Further structural characterization of the *Echinococcus granulosus* laminated layer carbohydrates: The blood-antigen P<sub>1</sub>-motif gives rise to branches at different points of the O-glycan chains

Gerardo Lin<sup>2</sup>, Adriane R Todeschini<sup>3</sup>, Akihiko Koizumi<sup>4</sup>, Jorge L Neves<sup>3,6</sup>, Humberto González<sup>5</sup>, Sylvia Dematteis<sup>2</sup>, Noriyasu Hada<sup>4</sup>, Jose O Previato<sup>3</sup>, Fernando Ferreira<sup>5</sup>, Lucia Mendonça-Previato<sup>3</sup>, and Alvaro Díaz<sup>1,2</sup>

<sup>1</sup>Cátedra de Inmunología, Departamento de Biociencias (Facultad de Química) e Instituto de Química Biológica (Facultad de Ciencias), Universidad de la República, Montevideo, Uruguay; <sup>2</sup>Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Cidade Universitária, 21941-902, Rio de Janeiro, Brazil; <sup>3</sup>Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan; and <sup>4</sup>Laboratorio de Carbohidratos y Glicoconjugados, Departamento de Química Orgánica, Facultad de Química/Facultad de Ciencias/Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

Received on November 13, 2012; revised on December 17, 2012; accepted on December 17, 2012

The glycobiology of the cestodes, a class of parasitic flatworms, is still largely unexplored. An important cestode species is *Echinococcus granulosus*, the tissue-dwelling larval stage of which causes hydatid disease. The *E. granulosus* larva is protected from the host by a massive mucin-based extracellular matrix termed laminated layer (LL). We previously reported (Díaz et al. 2009) that the blood-antigen P<sub>1</sub>-motif gives rise to branches at different points of the O-glycan chains described but in cases bear GlcNAcβ1-6 or Galβ1-4GlcNAcβ1-6 as ramifications on the core Galβ1-3 residue. We also obtained evidence that noncore Galβ1-3 residues can be similarly ramified. Thus, the new motif together with the previous information may explain all the glycan compositions detected in the LL by MS. In addition, we show that the anti-*Echinococcus* monoclonal antibody E492 *(Parasite Immunol 21:141, 1999)* recognizes Galβ1-4Galβ1-4GlcNAc (the blood P<sub>1</sub>-antigen motif). This explains the antibody’s reactivity with a range of *Echinococcus* tissues, as the P<sub>1</sub>-motif is also carried on non-LL N-glycans and glycolipids from this genus.

**Keywords:** cestode / *Echinococcus* / mucin / O-glycan / P<sub>1</sub>-blood group antigen

**Introduction**

The phylum platyhelmintha includes two branches of parasitic helminths, trematodes and cestodes. Both include important human and livestock parasites. Compared with trematodes (and in particular with the genus *Schistosoma*), cestodes are understudied, in spite of comprising the medically and economically important genera *Taenia* and *Echinococcus*. Transcriptomic and genomic data for these parasites are becoming available only recently (Olson et al. 2012; Parkinson et al. 2012), and the available glycochemical information on them is indeed very fragmentary (Persat et al. 1992; Khoo et al. 1997; Hulsmeier et al. 2002, 2010; Lee et al. 2005; Díaz et al. 2009; Paschinger et al. 2012) (reviewed in Wuhler and Geyer 2006).

The larval stage of *Echinococcus granulosus* is the causing agent of cystic echinococcosis (hydatid disease) in livestock and humans, while the *E. multilocularis* larva causes life-threatening alveolar echinococcosis in humans (Brunetti and White 2012; Nunnari et al. 2012). The *E. granulosus* larva takes the form of a fluid-filled subspherical structure (hydatid) that grows by concentric enlargement within host organ parenchymas. This larva is bounded by the hydatid wall, which comprises a thin inner layer of cells (germinal layer) and an outer, protective acellular layer termed the laminated layer (LL). The LL is a massive, mucin-based extracellular matrix,
present only in the genus *Echinococcus* (Díaz, Casaravilla, Allen et al. 2011; Diaz, Casaravilla, Irigoín et al. 2011). In *E. granulosus*, but not *E. multilocularis*, the LL comprises, in addition to the mucins, nano-deposits of the calcium salt of *myo*-inositol hexakisphosphate (Irigoín et al. 2002, 2004; Casaravilla et al. 2006).

Since the LL is extremely carbohydrate-rich and apparently devoid of nonmucin structural proteins (Casaravilla and Diaz 2010), *Echinococcus* larvae expose to the host mainly carbohydrate. Given that carbohydrates are central in the decoding of pathogens by the innate branch of the immune system, the structure of the LL mucin glycans is a very important input for viral *Echinococcus* immunology. The monosaccharide composition of the crude LL includes only galactose (Gal), *N*-acetylgalactosamine (GALNAc) and *N*-acetylglucosamine (GlcNAc) as detectable sugars (Kilejian, Sauer and Schwabe, 1962; Korc et al. 1967; Kilejian and Schwabe (1971)), suggesting that the dominant carbohydrates are mucin-type O-glycans, with a negligible contribution from N-glycans in particular. In our previous work (Díaz et al. 2009), we reported the structure of the major carbohydrates of the crude LL, which turned out to be indeed mucin-type O-glycans. In that work, we fractionated, by gel filtration as the first step, the LL oligosaccharide-alditols, released from mucin backbones by reductive β-elimination. The most abundant glycans comprised only two and three mono-carbohydrate residues, but larger glycans were present, in abundances that decreased with increasing size. We sub-fractionated the gel filtration fractions corresponding to glycans between two and six residues in size on a porous graphitized carbon column (PGC) and elucidated 10 major components. These were based on the conventional mucin-type O-glycan Core 1 (Galβ1-3GalNAc) or Core 2 (Galβ1-3GlcNAcβ1-6GALNAc). The Galβ1-3 residue of either core was elongated with a variable number of Galβ1-3 residues, thus forming a Galβ1-3 main chain (defined as including the mentioned core residue). This chain could be capped with a single Galα1-4 residue, depending on its length. Thus, capping seemed to be always absent for a main chain length of one residue, present or absent for a length of two residues, and always present for chain lengths of three or more residues. In combination with these main chain length and capping variants, Core 2-based glycans could be (or not) decorated on their core GlcNAc residue with the disaccharide Galα1-4Galβ1-4. The Galα1-4Galβ1-4GlcNAcP (P1) trisaccharide, which in the context of Core 2 or the new context found in the present work, can be used to deduce the presence of this motif in glycans of unknown structure.

**Results**

*The LL glycome is dominated by short glycans irrespective of host species*

The most common *E. granulosus* strain worldwide, the G1 or sheep strain is not well adapted to cattle as intermediate hosts. As a consequence, inflammatory resolution does not take place, and instead a granulomatous response chronically surrounds the parasite (reviewed in Diaz, Casaravilla, Allen et al. 2011). Since in our previous work (Díaz et al. 2009) we had only used material from cattle hosts, it was important to determine whether the observed predominance of short glycans, including the naked Cores 1 and 2, was representative of the parasite’s intrinsic biology or was instead a peculiarity of the host–parasite strain mismatch. Therefore, we compared by gel filtration the size distribution of the LL glycans previously released by reductive β-elimination, for samples from a number of host species. In spite of minor quantitative differences, the profiles were very similar (Figure 1), being dominated by fractions C and D, which contain the nondecorated Cores 1 and 2 plus a glycan consisting of Core 1 decorated with a single Galβ1-3 residue (Díaz et al. 2009). Of note, this analysis included so-called daughter hydatids, which develop within larger hydatids and are thus mostly shielded from host inflammatory mediators. For the major natural host, namely sheep, we also obtained hexose/*N*-acetylhexosamine compositions by MS for fractions C-J, encompassing glycans between two and nine residues in size (Supplementary data, Table S1): These were essentially the same as those observed previously for material from cattle. Also for material of sheep
origin, we further studied the same gel filtration fractions analyzed in depth in our previous report (Díaz et al. 2009). The PGC high pressure (or high performance) liquid chromatography (HPLC) profiles were the same as those previously observed for cattle-derived material and all the major glycans fully elucidated in the previous analysis were present as major components on the basis of MS2 analysis (data not shown; spectral data are indistinguishable from those published in Díaz et al. 2009). Therefore, in spite of the presence of long glycans, the LL glycome is dominated by short glycans, and this is intrinsic to the parasite’s biology, irrespective of how permissive the host is.

**Elucidation of LL glycans 6–8 residues in size reveals GlcNAcβ1-6 branches independent of Core 2, which can be further decorated with Galα1-4Galβ1-4**

In our previous work, we had sub-fractionated by HPLC and analyzed in depth only gel filtration fractions corresponding to the glycans of small molecular size (up to fraction G, containing glycans of five and six residues). As mentioned in the Introduction section, an in depth analysis of fractions corresponding to larger glycan sizes was necessary for determining the nature of HexNAc residues detected by MS1 that could not be explained by the structural motifs found in the previous work (Díaz et al. 2009). Therefore, we analyzed gel filtration fractions H, I and J (the latter two pooled together; all from cattle-host material).

Fractions H yielded a complex profile upon PGC HPLC (Figure 2). Its most abundant sub-fraction (H2) was deduced its chromatographic retention time and MS2 analysis (Supplementary data, Table S2) to contain a glycan identical to a component elucidated in our previous work (Díaz et al. 2009) as part of gel filtration fraction G (glycan G3; Galβ1-3Galβ1-3(Galα1-4Galβ1-4GlcNAcβ1-6)GalNAc-ol). Similarly, the latest-eluting major sub-fraction, H8, was deduced from its chromatographic retention time and MS2 spectrum (Supplementary data, Table S2) to contain a glycan identical to that in sub-fraction G7, elucidated in the previous work (Galα1-4Galβ1-3Galβ1-3Galβ1-3Galβ1-3GalNAc-ol).

Then three novel structures from this gel filtration fraction were fully elucidated (Figure 3 and Tables I and II). The glycan in sub-fraction H1 features two HexNAc residues in addition to the GalNAc-ol residue. It was elucidated as Galβ1-3Galβ1-3(Galα1-4Galβ1-4GlcNAcβ1-6)GalNAc-ol, i.e. a Core 2 glycan with a second GlcNAc residue generating a ramification on the core Gal residue. The main glycan in sub-fraction H3 (Galα1-4Galβ1-3Galβ1-3(Galα1-4Galβ1-4GlcNAcβ1-6)GalNAc-ol) is an analogue of structure G3 (Díaz et al. 2009) that has been capped with a Galα1-4 residue on its terminal Galβ1-3 residue, which follows previously observed structural trends. The glycan in sub-fraction H7 (Galα1-4Galβ1-3Galβ1-3(GlcNAcβ1-6)Galβ1-3GalNAc-ol) is related to that in sub-fraction H1 and also features a GlcNAcβ1-6 residue decorating the core Gal residue; however, it is based on Core 1 instead of Core 2, and its Galβ1-3 main chain has an additional residue. Finally for this fraction, one major peak (H6) could not be fully elucidated due to insufficient material. However, the combined MS2 and linkage analysis data (Supplementary data, Tables S2 and S3, respectively),
together with the extrapolation of structural features found in the remaining glycans, allow us to propose the structure Galpα1-4Galpβ1-3Galpβ1-3Galpβ1-3(GlcNAcpβ1-6)GalNAc-ol. Thus, this glycan is probably a homolog of glycan G2 elucidated in the previous work featuring an additional residue in the Galpβ1-3 main chain.

Fraction IJ gave a very complex chromatographic profile (Figure 2). Sub-fractions IJ1 and IJ3 were found to contain the same glycans found in sub-fractions H1 and H3, described in the previous paragraph, respectively (Figure 3). The major glycan in sub-fraction IJ8 was fully elucidated as Galpα1-4Galpβ1-3Galpβ1-3(GlcNAcpβ1-6)Galpβ1-3GalNAc-ol. This product is thus similar to that from sub-fraction H7 but bears the Galpα1-4Galpβ1-4 disaccharide as decoration of the branching GlcNAc residue. For sub-fraction IJ10, both MS² and linkage analysis data indicated the presence of mutually incompatible structural elements (Table I and Figure 3). Similarly, the ¹H nuclear magnetic resonance (NMR) spectrum of this sub-fraction presented two partially superimposed doublets for the anomeric proton corresponding to the core Galp residue, which collectively integrated to a single proton (Supplementary data, Figure S1, A). Further, the HSQC spectrum of this sub-fraction presented two pairs of crosspeaks for position 6 of the GalNAc-ol residue (Table II; Supplementary data, Figure S1, B); one pair of signals presented ¹³C shifts typical of a substituted position (71.1 ppm), while the other pair presented ¹³C shifts typical of a nonsubstituted position (63.4 ppm). Similarly, for position 6 of the core Galp residue, HSQC signals with ¹³C shifts corresponding both to a nonsubstituted (61.5 ppm) and to a substituted (70.0 ppm) position were observed (Table II). In sum, the data are fully consistent with the coexistence of two positional isomers differing in the placement of the single GlcNAcβ1-6 residue. These isomers have the structures Galpα1-4Galpβ1-3Galpβ1-3Galpβ1-3(GlcNAcβ1-6)GalNAc-ol (IJ10A) and Galpα1-4Galpβ1-3Galpβ1-3Galpβ1-3(GlcNAcβ1-6)Galpβ1-3GalNAc-ol (IJ10B). Thus, IJ10A is similar to the glycan from sub-fraction G2 elucidated in the previous work, but features a Galpβ1-3 main chain two residues longer. Glycan IJ10B is similar to the glycan from sub-fraction H7 described above but with one additional residue in the Galpβ1-3 main chain. For sub-fraction IJ2, although the amount of material obtained was not enough for NMR, the data from MS² (Supplementary data, Table S2), together with extrapolation of structural features found in the remaining glycans, allow us to propose the structure Galpβ1-3(GlcNAcβ1-6)Galpβ1-3(Galpα1-4Galpβ1-4GlcNAcβ1-6)GalNAc-ol: This glycan would result from the addition of a GlcNAcβ1-6 branch onto the core Gal residue of glycan G3 elucidated in the previous work (Díaz et al. 2009). With similar arguments, the glycan in sub-fraction IJ5 (Supplementary data, Table S2) is probably Galpα1-4Galpβ1-3Galpβ1-3(GlcNAcβ1-6)Galpβ1-3(Galpα1-4Galpβ1-4GlcNAcβ1-6)GalNAc-ol, i.e. an analog of the glycan in sub-fraction H1 with one more residue in the main chain. Sub-fraction IJ7 was found by MS to be heterogenous and to be dominated by a component indistinguishable by MS² (Supplementary data, Table S2) to that previously elucidated from the contiguous sub-fraction (IJ8). Sub-fraction IJ9, in spite of appearing as a symmetrical chromatographic peak, was determined by MS² (Supplementary data, Table S2) to contain at least two isobaric components, the elucidation of which was not possible.

In sum, six novel structures were fully elucidated, arising from five sub-fractions. The new glycans are based on the same set of structural motifs deduced from the smaller products previously elucidated (Díaz et al. 2009). However, they additionally show that the Core (1 or 2) Galp residue can be ramified with a GlcNAcβ1-6 residue, which can either be left without further decoration (H1, H7 and IJ10A) or can be further decorated with the Galpα1-4Galpβ1-4 disaccharide (IJ8), similar to what had been previously observed in the context of the Core 2 GlcNAc residue. In addition to the fully elucidated structures, a large number of glycans were studied by MS² only (H6, IJ2 and IJ5 mentioned above and remaining glycans on Supplementary data, Table S2). All the structures gleaned from these partial data appear to confirm the same motifs already mentioned. In a number of cases, and similar to the previous work (Díaz et al. 2009), glycans with Hex-ol reducing ends were found, which are probably due to peeling reactions.
Fig. 3. MALDI-TOF-TOF MS2 of permethylated oligosaccharide-alditols. The sub-fraction names are the same as given in Figure 2. Sub-fractions H1 and IJ1, originating from different gel filtration fractions, were found to contain the same glycans, and are presented together; the same applies to sub-fractions H3 and IJ3. All masses correspond to monoisotopic values of Na+ adducts. Masses shown are experimental ones, and they correspond to the expected masses to within 0.1 Da for pseudomolecular ions and to within 0.3 Da for fragments. Only fragment signals having a signal/noise ratio of at least 10 and an intensity corresponding to at least 3% of that of the strongest signal in the sample are presented. Names of fragments follow the nomenclature of Domon and Costello (1988), with the alditol residue having been assigned the subscript 1. Fragments arising from double (internal) breaks are given in terms of intersections of pairs of single-break fragments; thus, for example C3αxY2α″ describes the fragment defined by the intersection of fragments C3α and Y2α″. Structures shown correspond to deductions based on MS data only. The assignment of fragments is presented on the structures, and in some cases for need of clarity, on the spectra. For sub-fraction IJ10, MS2 as well as linkage analysis (Table I) and NMR data (Table II) indicated that two isomeric compounds were present in similar proportions. Hence, for this sub-fraction two structures are presented (named IJ10A and IJ10B), and signals that are diagnostic of either structure are shown in bold.
The 3,6-disubstituted GalNAc-ol residues were evidenced by the appearance of 1,4,5-trimethyl, N-methyl, 3,6-anhydro N-acetylgalactosaminitol, identifiable by its MS fragmentation pattern (Wieruszeski et al. 1987). No attempt was made to determine molar ratios between the different partially methylated acetylated methyl glycosides obtained as significant components. The signal for 3,6-disubstituted Gal in sub-fraction H3 may arise from a contaminating product probably containing this residue (see Supplementary data, Table S2). For sub-fraction IJ10, linkage analysis as well as MS2 (Figure 3) and NMR data (Table II) indicated that two isomeric compounds were present in similar proportions. The data for this sub-fraction are therefore presented as if for two separate components (named IJ10A and IJ10B), with signals that are diagnostic of either structure underlined.

Table I. Linkage analysis of selected purified products

<table>
<thead>
<tr>
<th>Sub-fraction</th>
<th>Result of linkage analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>terminal Galp, 4-subst Galp, 3,6-disubst Galp, terminal GlcNAcp, 3,6-disubstit GalNAc-ol</td>
</tr>
<tr>
<td>H3</td>
<td>terminal Galp, 4-subst Galp, 3-subst Galp, 4-subst GlcNAcp, 3,6-disubstit GalNAc-ol (3,6-disubstit Galp)</td>
</tr>
<tr>
<td>IJ8</td>
<td>terminal Galp, 4-subst Galp, 3-subst Galp, 3,6-disubstit Galp, 4-subst GlcNAcp, 3-substit GalNAc-ol</td>
</tr>
<tr>
<td>IJ10A</td>
<td>terminal Galp, 4-subst Galp, 3-subst Galp, terminal GlcNAcp, 3,6-disubstit GalNAc-ol</td>
</tr>
<tr>
<td>IJ10B</td>
<td>terminal Galp, 4-subst Galp, 3-subst Galp, 3,6-disubstit Galp, terminal GlcNAcp, 3-substit GalNAc-ol</td>
</tr>
</tbody>
</table>

The E492 monoclonal antibody recognizes the P1 blood-antigen motif

The anti-carbohydrate E492 monoclonal antibody, generated by immunizing mice with E. granulosus protoscoleces (the stages infective for the dog definitive host, found within hydatid tumors), purifies from protoscoleces themselves, and also from E. multilocularis metacestode, carbohydrate-rich fractions that contain T-independent antigens and other immunological active components (Baz et al. 1999, 2008; Dematteis et al. 2001; Cardozo et al. 2002; Walker et al. 2004; Mourglias-Ettlin et al. 2011). The binding of this antibody to crude E. granulosus materials had been previously shown to be inhibited by the synthetic disaccharide Galpα1-4Galp, but not by Galpα1-6Glcp or Galpβ1-4Glcp (Baz et al. 1999). As this antibody reacts with both E. multilocularis (Walker et al. 2004) and E. granulosus LL (our unpublished results), its candidate epitopes were narrowly constrained. Indeed, in terms of potential trisaccharide motifs, it could be reasoned that the antibody had to recognize either Galpα1-4Galpβ1-3Galp (the capped end of the LL glycan main chain) or Galpα1-4Galpβ1-4GlcNAcp (the P1 blood-antigen motif). We tested the reactivity of the antibody against five synthetic glycans (Koizumi et al. 2011) corresponding to the structures reported for the E. multilocularis Em2 mucin, with anomerities inferred from those reported by us for analogous E. granulosus glycans (Diaz et al. 2009). The antibody only bound glycan “K” (Figure 4A), with the structure Galpβ1-3 (Galpα1-4Galpβ1-4GlcNAcp)GalNAc, i.e. the only glycan in the panel comprising the P1 blood-antigen motif. Then three synthetic (nonbiotinylated glycans) were tested as soluble competitors of the binding to immobilized glycan K: As expected, the product with the same carbohydrate as glycan K inhibited binding, while a related compound with inverted anomericity in the terminal residue of the P1 motif did not (Figure 4B). Finally, a series of LL oligosaccharide-alditol sub-fractions from gel filtration fractions H and IJ were tested for inhibition (Figure 4C). These glycans were derived from sheep host material (Supplementary data, Table S4). For five of the sub-fractions tested (H1, H2, H3, IJ8 and IJ10), the full structures of the major components were known, on the basis of identical MS2 spectra and same HPLC retention orders as the fully elucidated glycans derived from bovine material. For these five sub-fractions, no inhibition was observed for components that do not carry the P1 motif (H1 and IJ10), while clear inhibition was observed for products carrying this motif (H2, H3 and IJ8). Of note, while in the synthetic glycans K and the glycans in sub-fractions H2 and H3 the P1 motif comprises the Core 2 GlcNAcp residue, in the glycan from sub-fraction IJ8 the same motif is carried as a branch decorating the core Gal residue. This suggests strongly that the antibody recognizes the Galpα1-4Galpβ1-4GlcNAcp trisaccharide as such, independent of the structure that it is decorating. A further four sub-fractions tested contained glycans for which only MS2 information was available (Supplementary data, Table S4): Of these, sub-fractions H4, H8 and IJ4 inhibited binding, while sub-fraction IJ7 did not. This correlated perfectly well with the presence vs absence of a Hex-Hex-HexNAc trisaccharide in the sequences deduced by MS2. This suggests that the Hex-Hex-HexNAc trisaccharide, found in many LL O-glycan structures (Diaz et al. 2009; Supplementary data, Table S2) may in all cases correspond to Galpα1-4Galpβ1-4GlcNAcp. Importantly, this deduction applies to the major component in sub-fraction H8, which by MS2 (Supplementary data, Table S4) has the sequence Hex-(Hex-HexNAc)Hex-HexNAc-ol: This indicates that the P1 trisaccharide can also decorate noncore main chain Gal residues.

Of note, although the Galpα1-4Galpβ1-3Galp trisaccharide was mentioned above as an initial E492 antibody epitope candidate, the synthetic glycan panel used did not comprise this trisaccharide as such. Instead, the panel contained a product (glycan H; Figure 4A) carrying the related motif Galpα1-4Galpβ1-3GalNAcp; this is a consequence of the fact that, in the E. multilocularis Em2 mucin but not in the E. granulosus LL, the Galpα1-4cap appears to be placed directly onto the core Gal residue. The fact that E492 antibody binding was not inhibited by sub-fraction IJ10, containing two related glycans ended in Galpα1-4Galpβ1-3Galp (Figure 4C), confirms that this trisaccharide is indeed not recognized by the antibody.

Discussion

In this work, we first showed that the E. granulosus LL glycome is dominated by small glycans (nondecorated Cores 1 and 2, Core 1 decorated with a single Galpβ1-3 residue) irrespective of whether the parasite develops in a permissive or restrictive host species. Small differences in abundances of specific glycans were detected, particularly in fractions H and IJ (six to eight residues in size; data not shown). We did not analyze a large enough panel of samples as to discriminate whether these differences arose from inter-individual or host species differences. We did however ascertain that all major glycans previously reported in material from the restrictive cattle host are equally major components in material from the permissive sheep host.

Six novel LL O-glycans were fully elucidated. These are, like the previously known glycans, based on either Core 1 or
Table II. NMR analysis of purified products

<table>
<thead>
<tr>
<th>Residues</th>
<th>Chemical shifts (ppm)</th>
<th>H-1</th>
<th>H-2</th>
<th>H-3</th>
<th>H-4</th>
<th>H-5</th>
<th>H-6</th>
<th>H-6′</th>
<th>NAc</th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
<th>C-6</th>
<th>NAc</th>
</tr>
</thead>
<tbody>
<tr>
<td>GalNAc-ol</td>
<td>3.75; 3.80</td>
<td>4.39</td>
<td>4.05</td>
<td>4.24</td>
<td>3.70</td>
<td>3.70</td>
<td>3.95</td>
<td>2.06</td>
<td>61.1</td>
<td>51.7</td>
<td>77.6</td>
<td>69.4</td>
<td>68.1</td>
<td>71.0</td>
<td>22.7</td>
<td></td>
</tr>
<tr>
<td>Galp(2α)</td>
<td>4.54 (8.4 Hz)</td>
<td>3.76</td>
<td>3.84</td>
<td>4.15</td>
<td>3.89</td>
<td>3.79</td>
<td>3.98</td>
<td>–</td>
<td>102.0</td>
<td>72.4</td>
<td>82.2</td>
<td>68.1</td>
<td>73.8</td>
<td>69.6</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>GlcNAcp(2β)</td>
<td>4.54 (7.7 Hz)</td>
<td>3.71</td>
<td>3.55</td>
<td>3.45</td>
<td>3.49</td>
<td>3.76</td>
<td>3.94</td>
<td>2.07</td>
<td>102.0</td>
<td>55.9</td>
<td>74.3</td>
<td>70.5</td>
<td>74.7</td>
<td>61.1</td>
<td>22.7</td>
<td></td>
</tr>
<tr>
<td>Galp(3α')</td>
<td>4.70 (7.8 Hz)</td>
<td>3.68</td>
<td>3.77</td>
<td>4.06</td>
<td>3.77</td>
<td>3.88</td>
<td>3.64</td>
<td>–</td>
<td>105.3</td>
<td>71.3</td>
<td>72.8</td>
<td>77.9</td>
<td>75.6</td>
<td>60.6</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>GlcNAcp(3α″)</td>
<td>4.58 (8.4 Hz)</td>
<td>3.70</td>
<td>3.55</td>
<td>3.45</td>
<td>3.49</td>
<td>3.78</td>
<td>3.94</td>
<td>2.07</td>
<td>102.5</td>
<td>55.9</td>
<td>74.3</td>
<td>70.5</td>
<td>76.1</td>
<td>61.0</td>
<td>22.7</td>
<td></td>
</tr>
<tr>
<td>Galp(4α')</td>
<td>4.98 (7.8 Hz)</td>
<td>3.86</td>
<td>3.94</td>
<td>4.04</td>
<td>4.38</td>
<td>3.83</td>
<td>*</td>
<td>–</td>
<td>100.6</td>
<td>69.7</td>
<td>69.7</td>
<td>69.7</td>
<td>71.4</td>
<td>61.1</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Continued
Table II. (Continued)

<table>
<thead>
<tr>
<th>Sugar</th>
<th>H7</th>
<th>IJ8</th>
</tr>
</thead>
<tbody>
<tr>
<td>GalNAc-ol</td>
<td>3.71; 3.78</td>
<td>3.72; 3.80</td>
</tr>
<tr>
<td>Galp(2)</td>
<td>4.55 (8.0 Hz)</td>
<td>4.40</td>
</tr>
<tr>
<td>Galp(3α)</td>
<td>4.68 (7.4 Hz)</td>
<td>3.76</td>
</tr>
<tr>
<td>GlcNAc(3β)</td>
<td>4.60 (8.7 Hz)</td>
<td>3.76</td>
</tr>
<tr>
<td>Galp(4α)</td>
<td>4.70 (7.9 Hz)</td>
<td>3.67</td>
</tr>
<tr>
<td>Galp(5α)</td>
<td>4.98 (3.4 Hz)</td>
<td>3.87</td>
</tr>
<tr>
<td>GalNAc-ol</td>
<td>3.74</td>
<td>4.16</td>
</tr>
<tr>
<td>Galp(3α)</td>
<td>4.18</td>
<td>3.82</td>
</tr>
<tr>
<td>GlcNAc(3β)</td>
<td>4.34</td>
<td>3.67</td>
</tr>
<tr>
<td>Galp(4α)</td>
<td>4.05</td>
<td>4.04</td>
</tr>
<tr>
<td>Galp(5α)</td>
<td>4.38</td>
<td>4.36</td>
</tr>
</tbody>
</table>

Continued
2. They differ from the previous glycans mainly in that the \(\text{Galp}\) \(\beta_1-3\) main chain is longer, reaching four residues in glycan $\text{IJ10A}$. In agreement with the previously observed trend, the \(\text{Galp}\) \(\beta_1-3\) main chain, which is at least two residues long in these glycans, is in all cases capped with \(\text{Galp}\) \(\alpha_1-4\). In contrast with the previously elucidated glycans, in four cases the Core \((1 \text{ or } 2)\) \(\text{Galp}\) \(\beta_1-3\) residue is di-substituted, carrying a \(\text{GlcNAcp}\) \(\beta_1-6\) residue in addition to the next \(\text{Galp}\) \(\beta_1-3\) residue of the main chain. The ramifying \(\text{GlcNAcp}\) \(\beta_1-6\) residue is in one case further decorated with the galabiose disaccharide, thus giving rise to the blood-antigen P1 motif observed in the previous work but in a different context, i.e. comprising the Core 2 \(\text{GlcNAc}\) residue. Thus, all the sixteen LL glycans fully elucidated to date are clearly related, as summarized in Figure 5.

The \(\text{GlcNAcp}\) \(\beta_1-6\) branching on the main chain, found in some of the newly elucidated glycans, helps explain the monosaccharide compositions detected in the LL by MS1 and not explained by previously known structures. Strictly, the new motif explains compositions comprising two HexNAc residues (excluding the reducing end \(\text{GalNAc}\)). However, MS1 data for gel filtration fraction N (containing glycans 13–18 residues in size) indicate compositions comprising 3 and 4 HexNAc residues (Díaz et al. 2009). The glycan in sub-fraction H8 (from sheep host) is deduced from MS2 data to have the sequence Hex-(Hex-Hex-HexNAc).

<table>
<thead>
<tr>
<th>Table II. (Continued)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th></th>
<th>$\text{Galp}(6\alpha)$</th>
<th>$\text{Galp}(5\alpha)$</th>
<th>$\text{Galp}(4\alpha)$</th>
<th>$\text{Galp}(3\alpha)$</th>
<th>$\text{Galp}(2\alpha)$</th>
<th>$\text{GlcNAc-ol}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{GalNAc-ol}^{\text{IJ10A}}$</td>
<td>3.71; 3.85</td>
<td>4.40</td>
<td>4.09</td>
<td>3.46</td>
<td>4.28</td>
<td>3.64</td>
</tr>
<tr>
<td>$\text{GalNAc-ol}^{\text{IJ10B}}$</td>
<td>3.71; 3.85</td>
<td>4.40</td>
<td>4.09</td>
<td>3.46</td>
<td>4.28</td>
<td>3.64</td>
</tr>
<tr>
<td>$\text{Galp}(2\alpha)^{\text{IJ10A}}$</td>
<td>4.55 (7.7 Hz)</td>
<td>3.72</td>
<td>3.85</td>
<td>4.17</td>
<td>3.77</td>
<td>3.76</td>
</tr>
<tr>
<td>$\text{Galp}(2\alpha)^{\text{IJ10B}}$</td>
<td>4.55 (7.7 Hz)</td>
<td>3.72</td>
<td>3.85</td>
<td>4.17</td>
<td>3.89</td>
<td>3.98</td>
</tr>
<tr>
<td>$\text{GlcNAc}^{(2\beta/3\beta)}$</td>
<td>4.60 (7.9 Hz)</td>
<td>3.72</td>
<td>3.56</td>
<td>3.46</td>
<td>3.46</td>
<td>3.77</td>
</tr>
<tr>
<td>$\text{GlcNAc}^{(2\beta/3\beta)}$</td>
<td>4.60 (7.9 Hz)</td>
<td>3.72</td>
<td>3.56</td>
<td>3.46</td>
<td>3.46</td>
<td>3.77</td>
</tr>
<tr>
<td>$\text{Galp}(3\alpha)$</td>
<td>4.69 (7.7 Hz)</td>
<td>3.75</td>
<td>3.88</td>
<td>4.22</td>
<td>3.72</td>
<td>3.78</td>
</tr>
<tr>
<td>$\text{Galp}(4\alpha)$</td>
<td>4.69 (7.7 Hz)</td>
<td>3.80</td>
<td>3.88</td>
<td>4.17</td>
<td>3.72</td>
<td>3.78</td>
</tr>
<tr>
<td>$\text{Galp}(5\alpha)$</td>
<td>4.72 (7.5 Hz)</td>
<td>3.66</td>
<td>3.76</td>
<td>4.05</td>
<td>3.77</td>
<td>3.90</td>
</tr>
<tr>
<td>$\text{Galp}(6\alpha)$</td>
<td>4.98 (3.9 Hz)</td>
<td>3.85</td>
<td>3.93</td>
<td>4.03</td>
<td>4.39</td>
<td>3.74</td>
</tr>
</tbody>
</table>

$^{13}$C shifts were obtained from HSQC spectra, and hence the shifts of the carbonyl carbons could not be obtained. Coupling constants are given between parentheses, only for H-1 atoms of monosaccharide residues. Residues within each structure are named analogously to the Domon and Costello (1988) nomenclature for fragments, assigning the (implicit) number 1 to the alditol residue. Correlations, from ROESY spectra, are indicated on the structures: Solid arrows indicate correlations between perfectly resolved protons, and dotted arrows those between a well-defined proton and a second proton whose signal is superimposed with that of a third proton. For sub-fraction IJ10, MS2 (Figure 3) and linkage analysis data (Table I) as well as NMR data (see also Supplementary data, Figure S1) indicated that two isomeric compounds (named IJ10A and IJ10B) were present in similar proportions. These two compounds differ only in the position of the \(\text{GlcNAc}\) residue. The residues substituted by this \(\text{GlcNAc}\) residue are therefore the only ones that present significant differences in chemical shifts between the two compounds: For these two residues, chemical shifts are given separately for the two compounds (\(\text{GalNAc-ol}^{\text{IJ10A}}, \text{GalNAc-ol}^{\text{IJ10B}}; \text{Galp}(2\alpha)^{\text{IJ10A}}, \text{Galp}(2\alpha)^{\text{IJ10B}}\)). Asterisk denotes that chemical shift values could not be obtained due to signal superimposition.
Hex-fraction reacts with the E492 monoclonal antibody
(Figure 4c), found in this work to be specific for the P1 motif,
it is deduced that in this glycan the P1 trisaccharide decorates
the second Gal residue from the reducing end along the main
chain. In other words, this suggests that the P1 motif can dec-
orate residues further out along the Galβp1-3 main chain from
the core residue. It is thus likely that the large glycans
detected comprising more than 2 HexNAc residues carry
GlcNAcβp1-6 and/or Galαp1-4GlcNAcβp1-6 ramifications on
different points along the Galβp1-3 main chain, possibly in combination with being Core 2-based. In sum, all
the monosaccharide compositions observed by MS1 in the LL
glycome (Diaz et al. 2009) can in all likelihood be explained
by the structural motifs found in the previous and present
works. These motifs are those summarized in our recent
review article (Diaz, Casaravilla, Irigoín et al. 2011), with the
precision that Hex-HexNAc branches on the Galβp(1-3)
main chain are indeed, as speculated, P1 motifs. Thus a
common simple description arises for the longer LL glycans,
in terms of a linear chain formed mostly by Galβp1-4, rami-
fied at different points with the P1 trisaccharide (linked to
the 6th position of the Gal, as well as the nonreducing terminal
GalNAc, residues). Galβp1-3 galactan chains are believed to
form hydrogen-bonded triple helices (Chandrasekaran and
Janaswamy 2002). This structure accommodates disaccharide
branches linked to the 6th position of the Gal residues, which
project out from the triple helix. It would be tempting to
speculate that at least stretches of the long LL glycans may
associate in an analogous fashion (with the P1 trisaccharides
sticking out) and thus contribute to the superior levels of LL
structure (Diaz, Casaravilla, Irigoín et al. 2011). It is,
however, not obvious how such an association would accom-
date the fact that the glycan chains are attached to apomu-
cin backbones.

Branching with GlcNAcβp1-6 residues is well known in
animal mucin O-glycans (Cheng and Radhakrishnan 2011).
Related glycosyl transferases catalyze the addition of GlcNAc
residues to synthesize Cores 2 and 4 and to ramify N-acetyl
lactosamine chains (blood group i), giving rise to blood group
I. These enzymes catalyze the addition of GlcNAcβp1-6 resi-
dues to the Gal residues at the reducing end of either a
GlcNAcβp1-3Gal disaccharide or Galβp1-3/4GlcNAcβp1-3
Gal trisaccharides. The structures of the newly elucidated LL
glycans suggest that the enzyme catalyzing addition of
GlcNAc branches to the Gal main chain transfers GlcNAc to
the reducing end residue of a Galβp1-3Gal disaccharide.
Indeed, the GlcNAcβp1-6 branches on the main chain in the
fully elucidated glycans (Figure 5) are placed on residues
that correspond to the reducing ends of Galβp-3 sequences
comprising two (H1), three (H7 and IJ8) or four (IIJ10B) residues.

In other words, in none of the cases is a GlcNAcβp1-6 branch
placed on a terminal Gal residue, or on a Gal residue substi-
tuted with the Galαp1-4 cap. This trend is further supported by
the data from partially elucidated structures; Supplementary
data, Tables S2 and S4). To the best of our knowledge, there
are no precedents of GlcNAcβp1-6 transferases that target
Gal–Gal disaccharides, or structures that suggest the existence
of such enzymes.

In our previous work, we had noted that the Galαp1-4
Galβp1-4 disaccharide was added to the Core 2 GlcNAc
residue in an all-or-not fashion, so that terminal Galβp1-4
GlcNAc disaccharides (N-acetyllactosamine motifs) were not
exposed. In the new glycans for which we have information,
whether full structure (Figure 5) of sequence by MS2
( Supplementary data, Tables S2 and S4), this rule still holds, for
the GlcNAcβp1-6 residues in the two contexts observed. In con-
trast to this situation, the LL glycome includes examples in
which O-glycan biosynthesis has stopped at essentially every
other possible step. Thus, glycans are present that bear non-
capped Galβp1-3 main chains (although this only happens for
short glycans), as well as nondecorated GlcNAcβp1-6 residues.
We, therefore, hypothesize that the cryptic nature of the termi-

al N-acetyllactosamine motif in the E. granulosus LL obeys a
selective force related to the decoding of this motif by host
receptors. As mentioned in our previous article, the terminal
N-acetyllactosamine motif is, however, present in the mucin puri-

fied from the E. multilocularis metacestode (Hulsmeyer et al.
2002).

Interestingly, the E. granulosus LL glycan structures bear
important similarities with those of certain O-glycans from the
Xenopus laevis egg jelly (Strecker et al. 1995; Guerardel et al.
2000). Indeed, these Core 1- or 2-based glycans bear either one
or two Galβp1-3 residues on the core Gal residue. Further, the
Galβp1-3 chain thus formed can be capped by Galαp1-4, though
capping takes place irrespective of chain length. Contrary to E. granulosus, the P1 motif is absent, and there is
decoration of the Galβp1-3 residues with fucose (Fuc) α1-3
and/or decoration of the Core 2 GlcNAc residue with Fucα1-3.

No biological function has been described for the P1 blood-
antigen motif, except that in mammalian glycolipids, it is the
target of bacterial shiga toxins (Jacewicz et al. 1986). The motif
is found in phylogenetically distant organisms, with an
apparently discontinuous distribution. In addition to
mammals, it is present in pigeons but apparently not other
avian (Takahashi et al. 2001; Suzuki et al. 2003), and there
are isolated reports of its presence in trematodes (Ben-Ismail
et al. 1981, 1982), nematodes (Prokop and Schlesinger 1965;
Ponce de León and Valverde 2003) and annelids (Prokop and
Schlesinger 1966). The motif is carried on glycolipids, of
course in humans, but also in Echinococcus itself (Dennis
et al. 1993). It is also carried on N-glycans, both in pigeons...
Takahashi et al. 2001; Suzuki et al. 2003) and in all likelihood, in *E. granulosus* protoscoleces (Khoo et al. 1997). *Echinococcus* LL carbohydrates are, to our knowledge, the only example in which the motif is carried on O-glycans. The fact that in *E. granulosus* the P1 trisaccharide is carried both on protoscolex N-glycans (Khoo et al. 1997) and on LL O-glycans surely explains why the E492 monoclonal antibody, raised as mentioned against protoscoleces (Baz et al. 1999), reacts against the LL. This antibody has been used, as also mentioned, to purify glycoprotein fractions used in immunological studies (Baz et al. 1999; Dematteis et al. 2001; Cardozo et al. 2002; Walker et al. 2004; Mourglia-Ettlin et al. 2011). The knowledge of the fine specificity of the antibody does not necessarily shed light on the molecular motif(s) relevant to the immunological activities of those fractions mentioned, even in terms of carbohydrates only, as glycans with unrelated motifs may be carried on the same glycoprotein. This is exemplified by the fraction from the *E. multilocularis* metacestode purified with the antibody, which also reacts with the lectin PNA (Walker et al. 2004), indicating that the glycan consisting of nondecorated Core 1 is present in this mucin fraction.

The P1 motif may not be immunogenic in humans infected with *Echinococcus*. In *E. multilocularis* infections, anticalbohydrate antibodies are predominantly directed against Galpα1-4Gal, but these recognize mostly the capped Gal “main chain” (i.e. Galpα1-4Galpβ1-3GalNAc) rather than the P1 trisaccharide (Koizumi et al. 2011; Yamano et al. 2012). Thus, infection antibodies can discriminate between two trisaccharides sharing the galabiose disaccharide at their nonreducing termini (although some cross-reaction based on the galabiose disaccharide does exist Koizumi et al. 2011; Yamano et al. 2012). An analogous situation probably takes place in *E. granulosus* infections: Siracusano et al. found that an LL mucin fraction containing P1 reactivity was precipitated by human infection sera in a manner inhibitable by α-Gal, yet the same sera did not react with P1+ red blood cells (Russi et al. 1974). This suggests that Galpα1-4Galpβ1-4Gal (as Galpα1-4Galpβ1-3GalNAc is found as mentioned only in *E. multilocularis*) may be a dominant carbohydrate motif in *E. granulosus*. Similar (but reciprocal) to the discrimination between trisaccharide motifs sharing the galabiose disaccharide by infection sera, the E492 antibody binds Galpα1-4Galpβ1-4GlcNAc but not Galpα1-4Galpβ1-3GalNAc or

---

**Fig. 5.** Summary of all fully elucidated oligosaccharide-alditol structures from the LL. Some of the possible biosynthetic relationships between the respective parent O-glycans (which may be assumed to be attached to apomucin backbones by GalNAcα1-Ser/Thr linkages) are indicated. Glycans are identified by the name of the sub-fraction in which they occur (Figure 2) (Díaz et al. 2009); the two glycans elucidated from fraction IJ10 were additionally given the letters A and B. Structures newly reported in the present article are given in bold.
Galpβ1-4Galpβ1-3Gal (Figure 4). At the same time, this antibody does not seem to discriminate between glycans presenting the Galpβ1-4Galpβ1-4GlcNAc motif in different contexts, suggesting that the epitope is limited to the P1 trisaccharide. This is broadly consistent with previous structural data indicating that three, or at most four, monosaccharide residues are accommodated in antigen-binding sites of anti-carbohydrate antibodies (Cygler et al. 1991; Bundle et al. 1994; Zdanov et al. 1994; Ramsland et al. 1994; van Roon et al. 2004).

Materials and methods
Parasite material
The major starting material consisted of walls of *E. granulosus* hydatids from natural infections in cattle, obtained and processed as previously described (Díaz et al. 2009). Hydatid walls from natural infections in sheep and pigs were a kind gift of Marcela Cucher and Mara Rosenzvit (Facultad de Medicina, Universidad de Buenos Aires, Argentina). A human hydatid surgical sample was obtained from the Laboratorio de Anatomía Patológica, Hospital Maciel, Montevideo, Uruguay (Dr. M. Roldán).

Release of O-glycans
Hydatid walls were freed of calcium inositol hexakisphosphate by treatment with EDTA-containing buffer and subjected to prolonged reductive β-elimination, and the resulting soluble oligosaccharide-alditols were then de-salted, as described in (Díaz et al. 2009).

Purification of O-glycans
Oligosaccharide-alditols were purified on BioGel P4 (BioRad, Hercules, CA) columns with refractive index detection, and the resulting fractions re-fractionated on PGC columns as described in (Díaz et al. 2009). PGC columns (Hypercarb; Thermo Fischer Scientific, West Palm Beach, FL) of 7 μm particle size, 4.6 mm bore and 20 cm (cattle-host material) or 15 cm length (sheep host material) were used. UV detection was carried out at 220 nm. Acetonitrile concentrations used for elution are indicated besides chromatograms in the Results section.

MALDI-TOF MS and MS–MS
Oligosaccharide-alditols were permethylated and processed for matrix-assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS) as described in (Díaz et al. 2009). MS–MS spectra, obtained and calibrated also as described, were interpreted with the help of the GlycoWorkBench software (Ceroni et al. 2008).

Nuclear magnetic resonance
Spectra were obtained on Bruker DRX 400 MHz, Bruker DMX 600 and Bruker Avance III 800 MHz at the Centro Nacional de Ressonância Magnética Nuclear, UFRJ, Brazil. Acquisition and calibration were as previously described, except that ROESY instead of NOESY spectra were acquired. For the determination of the *gluco vs galacto* configuration of residues, 1H displacements were compared with relevant ones from Sweet-DB (Loss et al. 2002) and our previous work (Díaz et al. 2009). Substitution positions were determined on the basis of Overhauser effect coupling between anomeric protons and protons on the substituted position in the neighboring residue, as well as the larger 13C displacements in the substituted positions with respect to similar unsubstituted carbon atoms.

Methylation analysis
This was carried out as previously described (Loss et al. 2002).

Synthetic glycans
The glycans Galpβ1-3(GlcNAcβ1-6)GalNAcα1-TMSEt (A), Galpβ1-3(Galβ1-4Galβ1-4GlcNAcβ1-6)GalNAcα1-TMSEt (E), and Galpβ1-3(Galβ1-4Galβ1-4GlcNAcβ1-6)GalNAcα1-TMSEt (F), in which TMSEt represents the 2-(trimethylsilyl)ethyl group, have been previously described (Koizumi et al. 2009). The biotinylated glycans Galpβ1-3(GlcNAcβ1-6)GalNAcα1-R (G), Galpα1-4Galpα1-3GalNAcα1-R (H), Galpα1-4Galpβ1-3(GlcNAcβ1-6)GalNAcα1-R (I), Galpβ1-3(Galβ1-4GlcNAcβ1-6)GalNAcα1-R (J) and Galpβ1-3(Galpα1-4Galpβ1-4GlcNAcβ1-6)GalNAcα1-R (K), in which R comprises a linker and a biotin group, have also been described (Koizumi et al. 2011). The products correspond to O-glycan structures reported in *E. multilocularis* (Hulsmeier et al. 2002) but generally share nonreducing terminal motifs with the *E. granulosus* analogs (Díaz et al. 2009). Products A/G and F/K correspond exactly to *E. granulosus* glycans E1 and G1 (Díaz et al. 2009), respectively.

Enzyme-linked immunosorbent assay
Plates were coated with 5 μg/mL streptavidin in phosphate buffered saline (PBS), blocked with CarboFree™ reagent (Vector Labs, Burlingame, CA) and then sensitized with biotinylated synthetic glycans at 2 μM in PBS. The E492 monoclonal antibody (mouse IgG3), purified by protein G from hybridoma supernatants, was incubated in the concentration range 1.25–10 μg/mL. An isotype-matched control antibody (Sigma, St. Louis, MI) was assayed in parallel. Carbohydrate-bound antibody was detected with anti-mouse IgG coupled to peroxidase (Sigma, St. Louis, MI). For competition ELISA, the same format (using only synthetic glycan K, which reacts with the antibody) was modified by including in the first antibody incubation step the nonbiotinylated synthetic glycans or native oligosaccharide-alditols. Native oligosaccharide-alditols for this assay were from sheep host LL: Glycans showing the same MS2 signals as previously elucidated glycans obtained for this assay were from sheep host LL: Glycans showing the same MS2 signals as previously elucidated glycans obtained from bovine host (and eluting from the PGC column in the same relative order) were taken to be identical to those elucidated glycans.

Supplementary data
Supplementary data for this article is available online at http://glycob.oxfordjournals.org/.

Acknowledgements
The authors are grateful to Madelón Portela (UBYPA, Institut Pasteur Montevideo) for capable and kind help with MALDI-TOF. This work was supported by CSIC, Universidad...
de la República, Uruguay [1 + D grant 2008 number 404 to A.D.]; and by PEDECIBA and AMSUD Pasteur through travel scholarships to G.L. GL, JOP, LMP and AD dedicate this article to the memory of Dr. Orlando Augusto Agrellos Fillho, our friend and collaborator.

Abbreviations

EDTA, ethylenediaminetetraacetic acid; Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylgluco-samine; HPLC, high pressure (or high performance) liquid chromatography; LL, laminated layer; NMR, nuclear magnetic resonance; NMR, nuclear magnetic resonance; PBS, phosphate buffered saline; PGC, porous graphitized carbon column.

References


