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Current status and future prospects of protein vaccine candidates against *Schistosoma mansoni* infection

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ABSTRACT

Schistosomiasis is an acute and chronic tropical parasitic disease caused by blood dwelling worm of the genus Schistosoma. It is the most destructive disease globally and is a major cause of morbidity and mortality for developing countries. Three main species of schistosomes infect human beings from which *S. mansoni* is the most common and widespread. Over the last several decades, chemotherapy using praziquantel has been a commonly used strategy for the treatment and control of schistosomiasis. However, control programs focused exclusively on chemotherapy have been challenging because of the frequency and rapidity of reinfection and these programs were expensive. Thus, new schistosomiasis control strategies will be needed.

Vaccination strategy would be an ideal tool for a significant and sustainable reduction in the transmission and disease burden of schistosomiasis. An effective anti schistosome vaccine would greatly contribute to decreasing schistosomiasis-associated morbidity via protective immune responses leading to reduced worm burdens and decreased egg production. Vaccine development is a long process that can take decades. There have been three candidate vaccines that have been produced by Good Manufacturing Procedure and entered human clinical trials for *S. mansoni* are Sm14, SmTSP-2, and Sm-p80. Other candidates that are in pre-clinical trials at various stages include paramyosin, Sm29, SmKI-1, and Sm23. Since the growth of several new technologies, including genomics, transcriptomics, microarrays, immunomic profiling, and proteomics, have helped in the identification of promising new target schistosome antigens. Therefore, this review considers the present status of protein vaccine candidates against *Schistosoma mansoni* and provides some insight on prospects vaccine design and discovery.

Contents

Abbreviations: Ab, Antibody; AE, Asparaginyl Endopeptidase; Ag, Antigen; CB, Cathepsin B; CD, Cathepsin D; CL3, Cathepsin L3; DNA, Deoxyribonucleic Acid; FA, Fatty Acid; FABP, Fatty Acid Binding Protein; GLA-Alum, Glucopyranosyl Lipid A Formulated in Aluminum; GLA-SE, Glucopyranosyl Lipid Adjuvant Stable Emulsion; IFN-γ, Interferon Gamma; Ig, Immunoglobulin; IL, Interleukin; KI, Kunitz Type Protease Inhibitor; LcP, Lipid Core Peptide; Pmy, Paramyosin; Sm, *Schistosoma mansoni*; Th, T-helper Cells; TSP, Tetraspanins; WHO, World Health Organization.

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1. Introduction

Schistosomiasis or bilharziasis is an acute and chronic tropical parasitic disease caused by a blood-dwelling worm of the genus Schistosoma. It is endemic in 77 countries with an estimated total of 230 million people are infected worldwide, and 732 million persons being vulnerable to infection worldwide (Colley et al., 2014; Gryseels et al., 2006). Schistosomiasis is the most destructive disease globally and the major cause of morbidity and mortality for developing countries (Magalhães et al., 2011). Three main species that infect human beings are *S. mansoni, S. haematobium,* and *S. japonicum* (Gryseels et al., 2006). *S. mansoni* is the most prevalent being endemic in 55 countries, which is the source of hepatic and intestinal (Barakat, 2013).

In Africa, an estimated 660 million people were at risk, which accounts for 90% of globally at-risk people and 201.5 million infected people were estimated to occur (Okwori et al., 2014). Schistosomiasis is the second most widespread parasitic disease in various nations in sub-Saharan Africa (Adenowo et al., 2015). Almost 300,000 people die annually from schistosomiasis in Africa alone (Olveda et al., 2013). In Ethiopia, an estimated 37.3 million people living in schistosomiasis endemic areas (Negussu et al., 2017).

Schistosoma parasites complete their life cycle in two different hosts i.e. humans and freshwater snails. Human gets an infection when they come to contact with water containing the infectious cercarial larvae. The parasite has two times circulation in the blood-the first is to lung and the second is to liver portal system where they mature into adults. Then develop to male-female worm pairs, and inhabit either in different vessels. The adult worms migrate to the mesenteric vessels and begin to lay eggs. Most of the eggs are carried upstream to the liver via the portal veins and its branches and get trapped in the pre-sinusoidal portal venules. Some of the eggs migrate and penetrate the intestines and shed in the stool (Colley et al., 2014; Olveda et al., 2013). Based on pathogenesis schistosomiasis can be classified as acute and chronic forms. Acute schistosomiasis occurs weeks to months after infection. Typical clinical presentation is a sudden onset of headache, myalgia, fever, eosinophilia, malaise, and abdominal pain (Ross et al., 2007). In chronic schistosomiasis, eggs are the cause of pathology, which elicits chronic inflammation that leads to tissue fibrosis and chronic morbidity (Burke et al., 2009).

Microscopic examination of excreta for the detection of the egg remains the gold standard for the diagnosis of schistosomiasis. For people living in low transmission areas serological, immunological, and molecular tests may be useful in showing exposure to infection (Utzinger et al., 2015). Over the last several decades, human treatment with praziquantel (PZQ) being the only effective medication and widely used strategy for the treatment and control of schistosomiasis. However, control programs focused exclusively on chemotherapy have been challenging because of the frequency and rapidity of reinfection. Furthermore, praziquantel has two main drawbacks which are its poor activity against immature stages and the emergence of praziquantel resistant schistosomes is a constant threat (Siddiqui and Siddiqui, 2017; Cioli et al., 2014).

WHO 2025 target is “elimination as a public health problem,” meaning that the treated region has reached ≤1% prevalence of heavy intensity infections among school-age children. An additional goal is to interrupt the transmission in the low prevalence region by 2025 (Organization WH. Schistosomiasis Progress Report (2001–2011) and Strategic Plan (2012–2020) World Health Organization Press, 2013). Early studies showed that improvements in adherence to treatment, development of potential vaccine candidates was the best option to improve the possibility of achieving WHO goals (Toor et al., 2018). There is general agreement among experts that sustainable reduction in the transmission and disease burden of schistosomiasis can only acquire through long-term protection via vaccination. An effective anti-schistosome vaccine would greatly contribute to decrease schistosomiasis-associated morbidity via protective immune responses leading to reduced worm burdens and decreased egg production. A schistosomiasis vaccine currently does not exist and substantial development effort would be needed (Siddiqui et al.,

2011; Mo and Colley, 2016). This review evaluates the current status in the development of protein vaccines against schistosomiasis as well as future prospects that may improve the availability of vaccines.

The objective of this review is to discuss the current status of protein vaccine candidates against *Schistosoma mansoni***.** In addition, it may provide a good highlight of future prospects for schistosomiasis protein vaccine development.

2. Methods

Data for the present review were identified and collected using electronic extensive literature search strategies of published sources. Electronic databases included PubMed, Google Scholar, Science direct, Hindawi, and others used for searching the literature. During writing this seminar, most articles were published within the past 10 years in different journals were selected as well as articles which were written in the English language were used. Articles written in other languages and not open accessed were not included. After searching fully articles and abstracts were screened and those relevant to the objective were reviewed. The EndNote referencing manager was used for reference purposes.

To identify relevant studies on *Schistosoma mansoni* vaccines, the search initially began with the text string "*Schistosoma mansoni* vaccines" and a combination of the following words in permutations were used to extract suitable studies "Schistosomiasis + General characteristics," "Schistosomiasis + epidemiology," "Schistosomiasis + vaccine," "*Schistosoma mansoni* + vaccine" and "*Schistosoma mansoni* + protein vaccines," "*Schistosoma mansoni* + vaccine candidates," and others. Research articles bibliographic details (authors, title, full source, document type, and addresses) have been downloaded and maintained in a file.

3. Current status of protein vaccine candidates against *Schistosoma mansoni* infection

Proteins are molecules made of amino acids. They form the basis of living tissues and coded by genes. Proteins are the building blocks of life that are responsible for the development, repair, and maintenance of cells. They provide a substantial amount of energy allowing the body to carry out essential tasks such as tissue maintenance and processes. Schistosoma proteins play a central role in biological processes like; to catalyze reactions in the body, transport molecules, and transmit messages from cell to cell. They are the crucial component that makes life possible with every living organism. The majority of immunogens are proteins and are more immunogenic than lipids and carbohydrates (Tan et al., 2008).

3.1. Fatty acid binding protein

Fatty acid-binding protein (FABP), is a 14 kDa cytosolic protein expressed in the basal lamella of tegument and gut epithelium of cercaria, schistosomula and adult stage of parasites. The parasite is completely dependent on its host for the synthesis of Fatty Acid (FA) and other lipid supplies because it lacks oxygen-dependent pathways essential for the synthesis/production of FAs and sterols. The FABP plays an important role in uptake, transport, and compartmentalization of host-derived FAs, which is important for the existence of the parasite. Fatty acid binding protein is the primary target for both vaccination and drug development in both humans and domesticated animals (McManus and Loukas, 2008; Damasceno et al., 2017).

A recombinant 14 kDa *S. mansoni* (rSml4) antigen (Ag) in the absence of adjuvant enabled to reduce *S. mansoni* worm burden up to 67% in outbred Swiss mice and up to 89% in white rabbits against challenge with cercariae. Additionally, rSm14 Ag has cross-reactive protective immunity for the animal parasite like *F. hepatica* and schistosomes. In the outbred Swiss mice model, complete protection against *F. hepatica* was provided by using the same rSm14 Ag. This result displayed that it is possible to develop a single vaccine that is effective against the two parasites (Tendler et al., 1996).

A phase 1 clinical trial of an rSm14 formulated with the synthetic glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE) adjuvant was conducted on 20 volunteer males from a non-endemic area of Brazil for schistosomiasis. Significant increases in Sm14 specific total IgG, IgG1, and IgG3 were observed 30 days after the first vaccination with specific IgG2 and IgG4 after 60 days. Additionally, 88% of vaccinated subjects had produced high titers of Sm14 specific IgG Antibody (Ab) after 90 days of the first injection. A complex mixture of Th1 and Th2 cytokines are released from PBMN cells after rSm-14 stimulation. Type-1 response characterized by IFN and TNF; these cytokines control the effects parasite eggs on the liver, a situation that is rapidly followed by a strong egg-induced Th2 response (IL-5, IL-10) which avoids serious consequences and protects the liver by downregulating the inflammatory mediators. The rSm14/GLA-SE product was a strongly immunogenic and safe vaccine paving the way for follow up phase 2 trials (Santini-Oliveira et al., 2016). Phase II trials to assess field-based immunogenicity and safety of Sm14 are planned in endemic areas of Brazil and Africa (Molehin et al., 2016).

3.2. Tetraspanin proteins

Tetraspanins (TSP) are four transmembrane domain proteins found on the surfaces of eukaryotic cells. They are highly abundant at the outermost membrane of the parasitic stage of the schistosome so, they are highly exposed to the host immune system. They are responsible for stability during biosynthesis and essential for assembly and maintenance of the TSP web. The two extracellular loops mediate specific protein-protein interactions with laterally associated proteins. The major TSP proteins in *S. mansoni* are Sm-TSP-1 and Sm-TSP-2. The full-length Sm-TSP-2 protein believed to have a role in both tegument creation and maturation as the structural morphology of the adult worm. Specifically, TSP-2 provided high levels of protection as a recombinant vaccine in

S. mansoni challenge animal models and relating with protective immunity in naturally resistant people (McManus and Loukas, 2008; Tebeje et al., 2016; Curti et al., 2013).

Vaccination of mice with the recombinant Sm-TSP proteins resulted in reductions of 57%, 65%, and 64% in rSm-TSP-2 and 34%, 69%, and 52% in rSm-TSP-1 for mean adult worm burdens, fecal egg counts, and liver egg burdens, respectively, for two independent trials. Immunoglobulin G1 and IgG2a Abs are the most dominant Ab responses. Besides, Sm-TSP-2 was strongly recognized by IgG1 and IgG3 Abs from naturally resistant individuals to schistosomiasis, and the vaccine efficacy in mice, emphasize the potential of this molecule as a safe and effective vaccine for human schistosomiasis (Tran et al., 2006).

Schistosoma mansoni TSP-2 with alum/CpG adjuvant showed 25–27% reductions in adult worm burdens and a 20–27% reduction in liver egg burdens. Improved protection level found with the chimeric fusion protein called Sm-TSP-2/5B, which is Sm-TSP-2 fused to the immunogenic 5B region of the hookworm aspartic protease expressed in *Escherichia coli. Schistosoma mansoni* TSP-2/5B vaccinated mice had 54–58% decreases in worm burden and 48–56% reduction in liver egg burdens. Increased production of IL-4, IL-10, IFN-γ and anti-Sm-TSP-2 specific Abs obtained from Sm-TSP-2/5B vaccinated animals. The two vaccines mounted strong Sm-TSP-2 specific IgG responses but higher mean Ab titers and significantly greater protection were in the group vaccinated with SmTSP-2/5B. In schistosomes and hookworms endemic area the chimeric Sm-TSP-2/5B Ag could be used to effectively and safely vaccinate people (Pearson et al., 2012).

The safety and immunogenicity of a candidate Sm vaccine were assessed in this phase I, double-blind trial on healthy adults from a non-endemic area. Recombinant Sm-TSP-2 vaccine formulated on aluminum hydroxide adjuvant (Sm-TSP-2/Al) with or without GLA-AF. Dose and adjuvant-related increases in serum IgG against Sm-TSP-2 were observed. The frequencies of anti-Sm-TSP-2 IgG responses were low among recipients of Sm-TSP-2/Al without GLA-AF, whereas the recipients of Sm-TSP-2/Al with GLA-AF developed significant increases in IgG to Sm-TSP-2. The study conclude that Sm-TSP-2/Al with or without GLA-AF was safe and well tolerated in a Sm-naïve population. A vaccine like the one under development may represent our best hope for eliminating schistosomiasis (Keitel et al., 2019).

Sabin vaccine institute product development partnership launched the schistosomiasis vaccine initiative in 2008 to advance the development and global accessibility of a low cost, safe, and effective vaccine for schistosomiasis. This project energy the development of Sm-TSP-2/Alhydrogel® schistosomiasis vaccine. It also uses recombinant protein technology and alum as an adjuvant. Phase 1 clinical trials for Sm-TSP-2 are ongoing in the United States and Brazil to determine toxicity, safety, and immunogenicity in healthy adults (Beaumier et al., 2013; Insitute, 2017).

3.3. Paramyosin

Paramyosin (Pmy) is a 97-kDa myofibrillar protein expressed on the muscle of adults, the tegumental surfaces of lung stage schistosomula, and in the penetration glands of cercariae. It has an important role in immune evasion by binding C8 and C9 proteins of the human, prevents complement activation at the terminal stage, and protects the parasite from complement-mediated damage. Additionally, Pmy inhibited the in vitro killing of the trypsin-sensitized schistosomula of *S. mansoni* by human complement. Paramyosin proteins either in a recombinant or native form used as a vaccine candidate for protection against schistosomiasis (McManus and Loukas, 2008; Deng et al., 2007).

Paramyosin, a *S. mansoni* myoprotein associated with human resistance to infection and reinfection is a candidate Ag to establish a subunit vaccine against schistosomiasis. A differential proliferative response was detected in persons resistant to reinfection compared to individuals susceptible to reinfection in response to Pmy peptide stimulation. Paramyosin peptides were also recognized by T cells of the individuals. These results suggest that these Pmy peptides are promising Ags to compose an anti-schistosomiasis vaccine (Fonseca et al., 2005).

Swiss Albino mice were immunized three times, two week intervals with 100 mg of purified Sm97 induced 44.1%, 59.1%, and 61% reduction in worm burden, intestinal egg loads, and granuloma size respectively, when compared to the infected control group. Protective immunity in mice was associated with high titers of specific anti-Sm97 IgG1 and IgG2 Abs (Diab and Aly, 2011).

3.4. Calpain

Calpain is a calcium-activated neutral cysteine protease that is localized to the tegument of all developmental stages and inner tegument membrane of adult worms. Calpain is a central antigenic protein in the surface membrane biogenesis and renewal, a mechanism by which schistosomes escape the harmful host immune response. Calpain inactivation inhibits the C3b component of complement and 5-hydroxytryptamine signaling pathways that brings an acceleration of surface membrane synthesis. The large subunit of calpain is called Sm-p80, which is expressed in all developmental stages of *S. mansoni* (Siddiqui and Siddiqui, 2017; Karmakar et al., 2014). So that, Sm-p80 has provided an excellent target for a schistosome vaccine because of its consistent immunogenicity, protective and anti-fecundity potentials, and important role in the immune evasion process (Ahmad et al., 2009a).

Prime boost Sm-p80 vaccination in mice showed a 49% reduction in worm burden and boosting with recombinant protein in the presence of resiquimod (R848) as an adjuvant showed 50% protection. Splenocytes secreted significant levels of IFN-γ and IL-2 which is TH-1 immune responses (Ahmad et al., 2010). By using Deoxyribonucleic Acid (DNA) based formulation, 84% decrease in egg production, and 59% worm burden reduction in mice was achieved. *Schistosoma mansoni* p80-pcDNA3 provoked strong immune responses that include IgG2a and IgG2b Ab isotypes in vaccinated animals. Splenocytes proliferated to produce IL-2, IFN-γ appreciably more TH1 response enhancing cytokines (Ahmad et al., 2009b).

Using recombinant protein (oligodeoxynucleotide 10,104,) as adjuvant Sm-p80 vaccine has conferred up to 70% reduction in worm burden and up to 75% decrease in egg production in mice. In the group of mice immunized with the prime-boost approach, a 57% reduction in worm burden and a 71% reduction in egg production was recorded. The vaccine elicited strong immune responses that included IgM, IgA, and IgG subtypes (Ahmad et al., 2009a). In baboons, 52–58% and 38–47% protection provided by Sm-p80 formulated with recombinant protein approaches and prime-boost approaches, respectively. A balanced pro-inflammatory and anti-inflammatory type of response was generated, which is indicative of increased prophylactic efficacy of vaccines (Ahmad et al., 2011).

A recent study conducted by Zhang et al. (2018) on the protective efficacy of the Sm-p80 vaccine formulated in synthetic Hexa-acylated lipid A derivative, glucopyranosyl lipid A formulated in aluminum (GLA-Alum) against *S. mansoni* infections in mice and baboons. The rSm-p80 + GLA-Alum immunization regimen provided worm burden reduction by 33.33–53.13% and 38% in vaccinated mice and baboons, respectively. Strong Sm-p80 specific IgG, IgG1, IgG2a and IgM and a mix of TH cells response were observed in all immunized animals (Zhang et al., 2018).

The longevity of Sm-p80 specific Ab responses was studied in mice and baboons. Strong Ab titers were detected in mice for up to 60 weeks after vaccination with the Sm-p80 recombinant vaccine (Sm-p80 + GLA-SE). In baboons, specific IgG was also detected in 5–8 years post-vaccination with the Sm-p80 DNA vaccine. Additionally, the transfer of Sm-p80 specific Ab was detected in umbilical cord blood and the baby. These results coupled with vaccine efficacy data in nonhuman primates further strengthens the evidence of Sm-p80 vaccine to be moved forward to human clinical trial development (Zhang et al., 2014).

Furthermore, Sm-p80 provides cross-species protection against *S. haematobium*. The test result showed that 64% reduction in tissue egg load and 48% reduction in worm burden was observed in hamsters vaccinated with recombinant Sm-p80/GLA-SE. Similarly, in baboons produces 25% reduction in adult worms, 64% egg load decrement in the urinary bladder, and 53% reduction in urine egg output. A balanced pro-inflammatory (TH17 and TH1) and TH2 type of responses were produced after vaccination. It indicates that the Sm-p80 vaccine will be effective against both intestinal and urinary schistosomiasis and thus will be greatly advantageous in reducing the overall burden of schistosomiasis (Karmakar et al., 2014).

Schistosoma mansoni p80 is the only vaccine candidate that has been tested for prophylactic, anti-fecundity and therapeutic efficacy in different preparations and immunization approaches (DNA vaccine alone; recombinant protein with adjuvants; and priming with DNA vaccine, followed by boosting with protein plus adjuvants) in three experimental animal models (mice, hamsters, and baboons) of infection and disease. Besides, cross-species protective efficacy reinforces Sm-p80 as an excellent vaccine candidate with the potential of providing relief for both intestinal and urinary schistosomiasis. Having concluded the evidence of different studies, it is projected that the recombinant Sm-p80/GLA-SE vaccine, "SchistoShield®," is now entering Phase I and II human clinical trials as soon as 2017 (Molehin et al., 2016).

3.5. Schistosoma mansoni 29 kilodalton protein

Schistosoma mansoni 29 kilodalton (Sm29) protein is a membrane-bounded Ag presents in the outer tegument surface of lung stage schistosomula and adult worms. It is one of the most expressed and exposed Ag (Chura-Chambi et al., 2013; Cardoso et al., 2008). Specific Abs against Sm29 like IgG1 and IgG3 were detected in sera of patients living in endemic areas of Brazil, and significantly higher levels of these Abs were found in individuals who are resistant to re-infection (Cardoso et al., 2006).

Mice immunization with rSm29 gives 51%, 50%, and 60% reduction in adult worm burdens, liver granuloma counts, and intestinal eggs, respectively. Elevated production of IFN-γ, TNF-α, and IL-12, and high concentration of specific anti-Sm29 IgG1, and IgG2a was related to protective immunity in mice (Cardoso et al., 2008). In addition, three doses of rSm29 were necessary to elicit significant protection level ranging from 26% to 48%, to induce significant production of IL-2, IFN-γ, IL-17, IL-4; and specific Abs like IgG, IgG1, IgG2a, and IgE; to increase the percentage of CD4+ central and effector memory cells. The results demonstrate that rSm29 retains its ability to induce protection in previously infected animals, reinforcing its potential as a vaccine candidate. Therefore, the vaccination of individuals from endemic areas can be effective against *S. mansoni* infection (Alves et al., 2015).

Mice immunized with Sm29 vaccine preparation containing alum as an adjuvant (Sm29Alum) showed a protection level between 29 and 37%. In contrast, immunization with the Mono-phosphoryl Lipid A adjuvant (Sm29MPLA) did not deliberate any protection against reinfection. Using two adjuvants similar proportions of CD4+ cells producing IL-4, IFN-γ, and IL-10 were observed in the spleens of mice. Both formulations elicit increased production of specific IgG1, IgG2a, and IgE Abs for rSm29. In particular, mice immunized with Sm29Alum formulation showed higher production of IgG, IgG1, and IgE after all immunization doses. Having concluded that, Sm29Alum can effectively protect against *S. mansoni* reinfection in mice (Alves et al., 2018).

Fusion protein consists of Sm14 and Sm29, designated as FSm14/29. Polyinosinic-poly cytidylic acid [poly (I:C)] adjuvanted and unadjuvanted FSm14/29 vaccinated mice showed a significant reduction of adult worm burden of 48.4% and 44.7% respectively. The liver egg burden was most significantly reduced by 82.8% and 73.5% in mice immunized with polyinosinic-polycytidylic acid adjuvanted and unadjuvanted fusion protein, respectively. Similarly, the intestinal egg count reduction was reached 72.8% and 76.6% for the adjuvanted and unadjuvanted FSm14/29 Ag, respectively. Adult worms recovered from immunized mice had a deleterious structural change. The adjuvant greatly improves the protection and combines the concept of using multi-Ag fusion proteins as vaccine candidates against *S. mansoni* (Mossallam et al., 2015).

Recombinant Sm29 is expressed in *Escherichia coli* and exposed to high hydrostatic pressure, it results in dissociate aggregated proteins, leading to refolding on a soluble conformation. It is important to produce a stably soluble and structured protein of rSm29 (73%) in high yield. The refolded Ag presented a protective effect against *S. mansoni* development in immunized mice. The procedure can be scaled-up, allowing industrial production of Sm29 (Chura-Chambi et al., 2013) (See Table 1.)

Table 1
Summary of current status of some protein vaccine candidates against Schistosomiasis.

Ag	Location	Function	Target species	Delivery system	Adjuvant	Host	Development Stage	References
FABP	Tegument and gut epithelium of cercaria, adult, and schistosomula	Absorbs, transports, compartmentalizes FAs from the host	*Sm*	IM	GLA-SE	Human	Phase I/II	(Santini-Oliveira et al., 2016; Molehin et al., 2016)
TSP2	Tegument apical membrane	Tegument formation and maturation of adult worm	*Sm*	–	Alum	Human	Phase 1	(Insitute, 2017)
Pmy	Muscle of adults, tegumental of schistosomula and in penetration glands of cercariae	inhibits complement activation; host immune evasion	*Sm*	IP	_	Swiss Albino mice	Pre-clinical	(Diab and Aly, 2011)
Sm-p80	Tegument of all stages and inner tegument of adult worms	Surface membrane biogenesis and renewal	*Sm*	IM	*GLA-Alum*	mouse, hamster &baboon	Pre-clinical	(Zhang et al., 2018)
Sm29	Tegument of adult and schistosomula	Unknown	*Sm*	_	Alum	BALB/c Mice	Pre-clinical	(Alves et al., 2018)
SmCB1 & SmC-L3	Parasite gut and somatic extracts	Feeding and degradation of host blood protiens	*Sm & Sh*	SC	_	Hamster & mice	Pre-clinical	(Tallima et al., 2017)

IM-Intramuscularly, IP- Intraperitoneally, SC- Subcutaneously, Sm- *S. mansoni, Sh- S.haematobium.*

3.6. Schistosoma mansoni Kunitz type protease inhibitor

Kunitz inhibitor is a 16-kDa Kunitz-type protease inhibitor (KI-1) present in the excretory-secretory products, tegument of adult worms, and the sub-shell region of eggs. *Schistosoma mansoni* KI-1 inhibits numerous proteases, which shows anti-inflammatory and anti-coagulant properties by this means supporting parasite survival in an extremely hostile environment and possibly play a critical role in host immune modulation. Secretion of SmKI-1 by schistosome eggs might importance for its pass through the intestinal wall into the gut lumen preceding to being excreted in the human stool to the external environment. As such they may represent novel vaccine candidates and/or drug targets for schistosomiasis control (Ranasinghe et al., 2015).

Recently rSmKI-1 vaccine trials were performed in mice showed a reduction of 23–33% in adult worms, 28–31% in intestinal eggs, 33–39% in fecal eggs and 20–43% in liver eggs. Also, rSmKI-1 significantly increased the production of IFN γ, IL-10, and IL-6 in vaccinated mice, maintaining a Th1/Th2 type balanced response. Recombinant SmKI-1 generated partial protection against *S. mansoni* in the murine model of infection and could be established as part of a combination vaccine with other vaccine candidates to provide a more solid level of protection (Ranasinghe et al., 2018).

3.7. Gut Proteases

Peptidases are important for the establishment and survival of medically important parasites, including *S. mansoni*. This helminth expresses some of the gut-associated peptidases that degrade host blood proteins as a means of nutrition, which are essential functions for the survival of the parasite. Blocking these essential processes is an important strategy for vaccine development. Several gut-associated cysteine peptidases have been identified in adult *S. mansoni*. It includes cathepsin B1, cathepsin D, cathepsin L, Asparaginyl endopeptidase (AE) and others (Tebeje et al., 2016; Sajid et al., 2003).

3.7.1. Schistosoma mansoni cathepsin-B1

Cysteine proteases are members of the papain family of proteases. The cathepsin family of proteases (cathepsins B and L) are common members of parasites cysteine proteases and had a lysosomal effect. It was able to interact with host molecules and play roles in parasitism, including migration, feeding and immune evasion (Smooker et al., 2010). *Schistosoma mansoni* cathepsin B-like endopeptidase 1 (SmCB1) is the most abundant in both the parasite gut and somatic extracts, which is secreted into the gut lumen of adult and also been found in the gastrodermal cells (Sajid et al., 2003). Hemoglobin, released from the ingested red blood cells, is degraded by cathepsin B (Skelly and Shoemaker, 2001).

In experiments, using different immunization doses of active SmCB1 induced 60%–73% protection level. Increased secretion of Th2- associated cytokines (IL-4, IL-5, IL-13) and high titers of IgG, IgG1, and IgG2b also showed on SmCB1 immunized mice (El Ridi et al., 2014). In the presence of synthetic oligodeoxynucleotide containing unmethylated CpG dinucleotides, recombinant CB conferred a 59%, 56%, and 54% decrease in worm burden, hepatic egg burden, and intestinal egg burden, respectively. Besides, it induces strong production of SmCB specific Abs, both IgG1 and IgG2, and significantly higher levels of main Th1 cytokines, IFN-γ and TNF-α. These results highlight the potential of SmCB/CpG as a vaccine candidate against schistosomiasis (Ricciardi et al., 2015).

Cysteine peptidases based vaccination was also effective against *S. haematobium* in outbred mice. A mixture of SmCB1 and *F. hepatica* cathepsin L1 (FhCL1) led to 70% and 60% reduction in *S. haematobium* worm burden and liver egg counts, respectively.

Mice vaccinated with SmCB1/FhCL1/rSG3PDH mixture displayed a highly significant reduction of worm burden by 72% and no eggs in the liver. There is a mixture of Th1- and Th2 related cytokines production. The highest serum Ab responses included IgG1, IgG2a, IgG2b, and IgA Abs specific to SmCB1 were detected in immunized mice (Tallima et al., 2015).

Schistosoma mansoni CB1 and cathepsin L3 (SmCL3) cysteine peptidases, alone and in combination, immunized hamsters induces a reproducible and highly significant reduction in worm burden (>70%) as well as in egg counts and viability. Animals produced lower levels of IL-5, IL-13, and IL-17 than unimmunized controls, with an impressive decrease in IFN-γ levels. Significantly elevated memory responses were predominantly recorded for IL-5 and IL-13, especially in hamsters immunized with SmCB1 plus SmCL3. The data showed that SmCB1 and SmCL3 are effective adjuvant free vaccines that induce protection in mice and hamsters against both *S. mansoni* and *S. haematobium* (Tallima et al., 2017).

3.7.2. Schistosoma mansoni Cathepsin D

Schistosoma species cathepsin D (CD) is a member of the aspartic protease category of hydrolases. In *S. mansoni*, CD plays an integral role at the beginning of the digestion cascade within the gastrodermis of the flukes. Transcripts for CD are present in all stages of the parasite and the enzyme is also capable of digesting immunoglobulin and serum components such as albumin (Dougall and Dougall, 2014). Cathepsin D knockdown schistosome by ribonucleic acid interference approaches was led to phenotypic changes including significant growth retardation in vitro and did not develop intestinal heme pigmentation, indicative of impairment of hemoglobin proteolysis and suppression of aspartic protease enzyme activity. These and other findings suggest that schistosome CD had an essential function in parasite nutrition and could be developed as a target for the novel schistosomal vaccine (Morales et al., 2008).

Two *S. mansoni* CD peptides formulated with lipid core peptide (LcP) adjuvant had a potential interruption of blood meal digestion and provoke a humoral response in mice. Using immunohistochemistry, showed that anti-LcP Abs bound to the native *S. mansoni* CD protein in the esophagus and anterior regions of the gastrodermis of adult flukes. Further development of LcPs containing multiple epitopes from this and another vaccine Ags should become a research priority (Dougall and Dougall, 2014).

3.7.3. Schistosoma mansoni asparaginyl endopeptidase (SmAE/Sm32)

is expressed in the gastro dermal cells of the schistosome gut and head glands of the cercariae. Possibly, Sm32 hydrolyzes proproteins involved in the degradation of host hemoglobin and it is the principal source of amino acids for the parasite. The importance of Sm32 as a novel vaccine candidate is based on the possibility of inducing Ab mediated inhibition of its catalytic activity. Preliminary evidence using inhibitors of these activities has shown a profound reduction on the oviposition of adult worms (Chacon et al., 2003).

Deoxyribonucleic acid (DNA) based vaccine technology was used to induce an immune response in mice against an Sm32. The Sm32 encoding DNA construct immunized mice developed Abs which recognized the native protein. This DNA vaccine led to an anti-fecundity effect. Based on this female worms of a challenge infection produced 37% fewer eggs than those growing in native mice. The results suggest that Sm32 may be a candidate Ag for the generation of an anti-pathology vaccine against schistosomes (Chlichlia et al., 2001).

4. Future prospective of protein vaccine candidates against *Schistosoma mansoni* infection

Vaccine development is a long process that can take decades. Three protein vaccine candidates that have been produced by good manufacturing procedures and entered safety and immunogenicity human clinical trials – Sm14 (Molehin et al., 2016), Sm-TSP-2 (Insitute, 2017), and Sm-p80 (Zhang et al., 2014). Phase I clinical trial of rSm14/GLA-SE products were a safe and strongly immunogenic vaccine against schistosomiasis in healthy individuals. Phase II trials to assess field-based side effect, immunogenicity and safety test was planned in endemic areas of Brazil and Africa for Sm14 and West Africa for West Africa (Santini-Oliveira et al., 2016; Molehin et al., 2016).

Sabin Vaccine Institute product development partnership develops Sm-TSP-2/Alhydrogel® schistosomiasis vaccine. Phase 1 clinical trials to determine immunogenicity, reactogenicity, and safety in healthy, non-infected adults are ongoing in the United States and Brazil (Insitute, 2017). It is anticipated that the recombinant Sm-p80/GLA-SE vaccine, "SchistoShield®," is now entering Phase I and II clinical trials as soon as 2017 (Zhang et al., 2014). Other candidates that are in pre-clinical trials at various stages include pmy, Sm29, SmKI-1, and Sm23 for *S.mansoni* infection (Colley and Secor, 2014).

The recent sequencing of the genomes of schistosome species and modern proteomics methods has empowered the discovery of many new possible vaccine and drug targets, as well as diagnostic biomarkers, using high-throughput and sensitive proteomics methods. Particularly, Immuno proteomics approaches are important to screen proteins from various schistosome species from both animal models and clinical sources. Besides, protein microarray and microplate immuno proteomics permit virtually unrestricted screening of any Ag, including potentially surface exposed hypothetical proteins from the predicted proteomes of schistosome species (Driguez et al., 2016). Since the growth of several new technologies, including genomics, transcriptomics, microarrays, and immunomic profiling has helped in the identification of promising new schistosome target proteins which are important for the survival of the parasite in the host, which can be recognized and induce protective host immune responses (Merrifield et al., 2016).

Combining different Ags can also result in higher levels of vaccine-induced protection. Targeting key biological functions of schistosomes such as nutrient uptake and metabolism, tegumental integrity, fecundity, and survival represent key potential sites to target the parasites for elimination through vaccination. Although, clustered regularly interspaced short palindromic

repeats (CRISPR) technology is a family of DNA sequences found within the genomes of prokaryotic organisms and a simple yet powerful tool for editing genomes which may provide a novel approach identifying specific protein encoding schistosome genes for vaccine candidate discovery. Schistosomiasis vaccine development has proven highly challenging and costly. The new funding is required to encourage the identification and generation of a schistosome vaccine Ag pipeline and to progress existing promising candidates into clinical trials (Tebeje et al., 2016).

5. Conclusion and recommendation

Schistosomiasis remains an important public health problem because it causes very high levels of morbidity and mortality in many parts of the world. Currently, treatment is totally dependent on praziquantel chemotherapy. As exclusive use of the drug may lead to the emergence of drug-resistant strains, as well as the frequency and rapidity of reinfection are the main challenging. To have a long-lasting impact, an integrated approach involving chemotherapy, snail control, vaccines, and other innovative tools will be required.

Development and placement of a vaccine as part of an integrated approach for control and prevention of schistosomiasis may have a synergistic profit when combined with chemotherapy, a vaccine should be considered as the next step in pursuing disease elimination. Currently, three protein vaccine candidates that have been produced by good manufacturing procedures and entered safety and immunogenicity human clinical trials – Sm14, Sm-TSP-2, and Sm-p80.

Before the development of a vaccine, it needs better understand of immunologic mechanisms of protection, recognize more target Ags and establish an integrated approach to prioritize vaccine candidates are important steps. Biologic description of parasite targets, validation of animal models for Ag discovery and vaccine evaluation, potential Ag polymorphisms that might affect vaccine efficacy, safety and regulatory issues all need to be considered early in the development process. Combining different Ags can also result in higher levels of vaccine-induced protection.

Vaccine development is a long process that can take several decades. Since funding for vaccine development for neglected tropical parasitic diseases is very limited, so increase fund and well and well thought-out approach is necessary. Additionally, defining a clear target product profile for a desirable schistosomiasis vaccine and taking advantage of new technologies and tools available for new protein discovery, vaccine delivery, process development, clinical research, and vaccine efficacy evaluation could all contribute to speeding up the progress. Together with all the present tools, a newly developed schistosomiasis vaccine tailored to fit program needs would help to achieve and sustain true disease control and eventual elimination.

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The authors declare no competing interests.

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