Review

Progress in the Development of a Vaccine against Schistosomiasis in China

Liu Shuxian, MD;* Song Guangchen;* Xu Yuxin;* Donald P. McManus, PhD;† and Peter J. Hotez, PhD‡

Schistosomiasis japonica, a zoonotic disease caused by the Asian schistosome or blood fluke, *Schistosoma japonicum*, is still recognized as a major public health problem in China and is, perhaps, the nation's most important parasitic disease. An estimated 1.5 million people are infected, and over 40 million people are at risk for the disease in the Yangtze River valley provinces.[1] Thousands of new cases of acute schistosomiasis have been reported in recent years from endemic areas. A variety of measures have been adopted in an integrated approach to control schistosomiasis japonica in China. These measures rely predominantly on mass chemotherapy with the drug praziquantel; however, efforts to control schistosomiasis in China with extensive use of praziquantel administered to infected patients have been thwarted by three major problems. First and foremost, high rates of reinfection with schistosomes occur within months following treatment, particularly in hyperendemic areas.[1,2] Second, there are potential and proven concerns about emerging praziquantel drug resistance.[1,2] Third, the reservoir of domestic animal hosts, including cattle, buffaloes, pigs, and sheep, represent a significant source for zoonotic transmission of *S. japonicum* to humans; these animal reservoirs are generally not targeted for mass chemotherapy.[1,2]

As an alternative approach to the control of schistosomiasis japonica, we have genetically engineered schistosome vaccine antigens that significantly reduce host morbidity when used as immunogens. The vaccines are potentially suitable for both humans and animal hosts that act as reservoirs. This review deals with the ongoing progress in the development of a schistosomiasis japonica vaccine in China. All studies were done with a Chinese strain of *S. japonicum*, originally collected in *Oncomelania hupensis hupensis* snails from Guichi county, Anhui province.

GOALS FOR VACCINATION

With the exception of nematode parasites belonging to the genera *Strongyloides* and *Capillaria*, parasitic helminths do not replicate in their mammalian hosts. For that reason, the amount of disease caused by parasitic helminth infection is usually proportional to the worm burden. Therefore, the observation that sterilizing immunity has never been achieved with an immunogen need not discourage us from attempts at developing helminth vaccines. Instead, the goals of vaccination are either to reduce the worm burden to the point below a threshold at which disease could result, or to elicit antidisease effects directly by immunization. In the case of acute hepatosplenic schistosomiasis, disease occurs when schistosome eggs are deposited in the liver and intestinal wall to cause fibrosis.

Our major aim is to reduce the morbidity of schistosomiasis either by reducing the number of schistosome worm pairs or by limiting egg deposition in the liver and intestine. An antidisease vaccine might also reduce fecal egg excretion; in the case of schistosomiasis japonica (but probably not in that of *S. mansoni* or *S. haematobium*), a vaccine could be used to immunize domestic animal reservoirs and limit egg excretion, which might otherwise result in zoonotic transmission to humans.

Live Attenuated Schistosome Vaccines

The initial approach to the development of a schistosome vaccine in China relied predominantly on live attenuated larval vaccines. Cercariae and schistosomula, attenuated with gamma rays, x-rays, or ultraviolet (UV) irradiation, have been reported to elicit protective immunity against schistosomiasis. Overall, immunization with radiation-

*Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai, China; †Queensland Institute of Medical Research, Brisbane, Queensland, Australia; ‡Yale University School of Public Health, New Haven, Connecticut, USA.

Ongoing schistosomiasis japonica vaccine studies are partially supported by a grant of the Tropical Medicine Research Center (TMRC) of the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health, Bethesda, MD, (NIH 1 P50 AI39461).

Address correspondence to Prof. Liu Shuxian, Chief, Department of Immunology, Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai 200025, China.

attenuated cercariae reduces the worm burden between 33% and 77% in laboratory mice. Vaccination of mice to cercariae of *S. japonicum* attenuated with 300, 400, and 500 μw/min/cm^2 resulted in a reduction of the worm burden of 49%, 69%, and 60%, respectively ($P < 0.05$).[3] Moreover, the reduction of eggs both in the liver (57–69%) and intestine (76–80%) tissues was significant ($P < 0.01$).[4] There was no correlation between the amount of resistance and whether single or multiple immunization had been carried out. Protection using UV-attenuated cercariae lasted 5 to 7 months and was noted to be associated with both humoral immunity and natural killer cells recovered from the spleen of immunized mice.[5] Comparable reductions in worm burden resulted when mice were immunized with gamma ray–irradiated cercariae of *S. japonicum*. The maximal protection against *S. japonicum* challenge-infections was obtained by immunizing mice with 500 cercariae exposed to 24 to 48 krad.[6] Both the number of proliferating T lymphocytes and the amount of serum IgG antibody recovered from vaccinated mice were noted to increase 14 days after immunization and peaked on day 28 post-challenge.[6,7]

Protection against challenge infections using radiation-attenuated schistosomes has also been observed in domestic pigs, sheep, and buffaloes. These domesticated animals represent a significant reservoir of *S. japonicum,* and their vaccination offers an approach to control schistosomiasis by interrupting its zoonotic transmission. Pigs and buffaloes, immunized with three doses each of 10,000 UV-irradiated (400 μw/min/cm^2) *S. japonicum* cercariae were protected to 90% against challenge infections of 1000 untreated cercariae ($P < 0.001$).[8] The challenge cercariae were administered either 2.5 or 6 months after porcine immunization and 90 days after bovine immunization. Just as in immunized mice, the fecundity of female schistosomes was reduced in both immunized pigs (over 90% reduction) and buffaloes (86% reduction; $P < 0.001$).[8,9]

Bovine intradermal immunizations using either cryopreserved irradiated (CI) or freeze/thawed (FT) schistosomula in the presence of bacillus Calmette-Guérin (BCG) were also effective in reducing the *S. japonicum* worm burdens compared with BCG controls. A single intradermal vaccination in buffaloes and cattle with 10,000 CI (20 krad) schistosomula provided 62% and 55% worm reduction, respectively ($P < 0.05$).[10] Intradermal vaccination with 30,000 FT schistosomula with BCG provided 57% protection ($P < 0.05$).[10] Comparable rates were obtained in CI and FT schistosomula-immunized sheep.[11] The intradermal immunizations elicited specific proliferative cellular immune responses as early as 4 weeks after challenge. The lymphoproliferative responses were transient and were significantly diminished at 8 weeks after challenge. Responses of IgG antibody developed somewhat later, at 6 weeks post-challenge, but were found to persist for as long as 14 weeks after the challenge.[10]

In addition to ionizing radiation, the alkylating mutagen N-methyl-N′-nitro-N-nitrosoguanidine (NTG) will also attenuate a multicellular organism, but without diminishing its immunogenicity.[12] In order to prepare the immunogen, cercariae are typically attenuated by exposing them to NTG at a concentration of 30 μg/mL for 15 minutes. Immunization of NTG-attenuated cercariae elicits high levels of protection as measured by reductions in the worm burden (77%; $P < 0.05$), and hepatic granulomas.[13,14] Prominent antibody and cellular immune responses can be measured after NTG-attenuated cercarial immunizations.[15]

Thus, the use of the live cercarial vaccines attenuated either by ionizing radiation or alkylating mutagens, offers an approach to reduce the worm burden and the hepatosplenic pathology in laboratory and domestic animals. The live vaccines are suitable for inducing protection in the laboratory, but they are generally not considered a practical means of vaccinating humans or domestic animals in the field. The reasons for this include: (1) the production costs and labor-intensive efforts required to obtain large numbers of cercariae from infected *Oncomelania* snails; (2) the difficulties in standardizing the dose of ionizing radiation in order to induce cercarial attenuation; (3) the requirement for cryopreservation in order to transport attenuated cercariae over long distances; and (4) the potential toxicity of administering a live vaccine. To circumvent these problems, our group and others in China have attempted to reproduce or even improve the protection afforded by attenuated cercarial vaccines by substituting chemically defined schistosomal antigens genetically engineered in bacteria or yeasts. To date, promising results have been obtained with three major classes of recombinant molecules, the enzyme glutathione S-transferase, triosephosphate isomerase, and paramyosin.

Glutathione S-Transferase (GST)

Pairs of adult schistosome worms, residing in intestinal veins, are exposed to high doses of toxic products when organic molecules enter the portal circulation from the host gut. Schistosomal GSTs are parasite-derived conjugating enzymes that presumably function to detoxify several classes of organic molecules. Some investigators have proposed that GSTs are a major target for praziquantel.[16] Each of the two major classes of schistosomal GSTs, the 26 kDa and the 28 kDa forms, in turn consists of several isoforms. Immunization of mice with the 26 kDa form (Sj26), which was genetically engineered from a Philippine strain of *S. japonicum,* resulted in modest (26 to 32%) protection upon challenge with a Chinese strain.[17–19] In order to improve on this result efforts were made to clone the corresponding cDNA from the Chinese strain. By glutathione agarose-affinity chromatography, both forms of GST were biochemically isolated from our

laboratory strain of Chinese *S. japonicum*.[20] Although a modest reduction in worm burden was also obtained by immunizing with these native products, we observed a striking reduction in the number of eggs excreted by female schistosomes recovered from their immunized murine hosts.[21] In order to follow up on these studies, we cloned a cDNA, coding for Sj26 from Chinese *S. japonicum* and expressed and purified the recombinant protein.[21] Immunization of mice with recombinant Sj26 (rSj26) with Freund's adjuvant resulted in a significant (71%, $P < 0.01$) reduction in the number of eggs excreted by female schistosomes with a corresponding reduction in liver granulomas. The worm burden was reduced by 29%.[21]

Therefore, immunization with Chinese rSj26 will elicit significant anti-fecundity effects in female schistosomes, which mature as a result of the challenge dose. Since each schistosome egg has the potential for causing a liver granuloma, the antifecundity effect of rSj26 vaccination will dramatically reduce the number of granulomas present in the liver. Thus, the disease resulting from acute hepatosplenic schistosomiasis can be eliminated by vaccination. To confirm the antidisease vaccine effect of rSj26 immunization, immunization and challenge experiments using rSj26 were undertaken in domestic animal reservoir hosts. Immunization with rSj26 using alum as the only adjuvant, resulted in a 54% reduction ($P < 0.05$) in the total number of eggs and a 72% reduction in the number of mature eggs in the livers of vaccinated pigs ($P < 0.01$).[22] These trials were subsequently confirmed in water buffaloes in which the pronounced antifecundity effects resulting from rSj26 immunization caused significant reductions in the number of eggs released into the feces and deposited in the liver, spleen, and intestinal mucosa and submucosa.[23] The hatching capacity of the schistosome eggs into viable miracidia was also impaired.[23] In order to improve the Chinese rSj26 vaccine, the cDNA was cloned and expressed in bacille Calmette-Guérin (BCG). For these studies, the cDNA was placed under the control of a *Mycobacterium tuberculosis* heat-shock protein-70-promoter prior to ligation into an *Escherichia coli / mycobacterium* shuttle plasmid (pBCG-2000).[24] The expressed rSj26 was found to represent approximately 18% of the total cellular protein. Large-scale field trials under natural conditions, using both purified rSj26 (expressed in *E.coli*) and BCG (expressing Sj26), are now underway in Hunan Province. These trials determine whether immunization of water buffaloes with these vaccines will reduce the excretion of eggs and the hepatosplenic schistosomiasis when the animals are allowed to graze in a hyperendemic area.

Paramyosin

Paramyosin is a major structural protein of thick filaments in invertebrate muscle. The absence of a homologous protein in vertebrates makes paramyosin a potentially useful candidate for a vaccine. The native 97kDa paramyosin was isolated from both adult Chinese schistosomes and its snail vector, *Oncomelania hupensis hupensis* using previously published methods,[25,26] and used as an immunogen in mice. Mice vaccinated with 20 μg of native paramyosin and Freund's adjuvant showed reductions in worm burden and egg excretion compared with adjuvant alone. Immunization with schistosomal paramyosin resulted in a 26% reduction in worm burden ($P < 0.01$) and a 40% reduction in egg excretion ($P < 0.05$), whereas immunization with snail paramyosin caused a reduction of 34% in worm burden ($P < 0.01$) and a 20% reduction in egg excretion, respectively.[18] Similar immunizations using BCG gave even less promising results.[25]

Sj31

A 31 kDa antigen of unknown function was isolated from adult Chinese schistosomes by ACA-54-super-gel chromatography and preparative gel electrophoresis.[28] Although immunization of mice with Sj31 did not reduce the worm burden upon challenge, it did result in a significant reduction, compared to controls, in fecal egg excretion (63–78%, $P < 0.01$), eggs in the liver (44%, $P < 0.01$), eggs in the intestinal wall (48%, $P < 0.01$), and eggs in the uteri of female schistosomes (53%, $P < 0.01$). In another laboratory *S. japonicum* 31/32 kDa proteins were also isolated on polyacrylamide slab gels and purified by elution from electrophoresis gels. Immunization with these proteins reduce the worm burden (28%; $P < 0.001$), the fecundity of female worms (71%; $P < 0.001$) and the size of egg granulomas in mouse livers (37–70%; $P < 0.001$).[29] Immunization resulted in a substantial increase in the number of nonviable eggs in the intestine and liver of immunized mice (59% and 66%) ($p < 0.01$).[28]

Triosephosphate Isomerase

Triosephosphate isomerase (TPI) isolated from adult Chinese *S. japonicum* by acetone precipitation and heat treatment,[16] was evaluated as a vaccine antigen in mice. Immunization of mice with TPI and Freund's adjuvant resulted in a significant reduction (58–60%, $P < 0.01$) in liver granulations. Some reduction in worm burden (21–25%, $P < 0.05$) was also obtained.[30] The TPI cDNA was obtained by a reverse-transcriptase polymerase-chain-reaction (RT-PCR) using total *S. japonicum* RNA as template and primers designed according to the published cDNA sequencing of *S. mansoni* TPI.[31] Expression of the TPI-cDNA of *S. japonicum* and evaluation of the vaccine potential for rTPI are in progress.

FUTURE STUDIES

The immunologic mechanisms leading to suppression of egg production and hatching induced by rSj26 and Sj31 remain to be elucidated. In addition, the duration of the observed antifecundity effects afforded by vaccination are not yet known. Studies are also underway to evaluate whether a "cocktail" of more than one antigen might improve existing subunit vaccines. Alternatively, immunizing with naked DNA coding for these antigens might obviate the need to isolate recombinant proteins or even improve protection by eliciting strong Th1 immune responses. Because DNA vaccines are more heat-stable than recombinant proteins, they are particularly attractive for use in the tropical and subtropical climates of China's Yangtze River valley provinces. Studies using GST and TPI DNA vaccines are in progress.

The genetic diversity of genes that code for vaccine antigens will also be investigated. For instance, although there is a high degree of predicted amino acid homology between the Sj26 genes of Chinese and Philippine strains of *S. japonicum*, there are substantial differences between the TPI genes of the two strains. The authors are currently investigating whether variations in cDNA and predicted amino acid sequences among different provincial strains are also sufficiently great to warrant producing designer-vaccines for each of the major Yangtze River valley provinces. For example, the rSj26 vaccine used in Anhui Province might one day differ from the one used in Sichuan Province.

Over the next few years we expect to obtain data on the efficacy of both rSj26 and rTPI vaccines currently being tested in water buffaloes under natural field conditions. Encouraging results in these studies will prompt investigations to determine whether veterinary use of the vaccines is sufficient to interrupt the transmission of schistosomiasis japonica to humans, or whether human vaccination will be required.

REFERENCES

1. WHO Technical Report Series 830. The control of schistosomiasis. Second report of the WHO Expert Committee, Geneva, Switzerland, World Health Organization, 1993: 50-76.
2. McManus DP. Helminth vaccines. Biotechnology 1992; 20:99-128.
3. Jiang CF, Wang Y, Tao J, et al. Experimental study of living vaccine against infection of *S. japonicum* with UV-attenuated cercariae of *S. japonicum* in mice. Chin J Zoonosis 1993; 9:17-20.
4. Lu FL, Han JJ, Li YL, et al. Study on resistance of mice vaccinated with UV-attenuated cercariae and schistosomula of *S. japonicum*. Chin J Parasitic Dis Contr 1996; 9:34-36.
5. Lu FL, Shi YE, Han JJ, et al. Dynamic changes of humoral immunity and NK cell activity in mice immunized with UV-attenuated cercariae of *S. japonicum*. Chin J Parasitic Dis Contr 1996; 9:93-95.
6. Li CM, Hu CR, Zhang GH, et al. Resistance against *S. japonicum* induced by immunization with highly γ-irradiated cercariae in mice. Chin J Schistoso Cont 1996; 8:12-16.
7. Xu ST, Wu H, Xu CB, et al. Immunity in mice vaccinated with cryopreserved and irradiated schistosomula of *S. japonicum*. Chin J Vet Sci Technol 1989; 12:8-10.
8. Shi YE, Jiang CF, Han JJ, et al. *Schistosoma japonicum*: an ultraviolet-attenuated cercarial vaccine applicable in the field for water buffaloes. Exp Parasitol 1990; 71:100-106.
9. Shi YE, Jiang CF, Han JJ, et al. Immunization of pigs against infection with *Schistosoma japonicum* using ultraviolet-attenuated cercaria. Parasitol 1993; 106:459-462.
10. Xu ST, Shi FH, Shen W, et al. Vaccination of bovines against Schistosomiasis japonica with cryopreserved, irradiated and freeze-thawed schistosomula. Vet Parasitol 1993; 47:37-50.
11. Xu ST, Shi FH, Wu H, et al. Tests to immunize sheep by freezing *Schistosoma japonicum* larva vaccines. Chin J Vet Sci Technol 1990; 20:19.
12. Johnson RP, Chi LW. Resistance to *Trypanosoma brucei* in mice following inoculation of NTG-treated trypanosomas. Am J Trop Med Hyg 1981; 30:952-954.
13. Chi LW, Liu SX, Audreus J, et al. Worm burden and lymphocyte response in mice immunized with N-methyl-N´nitro-N-nitrosoguanidine-attenuated cercariae of *S. japonicum*. Chin Med J 1988; 101:181-186.
14. Lui SX, Song GC, Nie JH, et al. Further study on the immunity induced by mutagenic NTG-attenuated cercaria of *Schistosoma japonicum*. Chin J Parasitol Parasitic Dis 1989; 7:89-92.
15. Lui SX, Song GC, Ding LY, et al. Humoral and cellular immunity in mice immunized with mutagenic NTG-attenuated cercaria of *Schistosoma japonicum*. Chin J Parasitol Parasitic Dis 1989; 7:267-270.
16. McTigue MA, William DR, Tainer JA. Crystal structures of a schistosomal drug and vaccine target: glutathione S-transferase from *Schistosoma japonica* and its complex with the leading antischistosomal drug praziquantel. J Molec Biol 1995; 246:21-27.
17. Liu SX. An overview of molecular parasitology in China. Chin Med J 1992; 105:91-96.
18. Liu SX, Song GC, Ding LY, et al. GST antigen from Chinese strain of *Schistosoma japonicum*. Chin J Parasitol Parasitic Dis 1992; 10:312-313.
19. Liu SX, Ding LY, Song GC, et al. The antigenicity of GST antigen extracted from Chinese strain of *Schistosoma japonicum*. Southeast Asian J Trop Med Public Health 1993; 24:61-64.
20. Liu SX, Song GC, Ding LY, et al. Comparative study on antigenicity and immunogenicity of 26-28kDa antigen and recombinant Sj26 (rSj26) of *Schistosoma japonicum*. Southeast Asian J Trop Med Public Health 1993; 24:65-69.
21. Liu SX, Song GC, Xu YX, et al. Immunization of mice with recombinant Sjc26 GST induces a pronounced anti-fecundity effect after experimental infection with Chinese *Schistosoma japonicum*. Vaccine 1995; 13:603-607.
22. Liu SX, Song GC, Xu YX, et al. Anti-fecundity immunity induced in pigs vaccinated with recombinant *Schistosoma japonicum* 26kDa glutathione-S-transferase. Parasite Immunol 1995; 17:335-340.
23. Liu SX, He YK, Song GC, et al. Anti-fecundity immunity to *Schistosoma japonicum* induced in Chinese water buffaloes (*Bos Buffelus*) after vaccination with recombinant 26kDa glutathione-S-transferase (roSic26GST). Vet Parasitol 1997; 69:39-47.
24. Cheng JH, Huangfu YM, Liong JQ, et al. Expression of the *S. japonicum* 26kDa antigen gene in mycobacteria and *Escherichia coli*. Acta Univ Med Tougji 1996; 25:173-179.

25. Lin JJ, Tian E, Fu ZQ, et al. The vaccination of BALB/c mice with *S. japonicum* paramyosin. Chin J Schistosomiasis Control 1996; 8:17-21.
26. Liu SX, Ding LY, Song GC, et al. Antigenicity induced by 24-26kDa and 97kDa protein "target antigen" of *Schistosoma japonicum*. Shanghai J Immunol 1989; 9:339-342.
27. Song GC, Xu YX, Liu SX. Protective immunity induced by native paramyosin isolated from adult worms of *Schistosoma japonicum* and snail muscle of *Oncomelania hupensis hupensis*. Ann Report Institute of Parasitic Dis, Chinese Academy of Preventive Medicine [Abstract] 1995:7-8.
28. Chen Y, Yi XY, Zhen XF, et al. Preliminary study on egg-resistant immunity in mice induced by *Schistosoma japonicum* adult worms 31/32kDa antigen. Chin J Schistosomiasis Control 1995; 7:72-74.
29. Shen DW, Li YL, Hang JJ, et al. Purification of 31/32kDa protein of adult *Schistosoma japonicum* and studies on their protective immunity. Chin J Parasitol Parasitic Dis 1993; 11:241-243.
30. Miao YX, Liu SX. The protective immunity in mice immunized with native TPI antigen from Chinese strain of *S. japonicum*. Chin J Parasitol Parasitic Dis 1996; 14: 257-261.
31. Miao YX, Liu SX, McManus DP, et al. Cloning and sequencing the cDNA gene encoding TPI antigen from the adult worms of *S. japonicum*. Chin J Parasitol Parasitic Dis 1997; 15:349-352.