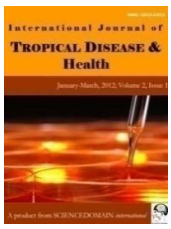

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Schistosomiasis: The Disease, Anti-*Schistosoma* Vaccine Candidates and Baboons as Ideal Models in Schistosomiasis Studies

C. A. Omedo Robin[1*]

[1]*Department of Medical Laboratory Sciences, Masinde Muliro University of Science and Technology, Box 190-50100, Kakamega, Kenya.*

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Schistosomiasis is still a major health hazard in developing countries. Acute form, Katayama's syndrome occurs weeks after the initial infection and manifestations include fever, cough, abdominal pain, diarrhea and hepatosplenomegaly. Chronic infection may cause granulomatous reactions and fibrosis in the affected organs. These reactions may result in: colonic polyposis with bloody diarrhea; portal hypertension with haematemesis and splenomegaly; cystitis and ureteritis with hematuria, which can progress to bladder cancer; pulmonary hypertension; glomerulonephritis; and CNS lesions. Cerebral granulomatous disease may be caused by ectopic *S. japonicum* eggs in the brain, and granulomatous lesions around ectopic eggs in the spinal cord from *S. mansoni* and *S. haematobium* infections may result in a transverse myelitis with flaccid paraplegia. At present the effective control method is chemotherapy with use of praziquantel as the drug of choice. But with the possibility of drug resistance and re-infection, the focus is on development of vaccine[s] that can reduce the incidence of the disease. Different *Schistosoma* antigens capable of inducing some

**Corresponding author: Email: omedoasaba@mmust.ac.ke, omedoasaba@yahoo.com;*

protection in experimental animals have been identified. The potential of these vaccine candidates need to be validated fully in humans. As a starting point, these antigens need to be tested in an animal model that is biologically and immunologically closely related to humans. Baboons may be the ideal models for this purpose as prelude for human clinical trials. This article reviews the disease schistosomiasis; anti-*Schistosoma* vaccine candidates; and also looks at baboons as ideal models in schistosomiasis studies.

Keywords: Antigens; baboons; control; model; pathology; praziquentel; protection; resistance; schistosomiasis; snails; vaccine; vector.

1. INTRODUCTION

Schistosomiasis is a parasitic disease caused by species of blood flukes of the genus *Schistosoma.* It affects human beings, domestic livestock and wild mammals [1]. The disease is a major health hazard in tropical countries. It is estimated to affect between 200 and 300 million people, particularly children who may acquire the disease by swimming or playing in infected water [2]. Majority of those infected are in sub-Saharan Africa, where it is a poverty related health problem and a major cause of morbidity [3].

Although it has a low mortality rate, it is often a chronic illness that can damage internal organs, and in children impair growth and cognitive development. 4.5 million disability-adjusted life years (DALYs) are lost on account of schistosomiasis world-wide [2], and an estimated 280,000 people die from this disease in sub-Saharan Africa each year [4]. Schistosomiasis may also be impacting the etiology and transmission of HIV/AIDS, TB and malaria, and vice versa [5,6]. In particular, the interaction between schistosomiasis and HIV/AIDS is of great concern, given the role of immune responses in both diseases and the geographic overlap in distribution; low $CD4^+$ T-cell counts resulting from HIV infection may increase susceptibility to schistosome infection and influence egg excretion [7].

Schistosoma haematobium, *S. mansoni* and *S. Japonicum* are the three important species that affect humans [8]. *S. haematobium* causes urinary schistosomiasis and occurs in Africa, Asia and rarely in Europe [9]. *S. mansoni* occurs in Africa, South and Central America, while *S. Japonicum* is restricted to the Far East counties, both cause intestinal schistosomiasis [2]. Other species, such as *S. intercalatum* and *S. mekongi* also affect humans, but to a lesser extent. *S. bovis, S. curassoni, S. margrebowiei, S. matheei* and *S. rhodhiani* infect animals; rarely infect human beings [10].

Schistosomiasis is endemic in 74 countries globally, and in Africa it is endemic in 46 out of 54 countries [11]. In Africa, more than 50% of morbidity cases associated with *S. mansoni* are in Tanzania, DR Congo, Nigeria and Kenya. Sudan and Somalia have very low endemicity for *S. mansoni*. *S. haematobium* infections are low in Ethiopia, Uganda, Madagascar, Burundi, Rwanda, Equatorial Guinea and Eritrea [4]. In Kenya, where about 1.5 Million people are affected, the species are: *S. haematobium* and *S. Mansoni* [4]. *S. haematobium* is found in the coastal counties, eastern and western Kenya [12], but *S. mansoni* is mainly found in Central and Western regions of Kenya [4].

The establishment of irrigation and other water resource development projects, increase in population, resettlement/migration, and other diseases in Africa have increased transmission, and also made it possible for schistosomiasis to spread to areas where it was not common [7,11,13]. The Diama dam on the Senegal River introduced *S. mansoni* to Mauritania and Senegal [7]. Egypt's Aswan Dam helped to eliminate *S. haematobium* from the Nile Delta but aided in establishing of *S. mansoni* in Upper Egypt. *S. mansoni* was introduced into Somalia and Djibouti due to population disarticulation [7]. Schistosomiasis and other water related diseases may become more acute as a result of the growing human population, and the ensuing demands on energy and food that will lead to expanded and intensified exploitation of water resources in Africa [11].

1.1 Diagnosis of Schistosomiasis

Microscopic identification of eggs in stool or urine is the most practical and standard method for diagnosis [14]. Stool examination is performed when infection with *S. mansoni* or *S. japonicum* is suspected and urine examined when *S. haematobium* is suspected. Eggs are identified by size and shape [7]. Eggs can be present in the stool in infections with all *Schistosoma*

species. The tests may be negative during the migration phase proceeding egg excretion [9]. Since eggs may be passed intermittently or in small amounts, their detection can be enhanced by repeated examinations and/ or concentration procedures [15]. Tissue biopsies (rectal biopsy for all species and urinary bladder biopsy for *S. haematobium)* may demonstrate eggs when stool and urine examinations are negative. Immunological methods used to detect schistosomiasis antibodies include: the indirect immunofluorescence test (IFT), indirect haemagglutination test (IHAT) and enzyme-linked immunosorbent assay (ELISA). ELISA is the commonly used test for the serological detection of *Schistosoma* infection, particularly in the diagnosis of egg-negative cases [9].

Molecular assays offer high sensitivity that is important when the parasitic load is low as observed in low transmission setting. These assays are useful in identifying species, determining the origin of the parasite by comparing the results with the international gene sequence databases, and assess unique or multiple introductions in a transmission focus. Real-time PCR is an alternative method for quantifying parasitic load, and 28S rRNA gene based nested PCR assay has been used to define *Schistosoma* species in migrants and international travellers [16].

1.2 The Morphology and Life Cycle of the Parasites

The adult males are creamish in colour, broader and bear a gynaecophoric canal. The females of the three species are darker, slender and relatively longer than the males, measuring 10-20mm long by 0.6mm wide [17]. The eggs of *S. mansoni* and *S. japonicum* are laid in the mesenteric blood vessels from where they penetrate into lumen of the intestine of the hosts and are passed out with faeces [18]. *S. haematobium* eggs are passed out in urine, rarely in faeces because they are laid in the walls of the urinary bladder [8]. Humans get infected after contact with freshwater that has free-swimming cercariae. The exposure to infected water is connected to agricultural, fishing, domestic and recreational activities [9].

Eggs passed in fresh water hatch into miracidia, under condition of light and warmth [19]. A miracidium swims actively until it encounters an appropriate snail or dies within 48 hours. Penetration into the vector snail is through the soft parts of the body. Once inside the snail, miracidia multiply asexually to yield the cercariae, which are shed from the snail between 4-6 weeks after infection depending on light and temperature (> 20-25°C) [9,17]. The optimum temperature for infection of the snails ranges from 20-30°C, which explains the tropical and subtropical distribution of schistosomiasis. Cercarial shedding occurs mainly during the warmest hours of the day, and one snail can shed about 1000-2000 cercariae daily, but the number decreases with time. The cercariae in fresh water can swim for about 72 hours, and penetrate the skin when they come into contact with the host [9]. The penetration process takes 5-15 minutes and cercariae lose their tails and become schistosomula [20].

The schistosomula enter the peripheral lymphatic or venous vessels and move to the lungs via the heart, appearing in the lungs 4-7 days after penetration. From the lungs they move to the portal vessels via the heart and mature into adult worms [2]. The adults, male and female then pair up, with the female lying in the gynaecophoric canal of the male. The worm pairs then migrate to their ultimate vascular beds, specifically, superior mesenteric veins (*S. mansoni*), inferior mesenteric and superior hemorrhoidal veins (*S. japonicum)*, or the vesical plexus and veins draining the ureters (*S. haematobium)* where the females begin to lay eggs. The life cycle continues when eggs come into contact with fresh water and hatch into miracidia [21,22]. Fig. 1 details the life cycles of three species of schistosome.

1.3 Host Preference and Tissue Localization of *Schistosoma*

Schistosoma mansoni affect humans and non-human primates [23]. The parasites may also develop to maturity in a number of experimental animals, referred to as permissive hosts, such as mice, hamsters and Rhesus monkeys. Rats are rarely infected [24,25]. The hosts for *S. japonicum* are humans and other mammals. Humans are the only reservoir of *S. haematobium* [9].

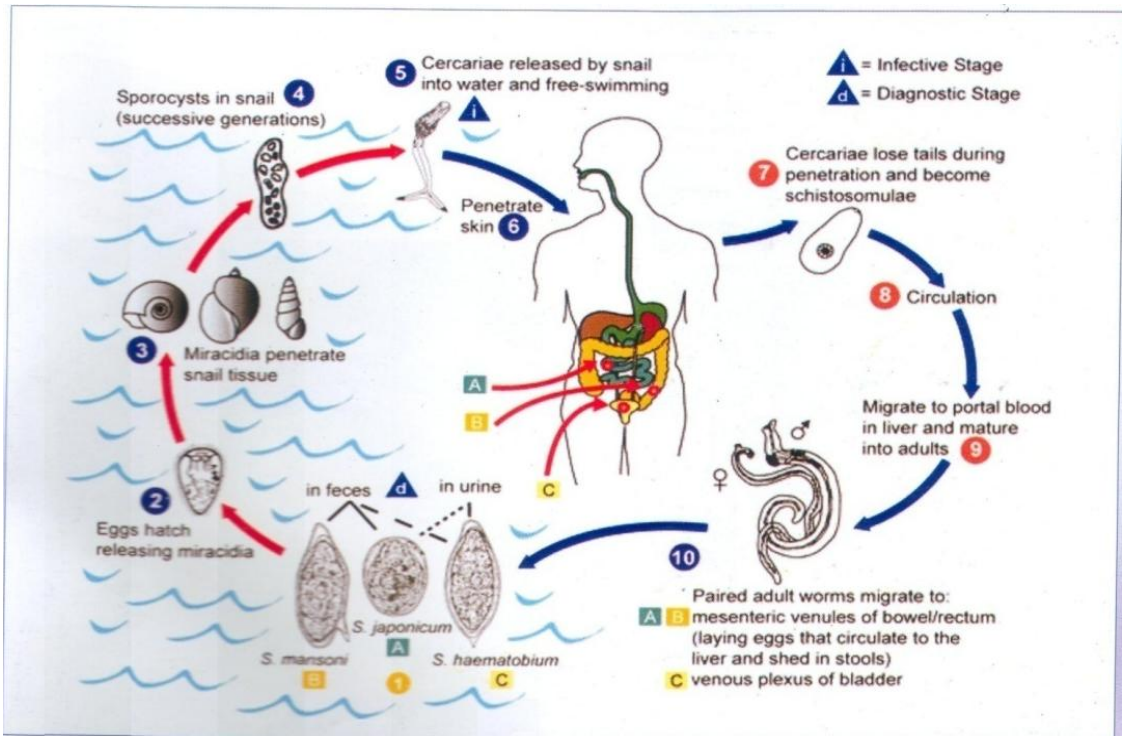

Fig. 1. The life cycle of *Schistosoma* parasites: adapted from Center for Disease Control. Laboratory Identification of Parasites of Public concern, 2nd edition, 2003

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Various snails act as intermediate hosts for different species of *Schistosoma* [4]. The geographical distribution of snails, therefore influence the endemicity of the parasite. *S. mansoni* is transmitted by species of the genus *Biomphalaria*. *S. haematobium* and *S. intercalatum* are transmitted by *Bulinus* species. In East Africa; *Biomphalaria pfeifferi* is the vector for *S*. Mansoni [21], and *Bulinus africanus and B. trancatus* complex are the vectors for *S. haematobium* [9]. *S. japonicum* is transmitted by *Oncomelania* species and *S. mekongi* by *Neotricula* species [7]. Snails live in well aerated water with vegetations. The vegetation serves as food and also supply leaf surface for egg deposition. Snails can survive at higher temperatures, but they thrive well at an optimum temperature range of 22-26°C. Their population decreases during the rainy season and increases in the drier and warmer months of the year. However, where water temperatures are more stable, some snail population, show no seasonal trends. But droughts and floods reduce their populations [6].

In tropical lakes the extraordinary growth of certain species of floating plants, particularly water hyacinth, water fern and water lettuce provide rich support for multiplication of vector snails, especially *Bulinus* and *Biomphalaria* species [2]. Snail dispersal along water-courses is also assisted by floating islets of vegetation. The spread of water hyacinth is having a severe economic impact on Eastern African lakes. Submerged vegetation, of which *Ceratophyllum demersum, Polygonum senegalense* and *Utricularia inflexa* are examples, may support large snail colonies, especially when the aquatic plants are growing vigorously. As it sometimes occurred in Lake Nasser, bottom algae can support a snail population that ensures the transmission of schistosomiasis [2].

2. PATHOLOGY

The pathological changes due to acute and chronic schistosomiasis in humans, non-human primates and mice are well documented [26]. Baboons infected with *S. mansoni* develop similar signs of acute human schistosomiasis [27]. The immune responses that develop following infection often proceed to cause pathological changes that are the primary cause of the illness [28]. The pathology is divided into three phases: the invasion, acute and chronic phase of the disease. The invasion phase is the actual penetration of the host by the cercariae and the following 2-7 days during which the cercariae migrate from the skin to the lungs [8]. This phase is marked by a moderate to intense skin reactions, "swimmer's itch" at the site of penetration depending on individual´s sensitivity to the parasites. Individuals in endemic areas show little or no reaction, while visitors develop marked skin rashes and urticaria [29].

The acute phase starts 6-12 weeks after infection, and coincides with peak egg deposition and the characteristic pathological manifestation in this phase, in moderate and heavy infections is an acute febrile reaction, referred to as the "Katayama syndrome" [29]. The symptoms includes: fever, fatigue, urticaria, cough and asthma-like symptoms. The eggs produced induce an inflammatory reaction in the liver and wall of the colon and rectum, with formation of granulomas where giant and epitheliod cells infiltrate around the egg and are held together by an extracellular matrix [30]. The intestine may have serosal nodular lesions called "sandy patches" or in severe cases polyps leading to pseudo-tumours or bilharziomas [26]. Mesenteric lymph nodes are remarkable enlarged [29]. Histologically, the acute granuloma is seen to consist of a centrally placed *Schistosoma* ovum surrounded by concentric layers of inflammatory cells, predominantly eosinophils [31]. Lymphocytes, macrophages, plasma cells and neutrophils are also present in the granulomas.

The chronic phase which is a sequel to modulation of the granulomatous inflammation, is characterised by reduction in granuloma size and diminished cellular infiltration particularly eosinophils. This stage is largely sub-clinical, but it contributes to long term debility associated with chronic schistosomiasis [32]. In some cases of schistosomiasis, the granulomas may fail to modulate resulting in the hepatosplenic disease [33]. This is the main cause of schistosomiasis related mortality. The reaction to the eggs in the liver may eventually cause the peri-portal fibrotic reaction known as "Symmers clay pipe stem fibrosis" which may lead to formation of varicose around the rectum, stomach and oesophagus [34,35]. In other cases the eggs can be lodged in the brain, spinal cord, lungs and the kidneys. Neurological schistosomiasis is due to eggs being trapped in the brain or spinal cord [36], and renal schistosomiasis manifest as nephropathy or glomerulonephritis [35]. Death due to *S. mansoni* infection is as a result of haematemesis, portal hypertension and occasionally upper gastro-intestinal haemorrhages [37]. Figs. 2A and 2B show the histopathology in the liver and colon of a baboon due to *S. mansoni* eggs.

Schistosoma haematobium in endemic areas affects mostly children of 5-15 years old. The skin penetration may also cause skin eruption and pruritus. The parasites migrate through blood to reach the venous plexuses and mature to the adult stage. During the first month of infection, a non-immune individual can present with general immune-allergic reactions during migration phase and early egg deposition [9]. Only a small proportion of cases will progressively develop chronic symptoms, which are more likely in people with heavy infections and recurrent exposure as seen in endemic settings. The infection can remain pauci-symptomatic for a long period but still cause progressive damage to the urogenital tract. Haematuria and dysuria are the main symptoms of urogenital schistosomiasis, and early complications are infections of the upper and lower urogental tract and lithiasis [9].

The chronic stage of the infection corresponds to urogenital complications as the female worm releases eggs which migrate through the wall of the bladder and the urogenital system. Some of the eggs are retained in the wall of the urogenital tract and they provoke a granulomatous inflammatory response, which is the main cause of pathology in the human host. While medical complications are progressive, microscopic haematuria is an early sign that should be investigated for individuals with known exposure to the parasite. Chronic infections are characterized by long term complications such as obstructive and fibrous lesions of the urogenital tract, chronic cystitis, calcification in the bladder and renal impairment. *S. haematobium* is considered a risk factor for cancer of the urinary bladder in endemic settings [9].

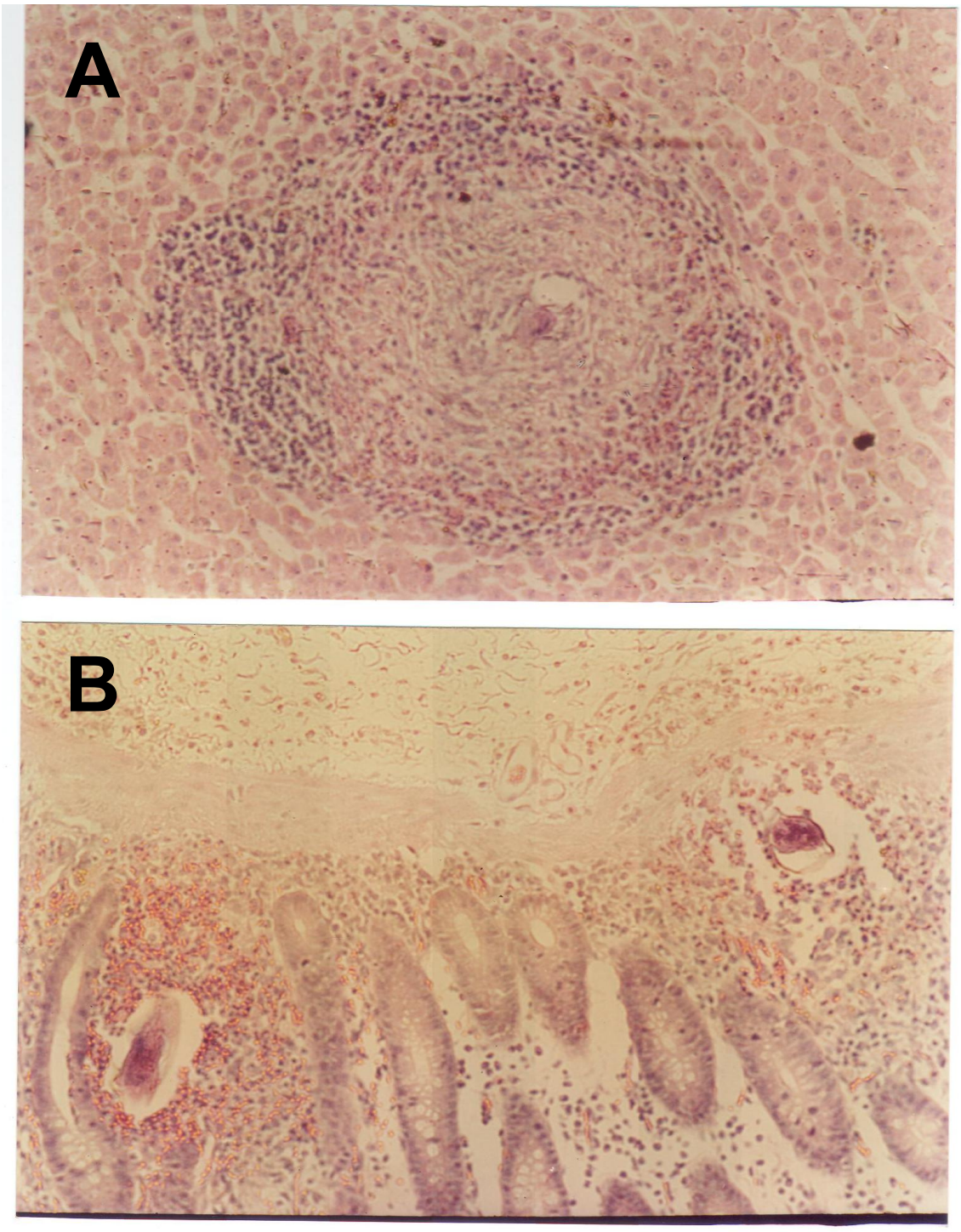

Fig. 2. A. Section of baboon liver showing egg-granuloma formed around remnant of the ovum in the center surrounded by mononuclear inflammatory cells and eosinophils, neutrophils and histiocytes. B: Section of baboon colon showing infiltration of inflammatory cells and presence of *S. mansoni* ova

2.1 Immune Responses to *Schistosoma* Infection

All stages of *Schistosoma* induce a very pronounced immunological response, with the more predominant being directed against the eggs [38]. The adult worms are largely unaffected due to development of evasive mechanisms which enable the worms to survive in the hostile environment of the host [37]. These evasive mechanisms of the adult parasite include induction of anti-inflammatory molecules [39], incapacitation of lymphoid cell function [40], coating of the host's surface antigens [41] and production of anti-oxidant enzymes among many other mechanisms [42].

Schistosomula are the most susceptible to immune attack both in-vitro and in experimental murine models. The surface of a schistosomulum has glycoprotein antigens that share carbohydrate epitopes with large polysaccharide antigens in the parasite egg. These antigens induce IgE and IgG dependent cellular cytotoxicity reactions involving eosinophils, macrophages, platelets and complement mediated cellular cytotoxicity [43]. Infection with *S. mansoni* has been shown to elicit both the cellular and humoral arms of the immune response. Cellular immune response to *S. mansoni* infection in baboons is largely of mixed Th1\Th2 phenotype, similar to the situation in the human hosts [44]. Studies to determine the role of antibodies in the immune response in baboons have demonstrated IgM as the predominant antibody in the primary response, while the secondary response is characterised by production of other classes specifically IgG and IgE [45].

Cytokine levels peak during acute *S. mansoni* infection, but decline with chronic infection and become almost undetectable after treatment [44]. Re-infection after treatment induces two to three fold increases in soluble egg antigen (SEA) specific interleukin-4(IL-4), IL-5, IL-10, IL-2 and transforming growth factor β (TGF-β) production. SEA-induced IFN-γ production does not increase with re-infection after treatment, but induced TGF-β production remains elevated as the infection become chronic and correlates with diminished hepatic granuloma size, implying its participation in the down-modulation [44]. SEA in baboons also induces lymphocytes proliferation during the acute phase of the disease [44].

3. CONTROL OF SCHISTOSOMIASIS

The main approaches that have been applied in an attempt to control schistosomiasis include: control of snail vectors, chemotherapy, improved sanitation, prevention of water contact and health education [1]. Molluscicides such as niclosamide have been widely used to control snails. However, this approach has several limitations. First, the chemicals are expensive and their application must be undertaken frequently. Some molluscicides are harmful to the handlers. Molluscicides also kill some of non-target beneficial molluscs and other organisms such as fish. These disadvantages have largely limited the use of molluscicides [9]. This is even important in areas where irrigation water is used for fishing, livestock, domestic purposes and even drinking [11,46].

Birds and large snails such as *Marisa carmuarietus* and *Tarebia granifera* that feed on other snails have been used to control the vector snails [17]. Other methods of control involve proper disposal of faecal and urinary waste in order to avoid contamination of the environment with schistosome ova [11]. The building of latrines would if practised ensure that this is achieved. But in practice, not every homestead in endemic areas has a latrine and in some cases the local population has cultural beliefs that are opposed to the use of latrines [11].

Chemotherapy has been the only successful method in controlling schistosomiasis. The drugs currently available for treatment of schistosomiasis are praziquantel, metrifonate, oxamnaquine and artemisinins [47]. Praziquantel is the drug of choice and all species are susceptible to it [22]. The efficacy of oxamnaquine and praziquantel is comparable, with a slightly better efficacy for praziquantel. Resistance to oxamniquine has however been observed in Brazil [48]. Even though schistosomiasis control can be approached with praziquantel alone, the possibility of developing resistance to this drug must be taken into account. Studies have shown that some schistosomes possess genes that carry a lesser susceptibility to praziquantel. Reduced cure rates were observed in Senegal due to very high re-infection rates with a relatively high number of developing immature parasite not susceptible to praziquantel [22,49]. *S. mansoni* isolates that tolerate high doses of praziquantel have also been characterized from Egyptians [50]. The other hiccup is that the drug is still out of reach

for many people in most endemic areas. Mass treatment does not also prevent re-infection [28,47]. Re-infection occurs rapidly in exposed populations in most areas of endemicity such that within a period of 6 to 8 months following chemotherapy, the prevalence returns to its baseline level. Due to these facts, it is clear that the most feasible long-term solution to schistosomiasis control is a protective vaccine [1].

3.1 Anti-Schistosome Vaccine

A reduction in worm numbers is the "gold standard" for anti-schistosome vaccine development, but as eggs are responsible for both pathology and transmission, a vaccine targeted on parasite fecundity and egg viability is also entirely relevant [51]. The effective vaccine should prevent the initial infection and reduce egg granuloma associated pathology [52]. Significant efforts have been made to develop a protective vaccine against schistosome infections, and several vaccine candidates have been identified [51,53–56], but as the efficacy of any of these against schistosomiasis remains uncertain, the identification and characterization of novel anti-schistosome vaccine molecules remains a priority [53]. The development of vaccine remains an important move in the control of schistosomiasis [57].

Vaccination with irradiated cercariae has consistently produced high levels of protection in experimental animals, but delivery problems, the need for a standardised product and safety considerations rule out this approach for use in human beings [28]. However, it appears feasible to develop them for veterinary application. Nevertheless, many *Schistosoma* antigens continue to be tested as possible vaccines. These vaccine candidates may not be the final answer to controlling schistosomiasis, but may provide measurable protection and since morbidity rather than sterile immunity is the target, only partially protective vaccine is required [58]. In view of the fact that the pathology in schistosomiasis is directly correlated to the number of *Schistosoma* eggs in the host, a vaccine can achieve its effect by offsetting parasite entry and development, and also by interfering with the production and delivery of eggs [1,58].

In this review, we look at some of the major vaccine antigens for schistosomiasis and their protective efficacies. Those that were independently tested under the umbrella of the TDR/WHO committee in the mid-1990s that have been the focus of attention for many years are also discussed briefly in this review. A number of potentially promising vaccine antigens from *S. mansoni* and, to a lesser extent, *S. haematobium*, have been discovered and published, but only a few, namely, BILHVAX, or the 28-kDa GST from *S. haematobium and* Sm14 a member of Fatty acid binding protein (FABP) family from *S. mansoni* have entered clinical trials (Sh28 GST at Phase III in Senegal http://clinicaltrials.gov/show/NCT00870649, and Sm14 at Phase I, sponsored by Oswaldo Cruz Foundation- Brazil).

3.2 Vaccine Candidates for *Schistosoma mansoni*

In mid 1990s under the coordination of UNDP/World Bank/ WHO special program for Research and Training in Tropical Diseases (TDR/WHO committee), six vaccine antigens from *S. mansoni*; Glutathione-*S*-transferase 28 (Sm 28-GST), paramyosin, Ir-V5, Triose phosphate isomerase (TPI), Sm 23 and Sm 14, were independently tested [59]. They yielded protective responses, but the stated goal of consistent induction of 40% or more protection was not achieved with any of tested antigens. The failure may be due to; a negative influence of insufficient antigen stability and the need to use standardized and effective adjuvant formulations or due to the fact that these antigens are not exposed onto the parasite surface, except Sm23 which is exposed on the apical membrane surface of the parasite, though not an abundant apical membrane protein on the parasite surface [60]. These antigens are not exposed to the host immune system and therefore do not induce the required protective responses in the host [61]. The failure to develop an efficacious schistosome vaccine can also be attributed in part to the complex immunoevasive strategies used by schistosomes to avoid elimination from their intravascular environment [1]. Nonetheless, the following arguments are in support of possibilities of developing effective vaccines against the various schistosome species [59]: 1) as mentioned above, irradiated cercariae regularly induce high levels of protection in experimental animals, and additional immunizations boost this protection further, 2) humans in endemic areas develop various degrees of resistance, both naturally and drug-induced, and 3) antihelminth recombinant vaccines against cestode platyhelminths have

been developed successfully and applied in veterinary practice [62]. Due to these arguments, a large number of schistosome antigens has been unearthed (utilizing the complete genome sequence), and additional candidates are now being found through proteomic and immunomic approaches.

3.3 Antigens from *S. mansoni*

3.3.1 Tetraspanins

These are four-transmembrane-domain proteins found on the surfaces of eukaryotic cells, including B and T cells. They have two extracellular loops; a short loop 1 of 17 to 22 amino acid residues (EC-1) with little tertiary structure and a larger, 70- to 90-amino acid residues loop 2 (EC-2), which has four or six cysteine residues that form two or three disulfide bonds. Although their functions are unknown, it is now apparent from proteomic studies that a family of tetraspanins is expressed in the schistosome tegument [61], and at least three of these show promise as vaccines. Sm23 is a tetraspanin expressed in the tegument of *S. mansoni* and is one of the independently tested WHO/TDR vaccine candidates [58]. Sm23 has been shown to be the most effective when given as a DNA vaccine [58,63], but not effective as a recombinant protein when formulated with alum. It induces 40-50% protection in mice [64]. Two *S. mansoni* tetraspanins SmTSP-1 and SmTSP-2 have also been discovered [65]. Both proteins are expressed in the tegument membrane of *S. mansoni* [66]. TSP-2 was identified as one of a subset of proteins that were biotinylated on the surfaces of live worms and subsequently identified using tandem mass spectrometry [61]. TSP-2 is produced high levels of protection as a recombinant vaccine in the mouse model of schistosomiasis, and both proteins were strongly recognized by IgG1 and IgG3 (but not IgE) from putative resistant (PR) individuals but not from chronically infected or unexposed individuals [66]. Both proteins induce good levels of protection (> 50% reductions in liver egg burdens) in immunized mice given an *S. mansoni* challenge [67], noted that TSP-2 provided protection in excess of 40% benchmark set by WHO for schistosome vaccine antigens to proceed to clinical trails. Another membrane-spanning protein, Sm29, has also been assessed as a vaccine candidate. Like TSP-2, Sm29 is preferentially recognized by antibodies from naturally resistant individuals compared with chronically infected individuals [66], although the extent of selectivity is not as great as that reported for TSP-2. Studies in mice have shown that recombinant Sm29 is more effective [68]. Other apical membrane proteins from the tegument that warrant attention as vaccines include the structural membrane proteins with large extracellular regions, such as annexin and dysferlin, and other proteins that are accessible to antibodies but with no homologues of known function, such as Sm200 [61]. Murine DNA vaccination with gene encoding Sm200 induced 38.1% protection [69].

3.3.2 SmTPI

The glycolytic pathway enzyme triose-phosphate isomerase (TPI) is found in the cells of each stage of the schistosome life cycle, and the *S. mansoni* enzyme (SmTPI) has long been targeted as a schistosomiasis vaccine candidate [59].

3.3.3 Sm28/Sh28Glutathione-*S*-Transferase, GST

Sm28-GST is an enzyme expressed in subtegumental tissues of most developmental stages of the parasite [70]. GSTs are thought to have detoxification role in the parasite. Vaccination of rats and hamsters with recombinant Sm28-GST resulted in significant reductions of worms [71]. Trials in Primates demonstrated antifecundity effect [72], and an anti-Sm28 monoclonal antibody showed antifecundity and anti-egg embryonation effects [73]. Clinical testing of Sh28-GST in people showed that vaccine was immunogenic and induced antibodies capable of neutralizing the enzymatic activity of the recombinant protein [74] and BILHVAX, or the 28-kDa GST from *S. haematobium* has entered Phase III clinical trial in Senegal (http://clinicaltrials.gov/show/NCT00870649. Institut National de la Santé Et de la Recherche Médicale, France).

3.3.4 Sm-p80 Calpain

This is a calcium-activated neutral cysteine protease. The calpain large subunit was first discovered from *S. mansoni* by immunoscreening of a lambda phage cDNA library with sera from infected humans [75]. Calpain is localized to the tegument surface and underlying musculature in adults and in the penetration glands and secretions of cercariae of both *S. mansoni* and *S. japonicum* and is

involved in surface membrane turnover [76] and associated with the inner tegument membrane [61]. Calpain has been shown to be the target of a protective $CD4^+$ T-cell clone that induce peritoneal macrophages from syngeneic recipients to kill schistosomula in vitro [77]. In addition, mouse recipients of this T-cell clone displayed significant resistance against cercarial challenge, making calpain the first vaccine antigen identified on the basis of T-cell reactivity.

A semi-purified Sm-p80 calpain protein induced 29 to 39% reductions in worm burdens [78]. Subsequent efforts to improve the efficacy of this vaccine have focused on DNA vaccine constructs in mice and baboons [79]. Sm-p80 antigen is a promising vaccine target because of its immunogenicity, protective efficacy and anti-fecundity effects observed in both experimental murine and non-human primate models of schistosomiasis [80]. In VR1020, a vector approved for human use, an Sm-p80 based DNA vaccine formulation conferred 46% (reduction in worm) and 28% (decrease in egg) in an olive baboon (*Papio anubis)* model. The vaccine elicited robust immune responses to specific antigen Sm-p80 in vaccinated animals. When stimulated in vitro with recombinant Sm-p80, peripheral blood mononuclear cells and sphenocytes from baboons vaccinated with Sm-p80-VR1020 produced higher levels of Th1 response enhancer cytokines (IL-2 and IFN-γ) than IL-4 and IL-10 which are Th2 response enhancing cytokines. A mixed Th1/Th2 type of T-cell and humoral responses was generated after immunization with Sm-p80VR1020 [80]. Prophylactic and antifecundity efficacies of Sm-p80 have also been tested using a variety of vaccine approaches in both rodent and nonhuman primate models. Kamarkar 2014 [81], evaluated the therapeutic efficacy of Sm-p80 using two different strategies and three Sm-p80-based vaccine formulations in baboons. Vaccine formulations were able to eliminate 10-36% of established adult worms; reduced egg retention in tissues (10-57%) and decreased the egg excretion in feces (13-33%). The ability of Sm-p80 to provide cross-species protection against *S. haematobium* challenge has also been evaluated in hamster and baboon models. In hamsters vaccinated with recombinant Sm-p80 admixed with glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE), a reduction in worm burden (48%) and in tissue egg load (64%) was observed. But, in baboons, the Sm-p80/GLA-SE vaccine produced a 25% reduction in *S. haematobium* adult worms and decreased the egg load in the urinary bladder by 64%. The egg output reduction in feces and urine was 40% and 53%, respectively, in vaccinated baboons. A balanced pro-inflammatory [Th17 and Th1] and Th2 type of response was generated after vaccination and appears indicative of augmented prophylactic efficacy. These data on cross-species protection coupled with the prophylactic, therapeutic and antifecundity efficacy against the homologous parasite, *S. mansoni*, reinforces Sm-p80 as a promising vaccine candidate. It is currently being prepared for GMP-compliant manufacture and for further pre-clinical development leading to human clinical trials. These results, it is highly expected that Sm-p80 vaccine may provide relief for both intestinal and urinary schistosomiasis and thus beneficial in reducing the overall burden of schistosomiasis [81].

3.3.5 Paramyosin (Sm 97)

Paramyosin is a 97-kDa myofibrillar protein with a coiled-coil structure, expressed on the surface tegument of lung-stage schistosomula in the penetration glands of cercaria and may function as a receptor for Fc [41]. The vaccine efficacy of paramyosin against *S. mansoni* was first described in the 1980s; mice immunized intradermally with *S. mansoni* extracts and *Mycobacterium bovis* BCG adjuvant were significantly protected against subsequent infection, and antibodies predominantly recognized paramyosin [82]. Vaccination of mice with native and recombinant paramyosin was then shown to provide modest (26 to 33%) but significant protection against challenge infection with *S. mansoni* [83].

3.3.6 Sm 22.6

This is also a tegumental protein. With its homologue in *S. japonicum* (Sj22.6), are involved in resistance to re-infection in endemic areas [57]. Immunization of mice with recombinant 22.6 formulated with Freund adjuvant resulted in 34.5% reduction in worm burdens, but Sm 22.6 formulated with alum failed to induce any protection against schistosomiasis but ilicited a regulatory response that was able to modulate allergic asthma in mice [69].

3.3.7 SmFABPs

The *S. mansoni* fatty acid binding protein (FABP), Sm14, is a cytosolic protein expressed in the basal lamella of the tegument and the gut

epithelium [84]. Sm14 has been assessed thoroughly as a recombinant protein vaccine and, to a lesser extent, as a DNA vaccine. Despite a high efficacy of recombinant Sm14 protein in mouse vaccine trials [85], Sm14 failed to induce protection levels of >40% when tested in other laboratories [86] and as part of the WHO/TDR-sponsored trials. Co-administration of recombinant Sm14 protein with either IL-12 [86] or tetanus toxin fragment C boosted protection. Immunization of mice with recombinant Sm14 expressed in *Mycobacterium bovis* BCG showed no induction of specific antibodies to Sm14, but splenocytes from vaccinated mice produced IFN-γ upon stimulation with recombinant Sm14. Moreover, mice that were vaccinated once with Sm14-BCG and then challenged with *S. mansoni* cercariae showed a 48% reduction in worm burden, which was comparable to that obtained by immunization with three doses of recombinant Sm14 protein [87]. Sm14 has entered human clinical trial (Phase I) in Brazil (sponsored by Oswaldo Cruz Foundation- Brazil).

3.3.8 Sm SOD, Sm GPX and Sm FILAMIN

Several studies of immunity in schistosomiasis, both in experimental models and human beings have shown that the larval stage is the most susceptible and the target for immune elimination [88,89]. The schistosomula elimination involves a cellular response that is potentiated by cytokines and/ or antibodies [89,90]. Once the parasites mature to adult worms and reach the portal circulation, they are able to evade the engendered immune response of the host through the evolution of several defence mechanisms [88,89,91]. The host cells involved in cytotoxic response against the schistosomula produce reactive oxygen species such as superoxide anion and hydroxyl radicals capable of attacking the parasite apical membrane and initiating a lipid peroxidation that set off a chain reaction that results in the death of the parasite [42]. The adult worms are able to evade immune elimination by expressing anti-oxidant enzymes Cu/Zn superoxide dismutase (SOD), Glutathione peroxidase (GPX) among other mechanisms [92,93]. Hong et al. [94] and Mei and LoVerde, [93] were able to demonstrate that the expression of these anti-oxidant enzymes are developmentally regulated such that the lowest gene expression and enzyme specific activity are in the larval stage and highest in adult worm. The anti-oxidant enzymes protect an organism from reactive oxygen species derived damage [95]. These enzymes have been immuno-localized to the tegument and gut epithelia of adult worms [93].

To provide direct evidence that antioxidant enzymes are important in immune evasion and thus viable candidate vaccines, DNA vaccination strategies have been used to evaluate the efficacy of DNA constructs containing genes encoding *S. mansoni* Cu/Zn cytosolic superoxide dismutase (Sm-CT-SOD), Signal peptide-containing *S. mansoni SOD (*Sm-SP-SOD) and *S. mansoni* glutathione-S-peroxidase (Sm-GPX) in *S. mansoni* infection murine models. After use of different doses of plasmid cDNA constructs containing genes that encode these enzymes, mice exhibited a significant level of worm burden reduction when challenged with *S. mansoni* cercariae, with Sm CT-SOD showing 54%, and Sm-GPX 43.4% protection [62]. In addition to the anti-oxidant enzymes, Sm-filamin a structural protein has been identified as a vaccine candidate that also consistently induces significant levels of protection in murine model of schistosomiasis. The targets of protective response due to filamin vaccination seem to be the larval and adult stages of the schistosome parasite [89].

To demonstrate whether the protection observed in mice can be replicated in baboons as a prelude to human vaccine trails, the author and others tested these three vaccine candidates (SmCT-SOD, Sm GPX and Sm-filamin, all as DNA vaccines) in olive baboons [*Papio anubis*]. These vaccines induced production of specific IgG against worm antigens, SEA and SWAP. CT-SOD and GPX showed slight anti-fecundity effect, but the reduction in the worm burden that had been reported in murine model was not observed in baboons vaccinated with these vaccines. These vaccines also did not alter the phenotype of the hepato-intestinal egg-associated pathology of *S. mansoni* infection in vaccinated baboons (no antipathology effect) (un-published report).

3.4 Vaccine Candidates for *Schistosoma japonicum*

The leading *S. japonicum* vaccine candidates (as recombinant protein and/or DNA vaccines) are membrane proteins, muscle components, or enzymes. This review looks at some of the vaccine candidates for *S. japonicum* that have been identified recently, giving a brief mention of their characteristics and efficacies.

3.5 Antigens from *S. japonicum*

3.5.1 Sj26GST

The GSTs are a group of enzyme isoforms that catalyze the detoxification of lipophilic molecules by thio-conjugation. Some encouraging data are available for the protective efficacy of Sj28GST against *S. japonicum* in different mammalian hosts in China [52,96]. But recent work has focused on the 26-kDa isoform. Recombinant Sj26GST (rSj26GST) induces a pronounced anti-fecundity effect as well as a moderate but significant level of protection in terms of reduced worm burdens in mice, sheep, cattle, and pigs following challenge infection with *S. japonicum*. Similar levels of vaccine efficacy were obtained in water buffaloes vaccinated with purified rSj26GST [96].

3.5.2 Paramyosin (Sj97)

Native and recombinant paramyosin (Sj97) proteins confer protection against *S. japonicum* in mice, water buffaloes, and other mammalian hosts [96]. Furthermore, recent studies of human antibody isotype [97] and Th2 cytokine responses to Sj97 add further support to this molecule as a leading vaccine candidate against *S. japonicum* [98].

3.5.3 SVLBP

An *S. japonicum* very-low-density lipoprotein binding protein (SVLBP; molecular size, 20 kDa) is a membrane associated and located in the teguments and subteguments of adult male schistosomes [99]. In a study that used affinity-purified recombinant SVLBP (rSVLBP) to vaccinate mice, the worm numbers and egg numbers recovered from the veins and livers of the immunized mice reduced by 33.5% and 47.6% respectively [100]. The antibody response in vaccinated mice was greatly increased: the titers of IgG1, IgG2a, and IgG2b of the vaccinated group were significantly higher than those of the controls [100].

3.5.4 SjTPI

Results of Chinese SjTPI (SjCTPI) plasmid DNA in mice have been good [101]. Therefore, Zhu *et al*., 2004 [102] examined the transmission-blocking potential in larger animals by determining its vaccine efficacy in naive pigs. Pigs vaccinated with SjCTPI DNA had adult worm burdens reduced. The reduction was more on worm burdens, i.e., 53.6% for SjCTPI alone and 59.6% for SjCTPI plus IL-12. Vaccination with SjCTPI DNA reduced liver egg numbers by 49.4%, and this response was significantly enhanced by the addition of IL-12 (65.8% reduction in liver eggs). In addition to the dramatic protective effects seen in vaccinated pigs, it was also noted that granuloma size was reduced 42% in both groups. Coimmunization with a DNA plasmid of SjCTPI fused to heat shock protein 70 (SjCTPI-Hsp70) and IL-12 DNA induced protective immunity against experimental *S. japonicum* infection in water buffaloes [103].

3.5.5 Sj23

Sj 23 is a tetraspanin integral membrane protein. The Chinese *S. japonicum* form (SjC23) has been shown to induce protection in mice as a synthetic peptide vaccine and as a plasmid DNA vaccine. Also induces protection in sheep, pigs, and water buffaloes. Vaccination with SjC23 DNA induces significant reductions in worm, egg burdens and the size of egg granulomas; thus, like SjCTPI, SjC23 produces an antipathology effect as well. The protective effect of the SjC23 plasmid DNA vaccine was enhanced with IL-12 in pigs [102] and mice [100,102] and by a CpG immunostimulatory sequence in mice [104]. As with the other candidate vaccines, extensive large animal field trials are now required to determine the precise protective potency of SjC23, with or without IL-12 or CpG.

3.5.6 Sj14

This is one of FABPs that are critical for schistosomes to take up fatty acids from host blood as essential nutrients and are thus prime targets for both vaccination and drug development. The 14-kDa FABP of Chinese *S. japonicum* (SjFABPc) has at least eight different variants encoded by a single-copy polymorphic gene, and it is particularly important to *S. japonicum* for uptake, transport, and compartmentalization of host-derived fatty acids, playing a vital role in the physiology and survival of the parasite. Several Chinese groups have obtained encouraging protection in mice by using SjFABPc, both as a recombinant protein and as a plasmid DNA vaccine.

3.5.7 Calpain

Immunization of BALB/c mice with purified recombinant *S. japonicum* calpain emulsified in

complete Freund's adjuvant resulted in significant reductions in the number of recovered worms and also in egg production per female worm. In addition, raised levels of inducible nitric oxide synthase expression were observed in immunized mice, while adhesion of peritoneal exudate cells also occurred in the presence of sera from immunized mice, suggesting the involvement of both cellular and humoral protective mechanisms. In addition, spleen cells from the immunized mice showed enhanced production of IFN-γ by activated $CD4^+$ T cells, and subsequent work with calpain-specific mouse T-cell hybridomas identified the T-cell epitope involved [105]. Localization studies have shown that calpain is present in the penetration glands and in the secretions of cercariae [106].

3.6 DNA Vaccination

The effectiveness of DNA vaccines against viruses, parasites and cancer cells has been demonstrated in animal models [89,107,108]. The DNA immunization induces both antigen-specific cellular and humoral immune response [109–111]. DNA vaccination strategies involve the incorporation of immuno-stimulatory sequences in the backbone of the plasmid, co-expression of stimulatory molecules, utilization of localization/secretory signals and utilization of appropriate delivery system [112]. The vaccines usually consist of plasmid vectors that contain DNA coding for a specific component of a disease-causing organism. The heterologous genes (transgenes) are inserted under control of a eukaryotic promoter, allowing protein expression in the mammalian cells [113]. To optimize the efficacy of DNA vaccines an appropriate choice of plasmid vector should be used. The basic requirements for the backbone of DNA vector are; a eukaryotic promoter, a cloning site, a polyadenylation sequence, a selectable marker and a bacterial origin of replication. With DNA vaccination, the actual production of immunizing proteins takes place in the vaccinated host [108].

DNA vaccination against schistosomiasis [114] has recently been investigated using a panel of plasmids encoding *Schistosoma* antigenic proteins such as Sjc 26 GST, Sj79 [55,115], Sm-CT-SOD and Sm-GPX [62], *S. japonicum* paramyosin [116], Sm 23, 28 GST from *Schistosoma mansoni* [114], and Sm-p80 [80]. Sj14 [117], Sj23 [100,102], Sm200 [69] etc. the list is long.

By the use of techniques such as immunomics, genomics, proteomics etc, new target antigens are being identified, and alternative vaccination strategies to improve vaccine efficacy are also being explored. Extracellular vaccine candidates need to be expressed in bacteria or eukaryotic expression systems. Many of the selected targets are likely to require processing through the endoplasmic reticulum by virtue of their expression sites in the parasite (i.e., secreted or anchored in the tegument), and this may prove challenging. An additional important consideration is that antigen identification and successful protective results are of little value if GMP cannot be applied for scaling up of production of any vaccine candidate [59]. The selection of a suitable adjuvant and delivery system to aid in the stimulation of the appropriate immune response is a critical step in the path to the development and employment of successful anti-schistosome vaccines, and a number of approaches have been tested, with some success.

3.7 Baboon as an Ideal Model in Schistosomiasis Studies

A number of animal species have been used as models to study the basic biology, immunology and pathogenesis of schistosomiasis [118]. The baboons are the most popular non-human primates in schistosomiasis research because of a multiplicity of qualities [119]. Baboons are similar to humans in their anatomy, genetics and immunological responses [120]. They acquire natural infections and are highly susceptible to experimental infections [30]. Baboons develop hepatic and intestinal pathology during the acute phase, modulate this pathology in chronic phase of the disease and acquire protective immunity as do humans [30,44,45,121]. In the wild, baboons show age-dependent prevalence of infection, with high rates of infection in juvenile baboons and young adults, a similar trend observed in humans [122]. Baboons are also abundant especially in East Africa, and are not an endangered species. They adapt readily in captivity and to changes in their environment; can give birth twice in 18 months and live up to 20 years; and can attain body weight of 20 kg [123]. It is easy to monitor the immune and disease progression in many different organs in individual baboons during the course of infection [44].

Baboons acquire immunity on vaccination. For example, immunization with irradiated cercariae

stimulates over 50% protections (worm, egg production and pathology) [17,121] which is associated with elevated serum levels of schistosome-specific IgG and IgE. Some protections to challenge infection following vaccination with recombinant antigens and DNA vaccines have also been reported in baboons [81,124]. The moderate size, possibility of repeated sampling, and ease of perfusion for recovery of adult worms make the baboon a good model for vaccine efficacy studies [123]. Baboons can also sustain repeated schistosome infections without any untoward lesions such as portal-caval shunting [125] and with subsequent development of acquired immunity and increased resistance to re-infection. Such re-infection episodes are characterized by reduced adult worm burden and granulomatous inflammation [44] which, as in humans, are associated with high serum levels of parasite specific IgE [45]. Therefore, for these reasons, vaccine candidates that have been tested in a baboon model and have shown encouraging results, such as those obtained with Sm-p80VR1020 DNA vaccination in baboons should be moved forward through development leading to human clinical trials.

Baboons have a few constraints that may limit their use in research, that include: the high costs involved in trapping and maintaining them in captivity; the data from wild caught baboons may vary due to their heterogeneous genetic background; and immunologic reagents suitable for baboon work are currently lacking [118].

4. CONCLUSION

Schistosomiasis is a chronic disease that is endemic in 74 countries in Africa, Asia and South America. Worldwide, an estimated 200 million people are infected, of which 20 million is assumed to suffer from more or less a severe form of the disease creating 4.5 million DALYs lost. At present the most effective control method is use of Praziquantel. But with the possibility of drug resistance and re-infection, the focus is on development of a vaccine that can significantly reduce the incidence of the disease. Many schistosome antigens that are capable of protecting experimental animals from challenge are being identified. These antigens need to be standardized and effective adjuvant formulation(s) selected. The potential of these vaccine candidates need to be validated fully in humans. As a starting point, these antigens, as native, recombinant or cocktail DNA vaccines need to be tested in an animal model that show the same characteristics of the disease as in humans. As mentioned above, the parasite biology, the pathology, immune response and immunity to *Schistosoma* infection in humans differ significantly from the murine model but correlate better with the disease in baboons. Therefore, baboons may be ideal models for this purpose as prelude for clinical trials. Vaccines that show good results in baboons should then be moved forward to human clinical trials.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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