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SOLUBILIZATION OF ANTIGENS OF S. MANSONI ADULT WORMS FOR THE PASSIVE HEMAGGLUTINATION TEST

PRELIMINARY REPORTS

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SUMMARY

In the preparation of **S. mansoni** antigens for the hemagglutination test, solubilization of components could be much improved by treating adult worms with 0.2 M NaOH and neutralizing with HCl. This procedure could yield about 10 times more antigen than the usual saline solution (0.15 M NaCl) extraction. Preliminary results with sera from 55 patients with chronic and 8 with acute schistosomiasis showed for the alkaline-extracted antigen a similar sensitivity to the saline extracted antigen. Specificity was also comparable, as 30 sera from normal individuals were tested. In this way it seems that antigenicity of **S. mansoni** components was not modified by the alkaline treatment which may then substitute efficiently the saline extraction.

INTRODUCTION

The hemagglutination test for **S. mansoni** schistosomiasis has been routinely used for seroepidemiological purposes [4,5,12] and even in the follow-up of patients after treatment [10,11]. To sensitize erythrocytes an adult worm aqueous extract is usually employed [3,4,9], adequately buffered and eventually made isotonic. In our experience, aldehyde-fixed red cells can be sensitized by saline soluble extracts both from fresh and lyophilized worms, but the former were observed to give better antigens. In either case worm components are little solubilized by 0.15 M NaCl extraction and after centrifugation a large residual pellet is always discarded. Not withstanding, in immunofluorescence tests performed in worm sections, almost the whole structure of worms, mainly males, is stained [7], indicating wide antigenicity. In this way we tried to improve parasite solubilization, with the help of alkaline solutions, as described for **T. cruzi** antigens [2]. Sensitivity and specificity of hemagglutination reagents prepared with extracts thus obtained were compared with the usual ones from worm saline-extracts.

MATERIAL AND METHODS

Alkaline soluble antigen (ASA) — Adult **S. mansoni** parasites were obtained by hepatic and portal perfusion of infected mice, washed in several changes of saline solution and stored at —70°C until used.

A suspension of about 25 worms per mililiter of 0.2 M — 0.3 M NaOH was ground in a tissue grinder or submitted to ultrasonic oscillation (Sonic Dismembrator, Quigley-Rochester, Inc. USA) for 1 minute in an ice-bath, and kept overnight at 4°C. After neutralizing to pH 7.0 with 2.0 M HCl, suspension was centrifuged at 3,000 r.p.m. for 10 minutes and the supernatant antigenic extract distributed in small aliquots and kept at —20°C.

Saline soluble antigen (SSA) — Worm antigens were extracted in 0.15 M NaCl solution, as

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previously described [3]. Protein concentrations of antigens were determined according to LOWRY et al. [8].

Sera — Blood samples were taken from 63 parasitologically proven cases of schistosomiasis, 55 from chronic and 8 from acute forms of the disease, according to criteria already described [7].

Hemagglutination test (HAT) — Cells were sensitized as previously described [2], with a few modifications. In brief, formalin-fixed human 0, Rh-negative red blood cells were treated with 1:15,000 (w/v) dilution of tannic acid for 15 minutes at 56.°C. After washing, cells were sensitized by incubating for 50 minutes at 37°C in an antigen dilution resulting in maximal reactivity of the cells. Antigen-coated erythrocytes were then fixed with 0.1% glutaraldehyde. Tests were performed in plastic plates with V-shaped wells (Auto-tray, Astec Inc., USA) with 50 microliters serum dilutions, from 1:20 to 1:20,480 in saline solution, and adding 25 microliters of a 2% sensitized cell suspension. Readings were done after incubating plates, in a moist chamber, at room temperature for 2 hours.

RESULTS AND DISCUSSION

Table I shows hemagglutination reagent yieldings for 6 batches of **S. mansoni** extracts, prepared at different times. It was observed that not only solubilization was improved by the alkaline extraction but also a more potent product resulted. Thus, about 3.5 times less protein were necessary to prepare maximal sensitized cells than for saline extracts. In this way, about 10 times more reagent could be prepared from the same amount of worms.

Apparently antigenicity was not affected by exposure to the NaOH solution. Table II illustrates the narrow correspondence of titers obtained between reagents prepared with the alkaline or the saline extract as the sera of patients with acute and chronic infections were tested.

In serum samples from 30 normal individuals, hemagglutination test with the "alkaline" reagent was negative, suggesting no basic changes in specificity in relation to the "saline" reagent. Such study is now being expanded to a large number of patients with different diseases other than schistosomiasis.

Another point to underline refers to a better stability of ASA in relation to SSA, when kept at 4°C, since no modifications were observed after several weeks, which is not the case for saline extracts. Probably this is due to inactivation by NaOH of enzimes or other factors. Although ASA revealed to be a good antigen for HAT tests, it was not as effective as SSA in immunodifusion tests, which could be expected considering the hydrolitic activity of NaOH.

Lyophilized worms have always yielded poor antigens in relation to fresh worms when saline extraction was used. However, in a preliminary observation, the alkaline extraction of lyophilized worms gave similar results to fresh worms.

Sera collected from residents of an endemic area of schistosomiasis mansoni are now under study, for a better evaluation of the ASA-sensitized hemagglutination reagent.

RESUMO

S. mansoni: **Solubilização dos antígenos de verme adulto para a reação de hemaglutinação passiva. (Nota prévia)**

No preparo do antígeno de **S. mansoni** para a reação de hemaglutinação passiva, a solubili-

TABLE I

Hemagglutination reagent yieldings for saline (SSA) or alkaline (ASA) **S. mansoni** extracts

Extract (from 25 worms/ml suspension)		Protein (mcg/ml)	Maximal sensitizing extract concentration (in mcg/ml protein)	Reagent yielding (in ml), per ml of worm suspension
type	batch			
SSA	I	540	108	5
	II	372	74	5
	III	355	38	10
ASA	I	1,520	15	100
	II	1,870	31	60
	III	1,800	12	90

TABLE II

Comparative hemagglutination titers obtained for **S. mansoni** saline-soluble (SSA) and alkaline soluble (ASA) reagents in serum samples from 8 patients with acute and 55 with chronic schistosomiasis

ASA \ SSA	20	40	80	160	320	640	1,280	2,560	5,120	10,240	20,480
20	①*	①									
40	①	① 3	6								
80		② 1	3	2							
160			① 4	5	1						
320			1	2	① 5	3	1				
640				5	2						
1,280					2	3					
2,560						1	1				
5,120						2		1		1	

* The encircled numbers indicate the distribution of acute cases

zação dos componentes do verme adulto melhorou consideravelmente pela adição de NaOH 0,2 M e neutralização com HCl. Este procedimento permitiu a obtenção de cerca de 10 vezes mais antígeno do que na extração usual feita com solução salina (NaCl 0,15 M). Os resultados preliminares obtidos em 55 soros de pacientes com formas crônicas de esquistossomose e em 8 com formas agudas mostraram sensibilidade semelhante à do antígeno de extrato salino. A especificidade foi também comparável quando 30 soros de indivíduos normais foram testados. O tratamento dos vermes adultos com solução alcalina parece não ter influído na antigenicidade, podendo com vantagem substituir o antígeno de extração salina.

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