Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87*, Suppl. I, 281-286, 1992

VACCINATION IN MURINE SCHISTOSOMIASIS WITH ADULT WORM DERIVED ANTIGENS – II. PROTECTIVE AND IMMUNE RESPONSE IN INBRED MICE

MIRIAM TENDLER; ROBERTO MAGALHÃES PINTO; ANTONIO DE OLIVEIRA LIMA*; WILSON SAVINO** & NAFTALE KATZ***

Departamento de Helmintologia, Instituto Oswaldo Cruz, Av. Brasil, 4365, 21045-900, Rio de Janeiro, RJ, Brasil *Fundação Ataulpho de Paiva, Rio de Janeiro, RJ, Brasil **Departamento de Imunologia, ***Centro de Pesquisas René Rachou – FIOCRUZ, Belo Horizonte, MG, Brazil

Previous work in our laboratory, mainly foccused the prospects of achieving resistance against Schistosoma mansoni *infection with adult worm-derived antigens in the form of a soluble extract (SE). This extract obtained by incubation of living adult schistosomes in saline, contains a large number of distinct molecules and was actually shown to be significantly protective in different outbred animals models such as Swiss mice and rabbits.*

It thus appeared worthwhile to investigate the potential protective activity of SE in different inbred strains of mice, known to be highly susceptible to the infection. Herein we present data showing that DBA/2 mice, once immunized with SE acquire significant levels of resistance to a S. mansoni *cercarial challenge. In addition, preliminary studies on the immune system of immunized animals revealed that, injection of SE caused no general inbalance of B or T cell responses.*

Key words: vaccination – *Schistosoma mansoni* – murine model – antigens

Schistosomiasis is a parasitic disease of major public health concern. Despite the development of active and relatively safe drugs, its is still spreading to new areas in some regions of the world. Hence, the development of efficient procedures to prevent infection and reinfection is essential. For this, a prerequisite is the search for specific antigens that mediate protective immunity.

To investigate and identify potentially protective antigens, different approaches have been used. "Concomitant immunity" hypothesis referring to the situation in which "invading schistosomula are destroyed by the immune response are unaffected" was the idea that has dominated the field of schistosoma immunology since it was originally stated (Smithers & Terry, 1965). This concept suggested the vulnerability of larval stages to immune attack, and subsequent work showed that the schistosomulum can be subjected to *in vitro* killing by antibody together with complement, or by cytotoxic cells (Smithers & Doenhoff, 1982). It was thus assumed that schistosomulum surface antigens were likely to be targets to protective immunity effector mechanisms, and it is easily understandable the large number of studies that have concentrated particularly on schistosomulum components as a rationale to the identification of antigens involved in the induction of protective immunity (reviewed by Smithers & Doenhoff, 1982).

Variability on the host's immune response to the parasite, as well as the immunogenic role and vulnerability of adult worms, were further analyzed more extensively and shown to be relevant for protective immunity to schistosomiasis (reviewed by Damian, 1984). In our laboratory, research on schistosomiasis mainly foccused the prospects of achieving resistance with adult worm-derived protective antigens in the form of a soluble extract (SE). This extract was obtained by incubation of living adult schistosomes in a chemically-defined medium (Tendler & Scapin, 1979) and contains a large number of distinct molecules. The rationale for our approach towards the identification of potential protective molecules was centered on two major ideas: the obtention

This work was supported in part by CNPq (Brazil) and UNDP/WORLD BANK/WHO Special Programme for Research and Trainning in Tropical Diseases.

of adult worm-derived secretory/excretory products as well as tegumental components, and the antigen-combined strategy. Actually, we demonstrated that in a partially permissive animal model such as the New Zealand rabbit, SE injected subcutaneously could generate high and long lasting protection against challenge infection, with reduction on worm burden of up to 90% (Tendler et al., 1982, 1986). It thus appeared worthwhile to investigate the potential protective activity of SE in a further animal model for schistosomiasis, namely the mouse, which is known to be highly susceptible to the infection. Herein we present data showing that DBA/2 mice, once immunized with SE acquire significant levels of resistance to a *Schistosoma mansoni* cercarial challenge. In addition, preliminary studies on the immune system of immunized animals revealed that, injection of SE caused no general inbalance of B or T cell responses.

MATERIALS AND METHODS

Reagents – Tritiated thymidine was purchased from New England Nuclear (Boston, USA); 2,4-dinitro-1-fluorbenzene (DNFB) and phytohemagglutinin-P (PHA) were Sigma products (Saint Louis, USA). PPD and BCG (Moreau strain) were obtained from Ataulpho de Paiva Foundation (Rio de Janeiro, Brazil).

Mice – Male DBA/2 and Swiss, 6-8 week-old mice were obtained from FIOCRUZ's animal house, and weighted 20-25 grams by the beginning of the experiments.

Parasites – LE strain of *S. mansoni* is currently maintained in our laboratory in Swiss mice and *Biomphalaria glabrata*. The snail colony is kept in a moluscary with continuous water flow system and acclimation conditions.

Preparation of worm-derived immunogenic extract – *S. mansoni* adult worms were recovered from the portal system of Swiss mice by retrograde perfusion with heparinized saline, 45 days post-infection (Pellegrino & Siqueira, 1956). After rinsing in PBS, living worms were left to stand at room temperature in fresh PBS for 2-3 h before being stored at –20 °C. Suspensions containing 1.0 g of worms in 10 ml PBS/batch were then tawed, filtered through a wire mesh and centrifuged at 10,000 g for 1 h at 4 °C (Tendler & Scapin, 1979). Protein content of each batch was assessed by the classical method of Lowry. This SE was employed for immunizing mice.

Immunization protocol – The basic immunization protocol applied consisted of 2 weekly subcutaneous/intradermal injections of SE (100 μg emulsioned in CFA) in the mouse foot pads, followed by an intra-peritoneal (i.p.) booster of 100 μg SE 3 weeks later. Age matched control groups were maintained untreated.

Challenge infection – Challenge protocol consisted of 100 ± 20 living cercariae, subcutaneously injected into the neck region of the animal according to Peters & Warren (1969), 30 days following the immunizing booster. Infection was performed at the same day on both immunized and untreated groups.

Protective assay – Immunized animals, challenged as described above as well as respective controls, had their worm burden evaluated 45 days post-infection. The degree of protection for each group was evaluated either by direct adult worm counts or as percentage of protection as follows: % protection = C – V/C x 100, where C = mean of parasites recovered from controls and V = mean of parasites recovered from vaccinated animals.

Plaque forming cell assay – Quantitative determination of splenic cells forming direct hemolytic plaques was accomplished using the Cunningham's method, slightly modified (Cunningham & Szemberg, 1968). Briefly, animals vaccinated 30 days before with 3 x 100 μg SE + CFA were subjected to an i.p. injection of 10^8 SRBC. Four days later, splenic cells forming direct hemolytic plaques were determinated. The results were expressed by the number of hemolytic plaques formed by 10^6 spleen cells after 60 minutes of incubation at 37 °C.

Mitogenic response – Mice from control or SE-immunized groups were sacrificed and their spleen cells were cultured in RPMI 1640, supplemented with 10 μg/ml gentamicin, 3 mM-glutamine, 0.05 mM 2-mercaptoethanol and 5% inactivated human serum. Spleen cell suspensions (4.10^5 cell/well in 0.1 ml) were then subjected to either PHA 5 μg or PPD 20 μg. After three days in culture, cells were pulsed with tritiated thymidine (1 μCi./well) and harvested for scintilation counting 20 h later.

Contact hypersensitivity to 2,4 DNFB – Mice were sensitized by skin painting with 50 μg of 2,4 DNFB on the razor shaved abdominal skin, and challenged 15 days later in

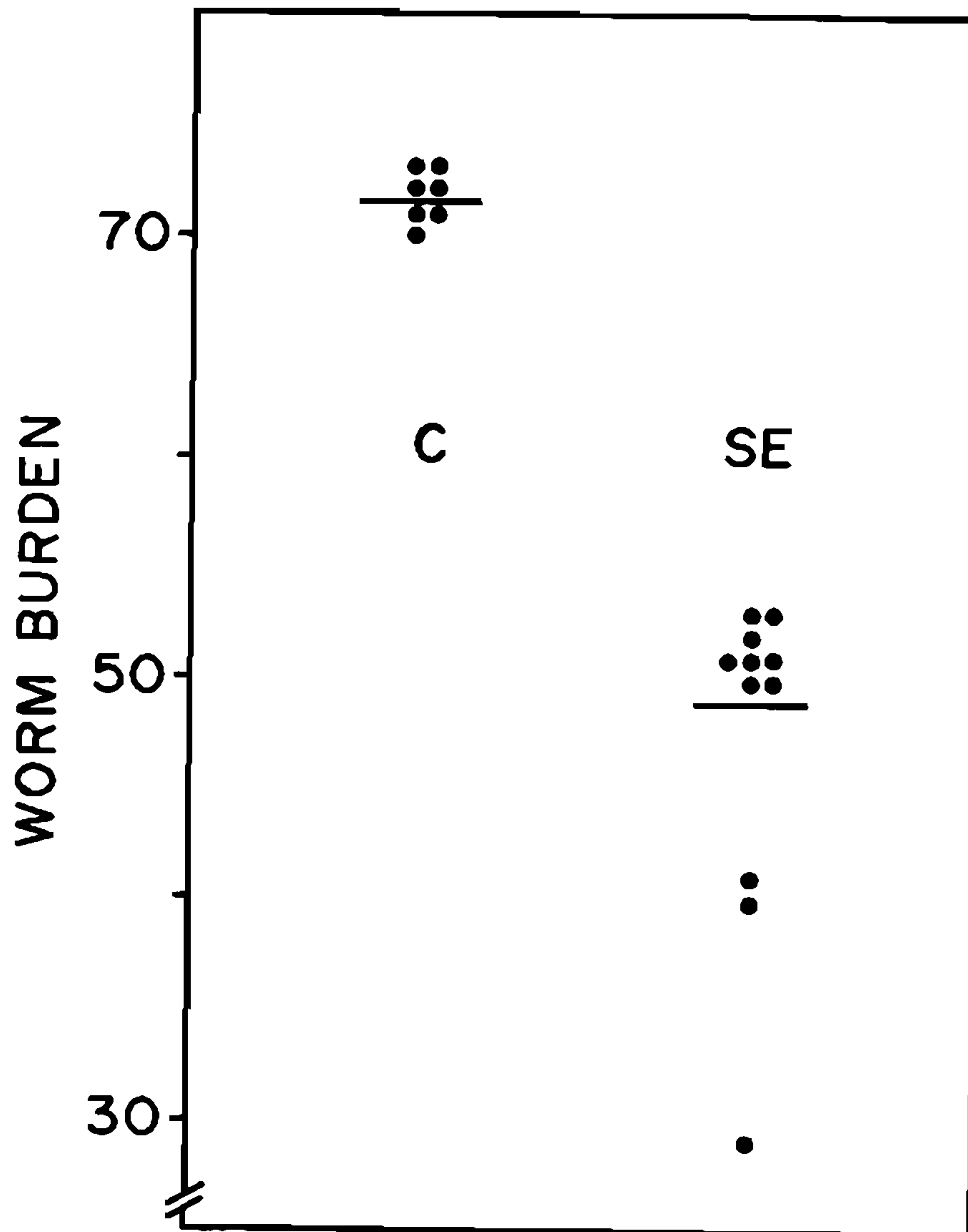

Fig. 1 – SE PROTECTIVE ACTIVITY IN DBA/2 MICE

GROUPS	TREATMENT	WORM BURDEN RECOVERY (mean ± SEM)	PROTECTION (%)	p
SE (vaccinated)	3X100μg SE + CFA	48.36±2.56 (11)	34.2	<0.0001
C (control)	PBS	73.43±0.84 (7)		

( ) nº of animals/group
% protection = 1 – (worm burden immune/control) x 100
Statistical significance by ANOVA probability by F test.
Challenge infection: 100 cercariae/animal (SE an C groups), 30 days after SE injection.

the ear, with 25 μg of the sensitizing agent. Ear thickness was measured (using a dial micrometer) 48 h after challenge and the data compared to those obtained just before the challenge.

Cutaneous hypersensitivity to PPD – Three weeks after an i.p. injection of viable BCG (100 μg), control and SE-immunized mice were intradermically tested with PPD 10 μg (in 10 μl) or saline, injected directly in the ear. Ear thickness was evaluated 48 h later as described above. Ear swelling was expressed as the difference (in mm) between the values recorded in the ear with PPD and the contralateral ear, injected with saline.

Statistical analysis – The Student's T test was applied when dealing with two experimental treatments, and one way analysis of variance, as fixed model for equality evaluation (Snedecor & Cochran, 1967).

RESULTS

Protective activity of SE in DBA/2 mice – Immunization with SE did promote a significant degree of resistance in DBA/2 mice against a cercarial *S. mansoni* challenge, performed 30 days post-booster. As depicted from the Figure, the number of adult worms recovered in controls ranged from 70 to 76, thus highly in contrast to the range 28 to 56 found in the SE-treated group, which led to a significant protection degree of 34.2%.

B and T cell responses are not affected by vaccination with SE – Direct plaque forming cells four days after immunization with sheep red blood cells, performed in both control and vaccinated mice, revealed no significant difference between the two groups; both being good responders in terms of IgM anti-SRBC production (Table).

Splenic T cell proliferation *in vitro*, in response to a lectin (PHA) and to an antigenic complex (PPD) also showed similar values in both control and SE-immunized animals as can be seen in the Table. In addition, *in vivo* delayed-type hypersensitivity to PPD as well as contact hypersensitivity to DNFB did not reveal any differences between control and vaccinated mice (Table).

DISCUSSION

The results presented in this brief report clearly demonstrate that an antigen-containing extract, derived from incubation of adult *S. mansoni* in saline, can confer a partial, yet highly significant, protection in DBA/2 mice challenged with 100 infective cercariae, 30 days after immunization. The first point meriting discussion concerns the differences in terms of protection degree, obtained in mice, as compared to previous data achieved in rabbits. In this latter partially permissive animal model to *S. mansoni*, immunization with SE, achieved a protection degree up to 100% (Tendler et al., 1986), thus contrasting with protection degree observed in this mouse model, which was inferior to 50%. The incompleteness of protective immune response in the murine model, has been widely observed (James, 1987) and there are several possible explanations for this phenomenon. Experimental systems are complex, involving host models, protective antigens and immune response thereof derived (Phillips & Colley, 1978).

One of the most critical aspects refers to host models, since earlier studies revealed a great diversity of effector and regulatory immune responses operating in mammal hosts, leading to quite diverse results on resistance-related immunity (reviewed by Smithers & Doenhoff, 1982; Damian, 1984). It is now generally accepted that, before the exact basis of protective immunity in man is clearly understood, no broad conclusions can be drawn

TABLE

Groups of animals vaccinated with 3 x 100 μg SE + CFA and respective non-immunized controls were submitted to the following treatments: A) I. P. immunization with 10^8 SRBC 4 days before tests for splenic PFC; B) I. P. immunization with 100 μg of viable BCG bacili and evaluation of cutaneous hipersensivity to PPD; C) Epicutaneous sensitization with 0.5 & 2,4 – DNFB to induce contact reactivity; D) SC – immunization with 100 μg BCG for *in vitro* evaluation of spleen lymphocyte blastogenesis

Treatment	Plaque forming cells[a] (10^6 spleen cells) ($\bar{X} \pm$ SD) (A)	Ear swelling (48 h)[a] to 10 μg PPD ($\bar{X} \pm$ SD) (B)	Ear swelling (48 h)[a] to 20 μg DNFB ($\bar{X} \pm$ SD) (C)	Splenic T cell proliferation[a] (CPM ± SD) (D) PPD (5 μg)	PHA (2 μg)
SE + CFA	2264 ± 178,2	0.48 ± 0.1	2.1 ± 0.2	7.113 ± 1227	12.982 ± 1856
Saline	3846 ± 183,6	0.42 ± 0.08	2.4 ± 0.4	6.689 ± 280	11.565 ± 886
–	NS	NS	NS	NS	NS

a: assays A, B, C, D, were performed in different groups consisting of 10 animals each (5 vaccinated and 5 controls). Immune response (as above) was evaluated 30 days after injection with SE.

from striking limited results imposed by particular models (Capron & Capron, 1986). Within the context of modulation of immunologically mediated resistance to *S. mansoni,* T cell-derived soluble regulatory factors, have been demonstrated (Abe & Colley, 1984; Chensue et al., 1983) as well as immunosupression mechanism regulated by idiotypically dependent pathways (Phillips et al., 1988).

It is noteworth to state, that the here reported data, showed that, at least for one unrelated antigen source, namely PPD, the immune response is not changed, thus suggesting that the immune system of SE-immunized mice is specifically modulated in respect to schistosome derived antigens. This situation appears to us a prerequisite for vaccine candidate antigens. Interestingly it is not found in SWAP + BCG vaccinated Balb/c mice that did not respond to PPD *in vivo* while BCG control mice did and a supressor mechanism generated in SWAP-stimulated Balb/c, was suggested to explain this finding (James et al., 1988).

Thus, our results should also be discussed in the context of data obtained in the murine model using other *S. mansoni*-derived antigens.

In reinfection experiments or immunization with radiation-attenuated cercariae, various mouse strains develop partial resistance against challenge infection and different immune responses have been indicated as potential effector mechanisms responsible for protection (reviewed by Smithers & Doenhoff, 1982). Recently, vaccination of mice with a purified 28 kd protein, has been shown to induce resistance associated with antibody response both *in vitro* and *in vivo* models. Contrastingly, in rats immunized with the same antigen, similar immune response was ellicited, although higher levels of protection were observed (Balloul et al., 1987). In other studies, vaccination with another purified antigen (Sm 97) in conjunction with BCG, induced a 32% mean protection against challenge infection in C57BL/6 inbred mice (Pearce et al., 1988). Resistance triggered by this antigen was strictly related to T-cell-dependent cell mediated immune response and the method of immunization was considered to be of outmost importance for protective immunity in this system (James et al., 1988).

Our studies have shown that SE contains a large number of distinct molecules recognized by sera from highly protected rabbits by means of immunoblotting (Tendler, 1987).

Actually we were able to identify so far, distinct SE-components namely paramyosin (97/95/78 kda complex) (Lanar et al., 1986), glutathione-S-transferase (p28), the heat shock protein (Hsp 70), a 13 kd protein bearing nucleotide sequence homology with mammalian myelin p2, cathepsin B and haemoglobinase (Tendler et al., 1990). The presence of a cytokeratin-related peptide with 59/60 kd as a further component of SE was also observed (Tendler et al., 1989).

Importantly, earlier studies, showed that in both permissive (mouse) and partially permissive (rabbit) animal models, subcutaneously-injected SE could generate a high and long-lasting protection. In addition, the same protocol promoted a strong humoral and cell mediated immune response in both outbred hosts (Tendler et al., 1982, 1986).

In addition in a systematic investigation on a series of distinct vaccination protocols in outbred mice, we were able to show that SE-induced protection, can be modulated by parameters such as dose of antigen, immunization/challenge interval and route of cercarial injection (Tendler et al., 1991).

However, regardless of mechanisms and models, the here reported results confirm and extend previous findings in two different host systems thus suggesting a potential relevance for the use of SE components as resistance-promoting antigens.

In fact the optimization of the expression of protective resistance to schistosomiasis is a major goal for vaccine development.

Finally, distinct experimental protocols in the murine model (both in inbred and outbred strains) are in progress to test which lymphocytes are involved in the modulation of immune response in infected mice as well as in animals vaccinated with total SE or with SE-derived selected potentially protective purified proteins, aiming to throw some more light on the understanding of protective immune response to schistosome infection.

ACKNOWLEDGEMENTS

To Dr M. Queiroz and H. Brascher for excellent technical assistance and L. C. Sauer-

braum Maia for statistical analysis and helpful suggestions.

REFERENCES

ABE, T. & COLLEY, D. G., 1984. Modulation of *S. mansoni* egg-induced granuloma formation. III. Evidence for an anti-idiotypic, I-J-positive, I-J-restricted, soluble T suppressor factor. *J. Immunol., 132:* 2084-2088.

BALLOUL, J. M.; GRZYCH, J. M.; PIERCE, R. J. & CAPRON, A., 1987. A purified 28,000 dalton protein from *Schistosoma mansoni* adult worms protects rats and mice against experimental schistosomiasis. *J. Immunol., 138:* 3448-3453.

CAPRON, M. & CAPRON, A., 1986. Rats, mice and men-models for immune effector mechanisms against schistosomiasis. *Parasitology Today, 2:* 69-75.

CHENSUE, S. W.; BOROS, D. L. & DAVID, C. S., 1983. Regulation of granulomatous inflammation in murine schistosomiasis. II. T suppressor cell-derived, I-C subregion-encoded soluble suppressor factor mediates regulation of lymphokine production. *J. Exp. Med., 157:* 219.

CUNNINHGAM, A. J. & SZEMBERG, A., 1968. Further improvements in the plaque technique for detecting single antibody forming cells. *Immunol., 14:* 599.

DAMIAN, R. T., 1984. Immunity in schistosomiasis: A holistic view. *Contemp. Top. Immunobiol., 12:* 359-420.

JAMES, S. L., 1987. Schistosoma spp. Progress towards a defined vaccine. *Exp. Parasitol., 67:* 247-252.

JAMES, S. L.; SALZMAN, C. & PEARCE, E. J., 1988. Induction of protective immunity against *Schistosoma mansoni* by a non-living vaccine. VI. Antigen recognition by non-responder mouse strains. *Parasite Immunol., 10:* 71-83.

LANAR, D.; PEARCE, E. J.; JAMES, S. L. & SHER, A., 1986. Identification of paramyosin as the schistosome antigen recognized by intradermally vaccinated mice. *Science, 234:* 593-596.

PEARCE, E. J.; JAMES, S. L.; HIENY, S.; LANAR, D. E. & SHER, A., 1988. Induction of protective immunity against *Schistosoma mansoni* by vaccination with schistosome paramyosin (Sm 97), a nonsurface parasite antigen. *Proc. Natl. Acad. Sci., 85:* 5678-5682.

PELLEGRINO, J. & SIQUEIRA, A., 1956. Técnica de perfusão para colheita de *Schistosoma mansoni* em cobaias experimentalmente infestadas. *Rev. Bras. Malariol. D. Trop., 8:* 589-597.

PETERS, P. A. & WARREN, K. S., 1969. A rapid method of infecting mice and other laboratory animals with *Schistosoma mansoni:* Subcutaneous injection. *J. Parasitol., 55:* 558.

PHILLIPS, S. M. & COLLEY, D. G., 1978. Immunologic aspects of host response to schistosomiasis: Resistance, immunopathology and eosinophil involvement. *Prog. Allergy, 24:* 49.

PHILLIPS, S. M.; PERRIN, P. J.; WALKER, D. J.; FATHELBAB, N. G.; LINETTE, G. P. & IDRIS, M. A., 1988. The regulation of resistance to *Schistosoma mansoni* by auto-anti-idiotypic immunity. *J. Immunol., 141:* 1728-1733.

SMITHERS, S. R. & DOENHOFF, M. J., 1982. Schistosomiasis, p. 527-607. S. Cohen & K. S. Warren (Eds) *Immunology of parasitic infection.* Blackwell Sci. Publ. Oxford.

SMITHERS, S. R. & TERRY, R. J., 1965. Acquired resistance to experimental infection of *Schistosoma mansoni* in the albino rat. *Parasitology, 55:* 711-717.

SNEDECOR, G. W. & COCHRAN, W. C., 1967. *Statistical Methods.* Iowa State University Press, Ames.

TENDLER, M. 1987. *Schistosoma mansoni:* Adult worm protective antigens. *Mem. Inst. Oswaldo Cruz, 82* (Suppl. IV): 125-128.

TENDLER, M.; KLINKERT, M. Q.; DIOGO, C. M. & SAVINO, W., 1990. Molecular characterization of an extract of *Schistosoma mansoni* with protective activity. *EOS J. Immunol. Immunopharmacol., 10:* 214-215.

TENDLER, M.; LIMA, A. O.; MAGALHÃES PINTO, R.; CRUZ, M.; BRASCHER, H. & KATZ, N., 1982. Immunogenic and protective activity of an extract of *Schistosoma mansoni. Mem. Inst. Oswaldo Cruz, 77:* 275-283.

TENDLER, M.; MAGALHÃES PINTO, R.; LIMA, A. O.; GEBARA, G. & KATZ, N., 1986. *Schistosoma mansoni:* Vaccination with adult worm antigens. *Int. J. Parasitol., 16:* 347-352.

TENDLER, M.; MAGALHÃES PINTO, R.; LIMA, A. O.; SAVINO, W. & KATZ, N., 1991. Vaccination in murine schistosomiasis with adult worm derived antigens: Variables influencing protection in outbred mice. *Int. J. Parasitol., 21:* 299-306.

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.