



Universiteit
Leiden
The Netherlands

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

Petralia, L.M.C.

Citation

Petralia, L. M. C. (2025, April 16). *Advancing helminth glycomics: structural specificity and immunogenicity of schistosomal and filarial glycans*. Retrieved from <https://hdl.handle.net/1887/4212211>

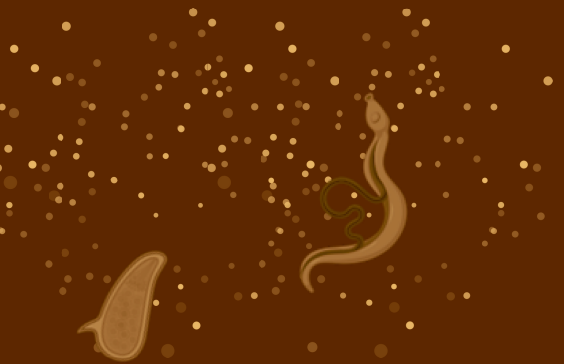
Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/4212211>

Note: To cite this publication please use the final published version (if applicable).





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 classes² (**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 relationships³. With an estimate of over 2 billion infected people, helminths are highly common infectious agents in developing countries⁴ 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 2030⁶, 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 malaria⁹.

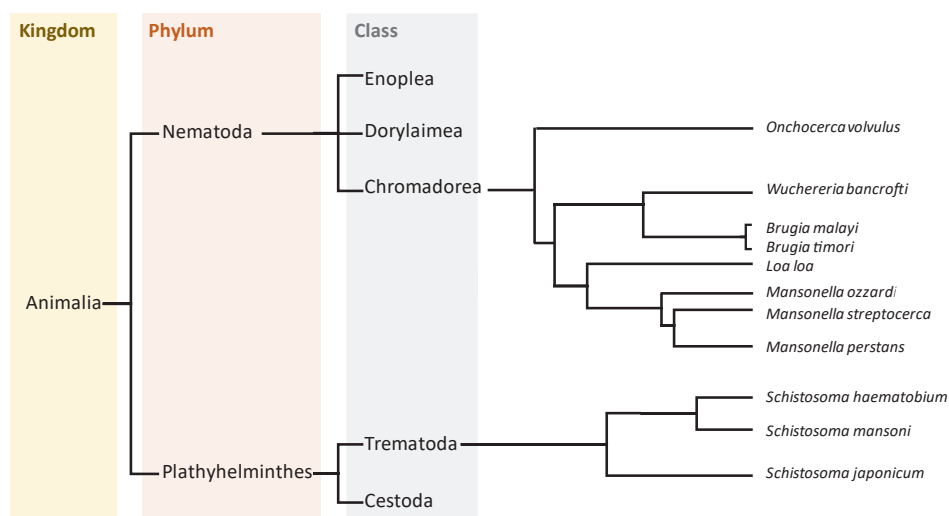


Figure I-1. Phylogenetic tree of selected helminths. Schematic representation of phylogenetic relationships between the major species of schistosomes and filarial nematodes infecting humans, which are the focus of the present thesis. Adapted from¹⁰ for filarial nematodes and from¹¹ for schistosomes. 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 hosts¹³ (**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 Indonesia¹⁴. *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 Caribbean⁹. *Biomphalaria* snails are also widely found in Africa and in the Arabian Peninsula¹⁵, 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 living¹⁹. Clean water, sanitation, and hygiene (WASH measures²⁰) 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 sunlight²³, 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 body²⁹. 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 starts¹⁴ 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* infections³³. Between 16 and 56 million women are affected by FGS³⁴, 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 birthweight³⁵, while MGS clinical symptoms include a range of erectile and prostatic problems³⁶. 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) programs⁶ aiming to contribute to schistosomiasis control and reduction of morbidity. As of today, this drug appears effective against all major *Schistosoma* species³⁸, 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 infections⁹. Thus, diagnosis of schistosomiasis can be challenging, although it is crucial to achieve proper drug administration and endemicity mapping⁴⁰. A range of diagnostic tests is available to detect *Schistosoma* infection⁴¹. 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 methods⁴². 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 specificity⁴⁰. 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 alternatives⁴¹. 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 option⁴³. 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 clearance⁴¹. 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 targets⁴¹. Assays to detect CAA and CCA have been developed in serum and urine and were tested and applied in endemic settings in many studies⁴⁸. CCA can be detected with a field-friendly lateral flow assay, the Point-Of-Care (POC)-CCA urine test, mainly for *S. mansoni* infections⁴⁹. 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 settings⁵⁰. Efforts to optimize these tools are still ongoing, specifically aiming to resolve specificity issues of the POC-CCA observed in infants and pregnant women⁵¹ and to develop a rapid diagnostic test for detection of CAA in resource-limited settings⁵² 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 worms⁶⁰. 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 filariasis⁶⁰. *Mansonella ozzardi* and *Mansonella perstans* occupy the abdomen serous cavity and thus are the causative agents of serous cavity filariasis⁶². 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 Asia⁶³, are responsible for approximately 10% of the 50 million LF cases²¹ while *W. bancrofti* cause the remaining cases throughout most of Sub-Saharan Africa, Madagascar, several Western Pacific Island nations, and

parts of the Caribbean⁶⁴. 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 lymphatics⁶⁴. 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 vector⁶⁶. 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 genitals⁶⁸.

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 blindness⁶⁹.

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 damage⁷¹. 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 loiasis⁷³.

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 pathology⁷⁴. 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 well¹⁰.

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)			Africa (West and Central)
		Onchocerca	Onchocerca volvulus	Simulium spp. blackflies	Subcutaneous tissues and skin nodules	Onchocerciasis	Subcutaneous filariasis	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 guidelines⁶⁵. 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 to 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 available⁹² and a dual version allowing simultaneous detection of IgG4 against Ov16 and Wb123 antigens has been developed recently⁹³. 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

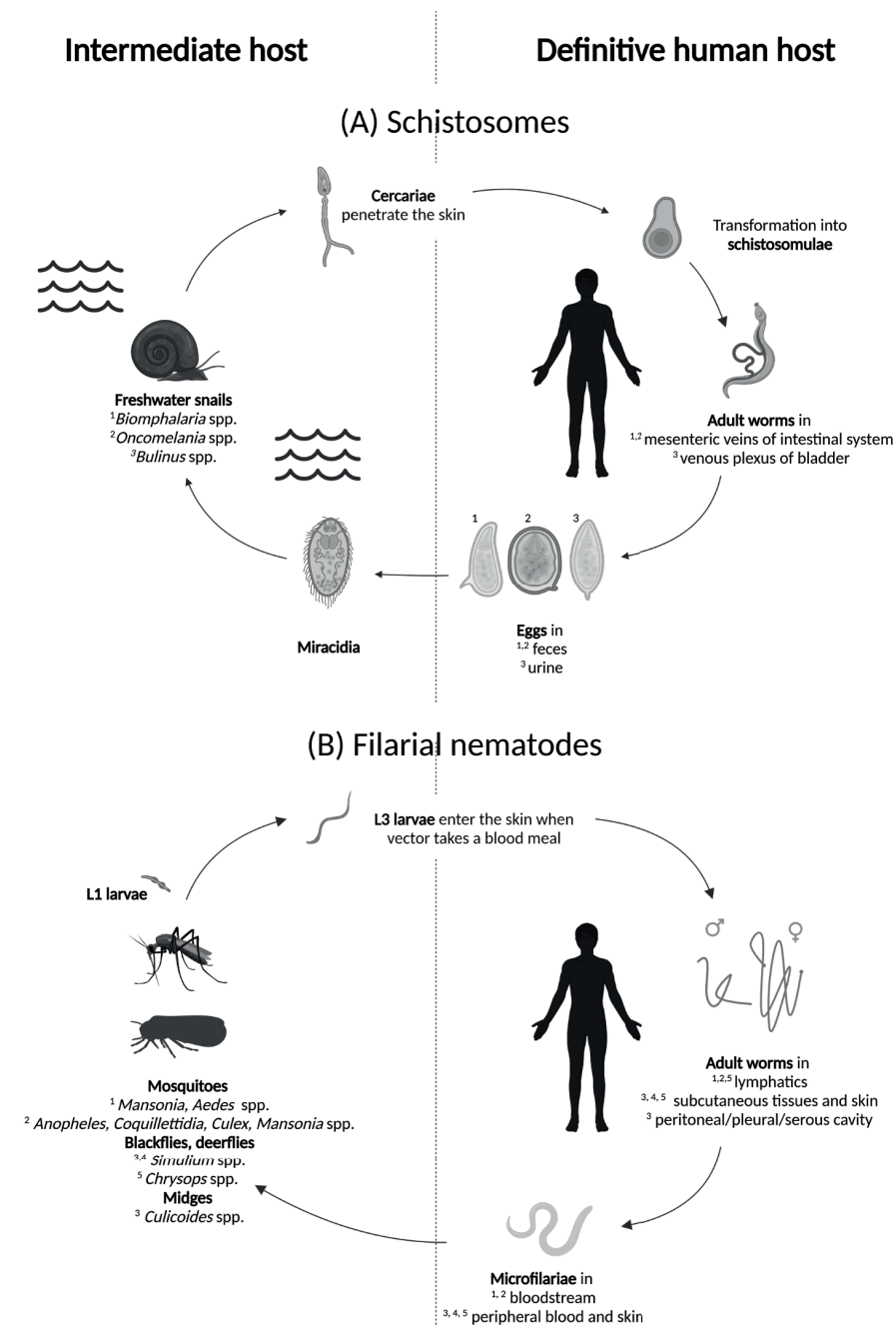


Figure I-2. Life cycles. Overview for (A) *Schistosoma* spp. with specifications for *S. mansoni* (1), *S. japonicum* (2) and *S. haematobium* (3) **and for (B) the human parasitic filarial nematodes** with specifications for *Brugia* spp. (1), *W. bancrofti* (2), *Mansonella* spp. (3), *O. volvulus*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

as a public health problem in 17 countries and onchocerciasis has been eliminated in 4 countries in the Americas⁶⁵. 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 infections⁹⁸ and has long been known in the veterinary context⁹⁹. 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 field³⁸. 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 exposure¹⁰⁰. Thus, identification of novel drug targets or prophylactic therapies are highly sought after³⁹. 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 development¹⁰⁶. 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 observed¹⁰⁷. 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 strengthened⁶⁵. Efficient mass distribution of anthelmintic 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 responses¹¹⁴. 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 evasion¹¹⁵.

In both schistosomiasis¹¹⁶ and filariasis¹¹⁷, 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 repair¹²⁰, 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 larvae¹²¹. Characteristic type-2 associated cytokines including interleukin (IL)-4, IL-5, IL-9, IL-10 and IL-13¹¹⁴ 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 cells¹¹⁵. 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 humans¹¹⁴. However, this immune hyporesponsiveness can also be detrimental to the host, with suboptimal responses to vaccines observed in individuals infected with helminths¹³² and increased susceptibility to other pathogenic infectious agents¹¹⁴.

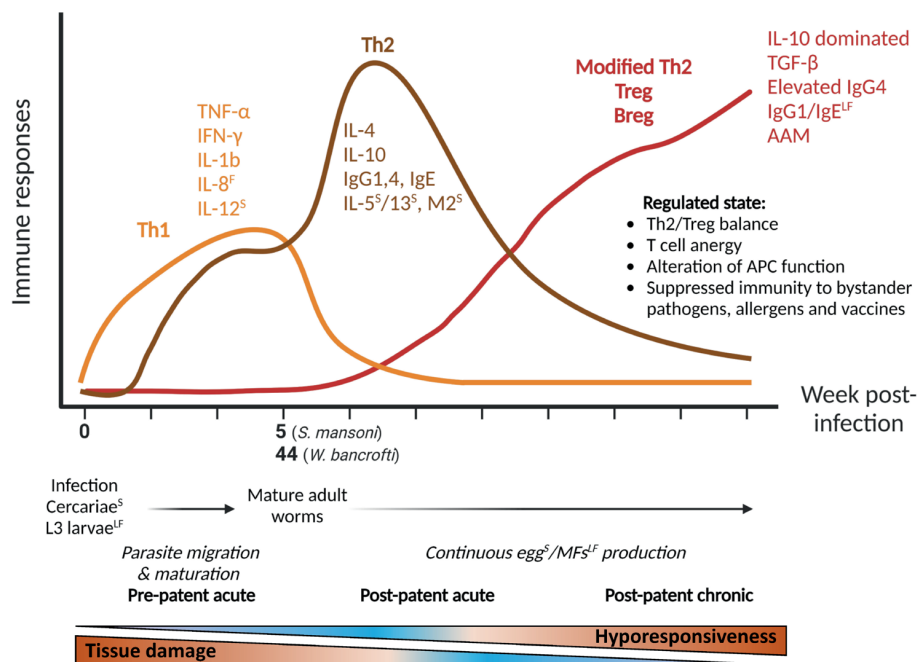


Figure I-3. Prototypical immune responses in schistosomiasis^(S) and filariasis^(F). Graph representing the immunological profiles resulting from infection with schistosomes or filarial nematodes. An initial proinflammatory T helper (**Th**)1 response is followed by a **Th2** response which builds up 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 from¹¹⁶ 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 coexist¹³⁴. 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 fungi¹³⁵. 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 Sowda¹³⁸. 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 individuals¹³⁹. 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 filariasis¹⁴⁰. When comparing the different subpopulations, AS microfilariaemic individuals show higher levels of IgG4 and those are associated with their hyporesponsiveness¹⁴¹, while elevated levels of IgG1-3 and IgE correlate with CP¹⁴². 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 reality¹⁴³, although the basis of such immunity is still unclear¹⁴⁴.

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, schistosomulae¹⁴⁹, as well as adult schistosomes¹⁵⁰, 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 host¹⁵².

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 infections¹³⁴. 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 antigens¹⁵⁵ 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 activation¹⁵⁹ 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-62¹⁶¹. Whether the ES-62 homologues expressed by the filarial parasites of humans share the same properties as *A. viteae* ES-62 must still be confirmed¹¹⁸. 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-drivers¹⁴⁸. Those include the major bioactive glycoproteins “IL-4 inducing principle of schistosome eggs”/alpha-1 (IPSE/α-1) and the hepatotoxic egg Omega-1¹⁴⁸. 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 activity¹⁶². 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 mixture¹⁶⁴. 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 macrophages¹⁶⁵. 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 damage¹⁶⁶, and SEA triggers the formation of neutrophils extracellular traps, some “web-like” structures that are suspected to cause indirect inflammation and local damage¹⁶⁷. 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-13¹⁶⁸. 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 repository¹⁸¹, 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 contiguity¹⁸². 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 challenge¹⁸². 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 diverse²⁰⁶, 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 matrix²⁰⁷. 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 polysaccharides²⁰⁸. 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 host²⁰⁸⁻²¹⁰, our knowledge of these molecules in the context of helminthiasis 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 carrier²¹¹. Specifically, the use of peptide-*N*-glycosidases permits the study of the major class of protein-linked glycans that are the asparagine (*N*)-linked glycans²¹² 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 portions²¹⁵, allowing the study of this subclass of glycolipids which is the main type of glycolipid found in animals²¹⁶. 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 residue²¹⁷. In that case, chemical methods have been optimized to achieve glycan release from this type of glycoconjugate²¹⁸. Upon release, glycans can be analyzed using mass spectrometry (MS), a technique that permits direct ionization of nonvolatile substances²¹⁹. The development of matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) MS have constituted real breakthroughs in the field of glycomics²²⁰. 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 glycans²⁰⁸, although parasitic worms have a largely conserved glycosylation machinery²²³ similar to that of other multicellular organisms²²⁸. Unlike bacteria²²⁹, 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 sugar²⁰⁸. Other monosaccharides and substituents have been reported in more specific instances such as glucuronic acid (GlcA) as part of the schistosome CAA⁴⁴ and of the filarial nematode *Dirofilaria immitis* N-glycans²³⁰, 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 acids²³³. 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 xylosylation²³⁴, i. e. the attachment of a β 1-2-linked xylose (Xyl) to the initial core-mannose, and presence of α 1-3 linked core Fuc in some life stages^{235,236} in addition to the α 1-6 core-Fuc that is also observed in mammals. Moreover, terminal GalNAc β 1-4GlcNAc or LacDiNAc (LDN) motifs are frequently found instead of the terminal Gal β 1-4GlcNAc or LacNAc (LN) motifs abundant in mammals²³⁷. Fucosylated LDN, rarely observed in mammals²³⁸, is highly expressed in schistosomes and form antigenic motifs containing α 1-3 linked Fuc residues of composition (Fuca1-3)GalNAc β 1-4GlcNAc, GalNAc β 1-4(Fuca1-3)GlcNAc or (Fuca1-3)GalNAc β 1-4(Fuca1-3)GlcNAc (F-LDN, LDN-F or F-LDN-F, respectively)^{208,239,240}. Moreover, with the synthesis of α 1-2 linkages between Fuc residues, yielding difucosylated (DF) motifs of composition Fuca1-2Fuc-R and even trifucosylated (TF) motifs of composition Fuca1-2Fuca1-2Fuc-R, schistosomes exhibit very specific fucosylation patterns that have not been reported in other species so far²⁰⁸. HexNAc substitution with such DF/TF motifs results in multifucosylated structures that are particularly ubiquitous in the glycosphingolipid (GSL) glycans of *S. mansoni* eggs²²⁵. More common glycan epitopes are also expressed by *S. mansoni* including the Lewis X (LeX) antigen of composition Gal β 1-4(Fuc α 1-3)GlcNAc which is also present in mammals²³⁴. Although not major

components of *S. mansoni* glycans, non-fucosylated LN terminated glycans are observed, in particular in the human life-stages of the parasite²²⁵. Stage- and sex-specificities²⁴⁰ 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 glycome²²⁵. 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 $-3\text{Gal}\beta 1-4(\text{Fuc}\alpha 1-3)\text{GlcNAc}1-$) for the CCA molecule⁴⁵ and repeats of GalNAcs substituted with GlcA forming $-6(\text{GlcA}\beta 1-3)\text{GalNAc}1-$ repeats for CAA⁴⁴.

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 region²⁴³.

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 well²⁴⁴ 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 species²³⁶. Importantly, the presence of xylosylation and structures carrying both $\alpha 1-6$ and $\alpha 1-3$ 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 patterns²⁴⁶, organ of residency and even disease outcome¹¹¹.

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 from²²⁴ 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 database²⁴⁸, 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*-linked²²⁷ and GSL glycans²²⁶ of *O. volvulus* that are summarized in **Figure I-4C**. Slightly more substantial knowledge of the *N*-glycans of parasitic nematodes of animals²²³ 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 residues²⁰⁸, mostly GlcNAc, but was also found attached to GalNAc in *D. immitis* *N*-glycans²³⁰. GlcNAc appears as a common terminal residue in filarial nematode *N*-glycans²²³. 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 antennae²³⁰ that can be PC-substituted. GSL glycans are known to be built on the arthrotypic core, which consists of GlcNAc β 1-3Man β 1-4Glc β 1 linked to the ceramide (cer) portion of the lipid molecule²²³. 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 β 1-4-linked GalNAc and terminal α -linked Gal, forming the structures of composition Gal α 1-3GalNAc β 1-4(PC-6)GlcNAc β 1-3(PE-6)Man β 1-4Glc1-1cer and Gal α 1-3GalNAc β 1-4(PC-6)GlcNAc β 1-3Man β 1-4Glc1-1cer. 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 phylum²²⁶. Before extensive glycomic studies were performed, immunomodulatory properties of filarial nematode glycans²⁴⁹, and more specifically of PC-substituted glycans, were known for a long time

already²²⁷. These have been particularly studied in the context of the ES-62 of *A. viteae*, which *N*-glycosylation has been fully characterized using targeted glycoproteomics²⁵⁰.

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 identification²⁵¹, 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 system²⁰⁸.

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 β 1-2 Xyl and terminal F-LDN-F motifs²⁵², also expressed in abundance by *S. mansoni* miracidiae²²⁵. 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 strain²⁵³. 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 cycle²²⁵, 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 antibodies²⁵⁴. 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-types²³⁷ or are part of the aberrant glycosylation of tumor cells²⁵⁵. 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-1¹⁶²). Other (helminth) glycan motifs have been shown to interact with host immune cells via their CLRs. Known examples include the recognition of GalNAc-terminated motifs²⁵⁸, such as LDN, by macrophage galactose-type lectin (MGL) or the binding of various mannose-containing glycans by the MR²⁵⁹. 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 interaction²³⁷.

Interaction with the host immune system via glycan binding proteins

1

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 polarization²⁶². 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 subset²⁶³. 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 anergy²⁶⁴.

As mentioned previously, *S. mansoni* egg-derived glycoprotein Omega-1, drives Th2 polarization by interacting with the MR via LeX-terminated *N*-glycans¹⁶². 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 properties²⁶⁵. 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 receptors²⁶⁶ 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 alone¹⁶¹, 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 proliferation¹¹⁶ and egg glycolipids have been shown to stimulate the production of IL-10, IL-6 and TNF- α from peripheral blood mononuclear cells²⁷¹. 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 infections²⁶⁷. In addition, the stage-specific Fuca1-3Gal β 1-4(Fuca1-3)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 determined²⁶⁹.

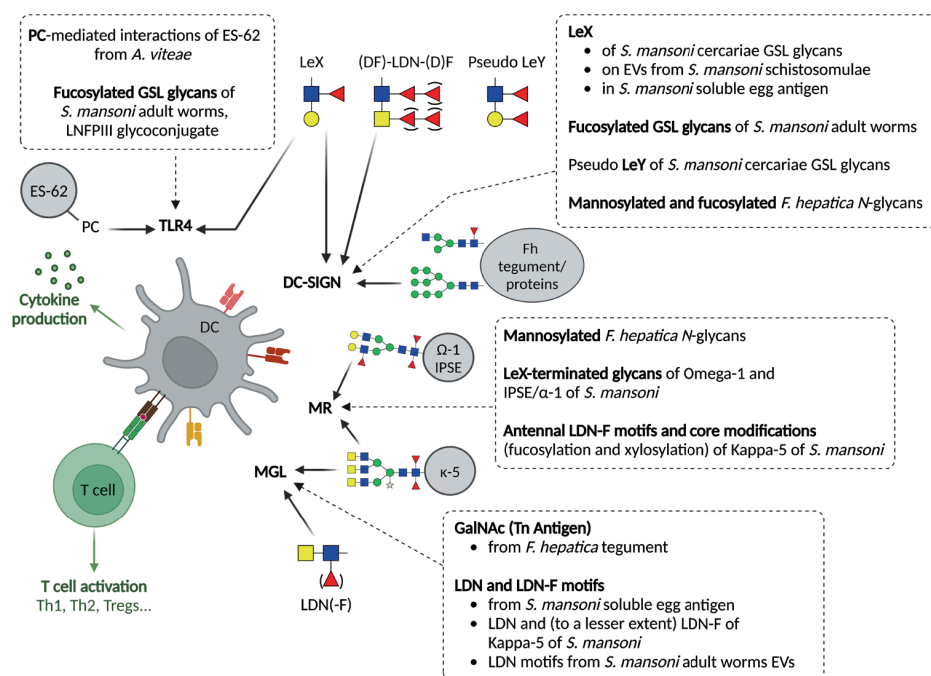


Figure I-5. Known interactions of filarial and trematode glycoconjugates with receptors of host dendritic cells (DCs). DCs are central players in the induction and maintenance of immune responses that detect pathogen-associated molecules, including helminth glycans, via Toll-like receptors (TLRs) and 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 from²³⁷ 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 known²⁷² 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 on²⁷³.

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 responses²⁷⁶. 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 proteins²⁷⁷. Screening of microarrays composed of synthetic glycans with sera from *S. mansoni*-infected individuals²⁷⁸ 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 β 1-2 core-Xyl have been positively associated with *S. mansoni* infections²⁸⁰. 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 children²⁷⁸. 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 epitopes²⁷⁴, 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 mice²⁸⁷, baboons²⁸⁸, 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-infection²⁸⁹, while IgG to β 1-2Xyl and α 1-3 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 <i>S. mansoni</i> infections		
Source	Structure	IgG	IgM	IgE
<i>S. mansoni</i> N-glycans, synthetic glycans	 Short, paucimannosidic glycans	(-)	(-)	(-)
<i>S. mansoni</i> N-glycans, synthetic glycans	 Core-Xylose	(+)	(-)	(+)
<i>S. mansoni</i> N-glycans	 LN	(-)	(-)	(-)
<i>S. mansoni</i> N-/O-/GSL glycans, synthetic glycans	 LeX	(-)	(+)	(-)
<i>S. mansoni</i> N-/GSL glycans, synthetic glycans	 LDN	(+)	(+)	(-)
<i>S. mansoni</i> N-glycans	 Fuc-GlcNAc	(+)	(++)	(-)
<i>S. mansoni</i> N-/O-/GSL glycans, synthetic glycans	 F-LDN(-F)	(++)	(++)	(-)
<i>S. mansoni</i> cercariae GSL glycans	 Pseudo LeY	(+++)	(+++)	(-)
<i>S. mansoni</i> 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 filariasis²⁹². 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 infection²⁹³. By causing aberrant expression of host glycosyltransferases and glycosidases, infection often affects the host glycan expression²⁹⁴. In that regard, serum is of great interest as most human serum proteins are glycosylated²⁹⁵, 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 glycans²⁹⁶, it has been shown that the human serum *N*-glycome profile is generally stable²⁹⁷, although gender and age-specific alterations have been reported^{298,299} as well as the impact of various conditions such as autoimmune disorders³⁰⁰ 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 unexplored²⁹¹.

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	LDN	LacDiNAc, GalNAc β 1-4GlcNAc
AS	Asymptomatic	LeX	Lewis X, Gal β 1-4(Fuca1-3)GlcNAc
Breg	regulatory B cells	LF	Lymphatic Filariasis
CAA	Circulating Anodic Antigen	LN	LacNAc, Gal β 1-4GlcNAc
CCA	Circulating Cathodic Antigen	LNFPIII	Lacto-N-Fucopentose III
Cer	Ceramide	Man	Mannose
CFA	Circulating Filarial Antigen	MDA	Mass Drug Administration
CLRs	C-Type Lectin Receptors	Mfs	Microfilariae
CP	Chronic Pathology	MGL	Macrophage Galactose-Type Lectin
DALYs	Disability-Adjusted Life Years	MR	Mannose Receptor
DCs	Dendritic Cells	MS	Mass Spectrometry
DC-SIGN	Dendritic Cell-Specific ICAM3-Grabbing Non-Integrin	NTDs	Neglected Tropical Diseases
DF	Difucosylated	PC	Phosphorylcholine
EN	Endemic Normals	PCR	Polymerase Chain Reaction
ES	Excretory/Secretory	PE	Phosphoethanolamine
EVs	Extracellular Vesicles	POC	Point-Of-Care
FGS	Female Genital Schistosomiasis	PRRs	Pattern Recognition Receptors
Fuc	Fucose	PTMs	Post-Translational Modifications
Gal	Galactose	PZQ	Praziquantel
GalNAc	N-acetylgalactosamine	RPA	Recombinase Polymerase Amplification
Glc	Glucose	SEA	Soluble Egg Antigen
GlcA	Glucuronic acid	TF	Trifucosylated
GlcNAc	N-acetylglucosamine	TGF-β	Transforming Growth Factor- β
GSL	Glycosphingolipid	Th	T helper
Hex	Hexose	TLRs	Toll-Like Receptors
HexNAc	N-acetylhexosamine	TNF-α	Tumor Necrosis Factor- α
HIV	Human Immunodeficiency Virus	Treg	regulatory T cells
IFN-γ	Interferon Gamma	UCP-LF	Up-Converting reporter Particle technology based, Lateral Flow
Ig	Immunoglobulin	UGS	Urogenital Schistosomiasis
IL	Interleukin	UPLC	Ultraperformance Liquid Chromatography
IPSE	IL-4 Inducing Principle of Schistosome Eggs	WASH	Clean water, sanitation, and hygiene
L3 larvae	Third stage larvae	WHO	World Health Organization
LAMP	Loop-Mediated Isothermal Amplification	Xyl	Xylose

References

1. Hotez, P. J. *et al.* Helminth infections: The great neglected tropical diseases. *Journal of Clinical Investigation* **118**, 1311–1321 (2008).
2. Hodda, M. Phylum Nematoda: a classification, catalogue and index of valid genera, with a census of valid species. *Zootaxa* **5114**, 1–289 (2022).
3. Gilbert A. Castro. Helminths: Structure, Classification, Growth, and Development. in *Medical Microbiology*. 4th edition. (ed. Baron S) 1–18 (University of Texas Medical Branch at Galveston, 1996).
4. Amin, A. S. M. al & Wadhwa, R. *Helminthiasis*. (StatPearls Publishing, Treasure Island (FL), 2022).
5. WHO - Neglected tropical diseases. https://www.who.int/neglected_diseases/diseases/en/.
6. WHO TEAM. *Ending the Neglect to Attain the Sustainable Development Goals: A Road Map for Neglected Tropical Diseases 2021–2030*. World Health Organization (2020).
7. Deshpande, A. *et al.* The global distribution of lymphatic filariasis, 2000–18: a geospatial analysis. *Lancet Glob Health* **8**, e1186–e1194 (2020).
8. CDC. Onchocerciasis - Epidemiology & Risk Factors. (2019).
9. McManus, D. P. *et al.* Schistosomiasis. *Nat Rev Dis Primers* **4**, 1–19 (2018).
10. Sinha, A. *et al.* Genomes of the human filarial parasites *Mansonella perstans* and *Mansonella ozzardi*. *Frontiers in Tropical Diseases* **4**, (2023).
11. Ebbs, E. T. *et al.* Phylogenomics and Diversification of the Schistosomatidae Based on Targeted Sequence Capture of Ultra-Conserved Elements. *Pathogens* **11**, (2022).
12. World Health Organization. Schistosomiasis. <https://www.who.int/news-room/fact-sheets/detail/schistosomiasis> (2022).
13. Ross, A. G. *et al.* Schistosomiasis. *New England Journal of Medicine* **346**, 1212–1220 (2002).
14. Gryseels, B., Polman, K., Clerinx, J. & Kestens, L. Human schistosomiasis. *Lancet* **368**, 1106–1124 (2006).
15. Lu, X. T. *et al.* Snail-borne parasitic diseases: An update on global epidemiological distribution, transmission interruption and control methods. *Infect Dis Poverty* **7**, 1–16 (2018).
16. Centers for Disease Control and Prevention. Schistosomiasis - Prevention and control. dc.gov/parasites/schistosomiasis/prevent.html (2020).
17. Koukounari, A. *et al.* The impact of single versus mixed schistosome species infections on liver, spleen and bladder morbidity within Malian children pre- and post-praziquantel treatment. *BMC Infect Dis* **10**, 1–14 (2010).
18. Gouvras, A. N. *et al.* The impact of single versus mixed *Schistosoma haematobium* and *S. mansoni* infections on morbidity profiles amongst school-children in Taveta, Kenya. *Acta Trop* **128**, 309–317 (2013).
19. WHO - World Health Organization. Schistosomiasis - Key facts. <https://www.who.int/news-room/fact-sheets/detail/schistosomiasis> (2023).

20. Grimes, J. E. *et al.* The roles of water, sanitation and hygiene in reducing schistosomiasis: A review. *Parasit Vectors* **8**, (2015).
21. WHO TEAM & Control of Neglected Tropical Diseases. *Ending the Neglect to Attain the Sustainable Development Goals: A Road Map for Neglected Tropical Diseases 2021–2030*. (2021).
22. Grimes, J. E. *et al.* The roles of water, sanitation and hygiene in reducing schistosomiasis: A review. *Parasit Vectors* **8**, (2015).
23. Nojima, H. & Sato, A. *Schistosoma mansoni* and *Schistosoma haematobium*: Emergence of schistosome cercariae from snails with darkness and illumination. *Exp Parasitol* **53**, 189–198 (1982).
24. Haas, W., Haeberlein, S., Behring, S. & Zoppelli, E. *Schistosoma mansoni*: Human skin ceramides are a chemical cue for host recognition of cercariae. *Exp Parasitol* **120**, 94–97 (2008).
25. Haeberlein, S. & Haas, W. Chemical attractants of human skin for swimming *Schistosoma mansoni* cercariae. *Parasitol Res* **102**, 657–662 (2008).
26. Brachs, S. & Haas, W. Swimming behaviour of *Schistosoma mansoni* cercariae: responses to irradiance changes and skin attractants. *Parasitol Res* **102**, 685–690 (2008).
27. Hockley, D. J. & McLaren, D. J. *Schistosoma mansoni*: Changes in the outer membrane of the tegument during development from cercaria to adult worm. *Int J Parasitol* **3**, 13–25 (1973).
28. Nelwan, M. L. Schistosomiasis: Life Cycle, Diagnosis, and Control. *Current Therapeutic Research* **91**, 5–9 (2019).
29. Nation, C. S., Da'dara, A. A., Marchant, J. K. & Skelly, P. J. Schistosome migration in the definitive host. *PLoS Negl Trop Dis* **14**, 1–12 (2020).
30. Colley, D. G., Bustinduy, A. L., Secor, W. E. & King, C. H. Human schistosomiasis. *The Lancet* **383**, 2253–2264 (2014).
31. Lackey, E. K. & Horrall, S. *Schistosomiasis*. (StatPearls Publishing, 2022).
32. Khalaf, I., Shokeir, A. & Shalaby, M. Urologic complications of genitourinary schistosomiasis. *World J Urol* **30**, 31–38 (2012).
33. Hotez, P. J., Engels, D., Gyapong, M., Ducker, C. & Malecela, M. N. Female Genital Schistosomiasis. *New England Journal of Medicine* **381**, 2493–2495 (2019).
34. Mazigo, H. D. *et al.* 'Female genital schistosomiasis is a sexually transmitted disease': Gaps in healthcare workers' knowledge about female genital schistosomiasis in Tanzania. *PLOS Global Public Health* **2**, e0000059 (2022).
35. Kjetland, E. F., Leutscher, P. D. C. & Ndhlovu, P. D. A review of female genital schistosomiasis. *Trends Parasitol* **28**, 58–65 (2012).
36. Kayuni, S. *et al.* A systematic review with epidemiological update of male genital schistosomiasis (MGS): A call for integrated case management across the health system in sub-Saharan Africa. *Parasite Epidemiol Control* **4**, e00077 (2019).
37. Christinet, V., Lazdins-Helds, J. K., Stothard, J. R. & Reinhard-Rupp, J. Female genital schistosomiasis (FGS): From case reports to a call for concerted action against this neglected gynaecological disease. *Int J Parasitol* **46**, 395–404 (2016).

38. Vale, N. *et al.* Praziquantel for schistosomiasis: Single-drug metabolism revisited, mode of action, and resistance. *Antimicrob Agents Chemother* **61**, (2017).
39. Cioli, D., Pica-Mattoccia, L., Basso, A. & Guidi, A. Schistosomiasis control: Praziquantel forever? *Mol Biochem Parasitol* **195**, 23–29 (2014).
40. Utzinger, J., Becker, S. L., van Lieshout, L., van Dam, G. J. & Knopp, S. New diagnostic tools in schistosomiasis. *Clinical Microbiology and Infection* **21**, 529–542 (2015).
41. Hoekstra, P. T., van Dam, G. J. & van Lieshout, L. Context-Specific Procedures for the Diagnosis of Human Schistosomiasis – A Mini Review. *Frontiers in Tropical Diseases* **2**, 1–10 (2021).
42. Weerakoon, K. G. A. D., Gobert, G. N., Cai, P. & McManus, D. P. Advances in the diagnosis of human schistosomiasis. *Clin Microbiol Rev* **28**, 939–967 (2015).
43. Hinz, R., Schwarz, N. G., Hahn, A. & Frickmann, H. Serological approaches for the diagnosis of schistosomiasis – A review. *Mol Cell Probes* **31**, 2–21 (2017).
44. Bergwerff, A. A. *et al.* The immunologically reactive part of immunopurified circulating anodic antigen from *Schistosoma mansoni* is a threonine-linked polysaccharide consisting of $\rightarrow 6$ -(β -D-GlcpA-(1 $\rightarrow$ 3))- β -D-GalpNAc-(1 $\rightarrow$ repeating units. *Journal of Biological Chemistry* **269**, 31510–31517 (1994).
45. Van Dam, G. J. *et al.* The immunologically reactive O-Linked polysaccharide chains derived from Circulating Cathodic Antigen isolated from the human blood fluke *Schistosoma mansoni* have Lewis X as repeating unit. *Eur J Biochem* **225**, 467–482 (1994).
46. Bassily, S., Mansour, M. M., Deelder, A. M., van Lieshout, L. & de Jonge, N. Assessment of cure in schistosomiasis patients after chemotherapy with Praziquantel by quantitation of Circulating Anodic Antigen (CAA) in urine. *Am J Trop Med Hyg* **44**, 323–328 (1991).
47. Kildemoes, A. O. *et al.* Rapid clearance of *Schistosoma mansoni* circulating cathodic antigen after treatment shown by urine strip tests in a Ugandan fishing community – Relevance for monitoring treatment efficacy and re-infection. *PLoS Negl Trop Dis* **11**, e0006054 (2017).
48. Corstjens, P. L. A. M. *et al.* Circulating anodic antigen (CAA): A highly sensitive diagnostic biomarker to detect active *Schistosoma* infections - improvement and use during SCORE. *American Journal of Tropical Medicine and Hygiene* **103**, 50–57 (2020).
49. Van Dam, G. J. *et al.* Diagnosis of schistosomiasis by reagent strip test for detection of circulating cathodic antigen. *J Clin Microbiol* **42**, 5458–5461 (2004).
50. Corstjens, P. L. A. M. *et al.* Tools for diagnosis, monitoring and screening of *Schistosoma* infections utilizing lateral-flow based assays and upconverting phosphor labels. *Parasitology* **141**, 1841–1855 (2014).
51. Casacuberta-Partal, M. *et al.* Specificity of the Point-of-Care urine strip test for *Schistosoma* Circulating Cathodic Antigen (POC-CCA) tested in non-endemic pregnant women and young children. *Am J Trop Med Hyg* **104**, 1412–1417 (2021).
52. FIND. Rapid test for precision mapping, and monitoring and evaluation of schistosomiasis control. <https://www.finddx.org/what-we-do/projects/rapid-test-for-precision-mapping-and-monitoring-and-evaluation-of-schistosomiasis-control-programmes/> (2021).
53. De Dood, C. J. *et al.* Refining diagnosis of *Schistosoma* haematobium infections: Antigen and antibody detection in urine. *Front Immunol* **9**, 1–9 (2018).

54. Ashton, R. A. *et al.* Accuracy of circulating cathodic antigen tests for rapid mapping of *Schistosoma mansoni* and *S. haematobium* infections in Southern Sudan. *Tropical Medicine & International Health* **16**, 1099–1103 (2011).
55. Midzi, N. *et al.* Use of circulating cathodic antigen strips for the diagnosis of urinary schistosomiasis. *Trans R Soc Trop Med Hyg* **103**, 45–51 (2009).
56. Hoekstra, P. T. *et al.* Diagnosis of Schistosomiasis without a microscope: evaluating Circulating Antigen (CCA, CAA) and DNA detection methods on banked samples of a community-based survey from DR Congo. *Trop Med Infect Dis* **7**, 315 (2022).
57. Rubaba, O., Chimbari, M. J., Soko, W., Manyangadze, T. & Mukaratirwa, S. Validation of a urine Circulating Cathodic Antigen cassette test for detection of *Schistosoma haematobium* in uMkhanyakude district of South Africa. *Acta Trop* **182**, 161–165 (2018).
58. Sanneh, B. *et al.* Field evaluation of a schistosome Circulating Cathodic Antigen rapid test kit at Point-Of-Care for mapping of schistosomiasis endemic districts in The Gambia. *PLoS One* **12**, e0182003 (2017).
59. Obeng, B. B. *et al.* Application of a Circulating-Cathodic-Antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of *Schistosoma haematobium* in urine samples from Ghana. *Ann Trop Med Parasitol* **102**, 625–633 (2008).
60. Pearson, R. D. MSD Manual - Filarial Worm Infections Overview. <https://www.msdmanuals.com/home/infections/parasitic-infections-nematodes-roundworms/filarial-worm-infections-overview>.
61. WHO. Lymphatic filariasis - Key Facts. <http://www.who.int/mediacentre/factsheets/fs102/en/> (2014).
62. Tangella, K. Serous Cavity Filariasis. <https://www.dovemed.com/diseases-conditions/serous-cavity-filariasis> (2022).
63. Nutman, T. B. & Kazura, J. W. Chapter 104 Lymphatic Filariasis. in *Tropical Infectious Diseases: Principles, Pathogens and Practice* 729–734 (Elsevier Inc., 2011). doi:10.1016/B978-0-7020-3935-5.00104-X.
64. CDC. Lymphatic filariasis. <https://www.cdc.gov/parasites/lymphaticfilariasis/> (2019).
65. World Health Organization. *Ending the Neglect to Attain the Sustainable Development Goals: A Road Map for Neglected Tropical Diseases 2021-2030*. <https://www.who.int/publications/i/item/9789240010352> (2020).
66. Taylor, M. J., Hoerauf, A. & Bockarie, M. Lymphatic filariasis and onchocerciasis. *The Lancet* **376**, 1175–1185 (2010).
67. Nutman, T. B. Insights into the Pathogenesis of Disease in Human Lymphatic Filariasis. *Lymphat Res Biol* **11**, 144–148 (2013).
68. Dreyer, G., Norões, J., Figueredo-Silva, J. & Piessens, W. F. Pathogenesis of lymphatic disease in bancroftian filariasis: a clinical perspective. *Parasitology Today* **16**, 544–548 (2000).
69. Brattig, N. W., Cheke, R. A. & Garms, R. Onchocerciasis (river blindness) – more than a century of research and control. *Acta Trop* **218**, (2021).
70. Lazdins-Helds, J. K., Remme, J. H. F. & Boakye, B. Focus: Onchocerciasis. *Nat Rev Microbiol* **1**, 178–179 (2003).

71. Forrester, J. V., Dick, A. D., McMenamin, P. G., Roberts, F. & Pearlman, E. Chapter 8 - Microbial infections of the eye. in *The Eye* 462-485.e1 (Elsevier, 2016). doi:10.1016/B978-0-7020-5554-6.00008-3.
72. Center for Disease Control and Prevention. CDC - Loiasis. <https://www.cdc.gov/parasites/loiasis/> (2023).
73. Jacobsen, K. H. *et al.* A call for loiasis to be added to the WHO list of neglected tropical diseases. *Lancet Infect Dis* **22**, e299–e302 (2022).
74. Mediannikov, O. & Ranque, S. Mansonellosis, the most neglected human filariasis. *New Microbes New Infect* **26**, S19–S22 (2018).
75. Lima, N. F., Veggiani Aybar, C. A., Dantur Juri, M. J. & Ferreira, M. U. *Mansonella ozzardi* : a neglected New World filarial nematode. *Pathog Glob Health* **110**, 97–107 (2016).
76. Ritter, M. *et al.* *Mansonella perstans* microfilaremic individuals are characterized by enhanced type 2 helper T and regulatory T and B cell subsets and dampened systemic innate and adaptive immune responses. *PLoS Negl Trop Dis* **12**, e0006184 (2018).
77. Boussinesq, M. *et al.* Clinical picture, epidemiology and outcome of *Loa*-associated serious adverse events related to mass ivermectin treatment of onchocerciasis in Cameroon. *Filaria J* **2**, 1–13 (2003).
78. Padgett, J. J. & Jacobsen, K. H. Loiasis: African eye worm. *Trans R Soc Trop Med Hyg* **102**, 983–989 (2008).
79. Vinkeles Melchers, N. V. S. *et al.* Projected number of people with onchocerciasis-loiasis coinfection in Africa, 1995 to 2025. *Clinical Infectious Diseases* **70**, 2281–2289 (2020).
80. Klion, A. D., Vijaykumar, A., Oei, T., Martin, B. & Nutman, T. B. Serum immunoglobulin G4 antibodies to the recombinant antigen, LL-SXP-1, are highly specific for *Loa loa* infection. *J Infect Dis* **187**, 128–161 (2003).
81. Kemp, C. & Roberts, A. Infectious Diseases: Filariasis - Bancroftian filariasis, Malayan filariasis, Loiasis (*Loa loa*), Onchocerciasis (river blindness). *J Am Acad Nurse Pract* **13**, 391–394 (2005).
82. Kelly-Hope, L. A., Thomas, B. C., Bockarie, M. J. & Molyneux, D. H. Lymphatic filariasis in the Democratic Republic of Congo; Micro-stratification overlap mapping (MOM) as a prerequisite for control and surveillance. *Parasit Vectors* **4**, (2011).
83. Akue, J. P. *et al.* Epidemiology of concomitant infection due to *Loa loa* and *Mansonella perstans* in Gabon. *PLoS Negl Trop Dis* **5**, (2011).
84. Mommers, E. C., Dekker, H. S., Richard, P., Garica, A. & Chippaux, J. P. Prevalence of *L. loa* and *M. perstans* filariasis in southern Cameroon. *Trop Geogr Med* **47**, 2–5 (1995).
85. Noireau, F., Carme, B., Apembet, J. D. & Gouteux, J. P. *Loa loa* and *Mansonella perstans* filariasis in the Chaillu mountains, Congo: parasitological prevalence. *Trans R Soc Trop Med Hyg* **83**, 529–534 (1989).
86. Centers for Disease Control and Prevention. Lymphatic Filariasis - Diagnosis. <https://www.cdc.gov/parasites/lymphaticfilariasis/diagnosis.html> (2018).
87. Centers for Disease Control and Prevention. Onchocerciasis - Diagnosis. <https://www.cdc.gov/parasites/onchocerciasis/diagnosis.html> (2019).

88. Centers for Disease Control and Prevention. Mansonellosis. <https://www.cdc.gov/dpdx/mansonellosis/index.html> (2019).
89. Centers for Disease Control and Prevention. Loiasis - Diagnosis. <https://www.cdc.gov/parasites/loiasis/diagnosis.html> (2015).
90. Pantelias, A., King, J. D., Lammie, P. & Weil, G. J. Development and Introduction of the Filariasis Test Strip: A New Diagnostic Test for the Global Program to Eliminate Lymphatic Filariasis. *Am J Trop Med Hyg* **106**, 56–60 (2022).
91. Steel, C. *et al.* Rapid *Wuchereria bancrofti*-specific antigen Wb123-based IgG4 immunoassays as tools for surveillance following mass drug administration programs on lymphatic filariasis. *Clinical and Vaccine Immunology* **20**, 1155–1161 (2013).
92. Nana-Djeunga, H. C. *et al.* Changes in Onchocerciasis Ov16 IgG4 Rapid Diagnostic Test Results Over One-Month Follow-up: Lessons for Reading Timeframe and Decision-Making. *American Journal of Tropical Medicine and Hygiene* **107**, 658–661 (2022).
93. Dolo, H. *et al.* Integrated seroprevalence-based assessment of *Wuchereria bancrofti* and *Onchocerca volvulus* in two lymphatic filariasis evaluation units of Mali with the SD Bioline Onchocerciasis/LF IgG4 Rapid Test. *PLoS Negl Trop Dis* **13**, (2019).
94. Lourens, G. B. & Ferrell, D. K. Lymphatic filariasis. *Nursing Clinics of North America* **54**, 181–192 (2019).
95. Wanji, S. *et al.* Mapping of lymphatic filariasis in loiasis areas: A new strategy shows no evidence for *Wuchereria bancrofti* endemicity in Cameroon. *PLoS Negl Trop Dis* **13**, 1–15 (2018).
96. Poole, C. B. *et al.* In Silico Identification of Novel Biomarkers and Development of New Rapid Diagnostic Tests for the Filarial Parasites *Mansonella perstans* and *Mansonella ozzardi*. *Sci Rep* **9**, (2019).
97. Amambo, G. N. *et al.* Application of loop mediated isothermal amplification (LAMP) assays for the detection of *Onchocerca volvulus*, *Loa loa* and *Mansonella perstans* in humans and vectors. *Frontiers in Tropical Diseases* **3**, (2023).
98. Osei-atweneboana, M. Y. *et al.* Phenotypic evidence of emerging ivermectin resistance in *Onchocerca volvulus*. *PLoS Negl Trop Dis* **5**, 1–11 (2011).
99. Prichard, R. K. Anthelmintic resistance in nematodes: extent, recent understanding and future directions for control and research. *Int J Parasitol* **20**, 515–523 (1990).
100. Cioli, D., Pica-Mattoccia, L., Basso, A. & Guidi, A. Schistosomiasis control: praziquantel forever? *Mol Biochem Parasitol* **195**, 23–29 (2014).
101. Taylor, D. W., Makepeace, B. L. & Lustigman, S. TOVA initiative. <https://www.riverblindnessvaccinetova.org/project/vaccine> (2020).
102. Hotez, P. J. *et al.* The Onchocerciasis Vaccine for Africa—TOVA—Initiative. *PLoS Negl Trop Dis* **9**, (2015).
103. Lustigman, S. *et al.* *Onchocerca volvulus*: The Road from Basic Biology to a Vaccine. *Trends Parasitol* **34**, 64–79 (2018).
104. Kalyanasundaram, R., Khatri, V. & Chauhan, N. Advances in Vaccine Development for Human Lymphatic Filariasis. *Trends Parasitol* **36**, 195–205 (2020).

105. Murthy, P. K., Tewari, P., Mandal, P. & Kushwaha, V. Immune response profiling of cocktails of *Brugia malayi* vaccine candidates DIM-1, Calponin and Troponin 1 in BALB/c mice. *Acta Parasitol* **68**, 929–936 (2023).
106. Molehin, A. J., McManus, D. P. & You, H. Vaccines for Human Schistosomiasis: Recent Progress, New Developments and Future Prospects. *Int J Mol Sci* **23**, (2022).
107. Zhang, W. *et al.* Sm-p80-based schistosomiasis vaccine: Double-blind preclinical trial in baboons demonstrates comprehensive prophylactic and parasite transmission-blocking efficacy. *Ann N Y Acad Sci* **1425**, 38–51 (2018).
108. Keitel, W. A. *et al.* A phase 1 study of the safety, reactogenicity, and immunogenicity of a *Schistosoma mansoni* vaccine with or without glucopyranosyl lipid A aqueous formulation (GLA-AF) in healthy adults from a non-endemic area. *Vaccine* **37**, 6500–6509 (2019).
109. Riveau, G. *et al.* Safety and immunogenicity of rSh28GST antigen in humans: Phase 1 randomized clinical study of a vaccine candidate against urinary schistosomiasis. *PLoS Negl Trop Dis* **6**, (2012).
110. Santini-Oliveira, M. *et al.* Schistosomiasis vaccine candidate Sm14/GLA-SE: Phase 1 safety and immunogenicity clinical trial in healthy, male adults. *Vaccine* **34**, 586–594 (2016).
111. McManus, D. P. *et al.* Schistosomiasis—from immunopathology to vaccines. *Semin Immunopathol* **42**, 355–371 (2020).
112. Janse, J. J. *et al.* Establishing the production of male *Schistosoma mansoni* cercariae for a controlled human infection model. *Journal of Infectious Diseases* **218**, 1142–1146 (2018).
113. Gass, K. Time for a diagnostic sea-change: Rethinking neglected tropical disease diagnostics to achieve elimination. *PLoS Negl Trop Dis* **14**, 1–6 (2020).
114. Gazzinelli-Guimaraes, P. H. & Nutman, T. B. Helminth parasites and immune regulation. *F1000Res* **7**, (2018).
115. Nutman, T. B. Looking beyond the induction of Th2 responses to explain immunomodulation by helminths. *Parasite Immunol* **37**, 304–313 (2015).
116. Dunne, D. W. & Cooke, A. A worm's eye view of the immune system: consequences for evolution of human autoimmune disease. *Nat Rev Immunol* **5**, 420–426 (2005).
117. Babu, S. & Nutman, T. B. Proinflammatory Cytokines Dominate the Early Immune Response to Filarial Parasites. *The Journal of Immunology* **171**, 6723–6732 (2003).
118. Bhoj, P., Togle, N., Khatri, V. & Goswami, K. Harnessing Immune Evasion Strategy of Lymphatic Filariae: A Therapeutic Approach against Inflammatory and Infective Pathology. *Vaccines (Basel)* **10**, (2022).
119. Gobbi, F. *et al.* New Insights on Acute and Chronic Schistosomiasis: Do We Need a Redefinition? *Trends Parasitol* **36**, 660–667 (2020).
120. Gieseck, R. L., Wilson, M. S. & Wynn, T. A. Type 2 immunity in tissue repair and fibrosis. *Nat Rev Immunol* **18**, 62–76 (2018).
121. Porthouse, K. H., Chirgwin, S. R., Coleman, S. U., Taylor, H. W. & Klei, T. R. Inflammatory responses to migrating *Brugia pahangi* third-stage larvae. *Infect Immun* **74**, 2366–2372 (2006).

122. Colley, D. G. & Secor, W. E. Immunology of human schistosomiasis. *Parasite Immunol* **36**, 347–357 (2014).
123. Babu, S. & Nutman, T. B. Immunology of lymphatic filariasis. *Parasite Immunol* **36**, 338–346 (2014).
124. Dafa'alla, T. H., Ghalib, H. W., Abdelmageed, A. & Williams, J. F. The profile of IgG and IgG subclasses of onchocerciasis patients. *Clin Exp Immunol* **88**, 258–263 (1992).
125. Ottesen, E. A., Skvaril, F., Tripathy, S. P., Poindexter, R. W. & Hussain, R. Prominence of IgG4 in the IgG antibody response to human filariasis. *J Immunol* **134**, 2707–12 (1985).
126. Allen, J. E. & Maizels, R. M. Diversity and dialogue in immunity to helminths. *Nat Rev Immunol* **11**, 375–388 (2011).
127. Nutman, T. B., Kumaraswami, V. & Ottesen, E. A. Parasite-specific anergy in human filariasis. Insights after analysis of parasite antigen-driven lymphokine production. *Journal of Clinical Investigation* **79**, 1516–1523 (1987).
128. Ottesen, E. A., Hiatt, R. A., Cheever, A. W., Sotomayor, Z. R. & Neva, F. A. The acquisition and loss of antigen-specific cellular immune responsiveness in acute and chronic schistosomiasis in man. *Clin Exp Immunol* **33**, 37–47 (1978).
129. Stadecker, M. J. The role of T-cell anergy in the immunomodulation of schistosomiasis. *Parasitology Today* **8**, 199–204 (1992).
130. Ottesen, E. A., Weller, P. F. & Heck, L. Specific cellular immune unresponsiveness in human filariasis. *Immunology* **33**, 413 (1977).
131. Smith, P. *et al.* *Schistosoma mansoni* Worms Induce Anergy of T Cells via Selective Up-Regulation of Programmed Death Ligand 1 on Macrophages 1. *The Journal of Immunology* **173**, 1240–1248 (2004).
132. Maizels, R. M., Pearce, E. J., Artis, D., Yazdanbakhsh, M. & Wynn, T. A. Regulation of pathogenesis and immunity in helminth infections. in *Journal of Experimental Medicine* vol. 206 2059–2066 (2009).
133. Angeles, J. M. M., Mercado, V. J. P. & Rivera, P. T. Behind enemy lines: immunomodulatory armamentarium of the schistosome parasite. *Front Immunol* **11**, (2020).
134. Everts, B., Smits, H. H., Hokke, C. H. & Yazdanbakhsh, M. Helminths and dendritic cells: Sensing and regulating via pattern recognition receptors, Th2 and Treg responses. *Eur J Immunol* **40**, 1525–1537 (2010).
135. Tesmer, L. A., Lundy, S. K., Sarkar, S. & Fox, D. A. Th17 cells in human disease. *Immunol Rev* **223**, 87–113 (2008).
136. Rutitzky, L. I., Smith, P. M. & Stadecker, M. J. T-bet protects against exacerbation of schistosome egg-induced immunopathology by regulating Th17-mediated inflammation. *Eur J Immunol* **39**, 2470–2481 (2009).
137. Mbow, M. *et al.* T-helper 17 cells are associated with pathology in human schistosomiasis. *Journal of Infectious Diseases* **207**, 186–195 (2013).
138. Katawa, G. *et al.* Hyperreactive Onchocerciasis is Characterized by a Combination of Th17-Th2 Immune Responses and Reduced Regulatory T Cells. *PLoS Negl Trop Dis* **9**, (2015).

139. Babu, S. *et al.* Filarial lymphedema is characterized by antigen-specific Th1 and Th17 proinflammatory responses and a lack of regulatory T cells. *PLoS Negl Trop Dis* **3**, (2009).
140. Adjobimey, T. & Hoerauf, A. Induction of immunoglobulin G4 in human filariasis: An indicator of immunoregulation. *Ann Trop Med Parasitol* **104**, 455–464 (2010).
141. Prodjinotho, U. F. *et al.* Pathological manifestations in lymphatic filariasis correlate with lack of inhibitory properties of IgG4 antibodies on IgE-activated granulocytes. *PLoS Negl Trop Dis* **11**, 1–25 (2017).
142. Kurniawan, A. *et al.* Differential expression of IgE and IgG4 specific antibody responses in asymptomatic and chronic human filariasis. *J Immunol* **150**, 3941–50 (1993).
143. Ravindran, B., Satapathy, A. K., Sahoo, P. K. & Mohanty, M. C. Protective immunity in human lymphatic filariasis: problems and prospects. *Med Microbiol Immunol* **192**, 41–6 (2003).
144. Nutman, T. B. & Kazura, J. W. *Lymphatic Filariasis. Tropical Infectious Diseases: Principles, Pathogens and Practice* (Elsevier, 2011). doi:10.1142/p048.
145. Sartono, E., Kruize, Y., Kurniawan, A., Maizels, R. & Yazdanbakhsh, M. Depression of antigen-specific interleukin-5 and interferon-gamma responses in human lymphatic filariasis as a function of clinical status and age. *J Infect Dis* **175**, 1276–1280 (1996).
146. Anuradha, R. *et al.* Circulating microbial products and acute phase proteins as markers of pathogenesis in lymphatic filarial disease. *PLoS Pathog* **8**, 1–9 (2012).
147. Anuradha, R. *et al.* IL-4–, TGF- β –, and IL-1–Dependent Expansion of Parasite Antigen-Specific Th9 Cells Is Associated with Clinical Pathology in Human Lymphatic Filariasis. *The Journal of Immunology* **191**, 2466–2473 (2013).
148. Acharya, S., Da'dara, A. A. & Skelly, P. J. Schistosome immunomodulators. *PLoS Pathog* **17**, (2021).
149. Skelly, P. J. & Alan Wilson, R. Making Sense of the Schistosome Surface. *Adv Parasitol* **63**, 185–284 (2006).
150. Hambrook, J. R. & Hanington, P. C. Immune Evasion Strategies of Schistosomes. *Front Immunol* **11**, (2021).
151. Smithers, S. R., Terry, R. J. & Hockley, D. J. Host antigens in schistosomiasis. *Proc R Soc Lond B Biol Sci* **171**, 483–494 (1969).
152. Bayne, C. J., Boswell, C. A. & Yui, M. A. Widespread antigenic cross-reactivity between plasma proteins of a gastropod, and its trematode parasite. *Dev Comp Immunol* **11**, 321–329 (1987).
153. Harnett, W. Secretory products of helminth parasites as immunomodulators. *Mol Biochem Parasitol* **195**, 130–136 (2014).
154. Hoerauf, A., Satoguina, J., Saeftel, M. & Specht, S. Immunomodulation by filarial nematodes. *Parasite Immunol* **27**, 417–429 (2005).
155. Van der Kleij, D. *et al.* A novel host-parasite lipid cross-talk. Schistosomal lyso-phosphatidylserine activates toll-like receptor 2 and affects immune polarization. *Journal of Biological Chemistry* **277**, 48122–48129 (2002).
156. Thomas, P. G. *et al.* Maturation of Dendritic Cell 2 Phenotype by a Helminth Glycan Uses a Toll-Like Receptor 4-Dependent Mechanism. *The Journal of Immunology* **171**, 5837–5841 (2003).

157. Al-Riyami, L. & Harnett, W. Immunomodulatory Properties of ES-62, a Phosphorylcholine - Containing Glycoprotein Secreted by *Acanthocheilonema viteae*. *Endocrine, Metabolic & Immune Disorders-Drug Targets* **12**, 45–52 (2012).
158. Buitrago, G., Duncombe-Moore, J., Harnett, M. M. & Harnett, W. Mini Review: Structure and Function of Nematode Phosphorylcholine-Containing Glycoconjugates. *Frontiers in Tropical Diseases* **2**, (2021).
159. Ahmed, U. K. *et al.* The carbohydrate-linked phosphorylcholine of the parasitic nematode product ES-62 modulates complement activation. *Journal of Biological Chemistry* **291**, 11939–11953 (2016).
160. Harnett, W. & Harnett, M. M. Immunomodulatory Activity and Therapeutic Potential of the Filarial Nematode Secreted Product, ES-62. *Adv Exp Med Biol* **666**, 88–94 (2009).
161. Harnett, W. & Harnett, M. M. Modulation of the host immune system by phosphorylcholine-containing glycoproteins secreted by parasitic filarial nematodes. *Biochim Biophys Acta Mol Cell Res* **1539**, 7–15 (2001).
162. Everts, B. *et al.* Schistosoma-derived omega-1 drives Th2 polarization by suppressing protein synthesis following internalization by the mannose receptor. *Journal of Experimental Medicine* **209**, 1753–1767 (2012).
163. Kaur, I. *et al.* Interleukin-4-inducing principle from *Schistosoma mansoni* eggs contains a functional C-terminal nuclear localization signal necessary for nuclear translocation in mammalian cells but not for its uptake. *Infect Immun* **79**, 1779–1788 (2011).
164. Everts, B. *et al.* Omega-1, a glycoprotein secreted by *Schistosoma mansoni* eggs, drives Th2 responses. *Journal of Experimental Medicine* **206**, 1673–1680 (2009).
165. Knuhr, K. *et al.* *Schistosoma mansoni* egg-released IPSE/alpha-1 dampens inflammatory cytokine responses via basophil interleukin (IL)-4 and IL-13. *Front Immunol* **9**, (2018).
166. Wu, C. *et al.* *Schistosoma japonicum* Egg Specific Protein SjE16.7 Recruits Neutrophils and Induces Inflammatory Hepatic Granuloma Initiation. *PLoS Negl Trop Dis* **8**, (2014).
167. Chuah, C. *et al.* Defining a pro-inflammatory neutrophil phenotype in response to schistosome eggs. *Cell Microbiol* **16**, 1666–1677 (2014).
168. Rumbley, C. A. *et al.* Activated Eosinophils Are the Major Source of Th2-Associated Cytokines in the Schistosome Granuloma. *The Journal of Immunology* **162**, 1003–1009 (1999).
169. Wilson, M. S. *et al.* Immunopathology of Schistosomiasis. *Immunol Cell Biol* **85**, 148–154 (2007).
170. Coakley, G. *et al.* Extracellular Vesicles from a Helminth Parasite Suppress Macrophage Activation and Constitute an Effective Vaccine for Protective Immunity. *Cell Rep* **19**, 1545–1557 (2017).
171. Drurey, C. & Maizels, R. M. Helminth extracellular vesicles: Interactions with the host immune system. *Mol Immunol* **137**, 124–133 (2021).
172. Buck, A. H. *et al.* Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity. *Nat Commun* **5**, (2014).
173. Kuipers, M. E. *et al.* Life stage-specific glycosylation of extracellular vesicles from *Schistosoma mansoni* schistosomula and adult worms drives differential interaction with C-type lectin receptors DC-SIGN and MGL. *Front Mol Biosci* **10**, (2023).

174. Kuipers, M. E. *et al.* DC-SIGN mediated internalisation of glycosylated extracellular vesicles from *Schistosoma mansoni* increases activation of monocyte-derived dendritic cells. *J Extracell Vesicles* **9**, (2020).
175. Sotillo, J. *et al.* Extracellular vesicles secreted by *Schistosoma mansoni* contain protein vaccine candidates. *Int J Parasitol* **46**, 1–5 (2016).
176. Montaña, K. J., Loukas, A. & Sotillo, J. Proteomic approaches to drive advances in helminth extracellular vesicle research. *Mol Immunol* **131**, 1–5 (2021).
177. Mekonnen, G. G. *et al.* *Schistosoma haematobium* extracellular vesicle proteins confer protection in a heterologous model of schistosomiasis. *Vaccines (Basel)* **8**, 1–20 (2020).
178. Abou-El-Naga, I. F. Emerging roles for extracellular vesicles in *Schistosoma* infection. *Acta Trop* **232**, 106467 (2022).
179. Wang, L. *et al.* Exosome-like vesicles derived by *Schistosoma japonicum* adult worms mediates M1 type immune- activity of macrophage. *Parasitol Res* **114**, 1865–1873 (2015).
180. Liu, J. *et al.* *Schistosoma japonicum* extracellular vesicle miRNA cargo regulates host macrophage functions facilitating parasitism. *PLoS Pathog* **15**, (2019).
181. Howe, K. L., Bolt, B. J., Shafie, M., Kersey, P. & Berriman, M. WormBase ParaSite – a comprehensive resource for helminth genomics. *Mol Biochem Parasitol* **215**, 2–10 (2017).
182. Doyle, S. R. Improving helminth genome resources in the post-genomic era. *Trends Parasitol* **38**, 831–840 (2022).
183. Chung, M. *et al.* Multispecies Transcriptomics Data Set of *Brugia malayi*, Its *Wolbachia* Endosymbiont wBm, and *Aedes aegypti* across the *B. malayi* Life Cycle. *Microbiol Resour Announc* **7**, (2018).
184. Wangiwatsin, A. *et al.* Transcriptome of the parasitic flatworm *Schistosoma mansoni* during intra-mammalian development. *PLoS Negl Trop Dis* **14**, 1–25 (2020).
185. Buddenborg, S. K., Lu, Z., Sankaranarayan, G., Doyle, S. R. & Berriman, M. The stage- and sex-specific transcriptome of the human parasite *Schistosoma mansoni*. *Sci Data* **10**, (2023).
186. Airs, P. M. *et al.* Spatial transcriptomics reveals antiparasitic targets associated with essential behaviors in the human parasite *Brugia malayi*. *PLoS Pathog* **18**, e1010399 (2022).
187. Choi, Y.-J. *et al.* A deep sequencing approach to comparatively analyze the transcriptome of lifecycle stages of the filarial worm, *Brugia malayi*. *PLoS Negl Trop Dis* **5**, e1409 (2011).
188. Sotillo, J. *et al.* In-depth proteomic characterization of *Schistosoma haematobium*: Towards the development of new tools for elimination. *PLoS Negl Trop Dis* **13**, (2019).
189. Sotillo, J., Pearson, M., Becker, L., Mulvenna, J. & Loukas, A. A quantitative proteomic analysis of the tegumental proteins from *Schistosoma mansoni* schistosomula reveals novel potential therapeutic targets. *Int J Parasitol* **45**, 505–516 (2015).
190. Kenney, E. T. *et al.* Differential Excretory/Secretory proteome of the adult female and male stages of the human blood fluke, *Schistosoma mansoni*. *Frontiers in Parasitology* **1**, (2022).
191. Winkelmann, F. *et al.* Comparative proteome analysis of the tegument of male and female adult *Schistosoma mansoni*. *Sci Rep* **12**, (2022).
192. Wang, T. *et al.* Proteomic analysis of the *Schistosoma mansoni* miracidium. *PLoS One* **11**, (2016).

193. Bennuru, S. *et al.* Stage-specific transcriptome and proteome analyses of the filarial parasite *Onchocerca volvulus* and its *Wolbachia* endosymbiont. *mBio* **7**, (2016).
194. Morris, C. P. *et al.* A Proteomic Analysis of the Body Wall, Digestive Tract, and Reproductive Tract of *Brugia malayi*. *PLoS Negl Trop Dis* **9**, (2015).
195. Moreno, Y. & Geary, T. G. Stage- and Gender-Specific Proteomic Analysis of *Brugia malayi* Excretory-Secretory Products. *PLoS Negl Trop Dis* **2**, e326 (2008).
196. Bennuru, S. *et al.* *Brugia malayi* excreted/secreted proteins at the host/parasite interface: Stage- and gender-specific proteomic profiling. *PLoS Negl Trop Dis* **3**, (2009).
197. Hewitson, J. P. *et al.* The secretome of the filarial parasite, *Brugia malayi*: Proteomic profile of adult excretory-secretory products. *Mol Biochem Parasitol* **160**, 8–21 (2008).
198. Hotterbeekx, A., Perneel, J., Vieri, M. K., Colebunders, R. & Kumar-Singh, S. The secretome of filarial nematodes and its role in host-parasite interactions and pathogenicity in Onchocerciasis-associated epilepsy. *Front Cell Infect Microbiol* **11**, 1–10 (2021).
199. Mersha, F. B. *et al.* Computational and experimental analysis of the glycoposphatidylinositol-anchored proteome of the human parasitic nematode *Brugia malayi*. *PLoS One* **14**, e0216849 (2019).
200. Zhu, L. *et al.* Molecular characterization of *S. japonicum* exosome-like vesicles reveals their regulatory roles in parasite-host interactions. *Sci Rep* **6**, (2016).
201. Nowacki, F. C. *et al.* Protein and small non-coding RNA-enriched extracellular vesicles are released by the pathogenic blood fluke *Schistosoma mansoni*. *J Extracell Vesicles* **4**, (2015).
202. Samoil, V. *et al.* Vesicle-based secretion in schistosomes: Analysis of protein and microRNA (miRNA) content of exosome-like vesicles derived from *Schistosoma mansoni*. *Sci Rep* **8**, (2018).
203. Harischandra, H., Yuan, W., Loghry, H. J., Zamanian, M. & Kimber, M. J. Profiling extracellular vesicle release by the filarial nematode *Brugia malayi* reveals sex-specific differences in cargo and a sensitivity to ivermectin. *PLoS Negl Trop Dis* **12**, 1–25 (2018).
204. Zamanian, M. *et al.* Release of Small RNA-containing Exosome-like Vesicles from the Human Filarial Parasite *Brugia malayi*. *PLoS Negl Trop Dis* **9**, (2015).
205. Zhu, S. *et al.* Release of extracellular vesicles containing small RNAs from the eggs of *Schistosoma japonicum*. *Parasit Vectors* **9**, 1–9 (2016).
206. Cummings, R. D. & Pierce, J. M. The Challenge and Promise of Glycomics. *Chem Biol* **21**, 1–15 (2014).
207. Varki, A. & Kornfeld, S. Historical Background and Overview. in *Essentials of Glycobiology* (ed. Cold Spring Harbor) (Cold Spring Harbor Laboratory Press, New York, 2022).
208. Hokke, C. H. & van Diepen, A. Helminth glycomics – glycan repertoires and host-parasite interactions. *Mol Biochem Parasitol* **215**, 47–57 (2017).
209. Nyame, A. K., Kwar, Z. S. & Cummings, R. D. Antigenic glycans in parasitic infections: Implications for vaccines and diagnostics. *Arch Biochem Biophys* **426**, 182–200 (2004).
210. Prasanphanich, N. S., Mickum, M. L., Heimburg-Molinaro, J. & Cummings, R. D. Glycoconjugates in host-helminth interactions. *Front Immunol* **4**, 1–22 (2013).

211. Taron, C. H. & Rudd, P. M. Glycomics: A rapidly evolving field with a sweet future. *NEB Expressions* 1–4 (2016).
212. Stanley, P., Moremen, K. W., Lewis, N. E., Taniguchi, N. & Aebi, M. N-Glycans. in *Essentials of Glycobiology* (ed. The Consortium of Glycobiology Editors) (Cold Spring Harbor Laboratory Press, New York, 2022). doi:10.1101/glycobiology.3e.009.
213. Tarentino, A. L. & Plummer, T. H. Enzymatic deglycosylation of asparagine-linked glycans: Purification, properties, and specificity of oligosaccharide-cleaving enzymes from *Flavobacterium meningosepticum*. in 44–57 (1994). doi:10.1016/0076-6879(94)30006-2.
214. Tretter, V., Altmann, F. & März, L. Peptide-N4-(N-acetyl- β -glucosaminyl)asparagine amidase F cannot release glycans with fucose attached $\alpha 1 \rightarrow 3$ to the asparagine-linked N-acetylglucosamine residue. *Eur J Biochem* **199**, 647–652 (1991).
215. Albrecht, S. *et al.* Comprehensive Profiling of Glycosphingolipid Glycans Using a Novel Broad Specificity Endoglycoceramidase in a High-Throughput Workflow. *Anal Chem* **88**, 4795–4802 (2016).
216. Varki, A., Cummings, R. & Esko, J. Glycosphingolipids. in *Essentials of Glycobiology* (ed. The Consortium of Glycobiology Editors) (Cold Spring Harbor Laboratory Press, New York, 2022). doi:10.1101/glycobiology.4e.11.
217. Brockhausen, I., Wandall, H. H., Ten Hagen, K. G. & Stanley, P. O-GalNAc Glycans. in *Essentials of Glycobiology* (ed. The Consortium of Glycobiology Editors) (Cold Spring Harbor Laboratory Press, New York, 2022). doi:10.1101/glycobiology.4e.10.
218. Saldoval, R. & Wilkinson, H. Current methods for the characterization of O-glycans. *J Proteome Res* **19**, 3890–3905 (2020).
219. Grabarics, M. *et al.* Mass Spectrometry-Based Techniques to Elucidate the Sugar Code. *Chemical Reviews Chem. Rev.* **122**, 8, 7840–7908 (2022).
220. Haslam, S. M. *et al.* Structural Analysis of Glycans. in *Essential of Glycobiology* (eds. Varki, A., Cummings, R. & Esko, J.) (Cold Spring Harbor Laboratory Press, New York, 2022). doi:10.1101/glycobiology.4e.50.
221. Riley, N. M., Bertozzi, C. R. & Pitteri, S. J. A Pragmatic Guide to Enrichment Strategies for Mass Spectrometry-Based Glycoproteomics. *Molecular & Cellular Proteomics* **20**, 100029 (2021).
222. Zauner, G., Deelder, A. M. & Wührer, M. Recent advances in hydrophilic interaction liquid chromatography (HILIC) for structural glycomics. *Electrophoresis* **32**, 3456–3466 (2011).
223. Wilson, I. B. H., Paschinger, K., Cummings, R. D. & Aebi, M. Nematoda. in *Essentials of glycobiology 4th Edition* (ed. Consortium of Glycobiology Editors) (Cold Spring Harbor Laboratory Press, Cold Spring Harbor (NY), La Jolla, California, 2022). doi:10.1101/glycobiology.4e.025.
224. Varki, A. & Kornfeld, S. Historical Background and Overview. in *Essentials of Glycobiology* (ed. Cold Spring Harbor) (Cold Spring Harbor Laboratory Press, NY, 2022). doi:10.1101/glycobiology.4e.1.
225. Smit, C. H. *et al.* Glycomic analysis of life stages of the human parasite *Schistosoma mansoni* reveals developmental expression profiles of functional and antigenic glycan motifs. *Molecular and Cellular Proteomics* **14**, 1750–1769 (2015).

226. Wuhrer, M. *et al.* Phosphocholine-containing, zwitterionic glycosphingolipids of adult *Onchocerca volvulus* as highly conserved antigenic structures of parasitic nematodes. *Biochemical Journal* **348**, 417–423 (2000).
227. Haslam, S. M. *et al.* Structural Studies of *N*-Glycans of Filarial Parasites - Conservation of phosphorylcholine-substituted glycans among species and discovery of novel chito-oligomers. *J Biol Chem* **274**, 20953–20960 (1999).
228. Schnaar, R. L. Glycobiology simplified: diverse roles of glycan recognition in inflammation. *J Leukoc Biol* **99**, 825–838 (2016).
229. Gagneux, P., Panin, V., Hennot, T., Aebi, M. & Varki, A. Evolution of Glycan Diversity. in *Essentials of Glycobiology* (ed. Essentials of Glycobiology [Internet]. 4th edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2022.) (New York, 2022). doi:10.1101/glycobiology.4e.20.
230. Martini, F. *et al.* Highly modified and immunoactive *N*-glycans of the canine heartworm. *Nat Commun* **10**, 1–18 (2019).
231. Reason, A. J. *et al.* Novel tyvelose-containing tri- and tetra-antennary *N*-glycans in the immunodominant antigens of the intracellular parasite *Trichinella spiralis*. *Glycobiology* **4**, 593–603 (1994).
232. Lochnit, G., Dennis, R. D. & Geyer, R. Phosphorylcholine substituents in nematodes: structures, occurrence and biological implications. *Biol Chem* **381**, 839–847 (2000).
233. Schiller, B., Hykollari, A., Yan, S., Paschinger, K. & Wilson, I. B. H. Complicated *N*-linked glycans in simple organisms. *Biol Chem* **393**, 661–673 (2012).
234. Khoo, K.-H., Huang, H.-H. & Lee, K.-M. Characteristic structural features of schistosome cercarial *N*-glycans: expression of Lewis X and core xylosylation. *Glycobiology* **11**, 149–163 (2001).
235. Hokke, C. H., Deelder, A. M., Hoffmann, K. F. & Wuhrer, M. Glycomics-driven discoveries in schistosome research. *Exp Parasitol* **117**, 275–283 (2007).
236. Khoo, K. H., Chatterjee, D., Caulfield, J. P., Morris, H. R. & Dell, A. Structural mapping of the glycans from the egg glycoproteins of *Schistosoma mansoni* and *Schistosoma japonicum*: identification of novel core structures and terminal sequences. *Glycobiology* **7**, 663–677 (1997).
237. van Die, I. & Cummings, R. D. Glycan gimmickry by parasitic helminths: A strategy for modulating the host immune response? *Glycobiology* **20**, 2–12 (2010).
238. Hirano, K. & Furukawa, K. Biosynthesis and Biological Significances of LacdiNAc Group on *N*- and *O*-Glycans in Human Cancer Cells. *Biomolecules* **12**, (2022).
239. Wuhrer, M., Koeleman, C. A. M., Deelder, A. M. & Hokke, C. H. Repeats of LacdiNAc and fucosylated LacdiNAc on *N*-glycans of the human parasite *Schistosoma mansoni*. *FEBS Journal* **273**, 347–361 (2006).
240. Wuhrer, M. *et al.* Gender-specific expression of complex-type *N*-glycans in schistosomes. *Glycobiology* **16**, 991–1006 (2006).
241. Meevissen, M. H. J. *et al.* Structural characterization of glycans on Omega-1, a major *Schistosoma mansoni* egg glycoprotein that drives Th2 responses. *J Proteome Res* **9**, 2630–2642 (2010).
242. Wuhrer, M. *et al.* IPSE/alpha-1, a major secretory glycoprotein antigen from schistosome eggs, expresses the Lewis X motif on core-difucosylated *N*-glycans. *FEBS Journal* **273**, 2276–2292 (2006).

243. Meevissen, M. H. J. *et al.* Targeted glycoproteomic analysis reveals that kappa-5 is a major, uniquely glycosylated component of *Schistosoma mansoni* egg antigens. *Molecular and Cellular Proteomics* **10**, (2011).
244. Nyame, A. K., Debose-Boyd, R., Long, T. D., Tsang, V. C. W. & Cummings, R. D. Expression of Le(X) antigen in *Schistosoma japonicum* and *S. haematobium* and immune responses to Le(X) in infected animals: Lack of Le(X) expression in other trematodes and nematodes. *Glycobiology* **8**, 615–624 (1998).
245. Khoo, K. H., Chatterjee, D., Caulfield, J. P., Morris, H. R. & Dell, A. Structural characterization of glycosphingolipids from the eggs of *Schistosoma mansoni* and *Schistosoma japonicum*. *Glycobiology* **7**, 653–661 (1997).
246. Rheinberg, C. E. *et al.* *Schistosoma haematobium*, *S. intercalatum*, *S. japonicum*, *S. mansoni*, and *S. rodhaini* in mice: relationship between patterns of lung migration by schistosomula and perfusion recovery of adult worms. *Parasitol Res* **84**, 338–342 (1998).
247. Berninsone, P. M. Carbohydrates and glycosylation. in *WormBook* (ed. The C. elegans Research Community) (2006). doi:10.1895/ wormbook.1.125.1.
248. The WormBase Consortium. WormBase ParaSite. <https://parasite.wormbase.org/species.html>.
249. Tawill, S., Le Goff, L., Ali, F., Blaxter, M. & Allen, J. E. Both Free-Living and Parasitic Nematodes Induce a Characteristic Th2 Response That Is Dependent on the Presence of Intact Glycans. *Infect Immun* **72**, 398–407 (2004).
250. North, S. J. *et al.* Site-specific glycoproteomic characterization of ES-62: The major secreted product of the parasitic worm *Acanthocheilonema viteae*. *Glycobiology* **29**, 562–571 (2019).
251. Oinam, L. & Tateno, H. Glycan profiling by sequencing to uncover multicellular communication: launching glycobiology in single cells and microbiomes. *Front Cell Dev Biol* **10**, (2022).
252. Lehr, T., Geyer, H., Maaß, K., Doenhoff, M. J. & Geyer, R. Structural characterization of N-glycans from the freshwater snail *Biomphalaria glabrata* cross-reacting with *Schistosoma mansoni* glycoconjugates. *Glycobiology* **17**, 82–103 (2007).
253. Lehr, T. *et al.* N-Glycosylation patterns of hemolymph glycoproteins from *Biomphalaria glabrata* strains expressing different susceptibility to *Schistosoma mansoni* infection. *Exp Parasitol* **126**, 592–602 (2010).
254. Smit, C. H. *et al.* Surface expression patterns of defined glycan antigens change during *Schistosoma mansoni* cercarial transformation and development of schistosomula. *Glycobiology* **25**, 1465–1479 (2015).
255. Blanas, A., Sahasrabudhe, N. M., Rodríguez, E., van Kooyk, Y. & van Vliet, S. J. Fucosylated antigens in cancer: An alliance toward tumor progression, metastasis, and resistance to chemotherapy. *Front Oncol* **8**, (2018).
256. Bogoevska, V. *et al.* CEACAM1, an adhesion molecule of human granulocytes, is fucosylated by fucosyltransferase IX and interacts with DC-SIGN of dendritic cells via Lewis x residues. *Glycobiology* **16**, 197–209 (2006).
257. Feinberg, H., Mitchell, D. A., Drickamer, K. & Weis, W. I. Structural basis for selective recognition of oligosaccharides by DC-SIGN and DC-SIGNR. *Science* (1979) **294**, 2163–2166 (2001).

258. van Vliet, S. J. *et al.* Carbohydrate profiling reveals a distinctive role for the C-type lectin MGL in the recognition of helminth parasites and tumor antigens by dendritic cells. *Int Immunol* **17**, 661–669 (2005).
259. Martinez-Pomares, L., Linehan, S. A., Taylor, P. R. & Gordon, S. Binding Properties of the Mannose Receptor. *Immunobiology* **204**, 527–535 (2001).
260. Yoshino, T. P. *et al.* Glycotope sharing between snail hemolymph and larval schistosomes: Larval transformation products alter shared glycan patterns of plasma proteins. *PLoS Negl Trop Dis* **6**, (2012).
261. Yoshino, T. P., Wu, X. J., Gonzalez, L. A. & Hokke, C. H. Circulating *Biomphalaria glabrata* hemocyte subpopulations possess shared schistosome glycans and receptors capable of binding larval glycoconjugates. *Exp Parasitol* **133**, 28–36 (2013).
262. Rodríguez, E. *et al.* *Fasciola hepatica* immune regulates CD11c+ cells by interacting with the macrophage gal/GalNAc lectin. *Front Immunol* **8**, (2017).
263. Ravidà, A. *et al.* *Fasciola hepatica* surface coat glycoproteins contain mannosylated and phosphorylated N-glycans and exhibit immune modulatory properties independent of the Mannose Receptor. *PLoS Negl Trop Dis* **10**, (2016).
264. Rodríguez, E. *et al.* *Fasciola hepatica* glycoconjugates immunoregulate dendritic cells through the dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin inducing T cell anergy. *Sci Rep* **7**, (2017).
265. Knuhr, K. *et al.* *Schistosoma mansoni* egg-released IPSE/alpha-1 dampens inflammatory cytokine responses viabasophil interleukin (IL)-4 and IL-13. *Front Immunol* **9**, (2018).
266. Meevissen, M. H. J. *et al.* Specific glycan elements determine differential binding of individual egg glycoproteins of the human parasite *Schistosoma mansoni* by host C-type lectin receptors. *Int J Parasitol* **42**, 269–277 (2012).
267. van Stijn, C. M. W. *et al.* *Schistosoma mansoni* worm glycolipids induce an inflammatory phenotype in human dendritic cells by cooperation of TLR4 and DC-SIGN. *Mol Immunol* **47**, 1544–1552 (2010).
268. Meevissen, M. H. J., Yazdanbakhsh, M. & Hokke, C. H. *Schistosoma mansoni* egg glycoproteins and C-type lectins of host immune cells: Molecular partners that shape immune responses. *Exp Parasitol* **132**, 14–21 (2012).
269. Meyer, S. *et al.* DC-SIGN mediates binding of dendritic cells to authentic pseudo-Lewis Y glycolipids of *Schistosoma mansoni* cercariae, the first parasite-specific ligand of DC-SIGN. *Journal of Biological Chemistry* **280**, 37349–37359 (2005).
270. van Die, I. *et al.* The dendritic cell-specific C-type lectin DC-SIGN is a receptor for *Schistosoma mansoni* egg antigens and recognizes the glycan antigen Lewis x. *Glycobiology* **13**, 471–478 (2003).
271. Van der Kleij, D. *et al.* Triggering of innate immune responses by schistosome egg glycolipids and their carbohydrate epitope GalNAcβ1-4(Fuca1-2Fuca1-3)GlcNAc. *Journal of Infectious Diseases* **185**, 531–539 (2002).
272. Dalton, J. P., Lewis, S. A., Aronstein, W. S. & Strand, M. *Schistosoma mansoni*: Immunogenic glycoproteins of the cercarial glycocalyx. *Exp Parasitol* **63**, 215–226 (1987).

273. Ravindran, B., Satapathy, A. K., Das, M. K., Pattnaik, N. M. & Subramanyam, V. R. Antibodies to microfilarial sheath in Bancroftian filariasis—prevalence and characterization. *Ann Trop Med Parasitol* **84**, 607–613 (1990).
274. Eberl, M. *et al.* Antibodies to glycans dominate the host response to schistosome larvae and eggs: Is their role protective or subversive? *Journal of Infectious Diseases* **183**, 1238–1247 (2001).
275. Kariuki, T. M., Farah, I. O., Wilson, R. A. & Coulson, P. S. Antibodies elicited by the secretions from schistosome cercariae and eggs are predominantly against glycan epitopes. *Parasite Immunol* **30**, 554–562 (2008).
276. Gao, C. *et al.* Glycan microarrays as chemical tools for identifying glycan recognition by immune proteins. *Front Chem* **7**, (2019).
277. De Boer, A. R., Hokke, C. H., Deelder, A. M. & Wuhrer, M. General microarray technique for immobilization and screening of natural glycans. *Anal Chem* **79**, 8107–8113 (2007).
278. van Diepen, A. *et al.* Differential anti-glycan antibody responses in *Schistosoma mansoni*-infected children and adults studied by shotgun glycan microarray. *PLoS Negl Trop Dis* **6**, (2012).
279. Van Diepen, A., Van Der Velden, N. S. J., Smit, C. H., Meevissen, M. H. J. & Hokke, C. H. Parasite glycans and antibody-mediated immune responses in *Schistosoma* infection. *Parasitology* **139**, 1219–1230 (2012).
280. Nkurunungi, G. *et al.* Microarray assessment of N-glycan-specific IgE and IgG profiles associated with *Schistosoma mansoni* infection in rural and urban Uganda. *Sci Rep* **9**, 1–12 (2019).
281. van Diepen, A. *et al.* Development of a *Schistosoma mansoni* shotgun O-glycan microarray and application to the discovery of new antigenic schistosome glycan motifs. *Int J Parasitol* **45**, 465–475 (2015).
282. Mickum, M. L. *et al.* Identification of antigenic glycans from *Schistosoma mansoni* by using a shotgun egg glycan microarray. *Infect Immun* **84**, 1371–1386 (2016).
283. Luyai, A. E. *et al.* Differential expression of anti-glycan antibodies in schistosome-infected humans, rhesus monkeys and mice. *Glycobiology* **24**, 602–608 (2014).
284. Vereecken, K. *et al.* Associations between specific antibody responses and resistance to reinfection in a Senegalese population recently exposed to *Schistosoma mansoni*. *Tropical Medicine and International Health* **12**, 431–444 (2007).
285. Fulford, A. J. C., Butterworth, A. E., Sturrock, R. F. & Ouma, J. H. On the use of age-intensity data to detect immunity to parasitic infections, with special reference to *Schistosoma mansoni* in Kenya. *Parasitology* **105**, 219–227 (1992).
286. Black, C. L. *et al.* Influence of exposure history on the immunology and development of resistance to human Schistosomiasis mansoni. *PLoS Negl Trop Dis* **4**, (2010).
287. Gong, W. *et al.* Protective immunity against *Schistosoma japonicum* infection can be provided by IgG antibodies towards periodate-sensitive or periodate-resistant glycans. *Parasit Vectors* **8**, (2015).
288. Michelle Yang, Y. Y. *et al.* Micro array-assisted analysis of anti-schistosome glycan antibodies elicited by protective vaccination with irradiated cercariae. *Journal of Infectious Diseases* **219**, 1671–1680 (2019).

289. Yang, Y. Y. M. *et al.* Specific anti-glycan antibodies are sustained during and after parasite clearance in *Schistosoma japonicum*-infected rhesus macaques. *PLoS Negl Trop Dis* **11**, (2017).
290. Luyai, A. E. *et al.* Differential expression of anti-glycan antibodies in schistosome-infected humans, rhesus monkeys and mice. *Glycobiology* **24**, 602–608 (2014).
291. Prasanphanich, N. S. *et al.* Anti-schistosomal immunity to core xylose/fucose in *N*-glycans. *Front Mol Biosci* **10**, (2023).
292. Mohanty, M. C., Satapathy, A. K., Sahoo, P. K. & Ravindran, B. Human bancroftian filariasis - A role for antibodies to parasite carbohydrates. *Clin Exp Immunol* **124**, 54–61 (2001).
293. Varki, A. Biological roles of glycans. *Glycobiology* **27**, 3–49 (2017).
294. Qin, R. & Mahal, L. K. The host glycomic response to pathogens. *Curr Opin Struct Biol* **68**, 149–156 (2021).
295. Clerc, F. *et al.* Human plasma protein *N*-glycosylation. *Glycoconj J* **33**, 309–343 (2016).
296. Hanić, M., Lauc, G. & Trbojević-Akmačić, I. *N*-glycan analysis by ultra-performance liquid chromatography and capillary gel electrophoresis with fluorescent labeling. *Curr Protoc Protein Sci* **97**, (2019).
297. Gornik, O. *et al.* Stability of *N*-glycan profiles in human plasma. *Glycobiology* **19**, 1547–1553 (2009).
298. Dall'Olio, F. *et al.* *N*-glycomic biomarkers of biological aging and longevity: A link with inflammaging. *Ageing Res Rev* **12**, 685–698 (2013).
299. Merleev, A. A. *et al.* A site-specific map of the human plasma glycome and its age and gender-associated alterations. *Sci Rep* **10**, (2020).
300. Maverakis, E. *et al.* Glycans in the immune system and The Altered Glycan Theory of Autoimmunity: A critical review. *J Autoimmun* **57**, 1–13 (2015).
301. Zhang, X.-L. & Qu, H. The Role of Glycosylation in Infectious Diseases. 219–237 (2021) doi:10.1007/978-3-030-70115-4_11.
302. Xie, Y. & Butler, M. Serum *N*-glycomic profiling may provide potential signatures for surveillance of COVID-19. *Glycobiology* **32**, 871–885 (2022).
303. Gudelj, I., Lauc, G. & Pezer, M. Immunoglobulin G glycosylation in aging and diseases. *Cell Immunol* **333**, 65–79 (2018).
304. Gornik, O., Pavi, T. & Lauc, G. Alternative glycosylation modulates function of IgG and other proteins — Implications on evolution and disease. *Biochim Biophys Acta* **1820**, 1318–1326 (2012).
305. Kobata, A. The *N*-linked sugar chains of human immunoglobulin G: their unique pattern, and their functional roles. *Biochim Biophys Acta* **1780**, 472–478 (2008).
306. De Vroome, S. W. *et al.* Serum *N*-glycome alterations in colorectal cancer associate with survival. *Oncotarget* **9**, 30610–30623 (2018).
307. Lin, S. *et al.* Serum immunoglobulin G *N*-glycome: a potential biomarker in endometrial cancer. *Ann Transl Med* **8**, 748–748 (2020).
308. Parikh, N. D. *et al.* Biomarkers for the early detection of hepatocellular carcinoma. *Cancer Epidemiology Biomarkers and Prevention* **29**, 2495–2503 (2020).

309. Gebrehiwot, A. G. *et al.* Exploring serum and immunoglobulin G *N*-glycome as diagnostic biomarkers for early detection of breast cancer in Ethiopian women. *BMC Cancer* **19**, (2019).
310. Hart, G. W. & Copeland, R. J. Glycomics hits the big time. *Cell* **143**, 672–676 (2010).
311. O'Regan, N. L. *et al.* Filariasis asymptomatically infected donors have lower levels of disialylated IgG compared to endemic normals. *Parasite Immunol* **36**, 713–720 (2014).
312. Adjobimey, T. & Hoerauf, A. Distinct *N*-linked IgG glycosylation patterns are associated with chronic pathology and asymptomatic infections in human Lymphatic Filariasis. *Front Immunol* **13**, 1–12 (2022).
313. Behrens, A. *et al.* Changes in canine serum *N*-glycosylation as a result of infection with the heartworm parasite *Dirofilaria immitis*. *Sci Rep* **8**, 1–9 (2018).