Mem. Inst. Oswaldo Cruz, Rio de Janeiro,
Vol. 83(4): 531-532, out./dez. 1988

RESEARCH NOTE

IN VITRO INHIBITION OF CELLULAR DIVISION BY TRIATOMINE'S HAEMOLYMPH

N. J. ALVARENGA & M. J. F. MORATO

Centro de Pesquisas René Rachou – FIOCRUZ, Av. Augusto de Lima, 1715, 30190 Belo Horizonte, MG, Brasil

It has previously been shown (Alvarenga et al., 1984, XI Reunião Anual sobre Pesquisa Básica em Doença de Chagas – Caxambu, MG, VE 43) that the addition of *Rhodnius prolixus* haemolymph to LIT culture medium of Y and CL strains of *Trypanosoma cruzi* promoted a decrease in the number of flagellates suggesting the presence of a cell growth inhibitor factor in the haemolymph of the insect.

Based on these findings we have decided to investigate the presence of this cell growth inhibitor factor in the haemolymph of different species of triatomines and also to test its effect on other cellular systems to verify whetther the action of triatomine's haemolymph would be restricted to culture forms of *T. cruzi.*

In the present investigation we have tested the effect of the haemolymph of *Dipetalogaster maximus, Triatoma infestans* and *R. prolixus* on culture forms of *T. cruzi* as weil as on human peripheral blood mononuclear cells (PBMN) proliferation after stimulation by Phytohemagglutinin P (PHA), (Difco Labs., Detroit, Michigan).

Haemolymph from fifth stage larva of the three triatomines species fed 72 hours before were collected from exiced leg with Pasteur pipette and maintained on ice. The haemolymph was then centrifuged (2.000 rpm/ 10 min at 4 °C) and the supernatant filtered in Millipore (0,45 μm) and stored at –20 °C until use. Culture forms of the Y_{P3} clone from the Y strain of *T. cruzi* (Morato et al., 1986, *Am. J. Trop. Med. Hyg., 35:* 505-511) in exponential growth were used in all experiments. Each experiment was performed in triplicate.

In the first experiment the haemolymph from the bugs were added in a final concentration of 1:8 (v/v) in LIT medium containing 2.500.000 flagellates/ml. The control tubes received equal volumes of PBS. The cultures were maintained at 27 °C and 72 and 96 hours later the number parasites/ml were counted using a Neubauer hemocytometer.

The effect of the haemolymph on the growth of human PBMN was also investigated. The blood was collected from normal (non-infected) donors, layered over a Ficoll-diatrizoated mixture (LSM) (Litton Biometrics, Inc., Kensiton, Maryland) and centrifuged (40 min/400 x g/20 °C). The PBMN layer was collected, washed 3 times with Minimum Essential Medium (MEM) (Gibco, Grand Island, New York) and ressuspended to a final concentration of 6 x 10^6 cells/ml of RPMI 1640 (Gibco). The PBMN were cultured in flatbotton tissue culture plates at a concentration of 150.000 cells per well in complete medium (91% RPMI; 1% L-glutamine stock of 2 mM), 3% antibiotic-antimycotic (100 x stock of 10.000 U penicillin, 10.000 μg streptomycin, 25 μg fungizone per ml), and 5% heat inactivated, normal human, AB, Rh^+ serum. Cultures were stimulated with 2,5 μg/ml of PHA either in presence and/or absence of different dilutions of triatomine's haemolymph and maintained at 37 °C in 5% CO_2 in air for 3 days, when each culture received 0,5 μCi of tritiated thymidine (specific activity: 2.0 Ci/mM). Cultured cells were collected 6 hours later on glass fiber filter paper using an automatic cell harvester and the retained radioactivity was determined by scintillation spectroscopy. Data were calculated as mean CPM of triplicate cultures, and presented as experimental CPM (E) – control (unstimulated) CPM (CPM = E-C). The percentage of inhibition was evaluated as follow: $\%\ \text{inhibition} = \frac{C - E}{C} \times 100$.

The results suggest that the haemolymph from *R. prolixus, D. maximus* and *T. infestans* are able to suppress *T. cruzi* growth (Table I) as well as inhibit PBMN proliferation after stimulation by PHA (Table II). Furthermore the data also show that *R. prolixus* and *D. ma-*

Research supported by CNPq.

Received July 26, 1988.
Accepted September 30, 1988.

ximus haemolymph have higher inhibitory activity on cellular division (*T. cruzi* and/or PBMN) than the haemolymph from *T. infestans*. No cellular lysis was observed in the cultures due to addition of haemolymph. One fact that corroborate with this observation is noted in Table II where the percentual of cell growth inhibition is higher for *D. maximus* and *T. infestans* haemolymph at a concentration of 1:512 than it is at 1:256 and 1:32 respectively.

TABLE I

Inhibition of *Trypanosoma cruzi* (Y_{P3} clone) culture growth in presence of triatomine's haemolymph

Insect haemolymph (concentration 1:8)	Number of flagellates/ml 72 hours	(% inhibition) 96 hours
R. prolixus	2.850.000 (71)	4.500.000 (79)
D. maximus	4.300.000 (75)	7.500.000 (65)
T. infestans	9.500.000 (4)*	7.000.000 (68)
Control	9.944.000	21.600.000

* Not significant.

TABLE II

Inhibition of human peripheral blood mononuclear cells proliferation after stimulation by Phytohemagglutinin in presence of triatomine's haemolymph

Triatomine species	% of cell growth inhibition						
	haemolymph concentration						
	1:8	1:16	1:32	1:64	1:128	1:256	1:512
R. prolixus	65	71	45	37	24	15	17
D. maximus	73	48	46	28	25	13	29
T. infestans	29	18	10	33	25	26	24

It is possible that differences in concentration of this growth inhibitory factor occur in the haemolymph of the three species of triatomines. We are now attempting to identify and isolate this factor from the haemolymph of different triatomines in order to clarify whether the factor is the same.

Acknowledgment – We would like to thank Dr Rodrigo Corrêa-Oliveira for his assistance in the preparation of this manuscript.