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A review Article

SCHISTOSOMIASIS: THE DISEASE, ANTI-SCHISTOSOMA VACCINE CANDIDATES AND BABOONS AS IDEAL MODELS IN SCHISTOSOMIASIS STUDIES

ABSTRACT

Schistosomiasis is a parasitic disease caused by blood flukes of the genus *Schistosoma.* Acute schistosomiasis (Katayama's fever) may occur weeks after the initial infection, especially by *S. mansoni* and *S. japonicum*. Manifestations include fever, cough, abdominal pain, diarrhea, hepatosplenomegaly and eosinophilia. Pathology is due to host response to egg antigens of the eggs lodged in tissues: 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. Chronic infection may cause granulomatous reactions and fibrosis in the affected organs, which may result in manifestations that include: colonic polyposis with bloody diarrhea; portal hypertension with haemato-emesis and splenomegaly; cystitis and ureteritis with hematuria, which can progress to bladder cancer; pulmonary hypertension; glomerulonephritis; and CNS lesions. At present the most effective control method is chemotherapy with use of praziquantel as the drug of choice. With the possibility of drug resistance and re-infection, the focus is on development of vaccine(s) that can significantly reduce the incidence of the disease. Many different *Schistosoma* antigens that are capable of protecting experimental animals against schistosomiasis have been identified. The potential of these vaccine candidates need to be validated fully in humans. As a starting point, the antigens need to be tested in an animal model that is biologically and immunologically closely related to humans. The 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.

Key words: Antigens; Baboons; Control; Model; Pathology; Praziquentel; Protection; Resistance; Schistosomiasis; Vaccine

Introduction

Schistosomiasis also known as bilharzia, bilharziosis or snail fever is a parasitic disease caused by several species of fluke of the genus *Schistosoma,* which affects human beings, domestic livestock and wild animals including non-human primates (Pearce, 2003). The disease is a major health hazard in tropical countries, especially in areas with water that is contaminated with freshwater snails which may carry the parasites. It is approximated to affect between 200-300 million people, particularly children who may acquire the disease by swimming or playing in infected water (Anon., 1985). More than 80% of those infected are found in sub-Saharan Africa, where it is a poverty related health problem and a major cause of morbidity (Chitsulo *et al.,* 2000).

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The parasites cause chronic morbidity in large numbers of infected individuals, with severe life threatening consequences in a small percentage of those affected. Although it has a low mortality rate, schistosomiasis often is a chronic illness that can damage internal organs and in children impair growth and cognitive development. Currently it is estimated that 280,000 people die from schistosomiasis in sub-Saharan Africa each year (van der Werf *et al*., 2003). Schistosomiasis is of major socio-economic and public health importance in endemic areas due to its adverse pathology, which ranks second to malaria (Pikes, 1987).

A number of *Schistosoma* species have been reported to affect humans and other mammals (Farah *et al*., 2000), but only three are of significant importance in human schistosomiasis. The three species are *Schistosoma haematobium*, S. *mansoni* and *S. Japonicum* (Cheesbrough, 1985). *S. haematobium* causes urinary schistosomiasis and occurs in Africa, Asia and Europe (Anon., 1993). *S. mansoni* which cause intestinal schistosomiasis occurs in Africa, South and Central America, while *S. Japonicum* the other causative agent of intestinal schistosomiasis is restricted to the Far East counties such as Philippine, China, Thailand, Laos and Cambodia (Anon., 1993). Other species, such as *S. intercalatum* and *S. mekongi* also affect humans, but to a lesser extent. Species such as *S. bovis, S. curassoni, S. margrebowiei, S. matheei* and *S. rhodhiani* infect animals but may also infect human being on rare occasions (Sturrock, 2001).

East African countries have higher incidences of *S. mansoni* infections than West African countries. More than 50% of morbidity /pathology cases associated with *S. mansoni* are found in Tanzania, DR Congo, Nigeria and Kenya. Sudan and Somalia have very low endemic for *S. mansoni*. *Schistosoma haematobium* infections are low in Ethiopia, Uganda and Madagascar (prevalence ≤ 1%). Burundi, Rwanda, Equatorial Guinea and Eritrea have also very low endemic for *S. haematobium* (van der Werf *et al.,* 2003). But building of water sources, increase in population, resettlement/migration, and disease have made it possible for schistosomiasis to spread to areas where it was not common (Gryseels *et al*., 2006; Li *et al.*, 2007). The Diama dam on the Senegal River introduced *S. mansoni* to Mauritania and Senegal (Gryseels *et al*., 2006). 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 (Gryseels *et al*., 2006).

In Kenya, where about 1.5 Million people are affected with schistosomiasis, the species are; *Schistosoma haematobium* and *S. Mansoni* (van der Werf *et al.,* 2003). *S. haematobium* is found in the coastal counties, eastern and western Kenya (King *et al*., 2003), but *S. mansoni* is mainly found in Central and Western regions of Kenya (Highton, 1974).

Diagnosis of schistosomiasis

Microscopic identification of eggs in stool or urine is the most practical and standard method for diagnosis (Feldmeier and Poggensee, 1993). 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 (Gryseels *et al*., 2006). 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 (Anon., 2014). Since eggs may be passed intermittently or in small amounts, their detection can be enhanced by repeated examinations and/ or concentration procedures (Elliott, 1996). The egg out-put can also be quantified by use of Kato-Katz technique or the Ritchie technique. Tissue biopsy (rectal biopsy for all species and urinary bladder biopsy for *S. haematobium*) may demonstrate eggs when stool and urine examinations are negative.

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Several widely used immunological methods are available for schistosomiasis antibody detection. The most important ones are the indirect immunofluorescence test (IFT), the indirect haemagglutination test (IHAT) and the 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 (Anon., 2014).

Molecular assays offer high sensitivity, which is important in the context of low parasitic loads as observed in low transmission setting. These assays can be useful to identify species (species specific DNA ampulification), determine 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. In addition a 28S rRNA gene based nested PCR assay has been used to define *Schistosoma* species in migrants and international travellers (Cnops *et al*., 2012).

The morphology and life cycle of the parasites

The males of *S. mansoni* are creamish in colour, broader, bear a gynaecophoric canal and measure 6-12mm long with a diameter of 1.1mm. The number of testes varies from 4-13 and these are anteriorly placed (Cheesbrough, 1981). The morphology of male *S. haematobium* is similar to that of *S. mansoni* except it is longer. *S. japonicum* has also similar morphology to *S. mansoni,* but like *S. haematobium* it is longer and narrower. The females of the three species are darker, slender and relatively larger than the males, measuring 10-20mm long by 0.6mm wide. The uterus is placed in the anterior half of the female (Yole, 1996).

The eggs of *S. haematobium* have a terminal spine, while those of *S. mansoni* and *S. japonicum* have lateral spine (Cheesbrough, 1981). The eggs of *S. mansoni* and *S. japonicum* are laid by the female adult worms in the mesenteric blood vessels from where they penetrate into lumen of the intestines of the definitive hosts and are passed out with faeces (Cheever and Andrade, 1967). *S. haematobium* eggs are passed out in urine, rarely in faeces because they are laid in the walls of the urinary bladder (Cheesbrough, 1981).

Mature eggs once passed with faeces or urine in fresh water hatch into female and male miracidia, under condition of light and warmth (Kassim and Gilberton, 1976). A miracidium swim actively by means of cilia until it encounters an appropriate snail or die within 48 hours. Penetration into the vector snail is through the soft parts of the body and is achieved by secretions from the anteriorly placed penetration glands of the parasite (Jourdane and Theron, 1987). Once inside the vector, miracidia lose their ciliated epidermal layer and develop into mother sporocysts (Cheesbrough, 1981). The mother sporocysts then become filled with germ balls and burst after 8 days releasing several germ balls. The germ balls migrate to the digestive glands and after a series of multiplications they develop into thin walled daughter sporocysts (Cheesbrough, 1981).

A further process of asexual multiplication takes place and the daughter sporocysts become filled with final larval stage, the cercariae, which emerge from the snail between 4-5 weeks after infection and are influenced by light (Yole *et al*., 1996). One snail can release about 1000-2000 cercariae daily, but these numbers decrease with time. The cercariae measure between 300-400µm in length and have a bi-forked tail (Cheesbrough, 1981). The cercariae once out of the snail may swim for about 12-14 hours, and when they come into contact with wet skin of the host, they penetrate between the hair follicles by means of anterior spines and capopystic

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secretion of the cephalic glands (Jourdane and Theron, 1987). The penetration process takes 5-15 minutes and cercariae lose their tails and become schistosomula (Cheesbrough, 1981).
The schistosomula enter the peripheral lymphatic or veinous vessels and are carried to the lungs via the heart and appear in the lungs 4-7 days after penetration. From the lungs they move to the portal vessels via the heart, where they grow into male and female adult worms (Anon., 2003). The adults, male and female then pair up, and remain in copula with the female lying in the gynaecophoric canal of the male. In this state, the worm pairs migrate to their ultimate vascular bed, 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. In *S. japonicum* and *S. mansoni*, a proportion of the eggs are carried to the lumen of the colon and removed through faeces while others remain attached to the gut wall and die. In *S. haematobium* eggs are concentrated in urine (Cheever, 1969). The life cycle continues when eggs come into contact with fresh water and hatch into miracidia (Lyons, 1978; Anon., 2003). Figure 1 detail the life cycles of three species of schistosome.

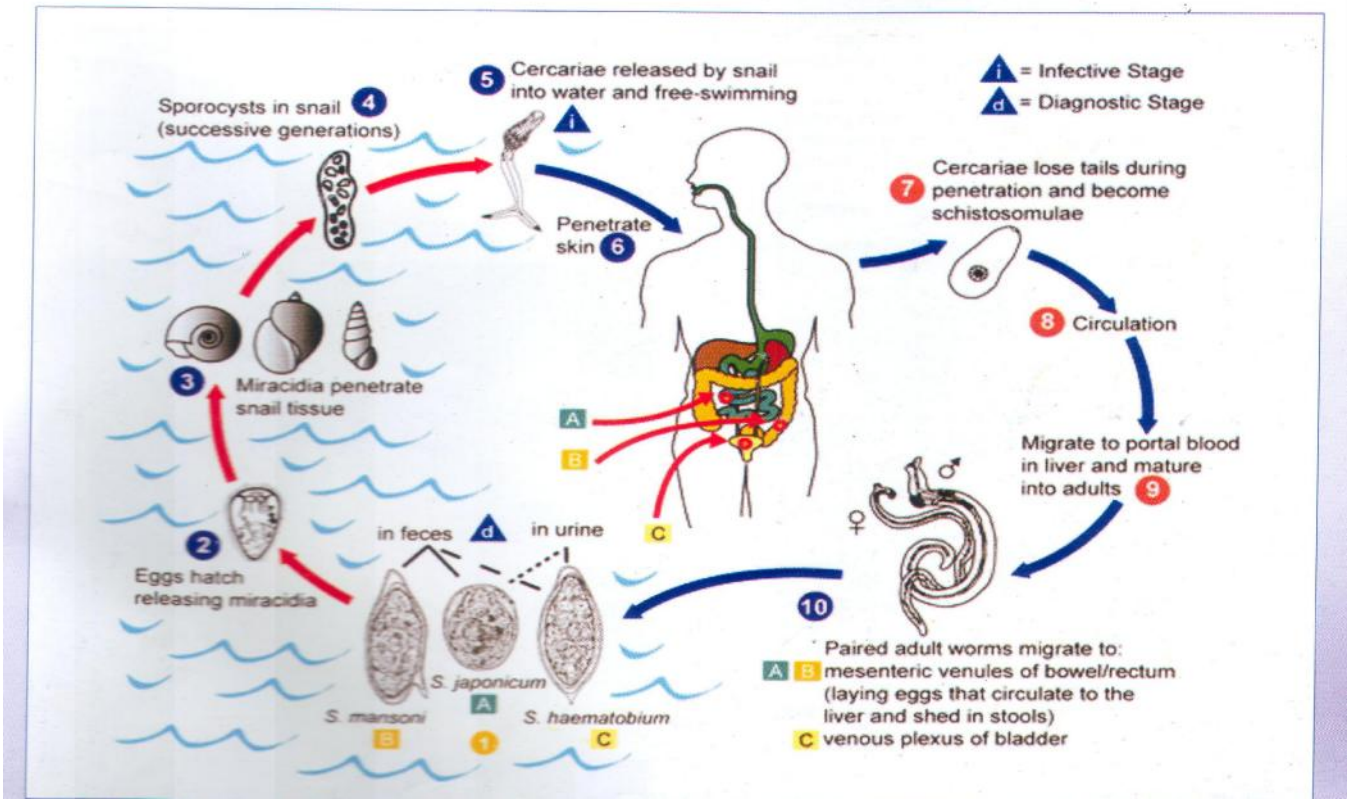

Figure1. The life cycle of *Schistosoma* parasites: adapted from Center for Disease Control. Laboratory Identification of Parasites of Public concern, 2nd edition, 2003.

Host preference and tissue localization of *Schistosoma*

Schistosomiasis due to *Schistosoma mansoni* is a chronic infectious disease of humans and other primates (Sturrock *et al*., 1978). Many primates are naturally infected. Miller, (1960), found out that in endemic areas, baboons, vervets and sykes are affected, suggesting presence of a true

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zoonosis. The parasites may also develop to maturity in a number of experimental animals, referred to as permissive hosts. These hosts include mice, hamsters and Rhesus monkeys. Rodents such as rats are rarely infected (Kuntz *et al*., 1971; Cheever *et al*., 1974). The hosts for *S. japonicum are humans and* other mammalian hosts (domestic livestock such as buffaloes [*Bubalus bubalis*], other bovines, porcines, ovines, and canines).

Snails of various groups act as intermediate hosts for different species of *Schistosoma* (van der Werf *et al.,* 2003). The geographical distribution of the snails, therefore influence the endemicity of the parasite. *S. mansoni* is transmitted by species of the genus *Biomphalaria*. In East Africa; *Biomphalaria pfeifferi* is the vector (Lyons, 1978). *S. haematobium* and *S. intercalatum* are transmitted by *Bulinus* species. In kenya, *Bulinus africanus and B. trancatus complex are the vectors for S. haematobium (Anon., 2014). S. japonicum is transmitted by Oncomelania* species and *S. mekongi* by *Neotricula* species (Gryseels *et al*., 2006). Snail vectors of *Schistosoma* live in well aerated water with vegetation. The vegetation serves as food and also supply leaf surface for egg deposition. Although snails can survive at higher temperatures, they thrive well at an optimum temperature range of 22-23°C (Malek, 1958). The population of Most snails fall during the rainy season and increase 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 (Malek, 1958).

In tropical lakes the extraordinary growth of certain species of floating plants, particularly *Eichlornia crassipes* (water hyacinth), *Salvinia auriculata* (water fern) and *Pistia stratiotes* (water lettuce), provide rich support for multiplication of vector snails, especially *Bulinus* and *Biomphalaria* species, and of insects (Anon., 1993). 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 (Anon., 1993).

In the host's body adult worms pair up, male and female with the female lying in the gynaecophoric canal of the male and the worm pairs migrate to their ultimate vascular bed. *S. mansoni migrate to the* superior mesenteric veins, *S. japonicum to* inferior mesenteric and superior hemorrhoidal veins or the vesical plexus and *S. haematobium* to veins draining the ureters. In these sites the females begin to lay eggs (Anon, 2014).

Pathology

The parasitological and pathological changes due to acute and chronic schistosomiasis in humans, non-human primates and mice have been documented (Warren, 1966; Sadun *et al.,* 1966; Cheever and Andrade, 1967; Myers *et al.,* 1970). Baboons infected with *S. mansoni* develop similar signs of acute human schistosomiasis, including eosinophilia and fever (Damian *et al.,* 1992). The immune responses that develops following infection with schistosomiasis often proceeds to cause pathological changes that are primary cause of the disease (Kariuki and Farah, 2005). The pathology of schistosomiasis may be divided into three main stages. These stages include; the invasion phase, the maturation of infection and the chronic phase of the disease. The invasion phase is the actual penetration of the definitive host by the cercariae and the following 2-7 days during which the cercariae migrate from the skin to the lungs (Cheesbrough, 1981). This phase is marked by a moderate to intense skin reactions called "Swimmer's itch" at the site

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of penetration depending on individual´s sensitivity to the parasites (Capron and Capron, 1994). Individuals in endemic areas show little or no reaction, while visitors develop marked skin rashes and urticaria (Clarke *et al*., 1970).

The acute phase starts 6-12 weeks after infection, and coincides with peak egg deposition and the characteristic pathological manifestation in this stage, in moderate and heavy infections is an acute febrile reaction often referred to as the "Katayama syndrome" (Clarke *et al.,* 1970). The symptoms includes; fever, fatigue, urticaria and eosinophilia. The eggs produced induce an inflammatory reaction in the liver and walls of the colon and rectum, with formation of granulomas where giant and epitheliod cells infiltrate around the egg and held together by an extra cellular matrix (Farah *et al*., 1997). The clinical signs at this stage are; abdominal pain and tenderness and intermittent dysentery or diarrhoea (King and Mohmoud, 1998). The intestines may have serosal nodular lesions called "Sandy patches" or in severe cases polyps leading to pseudo-tumours or bilharziomas (Cheever and Andrade, 1967). There is also a remarkable enlargement of the mesenteric lymph nodes (Clarke *et al*., 1970).

Histologically, the acute granuloma is seen to consist of a centrally placed *Schistosoma* ovum surrounded by concentric layers of inflammatory cells, predominantly eosinophils (Lenzi *et al*., 1987). 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 (Mitchnson and Oliviera, 1986).

In some cases of schistosomiasis, the granulomas may fail to modulate resulting in the hepatosplenic disease (Wyler and Telebian, 1997). 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 (Andrade, 1987). 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 (Pittella and Lana-Peixoto, 1981), and renal schistosomiasis manifest as nephropathy or glomerulo-nephritis (Andrade, 1987). Death due to *S. mansoni* infection is as a result of haemato-emesis, portal hypertension and occasionally upper gastro-intestinal haemorrhages (Dessein, 2003; Dune, 2003). Figure 2 and 3 show the histopathology in the liver and colon of baboons due to *S. mansoni* eggs.

Schistosoma haematobium in endemic settings 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 (Anon., 2014). 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 uro-genital tract (Anon., 2014).

The chronic stage of the infection of *S. haematobium* corresponds to uro-genital complications as the female worm releases eggs which migrate through the wall of the bladder and the uro-genital system. These retained eggs 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. Haematuria and dysuria are the main symptoms of uro-genital schistosomiasis, and

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early complications are infections of the upper and lower uro-gental tract and lithiasis (Anon., 2014). Chronic infections are characterized by long term complications such as obstructive and fibrous lesions of the uro-genital tract, chronic cystitis, calcification in the bladder and renal impairment. *S. Haematobium* is considered a risk factor cancer of the urinary bladder in endemic settings (Anon., 2014).

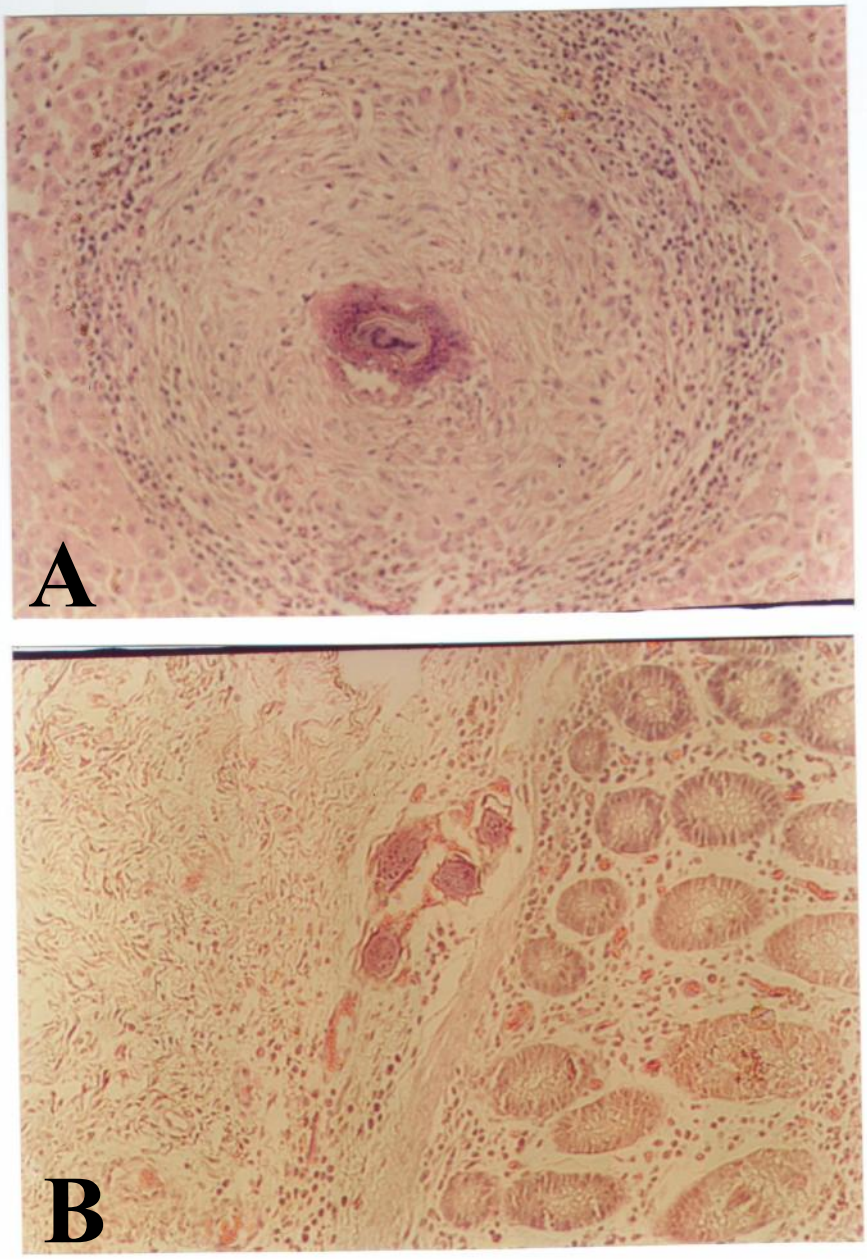

Figure2. Liver and large intestine of a baboon infected with *S. mansoni*. **A**: Section of liver showing a large portal egg-granuloma with an ovum surrounded by large numbers of eosinophils, neutrophils, histiocytes and lymphocytes. **B**: Section of the colon of same baboon having many *S. mansoni* eggs and infiltrated with inflammatory cells.

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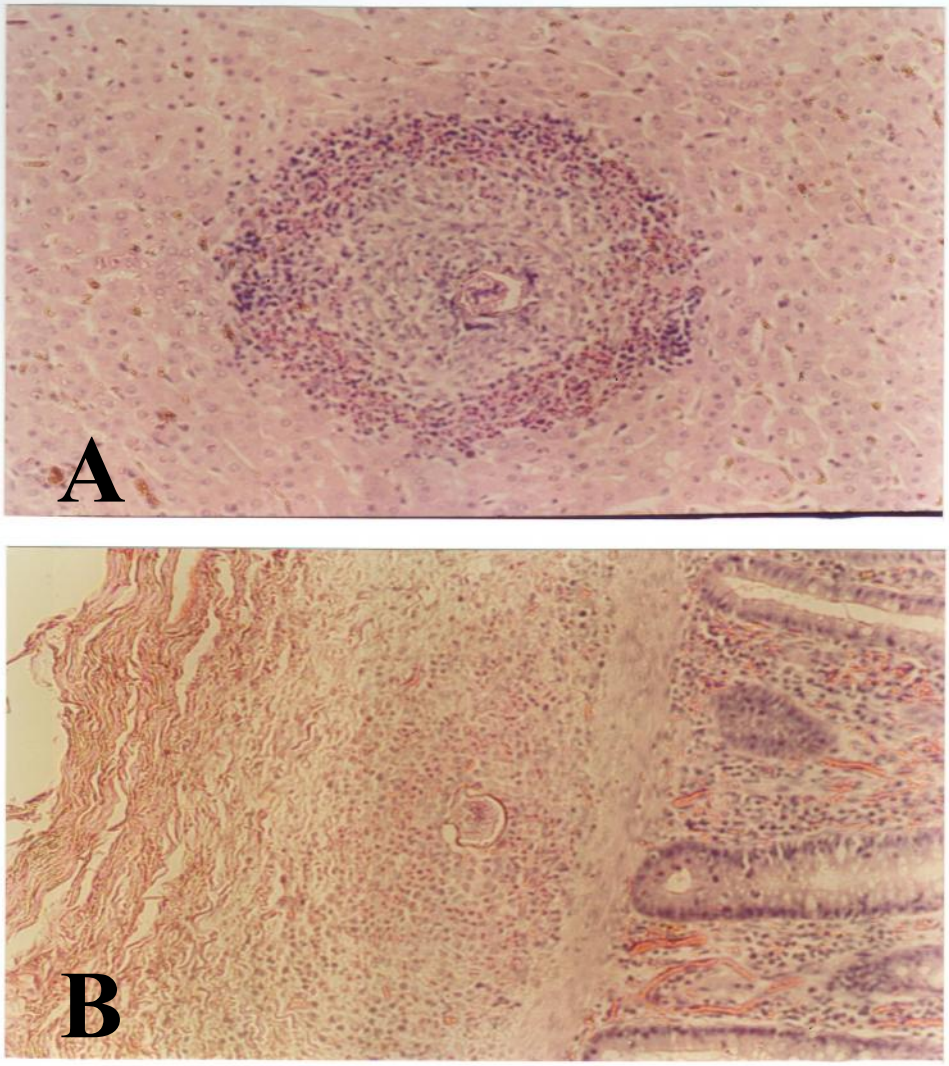

Figure3. The liver and colon of baboon infected with *S. mansoni*. **A**: Liver section showing egg-granuloma surrounded with inflammatory cells, mainly mononuclear cells and a few eosinophils. The granuloma is small. **B**: Section of the colon of same baboon showing an ovum, and also infiltrated with inflammatory cells.

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Immune responses to *Schistosoma* infection

All stages of *Schistosoma* induce a very pronounced immunological response, with the more predominant being directed against the eggs (Omer-Ali *et al.,* 1988). 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 (Dunne, 2003). These evasive mechanisms of the adult parasite include induction of anti-inflammatory molecules (Ramaswamy and Kumar, 2000), incapacitation of lymphoid cell function (Angeli *et al*., 2001), coating of the host's surface antigens (Loukas *et al.,* 2001) and production of anti-oxidant enzymes among many other mechanisms (LoVerde *et al.,* 2004).

Schistosomula are 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 (Capron *et al*., 1979; Gressner *et al.,* 1998).

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 (Mola *et al*., 1999). 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 (Nyindo *et al.,* 1999).

Cytokine levels peak during acute *S. mansoni* infection, but decline with chronic infection and become almost undetectable after treatment (Mola *et at.,* 1999). 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 (Mola *et al.,* 1999). SEA in baboons also induces lymphocytes proliferation during the acute phase of the disease (Mola *et al.,* 1999).

Control of schistosomiasis

Five main approaches have been applied in an attempt to control schistosomiasis. These include control of snail vectors, chemotherapy, improved sanitation, prevention of water contact and vaccination (Pearce, 2003). Molluscicides have been widely used to control snails. This approach has several limitations. First, the chemicals are expensive and their application must be undertaken frequently. Some molluscicides are harmful for example sodium chlorphenate cause skin irritation and hence dangerous to the handlers. Molluscicides also kill some of non-target beneficial molluscs and other organisms. These drawbacks have limited the use of molluscicides (Klump and Chu, 1987).

Biological methods have also been tried. Birds and large snails such as *Marisa carmuarietus* and *Tarebia granifera* that feed on other snails have been used to control the vector snails (Yole, 1996). The other methods of control involves proper disposal of faecal and urinary waste in order to avoid contamination of the environment with *Schistosoma* ova (CairnCross and Feachem, 1983). The building of latrines would if practised ensure that this is achieved. But in practice, not

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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 (CairnCross and Feachem, 1983).

Chemotherapy has been the only successful method in controlling schistosomiasis. The drugs currently available for treatment of schistosomiasis are praziquantel, metrifonate, oxamnaquine and artemisinins (Richter, 2003). Praziquantel is the drug of choice and all species that cause pathology in humans and other animals are susceptible to it (Anon., 1998). Apart from the original product, generic praziquantel is also available and because of this, the price of the drug has come down considerably (Richter, 2003). The efficacy of oxamnaquine and praziquantel is comparable, with a slightly better efficacy for praziquantel. Resistance to oxamniquine has however been observed in Brasil (Lambertucci *et al.,* 2000; Saconato and Atallah, 2000). Mirazid, the oleo-resin extract from myrrh of *Commiphora molmol* tree was established in Egypt as a novel drug against schistosomiasis and other parasitic diseases (Hamed and Hetta, 2005; Aly and Aly, 2006).

Even though schistosomiasis control can be approached with praziquantel alone, the possibility of developing resistance to this drug must be taken into account. This drug is effective against adult worms only and under vigorous selection in the laboratory, it has been possible to demonstrate that some schistosomes possess the genes that carry a lesser susceptibility to praziquantel. Reduced cure rates have also been observed recently in Senegal due to very high re-infection rates with a relatively high number of developing immature parasite not susceptible to praziquantel (Stalma *et al*., 1995; Fallon *et al*., 1995; Anon., 1998; Kussel and Hagan, 1999). *S. mansoni* isolates that tolerate high doses of praziquantel have been characterized from Egyptians (Ismail *et al*., 1996). The other problem is that in spite of recent reduction in price of praziquantel, the drug is still out of reach for many people in the endemic areas especially in Africa. Mass treatment does not also prevent re-infection (Richter, 2003; Kariuki and Farah, 2005). 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 reasons, it is clear that the most feasible long-term solution to schistosomiasis control is a protective vaccine (Pearce, 2003).

Anti-schistosome vaccine development

Although chemotherapy, especially with praziquantel has been successful in controlling schistosomiasis, it has not been able to prevent re-infection, which can be as often as once a year (Bergquist, 1998; Pearce, 2003). There is thus a need for a complementary tool with effect for the longer term and cost efficient, notably a vaccine. Vaccination can be targeted towards the prevention of infection or the reduction of parasite fecundity. 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 (Capron *et al.*, 2002). The effective vaccine should prevent the initial infection and reduce egg granuloma associated pathology (McManus *et al.*, 1998).

Recently, significant efforts have been made to develop a protective vaccine against *Schistosoma* infections, and several vaccine candidates have been identified (Ahmed *et al*., 2001; Da´dara *et al*., 2001; Zhang *et al.,* 2001; Dupre *et al*., 2001; Capron *et al*., 2001), 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 (Hanem *et al*., 2006). The development of vaccine remains to be an important long-term and a challenging goal in the control of schistosomiasis (Hafalla *et al*., 1999).

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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 (Kariuki and Farah, 2005). 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 (Bergquist, 1998). 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 (Bergquist, 1998; Pearce, 2003).

In this review, we look at some of the major vaccine antigens for schistosomiasis that have been the focus of attention for many years and newly described ones that show particular promise. 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 II and Sm14 at Phase I).

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 (Bergquist *et al*., 2005). They yielded protective responses but the stated goal of consistent induction of 40% or more protection was not achieved. The failure may be due to various reasons such as negative influence of insufficient antigen stability and need to use standardized and effective adjuvant formulations. The failure may also have been 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 (Wright *et al*., 1991), even though it is not one of the more abundant apical membrane proteins on the parasite surface. These antigens are not exposed to the host immune system and therefore do not induce the required protective responses in the host (Braschi and Wilson, 2006). 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 (Pearce and MacDonald, 2002). Nonetheless, the following arguments are in support of possibilities of developing effective vaccines against the various schistosome species (Bergquist *et al*., 2005): 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 (Craig *et al*., 2007). 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.

This review highlights, first some of the antigens from *S. mansoni* that have been tested as vaccine candidates for the last 10 years and later antigens from *S. japonicum*. The lead antigens include proteins expressed on the surfaces of the various stages of the parasite.

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Tetraspanins

Tetraspanins 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 (Braschi and Wilson, 2006), 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 (Bergquist and Colley, 1998). Sm23 has been shown to be the most effective when given as a DNA vaccine (Da'Dara, *et al*., 2003), but not effective as a recombinant protein when formulated with alum. It induces 40-50% protection in mice (Bergquist *et al*., 2002). Recently two new *S. mansoni* tetraspanins (SmTSP-1 and SmTSP-2) were discovered (Smyth *et al*., 2003). Both proteins are expressed in the tegument membrane of *S. mansoni* (Tran *et al*., 2006), and 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 (Braschi and Wilson 2006). TSP-2 in particular 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 (Tran *et al*., 2006). Both proteins induce good levels of protection (> 50% reductions in liver egg burdens) in immunized mice given an *S. mansoni* challenge. Knox, (2010), 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 (Tran *et al*., 2006), 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 (Loukas *et al*., 2007). 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 (Braschi and Wilson, 2006). Murine DNA vaccination with gene encoding Sm200 induced 38.1% protection (Fonseca *et al*., 2012).

SmTPI

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

Sm28/Sh28 Glutathione-*S*-Transferase, GST

Sm28-GST is an enzyme expressed in subtegumental tissues of most developmental stages of the parasite (Porchet *et al*., 1994). 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 (Balloul *et al.*, 1987). Trials in Primates demonstrated antifecundity effect (Boulanger *et al*., 1999), and an anti-Sm28 monoclonal antibody showed antifecundity and anti-egg embryonation effects (Xu *et al*., 1993). Clinical testing of Sh28-GST in people showed that vaccine was immunogenic and induced antibodies capable of neutralizing the enzymatic activity

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of the recombinant protein (Capron *et al*., 2005). Regrettably, the data on the efficacy of this vaccine in phase II clinical trials are not available.

Sm Calpain

Calpain 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 (Andresen *et al*., 1991). 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 (Siddiqui *et al*., 1993) and associated with the inner tegument membrane (Braschi and Wilson, 2006). 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 (Jankovic *et al*.,1996). 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.
The large subunit of calpain, called Sm-p80, was expressed in baculovirus, and the semipurified protein induced 29 to 39% reductions in worm burdens (Hota-Mitchell *et al*., 1997). Subsequent efforts to improve the efficacy of this vaccine have focused on DNA vaccine constructs, with and without Th1-type cytokine cDNAs, in mice and baboons (Siddiqui *et al*., 2003).

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 (Loukas *et al.,* 2001). 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 (Lanar *et al*., 1986). 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* (Pearce *et al*., 1988).

Sm22.6

This is also a tegumental protein. With its homologue in *S. japonicum* (Sj22.6), are involved in resistance to re-infection in endemic areas (Santiago *et al*., 1998). 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 (Fonseca e*t al*., 2012).

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 (Brito *et al*., 2002). 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 (Tendler *et al*., 1996), Sm14 failed to induce protection levels of >40% when tested in other laboratories (Fonseca *et al*., 2004) and as part of the WHO/TDR-sponsored trials. Co-administration of recombinant Sm14 protein with either IL-12 (Fonseca *et al.,* 2004) or tetanus toxin fragment C boosted protection. Immunization of mice with recombinant Sm14 expressed in *Mycobacterium bovis* BCG showed

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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 (Varaldo *et al*., 2004). Sm14 has entered human clinical trial (Phase I) in Brasil.

Sm SOD, Sm GPX and Sm FILAMIN

Two paradigms have emerged from the several studies of immunity in schistosomiasis, both in experimental animal models and human beings. One paradigm is that the larval stage (schistosomulum) is the most susceptible and the target for immune elimination (Smithers, 1982; LoVerde, 1998). The schistosomula elimination involves a cellular response that is potentiated by cytokines and/ or antibodies. The cells involved include monocytes, macrophages, eosinophils and platelets (Butterworth, 1984; LoVerde, 1998).

The second paradigm is that the adult worms once they reach the portal circulation seem to evade the engendered immune response through the evolution of several defence mechanisms (Smithers and Terry, 1969; Maizel *et al*., 1993; LoVerde, 1998). The cells involved in cytotoxic response against the schistosomula are thought to produce reactive oxygen species such as superoxide anion and hydroxyl radicals (LoVerde, 1998). 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 (Callahan *et al.,* 1988; Mei and LoVerde, 1987). Hong *et al.,* (1992) and Mei and LoVerde, (1997) demonstrated that expression of these anti-oxidant enzymes are developmentally regulated such that the lowest gene expression and enzyme specific activity are in the larval stages and highest in adult worms, the least susceptible to immune elimination. The anti-oxidant enzymes protect an organism from reactive oxygen species derived damage (James, 1994; LoVerde, 1998). These enzymes have been immuno-localized to the tegument and gut epithelia of adult worms, but not to the larval stages of the parasites (Mei and LoVerde, 1997).

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 (Shalaby *et al*., 2003). In addition to the anti-oxidant enzymes, Sm-filamin has been identified as a vaccine candidate that also consistently induces significant levels of protection in a 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 (LoVerde, 1998).

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 SEA and SWAP. CT-SOD and GPX showed anti-fecundity effect, but did not reduce the worm burdens. Also there was no reduction

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in histohepato-intestinal egg-associated pathology of *S. mansoni* infection in vaccinated baboons (un-published report).

Vaccine candidates for Schistosoma *japonicum*

Some of the leading *S. japonicum* vaccine candidates (as recombinant protein and/or DNA vaccines) are membrane proteins, muscle components, or enzymes.

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 (McManus, 2005; Wu *et al*., 2005). 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 (Wu *et al*., 2005).

Paramyosin (Sj97)

Native and recombinant paramyosin (Sj97) proteins confer protection against *S. japonicum* in mice, water buffaloes, and other mammalian hosts (Wu *et al*., 2005). Furthermore, recent studies of human antibody isotype (Nara *et al*., 2007) and Th2 cytokine responses to Sj97 add further support to this molecule as a leading vaccine candidate against *S. japonicum* (Leenstra *et al*., 2006).

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 (Fan *et al*., 2003). 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 (Gan *et al*., 2005). 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 (Gan *et al*., 2005).

SjTPI

Results of Chinese SjTPI (SjCTPI) plasmid DNA in mice have been good (Zhu *et al*., 2002). Therefore, Zhu et al. (Zhu *et al*., 2006) 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 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 (Yu *et al*., 2006).

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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 (Zhu *et al*., 2004) and mice (Gan *et al*., 2005; Zhu *et al*., 2004) and by a CpG immunostimulatory sequence in mice (Zhao *et al*., 2005). 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.

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.

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 (Osada *et al*., 2005). Localization studies have shown that calpain is present in the penetration glands and in the secretions of cercariae (Kumagai *et al*., 2005).

DNA vaccination

DNA vaccination is a vaccine methodology which is attractive and novel immunization strategy against a wide range of infectious diseases and tumours (Hanem *et al*., 2006). Injection of plasmid DNA as vaccine was first demonstrated to be effective using influenza as the model, where DNA construct containing a gene encoding nucleoprotein (NP) induced cytotoxic T-lymphocytes (CTLs) and cross-strain protection of mice (Ulmer *et al*., 1993). The effectiveness of DNA vaccines against viruses, parasites and cancer cells has been demonstrated in animal models (LoVerde, 1998; Tuteja, 1999; Seder and Gurunathan, 1999). The DNA immunization induces both antigen-specific cellular and humoral immune response (Ramsay *et al*., 1999; Alarcon *et al*., 1999; Gurunathan *et al*., 2000).

DNA vaccination strategies include 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 (Garmory *et al*.,

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2003). 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 (Davis, 1997). 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 (Gurunathan *et al*., 1998).

With DNA vaccination, the actual production of immunizing proteins takes place in the vaccinated host. This eliminates any risk of infection that has been observed with some live and attenuated virus vaccines. Other advantages of DNA vaccines are; production of long lived immune responses, vaccines for multiple diseases can all be given in a single inoculation, all DNA vaccines can be produced using similar techniques, DNA vaccines are extremely stable and candidate DNA vaccine can be recovered from diseased tissues (Gurunathan *et al*., 2000). DNA vaccination against schistosomiasis has recently been investigated using a panel of plasmids encoding *Schistosoma* antigenic proteins such as sjc 26 GST, Sj79 (Waine *et al.,* 1997; Zhou *et al*., 1999; Zhang *et al.,* 2001), Sm-CT-SOD and Sm-GPX (Shalaby *et al*., 2003), *S. japonicum* paramyosin (Yang *et al*., 1995) and Sm 23, 28 GST from *Schistosoma mansoni* (Dupre *et al*., 1997).

The use of novel 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 (Bergquist *et al*., 2005). 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 antischistosome vaccines, and a number of approaches have been tested, with some success.

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 (Farah *et al*., 2001). But the baboon is the most frequently used non-human primate in schistosomiasis research because of a multiplicity of qualities that make them more relevant models than rodents (Nyindo and Farah, 1999). Baboons are similar to humans in their anatomy, genetics and immunological responses (Villinger *et al.,* 2001). They acquire natural infections and are highly susceptible to experimental infections (Farah *et al.,* 1997). 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 (Farah and Nyindo, 1996; Farah *et al*., 1997; Mola *et al*., 1999; Nyindo *et al*., 1999). In the wild, baboons show age-dependent prevalence of infection, with high rates of infection in juvenile baboons and young adults, a similar characteristic observed in humans (Fulford *et al.,* 1999). Baboons are also abundant in Eastern Africa and are not an endangered species. They adapt readily in captivity and to changes in their environment; can give birth twice every 18 months and live up to 20 years; and attain body weight of 20 kg (Farah *et al.,* 2000). It is also easy to

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monitor the immune and disease progression in many different organs in individual baboons during the course of infection by performing some surgical manipulations (Mola *et al*., 1999).
Baboons acquire immunity on vaccination. For example, immunization with irradiated cercariae stimulates over 50% protection (Yole *et al*., 1996) which is associated with elevated serum levels of schistosome-specific IgG and IgE. In addition, vaccination with irradiated cercariae results in reduction of the intestinal pathology (Farah and Nyindo, 1996). Some protection to challenge infection following vaccination with recombinant antigens has also been reported in baboons (Soisson *et al.,* 1993). 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 (Farah *et al*., 2000). Baboons can also sustain repeated schistosome infections without any untoward lesions such as portal-caval shunting (Sturrock *et al*., 1984) 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 (Mola *et al*., 1999) which, as in humans, are associated with high serum levels of parasite specific IgE (Nyindo *et al*., 1999).
There are also a few constraints limiting the use baboons in schistosomiasis research, which 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 (Farah *et al*., 2001).

Conclusion

Schistosomiasis is a chronic disease that causes morbidity in large numbers of infected individuals, with severe life threatening consequences in a small percentage of those affected. Although it has a low mortality rate, schistosomiasis often is a chronic illness that can damage internal organs and in children impair growth and cognitive development. Currently it is estimated that 280,000 people die from schistosomiasis in sub-Saharan Africa each year. At present the most effective control method is chemotherapy with use of praziquantel as the drug of choice. 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 different schistosoma antigens that are capable of protecting experimental animals from challenge have been 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 is closely related to humans. As mentioned above, the parasite biology, the pathology, immune response and immunity to *Schistosoma* infection in humans differ significantly from the murine model of schistosomiasis but correlate better with the disease in baboons. Therefore, baboons may be ideal models for this purpose as prelude for clinical trials.

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