Stimulation of hamster and human lymphocyte cultures by soluble egg antigens (SEA) of *Schistosoma haematobium* and *S. mansoni**

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Summary
Soluble egg and adult worm antigen preparations of *Schistosoma haematobium* and *S. mansoni* were tested in hamster and human lymphocyte cultures. Primed lymphocytes from infected donors showed a marked blastogenic response to the homologous antigens. A cross-reactivity to the heterologous antigens was seen especially in *S. mansoni* infected hamsters.

Introduction

The role of soluble egg antigens (SEA) in the aetiology of liver granuloma formation in schistosomiasis is well known (reviewed by Warren, 1972). A cell-mediated immune response occurs around viable eggs secreting SEA or around bentonite particles coated with SEA. Furthermore SEA induce and elicit delayed hypersensitivity reactions in guinea-pigs as manifested by dermal reactivity, lymphocyte transformation and macrophage migration inhibition (Boros et al, 1973). Lymphocyte blastogenesis and macrophage migration inhibition were also studied in mice in the course of *Schistosoma mansoni* infections (Colley, 1972; Boros et al, 1975). Both *in vitro* assays are in good correlation with the granulomatous reaction seen *in vivo*, so that in late (chronic) infections a decline of the responsiveness *in vitro* goes parallel to the decreasing granulomatous response. Whether antibodies to SEA detected at this time may modulate the cellular response was recently discussed (Boros et al, 1975).

Experimental schistosomiasis has been less studied in hamsters, although this animal is a suitable host for all human schistosome species (Erickson et al, 1974).

Our preliminary results with hamster and human lymphocytes are part of a comparative study on *S. haematobium* and *S. mansoni* infections. In order to analyse the specificity of the cell-mediated immune response to homologous and heterologous schistosome antigen preparations, an *in vitro* assay for lymphocyte transformation has been designed.

Materials and methods

Experimental infection of Syrian hamsters

Male hamsters (60 g body weight) were infected with 100 or 300 cercariae of *S. haematobium* (strain isolated in Madagascar and maintained in *Bulinus obtusispira*) by paddling in a cercarial suspension, or by subcutaneous infection of 100 cercariae of *S. mansoni* (strain isolated in Liberia and maintained in *Biomphalaria glabrata*).

Antigen preparation

Eggs were collected by tryptic digestion of liver tissue obtained from hamsters infected with *S. haematobium* (about 16 weeks p.inf.) or with *S. mansoni* (8–9 weeks p.inf.) and purified by several filtration steps (Browne and Thomas, 1963). The eggs were homogenized in medium RPMI 1640 (Flow) with a glass tissue homogenizer. After centrifugation the supernatant (soluble egg antigens, SEA) was sterilized by filtration (Millipore 0·22 μ) and stored at -70° in small aliquots (Boros and Warren, 1970). Delipidized adult schistosome antigens (SAA) were prepared essentially as described by Chaffee et al (1954). A buffered saline extract of ether-extracted adult schistosomes was dialysed against medium RPMI 1640, filter-sterilized and stored in small aliquots at -70°C. Protein concentrations were determined according to Lowry et al (1951).

Cell suspension and culture conditions

Lymphocytes from *hamsters* were obtained by teasing lymph nodes. The cell suspension was filtered through glasswool. After two washes in Hanks (Flow) the cells were transferred in medium RPMI 1640 supplemented with Hepes (Flow, 0·03M), penicillin (Difco, 100 U/ml), streptomycin (Difco, 100 μg/ml), fresh l-glutamine (Flow, 20 mM) and 2·5% heat inactivated normal hamster serum (Meo, T., 1974, personal communication). *Human lymphocytes* were obtained from heparinized peripheral blood by density gradient centrifugation (Ficoll-Urografin d=1·077) as described by Bøyum (1968). The culture medium was the same as described above but included 10% inactivated human AB serum instead of hamster serum. The cells were cultured in round-bottomed tissue culture microplates (Cooke). The best results were obtained with a cell concentration of 2×10^6 viable hamster cells and 10^6 human lymphocytes per ml respectively.

The cultures were set up in quadruplicates (200 μl total volume per well) with various doses of SEA or SAA, without antigen and with a non-specific mitogen (PHA M, Difco) at a final dilution of 1%. The cultures were incubated at 37°C in a humid atmosphere of 5% CO_2 in air. The cultures were pulsed with 2 μCi per well of 3H-thymidine (NEN, specific activity 20 Ci/mM) at day 4 for 24 hours.

Cell harvesting and counting

The cells were collected from the culture wells on to glass fibre filters with a cell harvesting apparatus (Skatron), dried at 60°C and solubilized by adding 100 μl of hyamine-hydroxide (Calbiochem) per filter. The samples were counted after addition of 10 ml of scintillation fluid (Permablend III, Packard) in a liquid scintillation counter.

The degree of response is expressed in net cpm (=cpm of stimulated cultures minus cpm of unstimulated cultures) and reported as mean ± standard error (s.e.).
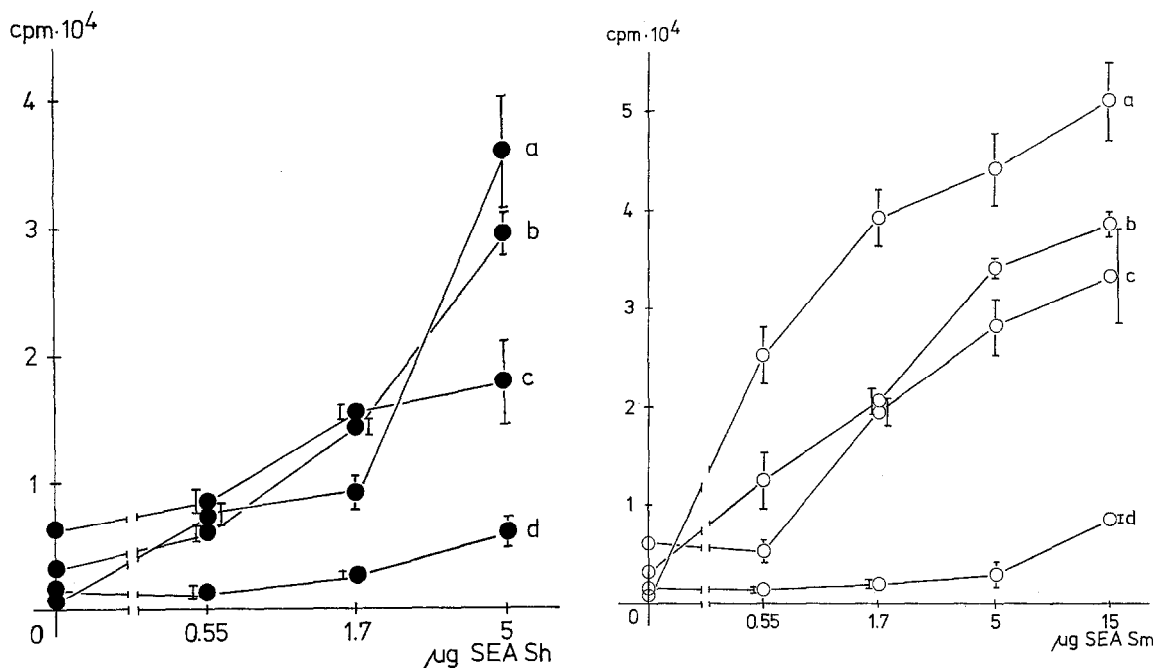

Fig. 1. Dose response curve for *S. haematobium*-infected hamsters (a=300 cercariae, 16 weeks post inf., b=100 cercariae, 23 weeks post inf.). Stimulation by homologous SEA (open circles) and heterologous SEA (closed circles).

Results

Hamster lymphocyte cultures

A marked blast transformation can be induced *in vitro* by SEA in lymphocytes from hamsters in which schistosome egg production has started. The effect of various antigen concentrations on the blastogenic response of *S. haematobium* infected hamsters is shown in Fig. 1. A maximal response to the homologous SEA is observed at an antigen dose of 5 µg protein per culture. With the same concentrations of heterologous SEA no stimulation was detectable. The blastogenic response of lymphocytes from hamsters infected with *S. mansoni* gets higher with increasing amounts of homologous and heterologous antigens (Fig. 2). At a given antigen concentration the stimulation was higher with the homologous SEA.

Lymphocytes from uninfected hamsters were also stimulated by antigen doses exceeding 5 µg protein per culture. In preliminary experiments the blastogenicity

of delipidized, soluble adult schistosome antigens (SAA) was compared to that of SEA (Table I). SAA-Sh* was less stimulatory than SEA-Sh. Despite a high stimulation of unprimed lymphocytes a significant response (P= <0·005) was observed with cells from hamsters infected with *S. haematobium*. Both antigens derived from *S. haematobium* and SEA-Sm showed a significantly higher stimulation in the homologous system. The difference in stimulation between adult and egg antigens of *S. mansoni* was less pronounced than between the Sh antigens. The results of lymphocyte transformation experiments using hamsters infected either with *S. haematobium* or *S. mansoni* are listed in Table II. The mean response of lymphocytes primed in a *S. haematobium* infection to the homologous SEA was 5·2 times higher than that to the heterologous antigens. The difference in stimulation was highly significant (26'562+ 4'764 cpm; P= <0·005).

*SAA-Sh: soluble adult worm antigens of *S. haematobium*

Table I – Blastogenicity of different antigens (protein concentration 5µg/culture) in hamster lymphocyte cultures (SEA: soluble egg antigens, SAA: soluble adult antigens)

Hamsters infected with	cpm of unstimulated cultures (mean±s.e.)	net cpm (mean±s.e.)[1]			
		SEA Sh	SAA Sh	SEA Sm	SAA Sm
S. haematobium	2,607±758	32,883±5,239*	14,644±2,227†	6,321±2,923*	n.d.
S. mansoni	2,874±756	13,807±4,705*	2,357±3,394†	19,888±5,187*	14,620±3,518
uninfected control	830±234	2,255± 967	4,518± 415	1,099± 344	1,167± 155

[1] net cpm=cpm of stimulated cultures – cpm of unstimulated cultures.
* t-test: P <0·05.
† t-test: P= 0·05.

Table II – Lymphocyte transformation induced by SEA (5 μg protein/culture) in individual hamsters infected with *S. haematobium* or *S. mansoni*

Hamsters infected with	SEA Sm net cpm[1] (mean±s.e.)	SEA Sh net cpm (mean±s.e.)	Difference in stimulation[2] cpm
S. haematobium			
Cc[3] wk[4]			
100 23	−88± 48	23,706±2,313	23,794
100 27	14,269±4,465	54,086±1,842	39,817
300 16	188± 68	24,778± 915	24,590
300 20	−372± 70	41,615±3,267	41,987
300 21	12,566±1,401	16,367±1,893	3,801
300 29	16,605±1,868	44,734±6,926	28,129
300 31	1,079± 216	24,898±3,247	23,819
mean±s.e.	6,321±2,923	32,883±5,239	26,562±4,764
	(t-test: P < 0·005)		
S. mansoni			
Cc wk			
100 11	43,254±3,557	34,913±4,382	8,341
100 11	22,185±2,625	11,705±3,128	10,480
100 11	30,818± 494	26,063±1,291	4,755
100 16	4,547± 821	1,166± 238	3,381
100 16	5,989±1,996	1,633± 204	4,350
100 16	14,854±1,361	11,225±1,519	3,629
100 16	17,569±1,621	9,944±2,175	7,625
mean±s.e.	19,888+5,187	13,807±4,705	6,080±1,034
	(t-test: P <0·02)		
uninfected control	602± 53	449± 54	
	1,759± 172	2,554± 565	
	937± 794	3,762± 595	
mean±s.e.	1,099± 344	2,255± 967	
	(t-test, n.s.)		

[1] net cpm=cpm of stimulated cultures – cpm of unstimulated cultures.
[2] Difference in stimulation=net cpm (homologous antigen) – net cpm (heterologous antigen).
[3] Cc=number of cercariae per animal.
[4] wk=weeks post infection.

Table III – Lymphocyte transformation induced by SEA (5 μg protein/culture) in patients infected with *S. haematobium* or *S. mansoni*

Patient	cpm of unstimulated cultures (mean±s.e.)	SEA Sm net cpm[1]±s.e.	SEA Sh net cpm±s.e.	Difference in stimulation[2] cpm
A.D. (Sh)	1,527± 69	11,871±664*	13,252±598*	1,381
A.R. (Sm)	1,252±114	6,582±211†	809±122†	5,773
N.N. (control)	788± 25	997±285‡	289± 39‡	

[1] and [2]: see Table II.
* t-test n.s.
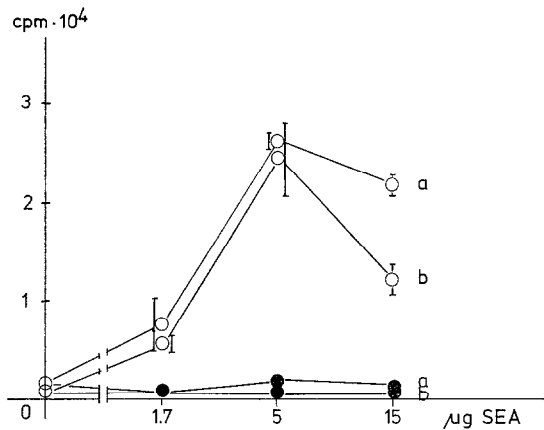† t-test: P <0·001.
‡ t-test: P <0·025.

Fig. 2. Dose response curve for *S. mansoni*-infected hamsters (a, b, c=100 cercariae, 11 weeks post inf.) and an uninfected hamster (d). Stimulation by homologous SEA (open circles) and heterologous SEA (closed circles).

In contrast in the *S. mansoni* infected hamsters the ratio between homologous and heterologous SEA was only 1·4 and the difference in stimulation was low ($6'080 \pm 1'034$ cpm, $P = <0·02$). The two antigens innuced a weak primary response of unprimed lymphocytes.

Preliminary experiments were also performed with human lymphocytes (Table III). In this experiment two patients and one uninfected donor were examined. A.D. had a reinfection with *S. haematobium* 20 months ago and eggs were found in the urine. His lymphocytes showed a highly significant blastogenic response compared to the uninfected donor but there was no significant difference between the two SEA tested. A.R. was lightly infected with *S. mansoni* for more than 16 months (stool examinations were negative; a few eggs were found in a rectal biopsy specimen). In this case the stimulation induced by the homologous SEA was significantly higher as compared to the heterologous SEA.

Discussion

A blast transformation of lymphocytes originating from *S. haematobium* and *S. mansoni* infected hamsters was induced *in vitro* by soluble antigens prepared from eggs and adult worms of both *Schistosoma* species. The response of lymphocytes to homologous SAA and SEA was consistently higher than the response to the corresponding heterologous antigen. The blastogenicity induced by SAA is lower than to SEA. Whether the reaction to SAA is due to common antigens of adult worms and eggs (as demonstrated by SADUN et al, 1965) or whether, in addition, stage specific adult blastogens are involved has to be clarified. The species specificity of these antigens is not absolute since primed lymphocytes reacted also with the heterologous antigen. Gel diffusion analyses have demonstrated a close antigenic relationship between these two schistosome species (CAPRON et al, 1966). Our results indicate that not only the humoral but also the cell-mediated immune response to the two schistosome antigens shows some cross-reactivity, especially when lymphocytes were sensitized in an active infection by *S. mansoni*. Lymphocytes from *S. haematobium*-infected hamsters showed none or only a moderate cross reactivity.

WARREN and DOMINGO (1970) studied the granulomatous reaction elicited by intravenous injection of eggs into sensitized mice. As in our experiments the cross-reactivity was prominent in *S. mansoni*-sensitized mice challenged with *S. haematobium* eggs. It has already been demonstrated that the duration and the severity of murine schistosomiasis mansoni has an influence on the cell-mediated immune response (COLLEY, 1972; BOROS et al, 1975). In order to evaluate these effects studies on the kinetics of the blastogenic response of hamsters infected lightly or heavily with *S. haematobium* and *S. mansoni* are under way. Furthermore purified egg antigens will be used to study the specificity of lymphocyte blastogenesis.

In conclusion we can say that the hamster, which is a suitable host for human schistosome species, is an adequate model for a comparative study of the cell-mediated immune response to schistosomes.

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Book Review

Cheebrough, M. & McArthur, J. (1976). *A laboratory manual for rural tropical hospitals. A basis for training courses.* Edinburgh, U.K.: Churchill Livingstone, xxiii+209 pp. (ISBN 0-443-01144-3. £2·50.)

In tropical developing countries the concept of community health is of great importance; communicable diseases constitute the bulk of medical practice but these can only be reliably diagnosed in laboratories. The publication of a manual, written by two authors with considerable experience in the field and aimed at training laboratory technicians, is welcome and will contribute greatly to improving standards in community health and in diagnosis in rural tropical hospitals.

The manual is divided into seven main sections: laboratory equipment; microscopy; haematology; blood transfusion; bacteriology; examination of stools, urine and other fluids; parasitology. In addition there are 11 pages of definitions of words used in the manual as well as an appendix of three pages in which instructions on preparing solutions and other practical information are included. The alphabetical index of nine pages makes the search for any information simple. The manual is well illustrated by clear line drawings and high quality photographs which enhance the clarity of the text and hence its practical usefulness. In the back cover of the manual there is a novel introduction: this is the inclusion of two strips of film with 16 colour transparencies and a collapsible viewer. The transparencies represent blood films of various types and some of the organisms to which reference is made as they appear under the microscope and are of very high quality. The generous spacing, the double column layout, the clarity of printing and the binding add to the value of the manual for daily practical use.

Most of the techniques employed in laboratories are covered and most of the organisms likely to be found in tropical countries are included. This manual should assist in the formation of reliable medical laboratory services in tropical rural areas and should create in laboratory workers an awareness of their opportunities and responsibilities. The authors have drawn widely on their own considerable practical experience and have certainly achieved their aim to produce a handbook of value to laboratory technicians and doctors working in rural areas. The remarkably low price of £2·50, made possible through financial assistance from several organizations, puts it within the reach of all for whom it is intended. It should not only be on the bench in all rural tropical hospitals but also in those departments and hospitals concerned in the training of those about to go to these areas.

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