Fuc(α1 → 3)GalNAc-: the major antigenic motif of *Schistosoma mansoni* glycolipids implicated in infection sera and keyhole-limpet haemocyanin cross-reactivity

Sven R. KANTELHARDT*[1], Manfred WUHRER*[1], Roger D. DENNIS*, Michael J. DOENHOFF†, Quentin BICKLE‡ and Rudolf GEYER*[2]

*Institute of Biochemistry, Faculty of Medicine, University of Giessen, Friedrichstrasse 24, D-35392 Giessen, Germany, †School of Biological Sciences, University of Wales, Bangor, Gwynedd LL57 2UW, Wales, U.K., and ‡London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, U.K.

The aim of the present study was the characterization of the dominant epitope present on *Schistosoma mansoni* glycolipids, which causes cross-reactivity of *S. mansoni* and *S. haematobium* infection sera with keyhole-limpet haemocyanin (KLH). To this end, the monoclonal antibody M2D3H was chosen for its similar behaviour in high-performance TLC immunostaining and inhibition-ELISA to infection sera. Individual, structurally defined oligosaccharides derived from *S. mansoni* egg glycolipids were tested for their binding to this monoclonal antibody by immunoaffinity chromatography. A terminal fucose residue linked in the (α1 → 3) position to *N*-acetylgalactosamine was found to be the common structural determinant of the four oligosaccharides binding to M2D3H. The Fuc(α1 → 3)GalNAc- motif also appeared to be the basis for the cross-reactivity with KLH, a phenomenon used in the serodiagnosis of *S. mansoni*, *S. haematobium* and *S. japonicum* infections.

Key words: immunoaffinity chromatography, serodiagnosis, trematode parasite.

INTRODUCTION

Schistosomiasis is a major human infection caused by parasitic trematodes and, after malaria, the most important parasitic disease worldwide. From over 200 million infected people, approximately 20 million are heavily afflicted, and 200000 die annually due to the pathology of this infection. Despite all investigations made to date, there is still no potent vaccine available [1].

Antibodies against glycans dominate the host response to schistosome larvae and eggs [2,3] and may play a role in protective immunity [2,4,5]. One of the major epitopes in *Schistosoma mansoni* infection is a determinant that is shared among various schistosome antigens, keyhole-limpet haemocyanin (KLH) and haemocyanins from different snails, e.g. the *S. mansoni* intermediate host, *Biomphalaria glabrata* [6,7]. This epitope is glycanic in nature [2,8,9], and has been shown recently to contain fucose [10]. It represents the basis for the application of KLH in serodiagnosis [11–13] and as a potential vaccine against schistosomiasis [14,15]. In addition, immunization experiments with an anti-idiotype antibody that mimics this cross-reactive epitope have been shown to have a protective effect [16]. Concerning *S. mansoni* antigens, this cross-reactive glycan structure has been found on the glycolipids of various life-cycle stages, and most prominently on egg glycolipids [9,10].

The structures responsible for this major cross-reactivity have not been identified so far, either on KLH or on *S. mansoni* antigens. As for KLH, no studies on the structural elucidation of its glycans have been published [17]. Although *S. mansoni* egg glycolipids have been structurally characterized as a 'pool' [18], it remains an object of speculation as to which motif in the large heterogeneity of structures represents the epitope which is cross-reactive with KLH.

In order to identify this epitope shared between schistosomes and KLH, we applied various monoclonal antibodies (mAbs) as well as individual, structurally defined glycan moieties derived from *S. mansoni* egg glycolipids. Both individual, structurally elucidated glycans and partially characterized mixtures of glycans were tested by immunoaffinity chromatography for the presence of the epitope. A comparison of the bound glycans allowed us to identify this highly antigenic structural motif on *S. mansoni* glycosphingolipids, which is mainly involved in the pronounced cross-reactivity of *S. mansoni* infection sera with KLH. On the basis of promising studies using KLH in serodiagnosis and vaccination, this elucidated structural determinant might have significant serodiagnostic potential, or could be of high value as a potential vaccine candidate.

EXPERIMENTAL

Antigens and antibodies

S. mansoni glycolipids were prepared as described previously [9]. KLH was generously given by Dr J. Markl (Institute for Zoology, University of Mainz, Germany). The mAbs M2D3H and C1C7 were produced as described previously [19,20]. The mAb E2 was generated in a *Balb/c* mouse after intraperitoneal injection of 30000 *S. haematobium* eggs prior to fusion of the spleen cells, and provided by Dr J. Schmitt (Department of Tropical Hygiene and Public Health, University of Heidelberg, Heidelberg, Germany). mAbs specific for GalNAc(β1 → 4)GlcNAc- (LDN), Gal(β1 → 4)[Fuc(α1 → 3)]GlcNAc- (Lewis X), GalNAc(β1 → 4)[Fuc(α1 → 3)]GlcNAc- (LDN-F) or GalNAc-(β1 → 4)[Fuc(α1 → 2)Fuc(α1 → 3)]GlcNAc- (LDN-DF) were provided by Dr A. Deelder (Leiden University Medical

Abbreviations used: dHex, deoxyhexose; HexNAc, *N*-acetylhexosamine; Hex, hexose; HPTLC, high-performance TLC; KLH, keyhole-limpet haemocyanin; LDN, GalNAc(β1 → 4)GlcNAc-; LDN-DF, GalNAc(β1 → 4)[Fuc(α1 → 2)Fuc(α1 → 3)]GlcNAc-; LDN-F, GalNAc(β1 → 4)[Fuc(α1 → 3)]-GlcNAc-; mAb, monoclonal antibody; MALDI–TOF-MS, matrix-assisted laser-desorption ionization–time-of-flight MS; PA, 2-aminopyridine.

[1] These authors contributed equally to this work.

[2] To whom correspondence should be addressed (e-mail rudolf.geyer@biochemie.med.uni-giessen.de).

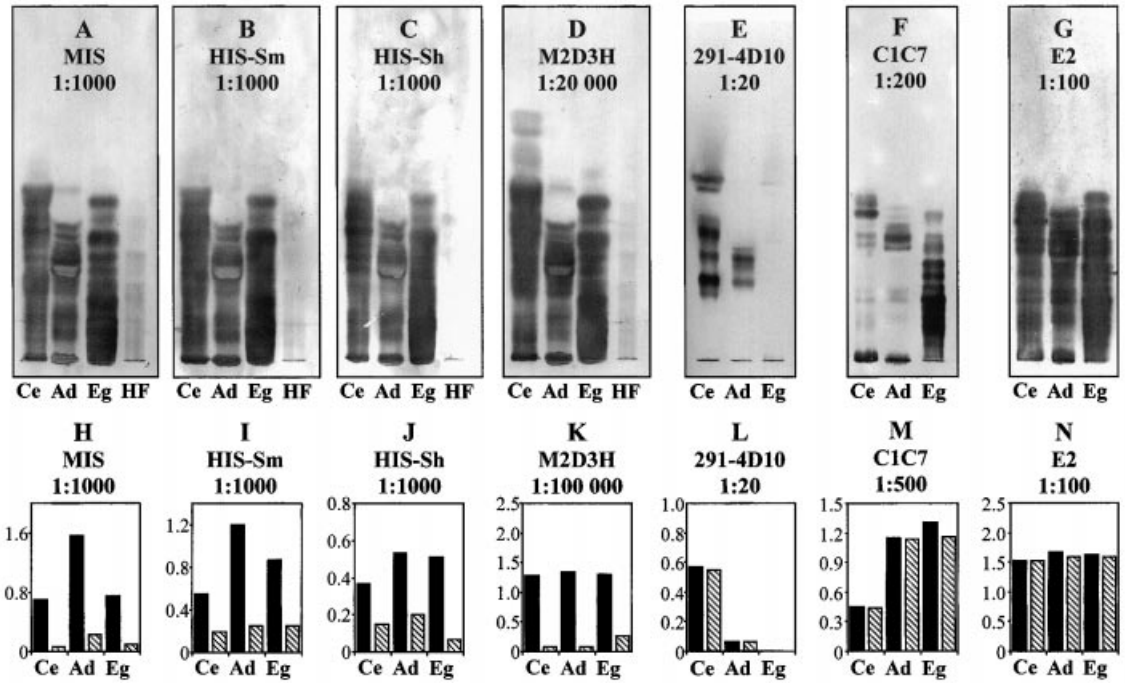

Figure 1 Comparison of the recognition of *S. mansoni* cercarial, adult and egg complex glycolipids in HPTLC-immunostaining and ELISA by various mAbs and infection sera

HPTLC-separated glycolipids were detected by immunostaining with pooled murine infection sera (MIS; **A**), sera from patients infected with *S. mansoni* (HIS-Sm; **B**) or *S. haematobium* (HIS-Sh; **C**) and four different mAbs (M2D3H, 291-4D10, C1C7 and E2; **D**–**G**). (**H**)–(**N**) ELISAs performed in the absence (black bars) or presence (striated bars) of KLH as inhibitor. The values along the *y*-axes in (**H**)–(**N**) refer to absorption at 450 nm. Ce, cercariae; Ad, adults; Eg, eggs; HF, egg glycolipids after HF treatment. The antibody dilutions are shown.

Center, Leiden University, Leiden, The Netherlands); mAbs 99-2A5-B and 100-2H5-A were directed against LDN, mAb 291-4D10 was directed against Lewis X, mAbs 114-5B1-A and 290-4A8 were against LDN-DF and mAbs 290-2E6 and 294-2A1 were against LDN-F + LDN-DF [21]. The human antisera used were obtained from the WHO/TDR reference serum bank for African schistosomiasis. The sera were collected from patients in Kenya and Sudan, and were selected on the basis of positive stool/urine egg counts for *S. mansoni* and *S. haematobium* respectively. Murine infection sera were obtained from mice with a low infection load several weeks after patency and used as a pool from eight mice. Oligosaccharides were enzymically released from *S. mansoni* egg glycolipids and characterized as their 2-aminopyridine (PA) derivatives [22] using the analytical methods and technical equipment described elsewhere [23].

High-performance TLC (HPTLC) and ELISA

S. mansoni glycolipids were analysed by HPTLC using chloroform/methanol/0.25 % (v/v) KCl (50:40:10, by vol.) as running solvent, followed by either orcinol/H_2SO_4 staining or HPTLC immunostaining, as described previously [9]. The amount of carbohydrate added per lane was 100 ng (see Figures 1E–1G) or 300 ng (see Figures 1A–1D). ELISA with *S. mansoni* glycolipids (10 ng of carbohydrate/well) as adsorbed antigens and KLH (10 μg/100 μl well) as an inhibitor or, alternatively, with adsorbed KLH tryptic peptides, was performed as outlined previously [10]. The secondary antibodies were alkaline phosphatase-conjugated goat anti-mouse Ig (Dako Diagnostics, Hamburg, Germany), goat anti-human Ig (Dianova, Hamburg, Germany) or goat anti-rabbit Ig (Sigma, Taufkirchen, Germany), all diluted 1:1000.

Hydrogen fluoride (HF) treatment and linkage analysis

Dried samples were treated with 48 % (v/v) HF at 4 °C for 48 h. HF was removed by a stream of nitrogen. Both *S. mansoni* egg glycolipids and KLH tryptic peptides, obtained after reduction and alkylation of the protein as described elsewhere [24], were defucosylated in this way [25]. HF-released monosaccharides were monitored by HPLC and fluorescence analysis as their anthranilic acid derivatives [10]. For linkage analysis, glycolipids and KLH tryptic peptides were permethylated and hydrolysed (in 4 M trifluoroacetic acid for 4 h at 100 °C). Partially methylated alditol acetates obtained after reduction and peracetylation were analysed by capillary GC-MS with a 0.25 mm × 60 m J + W DB5-bonded phase column (Agilent, Böblingen, Germany) and a temperature gradient of 130–200 °C at 1.5 °C/min and 200–290 °C at 4 °C/min [22].

Affinity chromatography

M2D3H mAb (40 μl of ascites fluid) was coupled with CNBr-activated Sepharose 4B (1 g; Amersham Biosciences, Freiburg, Germany) following the manufacturer's instructions. The material was packed in a Pasteur pipette plugged with glass wool. PA-oligosaccharide samples were applied to the column in 0.5 ml of Tris-buffered saline [25 mM Tris/HCl

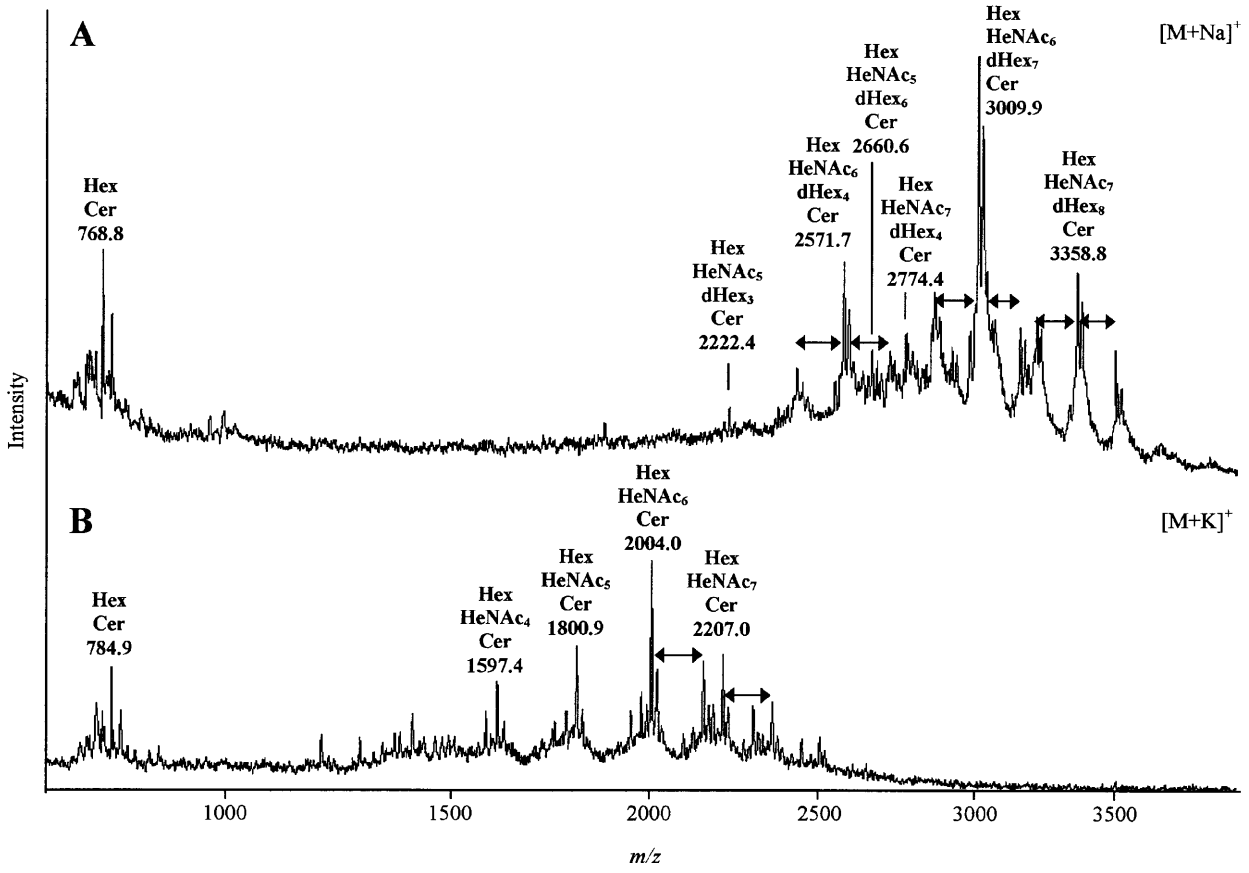

Figure 2 MALDI–TOF-MS of native and HF-treated *S. mansoni* egg glycolipids

Glycolipids before (**A**) and after (**B**) HF treatment were analysed by MALDI–TOF-MS and detected as their sodium or potassium adducts respectively. The dHex increments are marked by double-headed arrows. Cer, ceramide.

(pH 7.5)/100 mM NaCl] and incubated for 30 min at 4 °C. The column was washed with 5 ml of Tris-buffered saline and the flow-through was collected. For elution, 5 ml of 100 mM triethylamine, pH 11.5/150 mM NaCl was used. Both flow-through and eluate were applied separately to a 100 mg porous graphite-carbon cartridge (Thermoquest, Kleinostheim, Germany) for desalting. The cartridge was washed with 10 ml of water and PA-oligosaccharides were eluted with 5 ml of 25 % (v/v) aq. acetonitrile.

Detection of PA-oligosaccharides

PA-oligosaccharides, before and after affinity chromatography, were detected by matrix-assisted laser-desorption ionization–time-of-flight MS (MALDI–TOF-MS) [23] and monosaccharide composition analysis [26]. For detection of individual PA-oligosaccharides in the flow-through or eluate of the affinity column, fractions were applied to a porous 4.6 mm × 100 mm graphite-carbon HPLC column (Hypercarb; Hypersil, Runcorn, Cheshire, U.K.) and eluted at a flow rate of 1 ml/min at room temperature using fluorescence detection (at 310/380 nm). The column was equilibrated with 20 mM triethylamine/acetic acid, pH 5.0. A gradient from 0 to 30 % aq. acetonitrile in 50 min was applied for elution. Peak fractions were collected and analysed by MALDI–TOF-MS.

RESULTS AND DISCUSSION

Comparison of *S. mansoni* glycolipid antibody recognition

Murine *S. mansoni*- and human *S. mansoni*- as well as *S. haematobium*-infection sera detected various glycolipids of *S. mansoni* cercariae, adult worms and eggs in an HPTLC-overlay experiment (Figures 1A–1C). For all three life-cycle stages, most of the humoral immunoreaction with *S. mansoni* glycolipids appeared to be due to antibodies that cross-react with KLH, as revealed by inhibition-ELISA (Figures 1H–1J). Cross-reaction with KLH was also observed in the case of the mAb M2D3H (Figure 1K), whereas the other mAbs applied (Figures 1E–1G and 1L–1N) did not cross-react with KLH. The KLH-cross-reactive epitope defined by the mAb M2D3H was found to be distinct from the LDN, LDN-F and LDN-DF epitopes [21]; anti-LDN mAbs did not react with *S. mansoni* glycolipids, whereas mAbs recognizing LDN-DF or LDN-F exhibited an HPTLC pattern that differed largely from that observed for the infection sera, and their reactivity was not inhibited by KLH in inhibition-ELISA (results not shown). The antigenic determinants recognized by M2D3H and the KLH-cross-reactive antibodies in infection sera were shown to contain fucose, since chemical defucosylation of *S. mansoni* egg glycosphingolipids by HF treatment, monitored by MALDI–TOF-MS (Figure 2), resulted in the loss of immunoreactivity (Figures

Compound	Theoretical mass $[M+Na]^+$	
		bound by M2D3H
1	1036.4	**GalNAc(β1→4)**GlcNAc(β1→3)GalNAc(β1→4)Glc-PA **Fuc(α1→3)**┘
2	1182.5	**GalNAc(β1→4)**GlcNAc(β1→3)GalNAc(β1→4)Glc-PA **Fuc(α1→3)**┘ Fuc(α1→3)┘
3	1385.8	**GalNAc(β1→4)**GlcNAc(β1→3)GlcNAc(β1→3)GalNAc(β1→4)Glc-PA **Fuc(α1→3)**┘ Fuc(α1→3)┘
4	1385.8	**GalNAc(β1→4)**GlcNAc(β1→4)GlcNAc(β1→3)GalNAc(β1→4)Glc-PA **Fuc(α1→3)**┘ Fuc(α1→3)┘
		not bound by M2D3H
5	995.4	Gal(β1→4)GlcNAc(β1→3)GalNAc(β1→4)Glc-PA Fuc(α1-3)┘
6	1239.5	GalNAc(β1→4)GlcNAc(β1→3)GlcNAc(β1→3)GalNAc(β1→4)Glc-PA Fuc(α1→3)┘

Figure 3 ***S. mansoni*** **glycolipid-derived PA-oligosaccharides tested for binding to mAb M2D3H**

Structures **1** to **4** were bound by M2D3H, whereas **5** and **6** were not recognized (compare with Figure 4). The common structural motif Fuc(α1 → 3)GalNAc- occurring in all bound compounds is shown in **bold**.

1A–1D, lanes 'HF'). MALDI–TOF-MS revealed further that fucose removal was efficient, and the majority of the oligosaccharide backbones remained intact during HF treatment (Figure 2B). Removal of fucose residues by commercially available α-fucosidases was not possible (results not shown).

Affinity chromatography of individual glycolipid-derived glycans

In order to define the glycan epitope recognized by mAb M2D3H, individual structurally defined and pyridylaminated glycans (PA-glycans) derived from *S. mansoni* egg glycolipids [22] were tested for binding to M2D3H by immunoaffinity chromatography. The PA-oligosaccharide species applied are listed in Figure 3. The compounds were loaded on to a M2D3H-immunoaffinity column, and both flow-through and eluates were collected and tested for the respective PA-oligosaccharide by HPLC (for example, Figure 4A), MALDI–TOF-MS (Figures 4A–4E) and monosaccharide constituent analysis (results not shown). All three methods showed consistently that compounds **1** to **4** were bound to the M2D3H column, because they could only be detected in the eluate, whereas compounds **5** and **6** were not bound and were found exclusively in the flow-through fraction. Comparison of the bound structures revealed the common terminal motif Fuc(α1 → 3)GalNAc-. The Lewis X-containing structure **5** and compound **6**, which carries the terminal LDN-F motif, did not bind to M2D3H.

Immunoaffinity chromatography of glycan mixtures

Immunoaffinity chromatography of complex mixtures of glycans confirmed the results obtained above. The total pool of *S. mansoni* egg glycolipid-derived PA-oligosaccharides displayed a heterogeneous population of glycans with one hexose (Hex) and three to seven *N*-acetylhexosamine (HexNAc) residues, together with a varying degree of fucosylation (Figure 5A) [18,22]. Although present in this fraction, compounds **1** to **6** were not

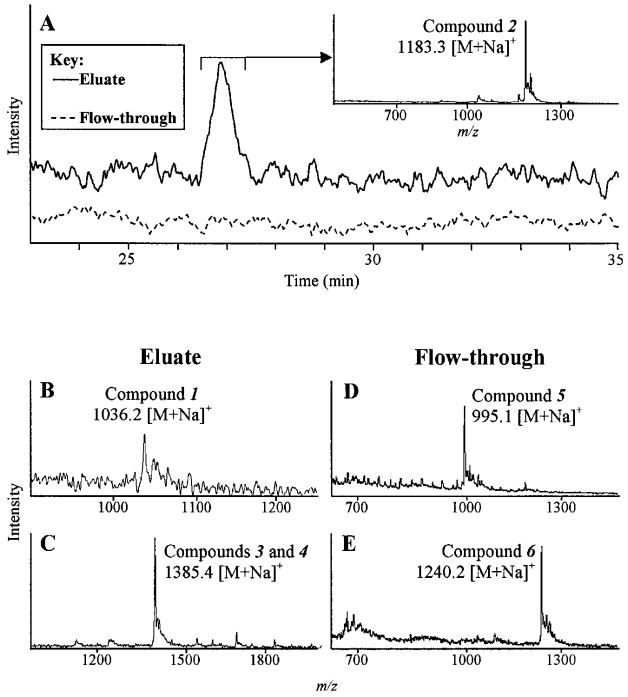

Figure 4 Immunoaffinity chromatography of individual ***S. mansoni*** **egg glycolipid-derived PA-oligosaccharides**

(**A**) Compound **2** was applied to the M2D3H-immunoaffinity column. Flow-through and eluates were collected and tested by HPLC and MALDI–TOF-MS (inset) for PA-oligosaccharides. Using the same approach, compounds **1**, **3** and **4** were detected in the eluate (**B** and **C**), whereas **5** and **6** were detected in the flow-through (**D** and **E**).

detected in the total mixture because of their low amounts. After applying this mixture to the immunoaffinity column, compounds **2** to **4** could now be detected in the eluate (Figure 5B), which confirmed their binding to M2D3H. As for the more complex

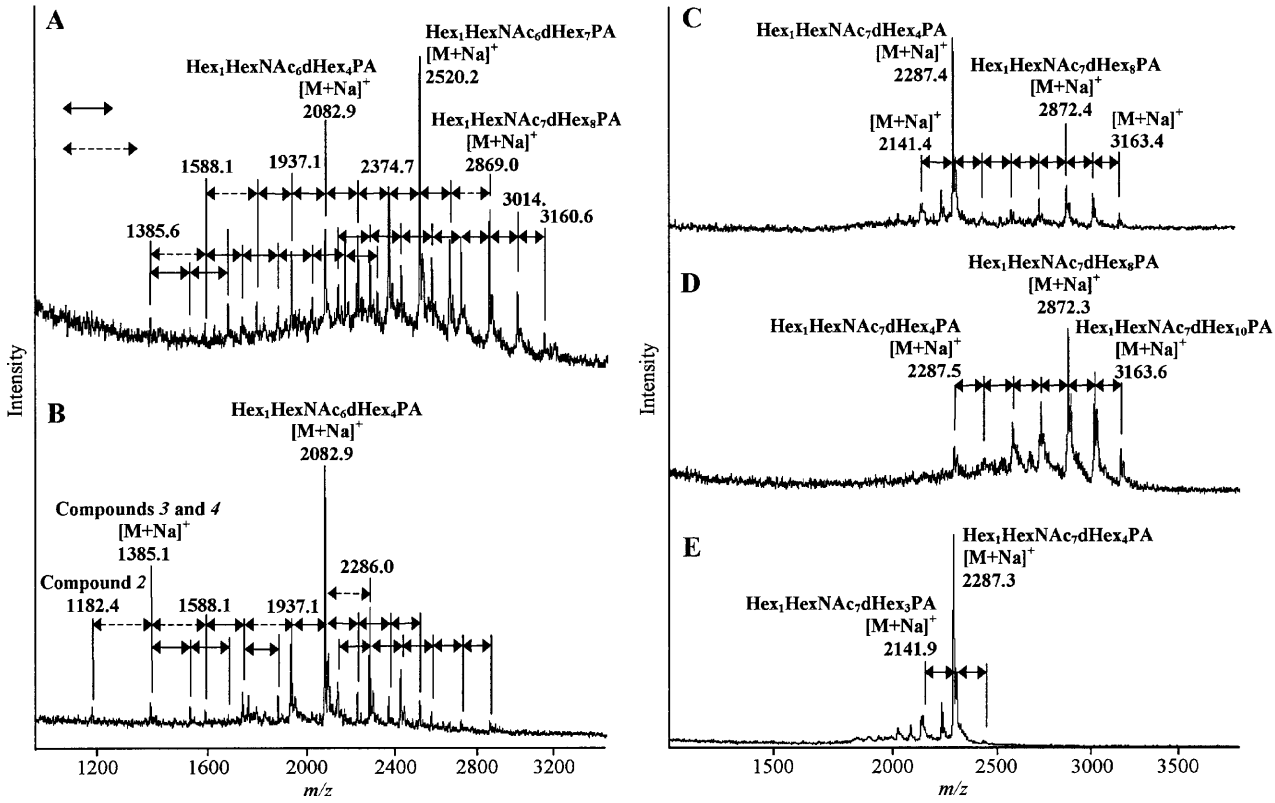

Figure 5 Immunoaffinity chromatography of PA-oligosaccharide mixtures derived from *S. mansoni* glycolipids

Comparison of MALDI–TOF mass spectra of the total PA-glycan fraction before affinity chromatography (**A**) and the corresponding eluate from the M2D3H affinity column (**B**), as well as of PA-oligosaccharide species with compositions of $Hex_1HexNAc_7dHex_{3-10}PA$ before affinity chromatography (**C**), the respective flow-through (**D**) and the eluate (**E**) from the M2D3H-immunoaffinity column.

structures dominating this fraction, one signal in MALDI–TOF-MS may represent several structural isomers carrying fucose residues in different positions. Highly fucosylated structures, such as the major species $Hex_1HexNAc_6dHex_7PA$ (where dHex represents deoxyhexose), prevailed in the total fraction (Figure 5A), whereas the dominant compound in the eluate was $Hex_1HexNAc_6dHex_4PA$ (Figure 5B). As a general scheme, species with a moderate degree of fucosylation bound to M2D3H, whereas the larger, more highly fucosylated structures did not bind. This conclusion was confirmed by immunoaffinity chromatography of glycolipid-derived PA-oligosaccharides with seven HexNAc residues, which contained between three and ten fucose residues (Figure 5C). Species with three or four fucose residues were found almost exclusively in the eluate (Figure 5E), whereas compounds with six to ten fucose residues did not bind to mAb M2D3H and were, therefore, collected in the flow-through (Figure 5D). Possibly, extensive fucosylation might prevent antibody binding in two ways: steric hindrance by di- or tri-fucosyl side chains at the adjacent GlcNAc residue, and/or capping of the Fuc(α1 → 3)GalNAc-epitope, yielding Fuc(α1 → 2)Fuc(α1 → 3)GalNAc-. The latter structure has been detected on both egg glycosphingolipids [18] and cercarial O-glycans [27].

Analysis of cross-reactive structures on KLH and *S. mansoni* glycolipids

Evidence for the presence of the Fuc(α1 → 3)GalNAc-structural motif on KLH was obtained by comparing KLH tryptic peptides before and after defucosylation by HF treatment [25] in ELISA and linkage analysis. HF treatment removed approx. 70 % of the total amount of fucose present (results not shown) and resulted in complete loss of monoclonal M2D3H immunoreactivity to KLH tryptic peptides (Figure 6A), whereas a polyclonal anti-KLH hyperimmune serum still bound to the HF-treated KLH peptides (Figure 6B), indicating that M2D3H-independent epitopes remained intact. Linkage analysis revealed 3-substituted GalNAc as the only GalNAc species present on KLH, in addition to a variety of GlcNAc derivatives (Figure 6C). In order to test on a structural level whether shared Fuc(α1 → 3)-GalNAc units provide a basis for the pronounced cross-reactivity between KLH and schistosome glycoconjugates, linkage data for KLH glycans were compared before and after chemical defucosylation. This HF treatment converted most of the 3-substituted GalNAc into terminal GalNAc (Figure 6D), thus identifying fucose as a substituent at C-3 of GalNAc and providing evidence for the presence of Fuc(α1 → 3)GalNAc units on KLH glycans. The degradation of the Fuc(α1 → 3)-GalNAc antigenic determinant by defucosylation correlated with the above-mentioned loss of M2D3H-immunoreactivity, indicating that this epitope is responsible for the M2D3H-defined cross-reactivity with schistosome glycoconjugates. Similar analysis of total *S. mansoni* egg glycolipids revealed HF treatment to release more than 90 % of the total amount of fucose (cf. Figure 2B), which similarly resulted in the loss of immunoreactivity (Figures 1A–1D, lanes 'HF') and the conversion of 3-substituted GalNAc into terminal GalNAc (results not shown). Taken together, the comparison of KLH glycopeptides and

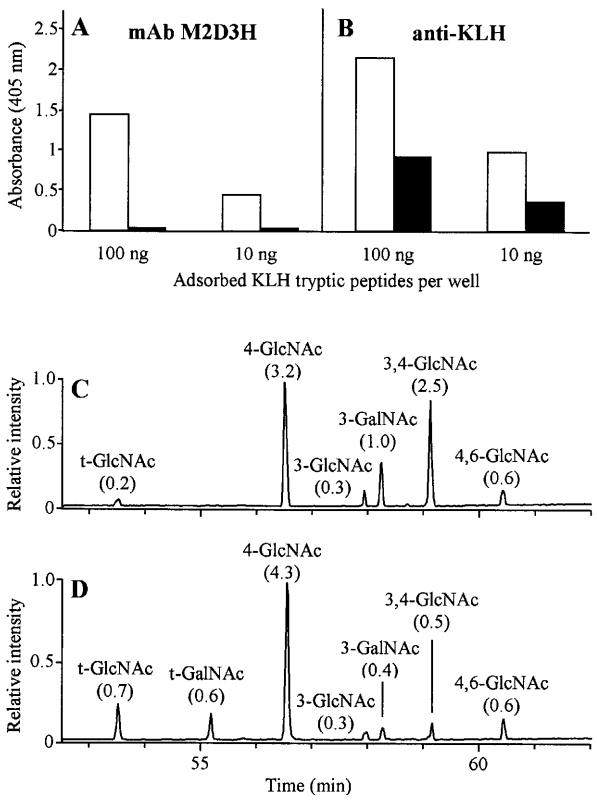

Figure 6 Evidence for the occurrence of the structural motif Fuc(α1 → 3)GalNAc- on KLH

KLH tryptic peptides before (open bars) and after (black bars) HF treatment were assayed by ELISA for their recognition by the mAb M2D3H (diluted 1:20000; **A**) and an anti-KLH rabbit hyperimmune serum (diluted 1:20000; **B**). (**C** and **D**) HexNAc species as revealed by linkage analysis of KLH tryptic peptides before (**C**) and after (**D**) HF treatment by selected-ion monitoring of partially methylated alditol acetates. Peak areas normalized to 1.0 = terminal GlcNAc (t-GalNAc) + 3-substituted (3-)GalNAc are shown in parentheses. 4-GlcNAc, 4-substituted GlcNAc, etc.

S. mansoni egg glycolipids by linkage analysis combined with chemical defucosylation provided evidence for the occurrence of the Fuc(α1 → 3)GalNAc motif on both types of molecules. Since this structural motif is recognized by the mAb M2D3H, it builds a basis for the cross-reactivity between KLH and schistosome glycoconjugates.

Conclusions

The carbohydrate motif Fuc(α1 → 3)GalNAc- has so far been identified on *S. mansoni* cercarial O-glycans [27] and egg glycosphingolipids [18,22]. Our data have shown that this structural epitope occurs on glycosphingolipids of all investigated life-cycle stages, and is recognized by both infection sera and the mAb M2D3H. In addition, this structure is present on KLH glycans and is responsible for serological cross-reactivity with KLH. Antibodies recognizing the Fuc(α1 → 3)GalNAc-epitope may also account for the pronounced cross-reactivity with KLH observed with sera from chimpanzees infected with *S. mansoni* [2]. The identified structural motif, however, clearly differs from the carbohydrate epitopes Lewis X, LDN, LDN-F and LDN-DF used recently for characterization of the humoral immune response in *S. mansoni*-infected humans [28] and chimpanzees [2].

The Fuc(α1 → 3)GalNAc- motif shared by schistosome glycoconjugates and KLH provides a basis for the potential of KLH in schistosomiasis vaccination and serodiagnosis. In order to corroborate this observation, the humoral response during infection to the Fuc(α1 → 3)GalNAc- moiety, presented, for example, as a neoglycoconjugate, should be characterized. Since this motif has not been identified on other pathogens so far, it could be tested further for its potential as a vaccine in comparison with intact KLH.

We thank Dr André Deelder and Dr R. Hokke (Leiden University Medical Center, Leiden University, The Netherlands), as well as Dr J. Schmitt (University of Heidelberg, Heidelberg, Germany), for their generous supply of mAbs. Part of the results were presented in August 2001 at the XVI International Symposium on Glycoconjugates in The Hague, The Netherlands. This study was supported by the Deutsche Forschungsgemeinschaft (SFB535, Teilprojekt Z1). The paper is in partial fulfilment of the requirements of S. R. Kantelhardt for the degree of Dr. med. at Giessen University.

REFERENCES

1 Doenhoff, M. J. (1998) A vaccine for schistosomiasis: alternative approaches. Parasitol. Today **14**, 105–109
2 Eberl, M., Langermans, J. A., Vervenne, R. A., Nyame, A. K., Cummings, R. D., Thomas, A. W., Coulson, P. S. and Wilson, R. A. (2001) Antibodies to glycans dominate the host response to schistosome larvae and eggs: is their role protective or subversive? J. Infect. Dis. **183**, 1238–1247
3 Omer-Ali, P., Magee, A. I., Kelly, C. and Simpson, A. J. G. (1986) A major role for carbohydrate epitopes preferentially recognized by chronically infected mice in the determination of *Schistosoma mansoni* schistosomulum surface antigenicity. J. Immunol. **137**, 3601–3607
4 Omer-Ali, P., Smithers, S. R., Bickle, Q., Phillips, S. M., Harn, D. and Simpson, A. J. G. (1988) Analysis of the anti-*Schistosoma mansoni* surface antibody response during murine infection and its potential contribution to protective immunity. J. Immunol. **140**, 3273–3279
5 van der Kleij, D., Tielens, A. G. and Yazdanbakhsh, M. (1999) Recognition of schistosome glycolipids by immunoglobulin E: possible role in immunity. Infect. Immun. **67**, 5946–5950
6 Dissous, C., Grzych, J. M. and Capron, A. (1986) *Schistosoma mansoni* shares a protective oligosaccharide epitope with freshwater and marine snails. Nature (London) **323**, 443–445
7 Grzych, J. M., Dissous, C., Capron, M., Torres, S., Lambert, P. H. and Capron, A. (1987) *Schistosoma mansoni* shares a protective carbohydrate epitope with keyhole limpet hemocyanin. J. Exp. Med. **165**, 865–878
8 Hamilton, J. V., Chiodini, P. L., Fallon, P. G. and Doenhoff, M. J. (1999) Periodate-sensitive immunological cross-reactivity between keyhole limpet haemocyanin (KLH) and serodiagnostic *Schistosoma mansoni* egg antigens. Parasitology **118**, 83–90
9 Wuhrer, M., Dennis, R. D., Doenhoff, M. J., Bickle, Q., Lochnit, G. and Geyer, R. (1999) Immunochemical characterisation of *Schistosoma mansoni* glycolipid antigens. Mol. Biochem. Parasitol. **103**, 155–169
10 Wuhrer, M., Dennis, R. D., Doenhoff, M. J. and Geyer, R. (2000) A fucose-containing epitope is shared by keyhole limpet haemocyanin and *Schistosoma mansoni* glycosphingolipids. Mol. Biochem. Parasitol. **110**, 237–246
11 Alves-Brito, C. F., Simpson, A. J. G., Bahia-Oliveira, L. M. G., Rabello, A. L. T., Rocha, R. S., Lambertucci, J. R., Gazzinelli, G., Katz, N. and Correa-Oliveira, R. (1992) Analysis of anti-keyhole limpet haemocyanin antibody in Brazilians supports its use for the diagnosis of acute schistosomiasis mansoni. Trans. R. Soc. Trop. Med. Hyg. **86**, 53–56
12 Yuesheng, L., Rabello, A. L. T., Simpson, A. J. G. and Katz, N. (1994) The serological differentiation of acute and chronic *Schistosoma japonicum* infection by ELISA using keyhole limpet haemocyanin as antigen. Trans. R. Soc. Trop. Med. Hyg. **88**, 249–251
13 Mansour, M. A., Ali, P. O., Farid, Z., Simpson, A. J. G. and Woody, J. W. (1989) Serological differentiation of acute and chronic schistosomiasis mansoni by antibody responses to keyhole limpet hemocyanin. Am. J. Trop. Med. Hyg. **41**, 338–344
14 Taylor, M. G., Huggins, M. C., Shi, F., Lin, J., Tian, E., Ye, P., Shen, W., Qian, C. G., Lin, B. F. and Bickle, Q. D. (1998) Production and testing of *Schistosoma japonicum* candidate vaccine antigens in the natural ovine host. Vaccine **16**, 1290–1298

15 Bashir, M., Bushara, H., Cook, L., Fuhui, S., He, D., Huggins, M., Jiaojiao, L., Malik, K., Moloney, A., Mukhtar, M., Ping, Y., Shoutai, X., Taylor, M. and Yaochuan, S. (1994) Evaluation of defined antigen vaccines against *Schistosoma bovis* and *S. japonicum* in bovines. Trop. Geogr. Med. **46**, 255–258
16 Grzych, J. M., Capron, M., Lambert, P. H., Dissous, C., Torres, S. and Capron, A. (1985) An anti-idiotype vaccine against experimental schistosomiasis. Nature (London) **316**, 74–76
17 Kamerling, J. P. and Vliegenthart, J. F. G. (1997) Hemocyanins. In Glycoproteins, vol. 2 (Montreuil, J., Vliegenthart, J. F. G. and Schachter, H., eds.), pp. 123–142, Elsevier Science
18 Khoo, K. H., Chatterjee, D., Caulfield, J. P., Morris, H. R. and Dell, A. (1997) Structural characterization of glycosphingolipids from the eggs of *Schistosoma mansoni* and *Schistosoma japonicum*. Glycobiology **7**, 653–661
19 Bickle, Q. D., Andrews, B. J. and Taylor, M. G. (1986) *Schistosoma mansoni*: characterization of two protective monoclonal antibodies. Parasite Immunol. **8**, 95–107
20 Bickle, Q. D. and Andrews, B. J. (1988) Characterisation of *Schistosoma mansoni* monoclonal antibodies which block *in-vitro* killing: failure to demonstrate blockage of immunity *in vivo*. Parasite Immunol. **10**, 151–168
21 van Remoortere, A., Hokke, C. H., van Dam, G. J., van Die, I., Deelder, A. M. and van den Eijnden, D. H. (2000) Various stages of *Schistosoma* express Lewis^X, LacdiNAc, GalNAcβ1–4 (Fucα1–3)GlcNAc and GalNAcβ1–4(Fucα1–2Fucα1–3) GlcNAc carbohydrate epitopes: detection with monoclonal antibodies that are characterized by enzymatically synthesized neoglycoproteins. Glycobiology **10**, 601–609
22 Wuhrer, M., Kantelhardt, S. R., Dennis, R. D., Doenhoff, M. J., Lochnit, G. and Geyer, R. (2002) Characterization of glycosphingolipids from *Schistosoma mansoni* eggs carrying Fuc(α1–3)GalNAc-, GalNAc(β1–4)[Fuc(α1–3)]GlcNAc- and Gal(β1–4)[Fuc(α1–3)]GlcNAc- (Lewis X) terminal structures. Eur. J. Biochem. **269**, 481–493
23 Wuhrer, M., Dennis, R. D., Doenhoff, M. J., Lochnit, G. and Geyer, R. (2000) *Schistosoma mansoni* cercarial glycolipids are dominated by Lewis X and pseudo-Lewis Y structures. Glycobiology **10**, 89–101
24 Liedtke, S., Geyer, H., Wuhrer, M., Geyer, R., Frank, G., Gerardy-Schahn, R., Zahringer, U. and Schachner, M. (2001) Characterization of N-glycans from mouse brain neural cell adhesion molecule. Glycobiology **11**, 373–384
25 Haslam, S. M., Coles, G. C., Morris, H. R. and Dell, A. (2000) Structural characterization of the N-glycans of *Dictyocaulus viviparus*: discovery of the Lewis^X structure in a nematode. Glycobiology **10**, 223–229
26 Anumula, K. R. (1994) Quantitative determination of monosaccharides in glycoproteins by high-performance liquid chromatography with highly sensitive fluorescence detection. Anal. Biochem. **220**, 275–283
27 Khoo, K. H., Sarda, S., Xu, X., Caulfield, J. P., McNeil, M. R., Homans, S. W., Morris, H. R. and Dell, A. (1995) A unique multifucosylated -3GalNAc β1–4GlcNAc β1–3Gal α1- motif constitutes the repeating unit of the complex *O*-glycans derived from the cercarial glycocalyx of *Schistosoma mansoni*. J. Biol. Chem. **270**, 17114–17123
28 van Remoortere, A., van Dam, G. J., Hokke, C. H., van den Eijnden, D. H., van Die, I. and Deelder, A. M. (2001) Profiles of immunoglobulin M (IgM) and IgG antibodies against defined carbohydrate epitopes in sera of Schistosoma-infected individuals determined by surface plasmon resonance. Infect. Immun. **69**, 2396–2401

Received 15 November 2001/30 April 2002; accepted 7 May 2002
Published as BJ Immediate Publication 7 May 2002, DOI 10.1042/BJ20011678