Spotlights on new publications

Sherif M Abaza

Medical Parasitology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

Corresponding Author: Sherif M. Abaza, **Tel.:** +20 1005243428, **E-mail:** smabaza@hotmail.com

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New vaccine candidates VIII
Schistosomiasis vaccines

Several *Schistosoma* vaccine candidates were investigated in pre-clinical animal model studies as well as clinical trials. They included the structural protein of the outer tegument (tetraspanin), calcium-activated protease calpain (p80), fatty acids binding protein (p14), tegument proteins (p23, and p29 or paramyosin), chemokine binding protein (CB), and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) for *S. mansoni*. For *S. japonicum*, glutathione transferase (GTT), and heat shock protein 70 (HSP70) were investigated. In spite of the huge number of publications, there is no licensed vaccine for animals or humans till date.

Review

In a review of the pre-clinical studies conducted on animal models utilizing *S. mansoni* vaccine candidates, **Emma L Houlder *et al.*** claimed that only three candidates were investigated over 20 times during the last three decades. They were two proteases [cathepsin B (CatB), and calpain (p80)], and p14. Variable results for efficacy outcomes were obtained; however, only proteases-based vaccines exhibited over 90% efficacy against *Schistosoma* adults. This was attributed to different study designs, formulations, and standardization processing. Four vaccine candidates are currently in progress for clinical phases of design and development. While only Bilhvax (*Sh*-28GST/Alhydrogel) reached phase III, *Sm*-14/GLA-SE, and The Human Schistosomiasis Vaccine (*Sm*TSP2/Alhydrogel) were in phase II, and SchistoShield (*Sm*-p80/GLA-SE) is still in phase I.

Similar to vaccine candidates, variable platforms were utilized; however, the most reported are protein-based systems, mainly recombinant proteins, followed by DNA vaccine systems. Recombinant vaccines are proposed in the majority of clinical trials since they allow precise targeting of immunogenic regions to elicit specific and robust immune response. Moreover, they are produced on a large scale, *i.e.*, significantly more cost-effective. In addition to their safety, they are the best applicable vaccines in clinical trials with highly standardized expression systems. Of note, future vaccine development against schistosomiasis should focus on optimizing the immunogenicity and efficacy of the vaccine candidates. On the other hand, adjuvants are most often changed in the studies of schistosome vaccine; however, alum was the most common adjuvant used followed by Freund. However, the reviewers claimed that the most efficacious adjuvants were Addavax, and Montanide. When combined with *Sm*-CatB, Addavax showed the highest reduction in worm burden (91%), followed by Montanide (76%).

The emulsion/toll like receptoe-4 (TLR4) agonist (GLA-SE) and the emulsion/TLR9 agonist (CpG) administered with *Sm*-p80 showed 91%, and 87% reduction of hepatic egg counts, respectively. Another emulsion/TLRs agonist (CFA) showed 89% efficacy against worms when given with *Sm*-14. In animal models, most vaccines were given in 3 doses (1 prime, 2 boosters) prior to schistosome challenge. The majority of vaccines were administered *via* the subcutaneous route, followed by intraperitoneal, and intramuscular routes. Compiled from **"Pre-clinical studies of *Schistosoma mansoni* vaccines: A scoping review." PLoS Negl Trop Dis 2025; 19(6):e0012956.**

Vaccine candidates
Compilation No. (1)

A previous proteomic study conducted by Zhong *et al.* (2022) observed significant high expression of *S. japonicum* glycosyltransferase (*Sj*GT) and nicastrin (*Sj*NCS) in single-sex males compared to mated males (Int J Parasitol 2022; 52:815–828). It is worth mentioning that GT plays a crucial role in synthesis of almost all sugar components (disaccharides, glycosides, glycoproteins, and glucans complex) that are essentially required for male fertility. On the other hand, NCS is required for synthesis of the transmembrane γ-secretase protein complex that also regulates male reproductive processes *via* LIN-12/notch signaling pathway. The investigators hypothesized that both proteins are linked with signals transmission from males to regulate females' reproduction during coupling. In the present compilation, the same group of investigators

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(**Bowen Dong** and his Chinese colleagues) utilized bioinformatic analysis to characterize their structure, *i.e.*, signal peptide regions, transmembrane domains, and protein interaction networks. Accordingly, *Sj*GT and *Sj*NCS were purified and expressed. Prediction of their tertiary structures using 3D models was performed to construct the recombinant forms of both proteins. Constructed forms were analyzed by Western blotting that confirmed their immunogenicity as demonstrated by their reactivity with antibodies raised against adults proteins.

Male BALB/c mice were subcutaneously injected by three doses, at two weeks intervals, of r*Sj*GT and r*Sj*NCS, separately, and combined. While the 1st dose was 50 µg/100 µl/mouse, the next two doses were 25 µg/100 µl/mouse. Two weeks after the last immunization dose, mice were percutaneously infected with 40±2 cercaria. Six weeks after infection, mice were sacrificed to determine worm burden and hepatic egg count, and estimate specific IgG levels using ELISA.

Results of *in silico* characterization of *Sj*GT and *Sj*NCS revealed absence of a signaling peptide, and transmembrane region in *Sj*GT, whereas *Sj*NCS possessed a signaling peptide [at amino acid (aa) residues 1–23], and a transmembrane region (at aa 658–680). In addition, their homology analysis demonstrated higher identity to *S. mansoni*, and *S. haematobium* (range 75-82%), and lower identity to *H. sapiens*, and *Mus musculus* (range 27-35%). The study also conducted phylogenetic analysis of *Sj*GT, and *Sj*NCS that showed distant evolutionary relationships, *i.e.*, human and mouse proteins were positioned on distinct branches from those of schistosomes. Since the expression profiles of GT were not available in the *S. mansoni* database, the investigators only analyzed the expression profile of *Sj*NCS in reference to *S. mansoni* RNA sequencing database. Results showed significant high levels in female gonadal, miracidia, and sporocysts, and was significantly up-regulated in male miracidia and sporocysts.

Compared to adjuvant- and PBS-immunized mice, the 1st immunization dose of r*Sj*GT and r*Sj*NCS induced significant elevated specific IgG levels that further increased after the 2nd dose, and remained at high levels after the last dose. Besides, immunization with r*Sj*GT and r*Sj*NCS exhibited significant reduction of female fecundity (36.73%, and 33.41, respectively), and decreased hepatic egg count (42.65, and 41.55%, respectively). Immunogenic properties of rGT, and rNCS were concluded and further studies were recommended to explore utilization of combined proteins as potential vaccine against schistosomiasis. To improved vaccine efficacy, the investigators also recommended utilization of advanced nanotechnology such as nanoparticle-based formulations (as delivery system) to enhance antigen-specific immunity. Compiled from **"Antibody responses and the vaccine efficacy of recombinant glycosyltransferase and nicastrin against *Schistosoma japonicum*." Pathogens 2025 Jan 14;14(1):70.**

Compilation No. (2)

There are over 40 different genes encoding tetraspanins (TSPs), the transmembrane proteins. Of note, TSPs have essential roles in tegument formation, maturation, and stability in several platyhelminths. In addition, they are involved in many cellular activities, *e.g.*, proliferation, differentiation, adhesion, and division. Structurally, they consist of 4 transmembrane domains, small and large extracellular loops (SEL, and LEL). It was reported that cysteine residues of LEL could serve as a distinctive signal motif allowing for specific protein-protein interactions with other proteins. Additionally, previous studies proposed TSPs as promising vaccine candidates for immunization against *E. granulosus*, and *O. viverrine*. Genomic studies of *S. haematobium* revealed 6 TSPs in its tegument and extracellular vesicles (*Sh*TSP2, *Sh*TSP4, *Sh*TSP5, *Sh*TSP6, *Sh*TSP18, and *Sh*TSP23). In the present compilation, **Angela Silvano *et al.*** selected to study three TSPs (2, 6 and 23) because of 3 issues; they are 1) expressed in all life stages of *S. haematobium*, in particular adults and schistosomula, 2) clustered together in the CD63 family, and 3) expressed in soluble forms. Therefore, the investigators hypothesized the ability of *S. haematobium* TSP-2, TSP-6 and TSP-23 to modulate host immune response.

To achieve their objective, the investigators prepared the recombinant forms of the selected TSPs to purify their LELs. The expressed protein of each LEL was identified and confirmed using SDS-PAGE, and Western blot, respectively. Meanwhile, the investigators isolated monocyte-derived dendritic cells (DCs), obtained from healthy donors who were never exposed to *S. haematobium* TSPs, to be *in vitro* cultured. Maturation of DCs was performed by their stimulation using the prepared LEL of the selected TSPs. To assess cytokine gene expression, the study stimulated pre-activated T CD^{4+} cells with mature DCs. To quantify cytokines expression, retro-transcriptase quantitative PCR was used.

In comparison to unstimulated DCs, only *Sh*TSP2, and *Sh*TSP6 induced significant concentration levels of maturation markers; 3-, and 7-fold increase of CD80, and CD83, respectively for *Sh*TSP2, and 2-, and 3-fold increase for *Sh*TSP6. In *Sh*TSP2 stimulation, the investigators recorded significant increased cytokines levels of pro-inflammatory (IL-6, and TNF), and regulatory (IL-10) cytokine. It also induced increased levels of IL-1β to a lesser extent.

A significant induction of Th1 and Th2 cytokine expression (IFNɣ, and IL23, respectively) in CD^{4+} T stimulated cells was recorded. Moreover, *Sh*TSP2 recorded a negligible, but significant, production of Th2 cytokine (IL-13). On the other hand, only significant increased level of IL-6 was recorded on stimulation with *Sh*TSP6, with induction of Th2 cytokines (IL-4, and IL-13) expression. It also increased levels of TNF and IL-1β, with results that were close to significance ($P \leq 0.10$).

Results revealed that *Sh*TSP2 had significant superior effects over *Sh*TSP6 to modulate human immune response, and the investigators decided to extend their study to investigate its potential use as a promising vaccine candidate. To assess dose-effect on DCs maturation and cytokine production, r*Sh*TDS2 at 3 different concentrations (1, 10, and 100 µg/ml) were incubated in DCs cultures, followed by assessment of the expression of maturation marker (CD83) and cytokines levels. Results revealed that 10 µg/ml concentration was optimal for stimulation assays, *i.e.*, non-significant increase at 1 µg/ml, and increased to significance level at 10 µg/ml, with no further increase at 100 µg/ml concentration. Similar results were observed in cytokine production. Using an endotoxin assay kit, investigators assessed possibility of residual contamination during preparation. Results revealed that r*Sh*TDS2 effects were independent without possibility of bacterial contamination. It is worth mentioning that *Sh*TSP2 has 69.6% sequence identity of *Sm*TSP2 that showed promising results in phase Ib clinical trials (Diemert *et al*., 2023; PLoS Negl Trop Dis; 17 (3):e0011236), and currently undergoing phase II trials. Compiled from **"*Schistosoma haematobium* tetraspanins TSP-2 and TSP-6 induce dendritic cells maturation, cytokine production and T helper cells differentiation *in vitro*." Microbes Infect 2025 Mar-Apr; 27(3):105439.**

Clinical trials

After the success of phase I clinical trial conducted in healthy volunteers residing in non-endemic area for schistosomiasis in Brazil, **Amadou Tidjani Ly** and his colleagues evaluated the vaccine candidate (*Sm*14) with glucopyranosyl lipid A (GLA-SE) as adjuvant. They conducted two randomized open-label clinical trials, on 30 adults, and 95 schoolchildren living in 3 villages in the lower Senegal River basin, an endemic region with high transmission. This location is characterized by high density and prevalence of the vector snails, and schoolchildren were re-infected, particularly by urogenital schistosomiasis, soon after mass treatment program. After recruitment of the study participants, baseline characteristics for each was performed including clinical examination, laboratory investigations (CBC, liver and kidney functions), and immunological profile (humoral and cellular immune responses).

In the 1st clinical trial, two cohorts of 15 male volunteers (18-49 y) were selected from one village. The primary cohort included participants with schistosomiasis that received treatment, while participants of the 2nd cohort were currently free from infection. The 2nd trial included schoolchildren residing in 3 villages divided into 3 groups: 1) healthy children (no. 15) from one village, 2) infected children (no. 40), 3) infected children who were not vaccinated, as control (no. 40). Recruited schoolchildren (8-11 y) were 42 boys, and 53 girls (32 infected by *S. mansoni*, and 48 infected with *S. haematobium*), among them 10 were infected with both species.

Praziquantel (PZQ) was administered to all adults before vaccination to ensure absence of infection which was confirmed by Kato–Katz, and urinary filtration methods. On day 0, adults of each cohort were vaccinated with 50 µg recombinant *Sm*14, while the 1st cohort received 2.5 µg GLA-SE adjuvant, the 2nd received 5 µg. Two boosters were administered 4, and 8 w later. Because results of the 1st trial showed similar or even stronger response with 2.5 µg dose, this dose was used in vaccination of schoolchildren. Each participant in the 1st two groups received three doses (50 µg each) with 2.5 µg GLA-SE, while those in the 3rd group were left unvaccinated as controls. Similarly, PZQ was administered 8-14 d before vaccination, and two boosters at one-month intervals.

Assessment of vaccine safety, as followed by international regulatory and ethical standards, included report of adverse events (AEs), and previously mentioned laboratory tests. Assessment of vaccine outcomes included determination of specific anti-*Sm*14 IgG antibodies, and isotypes IgG1, IG2, and IgG4. Besides, the study assessed the cell-mediated immune response using Luminex method, and intracellular flow cytometry approach to evaluate the subpopulations of B cells, T cells (CD^{4+}, CD^{8+}), and to determine cytokines (IFN-γ and TNF-α). Trained physicians observed the participants for any inflammatory responses at the injection site, regional and systemic symptoms, *e.g.*, pain, swelling, fever >38°C, headache, irritability, loss of appetite, and arthralgia. Adverse events (AEs) were categorized as grade 1 (easily tolerated), grade 2 (discomfort that requires no or minimal treatment); grade 3 (illness that prevents normal activities, and requires treatment); and grade 4 (life-threatening). It is worth mentioning that the study proposed a time set protocol for both clinical trials so that each participant knew the days of check-up and laboratory assessments.

Results showed safety outcomes in adults with no serious AEs. The most common reaction was local pain at the injection site, and its proportion decreased after subsequent vaccination. Only two participants had fever on the 7th d, and another two complained of headache and light local hyperemia in the vaccinated arm. There were no changes in the laboratory investigations except for slight elevation of liver function enzymes in few participants. Similar results were obtained in schoolchildren vaccination with more decreased proportions of local pain at the injections site after subsequent vaccination. All recorded AEs were of grade 1, except 2 participants who had vomiting.

Thirty days after the first dose in the 1st trial, significant increased levels of *Sm*14-specific total IgG were recorded, that remained high at least 120 d afterwards. In terms of isotype, the highest levels were observed for IgG1 and IgG3. Similarly, high cytokine production of IL-2 and IFN-γ levels were recorded, particularly after 8 w. Elevated levels were maintained up to the end of the trial (20 w).

For all vaccinated schoolchildren (healthy, and previously infected), a significant increase in total IgG was recorded after the 3rd dose with a gradual decrease over the subsequent follow-up time (12 m). Only 3 children did not respond at day 84 (or later), and 12 children had IgG levels below their baseline levels after 12 m. Regarding the previously infected, non-vaccinated children, their IgG levels did not show increase throughout the study. Generally speaking, 94% of vaccinated children showed significant increase in specific IgG antibodies. The major IgG isotype in vaccinated children was IgG1. In comparison to baseline profile, all vaccinated schoolchildren elicited robust cytokine responses, *i.e.*, significant increased levels of TNF-α, IFN-γ, and IL-2 production. The investigators concluded vaccine safety with highly immunogenic outcomes as demonstrated by significant protective potentiality against schistosomiasis. Accordingly, they recommended performing phase III clinical trials. Compiled from **"The *Sm*14 + GLA-SE recombinant vaccine against *Schistosoma mansoni* and *S. haematobium* in adults and school children: Phase II clinical trials in West Africa." Vaccines (Basel) 2025 Mar 16; 13(3):316.**