



Eliminating schistosomes through vaccination: what are the best immune weapons?

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The successful development of vaccines depends on the knowledge of the immunological mechanisms associated with the elimination of the pathogen. In the case of schistosomes, its complex life cycle and the mechanisms developed to evade host immune system, turns the development of a vaccine against the disease into a very difficult task. Identifying the immunological effector mechanisms involved in parasite attrition and the major targets for its response is a key step to formulate an effective vaccine. Recent studies have described some promising antigens to compose a subunit vaccine and have pointed to some immune factors that play a role in parasite elimination. Here, we review the immune components and effector mechanisms associated with the protective immunity induced by those vaccine candidates and the lessons we have learned from the studies of the acquired resistance to infection in humans. We will also discuss the immune factors that correlate with protection and therefore could help to evaluate those vaccine formulations in clinical trials.

Keywords: schistosome vaccine, protective immunity, schistosomiasis, effector mechanisms, immune response

INTRODUCTION

Vaccination is a great strategy to control and eradicate diseases (1) and there is no doubt that the development of a vaccine against schistosomiasis would have a massive impact on disease control and would be useful as a complementary tool to the disease eradication (2). Schistosomes in contrast to viruses and bacteria are complex parasites that pass through different life stages in different anatomic sites of its definitive host (3). The parasite has evolved to live for decades in the host, developing interesting strategies to evade host immune system [reviewed in Ref. (4)]. So developing an effective vaccine against schistosomes is a difficult task. But since the parasite does not replicate in its definitive host, even a partial reduction in parasite burden is believed to have an impact on disease control and eradication.

For decades, scientists have tried to develop an effective vaccine formulation against schistosomes, and although two candidates are under clinical trials, the search for new candidates and vaccine formulations are far from ending. During all those years of studies on schistosome vaccine development, some immunological mechanisms involved in parasite elimination have been proposed. Complement activation has been suggested to be involved in parasite elimination, but from what we know so far, only recently transformed schistosomes are killed by complement and 24 h schistosomula became refractory to death induced by complement activation (5). Since most of these studies on complement activation and parasite death were performed *in vitro*, the significance of complement activation to parasite death *in vivo* can be questioned. Schistosomes only get to host vessels 72 h after penetrating the host and by this time the parasite is already resistant to death induced

by complement (6). Indeed, many evasion mechanisms developed by the parasite have been described and give support to the idea that the activation of the membrane attack complex might not be the major mechanism involved in parasite elimination (7–10).

Antibody-dependent cellular cytotoxicity (ADCC) is another immune mechanism that has been associated with parasite elimination. In individuals living in endemic areas for schistosomiasis, ADCC involving IgE, IgG, eosinophils, monocytes, and platelets was associated with the acquisition of resistance to reinfection (11–13). In mice, ADCC has been highlighted as the immune mechanism involved in parasite death in animals immunized with Smp-80 and GST (14–16). However, eosinophils may not be the major cell involved in ADCC in mice, since deficiency in this cell did not result in any changes in worm and egg burden after infection, demonstrating that eosinophils do not play major roles in parasite death (17).

Regardless of the mechanisms involved, antibodies are key players in the protective immunity induced by vaccines. Immunization of mice deficient in B cells impaired the protective response induced in wild-type animals by vaccination with irradiated cercariae (18). Also, transference of sera from mice immunized with schistosomula tegument (Smtg) or Smp-80 to a naïve recipient induce partial protection against challenge infection (19, 20). Other evidence of the importance of antibodies in the protective immune response induced by vaccination comes from the studies of Hewiston and coworkers (21). They demonstrated that the protective immune response induced by attenuated cercariae was abrogated in CD154-deficient mice. CD40–CD154 interaction is involved in eliciting a humoral immune response dependent on

T cells (22). The inoculation of IL-12 together with the vaccine in these deficient mice restored all the cellular immune parameters in mice lung but failed to restore protection and antibody production (21).

Cellular immune responses are also important in parasite elimination. Immunization of C57BL-6 mice deficient on IFN- γ , and TNFRI impair or abrogate protection induced by vaccine (18, 23, 24). The role of IFN- γ and TNF- α in parasite killing seems to be related to nitric oxide production by macrophage. Immunization of mice deficient in the TNFRI with irradiated cercariae abrogates protection and impairs nitric oxide synthase (iNOS) expression in lung macrophages (24). Nevertheless, immunization of mice deficient in iNOS result only in partial reduction on the protective immunity induced by irradiated cercariae, indicating that nitric oxide is not the major factor involved in parasite death (25).

In BALC-c mice, deficiency in IL-4R expression abrogates protection induced by irradiated cercariae that can be restored by wild-type serum transference (26). Recently, protective immunity-associated Th2 profile was observed in outbred mice immunized with glyceraldehyde 3-phosphate dehydrogenase (SG3PDH) and peroxiredoxin (TPX) (27). IL-10 and IL-17 production seems to correlate negatively with protection. Blocking IL-10 with neutralizing antibodies enables protection against challenge infection in mice previously infected with *Schistosoma mansoni* and treated with praziquantel (28). In *S. japonicum* infection, blocking IL-17 with neutralizing antibodies enhances antibody production and protection in infected mice (29).

Although CD8+ cells are classically related to immune responses against intracellular pathogens, its role in schistosome elimination has been recently described (30). Immunization of mice with the *S. japonicum* 22.6/26GST coupled to Sepharose 4B bead induced a significant reduction in parasite burden that was associated with an increase in the number of activated CD8+ cells (30). These activated CD8+ cells were able to promote death of parasite carrying host MHC I molecules in its surface (30).

Coulson and Wilson (31) suggested that the major mechanism involved in parasite elimination after immunization with the irradiated cercariae vaccine was in fact the generation of an inflammatory focus in the lung of immunized mice that impairs parasite migration and therefore its transformation into adult worms (31). Evidence that support this hypothesis is given by histological examination of mouse's lungs which demonstrates that the parasites in the inflammatory foci were alive and when recovered from the lung and transferred to a naïve recipient they developed into adult worms (31, 32).

Besides all the knowledge generated and described so far, the majority of the studies on immune mechanisms involved in schistosome elimination have been performed using the irradiated cercariae strategy that for security reason are not used in human trials. Currently, researchers are developing vaccine formulations based on one or a cocktail of parasite antigens and although there are many studies on different vaccine strategies, little is known about the effective protective mechanisms. In this review, the immune components and mechanisms elicited by different vaccine strategies using subunit formulations containing promising parasite antigens will be described. We will evaluate whether there is (are) immune factor(s) that correlates with protection. And this

information might be used to rationally design a vaccine formulation and to suggest a strategy that better elicits these protective responses. These correlates of protection can also help to evaluate whether those vaccines are effective during clinical trials.

THE IMMUNE RESPONSE ELICITED BY PROMISING SCHISTOSOME ANTIGENS

Some of the schistosome antigens tested in pre-clinical trials emerged as promising candidates to compose an anti-schistosomiasis vaccine due to their ability to consistently induce protective immune responses in different animal models, under different formulations and vaccine strategies.

Two schistosome antigens are under clinical trials, the fatty acid-binding protein of 14 kDa from *S. mansoni*, Sm14 (33), and the glutathione-S-transferase of 28 kDa from *S. haematobium* (34). The *S. mansoni* tetraspanin 2, TSP-2, is now been produced under good manufacture practices (GMP) to soon be evaluated in Phase I clinical Trial (35).

GLUTATHIONE S-TRANSFERASE

The 28 kDa glutathione S-transferase of *S. mansoni*, Sm28GST, is one of the most promising vaccine candidates. Recognized as the enzyme glutathione S-transferase (36), Sm28GST was identified in tegument, parenchyma, and genital organs of schistosomula and adult worm (16, 37). In the vaccine protocol, Sm28GST purified protein was able to significantly reduce the worm burden in rats and mice (16). *In vitro* experiments suggested that this protection was related to the cytotoxic response since in the presence of anti-Sm28GST antibodies and mouse eosinophils, schistosomula can be killed through ADCC (15, 16). The importance of antibodies in worm elimination was evaluated by the passive transfer of specific antibodies which were able to induce protection against challenge infection (16). A study performed by Boulanger and colleagues (38) reinforced the immunoprotective potential of Sm28GST protein, by demonstrating that immunization of baboons with the recombinant protein together with aluminum hydroxide (Alum) confers up to 80% of protection.

Several studies demonstrated the importance of antibodies in parasite elimination in animals immunized with Sm28GST. Mouse immunization with one dose of rSm28GST plus bacillus Calmette-Guérin (BCG) or Alum, as adjuvants, conferred protection against *S. mansoni* infection, and induced significant production of specific IgG, IgA, and IgE (39). In another study, mice immunization with Sm28GST produced in recombinant *Mycobacterium bovis* BCG, regardless of the immunization route induced a vigorous production of IgG1, IgG2a, and IgG2b levels, which was associated with the neutralization of the Sm28GST enzymatic activity (40). Intradermal immunization with a DNA encoding Sm28GST, also induced a significant production of anti-Sm28GST IgG antibodies, mainly IgG2a and IgG2b, with an ability to kill schistosomula through ADCC mechanism (41). In addition to the antibodies, the cellular immunity is also critical to *Schistosoma* elimination. Mice immunized with the recombinant Sm28GST protein or with peptide derived from C-terminal region showed reduction in worm burden, in fibrosis, and in the number of eggs in the liver that were associated with high levels of IFN- γ (42, 43). In a DNA vaccine strategy, immunization of mice with Sm28GST co-delivered with

an IL-18-encoding plasmid, also induced a strong IFN- γ production and result in a significant reduction in egg and worm burden, reinforcing the importance of the Th1 response to the protection induced by Sm28GST (44).

One important feature of Sm28GST is the existence of cross-reactivity with other *Schistosoma* species, including *S. haematobium*, *S. japonicum*, and *S. bovis* (45). This property can be explored in the context of vaccine development which can act to eliminate different *Schistosoma* species at the same time. In this sense, it was demonstrated that immunization of primates with rSm28GST protect against heterologous infection with *S. haematobium* (46). The Sh28GST (GST protein derived from *S. haematobium*) has the ability to confer protection in monkeys that showed a reduction in worm fecundity (47). The results of the studies with schistosome GST as antigen in vaccine formulations clearly demonstrate the importance of antibodies for anti-fecundity effect and parasite elimination, through neutralization and ADCC mechanisms, respectively. It is important to note that this antigen can induce protection by reducing worm burden or female fecundity and thus this vaccine formulation is efficient to limit infection and pathology.

Sm14

Sm14 is a *S. mansoni* fatty acid-binding protein that might be involved in lipid uptake from the host (48). Due to its predicted function, Sm14 represent an interesting target for vaccines against schistosomes. Schistosomes are unable to synthesize fatty acids and sterols through *de novo* pathway and therefore require host lipids to maintain its complex membrane system and physiological function (49, 50). Sm14 is expressed in the cercariae, schistosomula, adult worm, and eggs and located in the parasite tegument and gut, both tissue that represent the interface between parasite and host (51).

The ability of rSm14 to protect against schistosomiasis was first demonstrated by Tendler and coworkers (52). In their study, immunization of mice with rSm14 alone or formulated with Freund's adjuvant (FA) induced protection levels ranging from 50 to 68% (52). In rabbits, rSm14 plus FA elicited 89% protection against challenge infection (52). Interestingly, Sm14 was also able to protect mice from *Fasciola hepatica* infection thus demonstrating its potential to be used in a vaccine formulation against both parasites (52).

In the case of Sm14, the protective immune response is dependent on IFN- γ and TNF- α production since in mice deficient in those cytokines, immunization with the recombinant form of Sm14 fails to induce protection (23). The importance of cellular components in the protective immune response elicited by Sm14 is also shown by the ability of epitopes from Sm14 to induce proliferative response in lymphocytes from resistant individuals and to induce protection in mice (53, 54). Immunization of mice with *Sm14* gene also induced significant protection associated with antibody production and increased production of IFN- γ by spleen cells and lung lavage cells (55). In a DNA vaccine strategy, the use of *IL-12* as adjuvant induced a significant production of IL-10 and nitric oxide and failed to induce antibody production and protection (55). The role of antibodies in the protective immunity induced by Sm14 cannot be ruled out. Although so far no direct

role for antibodies in parasite elimination have been described, all the successful vaccine formulation containing Sm14 induce significant antibody production (23, 54, 55). Therefore, the role of antibodies in the protective immunity induced by Sm14 is a key question that has to be addressed if they are to be used as correlates of protection in future clinical trials.

TETRASPANINS

Tetraspanins are members of membrane-spanning proteins containing four transmembrane domains, three short intracellular domains, and two extracellular loops (EC1 and EC2) (56). In schistosomes, tetraspanins are located in the outer tegument, thus in contact with host immune system (57). The EC2 loop mediates protein-protein interactions (58) that are important to tetraspanin role in the molecular organization of cell membranes. Through interaction with many proteins, tetraspanins form a complex termed as tetraspanin-enriched microdomains (TEM) (59). The importance of tetraspanins to parasite survival was recently demonstrated using interference RNA technique (RNAi). Silencing *Tsp-1* and *Tsp-2* transcription resulted in significant reduction in the number of worm that reaches maturity in the mammalian host. Also, schistosomula treated with *Sm Tsp-2* double strand RNA displayed vacuolated and thinner tegument, demonstrating TSP-2 role in maintaining tegument integrity (60).

Immunization of mice with a recombinant form of TSP-1 and TSP-2 resulted in 29–38 or 53–61% reduction in worm burden, respectively. The protection was associated with an increased titer for IgG1 and IgG2a antibody (61). Immunization of mice with a vaccine formulation containing TSP-2, Alum, and CpG as adjuvant elicited a protection level ranging from 25 to 27%. In this vaccine formulation, although significant titers of IgG antibodies were observed, there was no clear association between antibody levels and parasite burden and also there was no significant increased production of cytokine specific for TSP-2 immunized group. In contrast, an increased level of IFN- γ , IL-4, and IL-10 were observed in mice immunized with a chimerical protein containing TSP-2 and the 5B region of the hookworm aspartic protease Na-APR-1 (62). Besides the great result observed against *S. mansoni* infection, TSP-2 orthologs in *S. japonicum* do not represent a good vaccine candidate, since in this species TSP-2 is extremely polymorphic (63).

Smp-80

Another promising antigen is the large subunit of calpain, Smp-80. Schistosome calpain is composed of a smaller subunit of 28 kDa and a larger subunit of 78 kDa (64). The large subunit was described to be localized in the parasite surface (64). This subunit has proteolytic activity in the presence of Ca²⁺ (64) and plays an important role in immune evasion. Smp-80 is involved in the renewal/recycling of the parasite surface, an important evasion mechanism used by the parasite to escape from host immune system (65).

The large subunit of calpain has been evaluated in different vaccine formulations and strategies and the immune components produced in response to immunization have also been described. This antigen induced significant protection in mice, baboon, and hamster and protects against *S. mansoni*, *S. japonicum*, and

S. haematobium (66–68). Immunization of mice with naked DNA containing *Smp-80* gene resulted in 39% of reduction in worm burden, the use of *IL-2* and *IL-12* gene as adjuvant increased the protection level to 57 and 45%, respectively. This increased protection was associated with an increased production of specific IgG2a and IgG2b, increased proliferative response, and IFN- γ production (69, 70). The use of *GM-CSF* and *IL-4* gene as adjuvant resulted in 42 and 44% reduction in worm burden associated with increased production of IL-4, IgG, IgG1, and IgG2b in *GM-CSF* immunized animals and IL-4 and IgG3 in *IL-4* immunized group (70, 71). When the recombinant form of *Smp-80* is used as antigen either in its recombinant form or in a prime-boost regimen, higher titers of antibodies and significant production of IL-2 and IFN- γ are observed and this immune profile is associated with a reduction of 51 and 49% in worm burden (66). Therefore, the protective immune response induced by *Smp-80* seems to correlate to a Th1 profile with increased IFN- γ and antibody production especially IgG2a.

Indeed in mice immunized with *Smp-80*, antibodies play an important role in the elimination of the parasite. Transference of sera from *Smp-80* immunized mice to a naïve recipient result in 31–45% reduction in worm burden after a challenge infection (20). Complement seems not to play a major role in the parasite elimination once immunization of mice deficient in C3 factor with *Smp-80* did not result in significant reduction of the protection level observed in wild-type mice (72). ADCC instead seems to be the immune mechanism involved in parasite death, increased number of dead schistosomula was observed *in vitro* when these parasites were incubated with sera from *Smp-80* plus CpG immunized mice in the presence of lung lavage cells or lung cells (14). The increased percentage of dead parasites was associated with production of nitric oxide suggesting that the production of this molecule might be involved in ADCC-induced parasite killing (14).

Sm29

Sm29, other promising vaccine antigen, was identified by Cardoso and coworkers (73) using *in silico* analysis to identify in the *S. mansoni* transcriptome putative expressed proteins localized in the parasite tegument. The characterization of Sm29 demonstrated that this protein is expressed in the tegument of schistosomula and adult worm (74). The ability of Sm29 to induce protective immunity was assessed in mice with a vaccine formulation containing FA which resulted in a significant reduction of 51% in worm burden, 60% in intestinal eggs, and 50% in liver granuloma area, associated with a significant production of IgG, IgG1 and IgG2a-specific antibody, IFN- γ , TNF- α , and IL-10 cytokine production (74).

IMMUNE RESPONSE INDUCED BY MULTIVALENT FORMULATIONS

Schistosomes are complex parasites and thus the design of a multivalent vaccine against the parasite might enhance subunit vaccine efficacy. Recently, these multivalent vaccine formulations containing promising antigens have been tested. Sm29 was tested together with the SmTSP-2 in a multivalent chimeric recombinant vaccine in mice, aiming to enhance the single antigen vaccination efficacy. The vaccine formulation with a chimeric protein containing TSP-2

and the C-terminal part of Sm29 resulted in a small increase in the protection level induced by rSm29 alone from 20.36 to 34.83% (75), or by rTSP-2 alone from 27 to 34.83% (62, 75) when formulated with CpG-ODN plus alum. The chimeric protein induced a significant production of IgG and IgG2a-specific antibodies and a Th1 immune profile (75).

Another chimeric formulation combining Sm29 and Sm14 recombinant proteins was tested in vaccine protocol associated or not with poly (I:C)-adjuvant. Although immunization of Swiss mice with a subunit vaccine containing rSm14 or rSm29 alone did not induce significant reduction in adult worm, the vaccine formulation containing both rSm14 and rSm29 elicited 31 or 40% protection when it was formulated without adjuvant or with Poly (I:C), respectively (76).

The results observed in these multivalent formulations demonstrate that it is a promising strategy, but the choice of antigens that induce similar protective immune profile is a key step for the success of the vaccine formulation.

WHAT CAN WE LEARN FROM THE STUDIES IN HUMANS?

In endemic areas, the existence of naturally resistant individuals (77) that present persistently negative stool examination even if they are in constant contact with contaminated water enable the search for immune factors and biomarkers involved in resistance to *Schistosoma* infection.

Studies on human immune responses to Sm14, demonstrate that CD4+ T cells from naturally resistant individuals mounted a Th1-type of immune response to rSm14, based on IFN- γ and TNF- α production (78). Moreover, T-cell proliferative responses to rSm14 from these individuals were totally abrogated after treatment with anti-IFN- γ antibodies. These individuals also produce significant levels of IgG1 and IgG3 antibodies against Sm14, subclasses associated with parasite killing (79). Significant production of IgG1 and IgG3 specific for Sm29 and TSP-2 were also observed in individuals naturally resistant to *S. mansoni* infection (61, 80).

Human antibody responses to the large subunit of schistosomal calpain have also been associated with resistance. In humans infected with *S. japonicum*-presenting light infections, a strong reactivity to calpain was observed whereas in individuals with stronger infection, low reactivity to calpain was observed (81). In a study in an endemic area for schistosomiasis in Egypt, IFN- γ production in response to Sm14 and IgE and IgA antibodies against Sm28GST correlated with resistance to infection (82).

Grzych and colleagues (83) reinforce the pivotal role of the antibodies in protection. They show that IgA specific to Sm28GST were present in the sera from infected individuals before and after treatment with praziquantel and that these immunoglobulins have a key role in neutralizing the GST enzyme activity which resulted in impaired female fecundity.

Since 1998, Sh28GST recombinant protein plus aluminum hydroxide adjuvant has been tested in the human population. Partial results of the phase I clinical trials were published by Riveau and colleagues (34), in their study, no relevant side effects or toxicity following vaccine administration was observed. Humoral immune responses generated by the vaccine were characterized by high levels of IgG1 and IgG3 production and low levels of IgG2 and IgA. The cellular response profile was characterized

Table 1 | Summary of the protection levels and immune components elicited by immunization.

Antigen	Immunization strategy	Adjuvant	Protection level (%)	Humoral response	Cellular response	Reference
Smp-80	DNA vaccine	None	39		Prolif. IFN- γ , IL-4	(67–69)
		IL-2	57	↑ IgG, IgG2a and b	↑ Prolif. ↑ IFN- γ ↓ IL-4	
		IL-12	45	↑ IgG, IgG2a and b	↑ Prolif. ↑ IFN- γ ↓ IL-4	
		IL-4	44	↑ IgG3	Prolif. IFN- γ ↑ IL-4	
	Prime E boost	GM-CSF	42	↑ IgG, IgG1	Prolif. IFN- γ ↑ IL-4	
		Resiquimod (R848)	49	↑ IgG, IgG1, IgG2a and b, IgG3, IgA, IgM	IL-2 and IFN- γ	(70)
		Recombinant protein	Resiquimod (R848)	51	↑ IgG, IgG1, IgG2a and b, IgG3, IgA, IgM	IL-2 and IFN- γ
CpG-ODN	52.86		IgG, IgG1, IgG2b, IgG3, IgM	ADCC AND NO production	(14, 71)	
Sm14	Recombinant protein	None	25			(22)
		FA	25	↑ IgG2a		
		rIL-12	42.2	↑ IgG2a	Protection is dependent on IFN- γ and TNF- α production	
	DNA vaccine	None	40.5	IgG	IFN- γ by SC and BAL cells	(53)
Synthetic peptides	FA + Padre	26–36.7	IgG1, IgG2a	IFN- γ IL-10	(52)	
Sm29	Recombinant protein	FA	51	IgG1, IgG2a	IFN- γ , TNF- α , IL-10	(73)
		CpG-Alum	20	IgG, IgG1, IgG2a	IFN- γ	(74)
TSP-1	Recombinant protein	FA	29–38	IgG1, IgG2a	Not reported	(59)
TSP-2	Recombinant protein	FA	53–61	IgG1, IgG2a	Not reported	(59)
		Alum + CpG	25–27	IgG, IgG1 ^a	IL-4, IFN- γ , and IL-10 ^b	(60)
Sm28GST	Purified protein	FA	40–68.3	Not reported	Eosinophils (ADCC)	(16)
	Recombinant protein	Alum	46	Not significant	IL-2 and IFN- γ	(41)
	DNA vaccine	IL-18	23	Not significant	IFN- γ	(42)
Sh28GST	Recombinant protein	FA	77 (fecundity)	IgG and IgA	Not reported	(45)
		BCG	60	IgG		

^aNo association with protection;

^bproduction in response to infection and not to immunization; SC, spleen cells; BAL, broncho alveolar lavage; ↑ compared to vaccine formulation without adjuvant.

by significant production of IL-5 and IL-13, resulting in a Th2 predominant response. The ability of antibodies to inhibit the enzymatic Sh28GST activity was also observed, corroborating with experimental studies (34).

CONCLUSION AND FUTURE PERSPECTIVE

Understanding the immunological mechanisms involved in parasite elimination during an infection is a key step toward the development of an effective vaccine. Here, we reviewed the immune components activated under different formulations containing antigens described as promising candidates to compose an anti-schistosomiasis vaccine (summarized in Table 1). Although for some of the antigens the immune mechanism involved in parasite death have been demonstrated, for others they are still to be identified. The biological role the target antigen plays in the survival of the parasite and the immunological components elicited by a protective formulation gives us an indication of what immune mechanisms might be involved in parasite death. Yet determining

their involvement in protective immunity is still necessary. To address this question, the use of animals deficient in components of the immune system represents an interesting tool that should be further explored. Once those immune factors that correlate with protection are identified, they can be used as a biomarker of resistance in clinical trials.

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