the collision-induced dissociation product ions together with their corresponding precursor ions. The m/z values refer to the NaBD₄-reduced permethylated saccharitols.

branching point at m/z 285 (Fig. 3C and Table III, structure F). The product ions generated from the precursor ion m/z 331.2 (Table III, structure A) were identical to those observed in the MS-MS spectrum of HexGlcNAc-ol obtained from a bovine submaxillary mucin sample (data not shown).

Linkage Analysis of the Permethylated Oligosaccharitols—
The permethylated oligosaccharide alditols were subjected to acid hydrolysis, NaBH₄ reduction, peracetylation, and analyzed by GC-MS. The PMAAs were identified by their retention times and their characteristic electron impact mass spectra. The only deuterated PMAAs detected were the derivatives of terminal GalNAc-ol, 3-substituted GalNAc-ol, and 3,6-disubstituted GalNAc-ol, suggesting that GalNAc is the reducing end monosaccharide of all Em2(G11) O-glycans. 3-Substituted GalNAc-ol can be assigned to the branched structures D, E1, E2, and F (Table III). The NaBH₄-reduced PMAAs detected were the derivatives of terminal Gal, 4-substituted Gal, terminal GlcNAc, 4-substituted GlcNAc, and 4-substituted glucose (Fig. 4B). The latter component is most likely derived from a contaminating sugar. Trace amounts of glucose were also detected in the carbohydrate compositional analysis, which appeared in the sample as well as in the buffer only containing blank sample (see “Experimental Procedures”). The major peak in the total ion chromatogram corresponds to terminal Gal (Fig. 4A), derived from structures B–F (Fig. 5), whereas the PMAA of 4-substituted Gal accounts for only a minor signal compared with the derivative of terminal Gal (see Fig. 4A, peaks 1 and 3). Therefore, the main source of terminal Gal appears to be derived from structures D, B, and/or E1, because 4-substituted Gal only appears in structures C, E2, and F (Fig. 5). The PMAA of 3,6-disubstituted GalNAc-ol led to a significant signal relative to the PMAA of 3-substituted GalNAc-ol (Fig. 4A, compare peak 5 with peak 7), indicating that structures D, E1, and/or F are substantial components of the oligosaccharide alditol mixture. The slightly more intense signal of the PMAA corresponding to terminal GlcNAc compared with the signal for 4-substituted GalNAc suggests that structures D and/or E2 are more abundant than E1 and F (Fig. 4B). Because glycoform E2 contains 4-substituted Gal and the product ions from structure E2 in the MS-MS spectrum are much less intense than those from E1 (data not shown), it seems likely that the terminal GlcNAc of E2 contributes only marginally to the total amount of its PMAA (Fig. 4B, peak 6). Therefore, it appears that structures B and D constitute the most abundant glycoforms in the oligosaccharide alditol pool. These observations are in good agreement with the signal intensities seen in the ESI mass spectrum (Fig. 2B).
nal GalNAc-ol (5), 3-substituted Gal (6), 4-substituted Glc (7), and 4-substituted GlcNAc (8).

In the linear disaccharides and trisaccharides B and C, a 1–3 linkage between the terminal GalNAc-ol and the adjacent galactose may be postulated, because only PMAAs consistent with 3-substituted GalNAc-ol or 3,6-disubstituted GalNAc-ol have been detected. For this reason, the terminal GalNAc-ol of the branched structures D–F is likely to carry the Gal substituent similarly in position 3 and the additional GlcNAc residue in position 6. Hence, compounds B and C might be assumed to contain the common O-glycan core 1, whereas glycans D–F appear to be consistent with core 2 (21). The simultaneous presence of structural isomers, however, comprising GlcNAc1–3 and Gal1–6 linkages to the reducing GalNAc-ol cannot be ruled out.

Taken together, the results obtained from the GC-MS, MALDI-TOF-MS, and ESI-MS analyses suggest the structures depicted in Fig. 5 for the Em2(G11) O-glycans with B and D being the main constituents of the oligosaccharide alditol mixture.

**DISCUSSION**

From the results obtained in an earlier study on the partial characterization of Em2(G11), it was hypothesized that the antigen Em2(G11) does not contain a polypeptide moiety (5, 13). Lectin binding assays suggested the presence of glycans in this antigen with Gal, GalNAc, GlcNAc, and NANA as major constituents. This carbohydrate nature is correlated to the IgG3 subclass specificity developed by C57BL10 mice, characterized by their relative resistance to secondary alveolar echinococcosis, and furthermore to the observation that Em2(G11) induced the synthesis of low avidity IgG only including all isoatypes, and that it acts as a T-cell-independent antigen as shown by experiments with T-cell-deficient mice (5, 6). However, Lawton et al. (14) concluded on the basis of immunocross-reactivities of the Em2(G11) epitope with a peptide epitope of purified *E. multilocularis* alkaline phosphatase that part of the Em2(G11) antigen is of proteinaceous nature. To clarify the question of whether or not a polypeptide constituent is present in the Em2(G11) antigen and to obtain direct information on the carbohydrate component, aliquots of the sample were subjected to compositional analyses, which suggested the presence of mucin-type glycans being attached to a threonine- and proline-rich polypeptide. These findings are consistent with the previous results based on lectin binding assays as well as on earlier examinations on the nature of the laminated layer of the metacestode (15). Fucose or NANA was neither detectable by GC-MS compositional analysis nor by mass spectrometry of permethylated oligosaccharide alditols, indicating that the antigen O-glycans are composed solely of Gal and N-acetylgalactosamines. To obtain structural information regarding the O-glycans of the Em2(G11) antigen, O-glycans were released by reductive β-elimination. NaBH₄/NaBD₄ was used as a reducing agent to introduce a tag to the reducing monosaccharide of the saccharide alditols (Fig. 2A). Subsequently, the oligosaccharide mixture was permethylated, purified, and analyzed by mass spectrometric methods without prior separation of its components.

The MS-MS spectra revealed the presence of three linear structures (Table III, structures A–C) and four branched structures (Table III, structures D, E1, E2, and F). In the MS-MS spectra of the precursor ions *m/z* 984 and 780, minor diagnostic signals corresponding to linear glycoforms could also be observed, which might indicate the presence of additional Gal-GlcNAcGalNAc-ol and Gal-GlcNAcGalNAc-ol sequences in the O-glycan mixture. A further very weak ion at *m/z* 1392, consistent with the composition HexHexNAcHexNAc-ol, could also be observed in the MALDI-TOF and ESI-mass spectra. However, because of the low intensity of this signal, no MS-MS spectrum was recorded.

For the linkage determination, methylation analysis was employed. The proposed Em2(G11) O-glycan structures deduced from the GC-MS, MALDI-TOF-MS, and ESI-MS data gave rise to the hypothesis that they are synthesized via three divergent biosynthetic pathways starting with the initiation of O-glycosylation by means of a polypeptide GalNAc-transferase. Divergence of the pathway occurs after elongation of GalNAc with Gal in 1–3 linkage and a second time after the transfer of GlcNAc-GalNAc (Fig. 5). The presence of a Gal1–3GalNAc-ol linkage in structures B and C can be directly deduced from our data. Therefore, a Gal1–3GalNAc-ol linkage and consequently a GlcNAc1–6GalNAc-ol substitution are more likely to occur in the branched structures D–F than the reverse pattern.

Finally, the biosynthesis might be terminated with structures C, E2, and F. It is noticeable that these structures share a non-reducing end Gal1–4Gal1 motif, which might have a capping or masking function compared with that of sialylation or sulfation (16). Mucin-type core structures elongated with such digalactose motifs have not been described in the earlier literature. However, Gal repeats appear to be cestode-specific motifs. In glycosphingolipids isolated from the adult stage of *Metropoliathes coturnix*, metacestodes of *Toenia crassiceps* and *E. multilocularis* Galβ1–4Gal and Galβ1–6Gal sequences have been demonstrated, and the latter motif was suggested to constitute an epitope recognized by sera from alveolar echinococcosis patients (17–19). In this context, it would be informative to determine the epitope recognized by the MAbG11 as used in
are described in the paper. Nevertheless, the presence of an \( /H9251 \) lacto-N-3\[NANA\] available, and to date, no further elongated core 8 chronic bronchitis patient (27). However, to our knowledge, elevated carbohydrate antigen CA 19-tode stage of the parasite and by the frequent expression of core 1 as a terminal structure, also referred to as the epitope to be the most abundant ones of the Em2(G11) (reviewed in Ref. 22). A basic link among cancer and the metastasis and subject to vaccine development against cancer and inflammation. These glycans also play a role in immunodeficiencies like T-cell leukemia, Wiskott-Aldrich syndrome, and acquired immune deficiency syndrome (28). Hence, the high abundance of structure D within the Em2(G11) antigen might contribute to its immunomodulating properties during establishment of the infection with E. multilocularis metacestodes (reviewed in Ref. 3). One of the interesting remaining questions concerns the functional relationship between the individual structural components of the Em2(G11) and the TI-2 epitope composition of the antigen that may be experimentally addressed by epitope mapping. Possibly, the bulkiness of the branched saccharides together with the linear glycan structures may be important attributes in determining the tertiary structure of the Em2(G11) antigen, hence influencing the vesicle shape formation of the metacestode within the liver tissue. The findings of this study may provide a molecular basis to investigate the host-metacestode interplay in more detail.

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REFERENCES

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