Vaccination Against Schistosomiasis and Fascioliasis with the New Recombinant Antigen Sm14: Potential Basis of a Multi-Valent Anti-Helminth Vaccine?

Miriam Tendler, Monica Magno Vilar, Cristiana Alves Brito*, Nicolau Maués da Serra Freire*, Naftale Katz*, Andrew Simpson*/****

Laboratório de Esquistossomose Experimental, Departamento de Helmintologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Laboratório de Biologia Molecular, **Laboratório de Esquistossomose, Centro de Pesquisas René Rachou - FIOCRUZ, Av. Augusto de Lima 1715, 30190-002 Belo Horizonte, MG, Brasil, *** Universidade Federal Rural do Rio de Janeiro, RJ, Brasil

Molecular cloning of components of protective antigenic preparations have suggested that related parasite fatty acid binding proteins could form the basis of the well documented protective, immune cross reactivity between the parasitic trematode worms Fasciola hepatica *and* Schistosoma mansoni. *We have now confirmed the cross protective potential of parasite fatty acid binding proteins and suggest that it may be possible to produce a single vaccine that would be effective against at least two parasites,* F. hepatica *and* S. mansoni *of veterinary and human importance respectively.*

Key words: schistosomiasis - fascioliasis - vaccines - fatty acid binding protein (FABP)

Protective vaccination of Swiss outbred mice and rabbits with a soluble extract of adult *Schistosoma mansoni* (SE) resulted in 70 and 90% protection respectively, against cercarial challenge as previously reported (Tendler et al. 1986, 1991). One of the components of SE has been identified by gene cloning and sequencing as Sm14, a soluble lipid binding protein (Moser et al. 1991).

A recombinant form of the *S. mansoni*, fatty acid binding protein (FABP) rSm14 was produced (as a fusion protein with the 260 amino acids of the bacteriophage T7 major capsid protein under the control of the T7 gene 10 promoter) and used for experimental vaccination in outbred animals.

Protective activity of rSm14 was first assessed in NZ rabbits immunized and challenged with *S. mansoni* cercariae. The adult worm burdens in these animals was compared with positive controls vaccinated with SE, the saline extract within which Sm14 was originally identified (Moser et al. 1991) and non-vaccinated negative controls.

The results demonstrated that the recombinant molecule stimulated an extremely high level of protective immunity close to that stimulated by complete SE.

More extensive analysis of the protective activity of rSm14 was carried out in four large, independent experiments in Swiss mice. In all cases a high level of protection was achieved by vaccinating with rSm-14 which ranged from 50.6 to 65.7% with Freund's Complete Adjuvant and from 51.4 to 67.9% without adjuvant indicating that the inclusion of adjuvant is not necessary for the stimulation of protective immunity. Furthermore, as shown with rabbits, vaccination with rSm14 stimulated levels of protection that were not significantly different from those achieved with SE which was 53.9% without adjuvant and from 56.7 to 72.1% with adjuvant. Vaccination with the T7 capsid protein alone did not stimulate any measurable protection. Analysis of the distribution of worm burdens among the animals in the four experiments demonstrated that following vaccination with rSm14, up to 66% of the animals harboured 10 or fewer worms in contrast to control groups where the majority of animals had between 21 and 30 worms and where none had less than 11 worms.

Western blotting studies showed that rSm14 was specifically and strongly recognized by antibodies from mice and rabbits infected with *S. mansoni* as well as individuals from endemic areas. Furthermore, anti-Sm14 antibodies produced by immunizing New Zealand with rSm14 in the presence of Freund's Complete Adjuvant, strongly labeled sections of adult *S. mansoni* worms and reacted with a 14kDa molecule in Western blots.

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However, these antibodies did not label any cell of normal rabbit tissues including brain, heart, skeletal muscle, small intestine, pancreas, kidney, liver spleen, thymus or testis as assessed immunocytochemically.

In summary, rSm14 protected outbred Swiss mice and NZ rabbits by up to 67 and 89% respectively, against challenge with *S. mansoni* cercariae in the absence of adjuvant and without provoking any observable auto-imune response.

Hillyer and co-workers have isolated a low molecular weight *Fasciola hepatica* antigen fraction, that protects against both *S. mansoni* and *F. hepatica* infection in experimental animals (Hillyer 1984, 1985). An active component of the protective fraction was putatively identified by molecular cloning to be an antigen with homology to the *S. mansoni* fatty acid binding protein (Sm14) and termed Fh15 (Rodriguez-Perez et al. 1992). The coincidence of these results suggested that the pair of similar parasite proteins identified could provide a molecular basis for the observed immune cross reaction between the two parasites. By constructing molecular models of the two parasite proteins, based on the known crystal structures of homologous mammalian proteins, it was observed that both molecules adopt the same basic three dimensional structure as mammalian fatty acid binding proteins which consists of a barrel shaped molecule formed by ten anti-parallel B-pleated strands joined by short loops. Nevertheless, detailed examination revealed the likely presence of cross reactive, discontinuous epitopes principally derived from amino acids in the C-terminal portions of the molecules.

To test the protective potential of rSm14 against *F. hepatica*, Swiss mice immunized with rSm14 were orally challenged with *F. hepatica* metacercariae in two independent experiments. In both experiments detailed histopathological examination revealed 100% protection against parasite maturation and liver damage. The livers of all animals in each experiment showed that in all non-vaccinated animals complete parasite development and migration had occurred as indicated by extensive damage to the hepatic parenchyma and destruction of the hepatic lobes. In none of the vaccinated animals were parasites of any developmental stage observed nor were any intraperenchymal hepatic lesions indicative of successful parasite maturation found. Nevertheless, capsular cicatricial lesions were seen in all vaccinated mice indicating that immature parasites had successfully migrated to the liver but had not developed further.

We were thus able to show that a recombinant fusion protein containing the complete Sm14 polypeptide can stimulate a potent protective response against both *S. mansoni* and *F. hepatica* infection. The data suggest that Sm14 could form the basis of a single vaccine effective against both parasites. Such a multi-valent vaccine, aimed primarily for veterinary use against an economically important disease such as fascioliasis, may represent the most feasible route for producing a vaccine for human use against schistosomiasis which is endemic in developing countries.

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