Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2011, Article ID 503068, 9 pages
doi:10.1155/2011/503068

*Review Article*

# Computational Vaccinology: An Important Strategy to Discover New Potential *S. mansoni* Vaccine Candidates

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Received 1 June 2011; Accepted 12 August 2011

Academic Editor: Anne S. De Groot



The flatworm *Schistosoma mansoni* is a blood fluke parasite that causes schistosomiasis, a debilitating disease that occurs throughout the developing world. Current schistosomiasis control strategies are mainly based on chemotherapy, but many researchers believe that the best long-term strategy to control schistosomiasis is through immunization with an antischistosomiasis vaccine combined with drug treatment. Several papers on *Schistosoma mansoni* vaccine and drug development have been published in the past few years, representing an important field of study. The advent of technologies that allow large-scale studies of genes and proteins had a remarkable impact on the screening of new and potential vaccine candidates in schistosomiasis. In this postgenomic scenario, bioinformatic technologies have emerged as important tools to mine transcriptomic, genomic, and proteomic databases. These new perspectives are leading to a new round of rational vaccine development. Herein, we discuss different strategies to identify potential *S. mansoni* vaccine candidates using computational vaccinology.

## 1. Introduction

Schistosomiasis mainly occurs in developing countries and it is the most important human helminth infection in terms of global mortality. This parasitic disease affects more than 200 million people worldwide causing more than 250,000 deaths per year [1]. Furthermore, schistosomiasis causes up to 4.5 million DALY (disability adjusted life year) losses annually [2]. Current schistosomiasis control strategies are mainly based on chemotherapy but, in spite of decades of mass treatment, the number of infected people remains constant [3]. Extensive endemic areas and constant reinfection of individuals together with poor sanitary conditions in developing countries make drug treatment alone inefficient [4]. Many consider that the best long-term strategy to control schistosomiasis is through immunization with an antischistosomiasis vaccine combined with drug treatment [5]. A vaccine that induces even a partial reduction in worm burdens could considerably reduce pathology and limit parasite transmission [6].

The advent of technologies that allowed large-scale studies of genes and proteins had a remarkable impact on the screening of new and potential vaccine candidates of *Schistosoma mansoni*. Mass spectrometry- (MS-)based proteomics [7–10], transcriptome [11], and genome [12] of *S. mansoni* offered a vast repertoire of potential targets for vaccine and drug therapies. Despite this possibility to generate information about DNA and protein sequences, it remains an obstacle how to select them and which molecules would have the highest potential among thousands or hundreds of potential candidates. In this postgenomic scenario, bioinformatic technologies have emerged as important tools to mine transcriptomic, genomic, and proteomic databases. These new approaches have the potential to accelerate the identification

of new generation of vaccine candidates that may induce greater protection than the previous schistosome antigens studied to date [13, 14]. Specific algorithms allow the identification of molecules containing transmembrane domains, signal peptides, signal anchors, and other posttranslational modifications that can be used as predictors of excretory-secretory products or components exposed to the surface of the *S. mansoni* tegument [9, 13]. Additionally, predicting the peptides that bind to MHC class II molecules can effectively reduce the number of experiments required for identifying helper T-cell epitopes and play an important role in rational vaccine design [15].

The tegument is a dynamic host-interactive surface involved in nutrition, immune evasion and modulation, excretion, osmoregulation, sensory reception, and signal transduction [16, 17]. Consequently, the tegument is considered an important source of parasite antigens for the development of a schistosome vaccine. Currently, the most promising schistosome vaccine candidates are located in the tegument [18], such as TSP-2 [19] and Sm29 [20].

Throughout the next sections we will discuss and present the recent studies, approaches, and bioinformatics tools that have been used to search and validate new vaccine targets present in the tegument of *S. mansoni*.

## 2. Bioinformatic Approaches and Tools

The sequencing of the *S. mansoni* transcriptome [11, 12] and the development of proteomic and microarray technologies have dramatically improved the possibilities for identifying novel vaccine candidates [21, 22]. In the search for an effective schistosome vaccine, several available bioinformatic tools can be helpful and a rational design of possible vaccines has replaced the trial-and-error approach [23]. A first step for a rational vaccine design is the identification of target antigens. For *Schistosoma*, a potential vaccine should include proteins that are preferentially surface exposed and/or secreted ones, expressed in the intramammalian host [11]. The genes sequences can be obtain at the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/), and at the site of the whole schistosome genome sequencing project (http://www.SchistoDB.org). The proteins can be identified by proteomic analysis which has resulted in a remarkable understanding of the protein composition of the schistosome tegument [7–10, 24, 25]. If necessary, the target sequence can be translated using the *Translate* tool which allows the translation of nucleotide (DNA/RNA) sequence to a protein sequence (http://expasy.org/tools/dna.html). Based on their amino acid sequences, topology prediction to confirm the presence of transmembrane helices can be performed using *TMHMM* server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) or *SOSUI* (http://bp.nuap.nagoya-u.ac.jp/sosui/) and subcellular localization can be performed using the *WolfPSORT* (http://wolfpsort.org/aboutWoLF_PSORT.html.en). The identification of domains within proteins can, therefore, provide insights into their function and some databases are able to identify known functionally important sequence motifs that may not be identified on the basis of sequence homology by itself. Such searches can be performed using different tools as *Pfam* (http://pfam.janelia.org, http://pfam.sanger.ac.uk/search?tab=searchSequenceBlock), *InterPro Scan* which integrates search in *PROSITE*, *Pfam*, *PRINTS*, and other family, and domain databases (http://www.ebi.ac.uk/Tools/pfa/iprscan/) or *ScanProsite* that scans a sequence against *PROSITE* or a pattern against the UniProt Knowledgebase (Swiss-Prot and TrEMBL) (http://expasy.org/tools/scanprosite/). The prediction of either HLA-peptide binding or proteasomal processing of antigens can be predict for databases like *SYFPEITHI* (http://www.syfpeithi.de/Scripts/MHCServer.dll/EpitopePrediction.htm) epitope prediction algorithm, which comprises more than 7000 peptide known sequences binding class I and class II MHC molecules [26] or *NetChop* (http://www.cbs.dtu.dk/services/NetChop/) server that has been trained on human data only, and will therefore presumably have better performance for prediction of the cleavage sites of the human proteasome. However, since the proteasome structure is quite conserved, we believe that the server is able to produce reliable predictions for at least the other mammalian proteasomes [27]. Primary structure analysis can be performed at *ProtParam* (http://expasy.org/tools/protparam.html) tool which allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered sequence [28]. Post-translational modification prediction as N-glycosylation, Oglycosylation and signal peptide can be analyzed using the *NetNGlyc 1.0* (http://www.cbs.dtu.dk/services/NetNGlyc/), *YinOYang* (http://www.cbs.dtu.dk/services/YinOYang/) and *SignalP* (http://www.cbs.dtu.dk/services/SignalP/), respectively (Figure 1).

## 3. Tegument Proteins as Vaccine Candidates

*3.1. Tegument Surface Exposed Proteins.* Schistosome membrane-bound antigens have currently become frequent targets of vaccines studies. Upon publication of the transcriptome data and its scrutiny for genes with functions that would indicate their surface exposure and likely interaction with the host immune system, a series of novel genes were offered as potential vaccine candidates based on their functional classification by Gene Ontology [11, 29]. The use of bioinformatic programs to screen and select potential vaccine targets from the available *S. mansoni* sequence databases, such as transcriptomes, genome, and proteomics is an important strategy for the rational design of vaccines, allowing the prediction of antigens *in silico* [30]. These *in silico* analysis have lead to the selection of several schistosome vaccine candidates by different research groups and the protective effect of some tegument antigens that were already tested *in vivo* will be further discussed in this section and summarized in Tables 1 and 2.

In the context of searching for new protective tegument proteins, in 2006 Cardoso et al. [51] identified 34 proteins with membrane-bound protein motifs. At the same time other researchers across the world published the proteomic analysis of *S. mansoni* tegument [8, 9]. One of the first tegument proteins selected and studied, as a potential

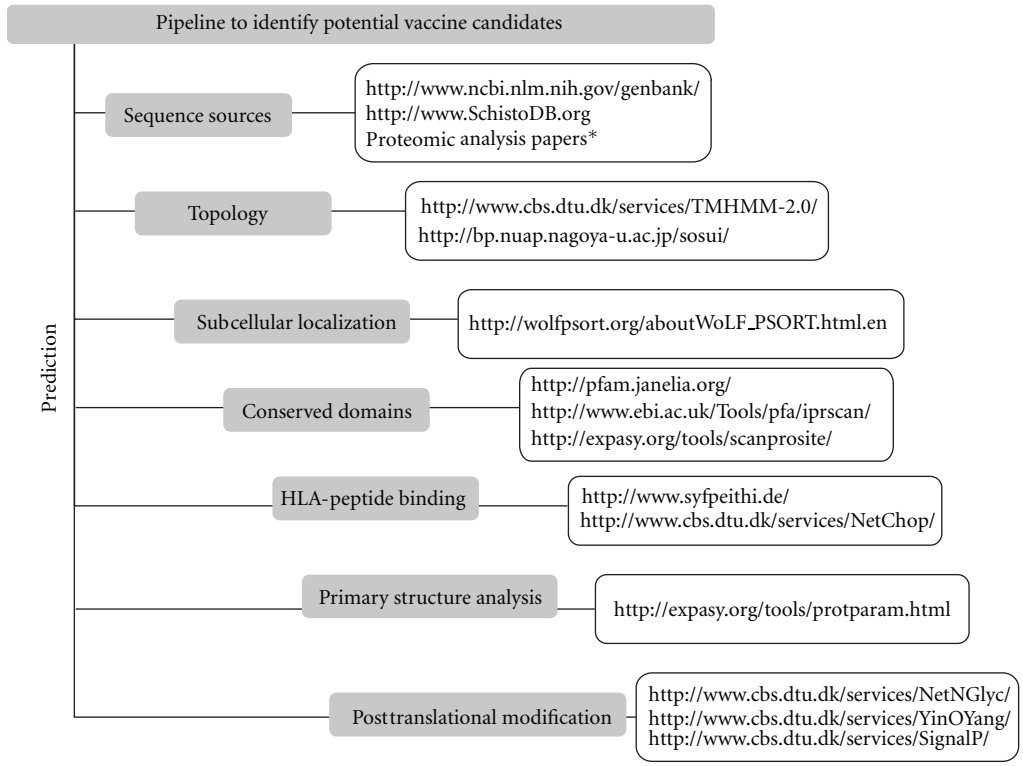


Figure 1: Most common methodology for investigation of potential vaccine candidates. Possible targets are predicted based on sequences databases and proteomic analysis. Further bioinformatic studies provide detailed information about protein primary structure, topology, subcellular localization, conserved domains, HLA-peptide binding, and posttranslational modifications.* [7–10].

Table 1: *S. mansoni* tegument surface exposed proteins evaluated as vaccine targets.

| Candidate | Vaccine type | Immunologic response | Humoral response | Eggs reduction | Worms reduction | Bioinformatic tool | Ref. |
|---|---|---|---|---|---|---|---|
| Sm-p80 | DNA vaccine (PcDNA) | Th1[a,b] | IgG, IgG2a and IgG2b | 84% | 59% | ND | [31] |
| TSP-2 | Recombinant protein | ND | IgG, IgG1 and IgG2a | 64% (liver) 65% (feces) | 57% | BLAST | [19, 32] |
| Sm29 | Recombinant protein | Th1[a,b] | IgG, IgG1 and IgG2a anti-Sm29 | 60% (intestinal eggs) | 51% | BLAST, InterPro Scan, SignalP 3.0, SignalP Neural, NetNGlyc 1.0, WolfPSORT, SOSUI, Compute pI/Mw tool, GOR IV. | [20, 21] |
| ECL (200 Kda protein) | DNA vaccine | ND | IgG, IgG1 > IgG2a | ND | 38.1% | ND | [33] |
| Sm 25 | Peptide vaccine | ND | IgG | No significative difference with control group | No significative difference with control group | ND | [34, 35] |

ND: not determined. Sm25 were tested in mice and rats, all the others antigens were tested in mice.

schistosome vaccine, was the schistosome membrane-bound protein Sm29 that was identified through membrane-bound motif search using bioinformatic analysis [20, 51].

Sm29 is a membrane-bound protein with predicted N-glycosylation and O-glycosylation sites, with unknown function and no homolog outside the *Schistosoma* genus [20, 21]. Recently, it was demonstrated that Sm29 is GPI-anchored on the tegument surface of *S. mansoni* and shaved off under phosphatidylinositol-specific phospholipase C (PiPLC) treatment [10]. Sm29 as recombinant

Table 2: Other *S. mansoni* tegument membrane proteins evaluated as vaccine targets.

| Candidate | Vaccine type | Immunologic response | Humoral response | Eggs reduction | Worms reduction | Bioinformatic tool | Ref. |
|---|---|---|---|---|---|---|---|
| Glutathione peroxidase | DNA prime-vaccinia virus boost | ND | ND | ND | 85% | ND | [36] |
| Sm21.7 | Recombinant protein | ND | ND | ND | 41–70% | ND | [37] |
| Cu/Zn superoxide dismutase | DNA vaccine | ND | ND | ND | 44–60% | ND | [36] |
| Filamin | DNA vaccine | Th1/Th2[a,b] | IgG, IgG2a, IgG2b, IgG1 | ND | 44–57% | ND | [38] |
| Sm fimbrin + Sm 21.7 | Multivalent DNA vaccine | ND | IgG | 41.5% (liver) 55.6% (intestine) | 56% | ND | [39] |
| Sm-p80 | DNA vaccine (VR1020) | Th1/Th17[b] | IgG | ND | 47% | ND | [40] |
| Sm 23 | DNA vaccine | ND | IgG | ND | 44% | ND | [41, 42] |
| Sm 21.7 | DNA vaccine | ND | IgG | 62% (liver) 67% (intestine) | 41.53% | ND | [43] |
| Fimbrin | Recombinant protein | ND | ND | ND | 39.4–41.6% | Sequenase version 2.0, PC/GENE 15.0 | [44] |
| Sm 22.6 | Recombinant protein | Th1/Th2[a,b] | IgG, IgG1 IgG2a | ND | 34.5% | BLAST | [45] |
| TSP-1 | Recombinant protein | ND | IgG, IgG1 and IgG2a | 52% (liver) 69% (feces) | 34% | BLAST | [19, 32] |
| Stomatin Like Protein-2 | Recombinant protein | Th1 | IgG, IgG1 > IgG2a | No significative difference with control group | 30–32% | BLAST and PSI BLAST, Pfam SPFH/Band 7 domains, SignalP 3.0, TMHMM, CSS-Palm, MitoProt program, Compute pI/Mw, ClustalX 1.83, TreeView program | [29] |
| Sm 20.8 | DNA vaccine | ND | ND | ND | 28.5–30.8% | ND | [46] |
| Sm28GST | DNA vaccine +plasmid containing IL-18 | Th1[a] | IgG* | 29.6% (liver) 27.5% (intestine) | 22.6% | ND | [47] |
| Dif 5 | DNA vaccine | ND | ND | ND | 22% | BLASTX, Gene Ontology Consortium Website, SMART, SignalP, TMHMM, and bigPI Predictor | [48] |
| SmIg | Recombinant protein | Th1/Th2[a,b] | IgG | ND | No significative difference with control group | ExPASy (Expert Protein Analysis System), SignalP 3.0, SOSUI, NetNGlyc 1.0, YinOYang, BLAST, INterPro Scan | [49] |
| Sm21.6 | Recombinant protein | Th1/Th2[b] | IgG, IgG1 > IgG2 | ND | No significative difference with control group | BLAST, Pfam program, Syfpeith, Expasy, Compute pI/Mw tool, SignalP 3.0, YinOYang, TMHMM | [50] |

ND: not determined; *no differences when compared with the group containing just the plasmid with Sm28. [a]Antibody, [b]cytokines. All antigens were tested in mice.

vaccine induced a Th1 type of immune response in rSm29-immunized mice, 50% reduction in liver granuloma, 60% reduction in intestinal eggs, and 51% reduction in adult worms [20].

Another group of proteins that are exposed on the tegument surface, hence potential vaccine candidates, are the integral membrane proteins tetraspanins TSP-1 and TSP-2 [19]. Tetraspanins cross the cell membrane four times and play multiple roles in mammalian cell signaling, such as interactions between immune effector cells and their ligands [32]. Vaccination of mice with these recombinant proteins resulted in 57% reduction in worm burden and 34% reduction in liver eggs for rTSP-1 and 64% reduction in worm burden and 52% reduction in liver eggs for rTSP-2 [19]. As mentioned before, the current most promising schistosome vaccine candidates are located in the tegument [18], such as TSP-2 [19] and Sm29 [20].

The calcium-dependent, neutral cysteine-protease (calpain) was previously purified from *S. mansoni* [52] and reported to be excreted/secreted [53] and present on the tegument surface [10]. The large subunit of calpain (Sm-p80) was tested as recombinant vaccine which provided a 29–39% reduction in worm burden in immunized mice challenged with *S. mansoni* [54]. This antigen (Sm-p80) was also evaluated in different protocols of immunization, such as DNA vaccine with Sm-p80 solely (39% of protection), combined with IL-2 (57% of protection), or associated with IL-12 (45% of protection) [55]. The authors attribute these results to the pivotal role of calpain on surface membrane biogenesis of schistosomes, as well as in the immune evasion process [55]. Other recent publications of this group reported higher levels of protection by Sm-p80 DNA-based vaccines, 59% reduction in worm burden in mice, decrease in egg production by 84%, and a predominant Th1 immune response [31]. Mice prime-immunized with DNA vaccine and boosted twice with rSm-p80 reduced up to 70% in worm burden and decreased egg production by up to 75% with an induction of a Th1 and Th17 cytokine profiles [31]. Sm-p80 was also cloned in VR1020, an FDA-approved vector for human use. Additionally, the protective efficacy of this vaccine formulation was tested in a murine model. Sm-p80-VR1020 vaccine formulation was able to induce 47% reduction in worm burden [40].

A glycosylphosphatidylinositol (GPI-anchored) protein with unknown function was initially identified in the tegument [56, 57] and termed later 200 kDa, Sm200, or ECL [33, 58, 59]. Brindley et al. [60] had suggested the use of this protein as immunogen to trigger the immune response against *S. mansoni* [60]. Furthermore, the immune and protective responses induced by immunization with plasmid DNA (ECL-encoded) complexed with protamine sulphate as adjuvant was evaluated. The protection elicited was 38.1% and the spectrum of the elicited immune response induced by the vaccine formulation without protamine were characterized by a high level of IgG ($IgG_1 > IgG_{2a}$) [33]. Sm200, like Sm29, was released from the tegument surface by an enzymatic shaving confirming its tegument surface localization [10].

Sm25, a major antigen on the tegument surface of *S. mansoni*, is a 25 kDa N-glycosylated glycoprotein that was previously reported to be palmitoylated [34]. Recently, it was demonstrated that Sm25 is a GPI-anchored protein on the tegument surface [10]. Differently from previous tegument surface antigens reported above that engendered substantial levels of protection as schistosomiasis vaccine candidates, the studies using rSm25 failed to protect mice against *S. mansoni* cercariae challenges, despite the induction of specific anti-Sm25 IgG [35].

*3.2. Other Tegument Membrane-Bound Proteins.* Some proteins present in the tegument of *S. mansoni*, regardless of being exposed on the surface, when used as vaccines have shown significant levels of protection in mice. The most relevant tegument proteins tested will be discussed below and are summarized in Table 2. Proteomic analysis of *S. mansoni* tegument [8, 9] provided the schistosome vaccinology field with promising candidates for vaccine design. Based on these new proteomic databases, it was selected a protein with unknown function that was termed SmIg because of the presence of an immunoglobulin domain [22]. SmIg tested as recombinant vaccine failed to induce worm burden reduction, but immunized mice had significant reduction of liver granuloma area and fibrosis content, showing a mixed Th1/Th2 type of immune response [49]. Sm22.6 gene, encoding a tegument protein, was first identified in a *S. mansoni* lung-stage cDNA library [45]. Further study has demonstrated two EF-hand motifs present in this molecule [50]. Sm22.6, a specific schistosome lung-stage protein, was tested as DNA and recombinant vaccine, but only the recombinant form provided reduction on adult worm burden (34%) and induced a mixed Th1/Th2 type of immune response [45]. A membrane-associated protein localized on the *S. mansoni* adult worm, Sm21.6, was also tested as recombinant vaccine. Sm21.6 showed 45% identity with Sm22.6, both possess EF-hand antigen from the family of EF-hand containing parasite proteins with sequence similarity to dynein light chain. Bioinformatic analysis predicted Sm22.6 as a soluble protein with neither signal peptide nor transmembrane domain, and confocal microscopy analysis revealed this protein as membrane-associated localized on the *S. mansoni* adult worm [50]. Mouse immunization with rSm21.6 induced a mixed Th1/Th2 cytokine profile and no protection against infection, but vaccination with rSm21.6 reduced by 28% of liver granuloma numbers, 21% of granuloma area, and 34% of fibrosis [50]. Further, a *S. mansoni* stomatin like protein-2 (SmStoLP-2) was demonstrated to be localized on the tegument and its recombinant form was also tested as a schistosomiasis vaccine [29]. The function of stomatins is still unknown. In erythrocytes, it may link stomatin or other integral membrane proteins to the peripheral cytoskeleton, playing a role in the regulation of ion channel conductance or in the organization of sphingolipids and cholesterol-rich lipid rafts [61]. Immunization with rSmStoLP-2 engendered 32% reduction in adult worm burden and a Th1 predominant immune response [29]. Dif5 gene, a possible homologue of human CD59/LY6, predicted to be GPI-anchored on the *S. mansoni* tegument surface conferred 22%

reduction in adult worm burden in mice immunized with the Dif5 gene as DNA vaccine [48].

Sm21.7, a protein that was localized on the tegument of *S. mansoni* by immunofluorescence techniques, was tested as recombinant [37] and DNA [43] vaccines. Similar to Sm22.6, Sm21.7 has a motif strongly homologous to the EF hand calcium binding domain; however, the change of the invariant glycine to glutamine in the calcium binding loop, makes this domain nonfunctional, as shown by the inability of Sm21.7 to bind calcium [62]. Sm21.7 was identified in cercariae, adults, and eggs whereas Sm22.6 and Sm20.8 were expressed in adults [63]. Sm21.7 as recombinant and DNA vaccines resulted in 41–70% and 41–53% reduction in the number of adult worms, respectively, following challenge by *S. mansoni* cercariae [37, 43]. Both forms of Sm21.7 vaccine revealed a decrease in the number, size, and cellularity of the granuloma in the liver of the vaccinated in comparison with unvaccinated mice [37, 43]. Sm21.7 DNA vaccine was also evaluated in association with the Sm fimbrin gene as DNA vaccine [39]. This Sm21.7/fimbrin vaccine showed 56% reduction in adult worm burden, 41% and 55% reduction in liver and intestine eggs, respectively. Sm fimbrin has homology to actin binding proteins, presents a calcium-binding site like in calmodulin molecules was localized on the tegument of adult worms and its recombinant form (solely) conferred 39.4–41.6% of protection in immunized mice [44]. Sm23, an integral membrane protein detected with antibodies in all stages of the parasite host forms, engendered different levels of protection (from 18% to 44%) depending on the vaccine formulation [41, 42, 64]. Sm23 is a 23 kDa integral membrane protein member of the "tetraspanin" family, possessing four hydrophobic putative transmembrane domains, some of them involved in signal transduction. Besides being an integral protein, Sm23 is additionally linked to the membrane by a glycosylphosphatidylinositol anchor, but is not released from the surface after cleavage with PiPLC [65]. This protein is expressed in all schistosome life stages examined and in several tissues, including the adult tegument [41]. Sm20.8, another tegument protein, is a member of a family of soluble tegument antigens that contain EF-hand motifs, it interacts with dynein light chain and is recognized as antigenic targets in protective antisera [46]. This Sm20.8 protein shows high homology to Sm21.7 and Sm22.6 [46]. In immunization studies rSm20.8 conferred 30% reduction in adult worm burden in mice [46].

Antioxidant enzymes have been shown to be interesting targets eliciting high levels of protection against *S. mansoni* challenging. Moreover, their localization on the tegument was previously demonstrated [66]. Mice immunized with cytosolic Cu-Zn superoxide dismutase and glutathione peroxidase as DNA vaccines showed reductions in worm burden at levels 41–70% and 85%, respectively, depending on the antigen and the vaccine formulation used in the studies [36]. The association of Cu-Zn superoxide dismutase or glutathione peroxidase with a partial sequence of the structural protein filamin, as DNA vaccines, resulted in protections ranging from 39% to 50% [38]. Another schistosome antioxidant enzyme, glutathione S-transferase (Sm28GST), when associated with IL-18 as DNA vaccine resulted in 28% reduction in egg laying and 23% reduction in worm burden in mice [47] and up to 59% reduction in worm burden in rats immunized with a single dose of rSm28GST, using either aluminum hydroxide or Bacillus Calmette-Guérin as adjuvants [67]. Sm28GST was extensively explored as a potential antigen against schistosomiasis in different immunization protocols and vaccine formulations [68–75]. These studies exploring Sm28GST as vaccine candidate and its evaluation in human clinical trials are revised in Capron et al. 2005 [76].

A strong evidence of the potentiality of *S. mansoni* tegument-bound antigens as vaccine candidates was recently demonstrated using an extract of tegument proteins from lung-stage schistosomula named Smteg [77]. Smteg-immunized mice showed a Th1 type of immune response associated with 48% reduction in worm burden, 65% reduction in liver eggs, 60% reduction in fecal eggs, and 41% reduction in liver granuloma [77].

## 4. Perspectives

Even though funding for vaccine development against schistosomiasis has dropped significantly, there is a common understanding within the scientific community that long-term effective disease control will benefit from the combination of vaccination and chemotherapy, plus sanitation and public health control measures [78]. The current vaccine candidates may prove not to be the most effective, but it is important to continue identifying new target antigens [14]. Consequently, the characterization and better understanding of the *S. mansoni* tegument composition, coupled with new bioinformatic approaches and tools will definitely initiate a new era for the development of schistosome vaccines. A recent publication identified several tegument-associated proteins released through enzymatic shaving [10]. This work presented a new repertoire of interesting and potential vaccine candidates to be explored, such as annexin [79], *S. mansoni* nucleotide pyrophosphatase/phosphodiesterase type 5 (SmNPP-5) [80], and an amino acid transporter, Schistosome permease 1 (SPRM1) [81]. Besides these surface exposed antigens, other potential tegument antigens to be further evaluated are the ones related to uptake or transport of nutrients, drugs, and other molecules across the tegument, such as aquaporins [82, 83]. Other candidates previously identified as tegument proteins, although not tested as vaccine, are the receptors such as the histamine receptors SmGPR-1 [84] and SmGPR-2 [85]. The major challenge to develop vaccines using defined and single antigens is finding molecules able to stimulate appropriate immune responses that can lead to resistance. However, a strategy that could accelerate the achievement of an effective antischistosomiasis vaccine would be the association of different recombinant antigens that previously resulted in partial protection or even the use of pools of antigens known as multivalent or multiepitope vaccines. This strategy was evaluated for some researchers using associations of *S. mansoni* synthetic peptides or even DNA-based vaccines but their approach did not engender higher levels of protection when compared to the use of a single antigen [39, 86]. This demonstrates that no additive or synergistic effects were obtained from

the different antigens selected in those studies but it could be related to the specific combination of antigens and/or the type of immune response resulted from this association and do not exclude the possibility of success using other parasite antigens. Taken together, all proteins discussed here, and many others identified by bioinformatic tools, could improve the search for an effective antischistosomiasis vaccine, and consequently, disease control.

## Acknowledgment

This paper was supported by CNPq, CNPq/INCT-Doenças Tropicais, FAPEMIG, CAPES/PROCAD, and CAPES.

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