Circulating gut-associated antigens of Schistosoma mansoni : biological, immunological, and molecular aspects

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Chapter 2

Schistosoma carbohydrates in host–parasite (immunological) interactions

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2.1. *Glycoconjugates in general – short review.*

Structural characteristics of glycoconjugates

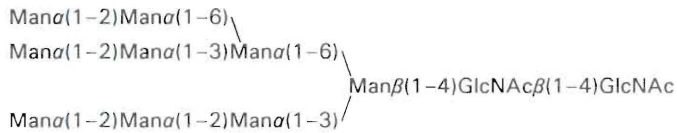
Glycoconjugates are molecules which contain one or more carbohydrate structures, like glycoproteins, glycolipids, proteoglycans or lipopolysaccharides. Glycosylation of proteins, the attachment of one or more carbohydrate chains (glycans) to a protein backbone, is a very common phenomenon shared by almost all organisms. The biological roles of these carbohydrate units include protection against proteolytic attack, induction and maintenance of the 3–dimensional conformation in a biologically active form, facilitation of the extracellular secretion, direction and modulation of the immune response, provision of ligand structures for cell–adhesive molecules (see recent reviews [46,98,181] and papers cited therein).

The major linkages described between the protein and the oligosaccharide or polysaccharide side–chains are N–glycosidic (to asparagine) or O–glycosidic (to serine or threonine). The major types of N–linked carbohydrate chains are summarized in Table 1, and seven types of core structures found in O–linked glycans of the mucin–type in Table 2. These core structures may be extended with poly–*N*–acetyllactosamine chains, containing repeating units consisting of Gal and GlcNAc [111]. These polysaccharide side–chains may be substituted with *e.g.* Fuc [165] and/or Neu5Ac (in terminal position) [109], and have been shown to carry various antigenic determinants such as I/i, ABO blood group and Lewis antigens (reviewed in *e.g.* [43,45]). Lewis x (Le^X, also called SSEA–1 or X–hapten [82], being the dominant epitope in lacto–*N*–fucopentaose–III, LNFP–III [75,93,174,182]) and sialyl–Le^X, present on granulocytes and monocytes, may serve as ligands for adhesion molecules (L–selectin and P–selectin) on endothelial cells and platelets [22,77,166,170], thus playing a critical role in the recruitment of leukocytes to inflammatory sites [84]. Besides

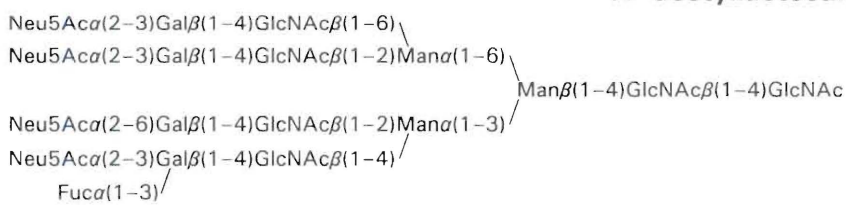
these roles in normal physiological processes, Le^x–containing glycoproteins and glycolipids are also considered to be onco–developmental antigens [43,59] and in some human neoplasms, the expression of Le^x or sialyl–Le^x appears to be associated with increased metastasis or tumor relapse [121,152,155].

Table 1. Examples of oligomannose, *N*–acetyllactosamine, hybrid and xylose–containing types of asparagine–linked carbohydrate chains.[a]

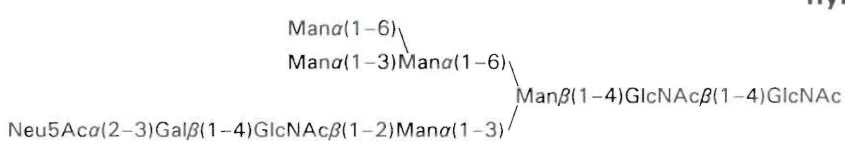
oligomannose type



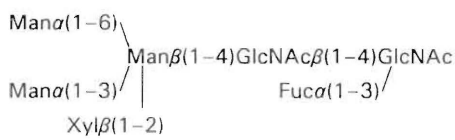
***N*–acetyllactosamine type**



hybrid type



xylose–containing type



[a] structures described *e.g.* in [4,81,95,117,180]

Proteoglycans are a class of glycoconjugates found in *e.g.* animal connective tissues and have as a common structural characteristic that they contain several linear polysaccharide chains (named glycosaminoglycans) generally composed of disaccharide repeating units of a uronic acid (GlcA or IdoA) and GlcNAc or GalNAc. Glycosaminoglycans are highly negatively charged at neutral pH and

most of them are sulphated, *e.g.* chondroitin sulphate and dermatan sulphate. The glycosaminoglycans are usually covalently bound to the protein backbone by Xyl–Ser linkages, but also GalNAc–Ser/Thr or GlcNAc–Asn linkages are found, dependent on the type of proteoglycan [169]. Functions of proteoglycans in which the carbohydrate chains are essential are *e.g.* interaction with complement components and inhibition of complement activation [145], regulatory functions (anticoagulant) in hemostasis [8], and stability of extracellular matrix [111].

Table 2. Core structures found in mucin–type serine– or threonine–linked oligosaccharides and polysaccharides.[a]

core 1 GalNAc–Ser(Thr) Galβ(1–3)/	**core 5** GalNAc–Ser(Thr) GalNAcα(1–3)/
core 2 GlcNAcβ(1–6)\ GalNAc–Ser(Thr) Galβ(1–3)/	**core 6** GlcNAcβ(1–6)\ GalNAc–Ser(Thr)
core 3 GalNAc–Ser(Thr) GlcNAcβ(1–3)/	**core 7** GalNAcα(1–6)\ GalNAc–Ser(Thr)
core 4 GlcNAcβ(1–6)\ GalNAc–Ser(Thr) GlcNAcβ(1–3)/	

[a] structures described *e.g.* in [64,172]

Glycoconjugates of parasites

The function and role of glycosylated structures in the interaction of pathogenic organisms like unicellular and multicellular parasites is becoming increasingly apparent. Glycoconjugates are involved in parasite membrane protection [44,150,191], specific interaction with some host cell–types [18,63,118,136,151,191], physical stability and protection of cercarial tegument [17,38,123,153], or masking of antigens the recognition whereof by the host immune system may result in damage to the parasite [38,160]. Since

the carbohydrate chains of the glycoconjugates appear to be directly involved in these interactions, the elucidation of the glycan structure may yield new insights into parasite glycoconjugate structure/effect relationships and into the biosynthetic pathways responsible for their formation. Several studies have indicated that parasite glycans may be highly immunogenic and thus can be exploited in vaccine development, as it has been shown that antibodies against these structures can mediate protective immunity [56,78,90,91,134,173].
The structural analysis of parasite glycoconjugates has often been hampered by the small amounts of purified material available. For characterization, peptides and proteins which are scarce can be obtained in theoretically unlimited amounts by recombinant DNA-technology, if the amino acid sequence or DNA code is known. However, only careful and laborious purification can yield sufficient material needed for carbohydrate structural analysis. For this reason many research groups have used (partially) purified antigen preparations and/or immunochemical or histochemical methods for studying these structures. These methods comprise specific antibody and lectin binding, to the structures themselves and/or in combination with various chemical and enzymatic carbohydrate degradation methods. A short description of the various techniques used follows below.

Structural analysis of glycoconjugate glycans

Biochemical techniques. To the biochemist, a variety of analytical techniques is available for the identification of the primary structure of carbohydrates isolated from glycoconjugates (reviewed in *e.g.* [74,98,99]). Among the techniques often used are monosaccharide analysis, enzymatic degradation methods, mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. A major advantage of MS is the very small amount of sample needed (as low as femtomoles [76]) while NMR spectroscopy needs more material (usually in the nanomole range), but is in contrast to MS non-destructive, allowing multiple analyses to be performed. Each technique yields specific information, and combination of the methods is mostly essential for a complete elucidation of a molecular structure. As an example, NMR spectroscopy can identify the type and sequence of sugar residues (Gal, Man, etc.) as well as the type of linkages, while MS can give size, composition (hexose, pentose, etc.) and linkage information. Enzymatic degradation methods, *e.g.* sequential exoglycosidase digestion [92,188], make use of the specificity of naturally occurring enzymes (purified or recombinant), sometimes in combination with sequential lectin-affinity chromatography [21,116]. These methods may also yield information on the identity of the monosaccharide residue as well as on the sequence and type of linkages. The introduction of radioactive (^{3}H, ^{14}C) or fluorescent labels makes detection highly sensitive, and are therefore widely applied in the analysis of minute amounts of parasite carbohydrates.

Lectin–based techniques. Lectins are sugar–binding proteins or glycoproteins of non–immune origin without enzymatic activity to the carbohydrate bound [94]. Various lectins show agglutination properties towards cells *in vitro* or precipitate glycoconjugates [36,50]. Many highly purified lectins of well–defined carbohydrate specificity (those applied in the analysis of schistosomal antigens are summarized in Table 3) are easily available, which has led to the widespread adoption of lectin techniques [4]. These techniques employ conjugates of lectins with a variety of fluorescent labels or enzymes, biotin–conjugates as well as immobilized lectins for affinity chromatography [21,48,51,104,116]. Because they do not usually enter cells, lectins can be used as probes to provide information about the location, abundance and function of glycoconjugates at cell surfaces [37,85,102,108,123,159,161,176,177]. The specific and reversible interactions between lectins and mono– or oligosaccharides form the basis for important affinity chromatographical methods used in isolation and fractionation of glycoconjugates and glycopeptides [21,105,116,131,167].

Table 3. Various lectins used for the analysis of carbohydrates in schistosomal antigens.[a]

Lectin		Carbohydrate specificity
Con A	*Canavalia ensiformis* (jack bean) (Concanavalin A)	α–D–Man > α–D–Glc > α–D–GlcNAc
DBA	*Dolichos biflorus* (horse gram)	α–D–GalNAc
GS–I	*Griffonia simplicifolia*	α–D–Gal,α–D–GalNAc
GS–II	*Griffonia simplicifolia*	α/β–D–GlcNAc
HPA	*Helix pomatia* (edible snail)	α–D–GalNAc
LBA	*Phaseolus lunatus* (lima bean)	α–D–GalNAc > Gal
LcH	*Lens culinaris* (lentil)	α–D–Man, α–D–Glc, α–D–GlcNAc
Lotus	*Lotus tetragonolubus purpureas*	α–L–Fuc
MAA	*Maackia amurensis*	Neu5Ac–α(2–3)–Gal
PNA	*Arachis hypogaea* (peanut)	D–Gal–β(1→3)–D–GalNAc > β–D–Gal
PWA	*Phytolacca americana* (pokeweed)	D–GlcNAc–β(1–4)–D–GlcNAc–β–(1–4)–D–GlcNAc
RCA–I	*Ricinus communis* I – 120 kDa (castor bean)	β–D–Gal
RCA–II	*Ricinus communis* II – 60 kDa (castor bean)	β–D–Gal, α/β–D–GalNAc
SBA	*Glycine max* (soybean)	α/β–D–GalNAc
SJA	*Sophora japonica*	α–D–GalNAc
UEA–I	*Ulex europaeus*	α–L–Fuc(1→x)[b]
WFA	*Wisteria floribunda* (Japanese wisteria)	α/β–D–GalNAc
WGA	*Triticum vulgaris* (wheat germ)	β–D–GlcNAc–β(1→4)–D–GlcNAc

[a] data based on *e.g.* [5,51,104,177].
[b] x may be any aglycon

Antibody-based techniques. There are close parallels between lectins and carbohydrate-specific monoclonal antibodies (McAbs), resulting in a similar spectrum of applications and methods. A major difference is that, in general, antibody binding to carbohydrate epitopes involves more than one sugar residue, and is not easily inhibited by a single monosaccharide or even by a disaccharide [13,179]. The maximum size of an antigen-binding site of an antibody is about 6 sugar or amino acid residues [19,24,127,179,186]. For the determination of overall carbohydrate recognition patterns in humoral immune responses polyclonal antibodies present in immune sera are often used. Another frequent application of antibodies is the immunohistochemical and immunocytochemical localization of antigens [25,26,71,72,96,135,163].

Partial destruction and inhibition. The above described techniques may also be applied to glycoprotein preparations which are partially degraded with respect to their carbohydrate or peptide portion. Methods generally used for degradation include chemical methods like sodiumperiodate treatment [5,91,126,187], or mild acid hydrolysis (for desialylation [137,175] and defucosylation [91,137,143,175,183]), as well as enzymatic methods [52,91,98]. The inhibitory effect of these treatments on the binding of antibodies or lectins yields information about the type of mono- or oligosaccharide involved. Inhibition can also be achieved by mono- or oligosaccharides themselves [19,90,100,105,157], by other antigenic preparations [57,124,144,179], or by other lectins, McAbs or polyclonal antibodies [58,100,144].

2.2. *Glycoconjugates of* Schistosoma

Parasites of the genus *Schistosoma* are complex organisms shown to contain and synthesize numerous glycoproteins and glycolipids, which include some completely unique carbohydrate structures. This chapter will give an overview, dealing in particular with glycoprotein carbohydrates which might interact with the immune system of the definitive host. As the methods employed in glycan analysis are summarized above, these will only be shortly referred to in the paragraphs below.

Schistosomula and adult worms

Purified lectins with defined specificity have been employed in a number of studies to determine which glycans in worm tissues and secretions were accessible for the host immune system. A summary of the lectins recognizing

carbohydrate structures on the tegument or in the gut of the schistosome is given in Table 4. The host is exposed to parasite gut-associated antigens when these enter the circulation after regular regurgitation of the undigested contents of the gut.

Table 4. Lectin binding to *Schistosoma* adult worms and schistosomula.[a,b,c]

Tegument	Gut	Reference
Con A, SBA, DBA, PNA, RCA-I, UEA-I	Con A, RCA-I, SBA, DBA, UEA-I, PNA	Beisler *et al.* (1984) [5][d]
Con A, RCA, SBA ±, PNA, UEA-I, Lotus	Con A, RCA, SBA, PNA, UEA-I, Lotus	Linder and Huldt (1982) [103]
Con A, LcH, SBA, RCA-I, RCA-II	n.d.[e]	Hayunga and Sumner (1986) [69]
PNA, DBA, Con A, SBA	n.d.	MacGregor *et al.* (1985) [108]
Con A, RCA-II, PNA, SBA, Lotus	n.d.	Simpson and Smithers (1980) [161]
Con A, UEA-I, SBA	n.d.	Murrell *et al.* (1978) [122]

[a] as various reports indicated that the binding of WGA is largely aspecific [5,105,122,159,161], this lectin has been omitted in this Table
[b] unless otherwise indicated, studies were performed in *Schistosoma mansoni*
[c] shaded lectins were also investigated but showed no binding
[d] studied in *Schistosoma japonicum*
[e] n.d. = not done

In some studies, the reduction in binding of several lectins to the schistosomulum surface in the course of development of the parasite indicated a decline in the number of exposed carbohydrate epitopes [138,139,159]. On the other hand, Con A-agarose chromatography showed the presence of 19 and 45 kDa surface glycoproteins in two-day old but not in younger schistosomula [138], while PNA-binding demonstrated a 170 kDa glycoprotein abundantly present on 4-week-old worms, but absent in pre-liver stages of the parasite [107]. The loss of lectin binding sites is increased after a short stay in the circulation of the host and appears to be due to antigen shedding rather than masking of carbohydrate epitopes [139].

As a more general phenomenon, antigen shedding has also been suggested by a decrease in surface-bound anti-carbohydrate antibodies with the development

of the parasite, resulting in an absence of antibodies recognizing lung schistosomula [97]. Shedding of surface antigens is one of the evasion mechanisms employed by the parasite to escape the host immune attack occurring through antibodies [10,97], or via the alternative complement activation route [112,113,154]. On the other hand, the parasite may evade the host immune response by masking of surface antigens through acquisition of host molecules on the tegument, as described by Clegg, Smithers and Terry in 1971 [20], as well as by others [49,156]. This phenomenon has been clearly linked with a decrease in binding to the schistosomula of host antibodies or complement components [73,146], while other studies also indicate some intrinsic changes in the tegument which render the parasite insusceptible to immune attack [14,31,120].

The presence of Con A–binding epitopes on tegument antigens as well as on gut antigens has been consistently shown (Table 4). The highest binding affinity of Con A is to the Man residue (Table 3), for which reason it is generally used to indicate the presence of N–linked carbohydrate structures (see Table 1) [21,116]. Binding of SBA and PNA to the parasite surface indicates the presence of GalNAc– and Gal–GalNAc–containing oligosaccharides, respectively (Table 3). These findings have been confirmed more recently by studies of Cummings and co–workers [128-131,167,168], using glycoconjugates synthesized by the worms after incubation *in vitro* with radiolabeled monosaccharides and combinations of the above described direct and indirect analytical methods (*e.g.* lectin–affinity chromatography, exo– and endo–glycosidase treatment). They were able to characterize a number of unique carbohydrate structures which are summarized in Table 5.

The presence of Le^x on the surface of schistosomula as detected by McAbs prepared from mice immunized with *S. mansoni* eggs [66,91], was described by Ko *et al.* (1990) [90]. Using sera of *S. mansoni* infected patients these investigators also found a positive reaction on sections of embryonic mouse head with a distribution similar to that of the SSEA–1 epitope (is identical to Le^x), from which they concluded that anti–Le^x antibodies are generated during human schistosomiasis infection. Recently, Köster and Strand (1994) described another McAb recognizing the Le^x epitope and showed that the epitope was expressed at the surface of schistosomula (immediately after transformation) and adult worms, as well as at the gut epithelium of adult worms [96].

In contrast with these reports on the presence of the Le^x trisaccharide in *Schistosoma* [90,128], several studies failed to show binding of the Fuc–specific lectins UEA–I and/or Lotus to schistosomula and adult worms (Table 4) [103,138,139,159,161], although both lectins were shown to recognize Le^x [163,167]. A possible explanation is that, due to conformational changes, steric hindrance, or epitope masking by other (glyco)proteins, the

Table 5. Schistosome carbohydrate structures described by Cummings and co-workers.

Carbohydrate structure	Reference
one-day cultured male worms	
O-linked terminal GalNAc O-linked terminal GlcNAc O-linked Gal-GalNAc	[128]
N-linked oligomannose-type oligosaccharides, ranging in size from $Man_7GlcNAc_2$ to $Man_9GlcNAc_2$	[130]
N-linked tri- and diantennary *N*-acetyllactosamine-type oligosaccharides, containing Man, Fuc, GlcNAc, and GalNAc[a]	
N-linked poly-Le^x, $(\rightarrow 3)$-β-Gal-$(1\rightarrow 4)$-[α-Fuc-$(1\rightarrow 3)$]-β-GlcNAc-$(1\rightarrow)_n$	[131]
N-linked biantennary, complex-type oligosaccharides, containing on both antennae the terminal sequence β-GalNAc-$(1\rightarrow 4)$-[$\pm\alpha$-Fuc-$(1\rightarrow 3)$]-β-GlcNAc-$(1\rightarrow 2)$-α-Man-$(1\rightarrow$	[167] [168]
glycosphingolipid with novel core β-GalNAc-$(1\rightarrow 4)$-Glc-ceramide	[110]
two-day cultured mechanically transformed schistosomula	[129]
O-linked GalNAc O-linked GlcNAc	
N-linked oligomannose-type oligosaccharides, ranging in size from $Man_6GlcNAc_2$ to $Man_9GlcNAc_2$	
N-linked complex-type oligosaccharides, containing Man, Fuc, GlcNAc, and GalNAc[b]	

[a] Neu5Ac could not be detected; GalNAc was β-linked in a terminal position
[b] Neu5Ac could not be detected

Fuc-containing epitopes are accessible to the lectins only in (partly) purified antigens but not if the epitopes are still present in worm tissues[1].

An interesting excursion on the interaction of fucosylated (Le^x and Le^y) oligosaccharides has recently been described by Harn and co-workers [182]. These authors show that these carbohydrates induce proliferation of human peripheral blood mononuclear cells (which include B cells) [65], as well as interleukin 10 production by isolated B cells ($B220^+$) of *S. mansoni*-infected

[1] Recently, in our laboratory using an IFA on sections of adult male *Schistosoma* worms fixed in Rossman's fixative, we found a strong reactivity of Lotus and a weak reactivity of UEA-I in the gut of the parasites (unpublished results). In Chapter 7 of this thesis it is demonstrated that circulating antigen CCA contains Le^x repeating units and that Lotus but not UEA-I binds to immunopurified CCA in ELISA [178]. In contrast, Carlier *et al.* (1980) have shown only a very weak binding of Lotus to partly purified M antigen (CCA), which might be due to a TCA-precipitation step in the preparation procedure causing a partial degradation of the Fuc-containing epitope [15].

and not of non-infected mice [182]. Moreover, they were able to show the presence of antibodies against these fucosylated oligosaccharides in the cerebrospinal fluid of schistosomiasis patients with cerebral disorders [47]. Based on these very new phenomena, the authors suggest a role of Le^x- and/or Le^y-containing oligosaccharides in the immunoregulation of the helper T cell response in schistosomiasis and maybe other chronic infectious diseases [182].

The presence of sialic acid (Neu5Ac) in schistosome glycoconjugates is a controversial issue. A number of researchers could not detect its presence in *in vitro* synthesized glycoproteins [128-131,168], while others found a minor amount in adult schistosome carcasses which they attributed to the presence of ingested erythrocytes [149]. In contrast, using immunochemical methods of lectin (PNA) or dye binding after neuraminidase treatment of the parasites, it was shown that some glycoconjugates in lung-stage schistosomula and adult worms contain sialic acid, as opposed to newly transformed schistosomula [115,159,161]. In the cercarial glycocalyx (surface coat), although negatively charged, Neu5Ac could not be detected [123].

The above described lectin-based studies demonstrate a large variety of carbohydrate structures on the surface of adult worms and schistosomula. Indeed, antigenic carbohydrate structures dominate at the surface of the schistosomulum indicated by absorption of more than 90 % of the schistosomulum-specific antibodies in infected mouse serum by an egg antigen preparation from which the protein antigens had been removed [134]. The cross-reaction between the carbohydrate structures of schistosome egg antigens and schistosomula surface antigens has formed the basis of a theory on the slow development of immunity observed in heavily-exposed children [9,10,12]. This experimentally well-supported hypothesis (recently reviewed in *e.g.* [11]) involves the development in young children of IgM and IgG2 antibodies which block the protective immune mechanisms of antibody-dependent cellular cytotoxicity (ADCC) by eosinophils and other cells. Polysaccharide antigens released by the large quantity of eggs deposited in the tissues elicit a predominantly thymus-independent antibody response resulting in especially IgM and IgG2 antibodies [3]. These so-called "blocking" antibodies may cross-react with carbohydrate epitopes on schistosomula surface glycoproteins, and not only fail to mediate ADCC reactions but also block "effector" antibodies like IgG1 and IgE. As the infection progresses and the child reaches adulthood the balance in the antibody response may switch to a predominantly protective type of response which results in the development of (partial) immunity.

Experimental and epidemiological evidence for this theory came from the groups of Capron, Butterworth, and recently of the group of Dessein. Capron and co-workers described a rat IgG2c McAb (isotype comparable with IgG3 in mice

[30,119,142] or IgG2 in man [3,79,89,158]) which inhibited a rat IgG2a McAb-mediated, eosinophil-dependent killing of schistosomula [54,55]. Mouse IgM McAbs recognizing cross-reactive egg polysaccharides and schistosomulum surface antigens as well as human IgM against the schistosomulum surface could effectively block the eosinophil-dependent killing of schistosomula by sera from infected humans [40,86]. Also in murine schistosomiasis blocking antibodies could be characterized [134,189,190] and it was shown that egg antigens elicit thymus-independent responses of the IgM and IgG3 isotypes [114]. The inhibitory effect on protection against schistosome infections of mice IgM antibodies which bind to egg and schistosomulum surface antigens might be limited to anti-carbohydrate antibodies as a number of studies indicate the presence of protective IgM responses or McAbs (in passive transfer experiments) which recognize egg and/or schistosomulum antigens [53,80,88,147,164].

After purification of individual IgG subclasses from schistosomiasis mansoni patient sera it was shown that IgG1 and IgG3 antibodies mediated eosinophil killing of schistosomula, that IgG4 consistently blocked killing, and that IgG2 would either mediate or block killing, depending on the state of activation of the eosinophils [87]. Epidemiological support for the blocking antibodies theory came mainly from the Kenyan reinfection studies of Butterworth *et al.* (1987,1988), who found that blocking antibodies of the IgG2 and IgM isotypes in the sera of young children prevented the expression of immunity [9,10]. Resistance to reinfection after treatment is associated with the presence of IgG isotypes other than IgG2 which recognize similar schistosomulum antigens [10], although Omer-Ali *et al.* (1989) obtained indications that, in general, antibodies against carbohydrate epitopes on the surface of schistosomula of *S. haematobium* do not have a major protective role in man [132]. Finally, Demeure *et al.* (1993) recently described that, in a population cross-section comprising all age-groups, anti-schistosomular IgG2 levels were predictive for susceptibility to reinfection [29].

The carbohydrate epitope originally defined by the protective rat IgG2a McAb produced by Capron and co-workers [33,54] (already mentioned above), is present on a 38 kDa schistosomula surface molecule and expressed in various molecular mass components in the different parasite stages of *S. mansoni* [32,33,35]. Interestingly, this epitope has been shown to be additionally synthesized by the freshwater snail *Biomphalaria glabrata* and may be implied in molecular mimicry between schistosomes and their invertebrate intermediate hosts [35]. Moreover, the epitope is present in products of other snails, including *Bulinus truncatus*, intermediate host for *S. haematobium* [35], as well as in keyhole limpet hemocyanin (KLH), synthesized by a marine mollusc *Megathura crenulata* [57]. The major immunogenicity of the 38 kDa carbohydrate moieties in mammalian hosts was confirmed in mouse and human

schistosomiasis [86,133]. The molecular structure of this carbohydrate epitope has not been elucidated (Dr. J.P. Kamerling, personal communication).

The above described role of schistosomulum surface carbohydrates in the development of a humoral immune response should be extended to the role of the excretory and secretory schistosome antigens. Indeed, in schistosome infections, high antibody titers are observed against parasite gut-associated antigens, which are regularly released into the circulation of the host [27,28,42,125]. Radiolabeling experiments showed that essentially all components released by the worm were recognized by antibodies in infected human sera, in contrast with most of the major (non-secreted) membrane and tegumental proteins [101]. It was therefore suggested that the adult worm is protected against immune attack by direction of the host's antibody response against released (glyco)proteins rather than against parasite surface antigens [101]. In passive transfer experiments, it was demonstrated that after administration of a mouse IgM McAb directed against the parasite gut epithelium, larger numbers of lung-stage schistosomula could be isolated than when an irrelevant IgM McAb or physiological salt was given [1]. The antigen recognized by this McAb was also found in high concentrations in *S. mansoni* eggs, was excreted in the urine of schistosomiasis patients and experimental animals [2,148] and appeared to have a polysaccharide nature, of which the antigenicity was readily destroyed by periodate, but not by protein-denaturing treatments [1]. In addition, a number of highly glycosylated gut-associated glycoproteins was described (among these CAA and CCA), which are summarized in Chapter 1 of this thesis. Although the immune response against those antigens is high, there appears to be so far no specific interaction with immune mechanisms, besides from those described in this thesis (Chapter 9,10).

Egg

The localization of glycans in schistosome eggs has been studied using lectins in similar procedures as for the carbohydrate structures found in schistosomula and adult worms (see above). A summary of the results of two studies is given in Table 6.

The cross-reactivity of carbohydrate epitopes on egg antigens with those on schistosomula surface antigens in relation to the occurrence of blocking antibodies has been discussed above. These epitopes were present on a 38 kDa schistosomulum surface molecule and also found on molecules of different M_r present in other schistosome life stages (miracidia, adult worm) or even in the intermediate snail host. A number of mouse IgM and IgG3 McAbs, which also reacted with antigens of about 38 kDa at the surface of *S. mansoni*

Table 6. Lectin binding to *Schistosoma* eggs.[a,b]

Miracidium	Vitelline membrane[c]	Vitelline space[d]	Reference
Con A, SBA ±, PNA, UEA-I ±	Con A, RCA-I	Con A, SBA, DBA, PNA, UEA-I	Beisler *et al.* (1984) [5][e]
RCA, SBA, PNA, UEA-I, Lotus [f]	[f]	[f]	Linder and Huldt, (1982) [103][f,g]

[a] as various reports indicated that the binding of WGA is largely aspecific [5,105,122,159,161], this lectin has been omitted in this Table
[b] shaded lectins were also investigated but showed no binding
[c] inside of egg shell
[d] containing hatching fluid (with miracidia excreta) which leaks through pories in the eggshell
[e] studied in *Schistosoma japonicum*
[f] no differentiation within egg was made
[g] studied in *Schistosoma mansoni*

schistosomula recognized a well-characterized polysaccharide egg antigen designated κ_3 [6,39,40]. Cross-reacting antigens were additionally found in a crude cercarial antigen preparation but not in adult worm homogenate. The IgM but not the IgG3 McAbs inhibited the killing of schistosomula by eosinophils in the presence of sera from infected humans [7,40]. However, passive transfer of the IgM McAbs to vaccinated mice at the time of challenge failed to produce *in vivo* blocking [6]. The polysaccharide κ_3 showed a molecular mass in the range of >750 – 70 kDa and was resistant to boiling and protease degradation, but sensitive to 50 mM periodate. It partially bound to Con A and remained unaffected by 0.1 M NaOH or 0.1 M HCl treatment. Cross-reactivity to other schistosome species was shown by rabbit anti-κ_3 antibodies which bound to the surface of *S. japonicum*, *S. haematobium*, and *S. bovis* schistosomula while also an egg antigen of *S. japonicum* was recognized [39].

Besides the blocking of cellular-dependent immune mechanisms, another effect of antibodies directed against egg carbohydrates was described by Simpson *et al.* (1990) who showed that the administration of a mouse anti-egg carbohydrate IgM McAb to mice intravenously injected with 2000 *S. mansoni* eggs resulted in larger lung granulomas than in mice which received no or an irrelevant McAb [162].

S. mansoni-infected T cell-deprived mice suffer from severe hepatocyte damage most likely caused by an egg-derived cationic glycoprotein antigen named ω_1. Together with another cationic glycoprotein, α_1, these antigens were recently purified from *S. mansoni* eggs and characterized with respect to their biochemical, immunological as well as hepatotoxic properties [41]. Ω_1 is a monomeric glycoprotein with a pI > 9.0 and a molecular mass of 31 kDa. The antigen α_1 consists of two immunologically cross-reactive dimers with a pI

ranging from 7.5 to 8.5 and molecular masses of 41 kDa and 36 kDa, each of which is composed of one unique and one common subcomponent. Since both antigens bound to Con A they might contain N-linked carbohydrate chains (Tables 3 and 1). The immunodominance of the carbohydrate epitopes was shown by the observation that antigenic activity against monospecific antisera was readily lost after oxidation with 50 mM periodate, but not affected by protease treatment. Mild acid hydrolysis destroyed the carbohydrate epitopes of ω_1 but not of α_1, while α_1 was more sensitive to NaOH treatment, suggesting the presence of O-linked glycans. Antibody-binding was abrogated after the antigens were heated at 100°C for 5 min.

Using an ELISA with sera from infected humans and mice, it has been shown that purified ω_1 is *S. mansoni*-specific and is a better marker than α_1 or unfractionated egg antigens to distinguish *S. mansoni* infection from other schistosome infections [41]. Passive transfer of monospecific anti-ω_1 sera into *S. mansoni*-infected, T cell-deprived mice completely prevented the occurrence of microvesicular hepatocyte damage in these animals, while monospecific anti-α_1 serum had no hepatoprotective activity [41].

Another common carbohydrate epitope identified by a mouse McAb (named 128C3/3, produced after immunization with cercarial antigens [171]) was found to be present on *S. mansoni*, *S. haematobium*, *S. japonicum* egg glycoproteins and glycolipids as well as on *S. mansoni* cercarial and adult worm antigens [96,184,185]. In addition, sera of both infected humans and mice contain large amounts of antibodies with a similar epitope specificity [184]. In an immunopurified glycolipid fraction (using the same McAb) the major immunogens were shown to be unique glycosphingolipids with repeating trisaccharide units containing internal Fuc residues substituted onto a novel glucosylceramide core structure [100]. The core structure was independently described by Makaaru *et al.* (1992) [110]. The largest antigen identified in the glycolipid preparation was interpreted to have the following structure[1]:

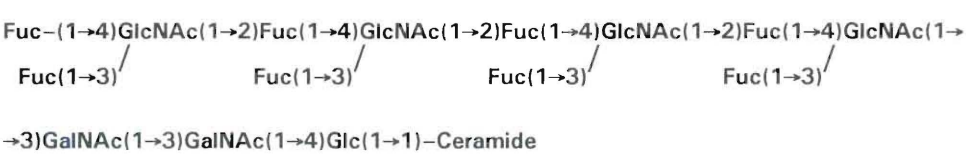


Figure 1. Chemical structure of a major carbohydrate antigen recognized by McAb 128C3/3.

[1] The anomeric configuration of the constituent monosaccharides is undetermined due to the analytical technique used.

Other smaller components have also been recognized by McAb 128C3/3 in which one or more Fuc(1→3) side chains were absent. The immunodominance of L-Fuc in the binding of the McAb to egg glycoproteins was demonstrated by inhibition with monomeric L-Fuc, while the McAb also bound to immobilized L-Fuc [100].

The same McAb 128C3/3 bound to a common epitope present on various polypeptides of the cercarial glycocalyx as well as in the tegument of cercariae and schistosomula [23]. The glycocalyx components were shed after transformation of cercariae to schistosomula, but two components of 170 and 180 kDa continued to be present in cercariae, in newly transformed schistosomula and in 36 h *in vitro* cultured schistosomula. The lectins Con A (to Man, Glc) and Lotus (to Fuc) also bound to these antigens as well as to a number of other high and low molecular mass components [23]. Inhibition studies later showed that in the binding of McAb 128C3/3 Fuc was dominant [100]. Remarkably, 128C3/3 and Con A but not Lotus were reactive with a 38 kDa glycoprotein from schistosomula which is possibly the same as the antigen recognized by the partially protective McAbs described by different groups, *e.g.* Dissous *et al.* (1982,1985) [33,34], Phillips *et al.* (1982,1986) [83,192], Bickle *et al.* (1987,1988) [6,40], or Harn *et al.* (1984,1987) [66,67]. Nevertheless, the epitopes recognized by these McAbs need not be the same, even though each of the McAbs bound to a carbohydrate moiety. Finally, the antigen recognized by McAb 128C3/3 appears to be present in the serum of *Schistosoma*-infected patients and an immunodiagnostic antigen-capture ELISA using this McAb has been described [68].

A number of egg glycoproteins (major serological antigens, $MSA_{1,2,3}$) showing immunodiagnostic potential were isolated from *S. mansoni* eggs by successive steps of Con A-affinity and ion-exchange chromatography [62,140,141]. MSA_3 bound weakly to Con A and appeared to be poorly glycosylated. MSA_1 and MSA_2 bound to Con A with higher affinity and PAS-staining, specific for carbohydrates, was also more intense. MSA_1 may be a dimer composed of two glycoproteins of 50 kDa each, while MSA_2 was found to be a heterogeneous lipoglycoprotein with a molecular mass of M_r 450 kDa. The M_r of MSA_3 was 80 kDa [140]. The major source of the antigens appeared to be the hatching fluid, containing miracidial excretory or surface coat products [62,140]. MSA_1 appeared to be both stage- and species-specific, while MSA_2 and MSA_3 showed partial cross-reactivity with cercarial but not with adult worm antigen, as well as with egg antigens from *S. haematobium* and *S. japonicum* [140]. Some evidence for strain variation of the antigen was provided by Hamburger *et al.* (1982) [60], who purified a glycoprotein from an Egyptian strain of *S. mansoni* (named major egg glycoprotein, MEG) using the same procedure as for MSA_1 from a Puerto Rican strain [141]. The glycoprotein from the Egyptian strain differed in molecular mass (70 kDa) and its reactivity with antibodies in

antigen–competitive radioimmunoassay was similar but not identical to that of MSA_1. In particular, MEG showed a high degree of cross–reaction with egg antigens from *S. haematobium* [60]. The major immunogenic epitopes of MEG appeared to be carbohydrates because various biochemical procedures as heating to 100°C for 1 h, treatment with 10 % TCA, 0.1 M NaOH, or 0.1 M HCl had no effect on the serologic reactivity of MEG, but treatment with 65 mM periodate resulted in a drastic loss of molecular mass and of serologic reactivity. Pronase treatment of MEG caused a limited fragmentation of the molecule and only a minor decrease in serum antibody recognition [61].

Minor carbohydrate–rich fragments of soluble egg antigens (<13 kDa and >10 kDa, isolated by ultrafiltration followed by dialysis) contained the same carbohydrate epitopes as MEG, but showed no immunopathological activity [106]. Having shown that a number of lectins (Con A, PNA, RCA II, Fuc) bound to MEG as well as to the smaller fragments, it was suggested that MEG consists of a polypeptide backbone to which several polysaccharide chains are bound [105,106].

Cercaria

A number of cercarial antigens showing cross–reacting carbohydrate epitopes with antigens on other life–stages have already been mentioned and described above. The results of localization studies employing lectins are summarized in Table 7, similar to the egg and worm carbohydrates.

As shown in Table 7, there appeared to be a considerable heterogeneity between the various studies in the types of lectins bound to cercarial structures. A number of these differences might be explained by species–specific expression of carbohydrate structures, *e.g.* between Beisler *et al.* (1984) [5], and Linder (1985) [102], but the discordant binding of Lotus to the cercarial heads is difficult to interpret. The components present in the excretion products of cercariae (as described by [102]) might partly be produced by the penetration glands causing overlap of the lectin–binding patterns, suggesting that enzymes used for skin penetration are glycosylated.

Using Bolton–Hunter radiolabeling of cercarial tegument structures, Hayunga *et al.* (1986) [70] identified a number of Con A–binding high and low molecular mass glycoproteins (180, 150, 43 and 30 kDa). These components were retained by 24 h cultured schistosomula, and supplemented by two other glycoproteins (66 and 57 kDa) [70].

Table 7. Lectin binding to *Schistosoma* cercariae and newly transformed schistosomula (≤ 3 h.).[a,b,c]

Head	Tail	Penetration glands	Excreta	Reference
Con A, SBA, DBA, UEA–I ±, PNA, RCA-I	Con A, SBA ±, DBA, RCA–I	SBA, UEA–I	n.d.[d]	Beisler *et al.* (1984) [5][e]
Con A, GS-I, RCA–I, SBA, HPA, WFA, UEA–I, Lotus, PNA, DBA	Con A, GS-I, RCA–I, SBA, HPA, WFA, UEA–I, Lotus, PNA, DBA	n.d.	Con A, GS-I, RCA–I, SBA, HPA, WFA, UEA–I ±, Lotus, PNA, DBA	Linder (1985) [102]
Con A, SBA, UEA–I [f,g]	n.d.	n.d.	n.d.	Murrell *et al.* (1978) [122][g]
Lotus	Lotus ±	n.d.	n.d.	Nanduri *et al.* (1991) [123]
Con A, PNA, Lotus	n.d.	n.d.	n.d.	Payares and Simpson (1985) [138][h]

[a] as various reports indicated that the binding of WGA is largely aspecific [5,105,122,159,161], this lectin has been omitted in this Table
[b] unless otherwise indicated, studies were performed in *Schistosoma mansoni*
[c] shaded lectins were also investigated but showed no binding
[d] n.d. = not done
[e] studied in *Schistosoma japonicum*
[f] 2 h schistosomula were weakly positive for SBA and UEA–I
[g] no differentiation within cercariae was made
[h] surface proteins of newly transformed schistosomula

Nanduri *et al.* (1991) [123] used lectin–affinity chromatography on immobilized Lotus lectin to isolate Fuc–containing carbohydrate structures from cercarial body and tail glycocalyx. Monosaccharide composition analysis of body glycocalyx showed that Fuc is the major sugar residue present, while in the tail Glc is predominant. Alkaline treatment indicated that the glycans are attached to the protein backbone via an O–glycosidic bond, both for bodies and tails. After reductive alkaline treatment, two glycan chains of 10.5 kDa and 5.6 kDa appeared to account for most of the glycocalyx components of both bodies and tails. The larger glycan was retarded on an anion–exchange column, thus being negatively charged, but Neu5Ac could not be demonstrated. Both body and tail glycocalyx were highly sensitive to mild acid hydrolysis, which for the body supports the presence of high amounts of Fuc [123]. These results confirm a

previous study by Caulfield *et al.* (1988) [16], who found a predominance of Fuc residues in the carbohydrate fraction and a relatively high amount of Thr and Ser in the amino acid fraction of the cercarial glycocalyx. Additionally, using a McAb which can be inhibited by monomeric L-Fuc (128C3/3, already been discussed in detail above) several smaller surface glycoproteins of cercariae have been identified [23]. The same McAb recognizes carbohydrate epitopes present on a variety of *S. mansoni, S. haematobium, S. japonicum* egg glycoproteins and glycolipids as well as *S. mansoni* cercarial and adult worm antigens [184,185]. These observations can be used as a clear illustration of a common feature in carbohydrate analysis, namely that the same glycan structure may be expressed throughout the schistosome life-cycle, and exhibit different biological roles, but that yet the overall glycolipid and glycoprotein structures are not conserved.

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