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IMMUNOPATHOLOGY OF HUMAN SCHISTOSOMIASIS MANSONI. I. IMMUNOMODULATORY INFLUENCES ON T CELL FUNCTION.[1]

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SUMMARY

Cell mediated immune response was studied in patients with recent and chronic **Schistosoma mansoni** infection. Precultured peripheral mononuclear cells showed significantly higher responses to **S. mansoni** adult worm antigen (SAWA) when compared to fresh cell preparations. The addition of each patient serum to the precultured cells reactions to SAWA or recall antigens demonstrated a strong inhibitory serum action, which was also noted on allogeneic cells derived from healthy subjects. The CD4 subset was the main responding cell to SAWA being this reactivity highly suppressed by the presence of the monocyte macrophage accessory cells. We stressed the simultaneous inhibitory action of humoral and cellular factors on the specific cell response to **S. mansoni**.

KEY WORDS: Immunoregulation; T cells; Human schistosomiasis.

INTRODUCTION

Cell-mediated immunity to **Schistosoma mansoni** remains a controversial issue. Reports on **in vitro** lymphocyte behavior suggest a disfunction of cell reactivity in chronically infected patients, which is in part attributed to serum factors[7,10]. Antibodies to soluble antigens seem to correlate with the presence of serum factors, particularly circulating immune complexes[5]. Although during the different phases of the disease, the infected individual is able to mediate cellular reactions like blastogenesis to polyclonal mitogens and soluble antigens, the suppression of such functions has been reported and partly related to parasitic antigens[7,10].

On the other hand, the suppressor effect possibly exerted by different cells has been documented, not only in **S. mansoni** infection but also in **S. japonicum**[16]. Thus, an inespecific cell suppressor action has been linked to the monocyte macrophage population which could be in part responsible for the immunological compromise observed in **S. mansoni** infected patients.

We have established a research protocol in Venezuelan infected population with **S. mansoni**, to explore simultaneously the different immunological variables which may regulate the mechanism to mount a T cell response to **S. mansoni**.

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MATERIAL AND METHODS

Patient population:

Fifteen patients infected with **Schistosoma mansoni,** ranged in age from 20 to 57 years, were investigated. The diagnosis was established following a clinical protocol already standarized at the Experimental Bilharzia Laboratory from the Tropical Medicine Institute of the Central University, Venezuela. Four patients recently infected (less than a year) and eleven in chronic phase (over a year) were evaluated (Table 1). Ten non-infected healthy individuals, matched by age and sex were selected as controls and studied simultaneously.

TABLE 1
Patient population
Immunoparasitological Features and Disease Stage

	Patient	Sex	Age	EPG*	CIC**
Recent Infection	1	F	32	100	28
	2	F	23	50	112
	3	M	44	50	30
	4	F	42	100	7
Chronic Infection	5	M	40	10	8
	6	F	42	10	90
	7	M	32	10	ND
	8	F	50	10	70
	9	M	38	10	25
	10	F	49	25	ND
	11	F	20	50	100
	12	M	30	ND	220
	13	F	57	75	ND
	14	F	33	50	ND
	15	F	45	10	ND

ND = Not determined
* : EPG = Egg per gram of stool
** : CIC = Circulating immune complexes (normal values: < 35μg/ml)
ELISA = All patients were positive
CPT = All patients showed a positive test

Parasitologic and immunodiagnostic tests:

Both groups of patients were investigated by intradermal test (Bilharzin)[1] and for the presence of antibodies to **Schistosoma mansoni** by the Circumoval Precipitin Test (CPT) and ELISA test as previously described[17,25]. Levels of circulating immune complexes (CIC) were measured by the Clq-solid phase assay as previously standarized[9]. Delayed type skin test to Soluble Adult Worm Antigen (SAWA), was also performed[15]. SAWA was obtained following the technique described by COLLERY et al. in 1977[7].

Stools from patients and controls were investigated for the presence of **Schistosoma mansoni** eggs by the Kato-Katz and the Formalin-ether techniques[13]. (Table 1).

Cells:

Peripheral blood mononuclear cells (PBMC) were obtained by centrifugation of heparinized blood samples on Ficoll-Hypaque density gradient as previously described[4]. The cell preparation was resuspended in RPMI 1640 (Grand Island Biological, N. Y., USA) supplemented with 1% glutamine (Gibco, Grand Island, N. Y., USA) 1% penicillin-streptomycin mixture and 10% pooled heat-inactivated normal human serum (NHS) or autologous serum (AS). The final PBMC suspension was then aliquoted and cultivated either fresh or following an overnight incubation period of 18 hours[2]. In all experiments, the viability of fresh and precultured cells was determined by trypan blue exclusion, being higher than 95% in all cases.

Isolation of lymphocyte subpopulation:

T and non-T lymphocyte subpopulations were fractionated by the sheep red blood cell rosetting (E-RFC) technique as described elsewhere[12]. The E-RFC fraction contained over 95% of T cells as assessed by Leu 4+ monoclonal antibody. The non-T cell fraction (non-T) showed less than 2% contamination of T cells. In order to obtain purified subsets of T cells, the panning technique was used to fractionate the E-RFC population into Leu 2 + (CD8) and Leu 3 + (CD4) subpopulations[5,6].

In relation to the non-T cell fraction adherent and non-adherent cells were separated by Percoll gradients, following the procedure of TIMONEN et al[23]. Briefly, seven clearly defined fractions were obtained. Fraction I contained 98% monocyte macrophage cells (m$\varnothing$) assessed by Giemsa stain, whereas pooled fractions III-V contained non-T cells and were free of adherent cells with less than 2% m$\varnothing$.

In vitro lymphoproliferative assays:

In order to determine T cell proliferative responses to streptokinase/streptodornase (SK

SD), tetanous toxoid (TT) and SAWA, the blast transformation test was employed[21]. Optimal concentration for each antigen was standarized prior to set the assays. The optimal SAWA concentration was established by a dose-response curve (Fig. 1). Cultures were carried out using flat bottom microculture plates (Falcon Plastics, Inc., USA), with a final volumen of 200 µl/well containing $1x10^5$ responding cells (PBMC, T and T cell subsets), different concentrations of monocyte or non-T cell fraction and the selected optimal concentration of the corresponding antigen (SAWA: 100 µg/ml, SK/SD: 100 U/ml, TT: 2 U/ml). The final serum concentration was 10% of either NHS or AS.

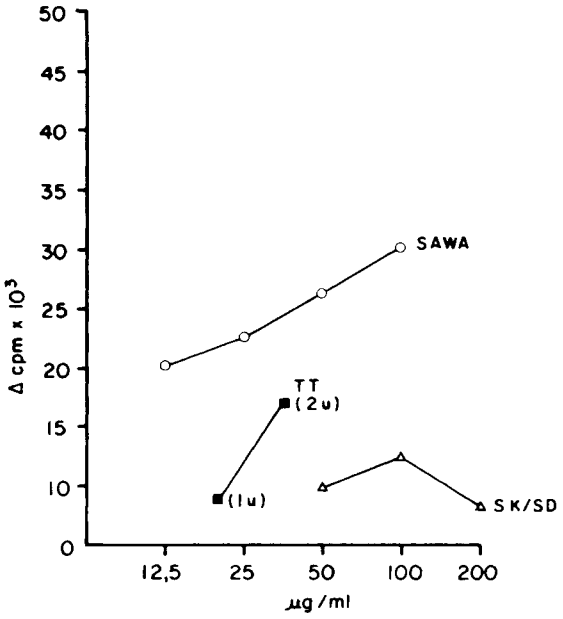

Fig. 1 — UP Optimal SAWA concentration. Dose-Response Curve. DOWN. TT and SK SD: Express in U/ml ○, □, △ mean Cpm 5 experiments. Expriments in NHS.

All cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 for 6 days and where pulsed with 1uCi of tritiated-Thymidine (New England Nuclear, MASS, USA) 16 hours before harvesting with a MASH II apparatus (Microbiological Associates, Bethesda, MD., USA).

Expression of results:

Results were expressed in counts per minute (Δ cpm). Percentage of inhibition was calculated following the formula:

$$\%\text{inhibition} = 1 - \frac{\Delta \text{ cpm AS}}{\Delta \text{ cpm NHS}} \times 100$$

Statistical analysis was performed by Student t test for paired and unpaired samples.

RESULTS

Parasitologic and immunodiagnostic tests:

Table 1 shows the immunoparasitological features from the group of patients. Intradermal test and CPT were positive in all patients. Fourteen patients showed a low parasitic load and 50% from 10 tested patients demonstrated high values of CIC.

Lymphoproliferative assays:

We first compared the blast-transformation of both fresh and precultured PBMC in response to SAWA. A significant greater response was obtained when PBMC were precultured.

Thus, in six experiments, (patients numbers 3, 4, 12, 13, 14 and 15) the precultured PBMC showed a mean response ± SD of 22906 ± 3971 Δ cpm, whereas using fresh cells, the response to SAWA was lower (15669 ± 3149 cpm). Therefore, precultured cells were employed throughout the research protocol[2, 14]. Blastogenic res-

TABLE 2
Responsiveness to SAWA in **S. mansoni** Infected Patients

Recently Infected		Chronically Infected	
1	18958	5	48130
2	16560	6	21654
3	26870	7	41884
4	25436	8	22106
X ± SD	21956 ± 4312	9	63734
		10	30062
		11	25349
		12	14312
		13	23499
		14	26512
		15	18560
		X ± SD	30527 ± 14110 (b)

Total group of patients X ± SD: 38242 ± 12878 (c) 10 matched controls X ± SD: 412 ± 270 (d)
Results Expressed in cpm.
a vs d $p < 0.005$
b vs d $p < 0.005$
c vs d $p < 0.005$

ponse to SAWA was obtained in the patient group but not in the controls (Table 2 (d)). The difference between recently infected and chronically infected patients was not significant (Table 2).

Immunomodulatory effect of Autologous Serum (AS):

The immunomodulatory influence of each patient AS on the proliferative responses to specific antigens was investigated. The mean response ± SD of six experiments to SAWA in the presence of NHS was 22911 ± 3975 Δ cpm while the response when the cells were cultured with AS was significantly lower (102 ± 51 Δ cpm) ($p < 0.005$), corresponding to a mean inhibition of 99.4% (Table 3). The strong inhibitory action of AS from patients was also noted when blast transformation was explored to recall antigens such as SK/SD and TT (Table 3). In addition, three patient sera also showed a significant inhibitory action on the lymphoproliferative responses of control T lymphocytes tested against SK/SD and TT (Table 4).

TABLE 3
Influence of autologous serum on the PBMC responsiveness to SAWA and recall antigens in **S. mansoni** infected patients

		Patients (N = 6) X ± SD	% SUPP.	P
SAWA	NHS	22911 ± 3975	99.4	< 0.005
	AS	102 ± 51		
SK SD	NHS	9456 ± 3338	84.4	< 0.005
	AS	1251 ± 719		
TT	NHS	2571 ± 892	85.7	< 0.005
	AS	335 ± 185		

Results expressed in Δ cpm
NHS: Normal Human Serum
AS: Autologous Serum
SAWA: Soluble Adult Worm Antigen
SK SD: Streptokinase Streptodornase antigen
TT: Tetanous Toxoid Antigen
% Supp: % of suppression

Lymphocyte subsets enumeration and its response to SAWA:

The assessment of total lymphocytes and T cell sub populations was investigated using Leu monoclonal antibodies; no differences between 7 patients (numbers 4, 6, 7, 8, 9, 10, 11) and controls were found (Table 5). Purified T cells (CD 4 and CD8 subsets) from 6 patients (numbers 4, 6, 7, 8, 9, 10) and 6 matched controls were tested against SAWA, SK SD and TT. The helper lymphocytes (CD 4) were the responding cells to SAWA and to recall antigens in both groups (Table 6).

TABLE 4
Influence of allogeneic patient sera on PBMC responsiveness to recall antigens

		Control 1	Control 2	Control 3
NHS	SK SD	15890	9358	7320
	TT	9235	3598	1483
APS	SK SD	2483	3066	1140
	TT	780	63	40
% SUPP	SK SD	93	78	93
	TT	99	99	99

Results expressed in Δ cpm.
APS: Allogeneic patient serum.
% SUPP.: % of suppression.

TABLE 5
T Lymphocytes subsets determined by monoclonal antibodies in patients with schistosomiasis mansoni

	Patients (N 7) X ± SD*	Controls (N 7) X ± SD*
LEU4+a (CD3)	1103 ± 385	1132 ± 437
LEU3+a (CD4)	648 ± 297	649 ± 319
LEU2+a (CD8)	570 ± 397	475 ± 287
RATIO LEU3+a (CD4) / LEU2+a (CD8)	1.39 ± 0.55	1.59 ± 0.50

* Number per mm³

Influence of accessory cells on the proliferation to SAWA:

Figures 2 and 3 represent the mean of three experiments (patients numbers, 13, 14, 15) which explored the influence of accessory cells on PBMC and T cell subsets response to SAWA. Non T cells inhibited the response of T cell and

TABLE 6
Purified T cell subsets responses to SAWA
SK SD and TT

		Patients (N = 6) X ± SD	Controls (N = 6) X ± SD
SAWA	CD4	8306 ± 1081	125 ± 71
	CD8	295 ± 102	20 ± 18
SK SD	CD4	7606 ± 833	10554 ± 2700
	CD8	358 ± 61	351 ± 156
TT	CD4	3623 ± 1929	3547 ± 1897
	CD8	297 ± 63	151 ± 49

Results expressed in Δ cpm
SAWA: Soluble Adult Worm Antigen
SK SD: Streptokinase Streptodornase
TT: Tetanous Toxoid

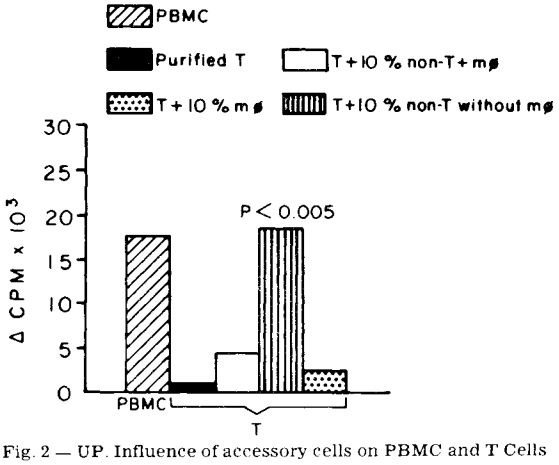

Fig. 2 — UP. Influence of accessory cells on PBMC and T Cells response to SAWA.
DOWN. Responding cells 1 x 10^5 T.

CD4 subset to SAWA. Removing of mØ from non-T cells allowed the responding cells to fully proliferate to SAWA. Furthermore, when responding cells (T and CD4) were cocultured with purified mØ a remarkable inhibition was observed.

DISCUSSION

Human cell-mediated immune responses (CMI) to **S. mansoni** have been widely investigated being the results subject of controversy[10]. Several reports have postulated different immune suppressive mechanisms, mediated either by parasite components, host cells or serum factors[8]. The majority of previous investigations referred to adult worm antigens probably based on the capability of this stage to evade the host immune response.

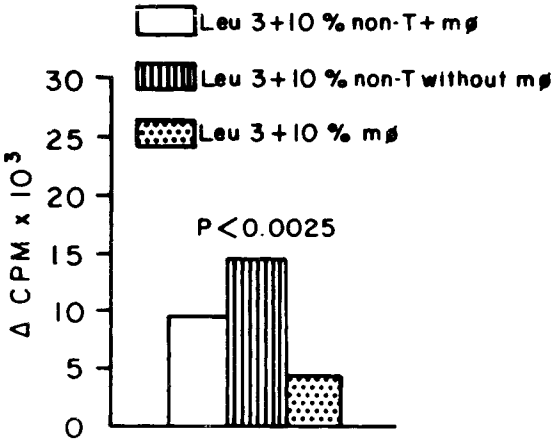

Fig. 3 — UP. Influence of accessory cells on T cell sub-sets response to SAWA.
DOWN. Responding cells 1 x 10^5 Leu 3+

In our investigation, the patients studied were adults, well nourished, all of them with low parasitic load and with the clinical expression of the intestinal or hepatointestinal forms of the disease (Table 1). Contrary to the findings by OTTENSSEN et al[19]., where low lymphoproliferative responses to SAWA were reported only in patients with chronic disease[19], we found that either recently or chronically infected patients exhibited a highly specific proliferative response to SAWA (Table 2), finding also demonstrated by GAZZINELLI et al[11]. This result was particularly evident when using precultured PBMC; thus in 6 selected patients, significant proliferative capability either to **S. mansoni** or to recall antigens was found when compared to fresh cells.

The effect of employing precultured cells in blast transformation test to mitogens or antigens has been previously documented by our laboratory in malignant disease and in other infectious models[2, 14]. The process of preculturing PBMC seems to allow the cell to shed membrane components which may interfere in the interaction between the antigenic molecule and the cell membrane receptors[20, 22]. Thus, in **S. mansoni** infections, the **in vitro** characterization of CMI may be better evaluated utilizing precul-

tured cells which will allow to express full responses to parasitic antigens.

In human Schistosomiasis mansoni there have been previous reports postulating the presence of different immunoregulatory elements such as parasite components or host related factors[18, 24]. COLLEY et al. reported a specific serum-induced suppressive effect on lymphocyte transformation to **S. mansoni** antigens[7]. More recently, GAZZINELLI et al. documented the existence of a suppressive serum effect but using PHA[11]. However, in our study, all six patients tested showed the ability to suppress both autologous (Table 3) or allogeneic blast (Table 4) transformation tests either to SAWA or to recall antigens. The probable nature of these serum factor remains to be elucidated.

On the other hand, in **S. japonicum** infection, coculture cells experiments to parasite antigens have suggested a probable CD8 lymphocyte mediated suppression[16]. In our functional studies using highly purified CD3 subpopulations the CD4 subset was the responding cell to SAWA and recall antigens (Table 6). Moreover, these illustrative experiments tend to indicate that instead of the CD8 subpopulation, the monocyte-macrophage cell lineage seems to be the suppressive cell subset (Fig. 2). Thus, in the absence of macrophages the proliferative responses of the purified CD4 to SAWA were enhanced (Fig. 3).

This suppressive influence of macrophages has previously been postulated using non-purified peripheral blood lymphocytes[24].

In conclusion, several kinds of modulatory influences may operate simultaneously in the **S. mansoni** host relationship. Serum and cell factors may substantially compromise the host capability to erradicate the parasitic load, decreasing the efficiency of the ongoing immune response to the parasite.

RESUMO

Imunopatologia da esquistossomose mansônica humana. I. Influências imunorregulatórias sobre a função T.

A resposta imune celular foi estudada em pacientes com infecção recente ou crônica por **Schistosoma mansoni.** Células mononucleares do sangue periférico pré-cultivadas reagiram significantemente a antígenos do verme adulto (SAWA) do **S. mansoni** quando comparadas à preparação contendo células frescas. A adição de soro autólogo às células pré-cultivadas resultou em inibição da reação frente a SAWA ou antígenos de memória; o mesmo efeito foi notado quando os soros de pacientes foram adicionados a culturas de células alogênicas obtidas de indivíduos normais. A subpopulação CD4 foi a principal população celular respondedora a SAWA, sendo que esta reatividade foi intensamente suprimida na presença de preparações purificadas contendo monócitos-macrófagos. Estes resultados sugerem a ação de fatores inibidores, tanto humorais como celulares, sobre a resposta imune celular específica ao **S. mansoni.**

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