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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[26].

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 (mØ) assessed by Giemsa stain, whereas pooled fractions III-V contained non-T cells and were free of adherent cells with less than 2% mØ.

### 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 $\mu$l/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 $\mu$g/ml, SK/SD: 100 U/ml, TT: 2 U/ml). The final serum concentration was 10% of either NHS or AS.

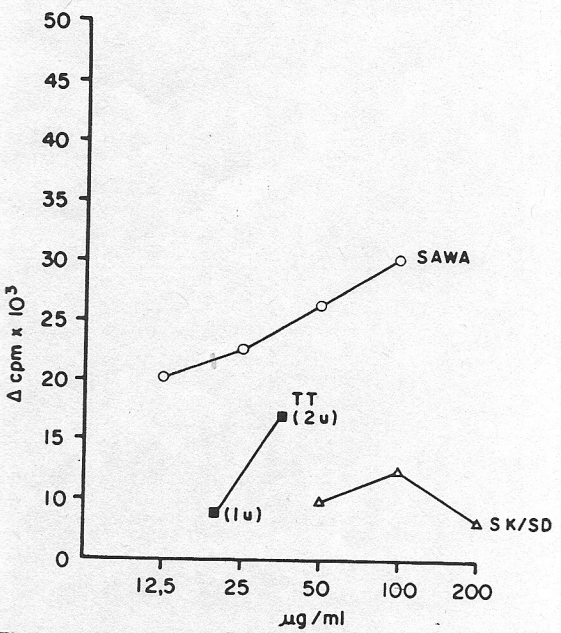


Fig. 1 — UP Optimal SAWA concentration. Dose-Response Curve. DOWN. TT and SK/SD: Express in U/ml ○, □, △ mean Cpm 5 experiments. Expriments in NHS.

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/LEU2+a (CD4) (CD8) | 1.39 ± 0.55 | 1.59 ± 0.50 |

* Number per $mm^3$

**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

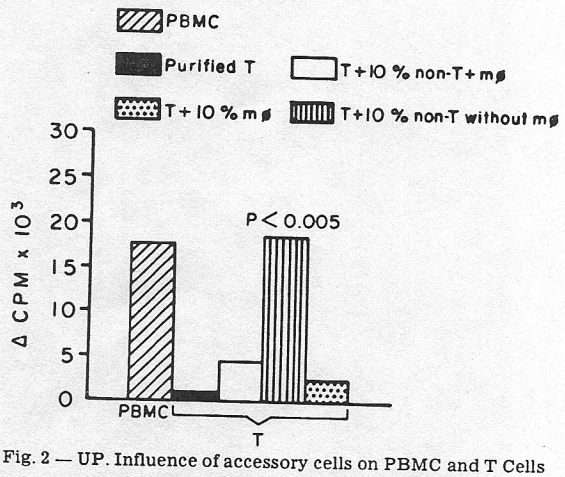


Fig. 2 — UP. Influence of accessory cells on PBMC and T Cells response to SAWA.
DOWN. Responding cells 1 x $10^5$ T.

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.

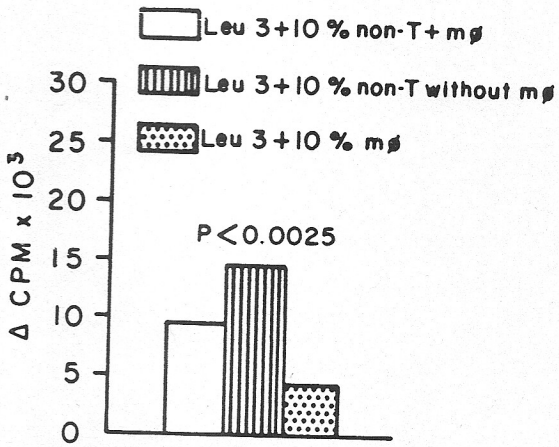


Fig. 3 — UP. Influence of accessory cells on T cell sub-sets response to SAWA.
DOWN. Responding cells 1 x $10^5$ Leu $3^+$

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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# IMMUNOPATHOLOGY OF HUMAN SCHISTOSOMIASIS MANSONI. II. NK ACTIVITY AND STIMULATION BY SPECIFIC ANTIGEN.

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## SUMMARY

Sixteen **S. mansoni** infected and untreated patients (5 with recent infection and 11 with chronic disease) were evaluated for their **in vitro** natural killer (NK) activity against the NK sensitive target K562 cell line. NK levels in 9 out of 11 patients (82%) with chronic disease were significantly lower (mean = 15 ± 6%), compared with patients recently infected (mean = 41 ± 9%, $p < 0.001$) and with the control group (mean = 38 ± 13%, $p < 0.001$). However, both patients and controls NK activity was stimulated by soluble adult worm antigens (SAWA), indicating that NK function even in the chronic stage of the infection is able to respond to the parasite antigens. These results suggest the possibility of NK cell participation as effector mechanism against **S. mansoni**.

**KEY WORDS:** Natural Killer cells (NK); **S. mansoni.**

## INTRODUCTION

Natural Killer (NK) cells are a subpopulation of lymphoid cells defined in several species, including man, by their spontaneous ability to lyse certain tumor target cells **in vitro**[11]. NK activity is found predominantly in a population of cells known as large granular lymphocytes (LGL)[19].

Increasing evidences indicate that in certain types of tumors and infection, NK cells behave as effector cells **in vivo**[18]. The levels of NK activity have been found elevated in children with malaria and in mice infected with **Trypanosoma cruzi** and **Toxoplasma gondii**, suggesting a possible effector role of these cells in parasitic disease[8, 9]. Studies in **Schistosoma mansoni** infected mice have shown contradictory results; some have demonstrated activation of the NK system during the acute phase of the infection, whereas other investigations have failed to show activation during the first four weeks of the infection[1, 2]. Currently, there is no evidence to support the participation of NK cells in any given aspect of Schistosomiasis or other helmintic infections. This prompted us to determine whether **S.mansoni**-infected patients with recent or chronic infection express altered levels of NK activity and the possible susceptibility of NK cells to be stimulated by **S. mansoni** adult worm antigens.

## MATERIALS AND METHODS

### Patients

Sixteen **S. mansoni** infected patients with a mean age of 36 years (range 18-50) were studied.

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Patients population was drawn from individuals admitted to the Bilharzia Experimental Laboratory at the Tropical Medicine Institute of the Central University of Venezuela.

The diagnosis was established following a standarized protocol (Table 1). Five patients were studied 6-12 months after primary exposure to the parasite and were classified as recent infections, and 11 individuals with documented infection longer than two years, were classified as patients with chronic disease. Two patients from the later group presented extra-hepatic granulomas diagnosed by biopsy, one in the cervix of the uterus and the second one in the rectum with acute proctitis. Blood samples were drawn and processed immediately after collection. Stool samples were studied by standard Kato and formol-ether techniques; the number of eggs was also determined by the Kato-Katz technique[14]. In addition, all patients were tested for the presence of specific antibodies against eggs or adult worms by intradermal[13], ELISA[21], and circumoval precipitin test[17]. All but one of the selected patients were parasitologically positive by at least 2 out of 3 immunodiagnostic procedures; furthermore, no other intestinal parasites but **S. mansoni** was detected in these patients. Sixteen non-infected healthy individuals, evaluated by parasitologic and immunological methods, matched by age and sex with the patients, were included as controls.

TABLE 1
Clinical and Immunoparasitological Features of Schistosomiasis Patients

| Type of infection | N° of patients | Sex (f m) | Age Range (years) | INTENSITY OF INFECTION Mean (range) eggs/gr. of feces | COPT* | ELISA** | Intradermal test *** |
|---|---|---|---|---|---|---|---|
| RECENT (6-12 months) | 5 | 2 3 | 23-45 | 65 (25-100) | 100 | 80 | 100 |
| CHRONIC (> 2 years) | 11 | 6 5 | 16-50 | 20 (10-50) | 100 | 91 | 80 |

* — COPT = % positive patients by circumoval precipitin test
** — ELISA = % positive patients by immunoenzymatic assay
*** — Intradermal Test = % positive patients by Bilharzina

### Cell Preparation

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood (10IU/ml sodium heparin) by centrifugation on Ficoll-Hypaque gradient as previously described[4]. PBMC was resuspended in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS) (Gibco, Grand Island, NY), 4/mM L-glutamine and 100IU/ml Penicillin-Streptomycin mixture.

Non-adherent peripheral blood lymphocytes (PBL) were obtained after depletion of adherent cells (mostly monocytes) and B cells by sequential incubation on plastic surfaces (30 min at 37°C) and nylon wool columns, following the Julius's Technique[12].

### Source of adult worm antigen

Soluble adult worm antigen (SAWA) was obtained according to COLLEY et al.[6]. Lyophilized adult worms were suspended in RPMI 1640 and homogenized in a teflon-glass homogenizer. The suspension was maintained at 4°C for 24 hours, frozen and thawed. Thereafter, the suspension was centrifuged at 12.000 g for 2 hours, the supernatant removed and sterilized by filtration through a 0.45$\mu$ pore diameter millipore filter. Protein concentration was determined according to LOWRY et al.[16]. The antigen was aliquoted and kept at -20°C until use.

### Stimulation of NK function with antigens

Two million cells (PBL) from patients and controls were stimulated with different concen-

trations of worm antigen or streptokinase/streptodornase (SK/SD), a common recall antigen; after 18 hours of incubation at 37°C, the cells were washed 3 times with culture medium and used as effectors in the NK cytotoxicity assay. Optimal stimulating doses for the adult worm antigen (2.5-5 $\mu$g/ml) was determined by mean of doses-response curves with a fixed number of cells.

### NK microcytotoxicity assay

A $^{51}$Cr-release assay was performed as previously described[5]. Briefly, the NK-sensitive target cells, K562, were labeled with 200$\mu$Ci of $NA^{51}CrO_4$ (New England Nuclear Boston, Ma.) for 1 hour at 37°C; the cells were washed three times and resuspended at the desired concentration in RPMI 1640 containing 10% FCS.

A fixed number of target cells (4 x $10^3$ in 0.1 ml) was mixed in triplicate with effector cells at different ratios in a 96 well round bottomed microtiter plate. Control wells were filled with 0.1 ml of target cells plus 0.1 ml of culture medium.

After incubation for 4 hours at 37°C, the plates were centrifuged at 400 g for 10 min, and 0.1 ml of supernatant were taken for determination of isotope release. Percent of specific release was calculated by the fórmula:

$$\% \text{ Specific release} = \frac{\text{Experimental release (cpm)} - \text{Spontaneous release (cpm)}}{\text{Total release (cpm)} - \text{Spontaneous release (cpm)}} \times 100$$

### Statistical Analysis

The Student's t-test for paired and non-paired data was used.

## RESULTS

NK activity was determined in the PBL of two groups of selected **S. mansoni** infected patients, 11 with chronic disease (> 2 years infection) and 5 recently infected (< 1 year infection). As shown in Fig. 1, the NK levels in 9 out of 11 patients (82%) with chronic disease was significantly lower (mean = 15 ± 6%), than that of recently infected (mean = 41 ± 9%; $p < 0.001$) and that of the control group (mean = 38 ± 13%; $p < 0.001$).

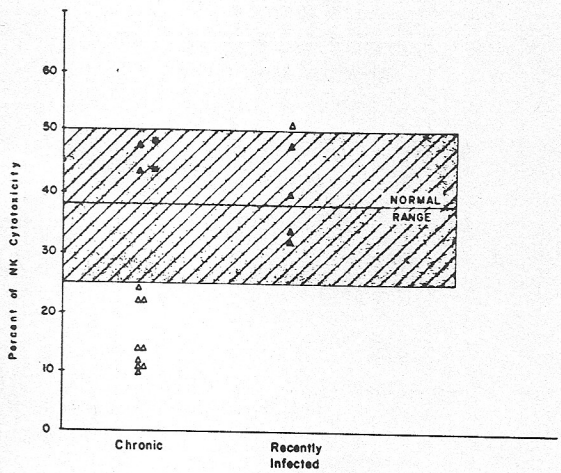


Fig. 1 — Basal NK activity in patients with **Schistosomiasis mansoni**, evaluated against K562 cell line in a 4 hours $^{51}$Cr release assay at 50:1 effector/target cell ratio.
* Patients with extra-hepatic granulomas.

It was interesting to note that the two remaining individuals with chronic disease and symptomatic extra-hepatic granulomas, showed a NK activity within normal range (also depicted in Fig. 1); differences observed among the recently infected patients and controls were not significant.

### Activation of NK function by adult worm antigen

In order to assess whether NK function was susceptible to **in vitro** activation by parasite antigens, non-adherent cells from patients and controls, were stimulated for 18 hours with SAWA and subsequently used as effector cells in the cytotoxicity assay.

As shown in Table 2, a significant activation of NK function was achieved in both patients and controls; this activation was consistently observed when cells from chronic patients were stimulated with 2.5 $\mu$g/ml of SAWA as compared with controls or recently-infected individuals. In the later group, the activation was detected only at the lower effector target cells ration (E/T) and the higher doses of the antigen (5.0 $\mu$g/ml).

TABLE 2
Effect of adult worm antigen on NK activity in schistosomiasis mansoni

| Group | Stimulus μg Ag/ml | Percentage of Cytotoxicity E/T Ratio 100:1 | 50:1 | 25:1 |
|---|---|---|---|---|
| Chronic | 0.0 | 29 ± 10 | 15 ± 6 | 9 ± 4(1) |
| | 2.5 | 47 ± 10(1) | 30 ± 14(2) | 23 ± 15(2) |
| | 5.0 | 42 ± 8(1) | 28 ± 10(2) | 21 ± 12(1) |
| Recently Infected | 0.0 | 58 ± 8 | 41 ± 9 | 30 ± 7 |
| | 2.5 | 60 ± 13 | 45 ± 11(3) | 32 ± 7(3) |
| | 5.0 | 62 ± 7 | 50 ± 4(2) | 36 ± 2(1) |
| Controls | 0.0 | 53 ± 12 | 38 ± 13 | 26 ± 11 |
| | 2.5 | 60 ± 12(3) | 45 ± 12(3) | 34 ± 13(3) |
| | 5.0 | 62 ± 11(2) | 47 ± 13(2) | 34 ± 12(1) |

NK activity was evaluated in basal condition and after estimulation of 2 x $10^6$ PBL (18h at 37°C) with 2.5 and 5.0 μg/ml of SAWA in a four hour $^{51}Cr$ release assay.

(1), (2), (3) represent $p < 0.001$, $p < 0.01$, $p < 0.025$ respectively obtained when compared the basal NK activity vs SAWA stimulated as calculated by the Student's t test for paired data.

### Activation of NK Function by SK/SD

Susceptibility of NK cells to be activated by a variety of antigen, including those of bacterial nature, allowed us to utilize the SK/SD recall antigen in order to evaluate the capability of the NK system in **S. mansoni** patients to respond to other stimulatory proteins[20]. As shown in Table 3, both patients with recent infection and controls responded with a significant increase of NK function when stimulated with a dosis of 100 μg/ml SK/SD antigen.

TABLE 3
"In vitro" stimulation of NK activity by the SK/SD recall antigen in patients with Schistosomiasis Mansoni

| Groups | Percent of Cytotoxicity Basal | SK/SD | P* |
|---|---|---|---|
| Patients (n = 4) | 58 ± 11 | 77 ± 16 | 0.025 |
| Controls (n = 4) | 61 ± 11 | 68 ± 9 | 0.05 |

2x$10^6$ PBL were estimulated (18 hour at 37°C) with 100 μg/ml of SK/SD antigen and tested for NK activity in 4 hours $^{51}Cr$ release assay.
Student's test for paired data.

## DISCUSSION

NK cells are considered a non-specific effector mechanism, susceptible of activation by a variety of viral, tumoral and some bacterial antigens[15, 20]. The reports on the participation of this effector mechanism against parasites are rather scant. In several models such as **Plasmodium chabaudi**[1], **Babesia microti**[1], **Trypanosoma cruzi**[8], and **Toxoplasma gondii**[9] increase in NK activity have been found. More recently, a NK-mediated killing of **T. gondii** was demonstrated in vitro[10]. Experimental studies in recent **S. mansoni** infected animals have shown activation of the NK function which decreases as the infection became chronic; in humans, no previous report has compared NK function in patients with recent and chronic **S. mansoni** infection. In addition, as far as we know, there is not previous evidence of NK activation by **S. mansoni** antigens.

The results presented here show that **S. mansoni** chronically infected patients have a significant depressed NK activity as compared with that of recently infected individuals and the control group. By contrast with other reports on NK function in Schistosomiasis, our patients were only infected with **S. mansoni** and the parasite burden was lower[7]. Furthermore, our results are in contrast with those of BARSOUM et al[3] who did not find alterations of the NK activity in chronic **S. mansoni** infected patients.

Although the presence of inactive or defective NK cells was not examined in this study, it was of interest to find that soluble antigen from adult forms of the parasite (SAWA) and SK/SD stimulated significantly the NK function in both groups of patients. These observations indicate that non-intrinsic defect exists at the NK cell level and also suggests the possibility of NK cells participation as effector mechanism against **S. mansoni** particularly during the early stages of the infection.

The susceptibility of the different stages of the parasite to human NK cells should be investigated in order to gain further knowledge on the possible protective role of this effector mechanism against **S. mansoni.**

BENARROCH, L. K.; NOYA, O.; NOYA, B.; BIANCO, N. E. & BLANCA, I. — Immunopathology of human schistosomiasis mansoni. II. NK activity and stimulation by specific antigen. **Rev. Inst. Med. trop. São Paulo, 30 (6):** 400-405, 1988.

## RESUMO

**Imunopatologia da esquistossomose mansônica humana. II. Atividade NK e estimulação por antígeno específico.**

Dezesseis doentes infectados e não tratados com **S. mansoni** (5 com infecção recente e 11 com doença crônica), foram submetidos à avaliação de atividade de células exterminadoras naturais (NK) "in vitro" frente a células-alvo de linhagem K562. Os níveis de atividade das células NK em 9 de 11 doentes (82%) com a infecção crônica foram significativamente menores (média = 15 ± 6%) quando comparados aos pacientes com infecção recente (média = 41 ± 9%, $p < 0,001$) e aos indivíduos do grupo controle (média = 38 ± 13%, $p < 0,001$). Porém, tanto nos doentes como nos controles, a atividade de células NK foi estimulada pelo antígeno solúvel do parasito adulto (SAWA), indicando que as células NK, mesmo na fase crônica da infecção, têm capacidade de responder ao antígeno dos parasitos. Estes resultados sugerem a possível participação das células NK no mecanismo efetor de defesa contra o **S. mansoni.**

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