Advancing helminth glycomics: structural specificity and immunogenicity of schistosomal and filarial glycans

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Chapter VI.

Summarizing discussion

Glycans are a wide range of structurally diverse molecules fulfilling a multitude of biological functions[1]. Their role in cellular communication and their recognition by a variety of glycan binding proteins make them key players in immunity. Glycans of parasitic helminths have been shown to have immunogenic and immunomodulatory properties[2,3]. Thus, they constitute possible diagnostic targets, as well as vaccine or drug targets, all very much needed tools to tackle the current challenges associated with parasitic infections of humans[4]. However, current knowledge of the glycans expressed by parasitic helminths is far from being comprehensive and neither is our understanding of their biological functions.

Filarial nematodes and schistosomes, responsible for filariasis and schistosomiasis, respectively, are amongst the parasitic helminths causing significant health burden in humans worldwide[5]. This thesis aimed to bridge some of the gaps in our knowledge of these parasites' glycobiology. The starting point of our studies largely involved structural characterization of schistosome (**chapter 2**) and filarial nematode (**chapters 3-4**) glycan repertoires, aiming to generate novel and comprehensive information about the parasite's glycosylation. Next, antigenicity of the characterized glycans was assessed by studying their recognition by host antibodies (**chapters 2-4**). Finally, the impact of filarial infection on the host serum *N*-glycome was examined (**chapter 5**), bringing novel insights into the host response during infection.

This discussion summarizes our findings addressing them from a broad parasitology perspective, discusses functional and biological implications, considers unanswered questions, and explores avenues for future investigations.

1. Structural characteristics of schistosome and filarial nematode glycans

As covered in **chapter 1** of this thesis, knowledge of helminth glycans is fragmented. In the past decades, substantial data has been gathered in the case of *Schistosoma mansoni* (**Figure D-1**) for which in-depth studies of several classes of glycans have been performed on various stages of the parasite, covering its life cycle[6,7]. This was not yet the case for other *Schistosoma* species nor for parasitic filarial nematodes of humans. Thus, we used mass-spectrometry (MS)-based methods to characterize several classes of glycans expressed by *S. haematobium* (**chapter 2**), *Brugia malayi* (**chapter 3**) and *Onchocerca volvulus* (**chapter 4**). **Figure D-1** constitutes a graphic summary of our findings and how they extend previous knowledge of these parasites' glycosylation. The various monosaccharide abbreviations used to describe the glycan structures throughout this section are listed in the symbol key inset of **Figure D-1**.

Schistosome and filarial glycan features

As expected, the characterized glycans encompassed features generally common to helminths such as truncated and mannosylated *N*-glycans, terminal (fucosylated) LDN (LacDiNAc, GalNAcβ1-4GlcNAc) and the absence of sialic acids[3]. Besides those common traits, schistosome- and filarial-specific glycan features are made particularly visible by their side-by-side representation in **Figure D-1**.

Glycan elements previously described in *S. mansoni* also identified in *S. haematobium* include modifications of the *N*-glycan core including β1-2-linked xylose (Xyl) and the stage-specific α1-3 core fucose (Fuc) that are common modifications in invertebrates, notably in plants[8] (**chapter 2**). In addition, *S. haematobium* also expresses the highly *Schistosoma*-specific di- and tri-fucosylation patterns Fucα1-2Fucα1-3-R (DF) and Fucα1-2Fucα1-2Fucα1-3-R (TF) linked to N-acetylhexosamine (HexNAc) residues both in protein-linked glycans (*O*-linked) and in the glycosphingolipid (GSL) glycans. The expression of terminal Galβ1-4GlcNAc (LacNAc, LN) and Galβ1-4(Fucα1-3)GlcNAc (Lewis X, LeX) motifs, endogenous to their mammalian host, is an important feature of schistosomes, with these motifs represented in abundance in various life stages and glycan classes in both *S. mansoni* and in *S. haematobium*. As detailed in this thesis introduction (**chapter 1**), LeX-containing glycoconjugates such as the egg glycoproteins omega-1 and interleukin-4-inducing factor from schistosome eggs (IPSE/α1)[9,10] have been shown to be key components in host immunomodulation, notably in the context of major T helper (Th) 2 drivers. The LeX motif, however, is not observed in filarial nematodes in our work (**chapters 3-4**), nor in most parasitic helminths currently studied[11], suggesting that the recognition of LeX by host immune cells via C-type Lectin Receptors (CLRs) are immunomodulation strategies specifically exploited by schistosomes.

As briefly mentioned above, LDN is another important termination of helminth glycans. While largely represented in the GSL glycans of both schistosomes and filarial nematodes (**Figure D-1**), terminal LDN is very restricted in the *N*-glycans of *O. volvulus* and is not detected in those of *B. malayi*. In contrast, a majority of the *N*-glycans in these two species are extended with terminal N-acetylglucosamine (GlcNAc) residues, often carrying the filarial nematode signature substituent, the zwitterionic phosphorylcholine (PC)[12] (**chapters 3-4**). Interestingly we also found PC attached to the α1–3-linked mannose of the *N*-glycan core in both *B. malayi* and *O. volvulus*, a feature that had not been described at publication of our study but has now also been identified in the *N*-glycans of the porcine whipworm *Trichuris suis*[13]. GSL glycans of both *B. malayi* and *O. volvulus* carry PC substituents in abundance. Notably, PC is frequently found on the GlcNAc part of the arthro-type GSL core (GlcNAcβ1–3Manβ1–4Glc1–1cer), on which the GSL glycans of filarial nematode are built[14]. PC is also observed linked to the HexNAc residues – both GlcNAc and GalNAc – extending this

core, with glycans carrying multiple PC substituents that can occupy a variety of positions in the GSL glycan backbone including terminal and subterminal ones (**Figure D-1**). We found the GSL glycans of both *B. malayi* and *O. volvulus* to be dominated by the PC-bearing zwitterionic glycan Galα1-4GalNAcβ1–4(PC-6)GlcNAcβ1–3Manβ1–4Glc1–1cer that has been suggested previously to be highly conserved throughout the nematode phylum[15]. Our glycan microarray studies corroborated this hypothesis since immunoglobulin (Ig) G binding to this glycan isolated from *B. malayi* was observed in all the various filarial nematode infection sera tested (**chapter 4**). Importantly, binding to this structure persisted after removal of PC using hydrofluoric acid (HF) treatment, indicating the broad recognition of the α-Galactose (Gal)-containing structure in all infection sera as well. Previous work has suggested the terminal Gal residue of the major GSL structure in *O. volvulus* to be α1-3 linked to GalNAc[15] but our studies suggested an α1-4 linkage based on the respective specificities of the galactosidases used (**chapters 3-4**). A fucosylated variation of this epitope was characterized in the GSL glycans of both *B. malayi* and *O. volvulus*, to which IgG binding was observed in corresponding infection sera, as well as in sera from *Mansonella perstans* infected individuals (**chapter 4**). In the GSL glycans of *O. volvulus*, α1-4 Gal was also found in a glucuronic acid (GlcA)-containing branched epitope of putative composition GlcAβ(Galα1-4)GalNAcβ1-4GlcNAc-R (**Figure D-1**), that could additionally be fucosylated, and was not observed in *B. malayi* (**chapter 3**) and has not been described in other helminths so far. This epitope could potentially constitute an *O. volvulus*-specific feature, and may yield antibody responses specific for onchocerciasis, but this has not been addressed in the present work.

Anionic helminth glycans

Generally, an important observation brought to light in this thesis, together with other recent studies, is the occurrence of acidic glycans in helminths, *i. e.* glycans containing anionic hexuronic acid (HexA) in their composition, in addition to neutral monosaccharides. GlcA has been long known as part of the repeating [-6(GlcAβ1-3)GalNAcβ1-] unit of the schistosome circulating anodic antigen (CAA)[16], a glycosaminoglycan-like structure excreted by adult worms. However, there has not been other evidence of acidic monosaccharides in parasitic helminth *N*- or *O*-glycans until recently, when a comprehensive *N*-glycomic study of the canine heartworm *Dirofiliria immitis* described numerous glucuronylated structures in the *N*-glycome of this parasite[17]. There, GlcA appeared to extend LDN motifs on *N*-glycan antennae, and we found HexA in a similar context in the *N*-glycans of *B. malayi* (**chapter 3**). Sensitivity to β-glucuronidase proved this HexA to be β-linked GlcA. In addition, we observed terminal GlcA residues in abundance in the GSL glycans of *B. malayi* (**Figure D-1**) where GlcA is β-linked to GalNAc in terminal position of various GSL glycan structures. Notably, the second most abundant ion species in the MS spectra of *B. malayi* GSL

glycans corresponds to the acidic glycan GlcAβGalNAcβ1–4(PC-6)GlcNAcβ1–3Manβ1–4Glc1–1cer. While this was the first report of GlcA in the GSL glycans of a parasitic filarial nematode, further research subsequently revealed that this feature is not unique to *B. malayi*. During our glycomic study of *O. volvulus*, we characterized GlcA-containing GSL glycans in which this residue was observed in a similar context as in *B. malayi*, with β-linked GlcA capping LDN (**chapter 4**). As mentioned above, we additionally observed the structures terminated with branched GlcA and α1-4 Gal in *O. volvulus*. Mutual expression of GlcA-containing glycans in various filarial nematodes was reflected by the cross-reactivity of antibody binding to our glycan microarrays. Sera from both *B. malayi* and *O. volvulus* infected individuals contained IgG targeting GlcA-terminated glycans isolated from *B. malayi*. In addition, *M. perstans* infected individuals also showed significantly higher IgG levels to this epitope than uninfected control sera, suggesting that *M. perstans* also incorporate GlcA in their glycoconjugates, although the class(es) of glycans that encompass terminal GlcA in this parasite is unknown.

Acidic glycans were also observed in schistosomes, with HexA residues identified in many GSL glycans (**chapter 2**). Similar to our study of filarial nematodes, β-glucuronidase activity indicated that these HexA residues are most likely β-linked GlcA, which is in line with identification of this residue in the schistosomal CAA[16]. Although the CAA molecule has been determined to be *O*-linked, we did not detect any GlcA-containing structures in the *S. haematobium* and *S. mansoni* protein-linked (*N*- and *O*-linked) glycans examined. Besides being class-specific, acidic GSL glycans were found to be stage-specifically expressed with their presence in adult worms and eggs but not in cercariae (**Figure D-1**). In the eggs of both *S. mansoni* and *S. haematobium*, we identified many structures containing a GlcA residue, invariably occupying the subterminal position and capped by a single GalNAc (**chapter 2**). In *S. haematobium* adult worms, GlcA-containing GSL glycans were much scarcer than in the eggs and the acidic residues were found in a different context, occupying the terminal position in the GSL backbone. Regardless of these variations, acidic glycan species appear to be more widespread in helminths than previously known, particularly in the GSL glycans.

This raises the question about the biological role of these acidic epitopes in host-parasite interactions. While GlcA is one of the major components of glycosaminoglycan chains of proteoglycans[18], reports of GlcA in the composition of mammalian *N*-linked or GSL glycans remain sporadic and restricted to specific instances, with, for instance, the expression of (often sulfated) GlcA-containing carbohydrates in the nervous system[19] and of a minor subset of GlcA-terminated plasma *N*-glycans in humans[20,21]. As discussed in **chapters 2 and 3**, GlcA could potentially be an invertebrate equivalent of anionic monosaccharides frequently capping mammalian glycans, such as sialic acids. This is most likely when GlcA is

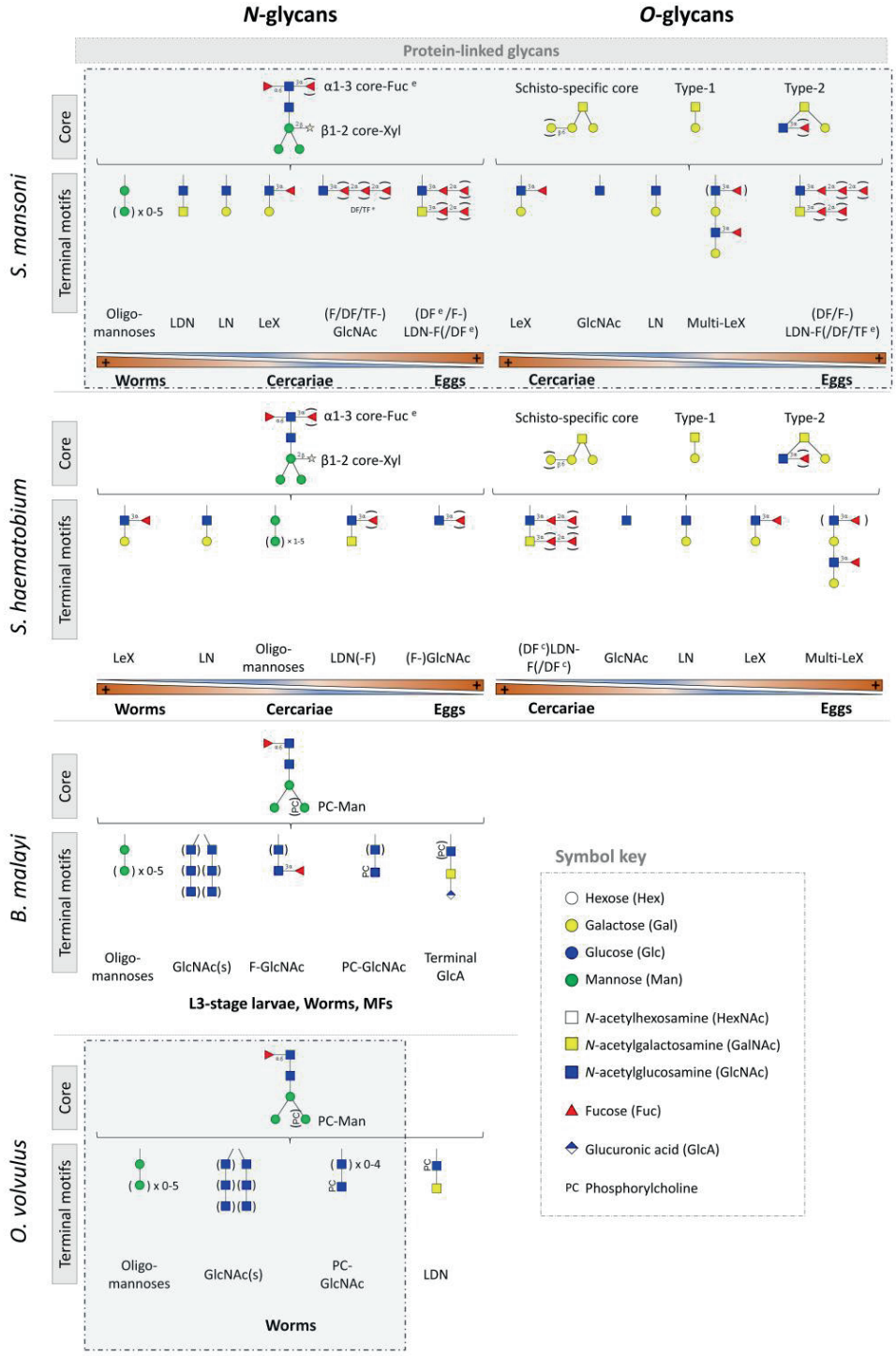
N-glycans
O-glycans
Protein-linked glycans
S. mansoni
Core
Terminal motifs
α1-3 core-Fuc e
β1-2 core-Xyl
Schisto-specific core
Type-1
Type-2
Oligo-mannoses
LDN
LN
LeX
(F/DF/TF-) GlcNAc
(DF e/F-) LDN-F(/DF e)
GlcNAc
Multi-LeX
(DF/F-) LDN-F(/DF/TF e)
Worms
Cercariae
Eggs
S. haematobium
(DF c)LDN-F(/DF c)
(F-)GlcNAc
B. malayi
PC-Man
F-GlcNAc
PC-GlcNAc
Terminal GlcA
L3-stage larvae, Worms, MFs
O. volvulus
LDN
Symbol key
Hexose (Hex)
Galactose (Gal)
Glucose (Glc)
Mannose (Man)
N-acetylhexosamine (HexNAc)
N-acetylgalactosamine (GalNAc)
N-acetylglucosamine (GlcNAc)
Fucose (Fuc)
Glucuronic acid (GlcA)
PC Phosphorylcholine

Figure D-1: Overview of the major *N*-glycan, *O*-glycan and GSL glycan motifs characterized in schistosomes and filarial nematodes.

Major glycan features identified in our glycomic studies of *S. mansoni* and *S. haematobium* (**chapter 2**), *B. malayi* (**chapter 3**), and *O. volvulus* (**chapter 4**), as indicated on the left of the figure. Previously known glycan features already characterized in *S. mansoni*[6] and *O. volvulus*[3,15,104] are shaded and framed. Glycans are represented using the Consortium for Functional Glycomics (CFG) nomenclature as detailed in the symbol key inset.
LDN = LacDiNAc; LN = LacNAc; LeX = Lewis X. Life-stages examined are specified and stage-specific glycan features of schistosome cercariae, worms and eggs are indicated with [c, w, e], respectively.

found in terminal position as seen in *N*-linked and GSL glycans of *B. malayi* (**chapter 3**), in the GSL glycans of *O. volvulus* (**chapter 4**) and in adult schistosomes (**chapter 2**). The fact that this residue is specifically expressed terminally in adult *S. haematobium*, a life-stage that is exclusively mammalian, encourages that hypothesis. Glucuronated proteoglycans and sialylated glycans are known to be involved in various immune processes in mammals[22–25], and it would be of major interest to investigate whether acidic helminth glycans interact with immune cell receptors and mimic the effect of anionic mammalian glycans to some extent. Another possible function for helminth acidic glycans, that would be in line with the presence of GlcA residues in schistosome eggs, is a role in interactions with the intermediate host, given that anionic glycans have been reported in mollusks[26] and insects[27,28]. Larval stages of *Anopheles gambiae* and *Aedes aegypti*, intermediate hosts for *W. bancrofti* and *Brugia* spp., respectively, have been found to express GlcA-containing *N*-linked and *O*-linked glycans[29]. In these mosquitoes, GlcA residues have however been reported to be linked to Gal, in terminal position of antennae of composition GlcAβ1-3Galβ1-3GalNAcβ1-4GlcNAc which constitutes a different epitope than the one we characterized in filarial nematodes, where GlcA extends LDN (**Figure D-1**). GlcA-containing glycans have not been reported in *Biomphalaria glabrata*, the only intermediate host of schistosomes for which glycomic data is available. In mammalian *N*-linked and GSL glycans, GlcA is mostly found as part of the human natural killer-1 carbohydrate epitope of composition HSO_3-3GlcAβ1-3Galβ1-4GlcNAc-R in lymphocytes and in the nervous system[19]. Similarly, when detected on human plasma *N*-glycans, GlcA has been found terminally, capping the Gal residue of LN antennae[20,21]. Thus, although this would have to be confirmed by further investigations, GlcA in terminal or subterminal position of HexNAc stretches, as reported in our studies (**Figure D-1**), could constitute structural contexts specific for helminth *N*-linked and GSL glycans. Notably, our glycan microarray studies revealed IgG responses to GlcA-containing glycans in filarial infections with *B. malayi*, *O. volvulus* and *M. perstans* (**chapters 3-4**), and this was also the case in schistosomiasis. IgG and IgM from schistosome-infected individual sera were found to bind the acidic GSL glycans expressed by *S. haematobium* and *S. mansoni* (**chapter 2**). Interestingly, differential IgG responses to GlcA-containing egg GSL glycans were observed in infection sera, as IgG levels to these acidic glycans were significantly higher in sera from *S. haematobium*-infected individuals than from non-endemic controls and from *S. mansoni*-infected individuals, making this feature discriminative between infection with one *Schistosoma* species or the other.

Species-specific glycan expression in schistosomes

Consistent with the differential IgG recognition of GlcA-containing epitopes, we identified major differences between the GSL glycans of *S. mansoni* and *S. haematobium* (**chapter 2** and **Figure D-1**).

Although we detected acidic GSL glycans in the eggs of both *S. haematobium* and *S. mansoni*, we showed the abundance of these structures to be much higher in *S. haematobium* eggs, with all major GSL glycans carrying a GlcA residue in subterminal position. Such structures were less prevalent in *S. mansoni* eggs, that are dominated by neutral GSL glycans (**chapter 2**), explaining why acidic GSL glycans had not been reported previously in schistosomes.

The (multifucosylated) structures in schistosome eggs were thoroughly characterized with the help of porous graphitized carbon nano-liquid chromatography (PGC-nano-LC) coupled to a tandem ion trap mass spectrometer, revealing differential patterns of fucosylation. In *S. mansoni* eggs, Fuc were found to form di- and tri-fucosylated motifs (DF/TF) that were systematically located terminally and thus associated with the subterminal GlcA residue, when present (**Figure D-1**). On the contrary, the few Fuc residues present on *S. haematobium* egg GSL glycans were found linked to HexNAcs directly extending the GSL glycan core, and never terminally nor in proximity of GlcA. We concluded that the dense fucosylation observed in *S. mansoni* probably masks the GlcA-containing epitope to some extent, limiting specific IgG formation to a GlcA-containing motif in *S. mansoni* infections, in contrast to *S. haematobium* infections where the non-fucosylated GlcA-containing epitope appears immunodominant.

In addition to these variations in the egg GSL glycans, we found that GSL glycans in all *S. haematobium* life-stages are built on a trihexosyl core extending the ceramide (cer) portion (**Figure D-1**), which contrasts with the *S. mansoni* core determined previously to be GalNAcβ1–4Glc1–1cer[30]. Another noteworthy structural glycan feature in *S. haematobium* cercariae, are the cercarial GSL glycans enriched in hexose residues containing a Galβ1-3Gal branching (**Figure D-1**) which contrast to *S. mansoni* that exclusively features linear GSL glycans.

All in all, our data indicate that the GSL glycan synthesis pathways differ substantially between *S. mansoni* and *S. haematobium*. Differences were less marked for the other classes of glycans examined, although considerable qualitative variations were shown to occur between the same life-stages from each species (**chapter 2**). Generally, *S. haematobium N*-glycans appear to be less fucosylated and less complex than those of *S. mansoni*, with the latter species expressing for example a higher amount of triantennary *N*-glycans. Substantially less *N*-glycans with core-xylosylation and terminal fucosylation are expressed in *S. haematobium* cercariae than in *S. mansoni*, resulting mainly in terminal LN motifs in the former species instead of the LeX motifs dominating in the latter (**Figure D-1**). Interestingly, similar observations have been made in previous work comparing *S. japonicum* glycans to those of *S. mansoni*. Multifucosylated *N*-linked and GSL glycans could not be detected in the eggs of *S. japonicum*[31,32] and xylosylated *N*-glycans were only found in *S. japonicum* eggs but in not in cercariae[33]. Although somewhat preliminary, these studies suggested a

distribution of these glycan motifs in *S. japonicum* resembling our findings in *S. haematobium*. Xylosylation is known to occur in mollusks, and specifically in *B. glabrata*[34,35], in which the abundance of glycans containing β1-2-linked Xyl has been associated with increased susceptibility of the snail strains towards *S. mansoni* infection[36,37]. Differences in the occurrence of the β1-2-linked Xyl modification in life stages that interact with the snail host between the different schistosome species may therefore be related to their specific snail-host compatibilities.

In any case, the distinctive glycobiology observed in schistosomes provides evidence that insights gained from studying one species may not necessarily apply to closely related ones. Thus, further research must seek to determine how the knowledge acquired from *S. mansoni*-based studies applies to the other schistosome species. This is particularly important with regards to understanding immune modulation mechanisms within the human host, that could impact prophylactic therapy or diagnostic strategies. It has been found that the field-friendly point-of-care test for the detection of the Circulating Cathodic Antigen (POC-CCA), has lower efficiency in detecting *S. haematobium* than *S. mansoni* infections, for which it has been fully validated[38–44]. The cause of the inconsistent performance of the test is unknown, but CCA is a carbohydrate antigen excreted by adult schistosomes constituted of LeX repeats[45]. Thus, there possibly are structural and/or quantitative differences in the expression of this molecule in *S. haematobium*. On a different note, previous work reported that the immune environment of granulomas caused by *S. mansoni* contains more eosinophils than neutrophils while it is dominated by neutrophils in *S. japonicum* infections[46], demonstrating that species-specific immunopathology occurs. Given that glycans, and more specifically LN and LDN-containing ones, have been shown to be involved in granuloma formation[47], one might speculate that differential glycosylation results in different immunopathology, notably in view of the differential egg glycosylation between *S. haematobium* and *S. mansoni* seen in our study. Importantly, a particular focus should be given to glycosylation in the context of vaccine candidates. Secreted products of early larval stages, including secretory proteins - thus, likely to be glycosylated - have been deemed promising vaccine candidates[48–50]. In such cases, it would be crucial to perform targeted glycoproteomic analysis of the native vaccine candidate in all major schistosome species infecting humans to assess possible species-specific glycosylation that might impact vaccine efficacy.

2. Glycans and host responses in schistosomiasis and filariasis

Antigenic helminth glycans

Due to their non-mammalian structural features many helminth glycans are antigenic in the definitive host. Unsurprisingly, antibodies to many known parasite glycans are present in infection sera. Detailed studies of anti-glycan antibody responses are of major interest. Firstly, the induction of an antibody response indicates the immunogenicity of the parasite glycan, and that it is exposed to the host immune system. Secondly, detection of serum antibodies to parasite antigens is a diagnostic strategy presenting potential advantages for sensitive and early detection of infection. In filariasis notably, it has been shown that filarial specific IgG4 antibodies are measurable while the circulating filarial antigen, which is currently targeted in the diagnostic test for *W. bancrofti*, is not (or not yet) detectable[51]. In **chapter 3** we showed that anti-glycan antibodies appear in the serum of *B. malayi*-infected rhesus macaques at an early stage of infection, since substantial IgG and IgM binding was already observed to a broad subset of antigenic *B. malayi* GSL glycans 5 weeks post-infection, and thus prior to microfilaremia. In schistosomiasis, antibodies to the cercarial tegument are detectable only a few days post-infection in mice[52] and recent data have shown that IgG and IgM to CAA are present 3-4 weeks post-infection in humans which could provide a promising angle for a specific and sensitive diagnostic[53]. There are however known limitations of antibodies as diagnostic tools, the main one being their persistence after pathogen clearance. Furthermore, specificity of the antibodies for their target is critical, as well as the specificity of the target itself. In that regard, species-specific features of schistosome and filarial nematode glycans may constitute useful targets.

Anti-glycan antibody responses in schistosomiasis

- **Antibody responses in schistosomiasis haematobium**

The work presented in **chapter 2** extended our knowledge of anti-glycan antibody responses in schistosomiasis mansoni to infections with *S. haematobium*. We reported IgG and IgM binding to a variety of motifs that are also antigenic in *S. mansoni* infections (**Table D-1A**). Unsurprisingly, *S. haematobium* infection sera were found to contain high levels of IgG and IgM to multifucosylated glycans, that have already been shown to be highly antigenic in *S. mansoni* infections. These observations are in line with our structural data showing that DF/TF glycan motifs were also expressed in *S. haematobium*, in particular by the cercariae (**Figure D-1**).

Table D-1: Anti-glycan antibody responses in schistosomiasis and filariasis

A

Glycan motif		IgG responses in infections with		IgM responses in infections with	
Source	Structure	*S. haematobium*	*S. mansoni*	*S. haematobium*	*S. mansoni*
S. haem & S. mans *N*-glycans	 Short, paucimannosidic glycans	(-)	(-)	(-)	(-)
S. haem & S. mans *N*-/GSL glycans	 LN	(-)	(-)	(-)	(-)
S. haem & S. mans *N*-/*O*-/GSL glycans	 LeX	(-)	(-)	(+)	(+)
S. haem & S. mans N-/GSL glycans	 LDN	(+)	(+)	(+)	(+)
S. haem & S. mans *N*-glycans	 Fuc-GlcNAc	(+)	(+)	(++)	(++)
S. haem & S. mans *N*-/*O*-/GSL glycans	 F-LDN(-F)	(++)	(++)	(++)	(++)
S. mans cercariae GSL glycans	 Pseudo LeY	(+++)	(+++)	(+++)	(+++)
S. haem & S. mans egg GSL glycans	 Multifucosylated (DF-LDN-DF/TF)	(+++)	(+++)	(+++)	(+++)
S. haem & S. mans egg GSL glycans	 GalNAc-GlcA	(++)	(-)	(+++)	(+++)

B

Glycan motif		IgG responses in infections with				
Source	Structure	*B. malayi*	*O. volvulus*	*M. perstans*	*L. loa*	*W. bancrofti*
B. malayi *N*-glycans	 Short, paucimannosidic glycans	(-)	(-)	(-)	(-)	(-)
B. malayi *N*-glycans	 PC-Man	(-)	(+) 1	(-)	(-)	(-)
B. malayi N-glycans	 Terminal PC-GlcNAc	(-)	(++) 1	(-)	(-)	(-)
B. malayi GSL glycans		(+++) 1,2	(+++) 1,2	(++) 1,2	(+)	(+)
B. malayi GSL glycans	 Term. GalNAc/LDN (+/-PC)	(+) 1,2	(+++) 1,4	(+) 1,2,4	(+)	(-)
B. malayi GSL glycans	 Term. α-Gal	(++) 1,2	(+++) 1,2,4	(+) 1,2,4	(+)	(+)
B. malayi GSL glycans	 Fuc-α-Gal	(+++) 1,2,4	(++) 1	(+) 1,2,4	(-)	(-)
B. malayi N-glycans	 Term. GlcA	(+) 1,2	(+) 1	(+) 1,2,4	(-)	(-)
B. malayi GSL glycans		(+++) 1,2,4	(++) 1,4	(++) 1,2,4	(-)	(-)
B. malayi GSL glycans	 Fucosylated HexNAcs (F-LDN-F,...)	(+++) 1,2,4	(++) 1,4	(++) 1,2,4	(-)	(-)

Glycan-microarrays constituted of native glycans (see “Source”) extracted from *S. haematobium* and *S. mansoni* (A) and *B. malayi* (B) were screened with sera from infected individuals, as detailed in column headers. Antibody binding (IgG or IgM, as specified) was measured using fluorescently labeled secondary antibodies. Binding to the glycan/glycan element specified in “Structure” is symbolized by “+” and orange shades, while “-” and blue colors indicate absence of binding comparable to uninfected controls. Darker orange shades indicate glycan motifs to which the highest levels of antibody binding have been observed in

Table D-1 ***(continued)*** our glycan-microarray assisted studies. When studied (in *B. malayi*, *O. volvulus* and *M. perstans* infections), specific IgG subclasses involved in glycan recognition are indicated in black next to the IgG symbol.

- **Species-specific IgG responses to GlcA-containing GSL glycans**

As mentioned previously, we found the GlcA-containing epitopes in *S. haematobium* and *S. mansoni* GSL glycans to be immunogenic with similarly high IgM responses observed in both infection groups, while high levels of IgG were reported in individuals infected with *S. haematobium* but not with *S. mansoni* (**Table D-1A**). These results were obtained by screening our glycan microarrays with a cohort of sera obtained from infected children. Thus, further investigation within different age groups is required to unravel the dynamics of antibody responses to this GlcA-containing glycan antigen. Nonetheless, the preferential IgG binding to the acidic epitope in *S. haematobium* infections constitutes a major finding, that, if validated in larger populations, could be exploited for detection of *S. haematobium* and species discrimination. This could complement the current diagnostic toolbox given that CAA detection, that has emerged as the gold standard diagnostic in terms of sensitivity over the past decades with the laboratory-based Up-Converting reporter Particle technology based, Lateral Flow (UCP-LF)[54], cannot distinguish between species. Mass drug administration (MDA) programs have achieved a substantial decrease of infection intensities in many areas[55], which results in a higher proportion of asymptomatic infections and in lower egg burdens. Consequently, there is a higher need for abilities to distinguish between species when eggs are not detectable, which is often the case due to day-to-day variation of egg secretion in infected individuals and to the general underperformance of microscopy of filtered urine for detection of light *S. haematobium* infections[56,57]. Thus, a *S. haematobium*-specific antibody detection assay based on a species-specific glycan antigen might prove useful for dedicated uses and for epidemiology studies. Furthermore, cases of urinary schistosomiasis caused by *S. haematobium* have occurred these past decades in China[58] and Europe[59], with outbreaks reported in France (Corsica)[60] and Spain[61]. In such non-endemic contexts, serology-based diagnostic tools would be highly valuable for population/traveler surveillance and mapping of risk-areas.

- **Function of anti-glycan antibodies: to be continued.**

Another question concerns the possible role that anti-glycan antibodies play in schistosomiasis. As mentioned in **chapter 1**, after years of being attributed subversive functions, it is now clear that schistosome glycans and the antibody responses they trigger are highly diverse. Studies using rhesus macaques[62,63] and baboons[64] as models have suggested a protective role for IgG to a specific subset of fucosylated LN and LeX-containing glycans. Interestingly, these studies have also pointed out possible

implications of specific IgG/IgM balances in immunity. Specifically, in both the rhesus macaques[62] and baboon models[64] high IgG and low IgM titers to glycan antigens appeared to be overall associated with resistance to (re)infection. It is interesting to note the differential recognition of the GlcA-containing GSL glycans by IgG and IgM in *S. mansoni* infection, although, to this epitope, we observed high levels of IgM and low levels of IgG (**Table D-1A**). As GlcA-containing GSL glycans were not included in the microarray-assisted studies in primate models, the role of antibodies to this subset of acidic glycans remains to be investigated.

The present study of *S. haematobium* glycans has contributed to revealing previously unknown features of the fascinating glycobiology of schistosomes, including the expression of acidic GSL glycans. Our glycan microarray-assisted study complemented these observations by bringing new insights on antibody responses to schistosome glycans. Importantly, GlcA-containing structures gave rise to a specific IgG response in schistosomiasis haematobium showing the impact that the absence or presence of parasite glycan motifs can have on specific host immunity. Much remains to be clarified, however, notably regarding the roles of these anti-glycan antibodies in schistosomiasis.

Deciphering the cross-reactivity of anti-glycan IgG responses in filariasis

- **Debunking the "overwhelming immunogenicity" of PC**

At the outset of the present studies, we expected to observe cross-reactive antibody responses in filarial infections of humans, based on the (limited) data suggesting shared glycan features throughout the nematode's phylum[3,12,15], in particular PC-containing structures. The idea of "extensive cross-reactivity among the antigens of helminthic parasites"[65] due to "heavy exposures to filarial antigens bearing the immunodominant epitope phosphocholine"[66] has indeed been widespread in the literature and collectively accepted since the 1980s[67–69]. To address the immunogenicity of PC in our studies, we included in our microarrays glycan fractions from which PC was removed using HF treatment, side-by-side with their native, PC-containing, versions (**chapters 3-4**). Interestingly, PC removal had little or no effect on IgG binding to the treated glycans, showing that binding was mainly not PC-driven. Similarly, at the most, there were only negligible IgG responses from infected individuals to certain PC-containing structures, such as PC-substituted *N*-glycans (**Table D-1B**). These observations challenge the idea of PC alone being responsible for an overwhelming IgG response in filarial infections and are in accordance with previous work in bancroftian filariasis. In these studies, anti-PC antibodies have been examined and no differences were found between infected individuals and controls[70], and it has

already been suggested that anticarbohydrate antibodies were recognizing non-PC determinants[71]. This also fits with the known immunomodulatory properties of PC and the idea that this substituent contributes to low antibody levels and poor lymphocyte responses observed in some filariasis patients[69]. This does not mean that PC-containing glycans are not antigenic, but that PC is not necessarily the most antigenic component of filarial nematode glycans, highlighting the importance and immunogenicity of other glycan motifs.

- **Pinpointing antigenic glycan motifs in filariasis**

When studying IgG responses from *B. malayi*-infected individuals, we recorded IgG binding to many *B. malayi* glycans. Notably, structures containing terminal α-Gal, GlcA and fucosylated epitopes were found to be highly antigenic (**chapter 3**). As documented in the first part of this discussion and in **Table D-1B**, cross-reactive IgG to various glycan motifs was found in the *O. volvulus*, *M. perstans, L. loa* and *W. bancrofti* infection sera examined on the *B. malayi* glycan microarray (**chapter 4**), confirming the existence of broadly shared antigenic glycans in filarial nematodes. This was the case for α-Gal terminated GSL glycans and for a subset of PC-containing GSL glycans to which IgG binding was observed in all five infection sera. In line with the above comment, we did not record IgG binding to the *N*-glycans, where PC has been abundantly found linked to mannose or GlcNAc, except in the *O. volvulus* infection sera. *O. volvulus* infection sera were generally highly reactive on the *B. malayi* glycan array and this cross-reactivity could be fully explained by the similarities in the *N*-linked and GSL glycans expressed by the two filarial nematodes (**Table D-1B**) revealed by the glycomic studies performed in **chapters 3-4**. A strong consistency between the structural data and the microarray results could be noted, with, for instance, higher IgG binding to LDN-containing GSL glycans from the *O. volvulus* infection group than the *B. malayi* infection group, in line with *O. volvulus* largely expressing GSL glycans terminated with LDN (**chapter 3**). The opposite trend applies to GlcA-containing glycans, consistent with a relatively higher abundance of acidic structures in the *B. malayi* glycome (**chapters 3-4**, **Table D-1B**). As hypothesized in **chapter 4**, perhaps *O. volvulus* infection triggers high titers of anti-glycan antibodies by producing higher amount of glycans or by presenting them on more immunogenic lipid/protein carriers. This could explain the instances where this infection group shows higher IgG levels on the *B. malayi* glycan array than the *B. malayi*-infected sera themselves. IgG responses to *B. malayi* glycans containing terminal GlcA and fucosylated motifs (α-Gal and HexNAcs) were exclusively observed in *B. malayi, O. volvulus* and *M. perstans* infection groups but not in the sera from individuals infected with *L. loa* and *W. bancrofti* (**Table D-1B**). It is plausible that these structural features are specific to a subset of filarial nematodes including the former species and excluding *L. loa* and *W. bancrofti*. However, this hypothesis needs to be confirmed using glycomic or glycogenomic

analysis as caution must be taken not to infer glycomic features of a parasite based on absence of antibody in the infected host, that could also result from a lack of accessibility of the antigenic glycan.

- **IgG subclasses**

In addition to total IgG, we also analyzed the different glycan-reactive IgG subclasses in *B. malayi, O. volvulus* and *M. perstans*-infected individuals (**chapter 4**). At the subclass level, the three infection groups followed similar trends with IgG1 and IgG2 being the major drivers of anti-glycan responses. Interestingly however, in *O. volvulus* infections, IgG1 appeared to be the subclass responsible for the high IgG levels reported, while in *B. malayi* and *M. perstans* infections, reactivity was more evenly distributed between IgG1 and IgG2.

In **chapter 3**, we reported a significant decrease of total IgG binding to most antigenic *B. malayi* glycans in sera of individuals post-treatment with diethylcarbamazine anthelminthic. Of all IgG subclasses involved in glycan recognition, IgG2 was the one to drop markedly and consistently across all individuals (formerly) infected with *B. malayi* (**chapter 4**). Thus, IgG2 to glycan antigens could constitute a promising diagnostic target for *B. malayi* infections and perhaps even provide a gain of specificity for detection of *B. malayi* and/or *M. perstans* infections.

- **IgM to glycan antigens**

IgG2 and IgM have been historically associated with carbohydrate antigens in parasitic infections, mainly by generalization of observations made in schistosomiasis but also according to limited data on bancroftian filariasis[71]. As discussed above, in our studies as well we identified a predominant role for IgG2 in the filarial infections studied, although IgG responses were far from being restricted to this subclass, with notably substantial IgG1 to glycan antigens reported as well (**chapter 4**). IgM reactivity to the *B. malayi*-glycan arrays, on the other hand, was very weak, not only from the *B. malayi* infection sera (**chapter 3**) but also from all the other filarial infection sera examined, including sera from *W. bancrofti*-infected individuals (data not shown). Furthermore, when studying the longitudinal cohort of *B. malayi*-infected rhesus macaques, we report a gradual decline of IgM binding to *B. malayi* glycans in the course of infection (**chapter 3**). IgM levels almost return to baseline 15 weeks post-infections in the macaque sera, suggesting that IgM, at least to the glycan antigens displayed on our microarrays, may not play any role during chronic filarial infections. In addition to their low intensities, the absence of significant differences between IgM responses from infected and control sera was notable. Thus, IgM responses to filarial glycan antigens appeared highly aspecific, matching observations of others[72].

Before our work presented in this Thesis, knowledge of the glycans expressed by filarial nematodes infecting humans was limited. Using glycan microarray-assisted studies, we provide information on the antigenicity of these molecules, additional to the recently acquired structural data (**chapters 3-4**). Our work highlighted common and differential antibody responses to *B. malayi* glycan antigens in five filarial nematode infections (**chapter 4**). In a field where glycans have been qualified as "cross-reactive determinants" for years, we observed substantial and, to some extent, specific IgG binding to glycan subsets, establishing their potential for diagnostic purposes. The dynamics of IgG2 binding pre- and post-treatment in *B. malayi* infections appear particularly promising for such applications. Substantial cross-reactive antibody responses were however observed, particularly between *B. malayi, O. volvulus* and *M. perstans* infection groups, an interesting clustering that does not follow the phylogenetic associations detailed in **chapter 1**. It must be noted that, being endemic in Asia, *B. malayi* is not co-endemic with *O. volvulus* nor with *M. perstans*, mainly present in Africa. Furthermore, cross-reactivity could also be exploited to engineer pan-diagnostics that could prove useful. As discussed in **chapters 3-4**, further validation work is required starting with extending this type of studies to larger cohorts and including the various subpopulations observed in filariasis that comprise asymptomatic, amicrofilariaemic and putatively immune individuals, in addition to microfilariaemic ones.

Serum *N*-glycoprofiling

In addition to glycans originating from the parasites, we studied the serum *N*-glycans of rhesus macaques infected with *B. malayi* (**chapter 5**). Using a validated hydrophilic interaction-ultra performance liquid chromatography (HILIC-UPLC)-based workflow in combination with MS, we monitored quantitative and qualitative changes of the serum *N*-glycome in this longitudinal cohort of animals. We report significant alterations of the serum *N*-glycan profile. These changes resulted in an overall gradual decrease in sialylation compensated by increases in galactosylated/mannosylated *N*-glycans, clearly visible 15 weeks post-infection. Most changes were monodirectional – *i. e.* specific *N*-glycans were either increased or decreased but did not fluctuate – and many were detectable as early as 5 weeks post-infection, which are interesting findings from a biomarker perspective. The timing of changes was consistent with observations made when screening our *B. malayi* glycan microarrays with the same set of sera, since substantial IgG and IgM responses to (glycan) antigens were also visible 5 weeks post-infection. Ig *N*-glycosylation significantly contributes to the total serum *N*-glycans and, consequently, increase of IgG and IgM resulting from the parasitic infection is expected to affect the total *N*-glycome profile. In a similar study performed during *D. immitis*

infection in dogs, increase in serum IgG titer was indeed deemed responsible for the majority of the marked alterations observed[73]. In our work, however, serum *N*-glycans not present in the IgG *N*-glycoprofile show alteration upon infection. Thus, an increase in Ig titers is unlikely to explain the totality of the changes reported, indicating that (many) additional serum glycoproteins must be impacted quantitatively and/or qualitatively by the filarial infection. Conducting glycoproteomic studies to determine which glycoproteins are affected, and how, would improve our understanding of the effect of filarial infections on the host. Furthermore, qualitative alterations of specific glycoproteins could constitute biomarkers of infection.

3. Helminth glycomics: future directions of research

The research presented in this thesis increased the number of helminth species for which structural glycomic data are available, contributing to our growing knowledge of helminth glycobiology (**chapters 2-4**). Complementary glycan microarray-assisted studies assessed the antigenicity of the characterized parasite glycans (**chapters 2-4**), while serum *N*-glycan profiling during establishment of filariasis (**chapter 5**), provide insights into the impact of infection on the host glycans. There is a lot more to investigate on the basis of this work, from functional implications of our observations to unresolved or lacking structural data. Between the many helminth species for which glycomic data are non-existent and neglected classes of glycoconjugates (*e.g.* glycosylphosphatidylinositol anchors, glycosaminoglycans or proteoglycans…), the list of glycan molecules waiting to be characterized is endless. Because technology is a major limiting factor in the structural characterization of glycans, this last section reviews the current state-of-the-art that has enabled the present work and touches on recent technological advances that are opening new areas of glycomic investigations.

Helminth glycomics: technological considerations

In view of its exceptional sensitivity, MS has been an invaluable tool for the analysis of glycans, notably with the emergence of Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF)-MS in the 90's[74]. This technique has been dominating the field with a wide range of applications and constitutes a robust method for structural characterization of glycans[75,76]. The use of tandem MS (MS/MS) combined with glycan sequencing techniques (**Figure D-2**) allowed us to accurately characterize the vast majority of the glycans studied in the present work. A known limitation of MALDI-TOF-MS is the difficulty to analyze sialylated and fucosylated glycan species due to the labile nature of their respective glycosidic bonds[77,78]. Chemical derivatizations of sialic acids, such as the one we applied in **chapter 5** for

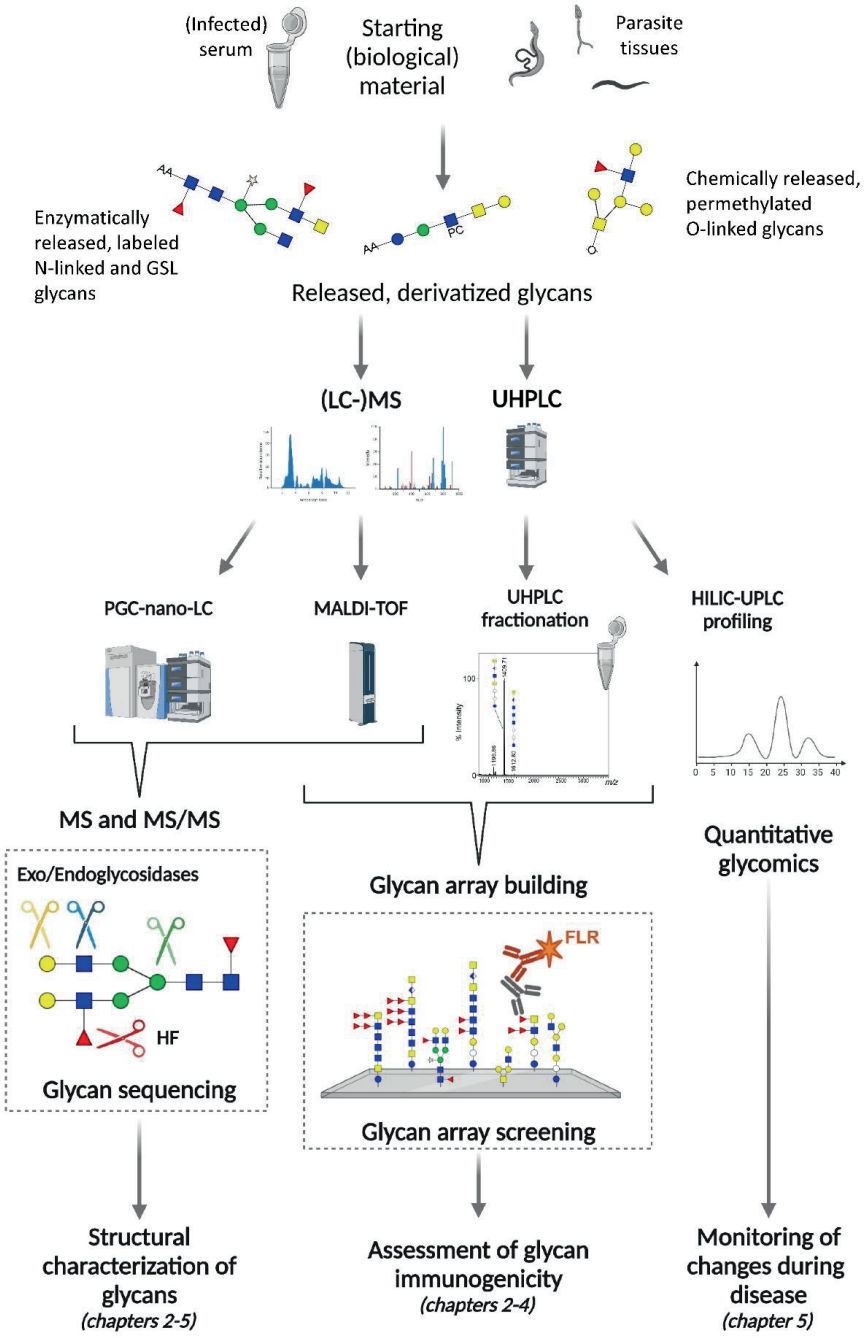

Figure D-2: Overview of the experimental workflows used in this thesis. General outlook of the complementary techniques used for glycomic and glycan microarray-assisted studies. Figure created with BioRender.com. Glycan examples are represented using the CFG nomenclature (see symbol key inset in **Figure D-1**).

the study of rhesus macaque serum *N*-glycome, offer a means to overcome the issue with sialic acid instability[79], while the use of negative-ionization mode is normally sufficient in stabilizing Fuc residues[80]. The main difficulty in our work lied in the numerous isomeric structures resulting from the variable Fuc distribution on the GSL glycans backbone (**chapter 2**). PGC-nano-LC-MS(/MS), a state-of-the-art technique achieving high-resolution separation of structurally similar glycans[81], was used to discriminate between these fucosylated isomers and to precisely assign the Fuc positions. Moreover, achieving separation of glycan structures was important to generate the glycan fractions for our glycan microarrays (**chapters 2-4**). To purify the pools of glycans extracted from parasite material, we used ultra-high performance liquid chromatography (UHPLC), a robust and efficient technique for the separation of fluorescently labeled glycans[82]. The same technology was part of the HILIC-UPLC workflow for *N*-glycan profiling used in **chapter 5** to study the serum *N*-glycome of the longitudinal cohort of rhesus macaques, providing quantitative data that could not have been obtained with MALDI-TOF-MS.

Thus, we used highly complementary technologies informative about structural, quantitative, and functional aspects of glycans (**Figure D-2**). Such glycomic studies could not have been conducted a couple of decades ago, when these workflows were not available. Although powerful, these methods present flaws and bottlenecks. They notably rely on the upstream release of glycans from their carrier (**Figure D-2**). There, enzymatic protocols provide undisputable advantages. In addition to accessibility and non-toxicity, they allow straightforward downstream labeling. Besides improving the ionization efficiency for MS detection and being used for attachment to surfaces[83] – glycan microarray slides, in our case – the stoichiometric attachment of one label per glycan via their reducing end permits quantitation using UV-absorbance or fluorescence intensity[84]. Released glycans, underivatized or labeled, can be subjected to glycan sequencing techniques, *i. e.* to treatments with reagents (*e. g.* HF) or glycosidases that degrade/cleave specifically certain monosaccharides. For instance, when all hexoses have the same monoisotopic mass (162.0528 Da) in the mass spectrometer, the use of galactosidases will reveal the presence of terminal galactose(s) together with their specific linkages[85]. This whole thesis advocates for how informative and powerful glycan sequencing tools, and particularly exoglycosidases, are for the elucidation of glycan structures. Unfortunately, their use is not compatible with certain derivatization procedures that modify the non-reducing end of glycans, such as permethylation. The latter is however a commonly used strategy to enhance the MS signal intensity and structural stability of glycans[84], notably when labeling is not possible, which is the case of chemically released *O*-glycans in our work (**Figure D-2**, **chapter 2**). For this reason, among others[86], broadly-specific enzyme(s) releasing *O*-linked glycans – ideally, equivalent to the peptide-N-glycosidases (PNGases) available for their *N*-glycan counterparts – would be of great help to the field. In the same vein,

although we currently benefit from a substantial toolbox of endo and exoglycosidases, some enzymes are not always (commercially) available, in particular when searching for enzymes acting on unusual, non-mammalian monosaccharides or substituents. Recently, high throughput functional metagenomic screening has been a successful approach for discovery of glycan-active enzymes[87–89], notably with the finding and characterization of a phosphodiesterase able to cleave off the PC substituent from filarial nematode glycans[90]. Thus, by enabling and facilitating glycan characterization or the release of glycans from less-studied classes of glycoconjugates of biological importance, development of new tools might open new glycomic horizons.

New perspectives: glycomics in the multi-omics era

Despite all the technological advances listed in the above section, in-depth glycomic studies of complex samples are, and will remain, somewhat cumbersome as data analysis requires manual curation steps. With the recent improvements of parasitic helminth genome quality mentioned in **chapter 1**, mining genomic data may be a way to indirectly obtain glycomic information and to circumvent shortcomings, for instance when biological starting material or advanced MS technology are not available. Major limitations to this approach are that glycan synthesis pathways in non-mammalian/non-model organisms are poorly characterized, and that functional genome annotations are largely incomplete, with more than half of protein-coding genes per helminth genome of unknown function[91]. Glycogenomic studies, *i. e.* linking glycome and genome data, when respective data are available, would allow us to better define the genes involved in glycan synthesis pathways in helminths. Glyco-genes of other species for which there is insufficient access to biological material could then be predicted by gene ontology. Furthermore, the transferases involved in the biosynthesis of parasite-specific glycan motifs, such as the glucuronyltransferases involved in the synthesis of acidic glycan motifs, or the schistosome fucosyltransferases making DF and TF, could potentially be targeted in therapeutic strategies if these glycan motifs prove to be essential for the parasite survival. Transcriptomic datasets which constitute a third layer of information could help identifying such glyco-active enzymes. For instance, it would be of interest to compare gene expression levels with regard to the variable inter-species and inter-life-stage expression of core-xylosylation, fucosylation and acidic epitopes reported in schistosomes in **chapter 2**, to understand the machinery involved in the making and regulation of these glycan motifs. Similarly, in view of the aforementioned clustering of *B. malayi*, *O. volvulus*, *M. perstans*-infected sera and the divergences from *W. bancrofti* and *L. loa*-infected ones upon glycan microarray screening in **chapter 4**, investigating the transcriptomes and glycogenomes of these filarial nematodes would be interesting. Generally, how the glycome relates to the genome is a recurring, and relevant, discussion in the field, and this thesis demonstrates clearly that genetically

Table D-2: How did this thesis advance the field?

	WHAT WAS PREVIOUSLY KNOWN?	KNOWLEDGE PROVIDED BY THIS THESIS	
		Study description	**Highlighted findings**
Schistosomiasis	Extensive glycomic data on *S. mansoni* [6]	Comprehensive glycomic study of *S. haematobium (Sh)* *N*-linked, *O*-linked and GSL glycans (**chapter 2**) In-depth structural characterization of acidic GSL glycans from *S. mansoni (Sm)* eggs (**chapter 2**)	**Differences between *Sh* and *Sm* :** • Trihexosyl core (*Sh*) instead of disaccharidic core (*Sm*) • Lower degree of fucosylation in *Sh* • Absence of multi-fucosylation (DF/TF) in *Sh* eggs • Higher abundance of acidic glycans in *Sh* associated with different fucosylation patterns **Similarities between *Sh* and *Sm* :** • Stage-specific expression of glycans, acidic GSL glycans in eggs • Shared *Schistosoma*-specific glycan motifs (*e.g.* DF, TF…)
	S. mansoni express antigenic glycans that trigger major antibody-responses in infected individuals[100–103]	Comparative study of anti-glycan IgG and IgM responses in *S. haematobium* (*Sh*) and *S. mansoni* (*Sm*) infections (**chapter 2**)	**Ab responses in *Sh* infection sera :** • IgG and IgM to many schistosome glycans • Acidic GSL glycans are antigenic • Higher levels of IgG to acidic GSL glycans in serum of *Sh*-infected individuals than in *Sm*
Filariasis	PC substituents are abundant in filarial nematode glycans[3,104] Structural data on the 3 major GSL glycans expressed by *O. volvulus* [15]	Comprehensive glycomic study of *B. malayi (Bm)* (**chapter 3**) and *O. volvulus (Ov)* (**chapter 4**) *N*-linked and GSL glycans	**Similarities of *Bm* and *Ov* glycans that contain:** • PC-substituted structures in abundance • Zwiterrionic αGal-terminated GSL glycans • GlcA-containing terminal epitopes • Fucosylated αGal and HexNAcs • PC-substituted mannose in *N*-glycans
	N/A	Study of anti-glycan antibody responses to *B. malayi (Bm)* glycans in a longitudinal cohort of *Bm*-infected rhesus macaques (**chapter 3**)	**Anti-glycan Ab responses in *Bm*-infected rhesus macaques:** • IgG and IgM to *Bm* glycans induced upon infection • Anti-glycan IgG and IgM already detected prior to microfilaremia • IgM peaks 5 weeks post-infection (wpi) and declines • IgG levels increased gradually and remained high 15 wpi

Filariasis	Infected individuals have elevated antibody levels to filarial (crude) antigen [71,72,105]	Study of anti-glycan antibody responses to glycan antigens isolated from *B. malayi (Bm)* in five filarial infections of human (**chapters 3-4**)	**Anti-glycan Ab responses in *Bm*-infected humans:** • Anthelminthic treatment leads to reduced anti-glycan IgG levels in *Bm* infected individuals • Total IgG response is driven by IgG1 and IgG2 • IgG2 showed a sharp decrease in response to treatment **IgG cross-reactivity in other filarial infection sera:** • Cross-reactivity to αGal and to certain PC-containing glycans observed in all infection sera • Cross-reactivity to many more glycans observed in *O. volvulus (Ov)* and *M. perstans (Mp)*-infection sera • Cross-reactivity in infections with Ov and *Mp* is mainly driven by IgG1 and IgG2
	The serum *N*-glycan profile is generally stable in mammals [106,107] but can be affected by various conditions including parasitic infection [73]	Serum *N*-glycan profiling in a longitudinal cohort of *B. malayi (Bm)*-infected rhesus macaques (**chapter 5**)	**Characterization of rhesus macaque serum *N*-glycans** • Increased knowledge on glycan expression in rhesus macaque sera • Definition of a healthy baseline for disease monitoring **Monitoring of rhesus macaque serum *N*-glycome during *Bm* infection** • Significant alteration of the serum *N*-glycan profile post-infection • Many different *N*-glycans are affected by *Bm* infection • Changes are mainly monodirectional and do not fluctuate over time

close organisms can differ in their glycomic traits. Thus, in the age of computational biology and artificial intelligence, there is a substantial amount of *in silico* work awaiting to be done that could significantly advance the field.

On a different note, studying intact glycoconjugates rather than released glycans would help gain a better understanding of glycosylation in the biological context. Targeted glycoproteomic studies have already contributed to major findings notably with the characterization of the immunomodulatory glycoproteins Omega-1, Kappa-5 and IPSE/α1 from *S. mansoni* eggs and the ES-62 from *Acanthocheilonema viteae*, all mentioned in **chapter 1**[92–95]. Glycoproteomic studies on larger sets remain rare but are emerging, including *N*-glycosite mapping in *Brugia malayi*[96] and the recent

characterization of *Fasciola hepatica* newly excysted juveniles' glycoproteins, shedding light on their complexity and heterogeneity[97]. As mentioned previously, glycoproteomic analysis of infected serum could be instructive, in view of the glycomic changes already reported (**chapter 5**). Similarly, concomitant characterization of the carbohydrate moieties and ceramide compositions of glycolipids can be highly informative. In *S. mansoni*, such analysis has revealed differences between cercariae and adult worms that could explain differential immune properties reported between both life-stages[98]. Undoubtfully, glycoproteomic and glycolipidomic studies would bring precious knowledge to better understand the fundamental roles that glycoconjugates play in nearly all aspects of pathophysiology, not to mention again the importance of considering molecules in their entirety for vaccine applications.

Finally, another promising method to study glycoconjugates in context is the growing field of MS imaging (MSI) allowing characterization of molecules *in situ*. Previous immunofluorescence work has shown marked spatial changes in glycan expression during the development of *S. mansoni* from cercariae to schistosomula[99]. However, such work is limited by the availability of anti-glycan antibodies targeting specific glycan motifs. Overcoming this limitation, MSI allows to investigate spatial expression of molecules, and successful MSI-based lipidomic studies have already been conducted in *S. mansoni*[108–110]. This technique can also be applied to tissues originating from the infected host, providing an opportunity to gain novel insights into the role of glycans at the host-parasite interface.

4. Concluding remarks

This thesis constitutes a comprehensive amount of work on glycans in the context of the major parasitic diseases that are schistosomiasis and filariasis. Descriptive structural work was complemented with immunological data obtained through glycan microarray and serum profiling studies. Efforts were made in untangling common features of helminthiasis from species-specific ones, which is particularly relevant for currently needed biomarker or diagnostic applications. **Table D-2** aims to summarize the work performed in the present studies and highlight our main findings.

There is still a lot to explore regarding glycans in helminthiasis, both structurally and regarding their biological implications. The advent of multi-omics technologies opens up unprecedented avenues to study glycans in a more comprehensive perspective potentially leading to a deeper understanding of the role of glycoconjugates in host-parasite interactions.

Abbreviations

CAA	Circulating Anodic Antigen
CCA	Circulating Cathodic Antigen
Cer	Ceramide
DF	Di-fucosyl, Fucα1-2Fucα1-3
Fuc	Fucose
Gal	Galactose
GalNAc	N-acetylgalactosamine
Glc	Glucose
GlcA	Glucuronic acid
GlcNAc	N-acetylglucosamine
GSL	Glycosphingolipid
Hex	Hexose
HexA	Hexuronic acid
HexNAc	N-acetylhexosamine
HF	Hydrofluoric acid
HILIC-UPLC	Hydrophilic interaction-ultra performance liquid chromatography
Ig	Immunoglobulin
IPSE/α1	Interleukin-4-inducing factor from schistosome eggs
LDN	LacDiNAc, GalNAcβ1-4GlcNAc
LeX	Lewis X, Galβ1-4(Fucα1-3)GlcNAc
LF	Lymphatic Filariasis
LN	LacNAc, Galβ1-4GlcNAc
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-Of-Flight
Man	Mannose
MS	Mass Spectrometry
MS/MS	Tandem MS
MSI	Mass Spectrometry Imaging
PC	Phosphorylcholine
PGC-nano-LC	Porous Graphitized Carbon Nano-Liquid Chromatography
pseudo-LeY	pseudo-Lewis Y, (Fucα1-3)Galβ1-4(Fucα1-3)GlcNAc
TF	Tri-fucosyl, Fucα1-2Fucα1-2Fucα1-3
Th	T helper
UHPLC	Ultra-High Performance Liquid Chromatography
Xyl	Xylose

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