

## SCHISTOSOMA MANSONI: PROTECTIVE ANTIGENS

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Since the pioneer works that introduced the basis of immunization as a powerful tool to prevent infectious diseases, the search of antigens that mediate protective immunity against parasites, has been a major goal of research in medical parasitology. It is thus envisioned that the development of effective vaccines could be relevant to control programs of largely endemic diseases.

Schistosomiasis is a major disease of public health importance (WHO, 1974) and the current status of continuous spreading of the endemy in some regions, in spite of the development of active and relatively safe drugs, reflects the clear need for the search of alternative measures to prevent reinfection and control transmission (Capron & Capron, 1986).

A variety of host species can develop partial resistance to *Schistosoma mansoni* following prior infection or immunization with radiation attenuated cercariae (Smithers & Doenhoff, 1982). The prior status of almost unsuccessful possibility to experimentally immunize against *S. mansoni* infection (Clegg & Smith, 1978) is now being replaced by a current enthusiasm on the possibility of producing a defined and effective vaccine against this parasite (Simpson & Cioli, 1987). Yet one of the major limitations is the incomplete degree of protection achieved in animals in most of experiments with purified and chemically defined parasite antigens. There is now a general consensus on the need to increase the level of protection induced by experimental immunoprophylaxis and it is envisioned that a successful vaccine should be based on a combined approach. The establishment of a good animal model for the development of an efficient vaccine against schistosomula, has been very hard to achieve (Capron & Capron, 1986). Possibly this may be based on the variety of methods of immunization used, and the remarkable diversity of immune effector mechanisms

against schistosomes. Future progress might depend on the understanding of the complex immunoregulatory events that modulate the evolution of natural schistosome infection and the exact basis of protective immunity in man.

In previous studies on the search of antigens that mediate protective immunity against schistosomes, we reported on the use of a "cocktail" of schistosome components (called SE) released early by incubation of live and freshly perfused *S. mansoni* adult worm in phosphate buffered saline, aiming at the extraction of metabolic components (excretory/secretory products) and those of structural origin (Tendler & Scapin, 1979; Kohn et al., 1979). Focusing on the high protection against cercarial infection, an experimental model was designed, in two different outbred animal hosts, the SW mouse and NZ rabbits, known to be fully susceptible and partial resistant to *S. mansoni* infection respectively.

In the New Zealand rabbit *S. mansoni* model, it was possible to establish a reliable pattern of percutaneous infections, with rather homogeneous adult worm loads, considering number and size of parasites and male/female proportion, for long period after infection (Tendler, 1985) and recent evidence suggests that the association of rabbit-*S. mansoni* may represent a new model of immunity for the disease (Almeida et al., 1987).

Immunization experiments performed in rabbits, with SE mixture, resulted in very high levels of protection upon challenge (Scapin et al., 1980; Tendler, 1980; Tendler et al., 1982) (90% mean worm burden reduction in immunized animals compared to sex and age matched normal controls, when challenged simultaneously with same number and pool of active cercariae-LE strain of *S. mansoni*). SW mice immunized with SE, have also shown to be significantly protected against challenge with normal cercariae and fully resistant to lethal infection (Tendler, 1985). To measure resistance, vaccinated and challenged animals, and

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the controls in parallel are submitted to hepatic and mesenteric perfusion for determination of adult parasite loads. The degree of protection is calculated by the difference in number of parasites recovered from control x vaccinated animals (Tendler et al., 1982).

It is however noteworthy that despite the strong immune response at both the humoral and cellular levels elicited by active vaccination with SE in the presence of different adjuvants, as early as 20-30 days after immunization (Tendler, 1985; Tendler et al., 1986) no direct correlation between worm burden reduction and the immune mechanisms involved in protection, could so far be identified, either in mice or in rabbits. It is likely, therefore, that the immunization with a mixture of antigens like SE emulsified in Freund's Complete Adjuvant (FCA) and administered subcutaneous/intradermally, is capable of inducing significant parasite killing mechanisms involving stimulation of both humoral and cell-mediated immunity against further infection.

To parallel *in vitro* evidences that antibodies formed against different developmental stages of the parasite are effective in eosinophil or complement dependent cytotoxicity assays (Grzych et al., 1982; Smith et al., 1982), the characterization of antigens recognized by sera from demonstrably immune hosts, is used to identify antigenic molecules concerned with protective immunity (Bickle et al., 1986; Horowitz & Arnon, 1985). Western blot experiments were undertaken to analyse the ab response of SE vaccinated (as above) rabbits. Probing SE antigens with a panel of anti-sera derived from rabbits immunized with the same scheme (SE-FCA), we were able to demonstrate in immunoblots, two distinct patterns of recognition of SE antigens in these individuals. Interestingly, some SE antigens were restrictedly recognized only by anti-sera from almost fully protected rabbits (90-100%), out of total SE antigens with molecular weights ranging from 20-100 kD. This finding enable us to identify two subsets of antigens in SE: one common to all individual rabbit antisera, and a second subset restricted to highly protected animals. Those two patterns were respectively named as Low and High protection patterns and used as "differential" antibodies. Taking advantage of these two patterns of recognition of SE components by polyclonal abs from rabbits that responded

"differentially" to the same immunization scheme, (probably on account of individual variation, expected to occur in outbred populations), the strategy of screening cDNA libraries with those sera was applied. On the constraint of the incomplete understanding of critical mechanisms of protective response in both experimental and human schistosomiasis, screening procedures adopted, frequently involve the use of infected human sera ("putative" immune or "susceptible" individuals of endemic areas [Carter & Colley, 1986] or selected monoclonal or polyclonal sera from immunized animals [Lanar et al., 1986; Balloul et al., 1987]).

In our initial attempts towards the molecular cloning of SE potentially relevant components, two cDNA libraries from whole adult worms of *S. mansoni* and *S. japonicum* constructed by Drs Klinkert, University of Heidelberg and Donnelson/Henkle, Iowa University, respectively, were screened, with duplicate filters differential screening. A parallelism could be drawn between the results of immunoblots. The detection of two different sets of clones, which could presumably correspond to the two different recognition patterns of rabbit anti-SE sera, opened an interesting experimental strategy for isolating SE protective antigens. In additional experiments aiming the identification of SE components, we compared in immunoblots, rabbit polyclonal anti-SE sera (High and Low protection) with a rabbit antiserum to purified schistosome paramyosin (kindly provided by Dr A. Sher, NIH). This protein is a recently defined molecule, partially protective against *S. mansoni* challenge infection in inbred mice (Lanar et al., 1986), of Mr (x10<sup>-3</sup>) 97, shown to be sensitive to proteolytic degradation to two major breakdown products of Mr (x10<sup>-3</sup>) 95 and 78 (Pearce et al., 1986).

The 97/95/78 kD complex was recognized by both High and Low protection anti-SE sera and monospecific anti-paramyosin sera. The "high" protection anti-SE sera recognized in addition to paramyosin, other polypeptides which remain to be further well characterized and assessed in terms of its protective activity and immunological role. The finding of paramyosin as component of SE, reinforces previous indirect immunofluorescence studies performed on sections of adult schistosomes with rabbit anti SE sera, that reacted with ags on the parasite surface and in between the

muscle layers (Mendonça et al., 1987), in a similar fashion as demonstrated for paramyosin (Pearce et al., 1986). This finding, paralleled also results of immunoscreening of cDNA libraries performed, similarly as mentioned before. Again, common paramyosin clones were isolated with both anti-paramyosin and anti-SE sera, with extra clones being recognized only by the latter rabbit sera (High protection). Among the other SE components of lower molecular weight, the 31/32 kD doublet, described as potential candidates for diagnosis of schistosomiasis (Klinkert et al., 1987) and recently identified as proteases located in the schistosome gut was also identified (Klinkert et al., 1988).

The incubation of freshly perfused schistosomes in a chemically defined media (PBS) was aimed at the extraction of early released antigens from live adult worms (specially excretory/secretory products and tegumental components). This strategy was envisioned on the view of former frustrating attempts to induce consistent resistance upon schistosomal infection with different crude extracts of *S. mansoni*, that theoretically could be depleted of relevant functional antigens. This premise was mainly influenced by the extraction procedures commonly adopted, that derived from the use of dead parasites. In fact, using SE emulsified in FCA (as preferential adjuvant) and administered through subcutaneous/intra-dermal route, we achieve a high and long term duration protection vaccine model design, based on the immunity raised by an antigen combined approach in two experimental animal hosts against *S. mansoni* infection. The rationale for the use of the rabbit model, unusual for protection trials, was held by the strategy of "tracking" potentially protective and discrete antigens in a partially resistant host (to be further tested in susceptible hosts) that could therefore "amplify" the immune response and effector mechanisms of parasite killing. In much as being the rabbits a known potent ab producer, this could also be envisioned as an important tool.

Studies on the induced immune response in vaccinated animals aiming the identification of the functional relevant SE protective components, site and mechanisms of parasite death and protection markers, were the focus of our efforts in the last years, but less information on the molecular composition of SE, as well as on

the identification and isolation of its protective components was available until recently. The identification of the mentioned two subsets of antigens and recombinant clones possibly related to the "contrasting" "High" and "Low" protection antibodies, recognizing different SE components, and the characterization of some of the cloned antigens (mainly paramyosin molecule), opened interesting new approaches to study the protective immune response in *S. mansoni* infections, molecular characterization of SE protection related components and purification of vaccine candidate antigens.

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#### REFERENCES

- ALMEIDA, M.; PINTO, R. M.; NORONHA, D.; KATZ, N. & TENDLER, M., 1987. Curative and protective activity in rabbits after reinfection with *S. mansoni*. A new model of immunity (submitted).
- BALLOUL, J. M.; SONDERMEYER, P.; DREYER, D.; CAPRON, M.; GRZYCH, J. M.; PEARCE, R. J.; CARVALHO, D.; LECOCQ, J. P. & CAPRON, A., 1987. Molecular cloning of a protective antigens of schistosomes. *Nature*, 326: 149-153.
- BICKLE, Q. D.; ANDREWS, B. J. & TAYLOR, M. G., 1986. *Schistosoma mansoni* characterization of two protective monoclonal antibodies. *Parasite Immunol.*, 8: 95-107.
- CAPRON, M. & CAPRON, A., 1986. Rats, mice and man-model for immune effector mechanisms against schistosomiasis. *Parasitology Today*, 2: 69-75.
- CARTER, C. E. & COLLEY, D. G., 1986. The molecular biology of schistosomes. *Parasitology Today*, 2: 84-85.
- CLEGG, J. A. & SMITH, M. A., 1978. Prospects for the development dead vaccines against helminths. *Adv. Parasitol.*, 16: 165-218.
- GRZYCH, J. M.; CAPRON, M.; BAZIN, H. & CAPRON, A., 1982. *In vitro* and *in vivo* effector function of rat IgG<sub>2a</sub> monoclonal anti-*S. mansoni* antibodies. *J. Immunol.*, 129: 2739-2743.
- HOROWITZ, S. & ARNON, R., 1985. Identification of *Schistosoma mansoni* antigens by means of biological active monoclonal antibodies. *Mol. Biochem. Parasitol.*, 15: 115-127.
- KLINKERT, M. Q.; FELLEISEN, R.; LINK, G.; RUPPEL, A. & BECK, E., 1988. Primary structures of SM 31/32 diagnostic proteins of *Schistosoma mansoni* and their identification as proteases. *Mol. Biochem. Parasitol.* In press.

- KLINKERT, M. Q.; RUPPEL, A. & BECK, E., 1987. Cloning of diagnostic 31/32 kDa antigens of *Schistosoma mansoni*. *Mol. Biochem. Parasitol.*, 25: 247-255.
- KOHN, A.; TENDLER, M.; FERNANDES, B. M.; PINTO, R. M. & CASTILHO, E. A., 1979. Schistosome motility in saline media. *Rev. Bras. de Pesq. Méd. e Biol.*, 12: 123-126.
- LANAR, D.; PEARCE, E. J.; JAMES, S. L. & SHER, A., 1986. Identification of paramyosin as the schistosome antigen recognized by intradermally vaccinated mice. *Sci.*, 234: 593-596.
- MENDONÇA, M.; LENZI, H. L. & TENDLER, M., 1987. *Schistosoma mansoni*: Migration patterns in normal and immunized Swiss Webster mice by means of autoradiographic analysis. *Mem. Inst. Oswaldo Cruz*, 82 (in press).
- PEARCE, E. J.; JAMES, S. L.; DALTON, J.; BARRAL, A.; RAMOS, C.; STRAND, M. & SHER, A., 1986. Immunochemical characterization and purification of SM-97, a *Schistosoma mansoni* antigen monospecifically recognized by antibodies from mice protectively immunized with a non living vaccine. *J. Immunol.*, 137: 3593-3600.
- SCAPIN, M.; TENDLER, M.; MESSINEO, L. & KATZ, N., 1980. Preliminary studies with a *Schistosoma mansoni* saline extract inducing protection in rabbits against the challenge infection. *Rev. Inst. de Med. Trop. São Paulo*, 22: 164-172.
- SIMPSON, A. J. G. & CIOLI, D., 1987. Progress towards a defined vaccine for schistosomiasis. *Parasitology Today*, 3: 26-28.
- SMITHERS, S. R. & DOENHOFF, M. J., 1982. Schistosomiasis. p. 527. In: S. Cohen & K. Wareen. *Immunology of parasitic infections*. Blackwell Scientific Publications, Oxford.
- SMITH, M. A.; CLEGG, J. A.; SNARY, D. & TREJ-DOSIEWICZ, A. J., 1982. Passive immunization of mice against *Schistosoma mansoni* with an IgM monoclonal antibody. *Parasitol.*, 84: 83-91.
- TENDLER, M., 1980. Estudo da atividade imunogênica e protetora de extrato antigênico de vermes adultos do *Schistosoma mansoni*. Tese de Mestrado, p. 75. Universidade Federal do Rio de Janeiro.
- TENDLER, M., 1985. *Schistosoma mansoni*: vacinação com antígenos de vermes adultos. Ph. D. Thesis p. 86. Universidade Federal do Rio de Janeiro.
- TENDLER, M.; PINTO, R. M.; LIMA, A. O.; GEBARA, G. & KATZ, N., 1986. *Schistosoma mansoni*: Vaccination with adult worm antigens. *Intern. J. for Parasitol.*, 16: 347-352.
- TENDLER, M. ? SCAPIN, M., 1979. The presence of *Schistosoma mansoni* antigens in solutions used for storing adult worms. *Rev. Inst. Med. Trop. São Paulo*, 21: 293-296.
- WORLD HEALTH ORGANIZATION, 1974. Immunology of schistosomiasis. *Bull. Wrld. Hlth. Org.*, 51: 553-595.