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REVIEW

Schistosome glycoconjugates in host-parasite interplay

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Schistosomes are digenetic trematodes which cause schistosomiasis, also known as bilharzia, one of the main parasitic infections in man. In tropical and subtropical areas an estimated 200 million people are infected and suffer from the debilitating effects of this chronic disease. Schistosomes live in the blood vessels and strongly modulate the immune response of their host to be able to survive the hostile environment that they are exposed to. It has become increasingly clear that glycoconjugates of schistosome larvae, adult worms and eggs play an important role in the evasion mechanisms that schistosomes utilise to withstand the immunological measures of the host. Upon infection, the host mounts innate as well as adaptive immune responses to antigenic glycan elements, setting the immunological scene characteristic for schistosomiasis. In this review we summarise the structural data now available on schistosome glycans and provide data and ideas regarding the role that these glycans play in the various aspects of the glycobiology and immunology of schistosomiasis.

Keywords: **antigenic glycans, immune modulation, anti-carbohydrate antibodies, helminth infections, schistosomes**

Introduction

One of the main parasitic infections in humans is schistosomiasis, also known as bilharzia, a disease caused by digenetic trematodes of the genus *Schistosoma*. Due to the lifecycle of the parasite, the disease is limited to tropical and subtropical areas where an estimated 200 million people are infected and 500–600 million people are at the risk of exposure. Although schistosomiasis is strongly associated with inadequate sanitation and water supply and thus in a perfect world could be effectively controlled, in reality the disease is spreading to new areas due to e.g. irrigation measures and migration.

Schistosomes have an intriguing and complex life cycle (Figure 1), the characteristics of which are essential for understanding the immunology of the host-pathogen interplay. The three major schistosome species infecting man are *Schistosoma mansoni* which is transmitted by snails of the genus *Biomphalaria* and occurs in Africa, the Middle-East and South-America, *Schistosoma japonicum* which is transmitted by snails of the genus *Oncomelania* and occurs in China and Southeast-Asia, and *Schistosoma haematobium* which is transmitted by snails of the genus *Bulinus* and occurs in Africa, Arabia and Asia-minor. The adult worms of approximately 1 cm long live in the mesenteric vessels (*S. mansoni, S. japonicum*) or in the vessels surrounding the bladder (*S. haematobium*). The female worm may produce up to hundreds of eggs per day, approximately one third of which migrate through the blood vessel wall and through the wall of the intestine or bladder to be excreted with faeces or urine, respectively. About two-thirds of the eggs are transported by the bloodstream to several organs, most notably the liver, where they become lodged. Here, by the excretion of a plethora of antigens including a variety of heavily glycosylated proteins through the porous eggshell, they provoke a strong granulomatous reaction which may lead to severe pathology and ultimately death of the host. When the eggs reach fresh water, a larval stage—the miracidium—hatches from the egg and will actively seek the intermediate host, a fresh water snail, where over a period of weeks it will asexually replicate via mother and daughter sporocysts. From the daughter sporocysts large numbers of so-called cercariae are released which consist of a body and a bifurcated tail, used to propel them through the water. When a host comes into contact with cercariae-infested water, the cercariae rapidly attach to the skin, shake off their tail and upon penetration into the skin transform into schistosomula, causing profound changes in their metabolism and antigenic structure. After a complex

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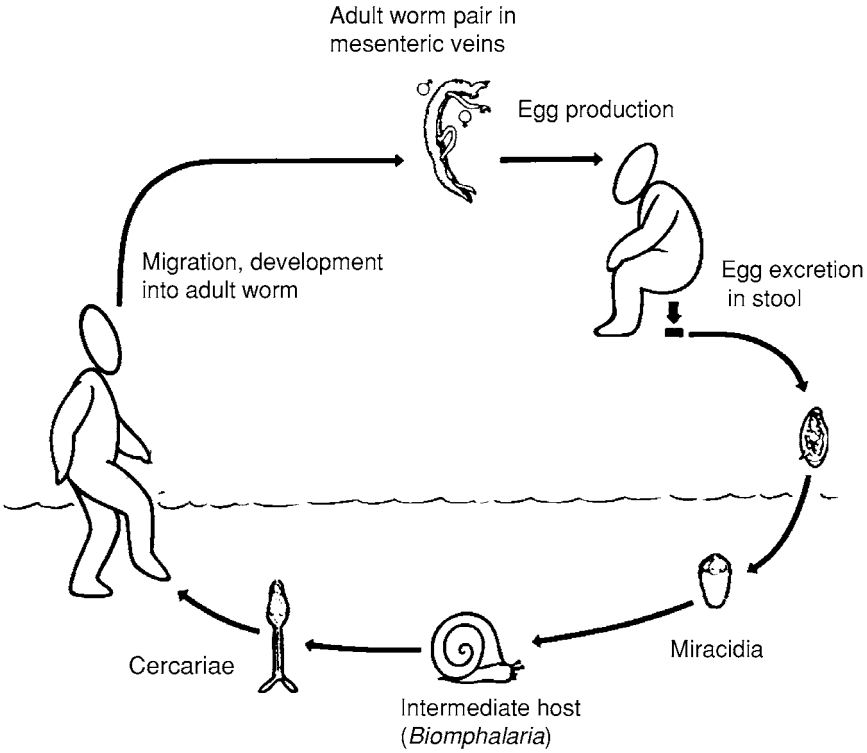

Figure 1. Life-cycle of *Schistosoma mansoni* (courtesy of Dr. A.M. Polderman).

migration pattern the schistosomula reach the mesenteric veins (*S. mansoni*, *S. japonicum*) or the urinary bladder plexus (*S. haematobium*) where in approximately 5 weeks they sexually mature, pair and start egg-production. Although the average schistosome probably lives for 5–10 years, already a surprisingly long period, there are cases where life-spans of 40 years have been reported.

Schistosomiasis is a disease with a spectral presentation, which is strongly influenced by the epidemiology in the various endemic foci and the resultant history of infection. Typically, an age-infection intensity plot will show a strong peak at the age of 10–15 years, after which a decline occurs, but with the majority of the population remaining infected life-long when untreated. Pathology is strongly influenced by the stage of the infection and the number of worms present, but may even in light infections with an ectopic localisation occasionally result in severe health problems. For further reading on human schistosomiasis see [1].

Schistosomiasis is receiving decreased research attention, probably because of the facts that the disease causes prolonged morbidity rather than rapid death, that there exists effective chemotherapy (Praziquantel) and that—like ten years ago—a vaccine might become available. In the not impossible scenario that resistance against Praziquantel would eventually develop, and no effective vaccine would yet be available, it would be prudent to have improved our knowledge of the molecular basis of the host-parasite interplay, which would hopefully allow identification of new, rational ways of intervention.

On the whole, it can be stated that schistosomes which were already afflicting ancient Egyptians are remarkably efficient parasites which have developed a range of evasion mechanisms to escape and modulate the efforts of their host to kill them. It is now increasingly recognised that glycoconjugates of the parasite play an essential role in many of these evasion mechanisms and form major activators and targets of the host's immune response.

Several reviews [2–4] have previously discussed various aspects of schistosome glycobiology. In the current review, we provide a summary of older data and discuss the most recent studies that have not been reviewed so far. First, we give an update on the structural features of schistosome glycoconjugate glycans, and subsequently discuss the functional and immunological aspects of these glycans in relation to the interaction between the schistosome and its definitive (mammalian) host.

Schistosome glycoconjugate structures

A large amount of structural data on schistosome glycosylation has become available in recent years primarily due to the application of newly emerging sensitive analytical techniques with emphasis on mass spectrometry [5]. Many primary structures and structural elements of schistosome glycans have now been chemically defined. In addition, histochemical methods have provided new information on the occurrence of glycan epitopes on proteins and lipids in the different developmental stages of schistosomes that complements the structural studies. Here, we

Table 1. Oligosaccharide structures and functional glycan elements of schistosome glycoproteins and glycolipids. It has to be taken into account that the distribution of these elements is not always exactly known, and that differential expression in the various schistosome species may occur. Please refer to the text for more details and the corresponding references

Abbreviation	*Structure*	*Occurrence*
LDN	GalNAcβ1-4GlcNAcβ1-	Widely expressed
LDN-F	GalNAcβ1-4GlcNAcβ1- Fucα1-3	Widely expressed
Le^X	Galβ1-4GlcNAcβ1- Fucα1-3	Widely expressed
polyLe^X	[-3Galβ1-4GlcNAcβ1-]$_n$ Fucα1-3	N-,O-glycans *worms*
pseudo Le^Y	Galβ1-4GlcNAcβ1- Fucα1-3 Fucα1-3	Glycolipids *cercariae*
CAA	-6GalNAcβ1-[6GalNAcβ1-]$_n$ GlcAβ1-3 GlcAβ1-3	CAA O-glycans *worms*
HexNAc-DF	GalNAcβ1-4GlcNAcβ1- Fucα1-3 Fucα1-3 $^{+/-}$Fucα1-2 Fucα1-2	Widely expressed
	GalNAcβ1-[4GlcNAcβ1]$_{1-3}$-4GlcNAcβ1-3GalNAcβ1-4Glcβ1-Cer Fucα1-3 Fucα1-3 $^{+/-}$Fucα1-3 $^{+/-}$Fucα1-2 Fucα1-2 $^{+/-}$Fucα1-2	Glycolipids *eggs*
	GalNAcβ1[-4GlcNAcβ1-3Galα1-3GalNAcβ1]$_{0-4}$-4GlcNAcβ1- Fucα1-3 Fucα1-3 $^{+/-}$Fucα1-2 Fucα1-2 $^{+/-}$Fucα1-2	O-glycans *cercariae*
core β2-Xyl core α3-Fuc	Manα1-6 $^{+/-}$Fucα1-6 $^{+/-}$Xylβ1-2Manβ1-4GlcNAcβ1-4GlcNAcβ1-Asn GlcNAcβ1-2Manα1-3 $^{+/-}$Fucα1-3	N-glycans *eggs, worms*
‘novel O-core’	$^{+/-}$Fuc-3 Gal-4GlcNAc-3Gal-6 GalNAc- Gal-6 Gal-3 Gal-4GlcNAc-3 $^{+/-}$Fuc-3	O-glycans *cercariae*

will list and discuss those structures and structural elements that are most likely involved in the interaction of schistosomes with their definitive host. An overview of the most important structural elements discussed in the text below is given in Table 1.

N-glycans

Schistosoma adult worm- and egg-derived glycoproteins contain typical N-linked oligomannose structures occurring in many eukaryotic and higher organisms [6]. Di-antennary N-glycans from adult worms carry terminal GalNAcβ1-4GlcNAc (LacdiNAc, LDN) and GalNAcβ1-4(Fucα1-3)GlcNAc (fucosylated LacdiNAc, LDN-F) elements [7,8]. More complex multi-antennary N-glycans that carry repeating units of Galβ1-4(Fucα1-3)GlcNAc (Lewis X, Le^X) in their antennae have also been found on adult worm glycoproteins [9]. Analysis of glycoproteins from 48 h old *S. mansoni* schistosomula indicated the presence of similar N-glycans as on adult worms [10].

Glycoproteins from *S. mansoni* and *S. japonicum* eggs contain oligomannose glycans similar to those from adult worms, and complex type glycans with Galβ1-4GlcNAc (LacNAc) as well as LDN elements constituting their backbones [11]. Egg glycoproteins also express truncated N-glycan core structures carrying β2-linked xylose and α3-linked fucose residues [11] that can also be found in some other helminths, plants and insects [12–14]. In addition, *S. japonicum* egg glycoproteins contain a β2-xylosylated, α3-, α6-difucosylated core structure so far undescribed in any other species [11]. Furthermore, in the same study, terminal $(HexNAc)_3$ stretches were found in the egg N-glycans of both *S. mansoni* and *S. japonicum*, with repeating fucose residues attached to the terminal HexNAc residue in the case of *S. mansoni*. Recently, it was reported that N-glycans of *S. japonicum* and *S. mansoni* cercariae are dominated by Le^X termini [15]. With respect to core decorations, it was concluded from the same study that in *S. japonicum* cercariae and adult worms, in contrast to eggs, no β2-xylosylation nor α3-fucosylation occurred. In the case of *S. mansoni*, N-glycans from cercariae, but not from adult worms, do carry core xylose.

O-glycans

Schistosomes synthesize O-glycans ranging from single O-linked GlcNAc residues or short (Galβ1-3)$_{0/1}$GalNAcα1-Ser/Thr mucin-type disaccharides on glycoproteins from *S. mansoni* schistosomula and adults worms [10,16] to very large and complex multi-fucosylated O-glycans from the cercarial glycocalyx [17]. These complex O-glycans contain unique structural elements of repeating GalNAcβ1-4GlcNAcβ1-3Galα1-3 units carrying (Fucα1-2)$_{0/1}$Fucα1-2Fucα1-3 sequences linked to the internal GlcNAc, and (Fucα1-2)$_{0/1}$Fucα1-3GalNAc terminal structures. These multimeric glycans have been shown to be based on conventional type 1 and 2 core structures [17,18]. In addition, another type of O-glycans have recently been described to occur on the *S. mansoni* cercarial glycocalyx that terminates with GlcNAc, LacNAc or Le^X carried by unusual diantennary-like core structures with multiple branched Gal residues [18]. O-glycans from *S. mansoni* but not from *S. japonicum* eggs were found to carry, just like the egg N-glycans and glycolipids, similar terminal multi-fucosylated HexNAc sequences as the cercarial O-glycans [11,17]. With respect to O-glycosylation of adult worm proteins, the two major gut-associated excreted antigens, circulating cathodic antigen (CCA) and circulating anodic antigen (CAA) have been analysed. *S. mansoni* CCA is O-glycosylated mainly with glycans carrying long linear multimers of the Le^X trisaccharide [19]. The highly negatively charged O-glycans of CAA are constructed of a polymeric β1-6-linked GalNAc backbone substituted with β1-3-linked GlcA residues [20]. Unfortunately, to our knowledge no other protein-specific chemical glycosylation analyses like those of purified CAA and CCA have been carried out on *Schistosoma* material so far.

Glycolipids

A great deal of structural information is now also available on schistosome glycolipids. In addition to simple galactose or glucose containing ceramides, glycolipids with a so-called schisto-core (GalNAcβ1-4Glc-ceramide) occur [21,22]. Extensions of the schisto-core can be complex, with either common structural elements found in N- and O-glycans, or with specific modifications found so far only on glycolipids. Detailed analyses of the major egg glycosphingolipids of *S. mansoni* and *S. japonicum* have demonstrated that these contain extensions with the repeating unit –4(Fucα1-2Fucα1-3)GlcNAcβ1- [23] terminating in the case of *S. mansoni* with (Fucα1-2)$_{0/1}$Fucα1-3GalNAcβ1- at the non-reducing end. *S. mansoni* cercarial glycolipids are dominated by terminal Le^X and Fucα1-3Galβ1-4(Fucα1-3)GlcNAc (pseudo Lewis Y, pseudoLe^Y) structures. These glycolipids are not expressed in the adult and egg stages [22]. Recently, the Fucα1-3GalNAc terminal element was demonstrated in *S. mansoni* egg glycolipids [24]. Stage specific expression patterns have not only been observed for certain glycan elements, but also for the ceramide part of schistosome mono- and dihexosides [25].

It should be noted that sialic acids, common terminal sugars of mammalian glycans, have never been chemically demonstrated as part of schistosome glycoconjugates. The only charged monosaccharide demonstrated so far in any schistosome N-, O- or lipid-linked glycan is the GlcA residue in the O-glycans of CAA.

Indirect studies on the structure and distribution of schistosome glycoconjugates

Histochemical studies probing schistosome glycosylation have been carried out using both anti-carbohydrate antibodies and lectins (reviewed in [2]). These indirect methods and direct structural analyses of glycosylation complement each other well. For our understanding of schistosome glycobiology, it is of great importance to not only determine which glycans occur, but also determine where they are spatially and temporally expressed; i.e. on which protein or lipid they occur if they are secreted or not, and if they are unique in their expression pattern or present rather as a common, shared constituent of different developmental stages.

As evident from the structural data presented in the previous paragraph many glycan structures, in particular terminal elements composed of specific combinations of two or more monosaccharides simultaneously occur as part of different classes of glycoconjugates, expressed by different developmental stages of schistosomes. As will be discussed later in this review, these elements often confer antigenic and immunological activities to glycoconjugates, and it is therefore clear that the immunological cross-reactivities between larval, adult and egg stages that have been observed from the very beginning of molecular schistosome research can, at least in part,

be explained by the expression of shared immunogenic glycan elements [26–30].

Anti-carbohydrate monoclonal antibodies (mAbs) have been obtained from hybridomas derived from *Schistosoma*-infected mice by several research groups and to increasing numbers of these mAbs defined glycan-epitope specificities are being assigned in recent studies [31–38]. These antibodies have been used for a number of studies providing information on the expression of their glycan-epitopes (Figure 2), and they are important tools for the immunodiagnostic detection of circulating worm and egg antigens [39–42].

In immunofluorescence assays (IFA), different anti-Le^X mAbs were found to be mainly reactive towards adult worm gut- and tegument-associated antigens, the egg shell, and the oral sucker of cercariae [32,33,38]. In the course of transformation of cercariae to schistosomula, Le^X epitopes appear in patches over the whole surface [33]. In addition, several anti-Le^X antibodies also recognised secreted antigens, and antigens taken up by Kupffer cells that surround worms and eggs in infected hamster livers, indicating that Le^X-containing glycoconjugates are secreted by the worms and eggs [38]. It should be noted that most of the mAbs reactive with the multimeric Le^X glycans on CCA [19,35] are not reactive with the monomeric Le^X group in the context of $(Le^X)_n$BSA [38]. This indicates that anti-Le^X mAbs can be grouped according to their specificity for longer (as present on CCA) or shorter/single Le^X repeats (B. Appelmelk and M. van Roon, personal communication). Thus, the observed differences in staining patterns with anti-Le^X mAbs may reflect the differential expression of mono- and multimeric Le^X sequences.

Very distinct IFA patterns were obtained using anti-LDN-DF mAbs which specifically stain the excretory system and flame cells of the adult worms, whole cercariae, and miracidia and their secretions [38,43]. A previously thoroughly studied anti-egg mAb 128C3/3, shows a staining pattern very similar to the anti-LDN-DF mAb 114-5B1, and also recognises various glycolipids, but it is not clear which is its exact epitope [28,44–46]. Anti-LDN mAbs also displayed unique staining patterns, mainly of adult worm tegument and miracidia [38], and different LDN-F recognising mAbs were tested that were reactive with adult worm tegument [37] or adult worm parenchyma, miracidia and partial cercariae [38].

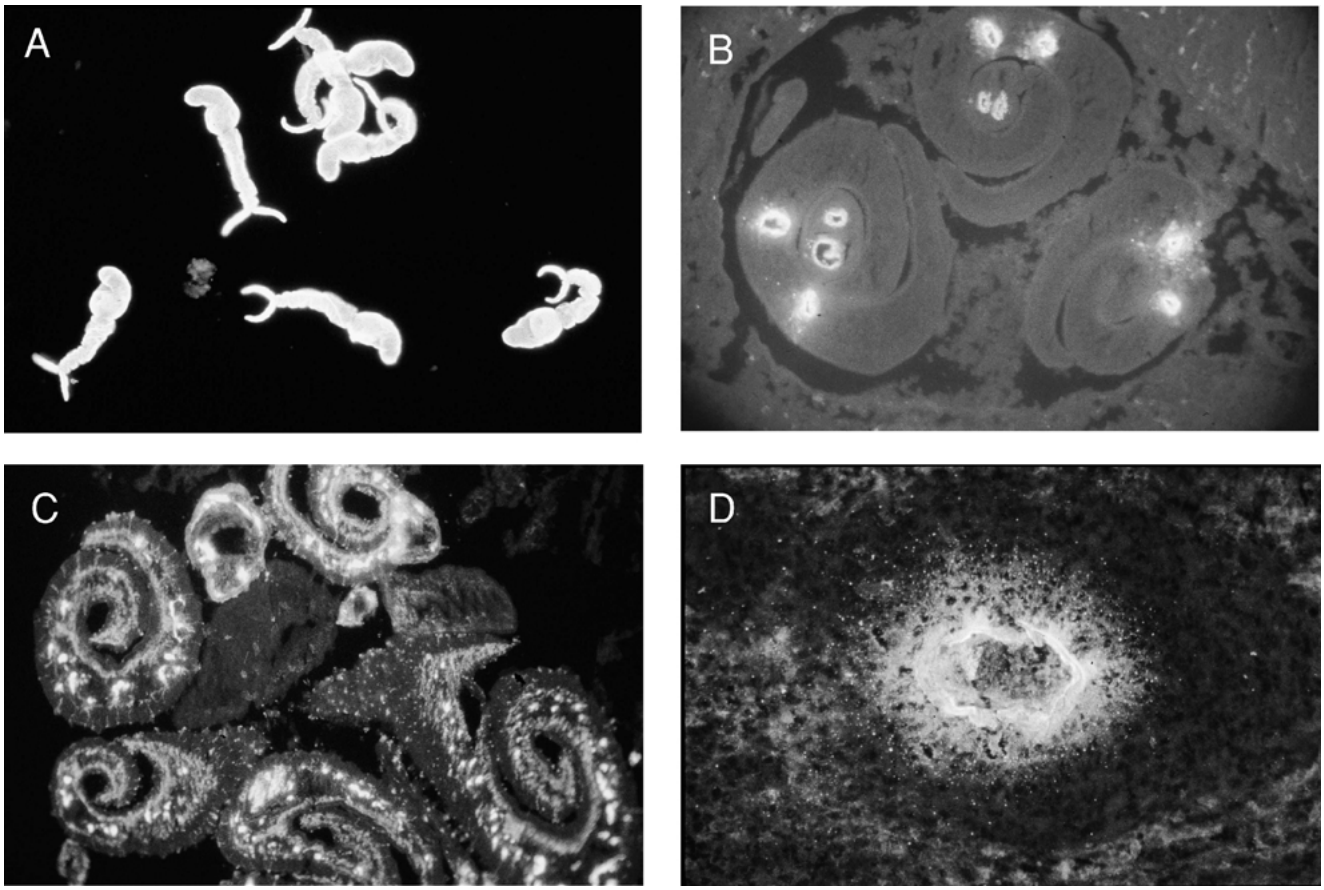

Figure 2. Examples of immunofluorescence patterns with different fluorescein-labelled anti-carbohydrate mAbs. (A) *S. mansoni* cercariae stained with mAb 114-5B1 (anti-LDN-DF); (B) Section of adult *S. mansoni* worms fixed in Rossman's fixative stained with mAb 147-1B4 (anti-CAA); (C) Frozen section of adult worms in *S. mansoni*-infected hamster liver stained with mAb 114-5B1 (anti-LDN-DF); (D) *S. japonicum* egg in frozen section of infected hamster liver stained with mAb 273-3E7 (anti-Le^X/CCA).

Other studies using mAbs or antisera in ELISA, Western blot or TLC overlay analyses have revealed several additional interesting aspects about the expression of glycan epitopes on schistosomes. It appears that Le^X and LDN epitopes occur on numerous different adult worm proteins when examined by Western blotting [36,47]. HPTLC-immunostaining of *S. mansoni* cercarial glycolipids with anti-Le^X mAbs has shown that Le^X epitopes occur on a broad range of lipids [22,48]. The mAb M2D3H, recently shown to be recognising the Fucα1-3GalNAc terminal element [24] stains many glycolipids of cercariae, adult worms as well as eggs and seems to recognise similar antigens as mAb 128C3/3 [48], but via different epitopes. Western blot analyses of *Schistosoma* proteins using antisera specific for core β2-xylose and core α3-fucose indicate the occurrence of these epitopes on many adult worm and egg proteins [49].

The availability of several panels of synthetic schistosome-related oligosaccharides, including fragments of the cercarial glycocalyx [50,51] and the O-glycans of CAA [52] has led to the definition of more anti-carbohydrate mAbs derived from *Schistosoma*-infected mice (H.J. Vermeer and A. van Remoortere, personal communication). These mAbs will be valuable tools for defining the distribution of glycan-epitopes on schistosome proteins and lipids.

In general, it can be concluded from all direct and indirect structural evidence obtained so far that the various developmental stages of schistosomes interacting with the definitive host express a wide variety of glycoconjugates. The carbohydrate elements carried by these glycoconjugates are in part shared by the different stages, but some occur as unique stage- or protein-specific elements. On the one hand, glycan structures may be identical or similar to endogenous glycans occurring in the mammalian hosts (e.g. Le^X, LDN, LDN-F), while on the other hand structural elements occur that are non-mammalian (e.g. LDN-DF, Fucα1-3GalNAc) and that may be specific for helminths, or for schistosomes. As can be inferred from the histochemical and blotting studies with mAbs, which show that different staining patterns for different mAbs of the same specificity for small synthetic oligosaccharides may occur, it is important to take into account that the underlying structure carrying the glycan-epitope may play a critical role in determining its exact immunogenic properties. In fact, presentation and multivalency is generally an important factor in carbohydrate-protein interactions [53].

The structural aspects of mixed glycoconjugate preparations of schistosomes have now largely been defined. It will be of critical importance for a better understanding of the role of glycans in the immunology of schistosomiasis to extend glycosylation analysis more towards specific (groups of) glycoconjugates that harbour certain biological activities.

Relation of schistosome glycosylation to other helminths

Many immunologically active carbohydrate structural elements expressed by schistosomes have also been detected in other helminths, the group of organisms including the nematodes, trematodes and cestodes. This is of particular interest in view of the common ability of helminth glycoconjugates to induce specific host responses discussed later in this paper. It is out of the scope of this review to extensively describe the glycoconjugates synthesised by other helminths (for a review on nematode glycosylation, see [54]), but we will briefly discuss a few features that may play a role in the helminth—host interactions.

The LDN backbone element seems to be common to most helminths (see reviews [4,54]). It has been shown by structural analyses that LDN and LDN-F occur on *Trichinella spiralis* glycoproteins where they form highly immunogenic Tyvulose-capped terminal epitopes [55–58]. *Haemonchus contortus* glycoprotein N-glycans contain typical highly fucosylated core structures [12,59]. These include the core α3-,α6-difucosylated structures also found in schistosomes. Characteristic for glycoconjugate antigens from filariae, including *Onchocerca volvolus*, *Brugia malayi* and *Wuchereria bancrofti*, is their shared immunogenic phosphorylcholine (PC) epitope, that is linked to GlcNAc residues in multi-antennary N-glycans [60–63]. The Tyvulose monosaccharide residue and the PC substituent on GlcNAc have never been demonstrated in schistosome glycans, but the PC group occurs as part of lipids in *O. volvolus* [64] and *Ascaris suum* [65].

Not surprisingly, carbohydrate epitopes are the main reason for the cross-reactivity observed between antigens from different helminths. Apart from the LDN and LDN-F groups [4], the α3-fucosylated and β2-xylosylated core structures of schistosome N-glycans form cross-reactive antigens with glycans from other helminths including *C. elegans*, *D. immitis* and *H. contortus* [49] and from insect and plant glycoproteins like bee venom phospholipase A2 and horseradish peroxidase [66,67]. Among these structures, the core α3-fucosylated epitopes are specific targets for a substantial amount of IgE from *S. mansoni* or *H. contortus*-infected animals [49] and the same epitopes are major contributors to IgE binding in plant glycoallergens [68]. In addition, it has been shown that keyhole limpet hemocyanin (KLH), a glycoprotein that can be used as a tool in serodiagnostic assays to monitor anti-schistosome antibodies [69,70], carries a fucosylated glycan recognized by mAb M2D3H directed against *S. mansoni* egg glycolipids [71].

Surprisingly, apart from the cattle parasite *Dictyocaulus viviparus* [72], no other helminths than *Schistosoma* express the Le^X epitope [47] that is widely expressed on schistosome glycoconjugates, and that may play a role in their immune modulating properties.

Schistosome glycosyltransferases

As evident from the incredible variety of glycoconjugates they produce, schistosomes must express a large number of glycosyltransferases (GlycT). Relatively few of these glycosyltransferases or glycosyltransferase activities have been described. Early reports have identified ManT and GalT activities towards lipids in adult worm surface membranes [73,74]. Furthermore, a β4-GalT that can synthesize LacNAc, but with

properties distinct from the mammalian β4-GalT [75], and a β4-GalNAcT of similar acceptor substrate specificity that catalises the formation of LDN [76] have been identified in *S. mansoni* adult worms. Of particular interest are the FucT's since many schistosome-derived oligosaccharides are dominated by multiple fucosylations. It may be expected that a lot of different FucT's are expressed to facilitate the creation of all the different Fuc to GlcNAc, Fuc to Gal, Fuc to GalNAc and Fuc to Fuc linkages that have been described so far. *S. mansoni* adult worms contain an α3-FucT activity that resembles human FucT V and VI with respect to its catalytic properties [77] and from a genomic library of *S. mansoni* worms an α3-FucT was cloned that is highly homologous in sequence to human and mouse FucT VII [78]. Another putative α3-FucT was cloned which displays moderate sequence identity with mammalian FucT III-VII and X [79]. Stage-specific measurement of activities of α3-FucT's towards various type-1 and type-2 based acceptor structures has revealed that the total FucT activity in eggs is 50-fold higher than in other stages and that multiple fucosylated products are formed using Galβ1-4GlcNAcβ1-3Galβ1-4Glc (LNnT) as acceptor, indicating the presence of at least several distinct FucT's [80]. In this last study it was also shown that the expression of the fucosylated epitope recognised by the anti-egg antigen mAb 128C3/3 could be blocked *in vivo* by a novel 1-iminosugar FucT inhibitor. In addition, an α2-FucT activity that is capable of forming the Fucα1-2Fuc linkage has been identified in cercariae of the 'bird schistosome' *Trichobilharzia ocellata* [81]. It is anticipated that one or more similar enzyme activities are present in the 'human schistosomes' that have now been shown to express Fucα1-2Fucα1-3HexNAc on various glycoproteins and glycolipids.

In humans, six different α3-FucT's, all catalising the formation of the same Fucα1-3-linkage to GlcNAc, but each with specific expression patterns or substrate specificities have been identified (reviewed in [82]). Similarly, many FucT's with specific properties and expression patterns may occur in schistosomes. In addition, novel types of linkages like Fucα1-3Gal in the pseudo Le^Y element or Fucα1-3GalNAc in the terminal unit of cercarial and egg glycoconjugates have been found that indicate the occurrence of unique schistosome FucT's yet to be identified. In addition, based on the structural data, many other glycosyltransferases including GlcAT and XylT must be present. It would help to enhance our understanding of the glycobiology of schistosomes if we get a more complete picture of the stage-specific expression of a larger set of glycosyltransferases.

Host immune responses to schistosome glycoconjugates

Humoral responses

It has been appreciated since more than two decades already that a substantial portion of the strong humoral immune response toward schistosomes is directed against carbohydrate epitopes on glycoconjugates presented or excreted by larvae, adult worms and eggs [83–86]. As already published in the early papers, and quite obvious bearing in mind the structural data presented in the previous paragraph, possible extensive cross-reactivity has to be taken into account when examining immunological responses to the glycan part of glycoconjugate antigens of the different life-cycle stages of schistosomes.

As determined by ELISA and surface plasmon resonance (BIAcore) methods using defined (neo-)glycoconjugates, sera of infected humans [87,88], primates [87,89] and rodents [47,90,91] contain IgM and IgG antibodies against the Le^X epitope. Part of these antibodies are directed against the linear Le^X multimer which is the immunodominant part of CCA [19]. However, anti-Le^X responses have usually been measured against Le^X in its monomeric form and anti-polyLe^X antibodies may not be detected using monomeric Le^X as a target [38]. It seems that in humans and primates anti-Le^X responses are relatively low compared to those in mice, but strong responses to CCA are present in *Schistosoma*-infected individuals. Similarly, the other major gut-associated worm antigen CAA gives rise to humoral responses to its O-glycans [92–95]. Also against the LDN and LDN-F epitopes IgM and IgG responses are present in sera of *Schistosoma*-infected mice [36,37], primates [89] and humans [88]. Interestingly, using the LDN-based difucosylated epitope GalNAcβ1-4(Fucα1-2Fucα1-3)GlcNAc (LDN-DF) antibody levels could be measured in sera of *S. mansoni*, *S. japonicum* and *S. haematobium*-infected individuals that were up to an order of magnitude higher than the anti-Le^X or anti-LDN responses [88]. Using a panel of glycocalyx-related synthetic oligosaccharides [51] similarly high antibody levels were measured against the Fucα1-3GalNAc element (A. van Remoortere and H.J. Vermeer, personal communication).

The observed high response to an epitope like LDN-DF may reflect its high immunogenicity compared to LDN(-F) and Le^X in mammalian hosts. Whereas the Le^X, LDN and LDN-F epitopes are all endogenous oligosaccharide sequences in humans and possibly in many other mammals [96–100], the Fucα1-2Fuc (DF) element has never been found in mammals and may be specific for schistosomes and related species. Nevertheless, also against these mammalian self-antigens responses are mounted in schistosome infections, and this may be related to the persistent presentation to the host's immune system, or the underlying antigens that present these epitopes. With regard to the occurrence of these auto-antibodies it has been shown that anti-Le^X IgG and IgM from *Schistosoma*-infected humans, primates and mice can induce antibodies mediating complement-dependent cytolysis of Le^X presenting host-cells *in vitro* [87,91,101]. It is not known whether this autoimmune effect plays a substantial role in the immunopathology of schistosomiasis *in vivo*. Furthermore, it remains to be determined if anti-LDN and LDN-F and possibly other *Schistosoma*-infection-induced antibodies against yet undefined host determinants can cause similar cytotoxic effects. In addition, one could speculate that auto-antibodies interact with host glycans involved in endogenous binding events like those mediated by

selectins or galectins, thereby potentially interfering with biological processes in the host.

Apart from IgG and IgM, in some cases also IgE and IgA responses to specific carbohydrate epitopes have been determined. A substantial amount of anti-Le^X response in *S. mansoni*-infected C57 mice is of the IgA subclass [90], and IgA and IgE directed against the LDN-F epitope is present in serum of infected mice [37]. An IgE response is also mounted against N-glycan core α3-Fuc- and β2-Xyl-containing epitopes on various schistosome proteins [49] and schistosome-induced IgE is recognising a glycolipid fraction from worms and eggs [102].

Interestingly, several older papers have already described that anti-carbohydrate antibodies are associated with immunity to schistosome infections [31,103]. Antibodies recognising a carbohydrate epitope that is cross-reactive with KLH have been associated with protection [104], and so has a Le^X-reactive monoclonal antibody [32]. Also in the case of other helminth infections, including those with *H. contortus* [105] and *T. spiralis* [106] glycan epitopes have been shown to induce protective antibodies. Immunisation of mice with a cercarial glycocalyx preparation gives rise to anti-carbohydrate antibodies that are cytotoxic to schistosomula *in vitro*, but not *in vivo*. An anti-glycocalyx IgM mAb was shown to inhibit the cellular cytotoxicity induced by other antibody isotypes in the mouse apparently by acting as a blocking antibody [107]. The occurrence of cross-reactive anti-carbohydrate antibodies of different subclasses may play an important role in the survival of the vulnerable schistosomulum stage. T-cell independent egg glycan antigens elicit antibody responses of the IgM and IgG2 type that are cross-reactive with antigens of schistosomula. These antibodies not only fail to mediate antibody-dependent cytotoxicity, but at the same time may block effector antibodies like IgG1 and IgE [108,109]. A similar theory can be inferred from the contrasting relationship of IgG4 and IgE to susceptibility to reinfection with schistosomes. IgE is implicated in immunity since adults, who are less susceptible than children, produce higher levels of specific IgE whereas children produce higher levels of IgG4 with similar specificity spectra towards crude antigens preparations [110–112]. It has been indicated that IgG4 could act as blocking antibody for IgE-mediated protective effects [110,113]. It is not clear to which extent the abovementioned observations include anti-carbohydrate responses, or if they are exclusively related to anti-peptide antibodies. However, IgE directed against the ceramidepolyhexoside fraction of worm lipids was shown to be negatively correlated with post-treatment infection intensity suggesting indeed a role for anti-glycoconjugate IgE [102].

In a longitudinal study monitoring anti-Le^X responses in mice it was shown that IgM response is detectable at two weeks post-infection, whereas IgG response can be detected from week five onwards, with decreasing responses during the course of the infection [91]. Similar response profiles were obtained for LDN and LDN-F specific IgM in chimpanzees, but to Le^X hardly any IgM nor IgG response was found [89]. The same observation was made in the case of human subjects [88]. In schistosome-infected chimpanzees IgG responses against LDN and LDN-F were present, but with variable intensities and time-course profiles for the different individual animals [89]. This study also showed that virtually all antibodies induced by vaccination with irradiated cercariae, which gives a moderate level of protection, are directed at glycan epitopes. Periodate treatment to destroy glycan epitopes of all antigen preparations tested (KLH, adult worm antigens, egg antigens and larval antigens) abrogated a large part of the response.

In summary, although there are several indications for the involvement of anti-glycan antibodies in protection, it is still largely unclear which role anti-glycan antibodies play in protective immunity, and how they exactly do this. Eberl et al. [89] have suggested that the massive anti-egg and worm glycan antibody responses may provide a smoke-screen to mask peptide epitopes, or larva-specific glycan epitopes that could mediate a protective response.

Non-humoral responses and immune modulation

A key feature of *Schistosoma* infections, and in fact of helminth infections in general, is that a typical T helper 2 (Th2) type immune response accompanied by specific cytokine profiles, high IgE production and eosinophilia is mounted in the definitive host. It has been shown that egg antigens (SEA) are particularly potent in stimulating this Th2 skewing in schistosomiasis. Although also other factors are involved, the glycan parts of glycoconjugates in SEA seem to be major immune modulators [114–117]. Periodate-treated SEA, devoid of functional glycans, loses its ability to restimulate SEA-sensitised lymphocytes to produce high levels of Th2 cytokines [117,118].

In the past few years, many studies have been undertaken to unravel the precise molecular aspects of this part of the schistosome-host interaction. Schistosome-related glycoconjugates and oligosaccharides, either as complex mixtures or as defined synthetic or purified compounds have been shown to interact with the immune system of experimental animals or humans. In many cases complex responses are observed, and data indicate—not surprisingly—that immune responses to schistosome glycoconjugates involve a multifactorial system in which various components of the worm or egg act to stimulate innate and acquired immune responses.

Several immunomodulatory mechanisms associated with schistosomiasis have been attributed to Le^X-containing glycoconjugates. Initial investigations have shown that Le^X in the context of Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc (LNFPIII) induces splenic B220+ B-1 cells from *S. mansoni*-infected mice to proliferate and to secrete IL-10 and PGE-2 associated with Th2 polarisation [119]. Notably, the expansion of these B220+ cells in murine schistosomiasis was also observed by injection with soluble egg antigens, but not in its deglycosylated form. The anti-inflammatory cytokine IL-10 has been related to the downregulation of strong inflammatory

immune responses, which allows a harmonious host-parasite co-existence by restricting immunopathology [120]. This is currently of additional interest since helminth-induced IL-10 has been associated with reduced incidence of allergic diseases that are also characterised by Th2 type immune responses [121,122]. Interestingly, IL-10 secretion by peritoneal B-1 cells was observed in response to LNFPIII and to SEA glycans in some, but not all mice strains. This indicates that a genetically restricted factor may play a role in the glycan-induced immunology/immunopathology of schistosomiasis [118].

The same oligosaccharide, but presented in a multimeric form as a LNFPIII-HSA neo-glycoconjugate, activates peripheral blood mononuclear cells (PBMCs) of schistosome-infected patients and induces IL-10 production, which is inversely associated with intensity of infection. The induction of the same responses is found with SEA, whereas pretreatment of PBMCs with free LNFPIII attenuates the SEA-induced IL-10 production [123]. Another example of the ability of particular glycoconjugates to direct specific immuneresponses in schistosomiasis was provided by Okano et al. [117] who reported that a Th2 type immune response is accompanied by the increased production of both total and specific IgE in a murine model of intranasal sensitisation with SEA. Although the carbohydrates on SEA are the major inducers of IgE production and recruitment of eosinophils, they are not the targets of the IgE response. The same effects were observed using LNFPIII-HSA [124]: a strong Th2 response and HSA-specific IgG and IgE were induced by LNFPIII-HSA a 1000-fold more potently than by HSA, but none of the antibodies were directed against LNFPIII. Lymphocytes of mock-immunised mice also responded to LNFPIII-HSA re-stimulation, but much less strong than those from sensitised mice. These immune modulating effects were not observed with Galβ1-4GlcNAcβ1-3Galβ1-4Glc-HSA (LNnT-HSA) indicating the importance of the fucosyl residue [124]. In contrast to Okano et al. [124], other groups have shown that IgE antibody resonses directed against schistosome glycan epitopes can be substantial [37,49,102]. Thus, oligosaccharide sequences involved in the induction of Th2 immune responses and high IgE production may be different from those that are the main targets of the humoral immune responses.

Another effect of an LNFPIII-conjugate used to emulate schistosome glycans, in this case LNFPIII-dextran, is the expansion of a peritoneal macrophage subclass ($Gr1^+$) from naïve mice that suppresses $CD4^+$ T-cells [125]. In a related study on the ability of dextran conjugates to expand $Gr1^+$ cells in relation to immune polarisation it was found that LNFPIII-dextran and, surprisingly, also LNnT-dextran could expand a population of $Gr1^+$ cells upon injection in mice and induce the production of the anti-inflammatory cytokines IL-10 and TGF-β [126]. Using an extended set of neo-glycoconjugates containing not only Le^X-carrying molecules, but also the characteristic Fucα1-2Fuc-containing LDN-DF unit, it was shown that this antigen is not only an important antibody binding target in schistosomiasis [38,88] but additionally it can stimulate innate cellular responses [127]. Compared to Le^X-BSA and LDN-BSA, the LDN-DF-BSA conjugate was a superior inducer of IL-10 production in PBMCs of naïve human donors. The same response of PBMCs was found upon treatment with egg glycolipids that are reactive with an anti-LDN-DF mAb whereas worm glycolipids, unreactive with the anti-LDN-DF mAb, do not give rise to IL-10 induction. Treatment of PBMCs with the free monovalent LDN-DF did not induce any significant effect. All studies taken together suggest that Le^X, LDN-DF and possibly other oligosaccharide structures can induce adaptive as well as innate (non-specific) immune responses.

It should be noted that although many of the characteristic features of helminth-induced immune responses have been attributed to Le^X-containing oligosaccharides, the dominant immunomodulatory molecules in schistosomiasis seem to be present primarily among the egg antigens. Le^X has been shown to occur on different glycoconjugates from each of the infectious, larval, adult and egg stages of the schistosome. Therefore it may be hypothesised that although the immunological effects measured with Le^X-containing neo-glycoconjugates are indeed similar to those induced by schistosomes, the actual glycoconjugates involved *in vivo* may be different. This viewpoint is further strengthened by the notion that none of the other parasites that induce similar Th2-skewed responses in the human host seem to express the Le^X structure. In addition, the underlying structure of the protein or lipid that carries the immunogenic glycans may be an important factor influencing the immunomodulatory properties of schistosome glycoconjugates.

A key to a better understanding of the glycan-induced immune effects will be the identification of the corresponding monocyte and lymphocyte receptors. In particular monocytes contain various C-type lectins that could be involved in binding of schistosome glycoconjugates [128]. Toll-like receptors, some of which have been shown to respond to carbohydrates may also be good candidates [129].

Glycoconjugates and granuloma formation

Formation of granuloma around tissue-trapped eggs is in fact the cause of the main pathology observed in schistosomiasis and potentially leads to severe fibrosis, hepatosplenomegaly and portal hypertension. The inflammatory granuloma reaction is a T cell-mediated event, also associated with the typical Th2 immune response and is induced by secreted egg antigens, including glycoconjugates [130–133]. Some recent investigations have further focussed on the role of glycoproteins in this complex event. The ConA binding fraction of SEA coupled to sepharose beads to mimic schistosome eggs, induced granuloma formation in a mouse model [134]. Adult worm antigens exerted the same effect to a lesser extent. A significantly stronger response against SEA-coated beads was induced after sensitisation with CCA and CAA, from which it was deduced that SEA-induced granuloma formation is primed by the immunogenic carbohydrates on CCA and CAA leading to an accelerated response. This would

suggest a role for Le^X-containing polymeric glycans. The same granuloma promoting effect was observed when mice are sensitised with Le^X of LNFPIII, although less pronounced [135].

Glycan-lectin interactions in schistosomiasis

Adhesion molecules have been implicated in several events in *Schistosoma*-host interactions, including granuloma formation. The role of adhesion molecules in pathogenesis of hepatic and intestinal schistosomiasis has recently been reviewed [136], and it has been shown that serum levels of soluble adhesion molecules including E-selectin are correlated with the different pathological manifestations of schistosomiasis [137]. It may be expected that the glycoconjugates expressed by schistosomes interact with host lectins, most notably of the selectin family which have Le^X-related oligosaccharides as their ligands [138], and which have also been shown to interact strongly with LDN-F [139]. Indeed it has been demonstrated that host-soluble L-selectin enters tissue-trapped eggs and binds to glycosylated antigens on the surface of miracidia [140], but that E- and P-selectin do not. This implies that miracidia express L-selectin ligands and potentially directly influence L-selectin-mediated processes. In parallel with the selectin-mediated endothelium-leukocyte interaction [141], it has been shown that adhesion of eggs to endothelial cells under flow conditions is E-selectin mediated, and that process could be blocked by an anti-Le^X antibody [142]. Even more remarkable in this parallel between host and parasite adhesion molecules is the finding that *S. mansoni* itself expresses selectin-like molecules with affinity for Le^X and sialyl-Le^X [143]. These schistosome lectins and their human glycan counterparts, as well as the opposite combination of human selectins and Le^X-containing schistosome glycoconjugates are all required as co-receptors in the antibody-dependent cell-mediated cytotoxicity of macrophages and eosinophils to schistosomula [143,144]. Interestingly, other helminths also express C-type lectins homologous to human lectins involved in immunological events [145].

Concluding remarks

Our knowledge of schistosome glycoconjugates and their interaction with the host is rapidly increasing and strongly indicates that glycans are major players in many aspects of the immunology and biology of schistosomiasis. Schistosome glycoconjugate research has been boosted by the availability of new sensitive and sophisticated techniques for structural analysis of glycans and the analysis of carbohydrate-protein interactions. Furthermore, the importance of glycoconjugates in the immunology of schistosome infections and immunology in general is increasingly appreciated, leading to an intensified attention to this particular field of research. The structural definition and availability of schistosome-related glycoconjugates will continue to be of great importance for anti-carbohydrate antibody characterisation and detection, interaction studies with host-lectins, the study of schistosome glycosyltransferases, and the investigation of the immunomodulatory activities of glycoconjugates.

It can be expected that the technological developments leading to further increased sensitivity and miniaturisation in the field of structural analysis of glycoconjugates will enable studies on minimal amounts of scarcely available parasite material. In combination with the efforts currently put in the field of schistosome genomics [146] and proteomics [147] this will undoubtedly increase our understanding of the interplay of the schistosome with the host at the molecular level. This will not only be essential for the rational design of drugs, vaccines and diagnostic tools but will clearly also have a bearing on our knowledge of the general principles of carbohydrate-mediated immune modulation with implications well beyond schistosomiasis.

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