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THE PRESENCE OF SCHISTOSOMA MANSONI ANTIGENS IN SOLUTIONS USED FOR STORING ADULT WORMS

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SUMMARY

Immunochemical aspects of **S. mansoni** antigens released in saline solutions used for storing adult worms were evaluated by immunoprecipitation methods (double immunodiffusion — ID and immunoelectroosmophoresis — IEOP) with antisera consisted of human sera from patients with schistosomiasis (hepato-intestinal form with viable eggs in the stools) and rabbit hyperimmune serum anti-worm saline extract. Several samples of saline extract were prepared with adult schistosomes stored frozen in 0.85% NaCl and PBS, for different periods of time. Immunoprecipitation reactions (1 — 2 bands with patients sera and 4 bands with rabbit immune sera) were observed with all the saline samples.

INTRODUCTION

Different culture media are used to incubate schistosomes for the study of morphological and physiological aspects (FLOYD & NOLLEN [2]; FETTERER et al. [3]) in the evaluation of anti-schistosomal drugs action (HILLMAN & SENFT [4]) and for the immunochemical study of schistosome antigens (MURRELL et al. [7]). However, extreme caution should be taken for the interpretation of results based on the incubated schistosomes in culture media, since recent papers (ERNST & MAY [1]) report the occurrence of extensive morphological changes in the tegument and in other structures of the worms, even after short incubation period in various routinely used culture media. The immunological and physico-chemical characterization of the **S. mansoni** antigenic components requires a large quantity of worms to supply adequate amounts of antigens. This is only feasible by collecting and stocking the material during certain period that may vary from a few days to several weeks (HOUSSAIN at al. [5]). For this purpose, different procedures are used such as lyophilization of the parasites and freezing of the worms, most frequently in salt solutions.

Since antigens may be eluted after simple agitation of whole worms in hypertonic KCl (MURRELL at al.[7]; SCAPIN & TENDLER [10]), in salt solution (SADUN et al.[9]), or in culture media, there exists a real possibility of loss of important antigens in salt solutions used for storage.

Our initial attempts to define the immunochemical properties of the antigens detected in saline storage solutions for keeping adult worm are here reported.

MATERIAL AND METHODS

S. mansoni (LE strain of Belo Horizonte, M.G.) was mantained in **Biomphalaria glabrata** and Swiss mice. Adult worms were harvested by perfusion after 6-8 weeks' of infection with saline (PELLEGRINO & SIQUEIRA [8]). The worms were rinsed briefly in two changes of saline and then frozen in a small volume of saline, at -20°C. Preparations of the extracts

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from the first sample of saline (S_1), were obtained from 265mg of adult male and female worms that were kept frozen in 2.0 ml of PBS (0,15M phosphate buffered saline, pH 6.8) for 26 days. Then the saline of the worms was changed, at room temperature, for 3 times at intervals of 7 days, in volumes of 2 ml. The worms were refrozen after each change of saline. The saline obtained after the 26 day period was labelled S_1A and the subsequent ones, after 7 day periods were called S_1B, S_1C and S_1D, respectively. Samples S_1B, S_1C and S_1D were also stored frozen. After the last change the worms were homogenized as previously described (SCAPIN & TENDLER [11]) and the resulting extract labelled E. Hom. The second sample of saline (S_2) was obtained from 900 mg of worms stored frozen in 10 ml PBS for 10-20 days. The worms removed from S_2 were not refrozen. This sample, therefore, also acted as a control to monitor the effect of freezing and tawing process on the worms of the first sample (S_1). All the samples were centrifuged at 10.000 g for 60 min. at 4°C, filtered through Millipore filter 0.22 μm and stored frozen.

Antiserum consisted of 53 human sera from patients with schistosomiasis (hepatointestinal form with viable eggs in the stools) from a Brazilian endemic area of Minas Gerais, and rabbit anti-schistosome sera. Rabbit anti-S_2 (RAS) were prepared in 2 rabbits injecting 3 doses of 0.6 ml of S_2 (diluted with 0,15M NaCl to 1mg protein/ml) emulsified in complete Freud's adjuvant, in the four footpads, over a 45 day period. Rabbit anti-mouse sera (RAMS) were obtained by the same above mentioned scheme. The sera were stored at-20°C.

The samples of the two saline extract (S_1 and S_2) were studied by immunoelectroosmophoresis (IEOP) as described by SCAPIN & TENDLER [11], and by double immunodiffusion (ID) on slides covered with 2 ml of 1% agar (Difco) in 0.04M Na Barbital Buffer at pH 8.4. The gels were photographed after staining with 0.25% Coomassie Brilliant Blue made up in 45% methanol and acetic acid. Protein determinations of the saline extracts were performed according to LOWRY et al.[6].

RESULTS

Reactions of immunoprecipitation (1-2 bands) were detected by IEOP as well as by ID between all S_1 samples and the sera from schistosome-infected patients. By ID we detected one precipitation band with antigenic identity and decreasing intensity according to the following sequence, among S_1A, S_1B, S_1C, S_1D and E. Hom. The protein content of the antigens was as follows: S_1A — 6.04mg/ml; S_1B — 1.21mg/ml; S_1C — 0.11mg/ml; S_1D — 0.24mg/ml; HE — 0.90mg/ml and S_2 — 3mg/ml.

From 53 sera of patients tested against S_2, in IEOP, 15 gave positive reactions (28%). The immunological analysis of S_2 was carried out in immunodiffusion test against RAS and RAMS sera, as shown in Fig. 1. The reaction with RAS was too intense to be precise as to the number of bands, but at least 4 or 5 bands were discernible.

DISCUSSION

The results indicate that all the studied saline samples contained antigens. In all five extracts (S_1) where the worms had remained in saline initially for 26 days followed by three successive periods of 7 days each, and in the final homogenate was observed by ID (in reaction with serum of patients) one band with antigenic identity and decreasing intensity (between the initial and the final extraction period), suggesting that only tentative quantitative conclusions can be drawn from that data alone. Specially noteworthy is the high protein content of the storage solutions.

In order to check on the possibility that the storage solution might contain antigens of host origin, we compared the results obtained in ID with rabbit anti-mouse serum (RAMS) and rabbit anti-S_2 serum (RAS). However based on the pattern of immunodiffusion reaction as shown in Fig. 1, we found no evidence to suggest the presence of contaminants of host origin.

Further studies are necessary to characterize and purify the antigens present in storage solution. As already mentioned, this is specially important because storage solutions are frequently discarded without being used for the preparation of antigens from schistosomes.

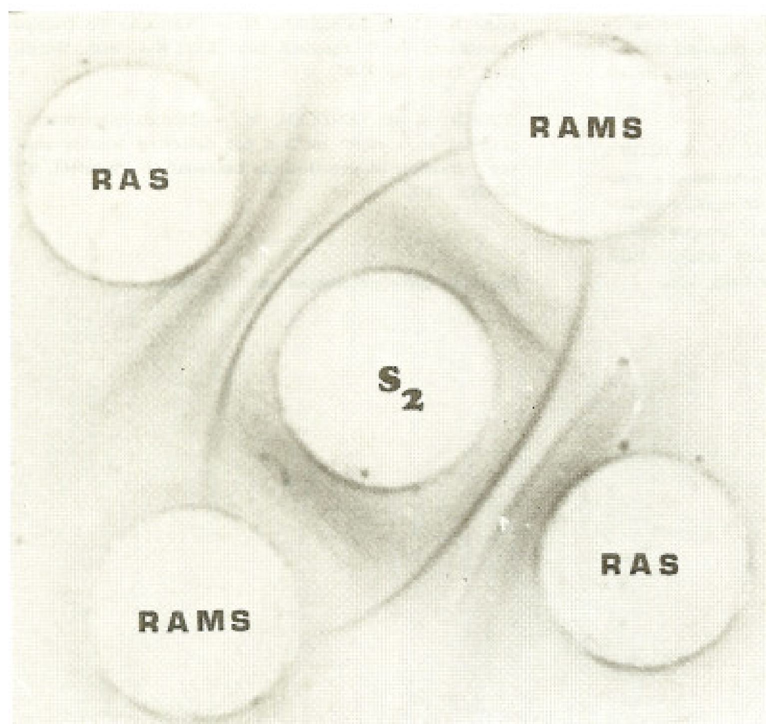

Fig. 1 — Immunodiffusion plate showing **S. mansoni** antigens in saline solution (S_2) used for storing adult worms when tested against rabbit anti-S_2 serum (RAS)

RESUMO

Presença de antígenos de Schistosoma mansoni **em soluções empregadas para a estocagem de vermes adultos**

Foram avaliadas características imunoquímicas de antígenos do **S. mansoni** detectados por métodos de imunoprecipitação em soluções salinas empregadas para a estocagem de vermes adultos. Como antissoro foram utilizados soros de doentes com esquistossomose (forma hepatintestinal com ovos viáveis nas fezes) e soro imune específico produzido em coelhos. Diversas amostras de extrato de salina, obtidos pela estocagem sob congelação, de vermes adultos em NaCl 0,85% e PBS, por diferentes períodos de tempo, foram estudadas por dupla imunodifusão (ID) e imunoeletroosmoforese (IEOF), tendo sido detectadas reações de imunoprecipitação (1-2 linhas com soro de doentes e 4 linhas com soro imune de coelhos) em todas as amostras.

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REFERENCES

1. ERNST, S. C. & MAY, L. M. — Morphological changes induced by short term maintenance of **Schistosoma mansoni** "in vitro". **Am. Soc. Parasitol. Ann. Meeting. Abs.** 150, 1975.

2. FETTERER, R. H.; PAX, R. A. & BENNETT, J. L. — **Schistosoma mansoni**: direct method for simultaneous recording of eletrical and motor activity. **Exp. Parasitol. 43:** 286-294, 1977.

3. FLOYD, R. D. & NOLLEN, P. M. — Effects of stressful conditions on the development and movement of reproductive cells in **Schistosoma mansoni. J. Parasitol. 63:** 87-90, 1977.

4. HILLMAN, G. R. & SENFT, A. W. — Anticholinergic properties of the antischistosomal drug hycanthone. **Amer. J. Trop. Med. Hyg. 24:** 827-834, 1975.

5. HOUSSAIN, R.; VANNIER, W. E. & MURRELL, K. D. — Hypersensivity to **Schistosoma mansoni** antigens. I — Immunochemical and biological characterization of an antigenic extract. **Immunochemistry 12:** 561-567, 1975.

6. LOWRY, O. H.; ROSENBROGHT, N. J.; FAN, A. L. & RANDALL, R. J. — Protein measurement with the Folin Phenol Reagent. **J. Biol. Chem. 193:** 265-275, 1951.

7. MURRELL, K. D.; VANNIER, W. E. & ALHIMED, A. — **Schistosoma mansoni:** antigenic heterogeneity of excretion and secretion. **Exper. Parasitol. 36:** 316-330, 1974.

8. PELLEGRINO, J. & SIQUEIRA, A. F. — Técnica de perfusão para colheita de **Schistosoma mansoni** em cobaias experimentalmente infectadas. **Rev. Soc. Brasil. Malariol. Doenças Trop. 8:** 589-597, 1956.

9. SADUN, E. N.; SCHOENBECHLER, M. J. & BENTZ, M. — Multiple antibody response in **Schistosoma mansoni** infections: antigenic constituents in eggs, cercariae and adults (excretion and secretions) determined by floculation reaction cross absortion and double diffusion. **Amer. J. Trop. Med. Hyg. 14:** 977-995, 1965.

10. SCAPIN, M. & TENDLER, M. — Extração de frações antigênicas de **S. mansoni** com KCl. **Rev. Soc. Brasil. Med. Trop. 9:** 91-93, 1975.

11. SCAPIN, M. & TENDLER, M. — Immunoelectroosmophoresis as a rapid method for detecting soluble antigenic fraction of **Schistosoma mansoni. J. Parasitol. 61:** 561-562, 1975.

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