An Overview on Schistosomiasis Infection among Nigeria School Age Children and Progress of Vaccine Development

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ABSTRACT: In terms of endemicity, public health and socio-economic burden, schistosomiasis remains one of the most widespread human parasitic diseases and is ranked second after malaria, especially in the Sub-Sahara Africa. Despite the worldwide success of chemotherapy, the disease continues to defy control efforts. The disease is endemic in 78 countries, infecting about 240 million people worldwide and more than 700 million people live under risk in endemic areas. Within Sub-Sahara Africa, Nigeria carries the heaviest burden with an estimated 29 million cases of infection. School children have been identified as the major victim of the disease and transmission is chiefly by the planorbid fresh water snails commonly found around sources of water such as streams, rivers, ponds and irrigation canals. A study carried out in Imo, for instance, revealed a high (71.4%) prevalence of schistosomiasis among school age children. The burden of schistosomiasis was high among children of migrant workers and they serve as reservoirs for transmission of the disease. Praziquantel, the only drug currently available for treatment, is effective to kill developing schistosomes, it does not prevent re-infection and continued extensive use may result in the future emergence of drug-resistant strains. As a result, vaccine strategies represent an essential component as adjunct to chemotherapy for the future control of schistosomiasis. Vaccination against schistosomes can either target prevention of infection or reduction of parasite fecundity. A reduction in worm numbers is the "standard" for anti-schistosome vaccine development, with the migrating

I:C adjuvant; 40.3, 68.2 and 57.9% reductions in adult worm burden, liver egg burden and intestinal eggs, respectively, whereas achieved, along with a reduction in granuloma size and number in livers of immunized mice (Ewaisha *et al.,* 2014). Another report showed that by fusing Sm29 and Sm14, a 48.4% reduction in worm burden was achieved in mice (Mossallam *et al.,* 2015). In a further advance, when the recombinant Sm29 was subjected to high hydrostatic pressure which dissociated the aggregated protein resulting in a successfully folded, soluble, stable and structured molecule, it was protective against *S. mansoni*, thereby paving the way for its industrial production down track (Chura-Chambi *et al.,* 2013).

4. ANTIOXIDANTS

The antioxidants Cu–Zn superoxide dismutase (SOD) and glutathione S peroxidase (GPX) induced ≥ 40% reduction in worm burdens when administered as DNA-based vaccines in *S. mansoni*-challenged mice (Shalaby *et al.,* 2003). Similarly, a recent study showed a protective effect against Schistosomiasis in baboons (Carvalho-Queiroz *et al.,* 2015). Vaccinated baboons with two different formulations of SOD (SmCT-SOD and SmSP-SOD) and one of GPX, with a protocol of priming with naked DNA and boosting with the respective recombinant antioxidant proteins encapsulated in polylactic acid microspheres, exhibited a robust immune response, which resulted in a reduction in worm numbers, and pronounced anti-pathology effect compared with control animals.

5. DIGESTIVE TRACT PROTEINS

S. mansoni worms ingest host blood which passes through the oesophagus before arriving at an area in the gut where many peptidases catalyse its proteolysis. Processing of the blood and the resulting uptake of nutrients are functions essential for the survival of the parasite (Tebeje *et al.,* 2016). Blocking these critical processes represents an important strategy for vaccine development and a number of digestive tract proteins, that are not recognized by host immune responses during normal infection, but are essential for parasite survival, have been tested as cryptic vaccine candidates (Figueiredo *et al.,* 2015). One example was the trialling of a soluble form of schistosome lysosome-associated membrane glycoprotein, located in the gastrodermis, which resulted in reduction in worm burden (16–25%) and in faecal eggs (52–60%); moreover, its insoluble form produced up to 38% reduction in liver egg burden (Nawaratna *et al.,* 2015). Another recent

study identified a number of esophageal secreted proteins, encoded by micro exon genes (MEGs), that are involved in the initial processing of ingested blood and these, along with lysosomal hydrolase, also localised to the oesophagus, may prove to be novel immune targets (Wilson *et al.,* 2015).

5.1 *S. japonicum* (Sj97)

Paramyosin is a myofibrillar 97 kDa protein present in the muscle layers and the tegument of schistosomes that has long been regarded as a vaccine candidate against both *S. japonicum* and *S. mansoni* infection (McManus *et al.,* 2008). An early study showed that mice vaccinated intra peritoneal with purified paramyosin (without use of an adjuvant) stimulated 62–86% resistances against *S. japonicum* cercarial challenge (Ramirez *et al.,* 1996). In addition to preventing infection, a longitudinal treatment-re-infection design study in Leyte, the Philippines showed that a Th2 bias in response to Sj97 predicted a longer time to human re-infection and lower re-infection intensity after treatment with PZQ (Leenstra *et al.,* 2006, Jiz *et al.,* 2015). Moreover, it was reported in the human Leyte cohort that individuals who produce IgE but not IgG4 in response to rSj97 had 77% lower re-infection intensity after 12 months of treatment with PZQ (Jiz *et al.,* 2009 and 2015).

5.2 *S. japonicum* 26 kDa GST *plasmid* DNA Vaccine (Sj26GST)

A *S. japonicum* 26 kDa GST plasmid DNA vaccine (Sj26GST) resulted in a significant reduction in worm numbers, hepatic and faecal eggs in vaccinated mice (Wei *et al.,* 2008). When the DNA vaccine was given in combination with interleukin 18 (IL-18), a potent IFN-γ inducing factor, the protective efficacy was improved. DNA vaccines have some advantages over other types of immunization but they have some limitations concerning the gene delivery system. A recent study reported on a novel nanoparticle formulation of the Sj26GST DNA vaccine; although there was no significant reduction in worm burden, a highly significant decline in tissue egg burden and fecundity of female worms were recorded (Mbanefo *et al.,* 2015). Toll-like receptor (TLR) 7/8 ligands (*e.g.* R848) and TLR 9 ligands (*e.g.* CpG oligodeoxynucleotides, or CpG) as adjuvants can increase vaccine effectiveness through activating the innate immune system and ultimately activating and directing the adaptive immune system. Such adjuvants have been shown to potentiate the activity of the Sj26GST DNA vaccine in mice by increasing splenocyte proliferation, elevating IgG,

schistosomulum stage likely to be the major vaccine target of protective immune responses. Over 100 schistosome candidate vaccine antigens have been identified, however, only three molecules, S. mansoni fatty acid binding protein (Sm14), S. mansoni tetraspanin (Sm-TSP-2) and S. haematobium glutathione S-transferase (Sh28GST), have entered human clinical trials. Schistosomes use fatty acid binding proteins (FABPs) to absorb, transport and compartmentalize fatty acids from the host and, because of this critical biological function, Sm14 has long been considered a potential vaccine candidate. Recombinant Sm14 (rSm14) provided up to 67% protection in terms of reduced S. mansoni worm burden in outbred Swiss mice without use of an adjuvant. Sh28GST, a 28 kDa glutathione S-transferase enzyme plays a role in fatty acid metabolism, prostaglandin D2 synthesis and may help the parasite to evade the host immune system. The enzyme present in S. mansoni (Sm28GST) has been tested extensively as a recombinant protein vaccine in various experimental models and has shown partial protection in terms of reduced worm burden, inhibition of female worm fecundity and reduction in egg viability. Sm-p80 Calpain is a calcium activated neutral cysteine protease. Prime-boost vaccination with Sm-p80, the large subunit of S. mansoni calpain, in combination with resiquimod adjuvant, resulted in 49% worm burden reduction, while 50% protection was achieved using the recombinant protein as primary and boost vaccine in mice. Vaccinomics is a powerful innovation which provides a foundation for searching critical determinants of immunity. It can promote both antigen discovery and design of novel vaccines for multi-cellular pathogens such as schistosomes. A recent vaccinomics approach for discovering novel schistosome antigens that may not be revealed by conventional proteomics involved both design and manufacture of an immunomics protein array, the first to be generated for a multi-cellular pathogen. This vaccine could be administered to children between the age of 3 and 12 years to prevent severe infection particularly in high risk population. This review summarizes the state of the art of schistosomiasis vaccine development.

Keywords: Schistosomiasis, Schistosoma Mansoni, S. Japonicum, Immune Response, Vaccine, Antigen Discovery.

1. INTRODUCTION

Schistosomiasis is a Neglected Tropical Diseases (NTDs) caused by parasitic worms of the genus *Schistosoma*. In terms of public health and socio-economic burden, Schistosomiasis remains one of the most widespread human parasitic diseases and ranked second after malaria, especially in the Sub-Sahara Africa (WHO 2016a). The disease is endemic in 78 developing countries, infecting almost 240 million people worldwide, and more than 700 million people live under risk in the endemic

areas. Twenty millions of these suffer severe consequences of the disease, 120 million are symptomatic and the rest are usually asymptomatic (Capron *et al.*, 2002, WHO 2016a). Estimates in 2014 showed that at least 258 million people required preventive treatment for Schistosomiasis. Ninety percent of those live in Africa (WHO 2016b). In Africa, Schistosomiasis is endemic in most rural settlement aligning coastal bodies. This is attributed to suitable environmental condition that favours proliferation of the intermediate host (planorbid snails); including river, creek, ponds, lakes and slow flowing water. This water is typically used for domestic activities and indiscriminate sewage disposal (Nwosu *et al.*, 2015). In Africa where the greatest prevalence of infection occurs, it has been estimated that 150,000 people die each year due to Schistosomiasis-related causes (WHO 2011). In Nigeria, one of the most severely affected countries in Africa, it is estimated that 101.28 million people are at risk of infection while 25.83 million are infected. A recent review by Angaye (2016) showed that Schistosomiasis occurs in all the states of the federation.

Schistosomiasis leads to a chronic and debilitating ill health (Enersen 2015, Uneke *et al.*, 2010). The estimates for morbidity in affected populations are high especially among school children between the ages of 6-15 years; children who swim and play in nearby lakes and irrigation channels, women who carryout household chores like fetching water, washing clothes and cooking utensils, fishermen and irrigation workers who always make contact with water for leisure, recreation or as a result of their profession expose themselves to the shedding freely swimming infective larvae of the parasite from the intermediate snail hosts (Okoli and Odaibo 1999, WHO 2010). School age children who live in areas with water bodies that harbour the vectors are most susceptible to the diseases (Okpala 2010, FMOH 2015). Urogenital Schistosomiasis is caused by *Schistosoma haematobium* and whereas intestinal Schistosomiasis is due to infection by *S. mansoni, S. japonicum, S. intercalatum* and S. *mekongi* (WHO 2016b).

Schistosomiasis is a major public health problem in Nigeria but has received little attention. Reasons for the significant burden of the disease includes: poverty, poor environmental sanitation and water supply and poor access to health care facilities. These communities are also hard to reach and therefore underserved by policies and programs aimed at controlling Schistosomiasis and other NTDs (Hotez *et al.*, 2007). There has been no sustained decrease in the prevalence of disease as the prevention, control and treatment programs in Nigeria have been suboptimal (WHO 2010). In 2013, more than 60 million people required treatment for Schistosomiasis, however, treatment programs were available for only a

few of the affected population (CIA 2015). The Carter Center along with other development and implementation partners work with the Federal Ministry of Health (FMOH) in Nigeria to treat school age children in priority states for the disease; however these control programmes are yet to have a noticeable effect on the prevalence of Schistosomiasis therefore raising doubts if control can be achieved (SU 2015). Human treatment with PZQ plays a central role in the control and prevention of Schistosomiasis, being the only effective drug currently available (Joseph *et al.,* 2010). However, the drug does not prevent re-infection and its exclusive use for the prevention and control of Schistosomiasis is problematic; having been used for more than three decades, the emergence of PZQ-resistant schistosomiasis a constant threat (Tebeje *et al.,* 2016). Other drawbacks of PZQ are its poor activity against immature schistosomes, resulting in sub-optimal outcomes during mass drug administration campaigns and, as its mechanism of action remains unclear, the design of alternative drug formulations has proven difficult (Cioli *et al.,* 2014). The commonly used molluscicide 'Niclosamide' is effective but to be able to achieve best results; the application has to be done at least twice a year. This is not affordable by the local communities (Oketch *et al.,* 1998). According to WHO (1965), Niclosamide is highly toxic to snails and eggs but difficult to formulate thereby requiring expertise before use. This compound is harmful to non-target organisms like the fish, non-biodegradable, not eco-friendly and there is probability that some resistance to niclosamide can be induced under extreme conditions of genetic selection of the snails (Sulivan *et al.,* 1984). In order to control and finally eliminate Schistosomiasis, a vaccine will likely be a key component of an integrated approach (*i.e.* involving mass chemotherapy, targeted molluscicides, environmental modification, health education, and improved sanitation and vaccination (Tebeje *et al.,* 2016). A transmission blocking vaccine for use in bovines could serve as a vital component in the control of *S. japonicum* (Ross *et al.,* 2013), whereas clinical vaccines against *S. mansoni* and *S. haematobium* need to be developed.

This review evaluates the prevalence of Schistosomiasis among school age children and the current status of schistosome vaccine development. Although complexity of the schistosome life-cycle with its various stages each expressing distinct antigens development, it provides a vehicle for identifying many alternative candidate molecules for vaccine development. The fact that different parasite stages reside in different host niches (larvae in the skin and lungs, adults in the liver and intestine or bladder capillaries) can help in designing potential vaccines to prevent the migration of schistosome parasites and their maturation to adult worms. Importantly,

the fact that schistosomes do not replicate in the definitive host makes partial reduction of the parasite burden sufficient to control Schistosomiasis, strengthening the argument for developing an effective vaccine as a control intervention (Hewitson *et al.,* 2014). When identifying a suitable vaccine candidate, it is prudent to select key schistosome molecules in the live parasite that are (a) exposed to the host immune system and (b) are essential for parasite survival. Such components may, for example, function in migration, immune evasion, nutrient uptake or attachment (Tebeje *et al.,* 2016). Adjuvant selection and mode of vaccine formulation and delivery are other important considerations in vaccine design and deployment as they can have a considerable impact on the protective effectiveness of the vaccine. It is well known that, in contrast to attenuated cercarial vaccines, other types, such as subunit vaccines, require an appropriate adjuvant to help stimulating the immune system. A number of adjuvants are available such as gels, emulsions, particulates, cytokines, microbial products (*e.g.* CpG, cholera toxin) and proteases. Adjuvants can overcome immune senescence in older individuals, prolong the immunological memory of a vaccine broaden the antibody repertoire and direct the immune system to a Th1 biased, Th2 biased or mixed Th1/Th2 responses (Knudsen *et al.,* 2016). For example, a Th1 driving adjuvant such as IL-12, administered with irradiated cercariae, provided up to 90% protection in murine Schistosomiasis (Lebens *et al.,* 2004, Stephenson *et al.,* 2014). Currently, in order to increase the protective efficacy of schistosome vaccines, a strategy of combining existing adjuvants with novel ones, developed based on emerging immunological targets, has been muted (Stephenson *et al.,* 2014). Some of the other challenges in Schistosomiasis vaccine development are the risk of an atopic IgE response to a candidate vaccine (Diemert *et al.,* 2012), a lack in understanding the nature of the immune response and the correlates of protective immunity in humans and other mammalian hosts, the transmission of other pathogens in Schistosomiasis endemic areas resulting in co-infected individuals which can impact on vaccine efficacy, and antigenic polymorphism (Mo *et al.,* 2014). Vaccine development or Vaccinomics is another powerful innovation which provides a foundation for searching critical determinants of immunity and can promote antigen discovery and the design of novel vaccines for complex pathogens such as the schistosomes. A recent vaccinomics approach for discovering novel schistosome antigens that may not be revealed by conventional proteomics involved the design and manufacture of an immunomics protein microarray, the first to be generated for a multi-cellular pathogen. This vaccine could be administered to children between the ages of 3 and 12 years to prevent

severe infection in a particularly high risk population. This review summarizes the current status of Schistosomiasis vaccine development.

2. PREVALENCE OF SCHISTOSOMIASIS AMONG SCHOOL AGE CHILDREN

Uchendu *et al.* (2017) conducted a study aimed towards describing the burden of Schistosomiasis and demographic characteristics among children of migrant workers residing in a rehabilitation home in Ibadan, Nigeria. A cross-sectional study was done using sixty six children and an interviewer-administered questionnaire was used to collect information on demographic and environmental characteristics of the children. The study revealed that the mean age of respondents was 11.8 ± 4.0 years and 57.6% were males. The prevalence of Schistosomiasis was 19.7% with preponderance among males (64.3%) and children aged 12 years and above (71.4%); 85.7% of infected children were from Kwara State; 78.6% waded in water body and 92.9% had red blood cells and pus cells on urine microscopy. The study concluded that the burden of Schistosomiasis was high among school age children of migrant workers and they serve as reservoirs for disease transmission. Also, in a study conducted by Birma *et al.* (2017), Kiri was selected for a comprehensive investigation. The objectives of the study were to determine the prevalence of urinary Schistosomiasis in different communities and the intensity of infection in terms of egg count/10 ml of urine, prevalence among gender and age groups and to relate infection with parent's occupation. A study on the prevalence of urinary Schistosomiasis was conducted in four villages around Kiri Lake in Shelleng Local Government Area, Adamawa State, Nigeria. Two hundred and thirty two urine samples were collected from four randomly selected primary schools from within the four study communities. Overall, prevalence of urinary Schistosomiasis was 48% (111/232), with males recording 49% (69/142) and females 47% (42/90). The total mean egg count (MEC) was 8.3. There was no significant difference in prevalence between males and females ($P > 0.05$). Prevalence was higher among age groups, with the 13–15 year old age group having the highest 62.96% (17/27) and the 4–6 year old age group had the lowest 37% (19/52). There was no statistically significant difference in prevalence among different age groups ($P > 0.05$). Infection was also high among children of fishermen 59.09% (13/22), followed by farmers' children 56.25% (45/80) and the least prevalence was among children of teachers 20% (3/15). Old Banjiram had the highest level of infection 91% (21/23), while Kwadadai had the lowest 36.8% (21/57) ($P < 0.05$). Old Banjiram and children in the 10–12 years age group had the highest MEC of 10.6 and 9.4 respectively. The study revealed a high prevalence of urinary Schistosomiasis among school age children.

3. *SCHISTOSOMA MANSONI* AND *S. HAEMATOBIUM* VACCINE CANDIDATES

Over 100 schistosome vaccine antigens have been identified, of which about a quarter have shown some level of protection in the murine models (Siddiqui *et al.,* 2011). Disappointingly, however, only three molecules, the *S. mansoni* fatty acid binding protein (Sm14), the *S. Mansoni* tetraspanin (Sm-TSP-2) and the *S. haematobium* glutathione S-transferase (Sh28GST), have entered human clinical trials with Smp80 (calpain) undergoing testing in non-human primates (Merrifield *et al.,* 2016). A recent report has suggested that the murine model of Schistosomiasis may be intrinsically flawed for pre-clinical testing of vaccine candidates as a result of the fragility of the pulmonary capillaries in mice which can prevent maturation of a large proportion of schistosome schistosomula upon challenge; which may lead to an incorrect assumption that vaccine antigen-induced acquired protective immunity has been generated (Wilson *et al.,* 2016).

3.1 *S. mansoni* (Sm14)

Schistosomes lack an oxygen-dependent pathway for the synthesis of sterols and fatty acids. Therefore, they are entirely dependent on the mammalian host to provide these essential lipids. Schistosomes use fatty acid binding proteins to absorb, transport and compartmentalize fatty acids from the host and, because of this critical biological function the Sm14 has long been considered a potential vaccine candidate (Tendler *et al.,* 2008). Recombinant Sm14 (rSm14) provided up to 67% protection in terms of reduced *S. mansoni* worm burden in out bred Swiss mice without the use of an adjuvant, and encouragingly, no autoimmune response was observed even though its structure is identical in basic form with the mammalian host homologues (Tendler *et al.,* 1996). It has been shown to be cross-species protective against both *S. mansoni* and *Fasciola hepatica* infection. Development of a dual vaccine effective against both fluke infections has great appeal in terms of human and animal health. The rSm14 with glucopyranosyl lipid adjuvant stable emulsion (GLA-SE) entered and successfully completed a Phase-1 clinical trial in healthy adult volunteers in Brazil, confirming its status as safe and immunogenic vaccine candidate (Santini-Oliveira *et al.,* 2016).

3.2 *Schistosome* 28 kDa *Glutathione S-Transferase*

Schistosome 28 kDa glutathione S-transferase enzymes (28GST) plays a role both in fatty acid metabolism and prostaglandin D2 synthesis and may help the parasite to evade the host immune system (Tebeje *et al.,* 2016). The enzyme present in *S. mansoni* (Sm28GST) has been tested extensively as a recombinant protein vaccine in various experimental models and has shown partial protection in terms of reduction of worm burden, inhibition of female worm fecundity and reduction of egg viability (Capron *et al.,* 2001). Along with Sm14, Sm28GST was one of the six *S. mansoni* antigens originally independently tested under the auspices of TDR/WHO (Bergquist *et al.,* 1998). The *S. haematobium* homologue, Sh28GST (Bilhvax), formulated with alum adjuvant, has undergone human clinical trials (Santini-Oliveira *et al.,* 2016, Bourke *et al.,* 2014). Cytokine production triggered by this vaccine candidate was shown to be influenced by factors such as host age, schistosome infection status and PZQ treatment history, therefore these features should be considered for determining efficacy of the GST-based vaccine during its testing in endemic communities (Bourke *et al.,* 2014). The Phase-1 and 2 clinical trials showed Bilhvax to be safe for both healthy and infected adults and children (Mo *et al.,* 2014). It was scheduled to complete a Phase-3 self-contained, randomized, double blind clinical trial in 2012 evaluating whether co-administration of the vaccine with PZQ could delay pathology due to *S. haematobium* infection in children. The trial results, however, have yet to be released which has raised doubts about the vaccine efficacy (Tebeje *et al.,* 2016).

3.3 *S. mansoni* Calpain (Sm-p80)

Calpain is calcium activated neutral cysteine protease (McManus *et al.,* 2008). Prime-boost vaccination (priming with DNA and boosting with recombinant protein) with Sm-p80, the large subunit of *S. mansoni* calpain, in combination with resiquimod adjuvant, resulted in 49% worm burden reduction, while 50% protection was achieved using the recombinant protein as primary and boost vaccine in mice (Ahmad *et al.,* 2010). With the same approach, but using a different adjuvant, oligo deoxy nucleotide (ODN), 70% worm burden reduction and 75% egg reduction was achieved with the Sm-p80 (Ahmad *et al.,* 2009). Moreover, a 58% worm burden reduction in baboons (*Papio anubis*) was reported recently with the Sm-p80-based vaccine adjuvanted with resiquimod and CpG ODN (Ahmad *et al.,* 2011). Using a different approach, a Sm-p80

DNA vaccine conferred 59% worm burden reduction and 84% decrease in egg production in mice (Ahmad *et al.,* 2009). In baboons the vaccine provided levels of protection against *S. mansoni* infection comparable to those achieved by the irradiated cercarial vaccine; moreover, antibodies and IFN-γ were shown to play an important role in the protective immunity generated in this non-human primate model (Zhang *et al.,* 2010, Ahmad *et al.,* 2009). Importantly, the recombinant Sm-p80 has also been shown to exhibit cross-species protection against *S. haematobium* in both hamsters and baboons (Karmakar *et al.,* 2014). Promisingly, enduring antibody titres were detected in mice at sixty weeks post-vaccination with recombinant Sm-p80, and Sm-p80-specific-IgG for was detected in baboons 5–8 years after initial vaccination with the Sm-80 DNA vaccine (Zhang *et al.,* 2014). It is anticipated that the recombinant Sm-p80/GLA-SE vaccine, "SchistoShield", will move forward to Phase-1 and 2 human clinical trials in 2017 (Karmakar *et al.,* 2014, Zhang *et al.,* 2014). Furthermore, it has also been shown that Sm-p80 has a therapeutic effect in vaccinated baboons through decreasing the numbers of established worms, reducing the retention of eggs in tissues, and decreasing the number of eggs excreted in faeces (Karmakar *et al.,* 2014).

3.4 *S. mansoni* Tetraspanins (Sm-TSP-2)

The tetraspanins are a group of proteins that are highly abundant in the schistosome tegument where they are found at the outer-most membrane of the intra-mammalian stage of the parasite, hence are highly exposed to the host immune system (Braschi *et al.,* 2006). The major *S. mansoni* tetraspanin are Sm-TSP-1 and Sm-TSP-2 with the latter conferring protection in *S. mansoni* challenge animal models and also correlating with protective immunity in naturally resistant people (Loukas *et al.,* 2007).

3.5 *S. mansoni* 29 (Sm29)

Sm29 is present in the tegument of adult worms and schistosomula and in its recombinant form it induces high level production of both the IgG1 and IgG3 isotypes among individuals resistant to infection and re-infection (Cardoso *et al.,* 2006). There are reports that recombinant Sm29 can prevent infection in animals previously exposed to *S. mansoni.* For example, 26–48% protection was observed in BALB/c mice that were previously infected with a Brazilian strain of *S. mansoni* and treated with PZQ (Alves *et al.,* 2015). Recently an increased level of protection was obtained through combining Sm29 with Sm14 in the presence of poly

I:C adjuvant; 40.3, 68.2 and 57.9% reductions in adult worm burden, liver egg burden and intestinal eggs, respectively, whereas achieved, along with a reduction in granuloma size and number in livers of immunized mice (Ewaisha *et al.,* 2014). Another report showed that by fusing Sm29 and Sm14, a 48.4% reduction in worm burden was achieved in mice (Mossallam *et al.,* 2015). In a further advance, when the recombinant Sm29 was subjected to high hydrostatic pressure which dissociated the aggregated protein resulting in a successfully folded, soluble, stable and structured molecule, it was protective against *S. mansoni*, thereby paving the way for its industrial production down track (Chura-Chambi *et al.,* 2013).

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5.1 *S. japonicum* (Sj97)

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5.2 *S. japonicum* 26 kDa GST *plasmid* DNA Vaccine (Sj26GST)

A *S. japonicum* 26 kDa GST plasmid DNA vaccine (Sj26GST) resulted in a significant reduction in worm numbers, hepatic and faecal eggs in vaccinated mice (Wei *et al.,* 2008). When the DNA vaccine was given in combination with interleukin 18 (IL-18), a potent IFN-γ inducing factor, the protective efficacy was improved. DNA vaccines have some advantages over other types of immunization but they have some limitations concerning the gene delivery system. A recent study reported on a novel nanoparticle formulation of the Sj26GST DNA vaccine; although there was no significant reduction in worm burden, a highly significant decline in tissue egg burden and fecundity of female worms were recorded (Mbanefo *et al.,* 2015). Toll-like receptor (TLR) 7/8 ligands (*e.g.* R848) and TLR 9 ligands (*e.g.* CpG oligodeoxynucleotides, or CpG) as adjuvants can increase vaccine effectiveness through activating the innate immune system and ultimately activating and directing the adaptive immune system. Such adjuvants have been shown to potentiate the activity of the Sj26GST DNA vaccine in mice by increasing splenocyte proliferation, elevating IgG,

IgG2a, IFNγ and TNFα levels, and preventing Treg-mediated immune-suppression (Wang *et al.,* 2013). In another development, Sj26GST alone, or in combination with the *S. japonicum* fatty acid binding protein, expressed in recombinant pseudorabies virus (PRV) Bartha-K61, induced significant levels of specific immunity and protection in mice and, importantly, sheep, emphasising the potential effectiveness of this live vector for vaccination against *S. japonica* in animal reservoirs (Wei *et al.,* 2010).

5.3 *S. japonicum* Triose-Phosphate Isomerase

The glycolytic pathway enzyme triose-phosphate isomerase (TPI) is expressed in all stages of the schistosome life-cycle and represents another targeted vaccine candidate for *S. japonica*. An early study showed that a *S. japonicum* (Chinese strain) TPI (SjCTPI) plasmid DNA vaccine (with or without an IL-12 DNA plasmid) protected pigs against challenge infection. Synergistic enhancement of immunogenicity and protection in mice against *S. japonicum* challenge was achieved with codon optimization and electroporation delivery of the SjTPI DNA (Zhu *et al.,* 2006), showing a similar level of protection as a replication-defective recombinant optimized SjTPI adenoviral vaccine (Dai *et al.,* 2014) which was enhanced using a heterologous prime-boost strategy (Dai *et al.,* 2015). A study, conducted in Chinese water buffalo with a DNA vaccine encoding SjCTPI alone or fused with bovine HSP-70 with booster immunizations co-administered using a plasmid encoding IL-12, resulted in a significant reduction in worm numbers, liver and faecal eggs and in faecal miracidial hatching (Da'dara *et al.,* 2008). SjCTPI, delivered by a heterologous "prime-boost" regimen, has been used to vaccinate bovines in China as part of a multi-component integrated control package (Gray *et al.,* 2014).

5.4 *S. japonicum* Insulin Receptors

S. japonicum possess two types of insulin receptors (SjIRs) which, on binding to mammalian host insulin, can activate the parasite's insulin pathway, which is pivotal for glucose uptake, growth, and maturation (You *et al.,* 2010). Recombinant ligands of both *S. japonicum* insulin receptor 1 and 2 (SjLD1, SjLD2), tested in vaccine/challenge trials in mice resulted in significant reductions in faecal eggs output, mature intestinal eggs and stunting of adult worms (You *et al.,* 2012, You *et al.,* 2015). The retardation in growth of the worms likely resulted from reduced glucose uptake (You *et al.,* 2012). Furthermore, knockdown of the SjIRs using

RNA interference (RNAi) resulted in their reduced expression coupled with a reduction in the transcription level of downstream genes within the insulin pathway that are associated with glucose metabolism and schistosome fecundity (You *et al.,* 2015), thereby reinforcing their vaccine potential.

5.5 *S. japonicum* (Sj14)

In early studies, Sj14 (fatty acid binding protein; SjFABP), the *S. japonicum* homologue of Sm14, generated no or only a limited level of protection (McManus *et al.,* 2008), but when given to mice as a DNA vaccine with a plasmid coding for IL-18 as adjuvant, the level of protection was increased substantially (Wei *et al.,* 2009). The latter study also showed that SjFABP + IL-18 increased the Th1 immune response by inducing a higher level of IFNγ and a lower level of IL-4 compared with mice vaccinated only with SjFABP alone (Wei *et al.,* 2009). Somewhat disappointingly, the Sj14 DNA vaccine, coupled with the Sj26GST a bivalent DNA vaccine, resulted in reduction in the level of protective compared to that obtained by the monovalent DNA immunization (Tu *et al.,* 2014).

5.6 *S. japonicum* (Sj23)

Sj23, like Sm-TSP-2, a member of the tetraspanin family, is a 23-kDa surface-exposed integral membrane protein expressed in all parasite stages. It was shown in BALB/c mice to elicit a rapid humoral immune response dominated by IgG2a antibodies, but not IgG1, and did not provide protection against cercarial challenge after priming with recombinant Semliki forest virus particles followed by a boost with the recombinant Sj23 (Jiang *et al.,* 2010). A subsequent report of mice vaccinated with purified recombinant protein LHD Sj23-GST (large hydrophilic domain of Sj23 fused with Sj26GST) in combination with one of three adjuvants (Freund's adjuvant, FA), Montanide ISA 206 or Montanide ISA 70 M), and parasite challenged, resulted in high-level production of LHDSj23-GST-specific IgG1, IgG2a and IgG3 antibodies and significant reductions in worm burden (Zhu *et al.,* 2012). In order to further improve the level of protection, a multivalent DNA vaccine comprising Sj23, glyceraldedyde-3 phosphate dehydrogenase (SjGAPDH), SjFABP and Sj26 was tested in mice which resulted in very high levels of protective efficacy in terms of reduction in worm burden (70.8%) and liver eggs (60.7%) (Zhu *et al.,* 2011). Another study in mice, using three cocktail DNA vaccines encoding Sj23, SjCTPI and NP30, boosted by electroporation *in-vivo* and a protein

vaccine boost to this regimen, resulted in 60% reduction in worm numbers and more than 60% reduction in the liver egg burden (Dai *et al.,* 2009).

6. NEW ANTIGEN DISCOVERY: A WAY FORWARD

New antigen discovery has been aided by major recent advances in schistosome genomics, transcriptomics and post-genomic technologies (Ricciardi *et al.,* 2015). Proteomics is another important and now widely used tool that can identify potential vaccine targets with focus on protein constituents of different schistosome sources such as host-parasite interface comprising tegument or gut. Studies on the tegument have used a number of procedures including biotin-labelling of live parasites and subsequent isolation and characterisation of the biotinylated proteins using tandem mass spectrometry (MS/MS) to identify surface-located proteins, and therefore those accessible to host antibodies. Proteomics of the schistosomegut and its contents has shed new light on the functionality of this important region of the parasite (Driguez *et al.,* 2016).

Coupled with other approaches such as metabolomics, interrogation of the schistosome proteome, particularly the surface, provides a mechanism to identify important clinically-relevant proteins and those having potential as new vaccine targets (De Sousa *et al.,* 2016). A recent vaccinomics approach for discovering novel schistosome antigens that may not be revealed by conventional proteomics involved the design and manufacture of an immunomics protein microarray, the first to be generated for a multi-cellular pathogen. Mostly surface-derived proteins (215 in total) from *S. japonicum* and *S. mansoni* were selected and they were produced using a rapid *in-vitro* translation system, and then printed as a vaccine discovery tool (Driguez *et al.,* 2010 and 2016, Mc William *et al.,* 2012). The reactivity of microarray proteins can be measured with anti-sera from human patients or Schistosomiasis-resistant/exposed animals using a labelled secondary antibody and a laser microarray scanner; highly reactive proteins are then assessed as putative vaccines. One application of the array used antibodies from acutely- or chronically-infected Chinese individuals with early/advanced *S. japonicum*, and subjects exposed, but stool negative for *S. japonicum* eggs, for screening. This resulted in identification of 25 immuno dominant antigens, including a number of vaccine candidates, transporters, tetraspanin-related proteins, and unannotated proteins (Driguez *et al.,* 2016). The array has also been screened for IgG subclassess and IgE responses, using sera from a human Brazilian cohort of putatively resistant (PR) and chronically *S. mansoni*-infected (CI) individuals

stratified by worm intensity levels (high, medium, low), determined by faecal egg counts, so as to identify antibody signatures reflective of protective *vs* non-protective immune responses (Gaze *et al.,* 2014). Probing for IgE responses allowed identification of antigens that might induce potentially deleterious hypersensitivity reactions if used as subunit vaccines in endemic populations so it was encouraging that the PR individuals did not mount an intense IgE response to these antigens compared with CI subjects. Using this immunomics-based approach to Schistosomiasis vaccine antigen discovery was further validated by identification of targets of protective IgG1 immune response in PZQ-induced resistant subjects exposed to *S. haematobium*; uncharacterized proteins and a number of recognised vaccine antigens (*e.g.* glucose transporters, tetraspanins, glutathione-S-transferases, calpain) were identified (Pearson *et al.,* 2015). The same report described use of sera from *Rhesus macaques* experimentally rendered resistant to *S. japonicum* infection to screen for antigen targets, and discovery of new and known vaccine candidates, including many recognized by the human subjects. Another important application has been the immune screening of the schistosome microarray with antibody secreting cell (ASC)-probes (Driguez *et al.,* 2010 and 2016, McWilliam *et al.,* 2012), generated from lymph nodes draining the sites of larval *S. Japonicum* migration (McWilliam *et al.,* 2013). This technique is especially advantageous for recognizing antigens with low immunogenicity (selective pressure may have an influence on important protective epitopes which evolve over time with low immunogenicity) or those only temporarily exposed to the immune response (McWilliam *et al.,* 2012). In one study, ASC probes (from skin and lung) and sera from semi-permissive rats and from susceptible mice were used to screen the array after infection and re-infection with *S. japonicum* (Driguez *et al.,* 2016). A total of 29 antigens, including a number of recognised vaccine candidates and several *S. japonicum* homologues of human Schistosomiasis resistance markers—the tegument allergen-like proteins—were differentially recognized by infected hosts from which eight proteins were prioritized as putative novel schistosome vaccine candidates and diagnostic antigens (Driguez *et al.,* 2016). In a related study, the protein microarray, screened with ASC probes generated from *S. Japonicum* infected rats, resulted in the identification of a novel antigen, termed *S. japonicum* Ly-6-like protein 1 (Sj-L6L-1) which shares structural and sequence features with the Ly-6 protein family and has several other features suggesting it is a promising vaccine candidate against developing larvae *S. japonicum* larvae (McWilliam *et al.,* 2014).

7. CONCLUSIONS

Schistosomiasis remains a substantial public health problem due to the very high levels of morbidity it causes in many parts of the world especially in Nigeria. School age children who live in areas with water bodies that harbour the snail intermediate host are most susceptible to the diseases and they serve as reservoirs for transmission of the disease. Currently, the treatment is entirely dependent on PZQ chemotherapy. As exclusive use of one drug may lead to emergence of drug resistant strains, development and deployment of a vaccine as part of an integrated approach for prevention and control of Schistosomiasisis is to be encouraged. Much of our current understanding of immunity and immune mechanisms against Schistosomiasis rely on studies conducted on mice, but vaccines based on studies performed only in mouse model could have undesirable effects if taken prematurely to human clinical trials. The recent concern raised about using mice for determining efficacy of vaccine candidates further reinforces the argument that additional critical examination of any identified candidate vaccine antigen, whether or not it has foundation in acquired immunity is essential, and that moving to studies using larger models such as rabbits, pigs or bovines in the case of *S. japonicum*, or non-human primates for *S. mansoni* and *S. haematobium,* is clearly necessary. Similarly, protection levels of many candidate vaccines show improvement after modification of antigen formulation and upon using improved delivery systems. Combining different genes or antigens can also result in higher levels of vaccine-induced protection with some exception. Targeting key biological functions of schistosomes such as tegumental integrity, fecundity, and nutrient uptake using RNAi represent key potential sites identify vaccine candidates (Gobert *et al.,* 2014). Although in its infancy, CRISPR technology may provide a novel approach identifying specific protein-encoding schistosomegenes for vaccine candidate discovery (Cai *et al.,* 2016). Schistosomiasis vaccine development has proven highly challenging and costly and new funding mechanisms are required to promote generation of anti-schistosome vaccines pipeline, similar to that in place for many other infectious diseases, and to progress the existing promising candidates into clinical trials. It is becoming apparent that mass drug administration alone will not eliminate Schistosomiasis and that a vaccine will likely be a key component of an integrated approach of any future Schistosomiasis control intervention toolbox.

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Effects of N

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ABSTRACT: *The aim of this stu
responses of natural plants, vitamins
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search was carried out on PubMed
Rosmarinus officinalis, vitamins, a
sativa and Pulicaria crispa which
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which were reported as anticancer
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products can be acted as immunomod
as therapeutics to evaluate the efficac

Keywords: Immunomodulatory,
Propolis, and Camel Milk.