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

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# Chapter I.

## General introduction

# 1. Helminth infections

Helminths are parasitic macroorganisms belonging to the two major phyla of nematodes (roundworms) or platyhelminths (flatworms)[1]. The latter include trematodes (flukes) and cestodes (tapeworms), while the nematode phylum is constituted of the Enoplea, Dorylaimea and Chromadorea classes[2] (**Figure I-1**). Helminths are broadly classified into these main groups and subgroups based on morphological aspects of egg, larval, and adult stages, as well as current molecular and developmental evidence, reflecting evolutionary relationships[3]. With an estimate of over 2 billion infected people, helminths are highly common infectious agents in developing countries[4] representing a significant burden for more than a quarter of the world's population. They strongly contribute to neglected tropical diseases (NTDs)[5], a group of conditions that mostly affect impoverished communities where they cause a large burden in terms of disability-adjusted life years (DALYs). As of today, over 20 NTDs are targeted in the World Health Organization (WHO) roadmap for 2030[6], including eight caused by helminths. Among these helminthiases are two filariasis - lymphatic filariasis and onchocerciasis - altogether affecting approximately 70 million people[7,8] and schistosomiasis, a disease resulting from infection with trematodes of the genus *Schistosoma*. With more than 200 million people currently infected worldwide, schistosomiasis is the most prevalent parasitic disease after malaria[9].

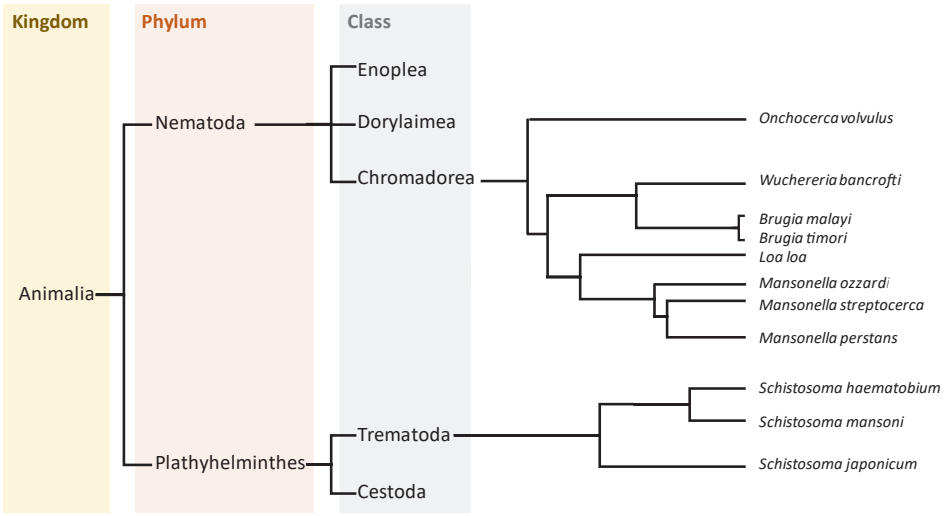


**Figure I-1. Phylogenetic tree of selected helminths.** Schematic representation of phylogenetic relationships between the major species of schistosomes and filarial nematodes infecting humans, which are the focus of the present thesis. Adapted from[10] for filarial nematodes and from[11] for schistosomes. Branch lengths are not drawn to scale.

## 1. a) Schistosomiasis

Several species of schistosomes infect humans, amongst which *Schistosoma mansoni*, *Schistosoma japonicum* and *Schistosoma haematobium* are the most widespread, with the two first species causing intestinal schistosomiasis while the latter is responsible for urogenital schistosomiasis (UGS)[12].

Endemicity of these species is correlated with their different intermediate hosts[13] (**Table I-1** and **Figure I-2A**). *S. japonicum*, transmitted by the aquatic gastropod *Oncomelania*, is restricted to Asia, causing intestinal schistosomiasis in China, the Philippines, and Indonesia[14]. *S. mansoni* infects freshwater *Biomphalaria* snails and is the only species endemic in South America where infections have been reported in Brazil, central America and in the Caribbean[9]. *Biomphalaria* snails are also widely found in Africa and in the Arabian Peninsula[15], where *Bulinus* species, which are host for *S. haematobium* are highly endemic as well. This leads to areas of co-endemicity of *S. haematobium* and *S. mansoni*[16] and to co-infections[17,18]. Altogether, schistosomiasis represents a major health issue on the African continent, where an estimated 90% of the infected people are living[19]. Clean water, sanitation, and hygiene (WASH measures[20]) are crucial contributors to control and elimination of human schistosomiasis. This includes mapping of risk areas via snail surveillance, snail control measures, and behavioral change by limiting contact with infested waters[21,22]. Transmission to humans happens when, in response to sunlight[23], infected freshwater snails release cercariae into their environment (**Figure I-2A**). These aquatic forms of the parasite use a variety of navigational mechanisms and chemotactic signals[24–26] to locate their mammalian host and head towards it. Upon encounter and subsequent skin penetration, cercariae transform into schistosomulae, thereby shedding their tails and undergoing substantial modification of their surface during this process with the replacement of the thick carbohydrate-constituted glycocalyx of the cercariae by a lipidic tegument[27,28]. Schistosomula next enter the bloodstream, where they mature, grow, and migrate through their host's body[29]. Upon maturation, male and female worms form pairs while migrating to their specific final destination. *S. mansoni* and *S. japonicum* reside in the mesenteric veins while the urogenital venules of the bladder plexus are the sites of oviposition of *S. haematobium*. At 5 to 7 weeks after the initial infection, the egg production starts[14] with hundreds (*S. haematobium*, *S. mansoni*) to thousands (*S. japonicum*) of eggs deposited daily by each worm pair.

Pathology originates from the eggs retained in tissues, mainly the liver and intestinal or urogenital tissues, leading to inflammation and granulomatous reactions[13,30]. In the intestines, this results in hyperplasia, ulceration, micro abscess formation and polyposis that can cause abdominal pain, diarrhea, blood in the stool, hepatosplenomegaly, fibrosis, portal hypertension and accumulation of fluid in the peritoneal cavity[9,31]. Eggs lodged in the urogenital system can result in polyps, nodules,

and fibrosis, which can progress into calcification of the bladder wall, causing obstruction, bacteriuria, and bladder cancer[31,32]. When trapped in the reproductive tracts, eggs can also cause Female and Male Genital Schistosomiasis (FGS/MGS), that are frequent complications of *S. haematobium* infections[33]. Between 16 and 56 million women are affected by FGS[34], mainly in sub-Saharan Africa, an inaccurate estimate reflecting the many misdiagnoses and misconceptions of FGS. FGS and MGS cause sexual health and reproductive problems. In women, those are going from gynecological disorders to infertility, ectopic pregnancies, abortion, premature birth and low birthweight[35], while MGS clinical symptoms include a range of erectile and prostatic problems[36]. Furthermore, FGS and MGS have been associated with an increased susceptibility to sexually transmitted diseases including the human immunodeficiency virus (HIV)[34,37].

The current drug of choice for schistosomiasis is praziquantel (PZQ), which is distributed to at-risk populations as part of mass drug administration (MDA) programs[6] aiming to contribute to schistosomiasis control and reduction of morbidity. As of today, this drug appears effective against all major *Schistosoma* species[38], which is an undeniable advantage. However, while schistosomicidal activity of PZQ on adult worms is effective in clearing current infections, treatment does not prevent reinfection. Thus, long term repeated administration of PZQ is required to lower the schistosomiasis prevalence and infection intensity in endemic areas[30,39].

In many cases, symptoms of schistosomiasis are mild and transient, relatively non-specific and shared with other infections[9]. Thus, diagnosis of schistosomiasis can be challenging, although it is crucial to achieve proper drug administration and endemicity mapping[40]. A range of diagnostic tests is available to detect *Schistosoma* infection[41]. Microscopical techniques detecting the presence of eggs in feces or urine specimens - Kato-Katz and urine filtration techniques, respectively - were the earliest diagnostic procedures developed. Despite limitations due to variations in daily excretion and uneven distribution of the eggs, they remain standard recommended methods[42]. Molecular assays based on the detection of *Schistosoma*-specific DNA, schistosome antigens or antibodies against schistosomes have been developed more recently, providing more sensitive alternatives although validation by large-scale studies is still needed for most of them[40,41]. The DNA-detection assays utilize polymerase chain reaction (PCR)-based techniques designed to amplify and quantify nucleic acids and show high levels of specificity[40]. Although they are generally demanding in terms of personnel training and technological means, some field-friendly versions such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) are emerging as promising alternatives[41]. Individuals infected with *Schistosoma* develop a variety of antibodies to different life-stages of the parasite, thus, detection of schistosome-specific antibodies in human plasma/serum is also a valid option[43]. However, the current tests suffer from cross-reactivity with

other helminths and are not informative regarding the intensity or the infection status (past or present) due to persistence of antibodies after parasite clearance[41]. Finally, two circulating antigens excreted by schistosomes into the host's circulation, the Circulating Cathodic Antigen (CCA) and the Circulating Anodic Antigen (CAA) are exploited for diagnostics. These antigens were first characterized several decades ago and shown to consist of highly specific carbohydrate chains[44,45]. They are regularly regurgitated into the bloodstream by living *Schistosoma* worms and their levels decrease rapidly after worm clearance[46,47], making them particularly appropriate diagnostic targets[41]. Assays to detect CAA and CCA have been developed in serum and urine and were tested and applied in endemic settings in many studies[48]. CCA can be detected with a field-friendly lateral flow assay, the Point-Of-Care (POC)-CCA urine test, mainly for *S. mansoni* infections[49]. The Up-Converting reporter Particle technology based, Lateral Flow (UCP-LF) CAA test allows highly sensitive and specific detection of CAA in serum and urine for infections with all major schistosome species in laboratory settings[50]. Efforts to optimize these tools are still ongoing, specifically aiming to resolve specificity issues of the POC-CCA observed in infants and pregnant women[51] and to develop a rapid diagnostic test for detection of CAA in resource-limited settings[52] which would be useful given the underperformance of the POC-CCA lateral flow to detect *S. haematobium* infections[53–59].

## 1. b) Filariasis

Filariasis refers to a group of vector-borne diseases caused by thread-like round worms[60]. Of the filarial nematodes, eight species infect humans causing different types of filariasis that are classified according to the habitat of the adult worms in their host. *Brugia malayi*, *Brugia timori* and *Wuchereria bancrofti*'s adult stages reside in the lymphatics and are responsible for lymphatic filariasis (LF)[61]. Located in subcutaneous tissues and the skin of their host, *Loa loa*, *Mansonella streptocerca* and *Onchocerca volvulus* cause subcutaneous types of filariasis[60]. *Mansonella ozzardi* and *Mansonella perstans* occupy the abdomen serous cavity and thus are the causative agents of serous cavity filariasis[62] . Filarial nematodes are transmitted by various insect vectors that are intermediate hosts for the parasite (**Table I-1**). Infected insects introduce third stage (L3) filarial larvae onto the skin or into the bloodstream of the human host. L3 larvae then migrate through the host body to the organ or tissue of residence of the adult worms. Upon reaching sexual maturity, females start producing circulating larval forms called microfilariae (Mfs) that are transmitted to the intermediate insect host when ingested upon feeding on an infected individual (**Figure I-2B**).

*Brugia* spp., exclusively found in Asia[63], are responsible for approximately 10% of the 50 million LF cases[21] while *W. bancrofti* cause the remaining cases throughout most of Sub-Saharan Africa, Madagascar, several Western Pacific Island nations, and

parts of the Caribbean[64]. Mosquitoes of the genera *Culex*, *Anopheles*, *Mansonia* and *Aedes*[65] can get infected when taking a blood meal and transmit L3 larvae to humans where they develop into adults and settle in the lymphatics[64]. Mfs produced by adult females migrate into lymph and blood vessels and circulate in the bloodstream in a periodical pattern matching the local feeding habits of their mosquito vector[66]. Despite potentially having thousands of circulating Mfs in their peripheral blood, an estimated two-thirds of the infected individuals are clinically asymptomatic or with only mild symptoms including dilated lymphatics and microscopic hematuria and/or proteinuria. However, about one third of the infected people develop severe pathology and endure significant lymphatic compromise and damage. This starts with acute adenolymphangitis, possibly accompanied by thrombophlebitis, and can lead to the chronic and most debilitating sequelae of LF [63,67] that are swelling of the upper or lower extremities resulting in elephantiasis of the leg or, in bancroftian filariasis, in hydroceles/lymphoedema of the genitals[68].

*O. volvulus*, causative agent of onchocerciasis, commonly known as river blindness, is transmitted by blackflies of the genus *Simulium* that have aquatic immature stages. Thus, they lay their eggs in streams or fast-flowing sections of rivers, threatening the access to fertile river valleys for the local populations[66,69,70]. Onchocerciasis affects about 20 million people in 31 countries of sub-Saharan Africa, in Yemen and in foci in six countries of Latin America. Adult worms residing in subcutaneous and deep tissues release thousands of microfilarial larvae that migrate to the skin and the eyes. Dying worms provoke inflammatory reactions contributing to nodule (onchocercomata) formation and to a variety of skin afflictions including intense itching, acute and chronic papular dermatitis, and lichenified onchodermatitis. Ocular lesions caused by Mfs can result in visual impairment and even in complete blindness. This extreme consequence of the disease makes onchocerciasis the second most frequent infectious cause of blindness[69].

Discomfort in the eyes is also a possible outcome of loiasis when adult *L. loa* worms are present under the conjunctiva of the eye, although they do not cause blindness or damage[71]. While many infections are asymptomatic, the deerfly (*Chrysops* flies)-transmitted parasite can cause painful and itchy subcutaneous oedema as well as muscle and joint pain (arthralgia)[72]. Localized inflammation, known as Calabar swellings is a common manifestation of the disease. *L. loa* Mfs spend most of their time in the lungs but also periodically enter the bloodstream, usually around midday. Very high microfilaraemia (Mfs density in the blood) is frequent in loiasis with fertilized females being able to release thousands of microfilariae a day. At least 10 million residents of central and west Africa are thought to have loiasis[73].

With hundreds of millions of people affected, mansonellosis ranks first in prevalence among human filariasis, but is also the least studied and the one with the least distinct pathology[74]. Infected individuals are often asymptomatic or present

aspecific clinical features that can include aches, pain, fever, headache, pruritis, corneal lesions and subcutaneous swellings as well as eosinophilia[75,76]. Biting midges (*Culicoides* spp.) and blackflies (*Simulium* spp.) are the vectors of *Mansonella* spp., the causative agents of mansonellosis. *M. ozzardi* and *M. perstans* are the two most widespread species with the former being highly prevalent in Latin American countries and Caribbean Islands, while the latter is predominantly found in sub-Saharan Africa although it is present in a few areas in South America as well[10].



**Table I-1. Major species of schistosomes and filarial nematodes infecting humans.**

| Phylum | Family | Genus | Species | Transmission vector/intermediary host | Organ of residence of adult worms in definitive (human) host | Causative agent of | | Endemic areas (geographic distribution) |
|---|---|---|---|---|---|---|---|---|
| Platyhelminthes | Schistosomatidae | *Schistosoma* | *Schistosoma haematobium* | *Bulinus* spp. snails | Pelvic venous plexus | Schistosomiasis | Urogenital | Africa and Middle East |
| | | | *Schistosoma japonicum* | *Oncomelania* spp. snails | Mesenteric veins of the bowel | | Intestinal | Asia (East and Southeast), Western Pacific |
| | | | *Schistosoma mansoni* | *Biomphalaria* spp. snails | | | | Africa, Middle East, and America (Central and South) |
| Nematoda | Onchocercidae | *Brugia* | *Brugia malayi* | *Mansonia* and *Aedes* spp. mosquitoes | Lymphatics | Lymphatic filariasis | | Asia (Southeast and East) |
| | | | *Brugia timori* | | Lymphatics | | | Indonesia |
| | | *Wuchereria* | *Wuchereria bancrofti* | *Anopheles, Coquillettidia, Culex* and *Mansonia* spp. mosquitoes | Lymphatics | | | Asia, Western Pacific, Africa, and America (Central and South) |
| | | *Mansonella* | *Mansonella perstans* | *Culicoides* spp. midges | Pleural or peritoneal cavity of the abdomen | Mansonellosis | Serous cavity filariasis | Africa and America (Central and South) |
| | | | *Mansonella ozzardi* | *Culicoides* spp. midges, *Simulium* spp. blackflies | Serous cavity of the abdomen | | | America (Central and South) |
| | | | *Mansonella streptocerca* | *Culicoides* spp. midges | Subcutaneous tissues (dermis) | | Subcutaneous filariasis | Africa (West and Central) |
| | | *Onchocerca* | *Onchocerca volvulus* | *Simulium* spp. blackflies | Subcutaneous tissues and skin nodules | Onchocerciasis | | Africa, Middle East and South America |
| | | *Loa* | *Loa loa* | *Chrysops* spp. deerflies | Subcutaneous tissues and lymphatics | Loiasis | | Africa (West and Central) |

Strategic interventions currently recommended by the WHO include preventive chemotherapy both for LF and onchocerciasis. Specifically, MDA of ivermectin is used for the latter, and various regimens of albendazole, diethylcarbamazine and ivermectin are administered for LF following the WHO guidelines[65]. However, risks of neurological serious adverse events are associated with administration of ivermectin in individuals with high *L. loa* parasitemia[77,78]. Thus, co-infections with two – or more – filarial parasites, a common occurrence in Africa due the concomitant presence of transmission vectors[79–85] (**Table I-1**), can be particularly problematic.

As of today, diagnosis of active infections with these filarial nematodes is still mainly achieved through microscopical detection of microfilariae in blood or skin samples[86–89]. For *L. loa*, the adult worm can also potentially be recovered from under the skin or the eye and then identified by a microbiologist or pathologist. These diagnostic techniques require well-trained clinicians and blood/skin biopsy sampling in accordance with the microfilariae periodicity, making them somewhat cumbersome and of low sensitivity. Due to the importance of robust diagnostics for achieving successful MDA programs, efforts to develop alternatives to microscopy have been made for LF and onchocerciasis. Serological techniques currently available include the Brugia Rapid test that measures serum immunoglobulin (Ig) G4 against *Brugia* spp. (Reszon Diagnostics International), the POC diagnostic filariasis test strip detecting *W. bancrofti* Circulating Filarial Antigen (CFA) in human blood (Abbott)[90] and the Bioline™ Lymphatic Filariasis IgG4 test detecting IgG4 antibodies generated in response to the *W. bancrofti* Wb123 antigen (Abbott)[91]. Similarly, both an ELISA and a POC rapid diagnostic test detecting IgG4 against *O. volvulus* antigen Ov16 (Bioline™, Abbott) are available[92] and a dual version allowing simultaneous detection of IgG4 against Ov16 and Wb123 antigens has been developed recently[93]. Certain limitations in the specificity and sensitivity of these tests have however been pointed out, specifically cross-reactivity of the filariasis test strip in areas of co-endemicity with *L. loa* and the difficulty to distinguish past from current infections due to the presence of sustained antibodies after parasite clearance with the Brugia Rapid test[94,95]. LAMP assays may offer convenient, field-applicable alternatives for the detection of *O. volvulus*, *L. loa*, *M. ozzardi* and *M. perstans*[96,97] but their deployment and validation are still awaited.

## 1. c) Current challenges associated with human schistosomiasis and filariasis

MDA is the strategy currently adopted by the WHO to control, prevent and treat schistosomiasis, onchocerciasis and lymphatic filariasis. Over the years, MDA programs have proven efficient in reducing the number of cases in many instances. Notably, thanks to the efforts driven by the WHO first road map, LF has been eliminated

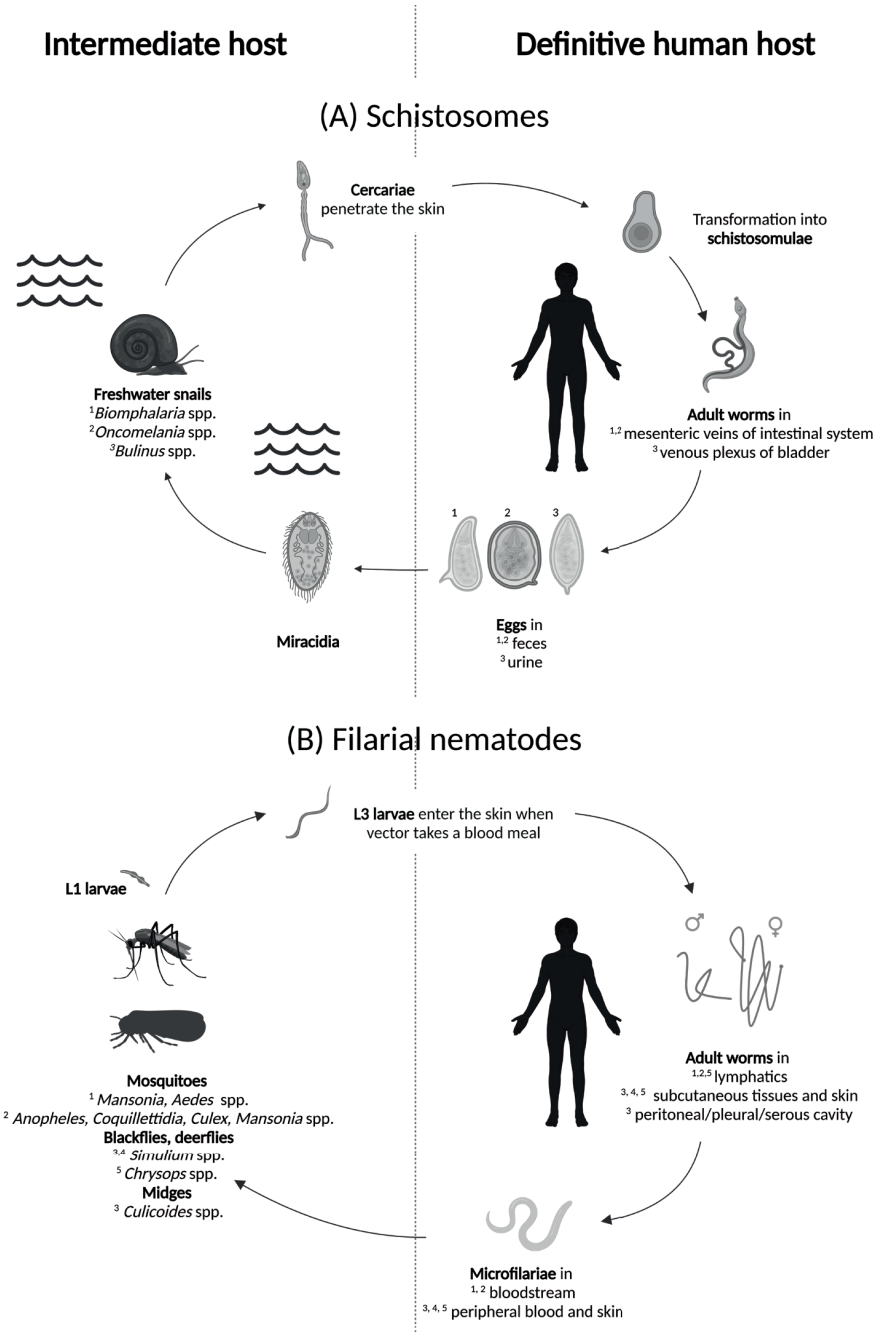


**Figure I-2. Life cycles. Overview for (A) *Schistosoma* spp.** with specifications for *S. mansoni* (1), *S. japonicum* (2) and *S. haematobium* (3) **and for (B) the human parasitic filarial nematodes** with specifications for *Brugia* spp. (1), *W. bancrofti* (2), *Mansonella* spp. (3), *O. volvulus* (4) and *L. loa* (5). See also **Table 1**. Figure created with BioRender.com adapted from information available from the Centers for Disease Control and Prevention (cdc.gov).

as a public health problem in 17 countries and onchocerciasis has been eliminated in 4 countries in the Americas[65]. Nonetheless, many of the targets set for 2020 in this earlier road map were not met and the new directive of the WHO for 2030 preconizes a variety of critical actions to be taken. These range from better scientific understanding to diagnostic needs and strengthening of MDA programs, in order to reach the current reduction and elimination targets for schistosomiasis as well as filariasis.

Current MDA strategies rely on a limited drug repertoire and, thus, are threatened by the risk of emerging resistance. Resistance against ivermectin, one of the drugs of choice for LF and onchocerciasis treatment, have been detected in *O. volvulus* in human infections[98] and has long been known in the veterinary context[99]. Fortunately, widespread resistance to PZQ, the main drug for schistosomiasis, has not been observed in nature so far, although reduced susceptibility has been identified in the lab and in the field[38]. Proof of actual resistance remain controversial, nonetheless suboptimal action of the drug against the immature stages of the parasite is a fact which further enforces the need for repeated administrations in endemic areas of frequent exposure[100]. Thus, identification of novel drug targets or prophylactic therapies are highly sought after[39]. Vaccines would be a game-changer in the fight against these NTDs, and promising research is ongoing, although at different stages of progress. There are ongoing efforts as part of the Onchocerciasis Vaccine for Africa Initiative (TOVA) to initiate phase one trials in African children[101–103], while vaccine candidates for brugian and bancroftian LF have been identified and tested in preclinical studies[104,105]. Several candidate antigens have been identified also in schistosomes, and vaccines using four of them – the *S. haematobium* 28-kD glutathione S-transferase (Sh28GST), the *S. mansoni* 14-kDa fatty acid-binding protein (Sm14), the *S. mansoni* tetracaine (Sm-TSP-2) and the *S. mansoni* calpain (Sm-p80) – have made it beyond preclinical phases stages and are in different phases of clinical development[106]. Particularly promising results were obtained with Sm-p80 vaccine in preclinical trials conducted in baboons where an Sm-p80-mediated preferential killing of adult female worms was observed[107]. Sh28GST-, Sm14- and Sm-TSP-2-based vaccines, on their hand, have all shown safety and immunogenicity in the different human populations on which initial phases of clinical trials have been conducted[108–110]. In addition, the recent establishment of a controlled human challenge infection model offers possibilities for testing schistosomiasis vaccines that were unimaginable previously[111,112].

Despite these developments, there is still a long road ahead of getting efficient and approved vaccines available. Meanwhile, the use of current therapeutic drugs must be optimized and strengthened[65]. Efficient mass distribution of anthelminthic treatments highly relies on joint diagnostic strategies. Although the validated diagnostic methods listed above have proven invaluable tools in control programs so far, their sensitivity and specificity are generally deemed insufficient for accurate mapping, informing decisions to stop community treatment and post-treatment

surveillance. According to the WHO, this is the case for schistosomiasis, LF and onchocerciasis. Concrete consequences of diagnostic insufficiency are for instance the need to extend LF control programs by several years, resulting in excessive use of medicines, or the absence of treatment of (millions of) people in hypo-endemic areas because of fear of risk of severe adverse events due to the absence of diagnostic for *L. loa*. Thus, lots of effort must be undertaken to provide the field with better diagnostic options. Not only will the sensitivity of these tools have to increase, but due to the huge geographical overlaps of NTDs (**Table I-1**), it will be crucial to improve their specificity and to develop multiplex platforms. The importance of considering the situation in its entirety when conceiving diagnostics, including the monitoring and evaluation tools available, has been emphasized lately[65,113].



## 1. d) Molecular landscape of host-parasite interactions and biological importance of glycans

From their development in invertebrate organisms to their establishment in mammals, schistosomes and filarial nematodes are known for surviving extensive periods of time in vastly different hosts. The search for novel diagnostics and therapies requires a strong knowledge of parasite biology and underlying molecular mechanisms of host-parasite interactions in schistosomiasis and filariasis.

### *Immune profiles of schistosomiasis and filariasis in humans*

Long-term investigative efforts have shed some light on various immune evasion strategies parasitic helminths have evolved to survive. Although each parasitic infection is different, decades of research have revealed that these parasites share the ability to downregulate their host immune responses[114]. In the mammalian hosts, a well-known feature is the skewing of the initial inflammatory immune response towards a T helper (Th) 2 or type-2 immunity accompanied by other strategies that ultimately allow parasite immune evasion[115].

In both schistosomiasis[116] and filariasis[117], the initial infection phase triggers type-1 inflammation which is defined by the activity of Th1 cells creating an environment rich in pro-inflammatory cytokines such as the tumor necrosis factor-α (TNF-α) and interferon gamma (IFN-γ). This attempt of the host to defend against the pathogens results in acute disease and undesirable tissue damage[118,119]. To avoid this outcome, a type-2 immunity, also involved in tissue repair[120], develops gradually. Studies of gerbils infected with *B. pahangi* have shown that the polarization of the host response towards a dominant Th2-like profile happens as early as seven days after the first encounter with infective L3 larvae[121]. Characteristic type-2 associated cytokines including interleukin (IL)-4, IL-5, IL-9, IL-10 and IL-13[114] are then produced by innate and adaptive Th2 cells. The type-2 environment is completely established, and peaks, when

egg laying and Mf release occurs in the case of schistosomiasis and filariasis, respectively (**Figure I-3**). This vigorous type-2 reaction is next modulated by regulatory T (Treg) and B cells as well as other (unidentified) immune cells[115]. Altogether, a new regulatory environment emerges, that is typified by increased levels of the regulatory cytokines IL-10 and transforming growth factor-β (TGF- β)[122,123], B cell class-switching to (antigen-specific) IgG1, IgG4 and IgE[124–126] and reduced antigen responsiveness[127–129] due to a muted/anergic lymphoproliferative response and dysfunctional antigen-presenting cells[127,130,131]. These features distinguish the "modified Th2 responses" triggered by helminths from the canonical Th2 responses typically observed in allergies, by missing the inflammatory components. For that reason, the immunosuppressive capacities of helminths have raised interest in view of their translational potential to tackle allergic, inflammatory, autoimmune, or metabolic disorders in humans[114]. However, this immune hyporesponsiveness can also be detrimental to the host, with suboptimal responses to vaccines observed in individuals infected with helminths[132] and increased susceptibility to other pathogenic infectious agents[114].

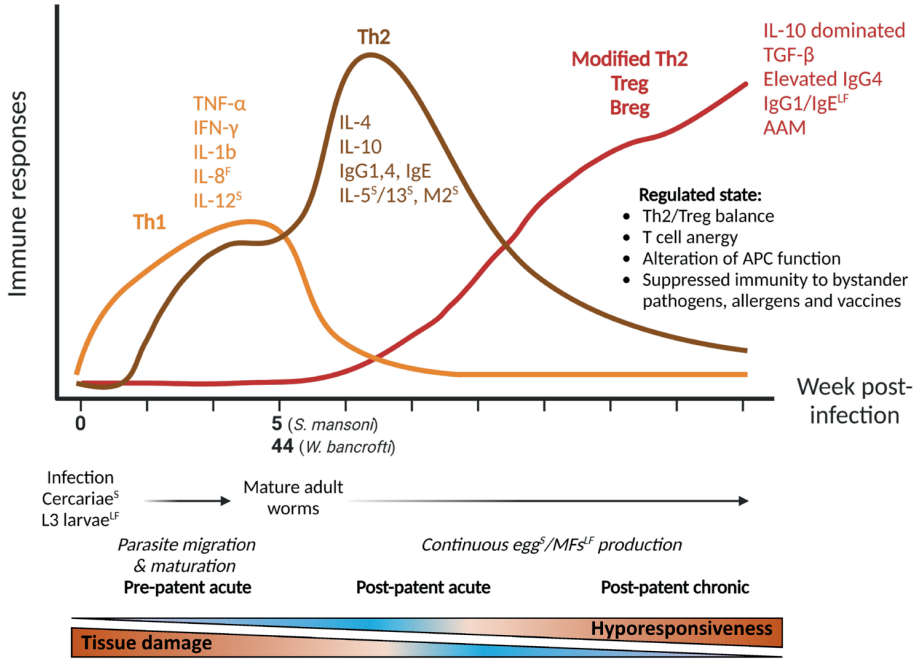


**Figure I-3. Prototypical immune responses in schistosomiasis(S) and filariasis(F).** Graph representing the immunological profiles resulting from infection with schistosomes or filarial nematodes. An initial proinflammatory T helper **(Th)1 response** is followed by a **Th2 response** which builds up upon exposure to cercariae and L3 larvae. This Th2 response is next regulated by specific populations of T and B cells driven by

interleukin (IL)-10, resulting in the expansion of regulatory T/B cell (T/Breg) population, themselves producing IL-10- and/or transforming growth factor (TGF)-β. Together with alternatively activated macrophages (AAMs) they participate in creating and maintaining the IL-10 dominated **Th2 modified** environment. Characteristic features – cytokine and immunoglobulin (Ig) production – are specified (TNF-α = tumor necrosis factor-α, IFN-γ = interferon-γ, M2 = M2 phenotype switch in macrophages, APCs = antigen-presenting cells). Figure created with BioRender.com adapted from[116] with details obtained from[115,122,123,126,133,134]

This regulated "Th2 modified" state is the hallmark of asymptomatic, chronic infections and constitutes the best compromise for host and parasite to coexist[134]. However, different disease outcomes correspond to different immune profiles. IL-17-producing CD4+ cells (or Th17 cells) are usually associated with responses to infection with extracellular bacteria and fungi[135]. In schistosomiasis, a higher percentage of Th17 cells has been observed in individuals with the most severe pathological reactions compared to those exhibiting lesser morbidity[136,137]. Similarly, it has been shown that elevated frequencies of Th17 and Th2 cells are instigating the development of severe hyperreactive onchocerciasis, also known as Sowda[138]. In LF, lymphedema patients have higher levels of the pro-inflammatory Th1 and Th17 antigen-associated cytokines

**Table I-2. Major immunological characteristics of the different subpopulations of individuals in LF-endemic areas.**

| | Patent asymptomatic infection (AS) | Chronic pathology (CP) | Endemic normal (EN) | Ref. |
|---|---|---|---|---|
| **Overt Clinical Symptoms** | No | Yes | No | 63 |
| **Patency** | MFs+ | MFs-* | MFs- | 63 |
| **T cell proliferation** | Low | High | High | 143 |
| **IFN-γ** | Low | High | High | 143,145 |
| **IL-5** | Low | High | - | 145 |
| **IL-10** | High | Low | Low | 123,143 |
| **IgG1-3** | Low | High | High | 142,143 |
| **IgG4** | High | High | Low | 142,143 |
| **IgG4/IgE** | High | Low | Low | 142,143 |
| **Inflammatory cytokines IL-1b, IL-12 and TNF-α** | Low | High | Low | 146 |
| **Th9 cells** | Low | High | Low | 147 |

***Most of the time**
**MFs+ = microfilariaemic, MFs- = amicrofilariaemic**
**IFN-γ = Interferon gamma, IL = interleukin, TNF-α = Tumor Necrosis Factor alpha, Th = T helper**

compared to asymptomatic individuals[139]. As mentioned above, LF can result in strikingly different outcomes. Most infected individuals are microfilariaemic carriers often free of disease manifestations (asymptomatic, AS) while a subgroup of individuals exhibits overt pathology in the form of lymphedema, hydrocele and/or elephantiasis and are often amicrofilariaemic (chronic pathology, CP). Studies of antibody responses to the parasite crude antigens have shown a general skewing of the humoral immune response toward IgE and IgG4 in filariasis[140]. When comparing the different subpopulations, AS microfilariaemic individuals show higher levels of IgG4 and those are associated with their hyporesponsiveness[141], while elevated levels of IgG1-3 and IgE correlate with CP[142]. Thus, elimination of circulating parasites is associated with higher IgE/IgG4 ratios and expansion of the other IgG subclasses (**Table I-2**). Interestingly, in endemic areas, a small percentage of the population remains free of demonstrable filarial infection. These putatively immune individuals, termed endemic normals (EN) suggest the existence of a protective immunity to be a reality[143], although the basis of such immunity is still unclear[144].

### *Molecular mechanisms of immune evasion*

Research work aiming to decipher the molecular mechanisms used by helminths to evade targeted immune responses from their host has been conducted over the years. Both schistosomes and filarial nematodes are known to utilize immune-resistant surface layers. A variety of ectoenzymes including alkaline phosphatase, phosphodiesterase and ATP diphosphohydrolase are expressed in the tegument of juveniles and adult schistosomes. These enzymes inhibit blood coagulation and are thought to prevent proinflammatory responses by interfering with signaling molecules and cleaving extracellular ATP, mitigating its proinflammatory tendencies[133,148]. The rapid shedding of the immunogenic cercarial membrane after skin penetration in mammals and formation of the heptalaminate surface membrane[27,28] is thus a crucial step of immune evasion. Next, schistosomulae maintain a continuous tegument turnover, which explains the inefficient immune response to this otherwise vulnerable life-stage. Furthermore, schistosomulae[149], as well as adult schistosomes[150], have been shown to coat themselves with host antigens that they incorporate onto their surface in a well-regulated process that is not a side-effect of the glycocalyx "stickiness"[151]. These strategies of molecular mimicry are important stratagems of helminths and are not limited to "stealing" host antigens. Carbohydrate molecules surface-expressed by the parasite are known to be crucial in those processes as suggested early on by the observation of shared carbohydrate epitopes at the surface of *S. mansoni* larvae with their *B. glabrata* snail host[152].

It has long been known that helminth-derived excretory/secretory (ES) products encompass immunomodulators[153,154]. ES products of various nature have been shown to display major immunomodulatory properties, notably by interacting with pattern

recognition receptors (PRRs) of a variety of immune cells such as epithelial/innate lymphoid cells, macrophages, eosinophils, basophils and dendritic cells (DCs)[114,123,148]. The latter are potent antigen-presenting cells that are pivotal in modulating the host response during helminth infections[134]. In schistosomiasis, it has been shown that signaling through toll-like receptors (TLRs), a well-studied class of PRRs, reduces the ability of DCs to produce IL-12 and promotes a polarization toward a Th2 immune response instead of the Th1 type. ES products implicated in the TLR2 and TLR4-priming of DCs include, respectively, phosphatidylserine lipid antigens[155] and the glycoconjugate lacto-N-fucopentose III (LNFPIII)[156]. Another well-studied case of Th2 induction via TLR4 is the one triggered by ES-62, a phosphorylcholine-containing glycoprotein of the nematode *Acanthocheilonema viteae*, a rodent parasite[157,158]. The mechanism of action of ES-62 has been minutely characterized in *A. viteae* and interaction of this glycoprotein with a variety of cells including B and T lymphocytes, macrophages and mast cells in addition to DCs have been highlighted. Notably, ES-62 inhibits the proliferation of $CD4^+$ T cells and conventional B cells and modulates the complement activation[159] to ultimately suppress Th1 proinflammatory responses[157,160]. The active component of these immunomodulatory properties has been shown to be the glycan moiety of ES-62[161]. Whether the ES-62 homologues expressed by the filarial parasites of humans share the same properties as *A. viteae* ES-62 must still be confirmed[118]. In schistosomiasis, eggs are central to the establishment of the polarized Th2 responses. Studies of the soluble fraction of schistosome eggs, termed Soluble Egg Antigen (SEA) have shown that egg products encompass potent Th2-drivers[148]. Those include the major bioactive glycoproteins "IL-4 inducing principle of schistosome eggs"/alpha-1 (IPSE/α-1) and the hepatotoxic egg Omega-1[148]. It has been shown that the glycan moieties of IPSE/α-1 and Omega-1 are essential for their uptake by DCs[162,163]. For Omega-1, this internalization is mediated by the mannose receptor (MR), a C-type lectin receptor (CLR) of DCs, and is key to its effector functions together with its ribonuclease activity[162]. Omega-1 is the primary egg component involved in Th2 skewing since the effect of this protein alone compares to the one observed from the whole *Schistosoma* soluble egg mixture[164]. IPSE/α-1, on its hand, binds immunoglobulins with highest affinity for IgE, and its interactions with basophils result in an increased production of IL-4 and IL-13, which is thought to stimulate an anti-inflammatory phenotype in macrophages[165]. Schistosome soluble egg products are also key players in immunopathology. In the early stage of hepatic granuloma formation, neutrophils recruited by specific egg proteins are deemed responsible for significant tissue damage[166], and SEA triggers the formation of neutrophils extracellular traps, some "web-like" structures that are suspected to cause indirect inflammation and local damage[167]. In the longer term, it has been observed that eosinophils take over the neutrophil population in *S. mansoni* infections, by infiltrating the granuloma in a Th2-driven process - mediated by IL-5 and IL-13[168]. Although granulomas are largely

detrimental to the host, they also constitute a barrier between the toxic egg secretions and the hepatocytes, thus acting as a host-protective function[133,169]. In addition to soluble proteins and lipids, EVs released by parasitic helminths have been recently highlighted as a means for the parasite to transmit immunoregulatory signals to host cells[114,170–172]. EVs are phospholipid bilayer membrane-enclosed vesicles capable of transferring a complex mixture of proteins, lipids, and genetic materials. The surface of EVs derived from *S. mansoni* schistosomulae and adult worms have been shown to be glycosylated and to interact with CLRs[173,174]. Although this field is still in its infancy, these studies have shown that EVs and their cargo encompass immunomodulators holding potential as diagnostic or drug/vaccine targets[175–180].

### *Latest advances and remaining areas of investigation: what about helminth glycans?*

The many immunological and molecular studies non exhaustively referred to above have considerably increased our general understanding of strategies employed by the parasite to evade its host immune system. Importantly, this work has shown a role for molecular key-players of diverse nature including lipids, soluble proteins, carbohydrates and EVs in these immunomodulatory mechanisms. Identifying the molecular targets that are unique to the parasite and/or essential for its survival is currently the major challenge in the search for (prophylactic) therapies.

This research has benefited from recent technological advances including progress in the fields of genomics, transcriptomics, and proteomics. With the development of high-throughput sequencing technologies, the genomes of over 180 different helminth species are currently available in the WormBase ParaSite repository[181], including those of *S. haematobium*, *S. japonicum*, *S. mansoni*, *B. malayi*, *B. timori*, *W. bancrofti*, *O. volvulus* and *L. loa*, although the quality of these genome assemblies and resources vary significantly in completeness and contiguity[182]. While efforts to compare genetic and transcriptomic variations, from single cells to populations are ongoing[183–187], the functional annotation of these genomes, still incomplete and inaccurate, remains a major challenge[182]. In parallel, in-depth proteomic studies have been performed for both schistosomes[188–192] and filarial nematodes[193–199], shedding light on specific life-stages, sexes, tissues and on a variety of ES products including EVs. Proteomic data are indeed available for the EVs of *S. japonicum*[200] and *S. haematobium*[177] adult worms, and for EVs released by several life-stages of *S. mansoni*[175,201,202] and *B. malayi*[203,204]. In addition, small RNA molecules, present in the cargo of *S. japonicum, S. mansoni* and *B. malayi* EVs, have also been examined[200–202,204,205].

Despite the significant advancements in genomics, transcriptomics, and proteomics, other areas, particularly the characterization of carbohydrate molecules,

have lagged and need more attention. (Chains of) carbohydrates, also called glycans, are indeed among the molecules of major importance at the host-parasite interface. One explanation for the lack of glycomic studies resides in the nature of glycans. These dynamic, non-template-based molecules are structurally highly diverse[206], reflecting their functional versatility. Glycans are indeed widely distributed in nature and take part in various biological processes. They are particularly prominent in complex multicellular organs and organisms, which require interactions between cells and the surrounding matrix[207]. Helminths are no exception and synthesize a large array of glycans, including glycans as post-translational modifications of proteins and as glycolipids (non-exhaustive illustration in **Figure I-4A**), as well as free oligosaccharides and polysaccharides[208]. Thus, while it is known that helminth glycans can be highly antigenic, are involved in immunomodulatory processes and are essential to the parasite survival in its host[208–210], our knowledge of these molecules in the context of helminthiases is still incomplete.

# 2. Helminth glycans

## 2. a) Structural specificities of helminth glycans

Although challenging, structural characterization of glycans has been made possible by the emergence of advanced technologies and analytical tools notably the production of dedicated reagents such as broad glycosidases allowing the release of the glycans from their carrier[211]. Specifically, the use of peptide-*N*-glycosidases permits the study of the major class of protein-linked glycans that are the asparagine (*N*)-linked glycans[212] by cleaving the bond between the saccharide chain and the asparagine residue[213,214]. Similarly, endoglycoceramidases allow the enzymatic release of the saccharide chains of glycosphingolipids from their lipidic portions[215], allowing the study of this subclass of glycolipids which is the main type of glycolipid found in animals[216]. Such an enzyme however does not exist for the *O*-linked glycans, a type of modification highly abundant on vertebrate mucin glycoproteins, where the glycan chain is attached to a serine or threonine residue[217]. In that case, chemical methods have been optimized to achieve glycan release from this type of glycoconjugate[218]. Upon release, glycans can be analyzed using mass spectrometry (MS), a technique that permits direct ionization of nonvolatile substances[219]. The development of matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) MS have constituted real breakthroughs in the field of glycomics[220]. Complementary to MS, chromatographic and electrophoretic methods such as ultraperformance liquid chromatography (UPLC), porous graphitized carbon (PGC) chromatography or capillary electrophoresis (CE) can be employed for glycan separation, enrichment and add a quantitative dimension to glycomic workflows[221,222].

The current literature suggests a large diversity within helminth glycans[208], although parasitic worms have a largely conserved glycosylation machinery[223] similar to that of other multicellular organisms[228]. Unlike bacteria[229], helminths use a relatively limited number of monosaccharides for glycan synthesis. Hexoses (Hex) incorporated in helminth glycans include galactose (Gal), glucose (Glc), mannose (Man) and N-acetylgalactosamine (GalNAc) and N-acetylglucosamine (GlcNAc) are the type of N-acetylhexosamine (HexNAc) residues that have been described so far. Helminth glycans also frequently contain fucose (Fuc) residues, the most common type of deoxyhexose sugar[208]. Other monosaccharides and substituents have been reported in more specific instances such as glucuronic acid (GlcA) as part of the schistosome CAA[44] and of the filarial nematode *Dirofilaria immitis N*-glycans[230], the highly specific tyvelose residue of *Trichinella spiralis*[231] or the phosphorylcholine (PC) substituent found in nematodes[158,232]. Absence of sialylation, however, constitutes a common feature of helminths and a clear difference with mammalian glycans that are rich in sialic acids[233]. Although expanding, glycomic knowledge of parasitic helminths remains patchy and highly uneven, varying significantly across different species and glycan classes.

### *Schistosome glycans*

Decades of research have contributed to substantial knowledge of *S. mansoni* glycosylation, making this parasite's glycome the best characterized of all helminths previously mentioned. Besides the lack of sialic acid, major differences with mammalian glycans have been observed. Specific modifications of the *N*-glycan core include xylosylation[234], *i. e.* the attachment of a β1-2-linked xylose (Xyl) to the initial core-mannose, and presence of α1-3 linked core Fuc in some life stages[235,236] in addition to the α1-6 core-Fuc that is also observed in mammals. Moreover, terminal GalNAcβ1-4GlcNAc or LacDiNAc (LDN) motifs are frequently found instead of the terminal Galβ1-4GlcNAc or LacNAc (LN) motifs abundant in mammals[237]. Fucosylated LDN, rarely observed in mammals[238], is highly expressed in schistosomes and form antigenic motifs containing α1-3 linked Fuc residues of composition (Fucα1-3)GalNAcβ1-4GlcNAc, GalNAcβ1-4(Fucα1-3)GlcNAc or (Fucα1-3)GalNAcβ1-4(Fucα1-3)GlcNAc (F-LDN, LDN-F or F-LDN-F, respectively)[208,239,240]. Moreover, with the synthesis of α1-2 linkages between Fuc residues, yielding difucosylated (DF) motifs of composition Fucα1-2Fuc-R and even trifucosylated (TF) motifs of composition Fucα1-2Fucα1-2Fuc-R, schistosomes exhibit very specific fucosylation patterns that have not been reported in other species so far[208]. HexNAc substitution with such DF/TF motifs results in multifucosylated structures that are particularly ubiquitous in the glycosphingolipid (GSL) glycans of *S. mansoni* eggs[225]. More common glycan epitopes are also expressed by *S. mansoni* including the Lewis X (LeX) antigen of composition Galβ1-4(Fuc α1-3)GlcNAc) which is also present in mammals[234]. Although not major

components of *S. mansoni* glycans, non-fucosylated LN terminated glycans are observed, in particular in the human life-stages of the parasite[225]. Stage- and sex-specificities[240] are striking features of glycan expression in *S. mansoni*. In-depth study of the *N*-linked, *O*-linked and GSL glycans expressed throughout the parasite life cycle has provided a unique and comprehensive coverage of this helminth glycome[225]. While a variety of elaborated, complex *O*-glycans are expressed in the cercariae and eggs, exhibiting some of the aforementioned terminal motifs, such structures have not been detected in adult stages[225,240], with the exception of the gut-associated antigens CCA and CAA. The latter are proteoglycan-like structures, carrying *O*-linked glycans comprised of LeX repeats (multimers of −3Galβ1-4(Fucα1-3)GlcNAc1−) for the CCA molecule[45] and repeats of GalNAcs substituted with GlcA forming −6(GlcAβ1-3)GalNAc1− repeats for CAA[44].



Moreover, in view of the strong immunomodulatory properties exhibited by ES products of *Schistosoma* eggs discussed above, the major glycoproteins of *S. mansoni* SEA have been characterized in targeted glycoproteomic studies. IPSE/α-1 and Omega-1 were both shown to carry LeX-terminated *N*-glycans with difucosylated cores[241,242], while Kappa-5, the third major glycoprotein of SEA that has been characterized, is *N*-glycosylated with LDN-terminated triantennary structures composed of a difucosylated and xylosylated core region[243].

**Figure I-4B** provides a visual overview of the different glycan motifs reported in *S. mansoni*. Given the absence of comprehensive glycomic studies performed for other *Schistosoma* species, significantly less is known regarding the glycans they express. Some early studies have described LeX-containing glycans in *S. haematobium* and *S. japonicum* adult worms as well[244] while others have focused on the glycans from *S. japonicum* and *S. mansoni* eggs[236,245] with mannosylated, truncated, and complex *N*-glycans observed in both species[236]. Importantly, the presence of xylosylation and structures carrying both α1-6 and α1-3 core-Fuc have also been reported as core-modifications of *S. japonicum N*-glycans. Additionally, *N*-linked and *O*-linked glycans carrying terminal LeX, LN and (fucosylated) LDN motifs were detected in the eggs of *S. japonicum*. GSL glycans of *S. japonicum* consisting of a Hex linked to the ceramide portion and extended by a chain of Hex and HexNAc residues, were found to be overall similar to those of *S. mansoni*[245]. However, none of the difucosylated terminal motifs found in *S. mansoni* were detected in *S. japonicum* eggs in those studies. This suggests that inter-species variation in glycan expression might occur, at least quantitatively, although this needs further confirmation given that the GSL glycans of *S. japonicum* have not been studied with the same depth as those of *S. mansoni* and that knowledge of *S. haematobium* glycans is even scarcer.

Differences might however be expected in view of the striking biological specificities of the different *Schistosoma* species in terms of their intermediate hosts, tissue migration patterns[246], organ of residency and even disease outcome[111].

**Figure I-4. Schistosome and filarial nematode glycan modifications (caption next page).**

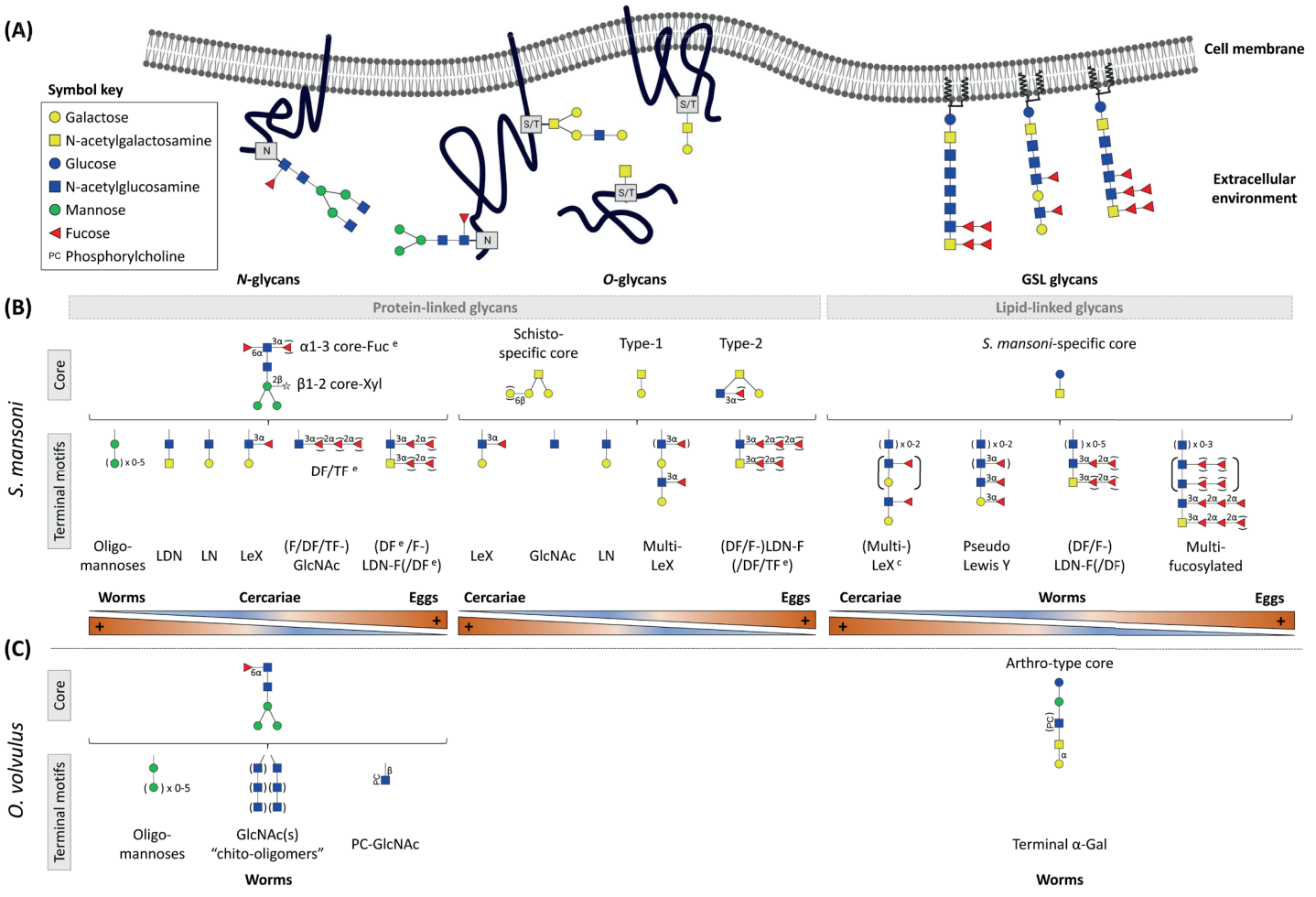

**Figure I-4. Schistosome and filarial nematode glycan modifications.** Graphic representation of *N*-glycan, *O*-glycan and GSL glycan modifications **in the cellular environment (A)** and overview of the major glycan motifs previously reported in **schistosomes (B)** and **filarial nematodes (C)**. Panel (A) was adapted from[224] and panels (B) and (C) were constructed based on previous glycomic studies of *S. mansoni*[225] and *O. volvulus*[208,226,227]. Stage-specific glycan features of *S. mansoni* are indicated with [c, e] when the motif is exclusively found in e = eggs and/or c = cercariae. Note that the CAA and CCA reported in adult worms are not represented in this figure. Figure created with BioRender.com. Glycans are represented using the Consortium for Functional Glycomics (CFG) nomenclature as detailed in the symbol key inset.



### *Filarial nematodes glycans*

Glycosylation of the non-parasitic model nematode *Caenorhabditis elegans* has been extensively studied over time[223,247]. Based on this work and extended genomic studies listed in the WormBase ParaSite database[248], nematodes are predicted to express many enzymes for glycoconjugate biosynthesis orthologous to those found in higher animals. However, in-depth studies focusing on parasitic filarial nematode glycans are rather scarce. In particular, virtually nothing is known regarding glycosylation of parasitic filarial nematodes of humans, with the exception of some partial data on the *N*-linked[227] and GSL glycans[226] of *O. volvulus* that are summarized in **Figure I-4C**. Slightly more substantial knowledge of the *N*-glycans of parasitic nematodes of animals[223] is available, including data on the filarial nematode *A. viteae*[227] and the dog heartworm *D. immitis*[230]. These studies have highlighted the generally abundant presence of glycoconjugates substituted with PC throughout the phylum[158,232]. PC has been reported as a substituent on HexNAc residues[208], mostly GlcNAc, but was also found attached to GalNAc in *D. immitis N*-glycans[230]. GlcNAc appears as a common terminal residue in filarial nematode *N*-glycans[223]. Some *N*-glycans named “chito-oligomers” carrying antennae extended by stretches of 1 to 5 HexNAc residues, most likely GlcNAc, have been found in *A. viteae*, *D. immitis, O. gibsoni* and *O. volvulus* [227] (**Figure I-4C**). In *D. immitis*, GalNAc was also found in HexNAc-terminated structures, forming LDN-containing antennae[230] that can be PC-substituted. GSL glycans are known to be built on the arthrotype core, which consists of GlcNAcβ1-3Manβ1-4Glcβ1 linked to the ceramide (cer) portion of the lipid molecule[223]. In the porcine parasite *Ascaris suum*, this core has been found to be substituted with PC and phosphoethanolamine (PE) and to be further extended with β1-4-linked GalNAc and terminal α-linked Gal, forming the structures of composition Galα1-3GalNAcβ1-4(PC-6)GlcNAcβ1-3(PE-6)Manβ1-4Glc1-1cer and Galα1-3GalNAcβ1-4(PC-6)GlcNAcβ1-3Manβ1-4Glc1-1cer. The latter structure has also been detected as a major species in *O. volvulus* GSL glycans (**Figure I-4C**), suggesting this zwitterionic glycan to be conserved throughout the phylum[226]. Before extensive glycomic studies were performed, immunomodulatory properties of filarial nematode glycans[249], and more specifically of PC-substituted glycans, were known for a long time

already[227]. These have been particularly studied in the context of the ES-62 of *A. viteae*, which *N*-glycosylation has been fully characterized using targeted glycoproteomics[250].

## 2. b) Glycans in host-parasite interactions

As cell surface molecules are able to mediate intercellular communication in diverse biological processes and to act as "surface markers" for cell identification[251], it is not surprising that glycans play major parts in host-parasite interactions. As covered above, there is much evidence of implications of glycans in the various strategies employed by the parasite to modulate and evade their host immune system[208].

### *Glycan mimicry and gimmickry*

Due to the expression of host-like features, such as the aforementioned LeX motif in *S. mansoni*, it has long been hypothesized that helminths utilize glycans for camouflage purposes. The shared glycosylation patterns between *S. mansoni* and its *B. glabrata* snail host mentioned earlier is probably the best evidence of the importance of glycans for molecular mimicry so far. Indeed, the hemolymph glycoproteins of a *B. glabrata* strain that is highly susceptible to *S. mansoni* has been found to exhibit many glycans with β1-2 Xyl and terminal F-LDN-F motifs[252], also expressed in abundance by *S. mansoni* miracidiae[225]. Interestingly however, glycomic analysis of the hemolymph from a resistant strain of *B. glabrata* showed significantly less of these glycan motifs compared with the susceptible strain[253]. Thus, this observation indicates that glycosylation impacts the snail susceptibility to schistosome infection and suggests that the parasite glycans might avoid or promote specific innate immune recognition by mimicking those of its snail host. With the drastic changes observed throughout its life cycle[225], it is undeniable that *S. mansoni* adapts its glycans to its environment. This is particularly striking during cercarial transformation, when specific and localized expression of glycans was observed using anti-glycan monoclonal antibodies[254]. LeX and LDN-F motifs only became surface-exposed on schistosomulae after transformation, indicating the biological importance of the regulation of the expression of these motifs in the definitive host. Interestingly, LeX-terminated glycans in humans are restricted to certain cell-types[237] or are part of the aberrant glycosylation of tumor cells[255]. This motif was found to interact with CLRs in the mammalian host (*e.g.*, with dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN) of DCs[256,257] or with the MR, in the case of *S. mansoni* Omega-1[162]). Other (helminth) glycan motifs have been shown to interact with host immune cells via their CLRs. Known examples include the recognition of GalNAc-terminated motifs[258], such as LDN, by macrophage galactose-type lectin (MGL) or the binding of various mannose-containing glycans by the MR[259]. Also in the intermediate snail host, interactions between schistosome larval transformation products and proteins from snail hemolymph have been reported and

shown to have a direct impact on the snail hemocyte glycans[260,261]. Thus, with these observations, it became clear that helminth glycans are not "invisible" to the host immune system, as a camouflage function would suggest, but that glycan-conjugated products of the parasite functionally target immune receptors, notably TLRs and CLRs giving rise to the more accurate concept of 'molecular gimmickry', which passes on the idea of active interaction[237].



### *Interaction with the host immune system via glycan binding proteins*

While it is clear that glycosylation is an important factor in the immunomodulatory properties of helminth glycoproteins, there are only a few instances where molecular details of glycan-receptor interactions have been elucidated.

It has been shown that MGL on the surface of monocyte-derived DCs binds to GalNAc-terminated glycans, including Tn antigens (αGalNAc-Thr/Ser), of the liver fluke *Fasciola hepatica*. This interaction contributes to upregulating the production of IL-10 and TNFα inducing Th2/Treg polarization[262]. In infected mice, MGL-expressing cells that produce various regulatory cytokines and markers – including IL-10 and TNFα – have been identified. It is likely that these cells expand specific Th2 and Treg cells and suppress Th1 polarization. In addition, *F. hepatica* expresses a variety of *N*-glycans including oligomannosidic structures in abundance, as well as truncated and complex type *N*-glycans, including a phosphorylated subset[263]. MR and DC-SIGN have been shown to bind tegumental glycans of the parasite[263,264], and the interaction between mannosylated and fucosylated *N*-glycans of DC-SIGN is required for the induction of a tolerogenic program enhancing TLR-induced IL-10 and IL-27p28 which ultimately results in T cell anergy[264].

As mentioned previously, *S. mansoni* egg-derived glycoprotein Omega-1, drives Th2 polarization by interacting with the MR via LeX-terminated *N*-glycans[162]. Egg derived IPSE/α1, that also carries LeX motif, is bound by this receptor as well and both proteins trigger DC-SIGN, although this binding appears not to be required for IPSE/α1 to exhibit anti-inflammatory properties[265]. Similarly, the LDN-F motif of kappa-5, also a component of SEA, is involved in its recognition by 3 CLRs (DC-SIGN, MR and the MGL) and by non-C-type lectin receptors[266] but the functional effects resulting from this binding (if any) are unknown. In the well-studied case of immunomodulation by the previously mentioned glycoprotein ES-62 of *A. viteae*, experimental work has shown that the inhibition of B and T lymphocyte proliferation observed in the presence of ES-62 could be broadly mimicked with PC conjugated to albumin or even PC alone[161], demonstrating the key-role of the PC-substituted glycan moiety of the protein.

Glycolipids too have immunoactive properties. Both egg and worm glycolipids of *S. mansoni* were found to activate natural killer T cell proliferation[116] and egg glycolipids have been shown to stimulate the production of IL-10, IL-6 and TNF-α from peripheral blood mononuclear cells[271]. Importantly, this activity could be attributed to the LDN-DF motifs present on these glycolipids, given that the same effect could be reproduced with LDN-DF neoglycoconjugate synthesized enzymatically. Fucosylated glycans of worm glycolipids also interact with DC-SIGN and TLR4 of DCs. Based on *in vitro* experiments, this activation of DCs could contribute to eliciting Th1 immune responses in schistosome infections[267]. In addition, the stage-specific Fucα1-3Galβ1-4(Fucα1-3)GlcNAc – or pseudo Lewis Y – motif expressed by *S. mansoni* cercariae glycolipid has been the first parasite-specific DC-SIGN ligand identified, but whether and how this interaction contributes to the parasites' immune evasion remains to be determined[269].

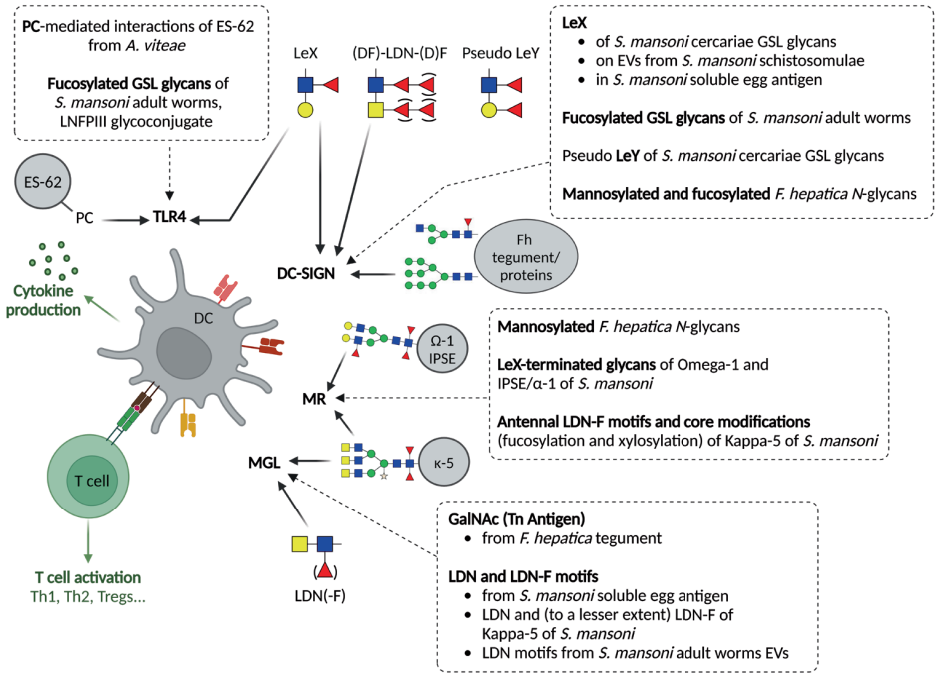


**Figure I-5. Known interactions of filarial and trematode glycoconjugates with receptors of host dendritic cells (DCs).** DCs are central players in the induction and maintenance of immune responses that detect pathogen-associated molecules, including helminth glycans, via Toll-like receptors (TLRs) and the carbohydrate-recognizing C-type lectin receptors (CLRs), including the dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN), the mannose receptor (MR) and the macrophage galactose-type lectin (MGL). Detection of parasite-associated patterns can trigger DC maturation, T cell activation, differentiation, and cytokine production. This figure was created with BioRender.com and adapted from[237] with data obtained from[118,134,162,173,174,258,262–264,266–270]. Glycan ligands are represented using the CFG nomenclature (see Symbol key inset in **Figure I-4**).

Finally, *S. mansoni* EVs exhibit stage-specific glycosylation with schistosomulae EVs carrying fucosylated glycans containing multiple LeX motifs and multifucosylated LDN contrasting with the LDN-dominated *N*-glycan profile of adult worm EVs[173,174]. Fucosylated schistosomulae EVs have been found to interact with DC-SIGN while adult worm EVs are recognized by MGL, showing that EV glycans influence their interactions with CLRs.

**Figure I-5** summarizes our current knowledge of the interactions between helminth glycans and DC receptors. While these studies clearly show the functional importance of specific glycan features of key parasite molecules, many more glycan-mediated immune mechanisms are still to be elucidated.



### *Anti-glycan antibody responses*

In addition to triggering or facilitating innate immune reactions, it is known that infected hosts raise antibodies to the parasite antigens, including glycans. Immunogenicity of the cercarial glycocalyx of schistosomes has been long known[272] and similarly, antibodies directed towards the sheaths of *W. bancrofti* microfilariae, and specifically binding to molecules of carbohydrate nature, sensitive to periodate treatment, have been reported early on[273].

Importantly, a dominant part of the serum antibody responses in human schistosomiasis has been shown to be directed to glycans[274,275] and the development of glycan microarrays has constituted a major breakthrough to study these anti-glycan antibody responses[276]. In these workflows, purified glycans from natural and/or synthetic sources are covalently immobilized, or "printed", onto glass slides that can then be incubated with a variety of (biological) samples containing carbohydrate-binding proteins[277]. Screening of microarrays composed of synthetic glycans with sera from *S. mansoni*-infected individuals[278] confirmed historical observations[209,279] with IgG and IgM binding observed to LDN, LDN-F, F-LDN and F-LDN-F motifs. IgM, but not IgG, to LeX were found in *S. mansoni* infection sera and IgE and IgG to β1-2 core-Xyl have been positively associated with *S. mansoni* infections[280]. In addition, antibodies to many "natural" glycans, including *N*-linked, *O*-linked and GSL glycans, extracted and purified from various life-stages of the parasite have been detected using so-called shotgun microarrays[278,281–283] and showed structures carrying multifucosylated LDN motifs to be highly antigenic (**Table I-3**). Interestingly, differential anti-glycan antibody responses have been observed between subgroups of individuals. Notably, IgG and IgM binding to glycan containing fucosylated LDN motifs has been shown to be higher in children than in adults infected with *S. mansoni*. IgM binding to LeX motifs is also higher in children[278]. Given that children are more susceptible to reinfection than adults[284–286], these findings raised the question of the role of these anti-glycan antibodies. Early studies have suggested that the abundant antibody response to glycan antigens in schistosomiasis acts as a smokescreen, subverting the host immune system from

more vulnerable peptide epitopes[274], in line with the initial idea of glycan being restricted to "camouflage" function. Sharpening of our understanding of these anti-glycan antibody responses has been provided by studies on animal models. These longitudinal studies have not only been highly informative regarding the dynamics of anti-glycan responses during disease establishment, but also suggested a protective role for glycan-binding IgG in vaccinated mice[287], baboons[288], in resistant brown rats and in self-cured rhesus macaques[289,290]. This helped decipher the complex picture caused by the diversity of glycans synthesized by the parasite and revealed a potential as vaccination targets for specific glycan subsets. Specifically, IgG towards *Schistosoma*-specific multi-fucosylated structures elicited and maintained in infected rhesus macaques has been associated with infection clearance and resistance to re-infection[289], while IgG to β1-2Xyl and α1-3 core fucose from brown rats showed the ability to kill schistosomula *in vitro*[291].

**Table I-3. Known anti-glycan antibody responses in *S. mansoni*-infected individuals.**

| Glycan motif | | Antibody responses in *S. mansoni* infections | | |
|---|---|---|---|---|
| Source | Structure | IgG | IgM | IgE |
| *S. mansoni N*-glycans, synthetic glycans |  Short, paucimannosidic glycans | (-) | (-) | (-) |
| *S. mansoni N*-glycans, synthetic glycans |  Core-Xylose | (+) | (-) | (+) |
| *S. mansoni N*-glycans |  LN | (-) | (-) | (-) |
| *S. mansoni N-/O-*/GSL glycans, synthetic glycans |  LeX | (-) | (+) | (-) |
| *S. mansoni N-*/GSL glycans, synthetic glycans |  LDN | (+) | (+) | (-) |
| *S. mansoni N*-glycans |  Fuc-GlcNAc | (+) | (++) | (-) |
| *S. mansoni N-/O-*/GSL glycans, synthetic glycans |  F-LDN(-F) | (++) | (++) | (-) |
| *S. mansoni* cercariae GSL glycans |  Pseudo LeY | (+++) | (+++) | (-) |
| *S. mansoni* egg GSL glycans |  Multifucosylated (DF-LDN-DF/TF) | (+++) | (+++) | (-) |

Glycan-microarrays constituted of synthetic or native glycans extracted from the parasite (see "Source") have been screened with sera from *S. mansoni*-infected individuals[278–282]. (+) and orange colors symbolize antibody binding to the glycan/glycan element specified (see "Structure"), while (-) and blue colors translate absence of binding compared to (uninfected) controls. Darker orange shades indicate glycan motifs to

which the highest levels of antibody binding have been observed in these glycan-microarray assisted studies.

As for structural glycomics, anti-glycan antibody studies have so far mainly focused on *S. mansoni*. In view of the possible glycosylation differences between *S. mansoni* and other schistosome species, it will be important to assess whether these findings translate to the other *Schistosoma* species causing infections in humans, most importantly when considering glycans for vaccine or diagnostic applications. Similarly, virtually nothing is known regarding antibody responses to glycan antigens in the context of filariasis although IgM and IgG2 binding to antigens of carbohydrate nature has been reported in bancroftian filariasis[292]. Interesting subclass-specific associations of IgG to (crude) filarial antigen have been reported for AS, CP and EN subpopulations in LF-endemic areas (**Table I-2**). Carbohydrate molecules present in filarial antigen have not been characterized and it is unknown whether they are targeted by these antibody responses.



### *Impact of (parasitic) diseases on host glycans*

In addition to parasite glycans, the glycans of the host may also play a role at the host-parasite interface. Host glycans are involved in direct interactions with infectious agents including defense mechanisms such as trapping pathogens via mucins or activating immune cells in response to infection[293]. By causing aberrant expression of host glycosyltransferases and glycosidases, infection often affects the host glycan expression[294]. In that regard, serum is of great interest as most human serum proteins are glycosylated[295], and the relative abundances of protein glycoforms can reflect alterations in health and disease. Many proteins carry *N*-glycans, thus, contributing to the serum *N*-glycome. Using UPLC profiling workflows, which constitute robust and quantitative platforms for the detection of fluorescently labeled glycans[296], it has been shown that the human serum *N*-glycome profile is generally stable[297], although gender and age-specific alterations have been reported[298,299] as well as the impact of various conditions such as autoimmune disorders[300] or infectious diseases[301,302]. In addition, studies of IgG, the most abundant human immunoglobulin and major serum glycoprotein, have shown the importance of the *N*-glycosylation of this antibody on its effector functions, having a direct impact on disease development and progression[303–305]. Thus, both serum and IgG *N*-glycans have been investigated as biomarkers for a variety of pathologies and have led to promising results for (early) detection of certain cancers[306–310]. Research into infectious diseases has been relatively limited so far, particularly for parasitic infections. Differences in IgG Fc glycosylation between asymptomatic, chronically affected, and non-endemic patients have been reported in bancroftian filariasis[311,312], and changes in the serum *N*-glycome of dogs have been

highlighted in the course of infection with the heartworm *D. immitis*[313]. Yet, glycomic changes during parasitic infections remain largely unexplored[291].

## 3. Scope of the thesis

The work presented here focuses on glycans in the context of schistosomiasis and filariasis. As described in the first part of the introduction, these two parasitic infections of humans largely differ in terms of causative agents, outcomes, and general biology. Nonetheless, current challenges associated with prevention and control of these NTDs are to some extent similar, particularly regarding the need for new drug/vaccine targets and for parasite detection with utmost sensitivity. This can only be achieved by increasing our fundamental understanding of helminth parasites and host-parasite biology.

Glycans, as addressed in this introduction, are carbohydrate molecules that play central roles in host-parasite interactions. Clearly, a better knowledge of the parasite glycans is required and this thesis aims to bridge some of the current gaps. Comprehensive glycomic studies of *S. mansoni* have provided substantial information on the glycosylation of this species. However, little is known about the other *Schistosoma* species, including the most common one, *S. haematobium* which is highly prevalent in sub-Saharan Africa and the Middle East. In view of major biological dissimilarities, differential glycosylation can be expected with *S. mansoni*. Therefore, **chapter 2** of this thesis explores the glycosylation of *S. haematobium* cercariae, adult worms and eggs. Structural characterization of *N*-linked, *O*-linked and GSL glycans was performed using a MS-based workflow in combination with glycan sequencing techniques to determine common features and differences between both *Schistosoma* species. In addition, a glycan microarray-assisted study of serum IgG and IgM responses was conducted to assess whether the quantitative and qualitative glycomic differences observed resulted in differential antibody responses in *S. haematobium* and *S. mansoni* infections.

Glycans of filarial nematodes of humans have been largely unexplored. Thus, **chapter 3** examines two major classes of glycans of *B. malayi*, one of the causative agents of LF. *N*-linked and GSL glycans of this parasite were characterized using our MS-based glycomic workflow and printed onto glycan microarrays to assess their antigenicity. The dynamics of serum IgG and IgM responses to the parasite glycans during establishment of infection were studied in rhesus macaques. In parallel, IgG and IgM responses in plasma from chronically infected humans were examined.

Knowledge of anti-glycan antibody responses during infection is not only crucial to gain a better understanding of host-parasite immune interactions but can also significantly help improve the current diagnostic methods. The question about the specificity of the antibody responses to antigenic glycan motifs is particularly crucial

since the limited data available in the literature on filarial nematode glycoconjugates has suggested certain glycan features to be shared throughout the phylum. This question is addressed in **chapter 4**, by screening microarrays constructed of glycans isolated from *B. malayi* with plasma from individuals infected with other filarial nematodes. Anti-glycan IgG responses in five major filarial infections of humans were compared to identify cross-reactive and infection-specific anti-glycan antibody responses. The *N*-linked and GSL glycans of *O. volvulus* were also characterized to better understand the cross-reactivity observed and validate our hypothesis regarding shared glycan epitopes between species. In addition to total IgG, the responses of the different IgG subclasses to the parasite glycans in selected filariases were investigated to clarify the role of glycan antigens in IgG subclass associations occurring in LF and onchocerciasis. Moreover, the four human IgG subclasses differ with respect to antigen binding specificity and immune properties, and so does their potential for diagnostic application.



Finally, not only the parasite glycans are of interest for diagnostic purposes at the host-parasite interface. **Chapter 5** focuses on glycans in host-pathogen interactions from a different angle, those of the host. In this chapter, UPLC profiling was used in combination with MS techniques to examine the impact of LF on the host serum *N*-glycome. *N*-glycan profiles in serum of humans and other mammals have been shown to be highly stable in healthy individuals, although affected by various (pathological) conditions. The *N*-glycan profiles of whole serum and IgG of healthy rhesus macaques, an important non-human primate model for LF as well as many other infectious and non-infectious diseases, were first determined. This defined healthy baseline was then used to monitor changes in the *N*-glycome of a longitudinal cohort of *B. malayi* infected rhesus macaques.

In conclusion, this body of work extends our knowledge of schistosome and filarial nematode glycans in terms of structural features and antigenicity. Specificity of host antibody responses to these glycans and changes in host serum *N*-glycosylation are investigated in a diagnostic perspective. Together, these chapters provide new insights into parasite glycobiology, show promise for future use of glycans and anti-glycan antibody responses for detection of parasitic infections and pave the way for further (glycomic) studies. A summary of these findings and future work directions are presented in **chapter 6**.

# Abbreviations

| | |
|---|---|
| **AAMs** | Alternatively Activated Macrophages |
| **AS** | Asymptomatic |
| **Breg** | regulatory B cells |
| **CAA** | Circulating Anodic Antigen |
| **CCA** | Circulating Cathodic Antigen |
| **Cer** | Ceramide |
| **CFA** | Circulating Filarial Antigen |
| **CLRs** | C-Type Lectin Receptors |
| **CP** | Chronic Pathology |
| **DALYs** | Disability-Adjusted Life Years |
| **DCs** | Dendritic Cells |
| **DC-SIGN** | Dendritic Cell-Specific ICAM3-Grabbing Non-Integrin |
| **DF** | Difucosylated |
| **EN** | Endemic Normals |
| **ES** | Excretory/Secretory |
| **EVs** | Extracellular Vesicles |
| **FGS** | Female Genital Schistosomiasis |
| **Fuc** | Fucose |
| **Gal** | Galactose |
| **GalNAc** | N-acetylgalactosamine |
| **Glc** | Glucose |
| **GlcA** | Glucuronic acid |
| **GlcNAc** | N-acetylglucosamine |
| **GSL** | Glycosphingolipid |
| **Hex** | Hexose |
| **HexNAc** | N-acetylhexosamine |
| **HIV** | Human Immunodeficiency Virus |
| **IFN-γ** | Interferon Gamma |
| **Ig** | Immunoglobulin |
| **IL** | Interleukin |
| **IPSE** | IL-4 Inducing Principle of Schistosome Eggs |
| **L3 larvae** | Third stage larvae |
| **LAMP** | Loop-Mediated Isothermal Amplification |
| **LDN** | LacDiNAc, GalNAcβ1-4GlcNAc |
| **LeX** | Lewis X, Galβ1-4(Fucα1-3)GlcNAc |
| **LF** | Lymphatic Filariasis |
| **LN** | LacNAc, Galβ1-4GlcNAc |
| **LNFPIII** | Lacto-N-Fucopentose III |
| **Man** | Mannose |
| **MDA** | Mass Drug Administration |
| **Mfs** | Microfilariae |
| **MGL** | Macrophage Galactose-Type Lectin |
| **MR** | Mannose Receptor |
| **MS** | Mass Spectrometry |
| **NTDs** | Neglected Tropical Diseases |
| **PC** | Phosphorylcholine |
| **PCR** | Polymerase Chain Reaction |
| **PE** | Phosphoethanoloamine |
| **POC** | Point-Of-Care |
| **PRRs** | Pattern Recognition Receptors |
| **PTMs** | Post-Translational Modifications |
| **PZQ** | Praziquantel |
| **RPA** | Recombinase Polymerase Amplification |
| **SEA** | Soluble Egg Antigen |
| **TF** | Trifucosylated |
| **TGF-β** | Transforming Growth Factor-β |
| **Th** | T helper |
| **TLRs** | Toll-Like Receptors |
| **TNF-α** | Tumor Necrosis Factor-α |
| **Treg** | regulatory T cells |
| **UCP-LF** | Up-Converting reporter Particle technology based, Lateral Flow |
| **UGS** | Urogenital Schistosomiasis |
| **UPLC** | Ultraperformance Liquid Chromatography |
| **WASH** | Clean water, sanitation, and hygiene |
| **WHO** | World Health Organization |
| **Xyl** | Xylose |

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