

A fucose-containing epitope is shared by keyhole limpet haemocyanin and *Schistosoma mansoni* glycosphingolipids

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Abstract

The glycolipids of *Schistosoma mansoni* adult worms, cercariae and eggs are recognised by schistosome infection serum and the monoclonal antibody M2D3H. The haemocyanin of the keyhole limpet, *Megathura crenulata*, is known to be immunoreactive to schistosomal infection sera and is, therefore, under investigation for the diagnosis of and vaccination against schistosomiasis. By dot-blot, inhibition-ELISA and inhibition-HPTLC immunostaining we have demonstrated that the M2D3H epitope is shared by both *S. mansoni* glycolipids and keyhole limpet haemocyanin (KLH). Analogously to the established epitopic importance of fucose to the immunorecognition of *S. mansoni* glycolipids, we have similarly defined the significance of the fucose residue(s) for the immunoreactivity between KLH and schistosomal infection serum and the monoclonal antibody M2D3H. Fucose was specifically removed from KLH by partial hydrolysis, monitored by ultrafiltration and carbohydrate component analysis. On removal of the fucose residue(s) the serological and immunological reactivity of KLH was greatly diminished, which implied that the fucose-containing M2D3H antigenic determinant was common to both *S. mansoni* glycolipids and KLH. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Haemocyanin of the keyhole limpet (*Megathura crenulata*; KLH) is in use or under consideration for many biochemical applications, due to its antigenic and immunogenic properties, such as, in the immunotherapy of various cancers and viral diseases [1,2]. KLH is also being used in antibody production assays for the detection of immunomodulatory activities [3]. In the field of schistosomiasis, KLH has been reported to be of value for the diagnosis of [4–6] and vaccination

Abbreviations: CDH, ceramide dihexoside; CTetH, ceramide tetrahexoside; CTH, ceramide trihexoside; HPLC, high-performance liquid chromatography; HPTLC, high-performance thin-layer chromatography; HRP, horseradish peroxidase; KLH, keyhole limpet haemocyanin; Le^x, Lewis^x; mAb, monoclonal antibody; MIS, murine infection sera; NS, normal mouse sera; TFA, trifluoroacetic acid..

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against [7,8] this disease. These effects are due, at least in part, to a shared carbohydrate epitope between haemocyanins from *M. crenulata* and other snails, e.g. from the *Schistosoma mansoni* intermediate host *Biomphalaria glabrata*, and a 38 kDa *S. mansoni* surface antigen [9,10]. This 38 kDa antigen has been defined by the monoclonal antibody (mAb) IPLSm1 [9,10] and has conferred protection against *S. mansoni* infection on passive transfer to rats [11]. Besides immunisation experiments with KLH vaccination [7,8], immunisation with an anti-idiotypic antibody against the IPLSm1 mAb, which should mimic the IPLSm1 epitope, was shown to have a protective effect [12]. Mouse infection serum as well as sera from patients with acute schistosomiasis strongly bind to the 38 kDa antigen on the schistosomular surface [13–15], and these immunoreactive antibodies may be responsible for the cross-reactivity with KLH. A carbohydrate-based cross-reactivity between KLH and serodiagnostic *S. mansoni* egg antigens has also been reported [16]. The T antigen, Gal(β 1–3)GalNAc–, which has been structurally determined, with the galactose either 3-*O*-methylated or α 2-fucosylated, on *Lymnaea stagnalis* haemocyanin [17] and detected by antibodies and lectins on KLH [2], has been speculated to be responsible for the recognition of KLH by schistosomal infection sera [18]. The structures of the antigenic determinant on KLH recognised by *S. mansoni* infection sera, however, have not been fully defined [18].

We have previously reported, that *S. mansoni* glycolipids from adult worms, cercariae and eggs are recognised by an anti-KLH hyperimmune serum [19]. As *S. mansoni* egg glycolipids are known to express predominantly fucosylated carbohydrate structures [20] and fucose is of acknowledged epitopic importance [19], we have analysed KLH in this study for the presence of fucose-containing epitope(s). By use of the mAb M2D3H, murine infection sera (MIS) and rabbit hyperimmune sera as well as by partial hydrolysis in combination with carbohydrate composition analysis, we show here that KLH comprises a fucose-containing epitope that is responsible for the cross-reactivity with *S. mansoni* glycolipids.

2. Materials and methods

2.1. Antigens, sera and antibodies

S. mansoni glycolipids were prepared as described earlier [19]. KLH was purchased from Sigma (Deisenhofen, Germany). The mAb M2D3H was produced and kindly provided by Q. Bickle, London School of Hygiene and Tropical Medicine, London University, UK [15]. The mAb 4D1 was kindly provided by B. Kniep, Institute for Immunology, Technical University — Dresden, Germany. Rabbit hyperimmune sera were prepared as previously described [21,22].

2.2. High-performance thin-layer chromatography (HPTLC)

HPTLC, orcinol/H₂SO₄-staining and immunostaining were performed as described elsewhere [19]. The amount of glycolipid applied per lane corresponded to 10, 10 and 1 ng of total carbohydrate for the adult worm, cercarial and egg life-cycle stages, respectively. For immunostaining, the following primary antibodies were applied: mAb M2D3H [15,19]; anti-Lewis^x (Le^x) mAb 4D1 [23]; anti-KLH rabbit hyperimmune serum [16]; anti-Sm480egg rabbit hyperimmune serum [22]; rabbit hyperimmune serum raised against a *Litomosoides sigmodontis* protein recombinantly expressed in *Escherichia coli* (negative control; kindly provided by Tarig Dafa'alla, Giessen University); a pool of sera from eight mice with chronic *S. mansoni* infection (MIS); and a pool of sera from four uninfected mice (normal mouse sera; NS). All rabbit antisera were prepared using Freund's adjuvants. Goat alkaline phosphatase-coupled, anti-mouse and anti-rabbit Ig (diluted 1:1000; Sigma) were used as secondary antibodies.

2.3. Dot-blot binding assay

PVDF membranes were wetted in methanol:water (1:1, by volume) and spotted with 2 μ l of each KLH sample (2 mg ml⁻¹). The membrane was air-dried, blocked with 2% bovine serum albumin in phosphate-buffered saline (100 mM sodium phosphate, pH 7.2, 100 mM sodium

chloride) containing 0.05% Tween 20 for 1 h at room temperature and incubated with the primary and secondary antibodies as described for HPTLC immunostaining [19]. Goat horseradish peroxidase (HRP)-conjugated, anti-rabbit Ig and rabbit HRP-conjugated, anti-mouse Ig (dilution 1:2000; Dako Diagnostics, Hamburg, Germany) were used as secondary antibodies [19].

2.4. Enzyme-linked immunosorbent assay (ELISA)

KLH was diluted in carbonate buffer (0.1 M Na_2CO_3 , pH 9.6), 100 μl of the appropriate dilution was applied to microtitre plate wells (Polysorb; Nunc, Wiesbaden, Germany) and incubated for 2 h at 37°C. The coated plates were washed twice with Tris-buffered saline (TBS; 25 mM Tris-HCl, pH 7.5, containing 100 mM sodium chloride) and blocked as described below. *N*-propanol-diluted glycolipids were applied at 20 μl to each well for coating. The air-dried plates were blocked by a 1 h incubation in 250 μl per well of 0.5% bovine serum albumin in TBS. Incubation with 100 μl per well of the first antibody (see Section 2.2) in TTBS-10-B (TBS 1:10 diluted, containing 0.05% Tween 20 and 0.25% bovine serum albumin) was performed for 1 h at 37°C. For inhibition experiments, the required amount of KLH was first added in 50 μl TTBS-10-B per well followed by the primary antibody in another 50 μl of TTBS-10-B. Plates were thoroughly shaken in order to mix the KLH inhibitor and primary antibody, and then incubated for 1 h at 37°C. After multiple washings with TBS, diluted 1:10 and containing 0.05% Tween 20, binding was detected following a 60-min incubation with 100 μl per well of goat HRP-conjugated, anti-rabbit Ig or rabbit HRP-conjugated, anti-mouse Ig (both 1:2000; Dako) secondary antibody in TTBS-10-B. Staining was performed with 100 μl per well of a ready-to-use liquid substrate system based on 3,3',5,5'-tetramethylbenzidine (Sigma) for 5–20 min and stopped with 100 μl per well of a 12.5% (weight per volume) sulphuric acid solution. Absorption was measured at 450 nm.

2.5. Partial hydrolysis

For partial hydrolysis, KLH (500 μg) was incubated in 1 ml of 10–2000 mM aqueous trifluoroacetic acid (TFA; Merck) at 80°C for 40 min, the resultant samples dried down in a centrifugal evaporator (Jouan, St Herblain Cedex, France), dissolved in 250 μl water, and aliquots were used for carbohydrate composition analysis (50 μg) and ELISA (50 ng). For centrifugal ultrafiltration, aliquots (50 μg) were diluted with 40 mM ammonium acetate, pH 6.0, to a volume of 250 μl and applied to an Ultrafree-MC filter with 10 kDa cut-off (Millipore, Eschborn, Germany). Ultrafiltration was performed for 90 min at $2000 \times g$. To the retained fraction (20 μl), 200 μl of 40 mM ammonium acetate, pH 6.0, was added and ultrafiltration was repeated. The retentate was recovered by several washings and subjected to carbohydrate composition analysis.

2.6. Carbohydrate composition and methylation analysis

For carbohydrate composition analysis, samples were routinely hydrolysed with 100 μl 4 M aqueous TFA at 100°C for 4 h [19]. Resulting monosaccharides were derivatised with anthranilic acid and analysed by HPLC with fluorescence detection [19]. For methylation analysis, KLH was permethylated and hydrolysed with 4 M TFA at 100°C for 4 h. Partially methylated alditol acetates obtained after sodium borohydride reduction and peracetylation were analysed by capillary gas chromatography/mass spectrometry, using a DB1 column (ICT, Bad Homburg, Germany) and the instrumentation and microtechniques described elsewhere [24].

3. Results

S. mansoni glycolipids are recognised by an anti-KLH rabbit hyperimmune serum on HPTLC immunostaining [19]. They also react with the mAb M2D3H and a group of hyperimmune sera, most of which were raised against *S. mansoni* egg glycoproteins [19]. These data suggested a com-

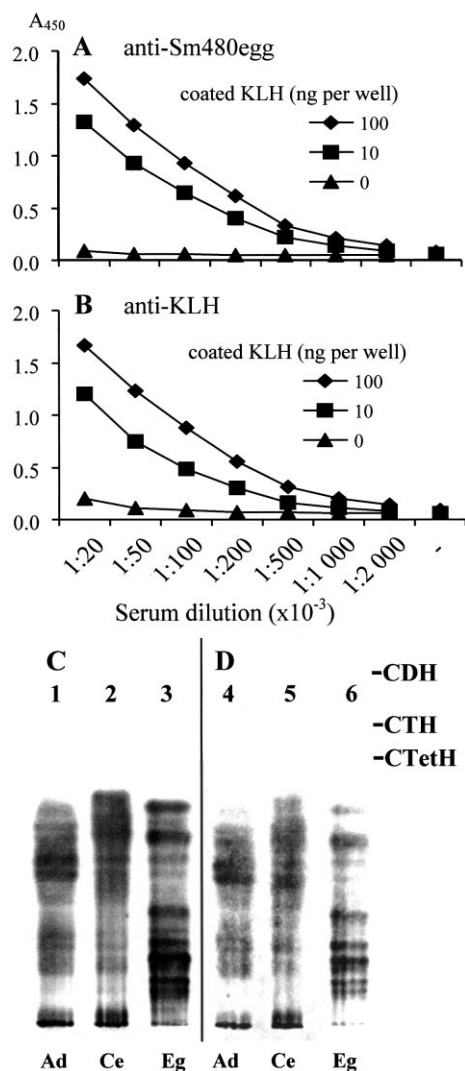


Fig. 1. Cross-reactivity of KLH with an anti-Sm480egg serum and of *S. mansoni* complex glycolipids with an anti-*B. glabrata* haemolymph serum. KLH (100, 10 or 0 ng)-coated microtitre plate wells were tested in ELISA with an anti-Sm480egg (A) or an anti-KLH serum (B) on dilution between 1:20 000 and 1:2 000 000. In control wells, the immune sera were omitted (–). The given data are the mean values of duplicate measurements. (C, D) HPTLC (running solvent, chloroform:methanol:0.25% aqueous KCl [50:40:10, by volume]). Glycolipids were visualised serologically with an anti-Sm480egg serum (dilution 1:2000; C) or an anti-*B. glabrata* haemolymph serum (dilution 1:500; D). Ad, adult worms; Ce, cercariae; Eg, eggs.

mon epitope in *S. mansoni* glycolipids, various egg *S. mansoni* glycoproteins and KLH. The Sm480egg hyperimmune serum [16], which is known to strongly react with *S. mansoni* glycolipids [19], recognised KLH as efficiently as the homologous hyperimmune serum (Fig. 1A and B). Both sera reacted in ELISA very strongly and showed significant binding even at a dilution of 1:1 000 000 (Fig. 1A and B). It is known that the cross-reactivity of snail haemocyanins with the 38 kDa *S. mansoni* surface protein does not only occur for KLH, but also for the haemocyanins of other molluscs [9], some species of which are known to be trematode intermediate hosts. This posed the question whether the haemolymph of the *S. mansoni* intermediate host, *B. glabrata*, also contained antigens that cross-reacted with *S. mansoni* glycoconjugates. For this purpose, a hyperimmune serum raised against *B. glabrata* haemolymph was tested by HPTLC immunostaining for the recognition of *S. mansoni* glycolipids (Fig. 1D) with the result that anti-haemolymph serum had a similar recognition pattern as anti-Sm480egg serum. A serum raised against an unrelated recombinant protein was used in parallel to exclude cross-reactivity due to antigenic determinants present in Freund's adjuvants, which showed no reaction. We, therefore, postulate that a common epitope is present not only on *S. mansoni* glycolipids and glycoproteins, but also on molluscan haemolymph glycoconjugates.

This cross-reactivity was further analysed by inhibition-experiments using KLH as inhibitor. The inhibition-ELISA assay was established using KLH as soluble inhibitor in a homologous system with KLH adsorbed to the ELISA plate and an anti-KLH hyperimmune serum used for detection (Fig. 2A). At 10 μ g of KLH per well (100 μ l), an almost total inhibition of binding was achieved. This inhibition assay was used to test, whether one of the epitopes known to occur on *S. mansoni* cercarial glycolipids (i.e. Le^x) is shared between these glycolipids and KLH. The binding of an anti-Le^x mAb to cercarial glycolipids, which express a high amount of the Le^x epitope [19,23], was not inhibited by KLH (Fig. 2B) and indicated that Le^x is not the epitope shared between schistosomal glycolipids and KLH. Binding of mAb

M2D3H, which is known to react with glycolipids of various life-cycle stages [19], to glycolipids of *S. mansoni* adult worms, cercariae and eggs was inhibited by soluble KLH (Fig. 2C, E and G). At a 1:50 000 dilution of mAb M2D3H, an inhibitor concentration of 1 μg KLH per well (100 μl) was sufficient to achieve over 50% inhibition of binding. These inhibition experiments clearly showed that the M2D3H-epitope, and not the Le^x-epitope, is shared between KLH and *S. mansoni* glycolipids of all three life-cycle stages. *S. mansoni* infection sera are also known to contain antibodies that give the same pattern of HPTLC immunostaining with *S. mansoni* glycolipids as the

mAb M2D3H and, thus, would seem to recognise the same epitope [19,23]. We, therefore, tested whether the binding of MIS to *S. mansoni* glycolipids could be similarly inhibited by soluble KLH (Fig. 4D, F and H). Using glycolipids of all three life-cycle stages, inhibition could be clearly demonstrated at serum dilutions of 1:1000–1:20 000 and KLH concentrations of 0.1–50 μg per well (100 μl ; Fig. 4D, F and H). With a KLH concentration of 10 μg ml⁻¹ and dilutions of infection serum from 1:1000 to 1:10 000, approximately 50% inhibitions were achieved. Cercarial glycolipids are known to express two different epitopes, the Le^x- and the M2D3H-epitope [19]. At the structural level, the Le^x-epitope has been shown to be chemically dominant at this life-cycle stage [23]. The availability of KLH as an inhibitor, which reduced binding to the M2D3H epitope, but did not affect binding to the Le^x-epitope, permitted differentiation of these two epitopes in the recognition of cercarial glycolipids by MIS (Fig. 2F). Because approximately 80% of the binding of infection serum to cercarial glycolipids could be inhibited by 50 μg KLH per well (100

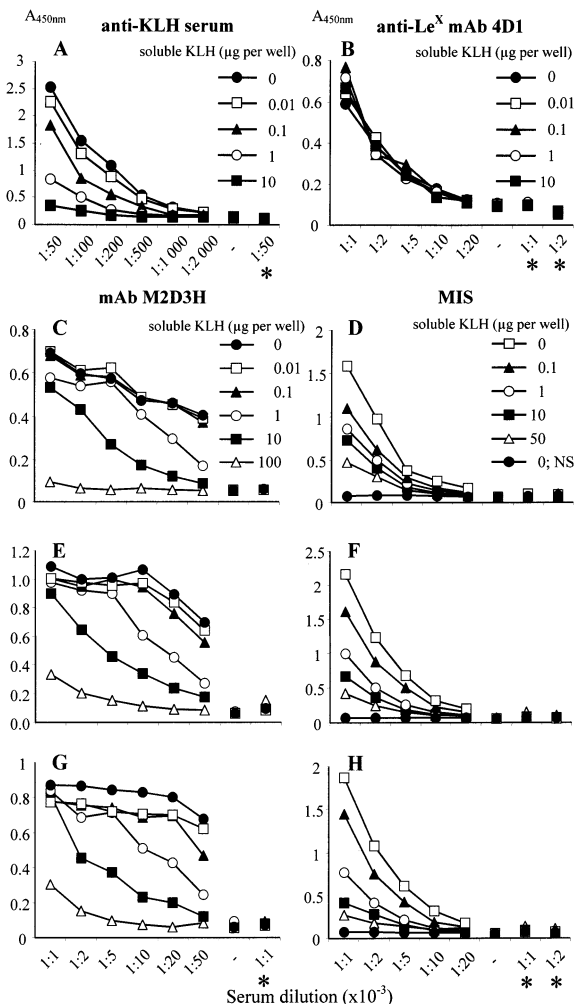


Fig. 2.

Fig. 2. Inhibition-ELISA using soluble KLH as the inhibitor. (A) Establishment of assay: The binding of an anti-KLH serum to adsorbed KLH was inhibited by soluble KLH. KLH was adsorbed at 50 ng per well. The serum was diluted from 1:50 000 to 1:2 000 000; (–) secondary antibody control (the immune serum was omitted); (*) control without antigen (anti-KLH serum 1:50 000). (B) Negative control: KLH was tested for its potential to inhibit binding of the anti-Le^x mAb 4D1 to *S. mansoni* cercarial glycolipids (50 ng carbohydrate per well). MAb 4D1 was applied at dilutions from 1:1000 to 1:20 000; (–) secondary antibody control (mAb was omitted); (*) controls without antigen (primary antibody 1:1000 and 1:2000). (C, E, G) Inhibition of mAb M2D3H binding to *S. mansoni* glycolipids. The mAb was diluted from 1:1000 to 1:50 000; (–) secondary antibody control (mAb was omitted); (*) non-antigen control (mAb M2D3H 1:1000). (D, F, H) Inhibition of MIS binding to *S. mansoni* glycolipids. MIS was diluted from 1:1000 to 1:20 000; (–) secondary antibody control (MIS was omitted); (*) non-antigen control (MIS 1:1000 and 1:2000). NS was also used as a control instead of MIS. Inhibition experiments were performed with various amounts of soluble KLH per well (0–100 μg) against *S. mansoni* adult (C, D), cercarial (E, F) and egg (G, H) complex glycolipids. The amount of glycolipid antigen applied per well was: (C, adult) 2 ng; (D, adult) 10 ng; (E, cercaria) 10 ng; (F, cercaria) 100 ng; (G, egg) 1 ng; (H, egg) 10 ng.

Table 1
Carbohydrate composition analysis of KLH fractions^a

KLH sample	Relative amount of monosaccharide						
	Fuc	Xyl	Glc	Man	Gal	GalN	GlcN
Native	0.7	0.05	0.15	0.8	0.7	0.3	1.0
Retained fraction, 0 mM TFA	0.75	0.05	0.15	0.7	0.55	0.25	1.0
Retained fraction, 1000 mM TFA	0.2	0.05	0.15	0.85	0.6	0.25	1.0
Retained fraction, 1500 mM TFA	0.25	0.1	0.1	0.75	0.65	0.2	1.0

^a KLH (50 µg) was analysed by carbohydrate composition analysis in its native form. Alternatively, KLH samples were partially hydrolysed using TFA of different molarity (40 min, 80°C) and fractionated by centrifugal ultrafiltration (10 kDa cut-off). The retentate was subjected to hydrolysis (4 M TFA, 100°C, 4 h) and the monosaccharide residues were detected by HPLC and fluorescence analysis as their anthranilic acid-derivatives. For each monosaccharide, the response factor was determined to allow their quantification. Relative amounts are given with GlcN = 1.0.

µl), most of the recognition by infection serum would appear to be due to the M2D3H-type epitope on cercarial glycolipids and only a maximum of 20% could be due to the Le^x epitope. Taken together, the experiments showed an inhibition of the binding of a mAb and MIS to *S. mansoni* glycolipids from all three life-cycle stages.

In another assay based on inhibition-HPTLC immunostaining, the inhibition of MIS, anti-KLH serum and mAb M2D3H binding by KLH to cercarial and egg complex glycolipids seemed to affect equally the recognition of all glycolipid bands (preincubation of primary antibodies with 1 mg ml⁻¹ KLH for 10 min prior to addition to HPTLC-plate; data not shown). In keeping with the inhibition-ELISA experiment (Fig. 2B), the binding of an anti-Le^x mAb to cercarial glycolipids was not inhibited by KLH. For the anti-Sm480egg hyperimmune serum, only partial inhibition of the binding to cercarial and egg glycolipids was observed.

We previously reported, that the recognition of glycolipids from *S. mansoni* adult worms, cercariae and eggs by the mAb M2D3H and MIS depended on the presence of fucose, as removal of this residue by partial hydrolysis or α -fucosidase treatment greatly reduced binding [19]. We used this partial hydrolysis approach to study KLH-binding of various hyperimmune and infection sera, and mAb M2D3H. Carbohydrate composition analysis of KLH gave a carbohydrate content of 1.7%. The dominant monosaccharide residues

found were glucosamine, fucose, mannose and galactose (Table 1). Glucosamine and galactosamine are assumed to be derived from the de-*N*-acetylation of *N*-acetylglucosamine and *N*-acetylgalactosamine during hydrolysis, respectively. Monosaccharide linkage analysis indicated the occurrence of terminal fucose (peak 1) and an internal, monosubstituted fucose (peak 2) in the relative amounts of 1.0: 0.7, respectively (Fig. 3). By pre-incubating KLH samples with increasing concentrations of TFA, the specific cleavage of fucose residue(s) was optimised. Released monosaccharide residues were derivatised with anthranilic acid without a further hydrolysis step and the products were separated by HPLC, detected by fluorescence and quantified (Fig. 4). When TFA concentrations from 10 to 1500 mM were used, fucose was the only monosaccharide to be released. When 2000 mM TFA was used, approximately 11% of the galactose content of the KLH sample was released as the monomer (Fig. 4). The proportion of monomeric fucose obtained when using 1000–2000 mM TFA was approximately 70% of that obtained by standard hydrolysis (Table 1). We then tested whether KLH glycans remained largely intact under these hydrolysis conditions, or were degraded to low-molecular mass fragments. For this, partially hydrolysed KLH was freed of low-molecular mass components by centrifugal ultrafiltration and the retained high-molecular mass fraction was analysed for its carbohydrate content. On com-

paring the retained fractions of untreated and TFA-treated KLH, fucose was the only monosaccharide to be dramatically depleted following partial hydrolysis (Table 1). Thus, the removal of fucose was the main effect obtained after partial hydrolysis of KLH, while the rest of the glycans appeared to remain largely intact and attached to KLH.

KLH obtained after fucose removal was investigated by ELISA and dot-blot analysis for its immunoreactivity to various sera and antibodies. Both ELISA and dot-blot experiments (Fig. 5A and B) showed that there was a large decrease in KLH immunoreactivity with infection serum,

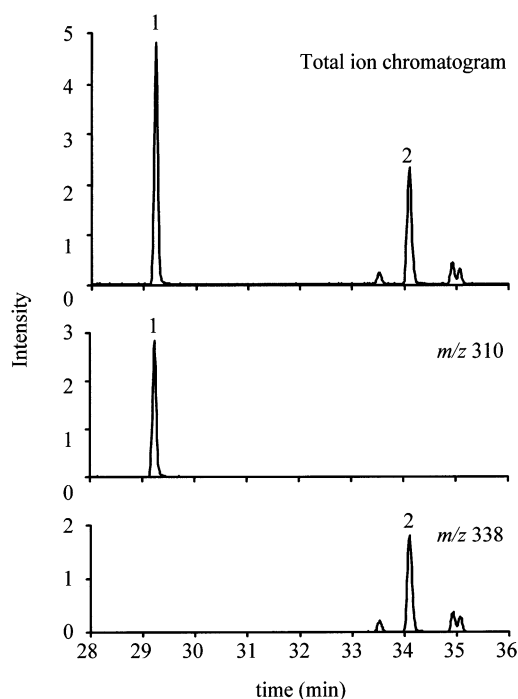


Fig. 3. Detection of terminal and monosubstituted fucose species by methylation analysis of total KLH. Partially methylated alditol acetates were separated by gas chromatography and registered in the positive-ion mode after chemical ionisation. In the total ion chromatogram, two major species were detected at retention times typical of terminal and internal fucose residues. Terminal fucose was identified by cochromatography with an authentic tri-*O*-methylfucitol standard and its characteristic pseudomolecular ion (m/z 310). The presence of internal, monosubstituted (i.e. di-*O*-methylfucitol) fucose was indicated by the pseudomolecular ion characteristic for a monosubstituted deoxyhexose species (m/z 338).

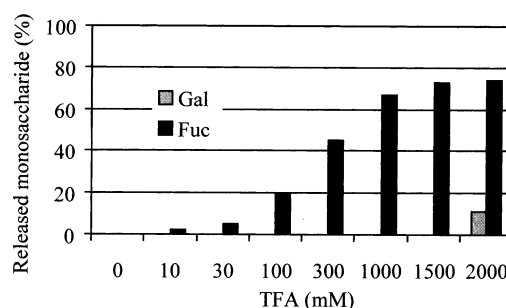


Fig. 4. Removal of fucose from KLH by partial hydrolysis as monitored by carbohydrate composition analysis. KLH samples were incubated with TFA of different molarity for 40 min at 80°C. The monosaccharides released were detected by HPLC and fluorescence analysis as their anthranilic acid-derivatives. The response factor for each monosaccharide residue was determined to allow their quantification. Fucose and galactose were the only monosaccharide residues detected. The fucose and galactose yield obtained for a KLH control sample after hydrolysis with 4 M TFA for 4 h at 100°C was set to 100%.

anti-Sm480egg hyperimmune serum and mAb M2D3H after treatment with 300 mM TFA and, more clearly, following the action of 1000 mM TFA. Anti-KLH serum, however, still showed a high degree of reactivity with KLH after partial hydrolysis in ELISA and dot-blot analysis. Whether the observed reduction in reactivity of the anti-KLH serum was due to the destruction of the M2D3H epitope or to thermal denaturation and destruction of protein antigenic determinants was not investigated further. The ELISA and dot-blot data indicated that partial hydrolysis almost completely destroyed the M2D3H-epitope, whilst other KLH-associated epitopes appeared to remain largely intact.

Taken together, the results of our experiments demonstrated the presence of a fucose-containing epitope on KLH glycans recognised by the mAb M2D3H as well as murine *S. mansoni* infection sera, which also reacted with *S. mansoni* glycosphingolipids.

4. Discussion

With the definition of the fucose-containing M2D3H epitope shared between *S. mansoni* gly-

colipids and KLH described in this study, an association has arisen between two areas of *S. mansoni* carbohydrate analysis, i.e. the studies performed on KLH as a serodiagnostic antigen or vaccine and the work on *S. mansoni* glycolipids, which has disclosed multiple facets for comparison. *S. mansoni* egg glycolipids have been structurally and immunochemically analysed [20,23], which established the importance of the fucose residue(s) for the M2D3H epitope. The IgE response to egg and adult worm glycolipids appeared to correlate with resistance to infection [25]. Adult worm glycolipids have been considered as possible serodiagnostic antigens [26] and, via the shared M2D3H epitope, this can now be correlated with the data on KLH as a diagnostic

antigen [4–6]. The reports of a common epitope present on *S. mansoni* glycolipids and egg-stage glycoproteins published by the groups of Strand [27–29], Dell [20,30] and our laboratory [19], now dovetail into each other with the recent finding of a common serodiagnostic epitope(s) on soluble egg antigens and KLH [16]. The finding of a common antigenic determinant on glycolipids from *S. mansoni*, *Schistosoma haematobium* and *Schistosoma japonicum* [28] overlaps with the application of KLH for the diagnosis [4–6] of and vaccination [7,8] against different schistosome species.

At the immunohistochemical level, the mAb M2D3H was observed to have a similar pattern of immunoreactivity on adult worm sections (unpublished results) to the mAbs 128C3/3 [29] and 114-5B1-A [31], both of which recognised the excretory system and its terminating flame cells, and showed pronounced reactivity with the cercarial surface and miracidia.

The observation of a shared epitope of the M2D3H-type between schistosome glycolipids and components in the intermediate host haemolymph (Fig. 1) is paralleled by that of the Le^x epitope, which is shared between cercarial glycolipids and the definitive host [19,23]. The Le^x epitope on cercarial glycolipids could be speculated to be a kind of reservoir, which is activated upon transformation, such that, Le^x is expressed at the schistosomular surface to give the invading parasite a host-like appearance. Glycolipid-bound Le^x thus fits into the concept of molecular mimicry of host-carbohydrate structures [23]. In the case of the M2D3H epitope, which at the glycolipid level is most strongly expressed in the egg-stage of *S. mansoni* [19], our observation of its cross-reactivity with intermediate host-glycoconjugates (Fig. 1) could be speculated to be an additional example of molecular mimicry to an intermediate host-carbohydrate epitope.

Speculation as to the nature of the KLH glycans can now be initiated using our carbohydrate composition data, which fit together with other results recently published [32]: the high relative amounts of glucosamine and mannose could indicate the presence of *N*-glycans, whereas the presence of galactosamine and galactose would fit the

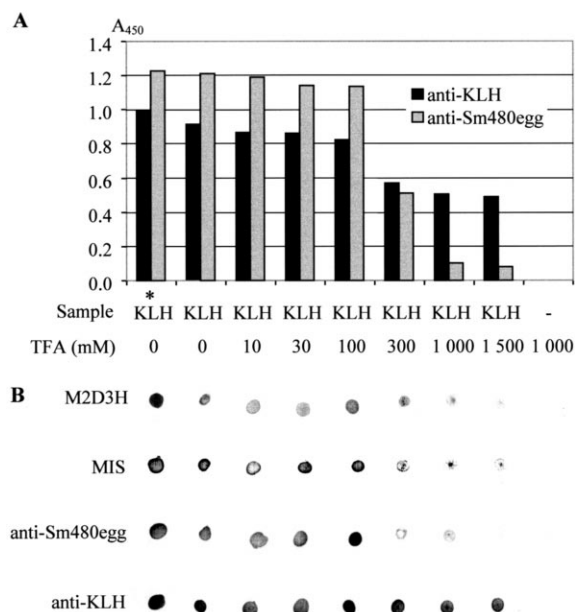


Fig. 5. Immunological detection of intact and partially hydrolysed KLH. Tests were performed with KLH after a 40-min partial hydrolysis at 80°C. The molarity of the TFA applied varied from 0 to 1500 mM and is given for each sample. As controls, native KLH was used without heat and TFA treatment (*) or 1000 mM TFA was incubated without KLH (-, 1000). For ELISA (A), the plates were coated with 50 ng KLH per well, and detection was carried out with anti-Sm480egg (1:50 000) and anti-KLH (1:50 000) sera. For the dot-blot (B), 4 µg KLH was applied per dot, and detection was performed with mAb M2D3H (1:1000), MIS (1:500), anti-Sm480 egg serum (1:5000) and anti-KLH serum (1:5000).

description of a possibly *O*-linked T antigen, which has been postulated in a recent study [32]. Our finding of fucose as part of an antigenic determinant on KLH poses the question as to how KLH glycans add to the picture of fucosylated antigens, which have been described for nematodes [33,34], trematodes [35], and various plant allergens [36].

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