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Review Article

Schistosomiasis Vaccine: Research to Development

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ABSTRACT

Due to their worldwide importance for human and animal health, schistosomes are in the focus of national and international research activities. Schistosomiasis is a major health problem and despite decades of research, only one effective drug, Praziquantel is currently available. Recent expansion of sequence databases on *Schistosoma mansoni* and *S. japonicum* has permitted a wealth of novel proteomic studies on several aspects of the organization and development of the parasite in the human host. Several proteomic studies in schistosomes have been performed in the past five years in an attempt to identify proteins involved in crucial processes for the parasite biology. Integration of proteomic data with those generated by microarrays is permitting a change of paradigm for the proposal of new targets for schistosomiasis treatment.

Keywords: Schistosome antigens, *S. japonicum* , *S. mansoni*, Nucleoproteins, DNA vaccine

INTRODUCTION

In 1852, Theodor Bilharz described for the first time a tropical parasitic disease (bilharzia, later termed schistosomiasis) caused by blood-dwelling trematode fluke worms of the genus *Schistosoma*. Five schistosome species infect humans; they are *Schistosoma mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum*, and *S. haematobium*. The first four species have well described associations with chronic hepatic and intestinal fibrosis and their attendant consequences. *S. haematobium* infections cause fibrosis, stricturing, and calcification of the urinary tract. A number of animal-specific schistosome species (e.g., *S. bovis* or *S. margrebowiei*) may occasionally accidentally infect humans. The cercaria-stage parasites of a large number [1]. In other African countries, where *S. haematobium* are endemic, severe intestinal involvement is not frequent. In China, a high incidence of colorectal cancer in regions with endemic *S. japonicum* was recorded, where patients with chronic *Schistosomiasis japonica* have a more than three times greater risk of developing colon cancer than those with no previous exposure to schistosomal infection[2]**.**

Unfortunately, schistosomiasis is a disease that primarily results from the lack of education and public health facilities, appalling unsanitary conditions, and poverty found in many underdeveloped nations. So long as such conditions persist, schistosomiasis will continue to plague humanity. Human exposure to freshwater in underdeveloped tropical and sub-tropical areas suffering from these problems is the major determinant to infection. The schistosome larvae (cercariae) can penetrate human skin or enter the human as he/she drinks infected water or uses it for personal hygiene. For example, the cercariae can easily infect workers who wade through the wet-rice fields in Central China without wearing anything to cover their legs and feet. Or it can infect a child in Ghana who uses a local lake to bathe because there is no running water or functional sewage system. The images below Figure (1) detail the life-cycles of the species of schistosomes.

Schistosomiasis causes significant morbidity and mortality in the developing world with recent studies indicating that the geographic extent and burden of the disease exceeds official estimates [3]**.** Praziquantel-based chemotherapy has achieved some success in controlling the disease but is not an optimal strategy due to its inadequate impact on reducing long-term transmission[3]**.** Despite the mass chemotherapy programs, schistosome reinfection rates and prevalence continue to be unacceptably high, with rebound prevalence and morbidity an inevitable consequence if ongoing interventions are not sustained [4]**.** Along with other options, long-term protection afforded by vaccination will be necessary for the future control and possible elimination of schistosomiasis. Mirazid, the oleo-resin extract from Myrrh of *Commiphora molmol* tree, was established in Egypt as a new drug against schistosomiasis and other parasitic diseases [5, 6]**.**

Schistosome antigens**:** The list of published schistosome antigens, now numbering in excess of one hundred, comes from various parasite stages with the schistosomulum surface membrane being the preferential target. Characteristics which are required for further development include: (1) significant reduction of worm burdens and/or egg production compared to controls shown consistently in two different, commonly used experimental animal species; (2) demonstrated induction of human cell-mediated and humoral immune responses;

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(3) inclusion of a sufficient number of protective epitopes to overcome MHC restriction in hosts and genetic variation of parasite strains; (4) compatibility with approved adjuvants and stability of vaccine formulations; (5) lack of gross side effects; (6) ease of incorporation into delivery programmes; and (7) ease of passage through regulatory authorities.

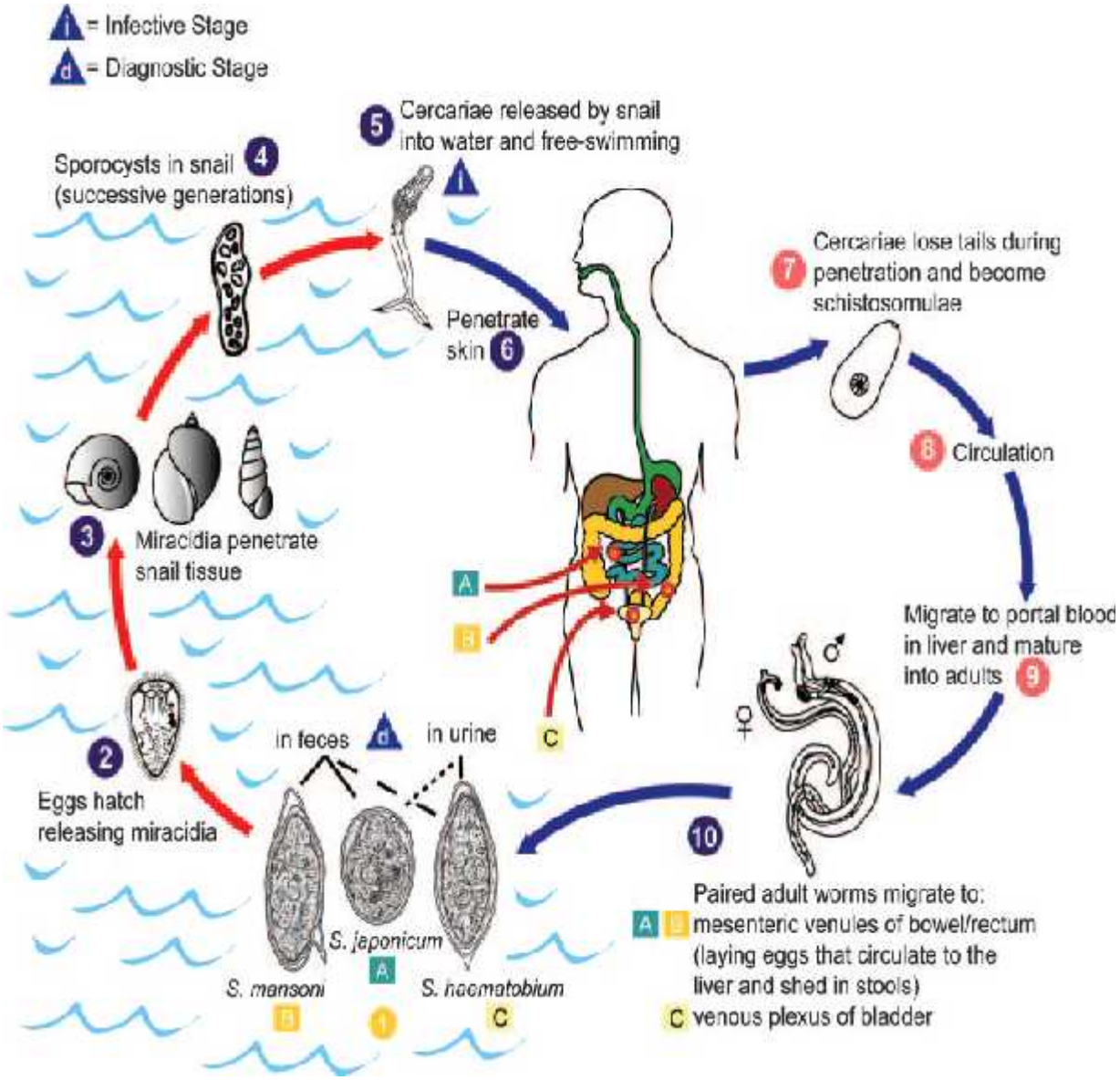
An effective vaccine against schistosomiasis would contribute to the current control strategy, mainly because it provides long lasting immunity against the disease. In the case of schistosomiasis, a sterilizing vaccine, although desirable, is not essential. Since schistosomes do not multiply within the final host, a vaccine that induces even a partial reduction in worm burdens could considerably reduce pathology, limit parasite transmission and be less expensive than repetitive drug treatment [7].

The major goal of research on the immune response to schistosomiasis is to develop a vaccine. Vaccines that can reduce schistosomiasis morbidity and mortality by lowering the intensity of infection or by modifying the immune response to parasite-derived antigens should be adopted for practical use even if they are not effective in complete elimination of the parasites [8]. Identification of schistosome antigens and their determinants that triggers the apparent protective immune response in resistant individuals could be a step towards the development of a vaccine against human schistosomiasis. Sm14 and paramyosin are among six *Schistosoma mansoni* antigens selected by WHO as candidate to compose a subunit vaccine against schistosomiasis [9]. Identifying immune dominant, possibly protective epitopes within a vaccine candidate antigen is extremely important, given the possibility to construct vaccines with relevant peptides from different candidate antigens.

The currently available vaccine antigens were discovered empirically using attenuated schistosome larvae, protective monoclonal antibodies, or by analysis of human antibody and cytokine responses to recombinantly- derived proteins[7]. These identified vaccine molecules may, however, lack the required efficacy because: 1) the vaccine-induced protective immunity generated in animal models may not translate to humans; 2) there is uncertainty about the type of human response most appropriate for protective immunity; and 3) the antigens may not be expressed on the schistosome apical surface, and will not therefore be exposed to the host immune system [1]. Key to the identification of new target vaccine molecules and high throughput antigen discovery are the recently published complete genomes of *Schistosoma japonicum* and *S. mansoni* [10,11] and related post-genomic research on the schistosome proteome, transcriptome, glycome and immunome [12,13]. The amalgamation of the information provided by these data sets, together with consideration of the host-parasite immune response in the field of immunomics, promises to result in more rapid and promising antigen discovery and the development of an effective vaccine for schistosomiasis [14-16].

Host–parasite endocrine interplay the highly evolved relationship between schistosomes and their hosts appears to include the exploitation of host endocrine and immune signals [17]. Host factors can trigger alternative developmental pathways that facilitate parasite survival in adverse conditions. Cytokines or cytokine-like proteins also appear to have key roles in the evelopment and maturation of schistosomes [18]. The transcriptome information from *S. japonicum* and *S. mansoni* further indicates this. Hu and co-workers compared the *S. japonicum* ESTs with homologues to mammalian sequences involved with (neuro) endocrine functions and identified a panel of schistosome genes with apparently similar roles to their mammalian counterparts [19]. In particular, *S. japonicum* shares sequences with humans and mice for insulin receptor or IGF-1 receptor, fibroblast growth-factor receptor 2 and neuropeptide Y2 receptor, which implies that S. japonicum could accept host hormone signals for cell proliferation and development, and/or suggesting the presence of an endogenous parasite endocrine system. Many sex-hormone-receptor-related proteins, including those encoding progesterone-receptor membrane component (PGRMC), small androgen-receptor-interacting protein, progesterone-receptor-associated p48 protein and progestin induced protein, were encountered in the *S. japonicum* EST data, suggesting that S. japonicum could produce hormones associated with mating and reproduction. Based on shared steroid-binding domains, schistosome receptors similar to PGRMC might accept sex hormones from their hosts. Somatostatin and thyroid hormone might influence growth, development and maturation of S. japonicum because the ESTs included homologues involved in the cell signaling that are pathways triggered by these neuro endocrine hormones. It is noteworthy that some members of the nuclear-receptor super family are involved in schistosome development [20]. More nuclear receptors and thyroid-hormone-associated proteins were identified among *S. mansoni* EST data [21]. The thyroid-hormone-interacting proteins 4, 12, 13 and 15 and thyroid-hormone-receptor-associated proteins Trap240 and Trap80, together with the reported effect of thyroid hormone on schistosome development. It is possible that *S. mansoni* uses an alternative tyrosine-rich protein as a substrate instead of thyroglobulin, if there is such an endogenous thyroid hormone [18].

Evading the host immune response mature schistosomes inhabit the mammalian bloodstream, potentially the most hostile immunological environment. They have evolved highly effective echanisms for evading the consequences of the cellular and humoral immune response that they provoke [22]. Helminthes are not equipped to outpace the immune system by faster cell division or rapid antigenic variation rather, their strategy appears to be assimilation, defusing aggressive immune reactions and inducing immunological tolerance to permit their long-term survival. Schistosomes exhibit an amazing diversity of ingenious mechanisms regulating their interactions with their intermediate and definitive host [23]. In immunocompetent hosts, they evade the immune response in several ways, including: (i) molecular masking, involving absorption of host antigens to the



Life cycle of S. mansoni, S. japonicum, and S. haematobium

parasite surface; (ii) expression of appropriate antigens by presenting epitopes similar, if not identical, to host molecules (antigen mimicry); and (iii) modification of the host immune response (by auto immune regulatory similarities) either directly, using its own molecules, or indirectly, by deregulating the host effector cells. Schistosomes might also use the signaling molecules of the host for growth and developmental control [24]**.** The new schistosome transcriptome sequences provide numerous leads for further investigation of molecular mimicry, antigen masking, antigen presentation, immune modification and immune inhibitors in this host–parasite relationship [25]**.** Antigens common to both vertebrate and invertebrate hosts were found in schistosomes, which support the notion of molecular mimicry used by schistosomes to avoid immune detection [26]**.**

Schistosomes can survive in the mammalian host for more than 30 years, developing a schistosome vaccine was never going to be an easy task [27]. Over the last four decades, there have been several scientific researches and review articles, but the results lack the required efficacy because: 1) the vaccine-induced protective immunity generated in animal models may not translate to humans; 2) there is uncertainty about the type of human response most appropriate for protective immunity; and 3) the antigens may not be expressed on the schistosome apical surface, and will not therefore be exposed to the host immune system **[1].** However, two more recent review articles were published. In 2009, a review article analyzing the use of live radiation-attenuated cercariae reported that the obtained high level of protection to a wide range of hosts offered great promise for human vaccine development [28]**.** In 2010, other reviewers

concluded that major hurdles have to be overcome in order to develop a suitable vaccine for field clinical trials, and optimistically added that better understanding of immune responses to schistosomiasis in both animal models and humans suggests that development of a vaccine is possible [29]**.**

Drugs or vaccines: From a pharmacological viewpoint, a potential drug target requires that the molecule malfunctions in the presence of the drug, whereas the vaccinologist would require that the chosen molecule be accessible to the immune response stimulated by vaccination. However, identifying such targets is not directly possible and would generally require high-through put screening. Immunization with whole or fractionated cDNA expression libraries has yielded promising results in recent years [30]**.** Even if irradiated cercariae consistently produce protection at this level, delivery problems, the need for a standardized product and safety considerations rule out this approach for human use [30]**.**

Schistosomiasis is one of the world's major public health problems in terms of morbidity and mortality, which is characterized by a marked egg-induced CD4+ T-cell, programmed granulomatous inflammation and cumulative fibrosis. The histopathological staging and collagen assessment for fibrosis showed that the cocktail PDDV presented an obvious down-regulation effect on hepatic fibrosis caused by chronic *S.japonicum* infection, and IFN- , IL-4 and IL-13 mRNAs in liver detected by RT-PCR [31]**.** The chronic morbidity in schistosomiasis is not due to the adult worms but is related to the T-cell-dependent immune responses in host, which is characterized by a marked egg-induced CD4+ T-cell programmed granulomatous inflammation and cumulative fibrosis, the main pathological manifestation in advanced schistosomiasis. The development of hepatic fibrosis, followed by cirrhosis, portal hypertension and other complications are the main causes of death to those people with advanced schistosomiasis. Immuno epidemiologic research and animal experimental findings showed that the hepatic granulomatous response to *Schistosoma japonicum* (*S. japonicum*) eggs began as a Th1-type response and then it was rapidly driven by egg antigens to a Th2-type dominant response, which suggests that the fibrotic process is highly dependent onTh2-type cytokines. Moreover, many studies presented the antifibrotic effect of interferon-gamma (IFN-) the main cytokine inTh1-type response [32]. Therefore, during the fibrotic progression, up-regulation of Th1-type cytokine responses in order to restore theTh1/Th2 balance may play a key role in slowing down or reversing the fibrosis. These factors are all known to be involved in the mechanism of fibrogenesis and they interact with Th1- or Th2-type cytokines in complicated ways. As we all know that development of an effective vaccine is a research priority while a multivalent (cocktail) antigen vaccine may be the way forward [33]**.** FourTh1-type epitopes for C57BL/6J mice from *S. japonicum* vaccine candidates, including P4 from Sj22.6 (22.6 kDa membrane protein), P6 from Sj28GST (28 kDa glutathione S-transferases), P18from SjTPI (triose phosphate isomerase), and P22from Sj97 (97 kDa paramyosin) [34- 36]**.**

Schistosomiasis vaccine requires a major research effort many factors justify making the investment needed. It appears that the immune system has been forced to balance responses associated with resistance to parasite invasion and against those that suppress the granulomatous reactions against eggs trapped in the host [37]. The coexistence of activated macrophages, different kinds of T-cells and antibodies, both effective ones and such which block protective responses accentuate the dualistic aim of the immune system [38].

Antigen Discovery**:** Antigens were found through simple disintegration and purification of tissues from adult schistosome worms or, for example, using the soluble egg antigen (SEA). When expression libraries became available for screening and when individual proteins could be linked to their encoding DNA, many new antigens were identified. The sequencing of the parasite genome and the generation of a large transcriptome database has greatly facilitated the finding of new information [39]. The new techniques permit preparations to be identified through blotting against protective sera and probed to identify the most antigenic components. The proteins in question can be chromatographically analyzed and the desired gel-bound band(s) identified, extracted and subjected to liquid chromatography followed by mass spectrometry. The resulting subset of proteins, selected on the basis of their involvement in the parasite biological processes, can then be produced in larger amounts for the usual, required efficacy testing in animal models.

Clinical trials: Clinical trials would permit the study of the underlying constituents of the consolidated response in the human host and even if current vaccine candidates may not be the final answer to controlling schistosomiasis, measurable protection will be provided and safety is assured by the availability of efficacious drugs with few side effects [37]**.** It should, in this context, be remembered that the pathology is directly correlated to the number of schistosome eggs in the host and a vaccine can achieve its effect both by offsetting parasite entry and development and by interfering with the production and delivery of eggs.

The rationale for schistosomiasis vaccine development can be summarized as follows: (1) since morbidity, rather than sterile immunity is the target, only a partially protective vaccine is required; (2) high-level protection is consistently realized with irradiated cercariae; (3) vaccines have proved an excellent means of cost-effective control of many infectious diseases; (4) rapid reinfection demands continuing treatment and drug delivery requires an infrastructure which must be both elaborate and reliable in the long term; (5) expanded chemotherapy programmes increase the risk of drug-resistance; and (6) a control approach based on chemotherapy followed by vaccination would integrate short-term effect with long-term protection. The design of a successful vaccine will be based not only on the most effective way of inducing

Advantages and disadvantages of protein vaccines

Advantages	Disadvantages
Safe and effective in use drugs	Drug resistance possible
Control programmes successful	Retreatment schedules required
Immunity acquired naturally	Still contended
Proof of principle shown beyond doubt (irradiated cercariae)	Approach not applicable for humans
Effective vaccine candidates exist to 70% protection reported	Protective mechanisms in (up humans largely unknown)
Due to focus on morbidity only partial immunity required	Required level of protection unknown
Absence of parasite replication the final (human) host	Reinfection contributes to in build-up of worm burdens
Several approaches available	Possibly difficult to combine

immunity but also on the technical feasibility of vaccine production [9]**.**

Antischistosome vaccine development: Schistosomes do not replicate within their mammalian hosts. Consequently, a non sterilizing naturally or vaccine-acquired immunity could significantly decrease human pathology and disease transmission. Vaccination against schistosomes can be targeted towards the prevention of infection and/or to the reduction of parasite fecundity. A reduction in worm numbers is the "gold standard" for antischistosome vaccine development, with the migrating schistosomulum stage likely to be the major vaccine target of protective immune responses [40]**.**

A schistosome eggs are responsible for both pathology and transmission, a vaccine targeted at parasite fecundity and egg viability also appears entirely appropriate. While they regularly induce 50 to 70% (over 90% in some cases) protection in experimental animals and additional immunizations boost this level further, it may be premature to pursue RA schistosome vaccines for human use, but their development for veterinary application is feasible. The concept is proven, and many of the requisite techniques, although they require refining and up scaling, are published. Although technically challenging, there is a case for promoting the development of a live, attenuated, cryopreserved schistosomulum vaccine for use against *S. japonicum* in buffaloes to reduce zoonotic transmission to humans in China [41]**.** If successful, the veterinary vaccine could provide a paradigm for the development of antischistosome vaccines for human use. In addition, while the *S. mansoni* RA vaccine model has enabled the dissection of different immune responses as putative effectors' mechanisms [42] and raised hopes for the development of molecular vaccines, this has not equated to advances in the development of recombinant vaccines. Independent testing of six candidate *S. mansoni* antigens (glutathione *S*-transferase 28 [Sm28-GST], paramyosin, Ir-V5, triosephosphate isomerase, Sm23, and Sm14) in the mid-1990s, orchestrated by a UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR/WHO) committee, resulted in protective responses being recorded, but the stated goal of consistent induction of 40% protection or better was not reached with any of the antigens tested, highlighting the possible negative influence of insufficient antigen stability and the need for standardized and effective adjuvant formulations [30]**.** Furthermore, of these six antigens, only one (Sm23) is exposed on the apical membrane surface of the parasite [43]**,** although it is not one of the more abundant apical membrane proteins on the parasite surface [44]. Also, the failure to develop an efficacious schistosome vaccine can be attributed in part to the complex immunoevasive strategies used by schistosomes to avoid elimination from their intravascular environment [22]**.** first, as discussed above, irradiated cercariae regularly induce high levels of protection in experimental animals, and additional immunizations boost this level further; second, as we have emphasized, endemic human populations develop various degrees of resistance, both naturally and drug-induced; and third, veterinary antihelminth recombinant vaccines against cestode platyhelminths have been developed successfully and applied in practice [45]**.** The optimism sparked by these arguments has resulted in the discovery of a large number of schistosome antigens (utilizing the almost-complete genome sequence), and additional candidates are now being found through proteomic approaches [46] these two dynamic areas of schistosome molecular biology are explored further below. However, antigen identification and successful protective results are of little value if recombinant proteins cannot be produced easily (and cheaply) with good manufacturing practice (GMP). Even the best protective results are no guarantee for ultimate success, and the scaling up of antigen production can be every bit as challenging as any immunological investigation. This was underscored when several of the frontline candidates chosen by the TDR/WHO committee discussed above had to be abandoned because, in addition to the low independent testing efficacy recorded, hurdles in consistent protein production could not be overcome. Nevertheless, as discussed below, there is still considerable interest in developing these and other molecules as antischistosome vaccines [44].

DNA vaccines: DNA vaccines generate both T-cell and B-cell (or antibody-mediated) immune responses and are thus particularly appealing for schistosome vaccine development. The preparation and production of DNA vaccines are convenient and cost-effective, and they can even be used in the field without a cold chain. Another advantage of applying DNA vaccines compared to other approaches is the possibility of targeting the in vivo expressed recombinant antigen to different cell compartments. Furthermore, methods such as prime boost regimens and the use of adjuvants (such as IL-12) in

combination with a DNA vaccine can enhance its protective effectiveness. The advantages and disadvantages of plasmid DNA vaccination, the strategies employed for DNA vaccine delivery, and technological and clinical advances in the area have been reviewed recently [47].

A vaccine for schistosomiasis that can be used in endemic areas would definitely complement drug treatment and other control strategies and will help immensely in reducing the levels of transmission of this disease. Several groups around the world have been working to develop a subunit vaccine for schistosomiasis but thus far none of them have achieved consistent and independently reproducible levels of protection which is acceptable for human use [48-50]. In this investigation, we report high levels of protection with Sm-p80 DNA vaccine constructed in VR1020 plasmid which is an FDA approved vector for human use. Previously, we have shown that Sm-p80 has significant promise as a vaccine candidate based on the data obtained from both murine as well as nonhuman primate models [51, 52]. In this study, Sm-p80-VR1020 DNA vaccination induced high titres of anti-Sm-p80 IgG, just 2 weeks after the initial immunization while the titres of IgG1 and IgG2a were considerably low even 6 weeks following the first vaccination. Conversely, the titres of IgG2b were found to be much higher compared to IgG1 and IgG2a. These findings further support our previous reports [53, 54]. The Th1 type of immune response after vaccination with DNA vaccine formulations.

A further supported by cytokine analysis in which Sm-p80-VR1020 immunization resulted in a Th1-skewed type of immune response as ascertained by high level of IFN-c and IL-2 and significantly low levels of IL-4 and IL-10 production by splenocytes following in vitro stimulation with recombinant Sm-p80. Further, high message levels of IL-18 were observed in vaccinated animals which are again indicative of a Th1-bias, because IL-18 is known to induce IFN-c production and other Th1 cytokines thus promoting a Th1 development and NK activity [55]. Up regulation of IL-15 in vaccinated animals is interesting; IL-15 possesses structural similarity to IL-2 and induces cell proliferation of natural killer cells. Overproduction of IL-15 has been shown to improve resistance to pathogens in other systems [56]. Additionally, induction of a Th17 type of immune response was also observed in these studies as characterized by the up regulation of TGF-b and IL-17 cytokines. Up regulation of IL-6 and TGF-b1 perhaps expanded Th17 immune responses leading to significant worm burden reduction. A significant increase in plasma levels of TGF-b and IL-17 cytokines in C57BL ⁄ 6 mice experimentally infected with S. mansoni and attributed the worm burden reduction to the increased levels of these cytokines [57]. Additionally, the down regulation of IL-4 cytokine, observed in this study, is in agreement with the published literatures that induction of IL-4 molecules suppresses the growth and maturation of Th1 and Th17 cells [58, 59]. It has been postulated that Th17 responses are likely to constitute an early immune response to several pathogens which could not be tackled properly by Th1 or Th2 type of immune response alone [60].

Partial CD25+ cell depletion fails to enhance the effectiveness of the schistosome vaccine, possibly due to IL-10 production by CD4+CD25- T cells, or other cell types, after CD25+ cell depletion during vaccination [61].

Peptide-DNA dual vaccine (PDDV): Plasmid DNA/cationic polymer complexes (so-called polyplexes) are attractive non-viral gene delivery systems for gene-therapy applications [62] and also have been mirrored in vaccine development [63]. For vaccine development, the concept PDDV was initiated by Wu *et al*., [64] the proper size is a prerequisite for efficient transfection by polyplexes [65, 66]. Here it is possible that the combination of both fast-releasing of epitope-based vaccine and slow-releasing of DNA vaccine could result in high effects of antifibrotic vaccine [67]. The cocktail PDDV was utilized to induce a dominant Th1-type response which is important in conferring immune lesion protection in C57BL/6J mice, the cocktail PDDV immunized mice showed lighter fibrosis as well as smaller size of non-confluent granulomas. Moreover, the amount of type I or III collagen was diminished, approximately in parallel by quantitative immune histochemistry and RT-PCR. It is suggested that the cocktail PDDV can attenuate the liver fibrosis and down-regulate the egg granulomas in the mice with chronic *S. japonicum* infection, which is probably due to that it induced the increased IFN- that exerts antifibrogenesis via inhibiting HSC activation, down regulating granulomatous inflammation and inducing the death of some eggs in liver tissue, the activated HSCs are well documented as the effector cells for hepatic fibrogenesis and also playing a contributory role in the granulomatous, fibrotic process induced by *S. japonicum* eggs, both in the murine model and in human disease [68]. By contrast, IFN- a predominant Th1-type cytokine, is a potent cytokine that exerts antiproliferative and antifibrogenic effects on HSC. IFN-_ can alter the phenotype of activated HSCs and regulate MMPs/TIMPs equilibrium to reduce extracellular matrix deposition in vivo by inhibition of HSC activation [69] and even killing the activated HSC in natural killer cell stimulatory receptor (NKG2D)- dependent and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-dependent manners [70]. The PDDV concept is first used in inducingTh1-type immune response and in antifibrotic research. It was also noted that immune adjuvant CpG ODN 1826 showed tender effect of anti-fibrosis, and the possible ways to improving the efficacy of the vaccine, cocktail PDDV, is now under investigation [36].

Paramyosin: The last 10 years of research into the development of a vaccine against schistosomiasis have been dominated by the search for an effective antigen for immunization. Paramyosin clearly has been shown to be one of the major targets. Indeed, paramyosin is one of the candidates selected by WHO for development of a vaccine against schistosomiasis. The wealth of information on paramyosin now at hand and the

availability of a variety of recombinant DNA constructs will facilitate the testing and evaluation of different approaches such as nucleic acid, live-virus, and adjuvantated recombinant protein vaccinations [71]**.** Cytokine IL-12, if administered as an adjuvant with a subunit vaccine such as paramyosin, might be highly effective in preventing infection by boosting both humoral and cell-mediated immune responses against schistosomes. For *S. juponicum* there is the additional possibility to test paramyosin- containing vaccines in a natural host, the water buffalo. These experiments are currently ongoing in collaboration with Chinese colleagues in tandem with pilot studies in laboratory mice [72]**.** Full length of DNA vaccine coding for paramyosin induced Th1-predominant response that presented an effect of anti-egg granulomas and the reduction of immune-pathological damage in C57BL/6J mice [67]**.**

S. japonicum triose-phosphate isomerase (SjTPI) DNA: The improvement in protection in a mouse model as measured by reductions in adult worm and liver egg burden by 50–60% with a single antigen- SjTPI DNA vaccine is very encouraging. As shown by human schistosomiasis, serious symptoms are generally associated with large numbers of parasites harbored within the body. A vaccine that can effectively reduce the worm burden by 50% or more may have a major impact on disease progression, including decreased morbidity. Furthermore, a recent study in water buffalo showed that a vaccine with 50% efficacy would reduce the transmission of the disease [73]. It was suggested that employment of such a vaccine in water buffalo, combined with praziquantel treatment, could result in significant decreases in transmission of the disease in the lake and marsh regions, based on mathematical models of transmission in these regions of China [74]**.** Furthermore, use of such vaccines in humans and in bovines, would lead to a dramatic decrease in transmission of schistosomiasis and a reduction in infection. Data presented in the current report support the notion that a codon optimized SjTPI DNA vaccine, when delivered by electroporation, may be one of such vaccines.

Immunological Control: Generating immunity through the use of vaccines is complex. In the presence of high prevalence, vaccine would not be given to naïve patients. Rather, those receiving the vaccine can be expected to have already been exposed and to experience repeated exposure to schistosomiasis after getting the vaccine. It is precisely the host immune response that gives rise to the granulomas responsible for the morbidity of schistosomiasis. Potentially, by triggering the production of immunity to various schistosomiasis antigens, the vaccine could promote the production of granuloma formation. In fact, however, progress is being made in phase 1 and 2 clinical trials of different vaccines [75]**.** Even without eradication of schistosomes from the environment, the vaccine appears to reduce susceptibility to re-infection. It is postulated that the vaccine's artificially-induced immunity is boosted by re-exposure to the not-yet-eradicated schistosomes. This suggests that immunogenicity may need to be assessed if and when schistosomes are eliminated [76]**.** Many trials of vaccinations are based on homologous or heterologous antigens. Bashtar *et al*. [7**7]** found that schistosomal worm and egg antigen had a potency role in protection against schistomiasis, while Hamed [78] postulated the immunization against schistosomiasis by using the excretory-secretory product of *Fasciola hepatica* worms.

Humphries and Yoshino [79] proposed that p38 mitogen-activated protein kinase play a role in *B. glabrata* immune signaling, which are known to be associated with stress and inflammatory signaling. In a comparative study, activated haemocyte p38 mitogen-activated protein kinase could also be detected using the anti-phosphorylated p38 antibody following cell treatment with anisomycin. The results suggesting fundamental differences in the role of p38 mitogen-activated protein kinase in signal transduction pathways between haemocytes and *B. glabrata* embryonic cells. Similar comparative studies, based on proteomics of either snail or parasite protein extracts are also beginning to reveal key molecules (such as mucin-like proteins from both parasite and snail) that may play a role in snail/schistosome compatibility [80]**.** A stress response, manifested by the modulation of genes encoding the stress response protein such as heat shock protein 70 may also underlie the snail-host/parasite encounter. Stress-related genes, heat shock protein 70 and reverse transcriptase were dramatically induced early in susceptible snails, but not in resistant/non-susceptible ones [81].

Most studies aimed towards deciphering differences in gene regulation between resistant and susceptible snails during the snail/schistosome encounter have focused mainly on this relationship in adult, but not juvenile snails. Age dependent variability in *B. glabrata* susceptibility to *S. mansoni* has been well documented with results showing that juvenile snails (even within the same stock) are, in general, more vulnerable than their adult counterparts to infection [80]**.**

Nucleoproteins: Nucleoproteins appear to play a role in the repair and regeneration of liver in mice infected with *S. mansoni*. Susceptible and resistant *Biomphlaria alexandrina* snails recorded significant improving levels of all parameters under investigation with more regard to the vaccine of the susceptible snails. More detailed studies are needed to ascertain whether dietary nucleotides might have a therapeutic effect on human liver cirrhosis or not. Therefore, the combination of susceptible snail's nucleotide with one of anti-schistosome drugs may be the aim for the future work in this field to evaluate the therapeutic possibility of such combination [82]**.** Nucleoproteins extracted from susceptible *B. Alexandrina* snails were more effective in protecting against schistosomiasis than those extracted from resistant ones. The results showed significant protection detected in worm and ova count reduction [83]**.**

Tegumental antigens: Adult worms tegument surface is such an obvious target for immune attack that several attempts have been made to induce protection in mice by

administration of purified membrane preparations. Several vaccination experiments were conducted with a variety of adjuvants, giving protection in the range 0-52% [84, 85]. *Sm*21.6 protein isolated from *S. mansoni* tegument proved to play an important role in reducing liver pathology. It was shown that mice vaccination with r*Sm*21.6 induced a mixed Th1/Th2 cytokine profile and r*Sm*21.6 was recognized by sera from individuals resistant to reinfection [86]. Codon optimization of *S. japonicum* TPI (*Sj*TPI) DNA vaccine can greatly improve its protective immunity as a viable schistosome vaccine candidate [73]. When pigs were immunized with UV-irradiated cercariae, results demonstrated that IFN-γ and IgG2 antibody production, as well as genes related to cytotoxicity are associated with the high level of protection.

Optimal vaccination required the induction of IFN-γ, IgG2 antibody related to Th1 responses and cytotoxicity effect [87]. Triton X-100 as detergent used for extraction of *S. mansoni* tegumental proteins was shown to potentiate the protective effect of the efficient tegumental antigen [88]. Nucleoproteins from susceptible and resistant snails were prepared according to the method of Nabih et al. [89]. Schistosomulae, in their first day in the host, are protected from the effect of blood components and cells. On reaching the blood and lymphatic capillaries, they shed the glycocalyx and undergo metabolic changes that end with replacement of the original tri-laminate tegumental membrane by a hepta-laminate double bilayer. Focusing of efforts on 6 days schistosomulae and their ESPs will accelerate steps towards a suitable convenient vaccine, to be established in clinical trials III.

Potential adjuvants and/or cocktail vaccines: Generation vaccines of *Fasciola gigantica* worms homogenate mixed with saponin on mice infected with *Schistosoma mansoni* were directed against infection and/or worm fecundity [90]. Currently there is a natural balance, tempering anti-schistosomal responses by stimuli down-regulating the granulomatous reaction against eggs in the tissue [91]. Role cytokine interaction in the development of pathology and immunity, looking for a way to induce maximum levels of immunity without enhancing egg-associated reactions [92]. Fasciola and Schistosoma worm antigens mixed with or without saponin as well as saponin alone succeeded to protect mice against *S. mansoni* infection with more potent effect of the separately saponin and Fasciola antigens, this protection is achieved by reduction in total, male and female worms as well as the levels of toxins elaborated by them [93], the role of these antigens in eliminating the product of oxidative stress and assistance in immune-mediated destruction of eggs that ameliorate the histopathological picture of the liver cells and preserve its function [93]. *Schistosoma mansoni* egg granuloma size reduction in liver section after vaccination with Fassiola or Schistosoma egg, in addition to Fassiola or Schistosoma worm with saponin antigens [94].

Schistosoma. japonicum GST: The use of levamisole as an adjuvant with *S. japonicum* GST-32, reduced worm and egg burdens as well as immunopathological complications associated chronic inflammation significantly in liver; and which were apparently associated with Th1-type response [95]. In two separate studies conducted in China, co-injection of recombinant plasmids and murine IL-18 containing *Sj*26- GST [96] and *Sj*14-FABP [97], enhanced protective effect against schistosomiasis *japonicum* as demonstrated by worm reduction and hepatic egg reduction rates. The results indicated that IL-18 may become a novel vaccine adjuvant for development of vaccines against schistosomiasis. Moreover, recombinant pseudorabies virus (PRV) Bartha-K61 vaccine strains expressing *Sj*26-GST and *Sj*14-FABP were constructed and evaluated for their ability to protect mice and sheep against *S. japonicum* challenge. The results indicated that the multivalent vaccine for *S. japonicum* can produce significant specific immunity and protection, and that PRV Bartha-K61 is an effective live vector for an animal schistosomiasis *japonica* vaccine [98].

As an adjuvant, HSP was used in two studies. Vaccination of water buffaloes with *Sj*C23-HSP70 and *Sj*C23-plasmids reduced worm burdens by 50.9% and 45.5%, respectively. In addition, mathematical modeling of *Sj*CTPI-HSP70 and *Sj*C23-HSP70 alone and in conjunction with human chemotherapy showed a significant reduction in transmission almost to the point of elimination [99]. In the second study, DNA vaccine of *Sj*GST combined with HSP70 induced reduction ~30% of worm burden and ~60% of egg burden in intestinal tissue of immunized mice. The potential adjuvant function of HSP70 suggested further studies for more characterization analysis of this regulatory molecule [100].

S. mansoni DNA vaccine: A study was conducted in Theodore Bilharz Institute, Cairo, Egypt to evaluate mice vaccination with multivalent *S. mansoni* DNA vaccine (*Sm*Fim-*Sm*21.7/ pBudCE4.1). The results revealed that it did not only induce a significant reduction in worm and egg burdens, but also significantly reduced hepatic granuloma size [101]. The efficacy of the schistosome DNA vaccine encoding the 23-kDa TSP, TPI and six fold-repeated genes of the complementarily determining region 3 (CDR3) was significantly enhanced by boosting *via* electroporation *in vivo* and/or with cocktail protein vaccines, leading to approximately 60% reduction for adult worm burden and greater than 60% for liver egg in mice [102].

Egyptian El-Ridi vaccine studies: In 1993, RA El Ridi and co-workers published their first article concerning schistosomiasis vaccines. Although they obtained 80% protection, using irradiated cercariae, many bands of *S. mansoni* soluble adult worm antigen (SAWA) were recognized by only few number of sera from vaccinated mice. Accordingly, they concluded that the potential vaccine should show its ability to evoke T cell immunity in the majority of vaccinated hosts [103]. In the following 2 years, with cooperation of Egyptian Organization for Biological Products and Vaccines (VACSERA) and NAMRU-3, it was possible to identify and characterize

SAWA bands that were recognized by sera obtained from immunized mice [104] and Egyptian children with early active schistosomiasis *mansoni* and/ or *hematobium* [105]**.** Results from the later study showed that the selected potential bands included glycoproteins. Meanwhile, it was concluded that the vaccine which induced partial immunity will become completely ineffective in the next cercarial exposure. The resistant larvae will mature and produce eggs, with consequent further down-regulation of T-cell responses and macrophage effectors' functions and persistent production of blocking antibodies. An effective vaccine for endemic areas should induce complete immunity, or have a very strong anti-fecundity effect, or both. The 62/60 kDa bands of *S. mansoni* SAWAs. The purified 62 and 60 kDa proteins were used for mice and rabbits immunization [106]. It was found that 62 kDa, identified as *S. mansoni* calreticulin, is a good T- and B-cell antigen and represents a potential vaccine candidate [107]. Based on previously published articles and in critical comment, she concluded that a candidate vaccine for schistosomiasis should be made of schistosome peptide(s) carrying T-helper-cell and B-cell epitopes (immunomes) in genetically diverse hosts [108]**.** Accordingly 6 peptides (A, B1, B, C, D and E), derived from the primary sequence of *S. mansoni* glyceraldehyde 3-phosphate dehydrogenase (SG3PDH) were used for BALB/c mice immunization. The results showed that the specificity of the induced immune responses is an important factor for the efficacy of synthetic peptide-based vaccine for schistosomiasis [109]**.** In 2003, El Ridi and coworkers had started to work on schistosome lung stage larvae (SLSL) by investigating the nature of the corn oil-mediated outer membrane changes responsible for antigen exposure in SLSL [110]**.** In subsequent publications they suggested the role of cholesterol and unsaturated fatty acids in sequestration of the surface protein antigens and natural attrition of SLSL, respectively [111,112]**.** The continuous laboratory studies on SLSL, conducted by El Ridi R and Tallima H, continued till 2008, when they found that *ex vivo* and *in vitro* grown SLSL express *S. mansoni* G3PDH on their surface membrane [113]**.** Then in 2009, they isolated 6-day-old *ex-vivo* SLSL from infected mice to be used for generation of ESPs, which elicited strong immune responses and significant protection against challenge infection in BALB/c mice. The investigators suggested their use as ideal potential vaccines, as these proteins have an essential role in development of local primary and memory immune response effectors that target, surround and pursue the *S. mansoni* larvae while penetrating lung capillaries [114]**.** This was followed by characterization of the cytokines response of ESPs (SG3PDH, 14-3-3-like protein, Cys peroxiredoxin and calpain) induced by *S. mansoni* schistosomula in the lung. Spleen cells from *S. mansoni*-infected mice were stimulated *in vitro* with the selected ESP, in a recombinant or MAP form. They found that larval ESPs elicited Th1 and Th17 type cytokines, and suggested that a balance between Th1, Th17, and Th2 cytokines is required for effective schistosome larval elimination [115].

Advantages and disadvantages of protein vaccines: Proteome-wide, selective expression of full-length proteins including insoluble and membrane proteins, Protein expression can be determined from C- and N terminal tags High throughput screening of antibody responses from experimentally immunized or naturally exposed individuals and animal models Small serum sample volume (1µl) required for immunoscreening, while the disadvantages were Some loss of protein tertiary structure and post-translational Modifications Reactivity is not directly comparable between microarray antigens Microarray protein levels cannot be quantified Antibodies against E. coli proteins in cell-free extract must be blocked [13,116].

CONCLUSIONS

The time has come to extend our knowledge about the immunological response to the vaccine candidates and their capability to protect against 100 Schistosomiasis Vaccine Development. Ethical considerations would be satisfied by the provision of protection and the assurance of safety through effective drugs with few side effects. As chronic schistosomiasis must be expected to be common in any study area the potential of exacerbation is, however, an issue to take into account [117]**.** Although we shall need to continue learning from animal models, in particular in relation to peptide/protein candidates and schistosome-related DNA vaccines, further progress will require shifting the focus to large-scale production and Phase I trial design. Many critical questions can only be answered by safety-first, well controlled clinical trials and it is now appropriate to move in that direction [118]. Building on the results from, and capacity developed in, Brazil, Egypt and Kenya, an alliance for research coordination and support dedicated to promoting vaccine candidates could achieve tangible results already in the short term.

RECOMMENDATIONS

Reviewing the vaccine studies in the last decade depicted two facts. First schistosomiasis vaccine development followed a long and tortuous road. Second, protection against schistosomiasis should not only reduce infection and protect from re-infection, but also accelerate immune responses in infected humans directed against granuloma-related pathology and/or worm fecundity. The major source of antigens that could serve as vaccine candidates is the surface tegument of both larvae and adults. The plasma membrane has documented transport functions and enzyme activities, and the exposed portions of the proteins may represent vulnerable points for attack. The ultimate vaccine might have to be a combination of two or more antigens and although attempts to combine full-length protein antigens from the same stage have not been encouraging, the possibility of synergistic action would increase by incorporating antigens from different developmental stages of the parasite. Once they are developed and employed, antischistosome vaccines will

not be a panacea. They need to be regarded as one component, albeit a very important one, of integrated schistosomiasis control programs that complement existing strategies, including active components of medicinal plants and health education [119,120]. This unprecedented accumulation of molecular data is allowing a more rational approach to propose vaccine candidates and drug targets.

Conflict of interest: Sanaa A. Ali and Manal A.Hamed None declared.

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