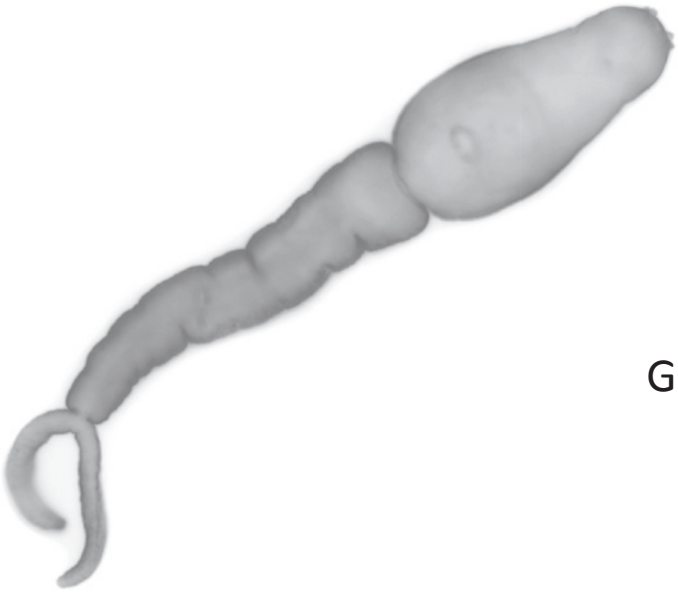


# Chapter 1

## General introduction

# Schistosomiasis

Schistosomiasis is a chronic and debilitating disease caused by parasitic helminths of the genus *Schistosoma.* Schistosomes are digenetic trematodes which have a snail intermediate host and a mammalian definitive host. Over 230 million people are infected worldwide and even more are at risk of infection, especially in developing countries in tropical and subtropical areas [1-3]. Approximately the same number of people suffers from residual morbidity in post-infection stages [4]. This leads to a total Disability Adjusted Life Years (DALY) of 1.7 million [2] and makes schistosomiasis a tropical disease with one of the highest impacts on public health. The disease is also often referred to as bilharzia, after Theodor Bilharz who discovered the disease in 1851.

Of all different schistosome species described, five are capable of infecting humans. Three of these species, *S. mansoni*, *S. haematobium* and *S. japonicum*, account for the majority of infections, whereas *S. mekongi* and *S. intercalatum* are less prevalent [1,4]. Transmission occurs through fresh-water snails of the genus *Biomphalaria* (*S. mansoni*) and *Bulinus* (*S. haematobium*), which live along the shores of lakes, rivers and other bodies of fresh water in large parts of sub-Saharan Africa, South America and parts of the Middle East. The third major schistosome species, *S. japonicum*, is endemic in Indonesia, China and South-East Asia and is transmitted through *Oncomelania* snails. Besides humans, *S. japonicum* is capable of infecting a wide range of other mammals, like dogs, pigs and cattle. The geographical distribution of the different *Schistosoma* species is dependent on the respective intermediate snail hosts and transmission is also highly dependent on the poor hygienic conditions in the endemic areas [1,4].

The term schistosomiasis generally refers to chronic schistosomiasis which has the biggest consequences for health and the highest socio-economic impact. Chronic disease is strongly linked to the production of schistosome eggs. Initially, egg-production leads to a strong polarization towards a T helper 2 (Th2)-type response [5] and periovular granulomas are formed by various immune cells, including macrophages, $CD4^+$ T-cells and eosinophils [6]. A regulatory response develops over time, down-regulating the Th2-responses and leading to decreased granuloma sizes [5]. Eventually, the granulomatous responses to the egg can lead to fibrosis and severe hepatic, intestinal and splenic damage, ultimately leading to death of the host [1,4]. Especially in children, where initial infections may already take place at a very young age (around 2 years) when they start to play in water contaminated with cercariae, the chronic inflammation associated with the granulomatous responses will also affect development, growth and cognitive function, further highlighting the enormous impact of schistosomiasis in endemic areas.

Schistosomiasis can be treated with the relatively cheap chemotherapeutic drug Praziquantel (PZQ), which is effective against all different *Schistosoma* species [4]. PZQ is believed to deregulate the membrane permeability for calcium-ions, thereby inducing muscle spasms and paralysis of the worms, which will eventually dislodge and die through the actions of the immune system, i.e. mainly by antibodies [7-9]. Although being very effective, juvenile worms and eggs are not affected by PZQ. Therefore, a later round of treatment is required to kill the remaining developing worms, and the egg-induced granulomatous responses are allowed to continue [10-12]. Another major drawback of PZQ is that

it does not induce rapid development of immunity and reinfections are very common. As a consequence of treatment and the slow development of immunity, infection intensity does decrease in adults, but in populations with frequent water contact high prevalence can persist [4,13-15]. Re-administration of drugs is therefore required on a regular basis, which demands an infrastructure that is often not available in developing countries. Drug treatment is therefore relatively costly and often impractical. Moreover, interrupted chemotherapy may lead to severe rebound morbidity [16]. Recently, due to intensified mass drug administration programs, there is growing fear of the development of drug-resistance [17]. Clearly, an effective prophylactic vaccine would be a welcome tool for control of schistosomiasis. Various animal models have shown that vaccination with radiation attenuated (RA) cercariae could lead to reductions in worm burden of up to 90% (reviewed in [18]). In combination with PZQ a vaccine might provide a durable and sustained reduction in morbidity and mortality of schistosomiasis, requiring a less demanding infrastructure [16,19,20] and therefore being more cost-efficient. Multiple candidate vaccine-targets have been proposed and some have made it into clinical trials, but no vaccines showing similar reductions in worm burden as the RA cercariae vaccine have been developed yet (reviewed in [16,21]).

## *Schistosoma* life cycle

For all different *Schistosoma* species the life-cycle is complex as well as intriguing, involving sexual replication inside the mammalian host and asexual replication in the molluscan host (Fig. 1) [1,4]. Humans become infected when they come into contact with water infested with free-living cercariae which are shed by the intermediate snail host, triggered by exposure to light [22]. Cercariae can find their host through chemotactic signals, mainly comprised of molecules released by the human skin (e.g skin-derived fatty acids and ceramides) [23,24] and can remain infective for up to three days [25]. Penetration of the human skin is facilitated by the secretion of various proteolytic enzymes [26,27]. During skin penetration cercariae transform into schistosomula, a process which is characterized by the loss of the bifurcated cercarial tail and remodeling of the outer surface of the larvae. The thick carbohydrate layer (glycocalyx) covering the cercariae is lost and surface membranes are replaced by a double lipid bi-layer, called the tegument [28]. Schistosomula migrate through the skin towards the vasculature, enter the blood circulation, pass through the lungs and subsequently reach their specific final destination in the vasculature. For *S. mansoni* and *S. japonicum* these are the mesenteric veins and for *S. haematobium* this is the venous plexus of the bladder. During migration through the circulation schistosomula mature into adult worms, which is accompanied by an enormous growth, especially after passage through the vasculature of the lungs. While maturing, male and female worms form pairs in which the male worm holds the female worm in his gyneacophoric canal [1,4]. Paired adult schistosome worms normally survive up to 3-10 years inside the human body, but extreme cases where adult worms survived for 40 years have been reported as well [29-31]. For their energy supply worms rely on the digestion of erythrocytes and the uptake of glucose through their teguments [32-34]. Since schistosomes are lacking an anus, digested erythrocytes are regurgitated into the blood flow [4]. Paired worms mate and then produce hundreds (*S. mansoni* and *S. haematobium*) up to thousands (*S. japonicum*) of eggs per day. Generally,

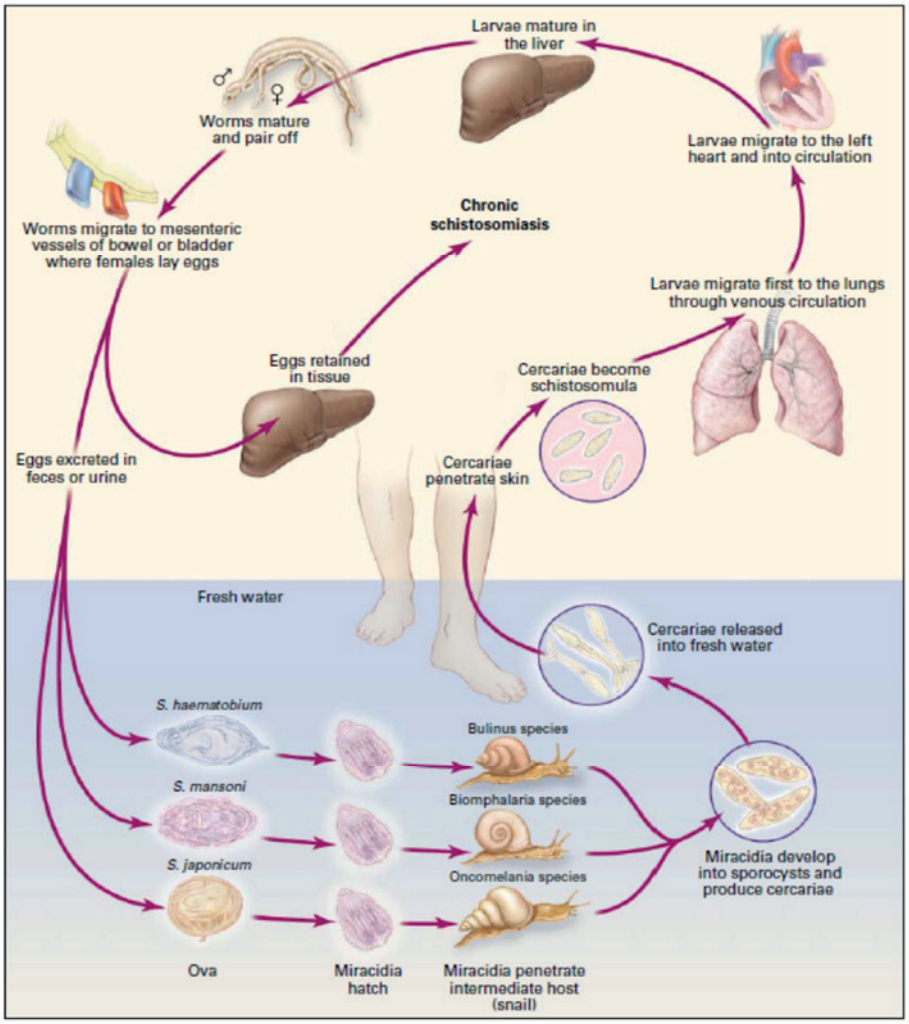



**Figure 1 Life cycles of *S. haematobium*, *S. mansoni* and *S. Japonicum***
(Adapted from Ross *et al.* 2002 [35])

it takes about 5-7 weeks from the start of infection to the onset of egg production [1]. Eggs are deposited in the vessel wall around the intestines and the bladder, from where they penetrate into the lumen of these organs and are eventually excreted with the stool and urine, respectively. However, about half of the eggs produced are carried away by the blood flow towards the liver, intestines and urinary tract, where they become trapped, leading to granulomatous responses causing the main pathology in schistosomiasis [1,4]. Eggs excreted via the stool or urine hatch when they come into contact with fresh water thereby releasing the miracidium, the larval stage capable of infecting the intermediate snail host. Following infection, miracidia develop into primary (mother) sporocysts, which in turn will replicate asexually inside the snail and give rise to secondary (daughter) sporocysts. Another round of asexual replication of these secondary sporocysts gives rise to new cercariae, which can infect a new human host.

## Molecular changes during parasite development upon infection of the definitive host

During parasite development schistosomes undergo several changes in morphology and adapt to the environmental niches where they reside. Transcriptome analysis of the schistosomula life stages revealed that genes involved in the morphological and behavioral changes associated with the schistosomula stages are differentially regulated. Genes associated with the free-living cercariae, e.g. those involved in high energy consumption were found to be downregulated in schistosomula, whereas genes involved in digestion of erythrocytes, tegument formation/turnover and those encoding proteases were upregulated [36-38]. Also numerous glycosyltransferases were differentially regulated, indicating that schistosomula undergo changes in glycosylation during development [37,39].

Because it is difficult to retrieve schistosomula from an infected host and to perform long-term *in vitro* cultures, proteomic analyses of schistosomula stages are very limited in number. Proteomics of soluble proteins and excretory/secretory preparations of schistosomula revealed that most of the identified proteins were expressed in other life stages such as cercariae and adult worms as well [40,41]. Some of the identified proteins have been suggested as vaccine candidates (e.g. glutathione-s-transferase (GST)) [40] and were able to reduce worm burdens by up to 50% after vaccination [41]. Only recently, a targeted analysis of the proteome of schistosomula tegument during development has been published [42]. Notably, three specific peptidases were found to be enriched in the tegument of specific schistosomula stages, suggesting a specific role for these enzymes in migration towards the vasculature. Furthermore, two specific proteins associated with the apical-membrane (which is in direct contact with the host) including GST and annexin, were enriched during schistosomula development and these proteins play a role in detoxification and in the structural integrity of the parasite outer membranes, respectively [43].

In addition to proteins, it has been shown that also glycans of schistosomes play an important role in the host-parasite biology, in particular due to their antigenic properties. As indicated by the differential expression of glycosyltransferases [37,39], glycans and glycan-motifs are also differentially regulated throughout schistosome development. However the exact changes in glycan structures during schistosomula and worm development are actually poorly characterized and the schistosomula and juvenile worm stages have never been thoroughly studied in terms of glycosylation. For those life stages that have been studied so far, the characteristic terminal motifs and core modifications for N-, O-, and glycosphingolipid (GSL)-glycans are summarized in Fig. 2. N-glycans with terminal Galβ1-4(Fucα1-3)GlcNAc (Lewis X or LeX)- and Galβ1-4GlcNAc (LacNAc or LN)-motifs (Table 1) are highly expressed by cercariae but largely disappear in 3 days old schistosomula, and adult worms eventually express mainly N-glycans with GalNAcβ1-4GlcNAc (LacDiNAc or LDN)-motifs [44-47]. Interestingly, female worms express more LN- and LeX-motifs (also as tandem-repeats) than male worms, while in male worms higher levels of GalNAcβ1-4(Fucα1-3)GlcNAc (LDN-F)-motifs (also as tandem-repeats) were seen [47,48]. This differential expression of glycans between males and females could provide a basis for the interaction of the sexes, or for the different manners in which they interact with the host [47]. Furthermore, all life stages during worm development expressed N-glycans with core($\alpha$6)-fucosylation,

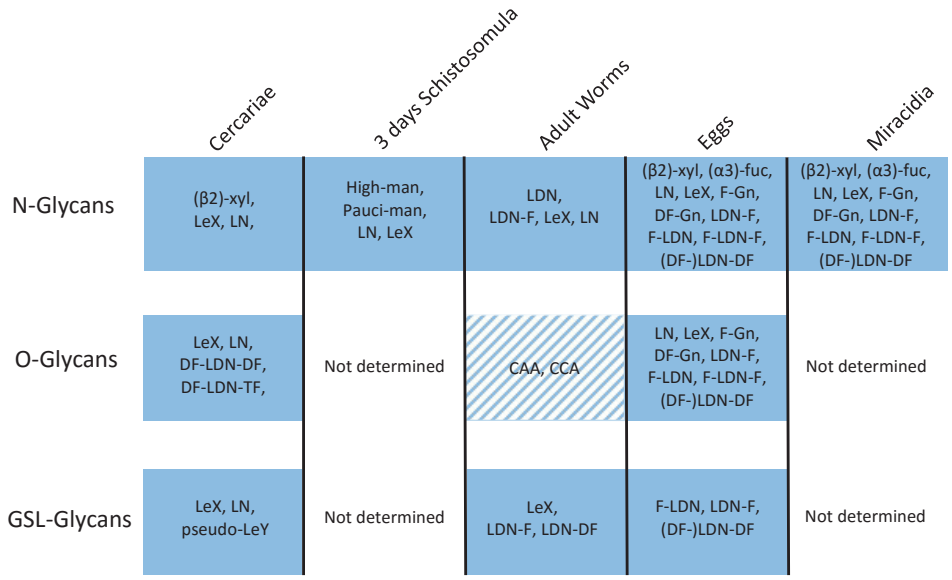
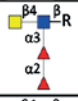
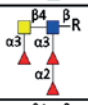
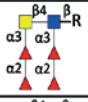
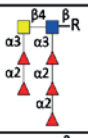
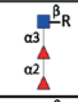
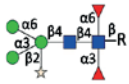



**Figure 2. Characteristic terminal glycan motifs and core-modifications for N-, O-, and GSL-glycans of *schistosoma* life stages.** Filled blue boxes indicate the presence of the respective glycan-class in the indicated life stage, striped blue boxes indicate that the respective glycan was only found in a specific non-somatic preparation of the indicated life stage. No fill indicates that the presence of the respective glycan class in the indicated life stage has not been determined yet. (β2)-xyl, core(β2)-xylosylation; High-man, high mannosidic; Pauci-man, pauci mannosidic, (α3)-fuc, core(α3)-fucosylation; Gn, GlcNAc; CAA, circulating anodic antigen; CCA, circulating cathodic antigen.

but core(β2)-xylosylation which was abundantly present only on cercarial N-glycans, gradually disappeared over time, being completely absent in adult worms [44,45,47]. N-glycans of eggs have been shown to express considerable amounts of multi-fucosylated LDN and GlcNAc as well as LeX and LN as terminal motifs [44,49]. Besides being extensively modified with core(α6)-fucose and core(β2)-xylose, also core(α3)-fucose could be detected within the egg N-glycans, sometimes on di-fucosylated core-structures [44,49]. Core(α3)-fucosylated N-glycans were mainly modified with LN and LeX [49] and with non-fucosylated LDN [44]. The major secretory egg-glycoproteins omega-1, IPSE/α1 and kappa-5 carry these terminal motifs on a difucosylated core as well [50-52], suggesting that these secretory glycoproteins may make an important contribution to the total egg N-glycan pool. Miracidia, which make out a major part of the mature eggs, display similar N-glycan structures as the mature eggs, but not the omega-1 and IPSE/α1 associated glycans [44].

O-glycans are abundantly present in cercariae with *S. mansoni* specific O-glycan cores (Galβ1-3(Galβ1-6)GalNAc) [53] carrying LN- and LeX-motifs, and core 1 (Galβ1-3GalNAc) and core 2 (Galβ1-3(GlcNAcβ1-6)GalNAc) O-glycans with LDN-motifs containing di-fucosyl (Fucα1-2Fucα1- or DF)- modifications which could be associated with the cercarial glycocalyx [53,54]. Notably, secretions of cercariae showed similar N- and O-glycans as total cercarial preparations, suggesting that a major part of the protein-linked glycans within this life stage is secreted [46]. Regular protein-linked O-glycans could no longer be detected in adult worms, but the gut-associated antigens CCA and CAA contain O-glycans with repeating LeX-motifs and repeating units of glucuronic acid-substituted GalNAc, respectively [55,56]. O-glycans expressed by the eggs are predominantly based on a mucin type 1 and type 2 core and they carry relatively large amounts of LN and LeX as well as fucosylated LDN-termini [49]. Egg secretions

**Table 1.** Terminal glycan motifs in *S. mansoni* glycoconjugates

| Glycan motif | Glycan structure | Structure in symbols |
|---|---|---|
| LacNAc /LN | Galβ1-4GlcNAcβ1- |  |
| Lewis X / LeX | Galβ1-4(Fucα1-3)GlcNAcβ1- |  |
| Pseudo Lewis Y / pseudo-LeY | Fucα1-3Galβ1-4(Fucα1-3)GlcNAcβ1- |  |
| LacDiNAc / LDN | GalNAcβ1-4GlcNAcβ1- |  |
| LDN-F | GalNAcβ1-4(Fucα1-3)GlcNAcβ1- |  |
| F-LDN | Fucα1-3GalNAcβ1-4GlcNAcβ1- |  |
| F-LDN-F | Fucα1-3GalNAcβ1-4(Fucα1-3)GlcNAcβ1- |  |
| LDN-DF | GalNAcβ1-4(Fucα1-2Fucα1-3)GlcNAcβ1- |  |
| F-LDN-DF | Fucα1-3GalNAcβ1-4 (Fucα1-2Fucα1-3)GlcNAcβ1- |  |
| DF-LDN-DF | Fucα1-2Fucα1-3GalNAcβ1-4 (Fucα1-2Fucα1-3)GlcNAcβ1- |  |
| DF-LDN-TF | Fucα1-2Fucα1-3GalNAcβ1-4 (Fucα1-2Fucα1-2Fucα1-3)GlcNAcβ1- |  |
| F-GlcNAc | Fucα1-3GlcNAcβ1- |  |
| DF-GlcNAc | Fucα1-2Fucα1-3GlcNAcβ1- |  |
| TF-GlcNAc | Fucα1-2Fucα1-2Fucα1-3GlcNAcβ1- |  |
| [a]Core-Xyl [a]Core(α3)-Fuc [a]Core(α6)-Fuc | Man(α1-3)(Manα1-6)(Xylβ1-2)Manβ1-4GlcNAcβ1-4(Fucα1-3)(Fucα1-6)GlcNAcβ1- |  |

Red triangle, fucose; yellow square, N-acetylgalactosamine; blue square, N-acetylglucosamine; yellow circle, galactose; green circle, mannose; white star, xylose.
[a]N-glycan core-modifications which can be present separately or combined

additionally showed O-glycans with the *S. mansoni*-specific core (Galβ1-3(Galβ1-6)GalNAc) and a novel type of core in which the glycans are linked to the protein via a Gal-residue [46]. Also the eggshell most likely contains different N-and O-glycans; however the precise composition of these glycans remains to be determined [57].

Finally, cercarial GSL-glycans also express terminal LN- and LeX-motifs, and specifically the Fucα1-3Galβ1-4(Fucα1-3)GlcNAc or pseudo-Lewis Y (LeY) motif [58]. GSL-glycans of adult worms showed structures with primarily LeX and (multi-)fucosylated LDN as terminal motifs [59]. In some cases, the GlcNAc-backbone of structures expressing fucosylated LDN as a terminal motif is further modified with fucose-residues (i.e. -4((Fucα1-2)$_{0\text{-}1}$Fucα1-3)GlcNAcβ1) [59]. In the egg GSL-glycans the (multi-)fucosylated LDN-motifs predominated and also here the GlcNAc-backbone is further modified with fucose-residues [60,61].

The presence of all these specific glycan-motifs during different stages of schistosome development has also been confirmed by western blot and thin-layer chromatography-immunostaining [62-64]. Although it is thus clear that schistosome glycosylation undergoes multiple changes during development, it is still largely unclear how glycan expression changes upon infection and larval development. Furthermore, studies on the spatial expression of specific glycan structures have mainly focused on the presence of terminal glycan motifs, in particular using immunofluorescence. These studies showed that LeX- and LDN- motifs were mainly present at the oral sucker of cercariae, while LDN-F- and GalNAcβ1-4(Fucα1-2Fucα1-3)GlcNAc (LDN-DF)- motifs covered the whole surface [64,65]. In schistosomula, LeX- and LDN-motifs were expressed all over the surface together with LDN-F and LDN-DF [64,66]. LDN- and LeX-motifs and to a lesser extent LDN-F were found at the tegument of the adult worms as well, whereas LDN-DF was predominantly found in the parenchyma and the excretory system [47,64,65]. On the eggshell multiple different terminal glycan motifs, including LDN, LDN-DF, (Fucα1-3)GalNAcβ1-4(Fucα1-3)GlcNAc (F-LDN-F)-, LDN-F and LeX are present, while the miracidium mainly presented LDN-DF and LDN-motifs [63,65]. Unfortunately, it is unknown which glycan structures are underlying these motifs and therefore it is not possible to determine which specific protein- or lipid-linked glycans are present at the surface of the tegument and eggshell. Since these studies have been performed with a restricted set of monoclonal antibodies, it may be expected that numerous other antigenic and non-antigenic glycan motifs are present in and on the different life stages of schistosomes.

## Antibody responses and immunity

Only after multiple rounds of infection and treatments immunity towards schistosomiasis may eventually develop [13-15]. Although reduced infection rates could in part be explained by reduced water contact by adults, (partial) immunity was also seen in people with continuing water-contact, i.e. fishermen [67]. A better understanding of the development of immunity might lead to the identification of new vaccine targets. Animal studies including non-permissive rats and macaques have pointed out that the elimination of the invading parasites proceeds via controlled immunological reactions, which might be translated to the human situation for identifying target vaccine candidates [6,68]. Antibody responses raised against schistosomes are closely associated with the development of protection, as in B-cell

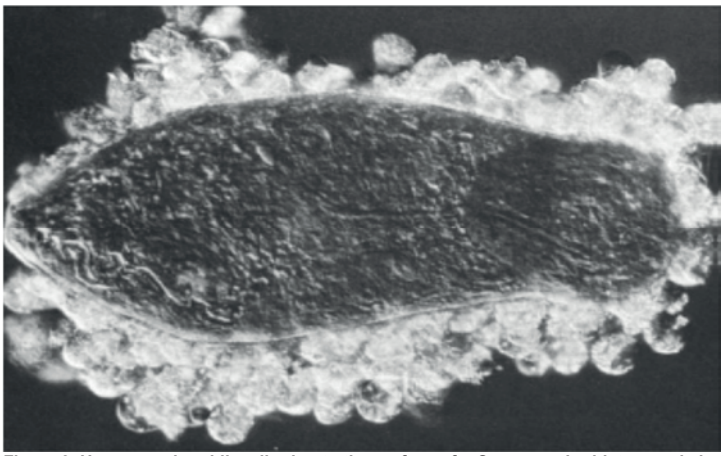


**Figure 3. Human eosinophils adhering to the surface of a *S. mansoni* schistosomula in the presence of infection serum.** (Adapted from Butterworth and Hagan 1987 [55])

deficient mice vaccination with RA-cercariae led to an increased susceptibility towards challenge infection [69,70]. Furthermore, serum obtained from animals (i.e. mice, rats and rabbits) which received vaccination with RA-cercariae can provide protection against infection in non-vaccinated animals, suggestive for a role of antibodies [71-73]. Two major effector mechanisms involving antibodies have been identified, i.e. antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated elimination. In ADCC, antibodies binding to the schistosome tegument trigger the release of lysosomal contents of effector cells, disintegrating the tegument and exposing underlying muscle layers [74]. Schistosomula are susceptible to this kind of immune killing during a relatively brief period after transformation [75,76]. Earlier studies revealed that antibodies can induce eosinophil-
mediated damage towards schistosomula (Fig. 3) [77-80] and that this damaging activity can be passively transferred by serum from infected to naïve animals [81]. Later on, a role for neutrophils, macrophages and platelets was identified as well [82-85]. A wide range of different antibody subclasses can be involved in ADCC, including IgE (in rats, baboons and humans) [82,85,86], IgA and IgG1,2 and 3 (in rats and humans) [87-89]. In humans, other antibody subclasses including IgM, IgG4 and under certain conditions IgG2, may however block the effects of the protective antibodies [89-91]. Furthermore, in human populations an increased IgE/IgG4-balance correlated with less susceptibility to re-infections [91-94]. The antibody subclass directed against a certain antigen is thus a very important determinant in the prediction of whether an antigen might be the target of a protective immune response and potentially a new vaccine candidate.

## Glycans as targets of antibody responses

Although most of the attention in the search for schistosome vaccine-candidates has been focused on protein antigens, antigenic glycans which are abundantly expressed throughout the schistosome life cycle may also be worth to be evaluated as potential vaccine targets. As discussed in

the above, glycans are present as protein-linked N- and O-glycans or glycosphingolipid- (GSL-) glycans in all different life stages of the parasite. They are expressed at the parasite surface as well as in secretions [44,46] and are therefore constantly exposed to the host immune system. Glycans are involved in multiple processes including immune modulation and activation [95-99]. For example, the LeX-motif [96,100] and core(α3)-fucose and core(β2)-xylose of egg N-glycans [97,101] were shown to be involved in the induction of Th2-responses. Furthermore, soluble egg antigens (SEA) and different egg glycoproteins such as omega-1 and IPSE/α1 depend on the interaction of glycans with different C-type lectin receptors including dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN), mannose receptor (MR) and macrophage galactose-type lectin (MGL) to exert their immunomodulatory functions [102-106]. Importantly, glycans also form major targets for antibody-responses in the infected host [44,64,65,107-113]. Antibody responses have been observed against almost all of the terminal glycan-motifs present in schistosomes, including LeX, LDN, F-LDN, LDN-F, F-LDN-F, LDN-DF and F-GlcNAc (Table 1). Antibodies against LeX, a glycan motif present in humans as well, have been observed in multiple studies in humans and animal models [64,107,110,114-117]. These anti-LeX antibodies were mostly of the IgM-isotype, but minor amounts of IgA and IgG were also detected [110,116,117]. Notably, responses toward monomeric LeX were generally lower than those towards multimeric LeX, suggesting that a specific motif can induce different antibody responses depending on the structural context [117]. Furthermore, IgM-responses were also observed against LDN- and LDN-F-motifs, which are present in the human host as well [113,115]. The most intense antibody responses seen in infected individuals were however raised towards F-LDN- and the schistosome-specific LDN-DF-motifs and those responses were mainly of the IgG-isotype [113,115]. The highest responses towards the latter type of motifs were generally observed in children, probably due to the higher infection intensities in this age-group. Isotypes of these responses may however vary depending on the age of the host and the schistosome-species [115]. In addition to the terminal schistosome glycan motifs, core modifications of schistosome N-glycans, including core(β2)-xylosylation, core(α3)-fucosylation are also targeted by IgG and by IgE [118,119].

Despite the prominent antibody responses against parasite-derived glycans in schistosome infected individuals there is still an ongoing debate about the precise role of these anti-glycan antibodies in schistosome infections and whether they do actually give rise to protective responses or not. It has been suggested that rather than conferring protection, these antibodies function as a smokescreen, which would prevent the generation of a protective response [107]. Furthermore, anti-glycan responses of the IgM and IgG2 isotypes recognizing glycans of schistosomula and eggs/miracidia have been negatively associated with protection [120,121] and the anti-glycan responses were mainly observed during the initial stages of infection, and not in the chronic stages of infection when protective responses are believed to be generated [107,108]. Nonetheless, several glycan-specific antibodies have demonstrated protective properties *in vitro*, including some against LDN- and LeX-motifs [64,114,116]. Other groups have shown that protective antibodies involved in ADCC were reactive against F-LDN-F-motifs shared with keyhole limpet hemocyanin [122,123] and LeX-motifs [124]. Furthermore, glycan-based vaccines have been developed against other infectious diseases [125], suggesting that a glycan-based vaccine against schistosomiasis might be feasible as well [66].

## Schistosomula – underexplored, but ideal targets

Schistosomula are considered the best stages to be targeted by vaccination as they are susceptible to antibody-mediated protective effector mechanisms early after transformation [75-80] and effective elimination of these early stages would furthermore prevent development of adult worms and consequently of egg-induced pathology and transmission of the disease. In numerous other helminth infections the larval stages have therefore been subject of vaccine studies, including different *Fasciola* species, cestodes and hookworms [126-131]. Also the relatively small size of these larvae compared to the adult worms could be considered as an advantage for effective elimination. However, relatively little is known about the precise targets (i.e. specific molecules) of (protective) antibody responses against schistosomes in general and for the schistosomula stages in particular. Especially the molecules which are in direct contact with the host, i.e. those at the tegument and in secretions, are of special interest as potential vaccine targets because they are directly accessible to the immune system. For example, mice vaccinated with the tegument of schistosomula showed an antibody-dependent and complement-mediated reduction in worm and egg counts upon challenge (40% and 65% reduction, respectively) [132,133]. Moreover, both protective IgG- and IgE-antibodies binding to the surface of the schistosomulum can be induced by secreted products of schistosomula, suggesting shared protective epitopes between the tegument and secretions [134-137]. Tegument and secretions have been shown to be abundantly glycosylated and antibodies are raised against these glycans [46,47,64,65,108,112,115]. Unfortunately, knowledge of schistosomula glycosylation is very limited. Consequently, little is known regarding which glycans could be possible targets of (protective) antibody responses, which could lead to further identification of possible vaccine targets. This further stresses the need to explore in more detail the developmental and spatial distribution of glycans expressed by these vulnerable early life stages and to identify those glycans that are targeted by antibodies in the infected host.

## Scope of the thesis

The studies presented in this thesis are aimed to generate a better understanding of the developmental N-, O-, and GSL-linked glycosylation of all life stages of *S. mansoni*, including schistosomula, and to identify glycans targeted by antibody responses in the infected host. These studies will thereby provide a basis for a better understanding of the anti-glycan antibody responses in schistosomiasis. To gain a more detailed and complete insight into the glycan structures present in the schistosomula stages of the schistosome life cycle and to determine how the glycan expression within these stages relates to other life stages, a comprehensive glycan profiling study was performed in **chapter 2**, addressing all major classes of glycosylation in all schistosome life stages, some of which have never been studied in detail before. The results of this profiling indicated some striking changes and shifts in the protein- and GSL-linked glycosylation during worm as well as egg development. Mature eggs also displayed a so far undescribed novel schistosome glycan element in the GSL-glycans containing a glucuronic-acid moiety which was characterized in more detail in **chapter 3**. Furthermore it was determined that this novel glycan-element is antigenic as antibody responses against this novel element were present in infected individuals.

The spatial distribution of glycans in schistosomula was addressed in **chapter 4** to determine which of the identified glycans are present on the schistosomulum tegument and are being exposed as targets to the host immune system. Using different anti-glycan monoclonal antibodies, which were characterized using microarrays of glycans isolated from different schistosome life stages, multiple changes in surface expressed glycans-classes and -motifs in the first few hours and days after infection were shown.

To further investigate which of these glycans were targeted by antibodies in an infected host, the local anti-glycan responses against migrating schistosomula in the lymph nodes of protected rats were studied by glycan microarrays in **chapter 5**. Distinct glycans were targeted by the antibody responses raised by migrating skin- and lung-stage schistosomula and, as shown in **chapter 4**, some of the glycans targeted are present at the surface of the developing schistosomula. Since the rats used in this study were protected against re-infection, a large pool of glycan targets potentially involved in protection was identified.

**Chapter 6** describes the anti-glycan responses in human populations for two distinct age-groups (children and adults) using glycan microarrays. Differential glycan recognition profiles were found for the different age groups that may reflect differences in disease characteristics, e.g. length of exposure and infection intensity, providing further insights into the role of schistosome glycans and anti-glycan antibody responses in relation to immunity.

Finally, **Chapter 7** summarizes the findings of this thesis and elaborates in more detail on the developmental glycosylation of *Schistosoma* and on glycan targets of the immune response in schistosomiasis.

## Reference List


1. Gryseels, B.; Polman, K.; Clerinx, J.; Kestens, L. Human schistosomiasis. *Lancet* **2006,** *368* (9541), 1106-1118.
2. Vos, T.; Flaxman, A. D.; Naghavi, M.; Lozano, R.; Michaud, C.*et al.* Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **2012,** *380* (9859), 2163-2196.
3. Chitsulo, L.; Loverde, P.; Engels, D. Schistosomiasis. *Nat. Rev. Microbiol.* **2004,** *2* (1), 12-13.
4. Colley, D. G.; Bustinduy, A. L.; Secor, W. E.; King, C. H. Human schistosomiasis. *Lancet* **2014,** *383* (9936), 2253-2264.
5. Pearce, E. J.; Kane, M.; Sun, J.; Taylor, J.; McKee, A. S.; Cervi, L. Th2 response polarization during infection with the helminth parasite Schistosoma mansoni. *Immunol. Rev.* **2004,** *201*, 117-126.
6. Pearce, E. J.; MacDonald, A. S. The immunobiology of schistosomiasis. *Nat. Rev. Immunol.* **2002,** *2* (7), 499-511.
7. Pax, R.; Bennett, J. L.; Fetterer, R. A benzodiazepine derivative and praziquantel: effects on musculature of Schistosoma mansoni and Schistosoma japonicum. *Naunyn Schmiedebergs Arch. Pharmacol.* **1978,** *304* (3), 309-315.
8. Doenhoff, M. J.; Cioli, D.; Utzinger, J. Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. *Curr. Opin. Infect. Dis.* **2008,** *21* (6), 659-667.
9. Brindley, P. J.; Sher, A. The chemotherapeutic effect of praziquantel against Schistosoma mansoni is dependent on host antibody response. *J. Immunol.* **1987,** *139* (1), 215-220.
10. Danso-Appiah, A.; de Vlas, S. J. Interpreting low praziquantel cure rates of Schistosoma mansoni infections in Senegal. *Trends Parasitol.* **2002,** *18* (3), 125-129.
11. King, C. H.; Olbrych, S. K.; Soon, M.; Singer, M. E.; Carter, J.; Colley, D. G. Utility of repeated praziquantel dosing in the treatment of schistosomiasis in high-risk communities in Africa: a systematic review. *PLoS. Negl. Trop. Dis.* **2011,** *5* (9), e1321.
12. Cioli, D.; Pica-Mattoccia, L.; Basso, A.; Guidi, A. Schistosomiasis control: praziquantel forever? *Mol. Biochem. Parasitol.* **2014,** *195* (1), 23-29.

13. Butterworth, A. E.; Fulford, A. J.; Dunne, D. W.; Ouma, J. H.; Sturrock, R. F. Longitudinal studies on human schistosomiasis. *Philos. Trans. R. Soc. Lond B Biol. Sci.* **1988,** *321* (1207), 495-511.
14. Ouma, J. H.; Fulford, A. J.; Kariuki, H. C.; Kimani, G.; Sturrock, R. F.; Muchemi, G.; Butterworth, A. E.; Dunne, D. W. The development of schistosomiasis mansoni in an immunologically naive immigrant population in Masongaleni, Kenya. *Parasitology* **1998,** *117 ( Pt 2)*, 123-132.
15. Wilkins, H. A.; Blumenthal, U. J.; Hagan, P.; Hayes, R. J.; Tulloch, S. Resistance to reinfection after treatment of urinary schistosomiasis. *Trans. R. Soc. Trop. Med. Hyg.* **1987,** *81* (1), 29-35.
16. McManus, D. P.; Loukas, A. Current status of vaccines for schistosomiasis. *Clin. Microbiol. Rev.* **2008,** *21* (1), 225-242.
17. Doenhoff, M. J.; Kusel, J. R.; Coles, G. C.; Cioli, D. Resistance of Schistosoma mansoni to praziquantel: is there a problem? *Trans. R. Soc. Trop. Med. Hyg.* **2002,** *96* (5), 465-469.
18. Bickle, Q. D. Radiation-attenuated schistosome vaccination--a brief historical perspective. *Parasitology* **2009,** *136* (12), 1621-1632.
19. Hotez, P. J.; Bethony, J. M.; Diemert, D. J.; Pearson, M.; Loukas, A. Developing vaccines to combat hookworm infection and intestinal schistosomiasis. *Nat. Rev. Microbiol.* **2010,** *8* (11), 814-826.
20. Capron, A.; Riveau, G.; Capron, M.; Trottein, F. Schistosomes: the road from host-parasite interactions to vaccines in clinical trials. *Trends Parasitol.* **2005,** *21* (3), 143-149.
21. McWilliam, H. E.; Driguez, P.; Piedrafita, D.; McManus, D. P.; Meeusen, E. N. Novel immunomic technologies for schistosome vaccine development. *Parasite Immunol.* **2012,** *34* (5), 276-284.
22. Nojima, H.; Sato, A. Schistosoma mansoni and Schistosoma haematobium: emergence of schistosome cercariae from snails with darkness and illumination. *Exp. Parasitol.* **1982,** *53* (2), 189-198.
23. Haas, W.; Haeberlein, S.; Behring, S.; Zoppelli, E. Schistosoma mansoni: human skin ceramides are a chemical cue for host recognition of cercariae. *Exp. Parasitol.* **2008,** *120* (1), 94-97.
24. Haeberlein, S.; Haas, W. Chemical attractants of human skin for swimming Schistosoma mansoni cercariae. *Parasitol. Res.* **2008,** *102* (4), 657-662.
25. Lawson, J. R.; Wilson, R. A. The survival of the cercariae of Schistosoma mansoni in relation to water temperature and glycogen utilization. *Parasitology* **1980,** *81* (2), 337-348.
26. Curwen, R. S.; Ashton, P. D.; Sundaralingam, S.; Wilson, R. A. Identification of novel proteases and immunomodulators in the secretions of schistosome cercariae that facilitate host entry. *Mol. Cell Proteomics.* **2006,** *5* (5), 835-844.
27. Minard, P.; Murrell, K. D.; Stirewalt, M. A. Proteolytic, antigenic and immunogenic properties of Schistosoma mansoni cercarial secretion material. *Am. J. Trop. Med. Hyg.* **1977,** *26* (3), 491-499.
28. 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.* **1973,** *3* (1), 13-25.
29. Warren, K. S.; Mahmoud, A. A.; Cummings, P.; Murphy, D. J.; Houser, H. B. Schistosomiasis mansoni in Yemeni in California: duration of infection, presence of disease, therapeutic management. *Am. J. Trop. Med. Hyg.* **1974,** *23* (5), 902-909.
30. Harris, A. R.; Russell, R. J.; Charters, A. D. A review of schistosomiasis in immigrants in Western Australia, demonstrating the unusual longevity of Schistosoma mansoni. *Trans. R. Soc. Trop. Med. Hyg.* **1984,** *78* (3), 385-388.
31. Payet, B.; Chaumentin, G.; Boyer, M.; Amaranto, P.; Lemonon-Meric, C.; Lucht, F. Prolonged latent schistosomiasis diagnosed 38 years after infestation in a HIV patient. *Scand. J. Infect. Dis.* **2006,** *38* (6-7), 572-575.
32. van Oordt, B. E.; van den Heuvel, J. M.; Tielens, A. G.; van den Bergh, S. G. The energy production of the adult Schistosoma mansoni is for a large part aerobic. *Mol. Biochem. Parasitol.* **1985,** *16* (2), 117-126.
33. Skelly, P. J.; Da'Dara, A. A.; Li, X. H.; Castro-Borges, W.; Wilson, R. A. Schistosome feeding and regurgitation. *PLoS. Pathog.* **2014,** *10* (8), e1004246.
34. Da'dara, A.; Krautz-Peterson, G.; Faghiri, Z.; Skelly, P. J. Metabolite movement across the schistosome surface. *J. Helminthol.* **2012,** *86* (2), 141-147.
35. Ross, A. G., Bartley, P. B., Sleigh, A. C., Olds, G. R., Li, Y., Williams, G. M., and McManus, D. P. Schistosomiasis. *N. Engl. J. Med.* **2002** 346, 1212-1220
36. Gobert, G. N.; Tran, M. H.; Moertel, L.; Mulvenna, J.; Jones, M. K.; McManus, D. P.; Loukas, A. Transcriptional changes in Schistosoma mansoni during early schistosomula development and in the presence of erythrocytes. *PLoS. Negl. Trop. Dis.* **2010,** *4* (2), e600.


37. Parker-Manuel, S. J.; Ivens, A. C.; Dillon, G. P.; Wilson, R. A. Gene expression patterns in larval Schistosoma mansoni associated with infection of the mammalian host. *PLoS. Negl. Trop. Dis.* **2011,** *5* (8), e1274.
38. Dillon, G. P.; Feltwell, T.; Skelton, J. P.; Ashton, P. D.; Coulson, P. S.; Quail, M. A.; Nikolaidou-Katsaridou, N.; Wilson, R. A.; Ivens, A. C. Microarray analysis identifies genes preferentially expressed in the lung schistosomulum of Schistosoma mansoni. *Int. J. Parasitol.* **2006,** *36* (1), 1-8.
39. Fitzpatrick, J. M.; Peak, E.; Perally, S.; Chalmers, I. W.; Barrett, J.; Yoshino, T. P.; Ivens, A. C.; Hoffmann, K. F. Anti-schistosomal intervention targets identified by lifecycle transcriptomic analyses. *PLoS. Negl. Trop. Dis.* **2009,** *3* (11), e543.
40. Curwen, R. S.; Ashton, P. D.; Johnston, D. A.; Wilson, R. A. The Schistosoma mansoni soluble proteome: a comparison across four life-cycle stages. *Mol. Biochem. Parasitol.* **2004,** *138* (1), 57-66.
41. El Ridi, R.; Tallima, H. Schistosoma mansoni ex vivo lung-stage larvae excretory-secretory antigens as vaccine candidates against schistosomiasis. *Vaccine* **2009,** *27* (5), 666-673.
42. 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.* **2015,** *45* (8), 505-516.
43. Tararam, C. A.; Farias, L. P.; Wilson, R. A.; Leite, L. C. Schistosoma mansoni Annexin 2: molecular characterization and immunolocalization. *Exp. Parasitol.* **2010,** *126* (2), 146-155.
44. Hokke, C. H.; Deelder, A. M.; Hoffmann, K. F.; Wuhrer, M. Glycomics-driven discoveries in schistosome research. *Exp. Parasitol.* **2007,** *117* (3), 275-283.
45. 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* **2001,** *11* (2), 149-163.
46. Jang-Lee, J.; Curwen, R. S.; Ashton, P. D.; Tissot, B.; Mathieson, W.; Panico, M.; Dell, A.; Wilson, R. A.; Haslam, S. M. Glycomics analysis of Schistosoma mansoni egg and cercarial secretions. *Mol. Cell Proteomics.* **2007,** *6* (9), 1485-1499.
47. Wuhrer, M.; Koeleman, C. A.; Fitzpatrick, J. M.; Hoffmann, K. F.; Deelder, A. M.; Hokke, C. H. Gender-specific expression of complex-type N-glycans in schistosomes. *Glycobiology* **2006,** *16* (10), 991-1006.
48. Wuhrer, M.; Koeleman, C. A.; Deelder, A. M.; Hokke, C. H. Repeats of LacdiNAc and fucosylated LacdiNAc on N-glycans of the human parasite Schistosoma mansoni. *FEBS J.* **2006,** *273* (2), 347-361.
49. 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* **1997,** *7* (5), 663-677.
50. Meevissen, M. H.; Wuhrer, M.; Doenhoff, M. J.; Schramm, G.; Haas, H.; Deelder, A. M.; Hokke, C. H. Structural characterization of glycans on omega-1, a major Schistosoma mansoni egg glycoprotein that drives Th2 responses. *J. Proteome. Res.* **2010,** *9* (5), 2630-2642.
51. Wuhrer, M.; Balog, C. I.; Catalina, M. I.; Jones, F. M.; Schramm, G.; Haas, H.; Doenhoff, M. J.; Dunne, D. W.; Deelder, A. M.; Hokke, C. H. IPSE/alpha-1, a major secretory glycoprotein antigen from schistosome eggs, expresses the Lewis X motif on core-difucosylated N-glycans. *FEBS J.* **2006,** *273* (10), 2276-2292.
52. Meevissen, M. H.; Balog, C. I.; Koeleman, C. A.; Doenhoff, M. J.; Schramm, G.; Haas, H.; Deelder, A. M.; Wuhrer, M.; Hokke, C. H. Targeted glycoproteomic analysis reveals that kappa-5 is a major, uniquely glycosylated component of Schistosoma mansoni egg antigens. *Mol. Cell Proteomics.* **2011,** *10* (5), M110.
53. Huang, H. H.; Tsai, P. L.; Khoo, K. H. Selective expression of different fucosylated epitopes on two distinct sets of Schistosoma mansoni cercarial O-glycans: identification of a novel core type and Lewis X structure. *Glycobiology* **2001,** *11* (5), 395-406.
54. Khoo, K. H.; Sarda, S.; Xu, X.; Caulfield, J. P.; McNeil, M. R.; Homans, S. W.; Morris, H. R.; Dell, A. A unique multifucosylated -3GalNAc beta 1-->4GlcNAc beta 1-->3Gal alpha 1- motif constitutes the repeating unit of the complex O-glycans derived from the cercarial glycocalyx of Schistosoma mansoni. *J. Biol. Chem.* **1995,** *270* (29), 17114-17123.
55. Bergwerff, A. A.; van Dam, G. J.; Rotmans, J. P.; Deelder, A. M.; Kamerling, J. P.; Vliegenthart, J. F. The immunologically eactive part of immunopurified circulating anodic antigen from Schistosoma mansoni is a threonine-linked polysaccharide consisting of --> 6)-(beta-D-GlcpA-(1 --> 3))-beta-D-GalpNAc-(1 --> repeating units. *J. Biol. Chem.* **1994,** *269* (50), 31510-31517.
56. van Dam, G. J.; Bergwerff, A. A.; Thomas-Oates, J. E.; Rotmans, J. P.; Kamerling, J. P.; Vliegenthart, J. F.; Deelder, A. M. 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.* **1994,** *225* (1), 467-482.

57. Dewalick, S.; Bexkens, M. L.; van Balkom, B. W.; Wu, Y. P.; Smit, C. H.; Hokke, C. H.; de Groot, P. G.; Heck, A. J.; Tielens, A. G.; van Hellemond, J. J. The proteome of the insoluble Schistosoma mansoni eggshell skeleton. *Int. J. Parasitol.* **2011,** *41* (5), 523-532.

58. Wuhrer, M.; Dennis, R. D.; Doenhoff, M. J.; Lochnit, G.; Geyer, R. Schistosoma mansoni cercarial glycolipids are dominated by Lewis X and pseudo-Lewis Y structures. *Glycobiology* **2000,** *10* (1), 89-101.

59. Frank, S.; van Die, I.; Geyer, R. Structural characterization of Schistosoma mansoni adult worm glycosphingolipids reveals pronounced differences with those of cercariae. *Glycobiology* **2012,** *22* (5), 676-695.

60. Wuhrer, M.; Kantelhardt, S. R.; Dennis, R. D.; Doenhoff, M. J.; Lochnit, G.; Geyer, R. Characterization of glycosphingolipids from Schistosoma mansoni eggs carrying Fuc(alpha1-3)GalNAc-, GalNAc(beta1-4)[Fuc(alpha1-3)]GlcNAc- and Gal(beta1-4)[Fuc(alpha1-3)]GlcNAc- (Lewis X) terminal structures. *Eur. J. Biochem.* **2002,** *269* (2), 481-493.

61. Khoo, K. H.; Chatterjee, D.; Caulfield, J. P.; Morris, H. R.; Dell, A. Structural characterization of glycophingolipids from the eggs of Schistosoma mansoni and Schistosoma japonicum. *Glycobiology* **1997,** *7* (5), 653-661.

62. Robijn, M. L.; Koeleman, C. A.; Wuhrer, M.; Royle, L.; Geyer, R.; Dwek, R. A.; Rudd, P. M.; Deelder, A. M.; Hokke, C. H. Targeted identification of a unique glycan epitope of Schistosoma mansoni egg antigens using a diagnostic antibody. *Mol. Biochem. Parasitol.* **2007,** *151* (2), 148-161.

63. Robijn, M. L.; Wuhrer, M.; Kornelis, D.; Deelder, A. M.; Geyer, R.; Hokke, C. H. Mapping fucosylated epitopes on glycoproteins and glycolipids of Schistosoma mansoni cercariae, adult worms and eggs. *Parasitology* **2005,** *130* (Pt 1), 67-77.

64. Nyame, A. K.; Lewis, F. A.; Doughty, B. L.; Correa-Oliveira, R.; Cummings, R. D. Immunity to schistosomiasis: glycans are potential antigenic targets for immune intervention. *Exp. Parasitol.* **2003,** *104* (1-2), 1-13.

65. van Remoortere, A.; Hokke, C. H.; van Dam, G. J.; van Die, I.; Deelder, A. M.; van den Eijnden, D. H. Various stages of schistosoma express Lewis(x), LacdiNAc, GalNAcbeta1-4 (Fucalpha1-3)GlcNAc and GalNAcbeta1-4(Fucalpha1-2Fucalpha1-3)GlcNAc carbohydrate epitopes: detection with monoclonal antibodies that are characterized by enzymatically synthesized neoglycoproteins. *Glycobiology* **2000,** *10* (6), 601-609.

66. Nyame, A. K.; Kawar, Z. S.; Cummings, R. D. Antigenic glycans in parasitic infections: implications for vaccines and diagnostics. *Arch. Biochem. Biophys.* **2004,** *426* (2), 182-200.

67. Kabatereine, N. B.; Vennervald, B. J.; Ouma, J. H.; Kemijumbi, J.; Butterworth, A. E.; Dunne, D. W.; Fulford, A. J. Adult resistance to schistosomiasis mansoni: age-dependence of reinfection remains constant in communities with diverse exposure patterns. *Parasitology* **1999,** *118 ( Pt 1)*, 101-105.

68. Wilson, R. A.; Langermans, J. A.; van Dam, G. J.; Vervenne, R. A.; Hall, S. L.; Borges, W. C.; Dillon, G. P.; Thomas, A. W.; Coulson, P. S. Elimination of Schistosoma mansoni Adult Worms by Rhesus Macaques: Basis for a Therapeutic Vaccine? *PLoS. Negl. Trop. Dis.* **2008,** *2* (9), e290.

69. Jankovic, D.; Wynn, T. A.; Kullberg, M. C.; Hieny, S.; Caspar, P.; James, S.; Cheever, A. W.; Sher, A. Optimal vaccination against Schistosoma mansoni requires the induction of both B cell- and IFN-gamma-dependent effector mechanisms. *J. Immunol.* **1999,** *162* (1), 345-351.

70. Anderson, S.; Coulson, P. S.; Ljubojevic, S.; Mountford, A. P.; Wilson, R. A. The radiation-attenuated schistosome vaccine induces high levels of protective immunity in the absence of B cells. *Immunology* **1999,** *96* (1), 22-28.

71. Mangold, B. L.; Dean, D. A. Passive transfer with serum and IgG antibodies of irradiated cercaria-induced resistance against Schistosoma mansoni in mice. *J. Immunol.* **1986,** *136* (7), 2644-2648.

72. Bickle, Q. D.; Andrews, B. J.; Doenhoff, M. J.; Ford, M. J.; Taylor, M. G. Resistance against Schistosoma mansoni induced by highly irradiated infections: studies on species specificity of immunization and attempts to transfer resistance. *Parasitology* **1985,** *90 ( Pt 2)*, 301-312.

73. Ford, M. J.; Bickle, Q. D.; Taylor, M. G.; Andrews, B. J. Passive transfer of resistance and the site of immune-dependent elimination of the challenge infection in rats vaccinated with highly irradiated cercariae of Schistosoma mansoni. *Parasitology* **1984,** *89 ( Pt 3)*, 461-482.

74. Glauert, A. M.; Butterworth, A. E.; Sturrock, R. F.; Houba, V. The mechansim of antibody-dependent, eosinophil-mediated damage to schistosomula of Schistosoma mansoni in vitro: a study by phase-contrast and electron microscopy. *J. Cell Sci.* **1978,** *34*, 173-192.

75. Wilson, R. A.; Coulson, P. S. Immune effector mechanisms against schistosomiasis: looking for a chink in the parasite's armour. *Trends Parasitol.* **2009,** *25* (9), 423-431.

76. Wilson, R. A.; Coulson, P. S. Schistosome vaccines: a critical appraisal. *Mem. Inst. Oswaldo Cruz* **2006,** *101 Suppl 1*, 13-20.

77. Butterworth, A. E.; Sturrock, R. F.; Houba, V.; Mahmoud, A. A.; Sher, A.; Rees, P. H. Eosinophils as mediators of antibody-dependent damage to schistosomula. *Nature* **1975,** *256* (5520), 727-729.
78. Butterworth, A. E.; Sturrock, R. F.; Houba, V.; Taylor, R. Schistosoma mansoni in baboons. Antibody-dependent cell-mediated damage to 51Cr-labelled schistosomula. *Clin. Exp. Immunol.* **1976,** *25* (1), 95-102.
79. Mahmoud, A. A.; Warren, K. S.; Peters, P. A. A role for the eosinophil in acquired resistance to Schistosoma mansoni infection as determined by antieosinophil serum. *J. Exp. Med.* **1975,** *142* (4), 805-813.
80. Butterworth, A. E.; Hagan, P. Immunity in human schistosomiasis. *Parasitol. Today* **1987,** *3* (1), 11-16.
81. Sher, A.; Smithers, S. R.; MacKenzie, P. Passive transfer of acquired resistance to Schistosoma mansoni in laboratory mice. *Parasitology* **1975,** *70 Part 3*, 347-357.
82. Joseph, M.; Capron, A.; Butterworth, A. E.; Sturrock, R. F.; Houba, V. Cytotoxicity of human and baboon mononuclear phagocytes against schistosomula in vitro: induction by immune complexes containing IgE and Schistosoma mansoni antigens. *Clin. Exp. Immunol.* **1978,** *33* (1), 48-56.
83. Kassis, A. I.; Aikawa, M.; Mahmoud, A. F. Mouse antibody-dependent eosinophil and macrophage adherence and damage to schistosomula of Schistosoma mansoni. *J. Immunol.* **1979,** *122* (2), 398-405.
84. Joseph, M.; Auriault, C.; Capron, A.; Vorng, H.; Viens, P. A new function for platelets: IgE-dependent killing of schistosomes. *Nature* **1983,** *303* (5920), 810-812.
85. Pancre, V.; Cesbron, J. Y.; Joseph, M.; Barbier, M.; Capron, A.; Auriault, C. IgE-dependent killing of Schistosoma mansoni schistosomula by human platelets: modulation by T cell products. *Int. Arch. Allergy Appl. Immunol.* **1988,** *87* (4), 371-375.
86. Capron, M.; Bazin, H.; Joseph, M.; Capron, A. Evidence for IgE-dependent cytotoxicity by rat eosinophils. *J. Immunol.* **1981,** *126* (5), 1764-1768.
87. Capron, M.; Capron, A.; Torpier, G.; Bazin, H.; Bout, D.; Joseph, M. Eosinophil-dependent cytotoxicity in rat schistosomiasis. Involvement of IgG2a antibody and role of mast cells. *Eur. J. Immunol.* **1978,** *8* (2), 127-133.
88. Capron, M.; Capron, A. Effector functions of eosinophils in schistosomiasis. *Mem. Inst. Oswaldo Cruz* **1992,** *87 Suppl 4*, 167-170.
89. Khalife, J.; Dunne, D. W.; Richardson, B. A.; Mazza, G.; Thorne, K. J.; Capron, A.; Butterworth, A. E. Functional role of human IgG subclasses in eosinophil-mediated killing of schistosomula of Schistosoma mansoni. *J. Immunol.* **1989,** *142* (12), 4422-4427.
90. Khalife, J.; Capron, M.; Capron, A.; Grzych, J. M.; Butterworth, A. E.; Dunne, D. W.; Ouma, J. H. Immunity in human schistosomiasis mansoni. Regulation of protective immune mechanisms by IgM blocking antibodies. *J. Exp. Med.* **1986,** *164* (5), 1626-1640.
91. Hagan, P.; Blumenthal, U. J.; Dunn, D.; Simpson, A. J.; Wilkins, H. A. Human IgE, IgG4 and resistance to reinfection with Schistosoma haematobium. *Nature* **1991,** *349* (6306), 243-245.
92. Viana, I. R.; Correa-Oliveira, R.; Carvalho, O. S.; Massara, C. L.; Colosimo, E.; Colley, D. G.; Gazzinelli, G. Comparison of antibody isotype responses to Schistosoma mansoni antigens by infected and putative resistant individuals living in an endemic area. *Parasite Immunol.* **1995,** *17* (6), 297-304.
93. Jiz, M.; Friedman, J. F.; Leenstra, T.; Jarilla, B.; Pablo, A.; Langdon, G.; Pond-Tor, S.; Wu, H. W.; Manalo, D.; Olveda, R.; Acosta, L.; Kurtis, J. D. Immunoglobulin E (IgE) responses to paramyosin predict resistance to reinfection with Schistosoma japonicum and are attenuated by IgG4. *Infect. Immun.* **2009,** *77* (5), 2051-2058.
94. Demeure, C. E.; Rihet, P.; Abel, L.; Ouattara, M.; Bourgois, A.; Dessein, A. J. Resistance to Schistosoma mansoni in humans: influence of the IgE/IgG4 balance and IgG2 in immunity to reinfection after chemotherapy. *J. Infect. Dis.* **1993,** *168* (4), 1000-1008.
95. Velupillai, P.; dos Reis, E. A.; dos Reis, M. G.; Harn, D. A. Lewis(x)-containing oligosaccharide attenuates schistosome egg antigen-induced immune depression in human schistosomiasis. *Hum. Immunol.* **2000,** *61* (3), 225-232.
96. Okano, M.; Satoskar, A. R.; Nishizaki, K.; Harn, D. A., Jr. Lacto-N-fucopentaose III found on Schistosoma mansoni egg antigens functions as adjuvant for proteins by inducing Th2-type response. *J. Immunol.* **2001,** *167* (1), 442-450.
97. van der Kleij, D.; van Remoortere, A.; Schuitemaker, J. H.; Kapsenberg, M. L.; Deelder, A. M.; Tielens, A. G.; Hokke, C. H.; Yazdanbakhsh, M. Triggering of innate immune responses by schistosome egg glycolipids and their carbohydrate epitope GalNAc beta 1-4(Fuc alpha 1-2Fuc alpha 1-3)GlcNAc. *J. Infect. Dis.* **2002,** *185* (4), 531-539.
98. Hokke, C. H.; Yazdanbakhsh, M. Schistosome glycans and innate immunity. *Parasite Immunol.* **2005,** *27* (7-8), 257-264.

99. van Die, I.; Cummings, R. D. Glycan gimmickry by parasitic helminths: a strategy for modulating the host immune response? *Glycobiology* **2010,** *20* (1), 2-12.

100. Thomas, P. G.; Carter, M. R.; Da'Dara, A. A.; DeSimone, T. M.; Harn, D. A. A helminth glycan induces APC maturation via alternative NF-kappa B activation independent of I kappa B alpha degradation. *J. Immunol.* **2005,** *175* (4), 2082-2090.

101. Faveeuw, C.; Mallevaey, T.; Paschinger, K.; Wilson, I. B.; Fontaine, J.; Mollicone, R.; Oriol, R.; Altmann, F.; Lerouge, P.; Capron, M.; Trottein, F. Schistosome N-glycans containing core alpha 3-fucose and core beta 2-xylose epitopes are strong inducers of Th2 responses in mice. *Eur. J. Immunol.* **2003,** *33* (5), 1271-1281.

102. van Die, I.; van Vliet, S. J.; Nyame, A. K.; Cummings, R. D.; Bank, C. M.; Appelmelk, B.; Geijtenbeek, T. B.; van Kooyk, Y. 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* **2003,** *13* (6), 471-478.

103. van Liempt, E.; Bank, C. M.; Mehta, P.; Garcia-Vallejo, J. J.; Kawar, Z. S.; Geyer, R.; Alvarez, R. A.; Cummings, R. D.; van Kooyk, Y.; van Die, I. Specificity of DC-SIGN for mannose- and fucose-containing glycans. *FEBS Lett.* **2006,** *580* (26), 6123-6131.

104. van Liempt, E.; van Vliet, S. J.; Engering, A.; Garcia Vallejo, J. J.; Bank, C. M.; Sanchez-Hernandez, M.; van Kooyk, Y.; van Die, I. Schistosoma mansoni soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation. *Mol. Immunol.* **2007,** *44* (10), 2605-2615.

105. Meevissen, M. H.; Driessen, N. N.; Smits, H. H.; Versteegh, R.; van Vliet, S. J.; van Kooyk, Y.; Schramm, G.; Deelder, A. M.; Haas, H.; Yazdanbakhsh, M.; Hokke, C. H. 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.* **2012,** *42* (3), 269-277.

106. Everts, B.; Hussaarts, L.; Driessen, N. N.; Meevissen, M. H.; Schramm, G.; van der Ham, A. J.; van der Hoeven, B.; Scholzen, T.; Burgdorf, S.; Mohrs, M.; Pearce, E. J.; Hokke, C. H.; Haas, H.; Smits, H. H.; Yazdanbakhsh, M. Schistosome-derived omega-1 drives Th2 polarization by suppressing protein synthesis following internalization by the mannose receptor. *J. Exp. Med.* **2012,** *209* (10), 1753-1767.

107. Eberl, M.; Langermans, J. A.; Vervenne, R. A.; Nyame, A. K.; Cummings, R. D.; Thomas, A. W.; Coulson, P. S.; Wilson, R. A. Antibodies to glycans dominate the host response to schistosome larvae and eggs: is their role protective or subversive? *J. Infect. Dis.* **2001,** *183* (8), 1238-1247.

108. 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.* **2008,** *30* (10), 554-562.

109. Hokke, C. H.; Fitzpatrick, J. M.; Hoffmann, K. F. Integrating transcriptome, proteome and glycome analyses of Schistosoma biology. *Trends Parasitol.* **2007,** *23* (4), 165-174.

110. Richter, D.; Incani, R. N.; Harn, D. A. Lacto-N-fucopentaose III (Lewis x), a target of the antibody response in mice vaccinated with irradiated cercariae of Schistosoma mansoni. *Infect. Immun.* **1996,** *64* (5), 1826-1831.

111. Nyame, A. K.; Leppanen, A. M.; DeBose-Boyd, R.; Cummings, R. D. Mice infected with Schistosoma mansoni generate antibodies to LacdiNAc (GalNAc beta 1-->4GlcNAc) determinants. *Glycobiology* **1999,** *9* (10), 1029-1035.

112. Nyame, A. K.; Leppanen, A. M.; Bogitsh, B. J.; Cummings, R. D. Antibody responses to the fucosylated LacdiNAc glycan antigen in Schistosoma mansoni-infected mice and expression of the glycan among schistosomes. *Exp. Parasitol.* **2000,** *96* (4), 202-212.

113. Naus, C. W.; van Remoortere, A.; Ouma, J. H.; Kimani, G.; Dunne, D. W.; Kamerling, J. P.; Deelder, A. M.; Hokke, C. H. Specific antibody responses to three schistosome-related carbohydrate structures in recently exposed immigrants and established residents in an area of Schistosoma mansoni endemicity. *Infect. Immun.* **2003,** *71* (10), 5676-5681.

114. Nyame, A. K.; Pilcher, J. B.; Tsang, V. C.; Cummings, R. D. Schistosoma mansoni infection in humans and primates induces cytolytic antibodies to surface Le(x) determinants on myeloid cells. *Exp. Parasitol.* **1996,** *82* (2), 191-200.

115. van Remoortere, A.; van Dam, G. J.; Hokke, C. H.; van den Eijnden, D. H.; van Die, I.; Deelder, A. M. Profiles of immunoglobulin M (IgM) and IgG antibodies against defined carbohydrate epitopes in sera of Schistosoma-infected individuals determined by surface plasmon resonance. *Infect. Immun.* **2001,** *69* (4), 2396-2401.

116. Nyame, A. K.; Pilcher, J. B.; Tsang, V. C.; Cummings, R. D. Rodents infected with Schistosoma mansoni produce cytolytic IgG and IgM antibodies to the Lewis x antigen. *Glycobiology* **1997,** *7* (2), 207-215.


117. van Roon, A. M.; Van de Vijver, K. K.; Jacobs, W.; Van Marck, E. A.; van Dam, G. J.; Hokke, C. H.; Deelder, A. M. Discrimination between the anti-monomeric and the anti-multimeric Lewis X response in murine schistosomiasis. *Microbes. Infect.* **2004,** *6* (13), 1125-1132.

118. Bardor, M.; Faveeuw, C.; Fitchette, A. C.; Gilbert, D.; Galas, L.; Trottein, F.; Faye, L.; Lerouge, P. Immunoreactivity in mammals of two typical plant glyco-epitopes, core alpha(1,3)-fucose and core xylose. *Glycobiology* **2003,** *13* (6), 427-434.

119. van Die, I.; Gomord, V.; Kooyman, F. N.; van den Berg, T. K.; Cummings, R. D.; Vervelde, L. Core alpha1-->3-fucose is a common modification of N-glycans in parasitic helminths and constitutes an important epitope for IgE from Haemonchus contortus infected sheep. *FEBS Lett.* **1999,** *463* (1-2), 189-193.

120. Butterworth, A.; Dunne, D.; Fulford, A.; Capron, M.; Khalife, J.; Capron, A.; Koech, D.; Ouma, J.; Sturrock, R. Immunity in human schistosomiasis mansoni: cross-reactive IgM and IgG2 anti-carbohydrate antibodies block the expression of immunity. *Biochimie* **1988,** *70* (8), 1053-1063.

121. Yi, X. Y.; Omer-Ali, P.; Kelly, C.; Simpson, A. J.; Smithers, S. R. IgM antibodies recognizing carbohydrate epitopes shared between schistosomula and miracidia of Schistosoma mansoni that block in vitro killing. *J. Immunol.* **1986,** *137* (12), 3946-3954.

122. Grzych, J. M.; Dissous, C.; Capron, M.; Torres, S.; Lambert, P. H.; Capron, A. Schistosoma mansoni shares a protective carbohydrate epitope with keyhole limpet hemocyanin. *J. Exp. Med.* **1987,** *165* (3), 865-878.

123. Geyer, H.; Wuhrer, M.; Resemann, A.; Geyer, R. Identification and characterization of keyhole limpet hemocyanin N-glycans mediating cross-reactivity with Schistosoma mansoni. *J. Biol. Chem.* **2005,** *280* (49), 40731-40748.

124. Ko, A. I.; Drager, U. C.; Harn, D. A. A Schistosoma mansoni epitope recognized by a protective monoclonal antibody is identical to the stage-specific embryonic antigen 1. *Proc. Natl. Acad. Sci. U. S. A* **1990,** *87* (11), 4159-4163.

125. Seeberger, P. H.; Werz, D. B. Synthesis and medical applications of oligosaccharides. *Nature* **2007,** *446* (7139), 1046-1051.

126. Pleasance, J.; Raadsma, H. W.; Estuningsih, S. E.; Widjajanti, S.; Meeusen, E.; Piedrafita, D. Innate and adaptive resistance of Indonesian Thin Tail sheep to liver fluke: a comparative analysis of Fasciola gigantica and Fasciola hepatica infection. *Vet. Parasitol.* **2011,** *178* (3-4), 264-272.

127. van Milligen, F. J.; Cornelissen, J. B.; Bokhout, B. A. Protection against Fasciola hepatica in the intestine is highly correlated with eosinophil and immunoglobulin G1 responses against newly excysted juveniles. *Parasite Immunol.* **1999,** *21* (5), 243-251.

128. Lightowlers, M. W. Cestode vaccines: origins, current status and future prospects. *Parasitology* **2006,** *133 Suppl*, S27-S42.

129. Fujiwara, R. T.; Loukas, A.; Mendez, S.; Williamson, A. L.; Bueno, L. L.; Wang, Y.; Samuel, A.; Zhan, B.; Bottazzi, M. E.; Hotez, P. J.; Bethony, J. M. Vaccination with irradiated Ancylostoma caninum third stage larvae induces a Th2 protective response in dogs. *Vaccine* **2006,** *24* (4), 501-509.

130. Bethony, J.; Loukas, A.; Smout, M.; Brooker, S.; Mendez, S.; Plieskatt, J.; Goud, G.; Bottazzi, M. E.; Zhan, B.; Wang, Y.; Williamson, A.; Lustigman, S.; Correa-Oliveira, R.; Xiao, S.; Hotez, P. J. Antibodies against a secreted protein from hookworm larvae reduce the intensity of hookworm infection in humans and vaccinated laboratory animals. *FASEB J.* **2005,** *19* (12), 1743-1745.

131. Cho-Ngwa, F.; Liu, J.; Lustigman, S. The Onchocerca volvulus cysteine proteinase inhibitor, Ov-CPI-2, is a target of protective antibody response that increases with age. *PLoS. Negl. Trop. Dis.* **2010,** *4* (8), e800.

132. Teixeira de Melo, T.; Michel de Araujo, J.; Do Valle, D. F.; Caliari, M. V.; Oliveira, S. C.; Coelho, P. M.; Fonseca, C. T. Immunization with newly transformed Schistosoma mansoni schistosomula tegument elicits tegument damage, reduction in egg and parasite burden. *Parasite Immunol.* **2010,** *32* (11-12), 749-759.

133. Teixeira de Melo, T.; Araujo, J. M.; Campos de Sena, I.; Carvalho, A. C.; Araujo, N.; Toscano, F. C. Evaluation of the protective immune response induced in mice by immunization with Schistosoma mansoni schistosomula tegument (Smteg) in association with CpG-ODN. *Microbes. Infect.* **2013,** *15* (1), 28-36.

134. Auriault, C.; Damonneville, M.; Verwaerde, C.; Pierce, R.; Joseph, M.; Capron, M.; Capron, A. Rat IgE directed against schistosomula-released products is cytotoxic for Schistosoma mansoni schistosomula in vitro. *Eur. J. Immunol.* **1984,** *14* (2), 132-138.

135. Auriault, C.; Damonneville, M.; Joseph, M.; Capron, M.; Verwaerde, C.; Billaut, P.; Capron, A. Defined antigens secreted by the larvae of schistosomes protect against schistosomiasis: induction of cytotoxic antibodies in the rat and the monkey. *Eur. J. Immunol.* **1985,** *15* (12), 1168-1172.

136. Verwaerde, C.; Auriault, C.; Damonneville, M.; Grzych, J. M.; Pierce, R.; Capron, A. IgG response of rats and humans to the released products of schistosomula of Schistosoma mansoni. *Parasitology* **1985,** *90 ( Pt 3)*, 509-518.

137. Damonneville, M.; Auriault, C.; Verwaerde, C.; Delanoye, A.; Pierce, R.; Capron, A. Protection against experimental Schistosoma mansoni schistosomiasis achieved by immunization with schistosomula released products antigens (SRP-A): role of IgE antibodies. *Clin. Exp. Immunol.* **1986,** *65* (2), 244-252.