

# Current Status of Vaccines for Schistosomiasis

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## INTRODUCTION

In 1852, Theodor Bilharz described for the first time a tropical parasitic disease (bilharzia, later termed schistosomiasis) caused by blood-dwelling trematode fluke worms of the genus *Schistosoma*. Five schistosome species infect humans; they are *Schistosoma mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum*, and *S. haematobium*. The first four species have well-described associations with chronic hepatic and intestinal fibrosis and their attendant consequences. *S. haematobium* infections cause fibrosis, stricturing, and calcification of the urinary tract. A number of animal-specific schistosome species (e.g., *S. bovis* or *S. margrebowiei*) may occasionally accidentally infect humans. The cercaria-stage parasites of a large number

of other non-human (particularly bird)-infecting schistosomes (e.g., *Trichobilharzia* sp.) may penetrate human skin but then die. These can give rise to an allergic condition called swimmer's itch, or cercarial dermatitis, a reaction caused by the release of antigens by the dying parasites in the skin.

Approximately 200 million people in 74 countries are infected with schistosomes; 120 million are symptomatic, and 20 million suffer severe illness (31, 129). Schistosomiasis is the most important human helminth infection in terms of morbidity and mortality; a recent meta-analysis assigned 2 to 15% disability weight to the disease (78). There is also emerging evidence that schistosome infections may impact the etiology and transmission of human immunodeficiency virus/AIDS (HIV/AIDS) (22, 23, 72, 73, 80), tuberculosis (23, 42–44), and malaria (14, 19, 39, 96, 119, 153), and vice versa. In particular, the possible interaction between schistosomiasis and HIV/AIDS is receiving increasing attention, given the role of immune responses in both diseases and the geographic overlap in

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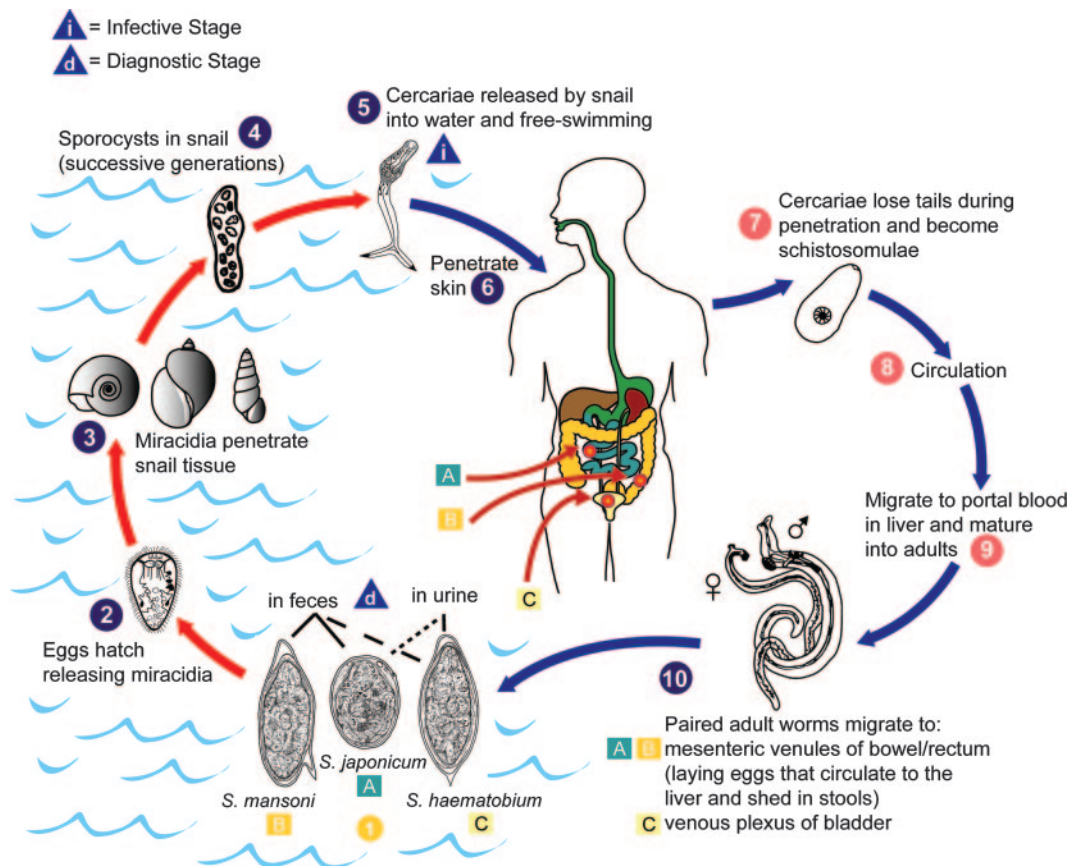


FIG. 1. Life cycle of *S. mansoni*, *S. japonicum*, and *S. haematobium*. (Reprinted from DPDx, the CDC website for parasite identification [<http://www.dpd.cdc.gov/dpdx/HTML/Schistosomiasis.htm>], with permission.)

distribution; low CD4<sup>+</sup> T-cell counts resulting from HIV infection may increase susceptibility to schistosome infection and influence egg excretion (54, 56, 74). Thus, schistosomiasis imposes a high socioeconomic burden on many affected developing countries.

*S. mansoni* occurs in much of sub-Saharan Africa, northeast Brazil, Surinam, Venezuela, the Caribbean, lower and middle Egypt, and the Arabian peninsula. *S. haematobium* is present in much of sub-Saharan Africa, the Nile valley in Egypt and Sudan, the Maghreb, and the Arabian peninsula. *S. japonicum* is endemic along the central lakes and River Yangtze in China, in Mindanao, Leyte, and some other islands in the Philippines, and in small pockets in Indonesia. *S. mekongi* occurs in the central Mekong Basin in Laos and Cambodia, and *S. intercalatum* is found in pockets in west and central Africa (56). Comprehensive reviews of the biology, epidemiology, diagnosis, treatment, and control of schistosomiasis are available (47, 48, 56, 78, 111, 129–131, 149, 179).

#### BIOLOGY, LIFE CYCLE FEATURES, AND TRANSMISSION

The schistosome life cycle is depicted in Fig. 1. Unlike other trematodes, schistosomes are dioecious (i.e., they have separate sexes), with the adults having a cylindrical body of 7 to 20 mm in length featuring two terminal suckers, a complex tegu-

ment, a blind digestive tract, and reproductive organs. The male's body forms a groove, or gynaecophoric channel, in which it holds the longer and thinner female (56, 129). All *Schistosoma* infections follow direct contact with freshwater harboring free-swimming larval forms of the parasite known as cercariae. Cercariae utilize an elastase proteolytic enzyme produced in the head region to penetrate the skin of humans or, in the case of *S. japonicum*, other mammalian hosts (domestic livestock such as buffaloes [*Bubalus bubalis*], pigs, sheep, and dogs). They shed their bifurcated tails and enter capillaries and lymphatic vessels en route to the lungs. After several days, the young worms, or schistosomula, migrate to the portal venous system, where they mature and unite. These worm pairs then migrate to their ultimate vascular bed, i.e., superior mesenteric veins (*S. mansoni*), inferior mesenteric and superior hemorrhoidal veins (*S. japonicum*), or the vesical plexus and veins draining the ureters (*S. haematobium*). Egg production commences 4 to 6 weeks after infection and continues for the life of the worm, which can be up to 15 years in the definitive host. Eggs are deposited in the vein lumen. The females produce hundreds (*S. mansoni*, *S. haematobium*, and *S. intercalatum*) to thousands (*S. japonicum* and *S. mekongi*) of eggs per day. Eggs pass into the host tissues, and then many pass through the intestinal or bladder mucosa and are shed in the urine (*S. haematobium*) or feces (all other species). It is the deposition in mucosae and tissues (the liver, in particular) of these eggs

and the ensuing immune response that are responsible for the pathology and disease associated with schistosomiasis. The life cycle is completed when the eggs passed in the feces hatch, releasing miracidia that, in turn, infect specific freshwater snails (*S. mansoni* infects *Biomphalaria* sp., *S. haematobium* and *S. intercalatum* infect *Bulinus* sp., *S. japonicum* infects *Oncomelania* sp., and *S. mekongi* infects *Neotricula* sp.). After two generations of primary and then daughter sporocysts within the snail, asexually produced cercariae are released.

Schistosomiasis transmission arises from agricultural practices and water resource manipulation, particularly if there is poor sanitation and substantial water contact. Environmental changes linked to water resource development, population growth, migration, and disease have facilitated the recent spread of schistosomiasis to areas where it is not endemic (56, 91, 129, 130). The Diama dam on the Senegal River introduced *S. mansoni* to Mauritania and Senegal (56). Population displacement has introduced *S. mansoni* into Somalia and Djibouti (56). Egypt's Aswan Dam virtually eliminated *S. haematobium* from the Nile Delta but facilitated the establishment of *S. mansoni* in upper Egypt (56). The Three Gorges Dam is currently being built on China's Yangtze River, between two areas where schistosomiasis is endemic (91, 129, 130), and the Chinese Ministry of Health is currently evaluating any potential impact on schistosomiasis transmission as a result of the dam (130, 149).

### THE ARGUMENT FOR ANTISCHISTOSOME VACCINES

In spite of remarkable chemotherapeutic progress and the existence of highly effective molecules such as the acylated quinoline-pyrazine praziquantel (PZQ), there is, as previously noted, still a spreading of schistosomiasis into new areas. After over 20 years of experience, it is generally agreed that chemotherapy, although the mainstay of current schistosomiasis control programs (13, 48, 79, 149), does have some limitations. In particular, mass treatment does not prevent reinfection. This 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. Furthermore, efficient drug delivery can require a substantial infrastructure to regularly cover all parts of an area of endemicity. This can make chemotherapy an expensive and often impractical approach. Although there is not yet clear-cut evidence for the existence of PZQ-resistant schistosome strains, decreased susceptibility to the drug has been observed (40, 56), and in view of renewed efforts to control schistosomiasis in high-burden areas, particularly in Africa, by large-scale use of PZQ, there is increasing concern about parasite resistance developing (48). In the case of *S. japonicum*, despite widespread use of PZQ, especially in China, there is no evidence of PZQ resistance, but an additional challenge is that transmission control necessitates interventions targeting animal reservoirs, particularly buffaloes (56, 57, 130). Furthermore, in situations of ongoing high transmission and interrupted chemotherapy campaigns, severe "rebound morbidity" in terms of hepatosplenic disease is now well documented for schistosomiasis, contributing to the disease burden (78, 129). As a result, vaccine strategies represent an essential component as an adjunct to chemotherapy for the future control of schistoso-

miasis. An improving understanding of the immune response to schistosome infection, both in animal models and in humans, suggests that development of a vaccine is possible. Critical questions are whether humans (and reservoir hosts, in the case of *S. japonicum*) develop immunity to schistosome infection and whether protective immunity can be induced in experimental animals, other natural hosts (such as bovines, in the case of *S. japonicum*), and humans.

This review considers aspects of antischistosome protective immunity that are important in the context of vaccine development. The current status in the development of vaccines against the African (*S. mansoni* and *S. haematobium*) and Asian (*S. japonicum*) schistosomes is then discussed, as are new approaches that may improve the efficacy of available vaccines and aid in the identification of new targets for immune attack. Recent, comprehensive reviews of the area are available (9, 10, 25–27, 61, 81, 87, 95, 99–101, 115, 145, 163, 166).

## THE IMMUNE RESPONSE IN SCHISTOSOMIASIS

### Basic Considerations in Immunopathology

Most chronic morbidity in schistosomiasis is not due to the adult worms but is related to the T-cell-dependent immune response of the host, which is directed against schistosome eggs trapped in tissues, mainly in the liver and intestines in the case of the intestinal forms (*S. japonicum* and *S. mansoni*) and in the bladder in the case of *S. haematobium*. The trapped eggs secrete a range of molecules leading to a marked CD4<sup>+</sup> T-cell programmed granulomatous inflammation involving eosinophils, monocytes, and lymphocytes, akin to a form of delayed-type hypersensitivity. Granulomas are also characterized by collagen deposition, and with the intestinal schistosomes, severe hepatic periportal (Symmer's) fibrosis occurs. Much of the morbidity and mortality associated with this disease is attributable directly to the deposition of connective tissue elements in affected tissues. In mice, a predominantly T-helper 1 (Th1) reaction in the early stages of infection shifts to an egg-induced Th2-biased profile, and imbalances between these responses lead to severe lesions (114, 118, 138, 143, 161, 167). A notable accomplishment in the past few years was the identification of interleukin-13 (IL-13) and the IL-13 receptor complex as central regulators of disease progression in schistosomiasis (105, 125, 167). Similar regulatory control could be at the basis of fibrotic pathology in humans (1), although this has not yet been established. This area is explored further below.

### Effector Mechanisms and Expression of Immunity in Animal Models of Schistosomiasis

A number of recent reviews have considered the immunobiology of schistosomiasis, including the nature of the host innate and adaptive responses to schistosomes and strategies used by the parasites to manipulate such responses (1, 26, 105, 114, 118, 138, 167). Much of our understanding of the mammalian immune response to schistosomes is based on the use of gene-disrupted (knockout) mice (51, 86, 125, 143, 167) and the immunization of mice, nonhuman primates, or other mammalian hosts with UV- or  $\gamma$ -irradiated cercarial vaccines, with or



without a subsequent challenge infection with nonattenuated cercariae (12, 41, 59, 75, 76, 132, 146). The attenuated larvae fail to mature into adult worms and do not produce eggs, so any results obtained are not confounded by egg-induced liver pathology. An even greater effect of triggering high-level resistance against schistosome reinfection has been shown for mice treated with artemether, a methyl ether derivative of dihydroartemisinin, followed by challenge (11). This model may provide an alternative approach to irradiated vaccines for dissecting different immune responses as putative effector mechanisms during schistosome infection and protective responses against reinfection.

In general, these studies have established that T-cell-mediated immunity is fundamental to acquired resistance to schistosomes in mice. Much of this protection was shown to be mediated by activated macrophages and, together with studies of cytokines, suggested that a vaccine that induced macrophage-activating Th1 cytokines (gamma interferon [IFN- $\gamma$ ] and IL-2) may be beneficial in preventing schistosomiasis. However, repeated vaccination with irradiated cercariae produced incremental increases in Th2-mediated (IL-4 and IL-5 predominance) protection, which was transferable to nonvaccinated animals. Studies using B-cell-deficient and cytokine-deficient mice demonstrated that successful antischistosome vaccination required induction of strong Th1 and Th2 responses. Following infection by normal or radiation-attenuated cercariae, the predominant early immune response was Th1 mediated and aimed at the adult worm. Following egg deposition in tissues (at 6 weeks postinfection for *S. mansoni* and 4 to 5 weeks postinfection for *S. japonicum*), the Th1 response was diminished, being replaced by a prominent Th2-mediated phase. Indeed, it appears that egg antigens are able to directly suppress the Th1 response (116, 118), a phenomenon which may also occur in humans. The Th2 response results in an increase in serum IL-5, massive bone and blood eosinophilia, and a granulomatous response aimed at the egg, resulting in collagen deposition, tissue fibrosis, and the disease manifestations of schistosomiasis. The precise role of eosinophils in the disease process in the mouse model of infection remains undetermined (141). The complexity of immune regulation and T-cell regulation in schistosome infection in mice is well recognized (98, 114, 143, 161), and this was further illustrated by a recent study by Walsh et al. (157), who highlighted a specific role for CTLA-4<sup>+</sup> but not CD25<sup>+</sup> cells in the regulation of Th2 responses in helminth infection. Furthermore, whereas the cytokine interplay during the development of protective immunity to the radiation-attenuated (RA) schistosome vaccine has been characterized extensively over recent years, the role of costimulatory molecules in the development of cell-mediated immunity is much less well understood. The importance of CD40/CD154 in vaccine-induced immunity was recently demonstrated (60), as it was shown that CD154<sup>-/-</sup> mice exposed to RA schistosomes developed no protection to challenge infection, suggesting that protective immunity to the RA schistosome vaccine is CD154 dependent but is independent of (IL-12 orchestrated) cellular immune mechanisms in the lungs.

As referred to earlier, in the case of *S. japonicum*, zoonotic transmission adds to the complexity of *S. japonicum* control programs but provides a unique opportunity to develop a transmission-blocking veterinary vaccine to help prevent hu-

man infection and disease. However, studies of protective immunity in bovine schistosome infections are few (101), and consequently, our knowledge of the immunology of schistosome infections in buffaloes and cattle is extremely limited. This is particularly the case for water buffaloes, for which immunological reagents for studying immune responses are scarce. Recent PZQ treatment and reinfection studies of bovines infected with *S. japonicum* in China have indicated that age-related resistance occurs in buffaloes but not cattle (159). Whether this self-cure phenomenon has an immunological basis has yet to be determined. Additional studies on the immunology of buffaloes and cattle represent an important area for future research and will be essential in selecting *S. japonicum* vaccine antigens and in defining the optimum route of immunization.

### Effector Mechanisms and Clinical Expression of Immunity in Human Schistosomiasis

Numerous longitudinal cohort studies of reinfection rates following curative drug treatment have shown that people living in areas where schistosomes are endemic acquire some form of protective immunity after years of exposure to *S. mansoni*, *S. haematobium*, or *S. japonicum* (27, 56, 107, 129, 130). However, age-related innate resistance mechanisms may also play an important part in the epidemiology of schistosomiasis (26, 56). Immune correlative studies in various parts of the world suggest that acquired antischistosome protective immunity after curative drug therapy is mediated (although not exclusively) by a Th2 response, orchestrated by immunoglobulin E (IgE), against adult and larval antigens which stimulate eosinophils to release cytotoxins targeting schistosomula (26, 27, 56). Despite the protective role of IgE, high levels of IgG4 are also produced during infection, potentially blocking the protective effects of other immunoglobulins (24). Subsequently, it was shown that immunity to reinfection is more closely related to the IgE/IgG4 balance than to the absolute level of each isotype (24). The opposing effects of IgE and IgG4 could not be dissociated in the analysis, indicating that these isotypes probably antagonize each other in terms of protection (24). Although both IgE and IgG4 responses initially depend on IL-4 and IL-13 production, the production of IgG4 antibodies is regulated in an antigen-specific context by IL-10 and IFN- $\gamma$  produced by Th0 cells (24). This supports the view that IgE and IgG4 can be dissociated, in spite of their reported dependence on IL-4. The putative role of IL-10 in the preferential induction of an IgG4 response should be placed in the broader perspective of the general properties of this cytokine. Indeed, it is now well established that IL-10 prevents antigen-presenting cell-dependent IgE synthesis and that IgE-dependent cytokine release from host cells causes activation of eosinophils as well as IL-5 release (24). The clinical expression of immunity to schistosome infection is obviously not determined simply by the mere balance between IgE and IgG4 antibodies. One cannot exclude the participation of additional mechanisms, such as a potential protective role of IgA antibodies in human schistosomiasis, supported by a series of correlation studies from several parts of the world; the effector functions of IgA antibodies may be associated with a decrease in female worm fecundity and egg viability (24).

In our opinion, the development of a vaccine for schistosomiasis that is dependent on IgE would potentially be problematic and would likely be impeded by regulatory and safety issues due to potential anaphylaxis induced by vaccination. Therefore, looking to the immune responses of chronically infected individuals, and even those who become refractory by producing IgE after drug treatment, should be approached with caution. Perhaps the most important clue of all towards understanding protective immunity to schistosomiasis is the naturally acquired immunity displayed by some individuals in Brazil in the absence of prior drug treatment (32, 155, 156). This small but well-defined cohort is referred to as endemic normals (32) or, more recently, putative resistant (PR) individuals (147). These individuals are resistant to infection despite years of exposure to *S. mansoni* and are defined as follows: (i) negative for over 5 years for *S. mansoni* infection based on fecal egg counts, (ii) never treated with antihelminthic drugs, (iii) continually exposed to infection, and (iv) have maintained vigorous cellular and humoral immune responses to crude schistosome antigen preparations (32, 33, 155, 156). PR individuals mount vigorous but very different (compared to those of chronically infected patients) immune responses to crude *S. mansoni* extracts from schistosomula (using detergent to solubilize the tegument) and adult worms (24, 155, 156). In response to stimulation with these antigens, peripheral blood mononuclear cells from PR individuals secrete both Th1- and Th2-type cytokine responses (6, 24), while chronically infected individuals make a Th2-type response (128). It is the Th1 response (particularly IFN- $\gamma$ ) to schistosomulum antigens that is thought to be the key to resistance to schistosomiasis in these subjects (32). Indeed, recent studies described the use of PR individuals to select two new vaccine antigens that are expressed in the tegument membrane of *S. mansoni*, namely, SmTSP-2 (147) and Sm29 (28). Both proteins were preferentially recognized by sera from PR individuals as opposed to sera from chronically infected patients, supporting the potential of the PR immune response to guide discovery of tegument plasma membrane proteins as recombinant vaccines (95).

Although the immune responses of resistant cohorts have been characterized, we still know very little about the protective mechanisms required to engineer an efficacious recombinant vaccine for human schistosomiasis. Contrasting and conflicting data have been presented from the mouse model and from human field studies. For example, activation of predominantly Th1 cells by schistosomulum antigens correlates with naturally acquired protection of PR individuals (who are exposed to the parasite but are not infected and have never been treated with PZQ) (32). On the other hand, partial resistance can be induced in some adult individuals with repeated PZQ treatment, and this correlates with a predominantly Th2 response (158). In mice, recombinant vaccines conferring various levels of protection induce different immune response phenotypes. This is influenced at least in part by the properties of the adjuvants used or the intrinsic immunogenicity of the respective proteins, but a general consensus is lacking. Studies using the RA cercaria model in mice suggest that protection can be induced with either a mixed Th1/Th2 response, a polarized Th1 response, or even a polarized Th2 response (reviewed in reference 59). Given that antibodies alone can confer protection in this model (70), perhaps the phenotype of the response,

and even the isotype/subclass of antibody produced, is not of prime importance. Most commercially available vaccines rely specifically on the induction of neutralizing antibodies that block the function of their target protein(s). This appears to also be the case for other helminth vaccines that are showing promise in preclinical studies, where neutralizing antibodies block proteins that have pivotal roles in tissue migration or digestion of the blood meal (93).

An understanding of immune regulation in human schistosomiasis is essential if schistosome vaccines are to be delivered to previously infected individuals. As emphasized above, experimental schistosome infections of laboratory animals, particularly mice, have contributed significantly to our understanding of the immunobiology of infection, particularly the mechanisms associated with egg-induced granuloma formation and subsequent fibrosis (reviewed in reference 1). Immune mechanisms elucidated in experimental models of schistosomiasis are not easily investigated in humans for ethical and logistical reasons, so available knowledge on human responses to schistosomes falls far short of what is known for mice (1). Furthermore, caution is required in extrapolating and interpreting results from murine experiments because, in many respects, the infection is dissimilar to the clinical situation, where there are a number of potentially confounding factors relating to exposure, infection/reinfection, coinfections, host and parasite genetics, nutritional status, and environmental modifiers that cannot be controlled, or even adequately assessed (1). Studies undertaken with experimental models of schistosome infection need to be validated fully in humans, which will prove challenging, as the immune regulatory mechanisms operating are clearly so complex. Nevertheless, there is accumulating evidence indicating that at least some features of the immune response evoked in infected humans are similar to those in mice (reviewed in reference 1).

#### STRATEGIES FOR ANTISCHISTOSOME VACCINE DEVELOPMENT

Schistosomes do not replicate within their mammalian hosts. Consequently, a nonsterilizing naturally or vaccine-acquired immunity could significantly decrease human pathology and disease transmission. Vaccination against schistosomes can be targeted towards the prevention of infection and/or to the reduction of parasite fecundity. A reduction in worm numbers is the "gold standard" for antischistosome vaccine development, with the migrating schistosomulum stage likely to be the major vaccine target of protective immune responses (99, 163). However, as schistosome eggs are responsible for both pathology and transmission, a vaccine targeted at parasite fecundity and egg viability also appears entirely appropriate. While they regularly induce 50 to 70% (over 90% in some cases) protection in experimental animals and additional immunizations boost this level further, it may be premature to pursue RA schistosome vaccines for human use, but their development for veterinary application is feasible. The concept is proven, and many of the requisite techniques, although they require refining and upscaling, are published. Although technically challenging, there is a case for promoting the development of a live, attenuated, cryopreserved schistosomulum vaccine for use against *S. japonicum* in buffaloes to reduce zoonotic transmis-

sion to humans in China (99). If successful, the veterinary vaccine could provide a paradigm for the development of antischistosome vaccines for human use.

In addition, while the *S. mansoni* RA vaccine model has enabled the dissection of different immune responses as putative effector mechanisms (59) and raised hopes for the development of molecular vaccines, this has not equated to advances in the development of recombinant vaccines. Independent testing of six candidate *S. mansoni* antigens (glutathione *S*-transferase 28 [Sm28-GST], paramyosin, Ir-V5, triosephosphate isomerase, Sm23, and Sm14) in the mid-1990s, orchestrated by a UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR/WHO) committee, resulted in protective responses being recorded, but the stated goal of consistent induction of 40% protection or better was not reached with any of the antigens tested, highlighting the possible negative influence of insufficient antigen stability and the need for standardized and effective adjuvant formulations (9). Furthermore, of these six antigens, only one (Sm23) is exposed on the apical membrane surface of the parasite (165), although it is not one of the more abundant apical membrane proteins on the parasite surface (17). Also, the failure to develop an efficacious schistosome vaccine can be attributed in part to the complex immunoevasive strategies used by schistosomes to avoid elimination from their intravascular environment (118). Nevertheless, convincing arguments still support the likelihood that effective vaccines against the various schistosome species can be developed (9); first, as discussed above, irradiated cercariae regularly induce high levels of protection in experimental animals, and additional immunizations boost this level further; second, as we have emphasized, endemic human populations develop various degrees of resistance, both naturally and drug-induced; and third, veterinary antihelminth recombinant vaccines against cestode platyhelminths have been developed successfully and applied in practice (35). The optimism sparked by these arguments has resulted in the discovery of a large number of schistosome antigens (utilizing the almost-complete genome sequence), and additional candidates are now being found through proteomic approaches (16, 17); these two dynamic areas of schistosome molecular biology are explored further below. However, antigen identification and successful protective results are of little value if recombinant proteins cannot be produced easily (and cheaply) with good manufacturing practice (GMP). Even the best protective results are no guarantee for ultimate success, and the scaling up of antigen production can be every bit as challenging as any immunological investigation. This was underscored when several of the frontline candidates chosen by the TDR/WHO committee discussed above had to be abandoned because, in addition to the low independent testing efficacy recorded, hurdles in consistent protein production could not be overcome. Nevertheless, as discussed below, there is still considerable interest in developing these and other molecules as antischistosome vaccines. Compromises may be necessary, however, because as emphasized by Bergquist and colleagues, "we might eventually have to settle for moderately effective antigens, but where the scaled-up GMP versions do not pose a problem. Despite the extra costs of scaling-up production of all antigens under con-

sideration, it is a selection criterion in assessing vaccine candidacy that cannot be avoided" (9).

A number of recent studies, particularly on *S. japonicum* (see below), have utilized plasmid DNA vaccines to deliver protective antigens. DNA vaccines generate both T-cell and B-cell (or antibody-mediated) immune responses and are thus particularly appealing for schistosome vaccine development. The preparation and production of DNA vaccines are convenient and cost-effective, and they can even be used in the field without a cold chain. Another advantage of applying DNA vaccines compared to other approaches is the possibility of targeting the in vivo expressed recombinant antigen to different cell compartments. Furthermore, methods such as prime-boost regimens and the use of adjuvants (such as IL-12) in combination with a DNA vaccine can enhance its protective effectiveness. The advantages and disadvantages of plasmid DNA vaccination, the strategies employed for DNA vaccine delivery, and technological and clinical advances in the area have been reviewed recently (18, 148).

## CURRENT STATUS OF VACCINE DEVELOPMENT FOR *S. MANSONI* AND *S. HAEMATOBIIUM*

### Basic Considerations

Given the enormous burden of disease related to schistosomes, relying solely on existing disease control methods, i.e., mass and repeated treatment of exposed populations with the antihelminthic PZQ, is unlikely to be feasible. Vaccines in combination with other control strategies, including the use of new drugs, are needed to make elimination of schistosomiasis possible (145). Despite the discovery and publication of numerous potentially promising vaccine antigens from *S. mansoni* and, to a lesser extent, *S. haematobium*, only one vaccine, namely, BILHVAX, or the 28-kDa GST from *S. haematobium*, has entered clinical trials (26). Published data are not available on the clinical efficacy of this vaccine, but nonetheless, it is disappointing that other vaccines have not progressed to this stage. Below, we review the most recent and pertinent data on the major vaccine antigens for schistosomiasis; some have been the focus of attention for many years, while others are newly described but show particular promise.

### Major Candidate Vaccines and Their Protective Efficacies

Table 1 summarizes the data for some of the most promising *S. mansoni* vaccine antigens discovered in the last 10 years, as well as those that were independently tested under the umbrella of the TDR/WHO committee in the mid-1990s (8); the latter group has been reviewed extensively elsewhere (26, 87, 115).

**Tetraspanins.** Tetraspanins are four-transmembrane-domain proteins found on the surfaces of eukaryotic cells, including B and T cells. They have two extracellular loops, including a short loop 1 of 17 to 22 residues (EC-1) with little tertiary structure and a larger, 70- to 90-residue loop 2 (EC-2), which has four or six cysteines that form two or three disulfide bonds (Fig. 2). In general, the extracellular loops mediate specific protein-protein interactions with laterally associated proteins or, in some cases, known ligands (reviewed in reference 90).

TABLE 1. *S. mansoni* recombinant proteins that correlate with resistance in human studies and/or have shown vaccine efficacy in animal models

Protein or cDNA	Location in adult worm	Identity	Protection of vaccine in mice (target stage [vaccine component]) <sup>a</sup>	Protective role in humans <sup>b</sup>	Reference(s)
SmTSP-2 <sup>c</sup> (tetraspanin D)	Tegument apical membrane	Tetraspanin integral membrane protein	++ (worms [recombinant protein]), ++ (eggs [recombinant protein])	Yes (PR), IgG1/IgG3	137, 147
SmTSP-1	Tegument apical membrane	Tetraspanin integral membrane protein	+ (worms [recombinant protein]), ++ (eggs [recombinant protein])	No	147
Sm29 <sup>c</sup>	Tegument apical membrane	Unknown, but has C-terminal transmembrane domain	++ (worms [recombinant protein]) <sup>d</sup>	Yes (PR), IgG1/IgG3	28
Sm23 <sup>c</sup>	Tegument apical membrane	Tetraspanin integral membrane protein	+ (worms [multiantigenic peptide {MAP}], + (worms [plasmid DNA])	Yes (DIR), IgG3 to MAP-3	3, 37, 127
Sm-p80	Associated with tegument inner membrane	Calpain—neutral cysteine protease	+ (worms [plasmid DNA]), ++ (worms [plasmid DNA including cytokines])	ND	17, 134
Sm14 <sup>c</sup>	Whole body, cytosolic	FABP	++ (recombinant protein)	Yes (DIR)	3, 50, 106, 144, 151
Sm28-GST <sup>c</sup>	Whole body	GST	+ (worms [recombinant protein] and eggs)	Yes (DIR)	3, 120
Sm28-TPI <sup>c</sup>	Unknown for adults, but located in tegument of newly transformed somula	TPI	+ (worms [transfer of anti-TPI monoclonal antibody])	Yes (DIR), IL-5 to MAP-4, IgG2 to MAP-4	3, 126, 127
Sm97 paramyosin <sup>c</sup>	Tegument of schistosomula and musculature of adults	Paramyosin	+ (worms [recombinant and native proteins])	Yes (PR IgG, DIR IgE)	3, 33, 97, 117
CT-SOD	Tegument and gut epithelia	Cytosolic Cu-Zn SOD	++ (worms [plasmid DNA])	ND	104, 133

<sup>a</sup> As reported in initial publications from inventors' laboratories. +, 30 to 50% reductions in worm and liver egg burdens; ++, >50% reductions in worm and liver egg burdens.

<sup>b</sup> PR, protective role in studies with PR subjects; DIR, protective role in studies with drug-induced resistant subjects; ND, not determined.

<sup>c</sup> Identified in tegument outer membranes from biotinylated worms by using proteomics (17).

<sup>d</sup> Costa Oliveira, personal communication.

<sup>e</sup> Vaccine efficacy was tested independently (8), and adult worm reductions did not exceed 40%.

The four transmembrane domains provide stability during biosynthesis and are crucial for assembly and maintenance of the tetraspanin web, a scaffold by which many membrane proteins are laterally organized (4). Although their functions are unknown, it is now apparent from proteomic studies that a family of tetraspanins is expressed in the schistosome tegument (16, 17, 150), and at least three of these show promise as vaccines (Table 1). Sm23 is a tetraspanin (165) expressed in the tegument of *S. mansoni* and is one of the independently tested WHO/TDR vaccine candidates (8). Sm23 is most efficacious when delivered as a DNA vaccine (36) and does not confer protection as a recombinant protein when formulated with alum. More recently, a reporter-based signal sequence capture technique was used to identify two new *S. mansoni* tetraspanins (SmTSP-1 and SmTSP-2) (137). Both proteins are expressed in the tegument membrane of *S. mansoni* (147) (Fig. 1), and TSP-2 was identified as one of only a subset of proteins that were biotinylated on the surfaces of live worms and subsequently identified using tandem mass spectrometry (17). TSP-2 in particular provided high levels of protection as a recombinant vaccine in the mouse model of schistosomiasis, and both proteins were strongly recognized by IgG1 and IgG3 from PR individuals but not from chronically infected people (147).

In addition to TSP-2, two more tetraspanins were identified

from the outer teguments of biotinylated *S. mansoni* adults (17), and both are clearly now vaccine targets (95). The extracellular loops of TSP-2 can be expressed at very high levels in soluble and stable form in both yeast and bacterial cells (M. Tran, M. Pearson, and A. Loukas, unpublished data) (Fig. 1), overcoming a major (and costly) impediment to the development of many vaccine antigens.

**Other membrane proteins.** The next few years will hopefully see the assessment of some of the newly identified tegument plasma membrane proteins from *S. mansoni* (17). Other than the tetraspanins (Sm23 and SmTSP-2), only one of these membrane-spanning proteins, Sm29, has been assessed as a vaccine. Like TSP-2 (147), Sm29 is preferentially recognized by antibodies from PR compared with chronically infected individuals (28), although the extent of selectivity is not as great as that reported for TSP-2. Moreover, preliminary trials in mice suggested that this protein is an efficacious recombinant vaccine (95; S. Costa Oliveira, personal communication), lending further support to its development as a recombinant vaccine. Other apical membrane proteins from the tegument (17) that warrant attention as vaccines include the structural membrane proteins with large extracellular regions, such as annexin and dysferlin, and other accessible (to antibodies) proteins with no homologues of known function, such as Sm200.



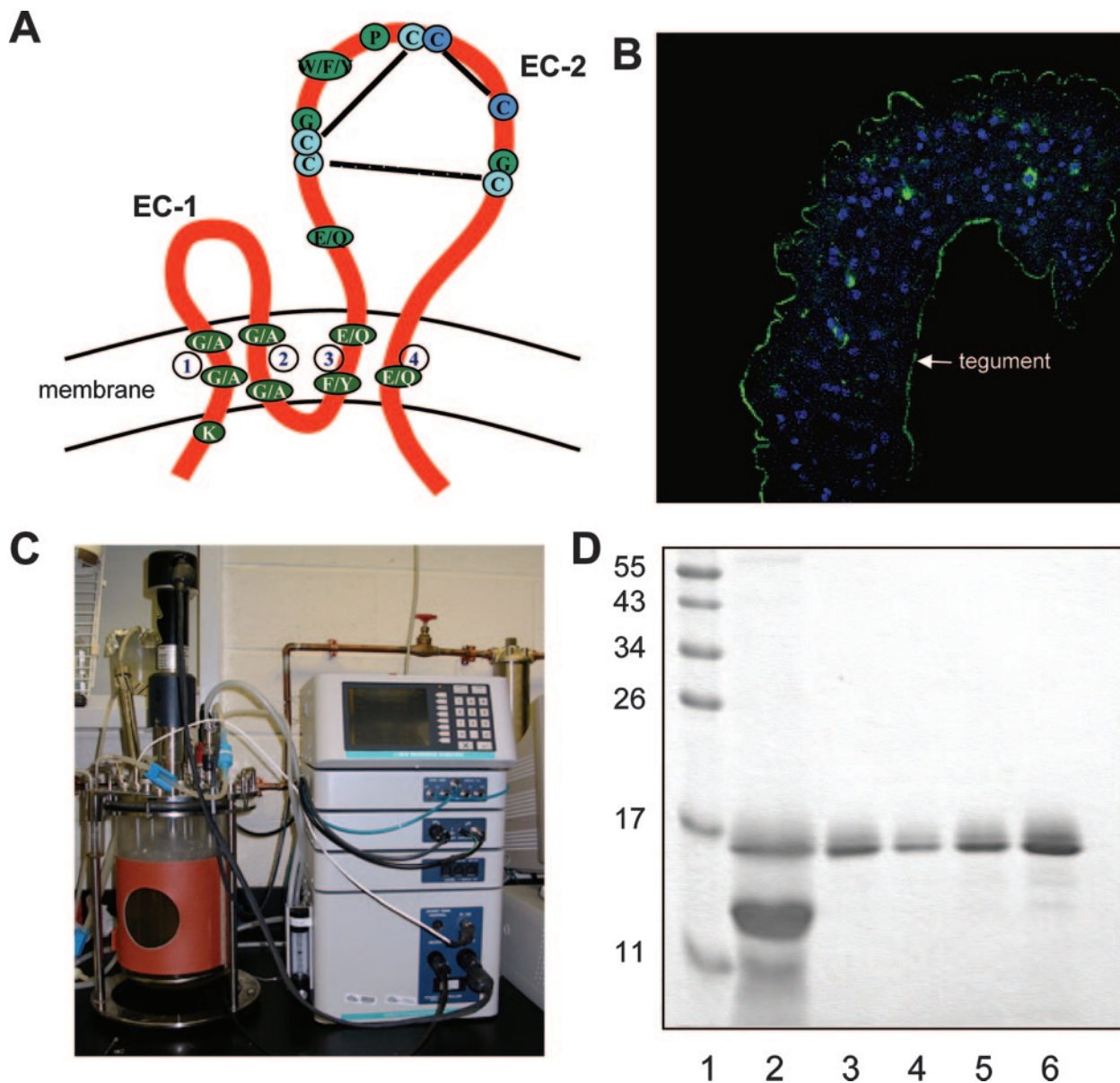


FIG. 2. Predicted structure and surface localization of the SmTSP-2 tetraspanin vaccine antigen from *S. mansoni* and fermentation and subsequent purification of the recombinant protein. (A) SmTSP-2 is predicted to span the membrane four times, presenting small (EC-1) and large (EC-2) extracellular loops to the external environment. (B) Antibodies to recombinant SmTSP-2 EC-2 localized the expression to the tegument outer membrane of adult *S. mansoni*. (Reprinted from reference 147 with permission from Macmillan Publishers Ltd.) (C) Fermentation of recombinant SmTSP-2 EC-2 as a secreted protein in the yeast *Pichia pastoris* produces recombinant protein at yields in excess of 100 mg/liter of purified protein (M. Tran and A. Loukas, unpublished data). (D) Lane 1, molecular weight markers; lane 2, supernatant from yeast culture expressing SmTSP-2 EC-2; lanes 3 to 6, eluates from immobilized metal-affinity chromatography column containing purified recombinant SmTSP-2 EC-2.

**Sm28/Sh28 GST.** Sm28-GST has GST properties and is expressed in subtegumental tissues of most developmental stages of the parasite (120). Vaccination of semipermissive rats and permissive hamsters with recombinant Sm28-GST resulted in significant reductions of worms (7), kick-starting a 20-year program on Sm28- and Sh28-GSTs as vaccine antigens. Primate trials were conducted and showed an antifecundity effect (15), and an anti-Sm28 monoclonal antibody showed antifecundity and anti-egg embryonation effects (168). This led to the clinical testing of Sh28-GST in people and the description

of its immunogenicity and induction of antibodies capable of neutralizing the enzymatic activity of the recombinant protein (26, 27). Unfortunately, there are no data available on the efficacy of this vaccine in phase II clinical trials.

**Smp80 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 (5). Calpain was immunolocalized to the tegument and underlying musculature of adult worms and was shown to be involved in surface mem-



brane turnover (135) and to be associated with the inner tegument membrane (17). Calpain was shown to be the target of a protective CD4<sup>+</sup> T-cell clone that induced peritoneal macrophages from syngeneic recipients to kill schistosomula in vitro (69). 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 (65). 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 (134).

**SOD.** Granulocytes release oxygen radicals that are toxic for *S. mansoni*, and exogenous superoxide dismutase (SOD) inhibited granulocyte toxicity for egg metabolic activity and hatching (77). A cDNA encoding a SOD with a signal peptide was cloned from *S. mansoni*, and its protein product was recognized by sera from infected humans (136). A cDNA encoding a cytosolic SOD (CT-SOD) was then identified (63), and both SODs were immunolocalized to the tegument and subtegumental tissues (64, 104). Proteomic studies have since shown that SOD is localized below the tegument plasma membrane (16, 150). Vaccination experiments using the recombinant SOD proteins have not been reported, but CT-SOD and a partial sequence encoding the structural protein filamin showed promise as DNA vaccines, resulting in significant reductions in adult *S. mansoni* in a murine challenge model (133).

**Paramyosin.** Paramyosin is a 97-kDa myofibrillar protein with a coiled-coil structure and is found exclusively in invertebrates. It is expressed on the surface tegument of lung-stage schistosomula in the penetration glands of cercariae (reviewed in reference 55) and may function as a receptor for Fc (94). 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 (85). 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* (117).

**FABPs.** 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 (21). 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 (144), Sm14 failed to induce protection levels of >40% when tested in other laboratories (49) and as part of the WHO/TDR-sponsored trials (8). Coadministration of recombinant Sm14 protein with either IL-12 (49) or tetanus toxin fragment C (2) 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- $\gamma$  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 compa-

table to that obtained by immunization with three doses of recombinant Sm14 protein (151).

## CURRENT STATUS OF VACCINE DEVELOPMENT FOR *S. JAPONICUM*

### Basic Considerations

Vaccine development against *S. mansoni* and *S. haematobium* necessitates the use of clinical vaccines for human application. The zoonotic transmission of schistosomiasis japonica allows for a complementary approach for *S. japonicum* involving the development and deployment of a transmission-blocking veterinary vaccine in livestock animals, particularly bovines (58, 101, 175). The vaccine would be used in reservoir hosts of *S. japonicum* to potentially reduce transmission to humans. Bovines (cattle and water buffaloes) are the major reservoirs for *S. japonicum* infection in China, with estimates that 90% of egg contamination comes from this source (29). Schistosomiasis japonica was once highly prevalent in other domestic animals in China, such as pigs, but in recent years, these animals have been of less importance because they are usually restricted to pens, with limited access to the marshland areas. Sheep and goats are also infected, but to a far lesser extent, and as wild animals become rarer, their involvement in transmission can probably be ignored (29).

The results of control technology advances, including the success of recent World Bank inputs, were used by Williams et al. (160) to mathematically model prospects for the future control of schistosomiasis in China. Another mathematical model of the dynamics and control of *S. japonicum* transmission on Bohol Island, Philippines, was developed by Ishikawa et al. (68). Furthermore, a bovine drug intervention trial (57) was recently concluded in communities of schistosome endemicity in Jiangxi Province, China, which indicated that buffaloes are responsible for 75 to 80% of schistosomiasis transmission in the marshland areas, underpinning the rationale for developing a veterinary vaccine against *S. japonicum*.

As with the African schistosomes (*S. mansoni* and *S. haematobium*), a human vaccine may be required for use against schistosomiasis japonica in the Philippines, given that the epidemiological studies that have been carried out there have emphasized the involvement of humans, not animal reservoirs, as the principal cause of *S. japonicum* transmission (13).

### Major Candidate Vaccines and Their Protective Efficacies

Considerable efforts have been aimed at the identification of relevant *S. japonicum* antigens that may be involved in inducing protective immune responses, with a view to developing them further as viable vaccines. Vaccination can be targeted either towards the prevention of schistosome infection or to the reduction of parasite fecundity. A reduction in worm numbers is the gold standard for antischistosome vaccine development, but because schistosome eggs are responsible for both pathology and transmission, a vaccine targeted at parasite fecundity and egg viability is also relevant.

Some of the leading *S. japonicum* vaccine candidates (as recombinant protein and/or DNA vaccines) are discussed below; the protective efficacies of these and other molecules in

TABLE 2. *S. japonicum* protein vaccines that have shown efficacy in the mouse model and in reservoir hosts of schistosomiasis japonica<sup>a</sup>

Antigen (native or recombinant protein)	Abbreviation	Size (kDa)	Stage	Biological function	Worm burden reduction <sup>b</sup> (%)	
					Mouse	Other hosts
Paramyosin (native)	Sj97	97	Schistosomula, adults	Contractile protein plus others	27–86	31–48 (sheep/cattle)
Paramyosin (recombinant)	Sj97	97	Schistosomula, adults	Contractile protein plus others	20–60	17–60 (water buffaloes/pigs/sheep)
Paramyosin (recombinant fragments)	Sj97	97	Schistosomula, adults	Contractile protein plus others	33–77	
TPI (native)	SjTPI	28	All stages	Enzyme	21–24	
Integral membrane protein (recombinant)	Sj23	23	Adults	Membrane protein	27–35	32–59 (water buffaloes/cattle/sheep)
Aspartic protease (recombinant)	SjASP	46	All stages	Digestion of hemoglobin	21–40	
Calpain large subunit (recombinant)	Calpain	80	All stages	Protease	40–41	
28-kDa GST (recombinant)	Sj28GST	28	All stages	Enzyme	0–35	33–69 (water buffaloes/sheep)
26-kDa GST (recombinant)	Sj26GST	26	All stages	Enzyme	24–30	25–62 (water buffaloes/cattle/pigs/sheep)
Signaling protein 14-3-3 (recombinant)	Sj14-3-3	30	All stages?	Molecular chaperone	26–32	
FABP (recombinant)	Sj14	14	All stages?	Binds fatty acids	34–49	32–59 (rats/sheep)
Serpin (recombinant)	Serpin	45	Adults	Serine proteinase inhibitor	36	
SVLBP (recombinant)	SjSVLBP	20	Adult males	Binds lipoproteins	34	
Ferritin (recombinant)	SjFer	450	All stages?	Iron storage	35 <sup>c</sup>	

<sup>a</sup> Adapted from reference 99 with permission from Blackwell Publishing, with additional data from references 53 and 172.

<sup>b</sup> Egg reduction (in feces and/or liver) was also recorded for many of the candidates. When evaluated, reduced egg-hatching capacity of *S. japonicum* eggs into viable miracidia occurred with some vaccines.

<sup>c</sup> Mucosal immunization.

different host animals are summarized in Tables 2 and 3. The majority are membrane proteins, muscle components, or enzymes, and further details of the characteristics and efficacies of these and other vaccine candidates can be found elsewhere (99, 101, 166).

**Sj26GST.** The GSTs are a group of enzyme isoforms that catalyze the detoxification of lipophilic molecules by thio-conjugation. In light of their physiological importance, a number of research groups have investigated the potential of the GSTs as vaccine targets for *S. mansoni* and *S. haematobium* (see above). Some encouraging data are available for the protective efficacy of Sj28GST against *S. japonicum* in different mammalian hosts in China (99, 166) (Tables 2 and 3), but more recent work has focused on the 26-kDa isoform. Recombinant Sj26GST (rSj26GST) induces a pronounced antifecundity 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* (166). Similar levels of vaccine efficacy were obtained in water buffaloes vaccinated with purified rSj26GST (166). Anti-Sj26GST antibodies were produced in the immunized water buffaloes, and following challenge with *S. japonicum* cercariae, the typical antifecundity effect was manifest, characterized by significant decreases in fecal egg output and in eggs deposited in host tissues, with those in the liver and intestine being reduced about 50%. In addition to the antifecundity effect, rSj26GST reduced the egg-hatching capacity of *S. japonicum* eggs into viable miracidia by nearly 40%. Recent field trials have demonstrated that the protective effect of the rSj26GST vaccine against *S. japonicum* can be maintained in cattle and water buffaloes for at least 12 months (99, 166).

**Paramyosin (Sj97).** Paramyosin is a 97-kDa myofibrillar protein with a coiled-coil structure and is found exclusively in invertebrates. It is expressed on adults and the tegumental surfaces of lung-stage schistosomula and appears to be multifunctional. It may act as a receptor for Fc (94), and an exogenous form inhibits activation of the terminal pathway of complement, implying an important immunomodulatory role in schistosomiasis (55). Native and recombinant paramyosin (Sj97) proteins confer protection against *S. japonicum* in mice, water buffaloes, and other mammalian hosts (166). Furthermore, recent studies of human antibody isotype (109) and Th2 cytokine responses to Sj97 add further support to this molecule as a leading vaccine candidate against *S. japonicum* (89). Unfortunately, a major challenge with Sj97 is its poor expression in soluble form, probably due to its coiled-coil structure and its large size. To improve its expression and to identify protective epitopes on paramyosin, the published Sj97 (Chinese) cDNA sequence was recently redesigned using *Pichia* codon usage and divided into four overlapping fragments (fragments 1, 2, 3, and 4), of 747, 651, 669, and 678 bp, respectively (172). These gene fragments were synthesized and expressed in *Pichia pastoris* (fragments 2 and 3) or *Escherichia coli* (fragments 1 and 4). The recombinant proteins were produced at high levels and purified, and BALB/c mice were immunized with the purified proteins formulated in the adjuvant Quil A. The protein fragments were highly immunogenic, inducing high, though variable, enzyme-linked immunosorbent assay antibody titers, and each was shown to resemble native paramyosin in terms of its recognition by the antifragment antibodies in Western blots. Promising protective efficacy in terms of significant reductions in worm burdens, worm pair numbers, and liver eggs in vacci-

TABLE 3. *S. japonicum* DNA vaccines that have been tested against *S. japonicum* in the mouse model and in reservoir hosts of schistosomiasis japonica<sup>a</sup>

Host	Vector	Antigen	Method of immunization <sup>b</sup>	Worm burden reduction (%)	Liver egg burden reduction (%)
Mouse	VR1020	Sj22 (22-kDa tegument antigen)	Gene gun	0	0
	pcDNA1/Amp	Sj26	IMI	0	Not evaluated
	pcDNA1/Amp	Sj97 (paramyosin)	IMI	35–40	50–80
	VR1020	Sj62, Sj28-GST, Sj23, and Sj14-3-3	IMI (cocktail vaccine with or without IL-12 plasmid)	40	Not evaluated
	pcDNA3.1	Sj23	IMI with or without IL-12 plasmid	27–35	22–28
	pcDNA3.1	Sj23	IMI with or without CpG immunostimulatory sequence	28–35	22–27
	pcDNA3.1	SjTPI	IMI with or without IL-12 plasmid	30–33	44–53
	pVIVO2	Sj14	IMI	40	NA <sup>c</sup>
	pVIVO2	Sj23	IMI	38	NA <sup>c</sup>
	pVIVO2	Sj14/Sj23	IMI	52–54	NA <sup>c</sup>
Pig (laboratory trial)	pcDNA3.1	Sj23/Sj14	IMI	41–53	NA <sup>c</sup>
		Cocktail of Sj14/Sj23 and Sj23/Sj14	IMI	59	NA <sup>c</sup>
		SjTPI	IMI with or without IL-12 plasmid	46–60	49–66 <sup>c</sup>
		SjTPI	IMI with or without HSP-70 plasmid and IL-12 plasmid	42–51	42–62 <sup>d</sup>
		Sj23	IMI with or without IL-12 plasmid	29–59	48–56
		Sj23	IMI	42	29–56
		Sj28GST	IMI	30–65	43–72
		Sj23	IMI	0	30–60
		Sj28GST	IMI	16	30–60
		Sj23	IMI	38	38
Water buffalo (laboratory trial)	VR1020	Sj28GST	IMI	39	19
	VR1020	Sj23	IMI	33	34
	VR1020	Sj28GST	IMI	44	19
	VR1020	Sj23 + Sj28GST	IMI	38	48
	VR1020	Sj31 (VRSj31) + Sj32 (VRSj32)	IMI plus boost with rSj31, rSj32, and complete Freund's adjuvant	21–32	48–52 <sup>d</sup>
Water buffalo (field trials)	VR1020	Sj28GST	IMI	39	19
	VR1020	Sj23	IMI	33	34
	VR1020	Sj28GST	IMI	44	19
	VR1020	Sj23 + Sj28GST	IMI	38	48
	VR1020	Sj31 (VRSj31) + Sj32 (VRSj32)	IMI plus boost with rSj31, rSj32, and complete Freund's adjuvant	21–32	48–52 <sup>d</sup>
Cattle (field trials)	VR1020	Sj28GST	IMI	39	19
	VR1020	Sj23	IMI	33	34
	VR1020	Sj28GST	IMI	44	19
	VR1020	Sj23 + Sj28GST	IMI	38	48
	VR1020	Sj31 (VRSj31) + Sj32 (VRSj32)	IMI plus boost with rSj31, rSj32, and complete Freund's adjuvant	21–32	48–52 <sup>d</sup>
Goat (laboratory trial; prime-boost)	VR1020	Sj28GST	IMI	39	19
	VR1020	Sj23	IMI	33	34
	VR1020	Sj28GST	IMI	44	19
	VR1020	Sj23 + Sj28GST	IMI	38	48
	VR1020	Sj31 (VRSj31) + Sj32 (VRSj32)	IMI plus boost with rSj31, rSj32, and complete Freund's adjuvant	21–32	48–52 <sup>d</sup>

<sup>a</sup> Adapted from reference 99 with permission from Blackwell Publishing, with additional data from references 142, 170, 171, 173, 174, and 178.

<sup>b</sup> IMI, intramuscular immunization.

<sup>c</sup> Granuloma size was reduced. NA, not available.

<sup>d</sup> Fecal egg reduction and/or reduced egg-hatching capacity of *S. japonicum* eggs into viable miracidia was also recorded.

nated mice resulted, but there was no apparent correlation between the antibody titers generated and protective efficacy, as all fragments produced effective but similar levels of protection. These fragments now need to be tested further for protective potency, both separately and in combination, in larger animals, including water buffaloes. Full-length DNA vaccines coding for Sj97 have been shown to induce protective immunity in mice (30), confirming previous studies (99).

**SVLBP.** An expressed sequence tag of *S. japonicum* encoding an *S. japonicum* very-low-density lipoprotein binding protein (SVLBP; molecular size, 20 kDa) was reported to be membrane associated and located in the teguments and subteguments of adult male schistosomes (45). Given that SVLBP may play an essential role in lipid acquisition by the parasite and/or in signal transduction pathways, its further investigation for development as a novel antischistosomal intervention was

warranted. Accordingly, Gan et al. (53) 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 were 33.5% and 47.6% lower, respectively, than those from control mice. There was also a marked increase in the antibody response in vaccinated mice: the titers of IgG1, IgG2a, and IgG2b of the vaccinated group were significantly higher than those of the controls. In a comparison of the reactivities of sera from healthy individuals and patients with rSVLBP, recognition patterns against this parasite tegumental antigen varied among different groups of individuals. Notably, the average titer of anti-rSVLBP antibody in sera from fecal egg-negative individuals was significantly higher than that in sera from fecal egg-positive individuals, which may reflect SVLBP-specific protection. These results suggest that the parasite tegumental protein SVLBP is a promising candi-



date for further investigation as a vaccine antigen for use against schistosomiasis japonica, but further testing is now required to assess its true value.

**Serine protease inhibitor (serpin).** Serine proteinase inhibitors (serpins) represent an important superfamily of endogenous inhibitors that regulate proteolytic events active in a variety of physiological functions. Yan et al. (169) used immunological screening of an *S. japonicum* adult worm cDNA expression library with sera of *Microtus fortis*, a naturally resistant rodent host, and identified one clone that encoded a sequence homologous to those of the serpin superfamily. The full-length sequence encoding *S. japonicum* serpin was amplified from adult worm cDNA by using 5' rapid amplification of cDNA ends-PCR (5' RACE-PCR) and subsequently cloned into the prokaryotic expression vector pET28c. The full-length *S. japonicum* serpin fusion protein with a His tag was expressed in *E. coli*, purified by affinity chromatography, and used to immunize rabbits. The *S. japonicum* serpin is located on the tegument in *S. japonicum* adult worms. C57BL/6 mice immunized with *S. japonicum* serpin induced the production of high levels of specific IgE and IgG1 subclass antibodies as well as a marked IL-4 response. Lymphocyte surface marker analysis revealed the proliferation of CD19-expressing B cells, indicating a predominant Th2-type response to *S. japonicum* serpin. Immunized mice developed moderate protection against infection with *S. japonicum*, as demonstrated by 36 and 39% reductions in the recovery of adult worms and eggs, respectively. Further vaccine-challenge experiments with *S. japonicum* serpin, modifying the delivery of the vaccine and testing different adjuvant formulations, will enable a better assessment of its potential as a vaccine candidate.

**SjTPI.** 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. Since the two schistosome TPI sequences are very similar (84% identity), it was logical to assess the protective efficacy of *S. japonicum* TPI (SjTPI). Encouraging results were obtained with Chinese SjTPI (SjCTPI) plasmid DNA in early experiments on mice (177), but to examine the transmission-blocking potential in larger animals, Zhu et al. (178) determined its vaccine efficacy in naive pigs. Pigs were vaccinated with the TPI DNA plasmid, alone or in conjunction with IL-12 plasmids, via intramuscular injection. Pigs vaccinated with SjCTPI DNA alone had adult worm burdens that were reduced 48.3%; a further decrease in adult worm burdens was not seen in the group vaccinated with SjCTPI DNA in conjunction with IL-12 (46.2% reduction). The SjCTPI DNA vaccines had a more pronounced effect on reducing female 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 (170). Although these data are encouraging, further extensive experimental and field-based nat-

ural challenge trials on bovines, particularly water buffaloes, in China are now required to determine whether vaccination with the SjCTPI DNA vaccine will likely reduce transmission by reducing adult worm burdens and worm egg output, with simultaneous reduction of hepatic egg-associated pathology.

**Twenty-three-kilodalton integral membrane protein (Sj23).** As with TPI, the tetraspanin integral membrane protein (Sm/Sj23) was identified as a major vaccine candidate some years ago, first against schistosomiasis mansoni and then against schistosomiasis japonica. The Chinese *S. japonicum* form (SjC23) was initially shown to induce protection in mice as a synthetic peptide vaccine and then, as a plasmid DNA vaccine, also induced protection in mice, sheep, pigs, and water buffaloes. Overall, the results from extensive plasmid DNA vaccine studies indicated that vaccination with SjC23 DNA not only induced significant reductions in worm and egg burdens but also significantly reduced the size of egg granulomas; thus, like SjCTPI, SjC23 produced an antipathology effect as well. The protective effect of the SjC23 plasmid DNA vaccine was enhanced with IL-12 in pigs (175) and mice (53, 176) and by a CpG immunostimulatory sequence in mice (173). 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.

**SjFABP (Sj14).** Like other parasitic helminths, schistosomes are unable to synthesize long-chain fatty acids or sterols and hence are completely dependent on the host for these components. FABPs 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. Especially noteworthy are the studies by Liu et al. (92), who expressed SjFABPc in *E. coli* and in baculovirus/silkworm systems. The recombinant protein from *E. coli* was a 41-kDa GST fusion protein (rSj14/GST), which could be purified by glutathione-agarose affinity chromatography, with a yield of 25 mg/liter *E. coli* culture. The recombinant protein from the baculovirus/silkworm system was an 18-kDa fusion protein (rSj14/His), which could be purified by Ni-nitrilotriacetic acid resin chromatography, with a yield of 3.5 mg per silkworm larva. Both rSj14/GST and rSj14/His were recognized by *S. japonicum*-infected mouse sera and anti-rSj14/GST mouse sera in Western blots. The purified recombinant protein was immunogenic in several mammalian host species, and 34.3%, 31.9%, and 59.2% worm reductions were obtained in Sj14/GST-vaccinated Kunming mice, Wistar rats, and sheep, respectively, compared to nonvaccinated control groups. Worm reductions of 48.8% and 49.0% were recorded for BALB/c mice immunized with Sj14/His compared to nonvaccinated and BCG-vaccinated groups, respectively. Taken together, these results emphasize

the promise of SjFABPc as a candidate vaccine for schistosomiasis japonica, particularly as no adjuvant was used in the rat and sheep vaccination experiments.

Another group (174) studied the protective efficacy of SjFABPc as a DNA vaccine enhanced by IL-12 in mice challenged with *S. japonicum*. They showed that IL-12 drives the immune response toward a Th1 direction and enhances the protective effect of the vaccine. Bivalent DNA vaccine constructs encoding SjFABPc and Sj23 provided higher levels of protective efficacy against *S. japonicum* in mice than those obtained with the univalent DNA vaccines (171).

**Calpain.** Calpain is efficacious against *S. mansoni* (see above) and is also recognized as an encouraging vaccine candidate against schistosomiasis japonica (110). When BALB/c mice were immunized with purified recombinant *S. japonicum* calpain emulsified in complete Freund's adjuvant, significant reductions in the number of recovered worms (Table 2) and also in egg production per female worm were observed. Furthermore, 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- $\gamma$  by activated CD4<sup>+</sup> T cells, and subsequent work with calpain-specific mouse T-cell hybridomas identified the T-cell epitope (EQLKIYAQRC) involved (112). Localization studies have shown that calpain is present in the penetration glands and in the secretions of cercariae (83).

#### NEW ANTIGEN DISCOVERY FOR VACCINES AGAINST SCHISTOSOMES

The current *Schistosoma* vaccine candidates may prove not to be the most effective. It is important to identify new target antigens and to explore alternative vaccination strategies to improve vaccine efficacy. The available schistosome antigens and prototype vaccine formulations induce 40 to 50% protection in animals, at best, using the standard readouts of reduced worm burden or egg production and viability. This apparent efficacy ceiling (for antigen combinations as well) has proved a significant roadblock to success. Accordingly, the current model vaccines may not be sufficiently protective or characterized by reproducible efficacy. Difficulties in obtaining good expression levels and in scaling up production according to good laboratory practice/GMP standards for the limited number of antigens selected have turned out to be another major obstacle. Some frontline candidates have suffered from difficulties in scale-up production according to good laboratory practice/GMP standards and have been dropped. The feasibility of large-scale production should be a prime selection criterion in assessing the vaccine candidacy of schistosome antigens (9).

Mining and functional annotation of the greatly expanded *S. mansoni* (154) and *S. japonicum* (67) transcriptomes and their public accessibility through public databases (<http://verjo18.iq.usp.br/schisto/>, <http://www.genedb.org/genedb/smansonii/index.jsp>, and <http://lifecenter.sgst.cn/en/schistosomaDispatch.do?disName=intro>), in combination with postgenomic technologies, including DNA microarray profiling, proteomics, glyco-

mics, and immunomics, have the potential to identify a new generation of potential vaccine target molecules that may induce greater potency than the current candidate schistosome antigens. Perhaps the most important advance in postgenomics for schistosomiasis has been the successful application of RNA interference (RNAi) to schistosomes (20, 82). These studies have had (and will continue to have) an enormous impact on our ability to determine the functions of schistosome genes/proteins and which ones are essential for survival and reproduction. Silencing the expression of numerous *S. mansoni* genes has resulted in phenotypic changes (34, 52, 84), highlighting their importance as targets for vaccines and new drugs. Genome-wide RNAi has been used to assess the functions of most genes from the free-living nematode *Caenorhabditis elegans* (reviewed in reference 122), and the eventual application of this technology to schistosomes will revolutionize the way we search for (and test) vaccine and drug targets.

Molecules containing signal peptides and signal anchors as predictors of excretory-secretory products, including enzymes, and components exposed on the schistosome epithelial surfaces (including receptors) that interact directly with the host immune system are highly relevant targets for schistosome vaccines (28, 71, 95, 137). The burgeoning area of schistosome genomics and postgenomic research has been reviewed extensively (62, 66, 102, 103, 123, 162, 164), but one important point that needs to be made is that the majority of studies have been undertaken on *S. mansoni* and *S. japonicum*; there is almost a complete absence of transcriptome/genome information for *S. haematobium*, and this is clearly an important area for future study.

There is an abundance of reports on schistosome antigens (from different anatomic locations within different stages of the parasite) that provide in the vicinity of 30% reductions in adult worm burdens. The tegument is where many researchers have focused their efforts, but it is those few tegument proteins which are truly exposed to the host immune system in a live worm—the tegument plasma membrane proteins—which, in our opinion, should be a major focus for future vaccinology efforts (95). Where investigated, membrane-spanning proteins of the tegument, e.g., the tetraspanins and Sm29, have shown great promise (Table 1). This subset of exposed proteins (16, 17), which present extracellular regions of various sizes outside the cell, should attract much more attention in the future, and we advocate that efforts of schistosomiasis vaccine laboratories would be better invested in developing methods to produce and deliver schistosome surface antigens (see below) or secreted molecules than in continuing to identify new intracellular antigens that show modest protection at best.

#### ANTIGEN FORMULATION AND DELIVERY OF VACCINES AGAINST SCHISTOSOMES

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 (9).

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. Traditional approaches have seen Freund's adjuvants used when antigens are first being assessed as vaccines in the mouse model. It must be remembered, however, that Freund's complete adjuvant, although the mainstay of immunological adjuvants in research for decades, is not suitable for human application, as it can produce a number of undesirable side effects that include the formation of local inflammatory lesions at the site of the injection that can result in chronic granulomas and abscesses. Once efficacy has been proven with Freund's adjuvants, other adjuvants, particularly those that are licensed (or have the potential for licensing) for human use, should be used to formulate an antigen. Less conventional or less widely used approaches have been explored as adjuvants for schistosome vaccines, including live *Salmonella* (113), tetanus toxin (2), filamentous phages (124), recombinant *Mycobacterium bovis* BCG (151, 152), nanoparticles (46), and various methods of mucosal delivery (88, 121, 140).

Before a well-informed decision can be made on adjuvant selection, a comprehensive understanding of the desired immune response (phenotype) is necessary. This, in turn, implies that the immune parameters required to obtain optimal protection are known. For human schistosomiasis, this is not the case. For example, very few people develop natural resistance to the parasite in the absence of repeated antihelminthic therapy (see previous section on PR individuals). We advocate the use of such cohorts to guide vaccine development (both antigen discovery and the phenotype of the protective response), but in reality, a schistosome vaccine will be delivered as part of an integrated control package that involves PZQ treatment before vaccination. Therefore, should we look more to the people who develop resistance to reinfection after PZQ therapy (158)? These two groups of individuals have very different immune responses to different antigens on different stages of the parasites (32, 108, 158). All of this information is relevant, albeit complicated, to deciding how best to formulate and deliver a vaccine for human schistosomiasis. If we are to target Toll-like receptors (TLRs) on antigen-presenting cells that induce a Th1 response, such as TLR-9, then adjuvants such as unmethylated CpG dinucleotides are attractive, and although not yet widely used for schistosomiasis vaccinology, these adjuvants are showing promise for experimental vaccines against other parasites (38). Indeed, the PR individuals identified in Brazil (32), who were utilized to identify two new tegument antigens (28, 147), mount a vigorous Th1 response to schistosomulum surface antigens, making CpGs a potentially attractive adjuvant for these vaccines. CpGs are being used in conjunction with more conventional adjuvants, such as alum, which induces a more Th2-like immune response. For the diphtheria-tetanus-pertussis vaccine, which is currently formulated with alum, the addition of CpGs reduced the total IgE levels and increased anti-pertussis toxin IgG2a in comparison with the ordinary diphtheria-tetanus-pertussis-alum vaccine (139). If a mixed Th1/Th2 response is optimal for a schistoso-

miasis vaccine, combination adjuvants such as alum-CpG seem to be a suitable way forward.

## CONCLUSION

Taking the breadth of consolidated international efforts to generate antischistosome vaccines, there is considerable optimism that these endeavors will prove successful. In our opinion, the most recent quantum leaps forward in schistosomiasis vaccinology have been the integrated genomic and proteomic studies that have now equipped us with all the information (for antigen selection at least) we need to choose the best antigens for a schistosomiasis vaccine. Again, in our opinion, we emphasize that the apical membrane proteins expressed on the surfaces of the schistosomulum and the adult worm are the logical vaccine targets on which to focus, and recent published data with some of these proteins support this hypothesis (28, 95, 147). Moreover, there are mRNAs encoding novel, putatively secreted proteins without known homologues that are lodged in the tegument membrane (16, 17), and these have yet to be explored. Indeed, there are very few descriptions of schistosomiasis vaccine trials with proteins that are completely unique to schistosomes and do not share sequence identity with any other proteins.

Once they are developed and employed, antischistosome vaccines will not be a panacea. They need to be regarded as one component, albeit a very important one, of integrated schistosomiasis control programs that complement existing strategies, including chemotherapy and health education. Although debatable, PZQ resistance is either here or on the horizon, and the need for vaccines is now more pressing than ever.

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