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COMUNICAÇÃO

HUMAN IMMUNE RESPONSE TO TRIATOMINE EMBRYO EXTRACT

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Dipetalogaster maximus *embryo extracts were used to stimulate peripheral blood mononuclear cells (PBMC) and in ELISA with sera either from* Trypanosoma cruzi *infected or non-infected individuals. The results showed that there was significant proliferative response and high antibody titers in sera of chagasic patients.*

Key-words: Dipetalogaster maximus. Trypanosoma cruzi. *Triatomine embryo extract. Chagasic immune response.*

Dipetalogaster maximus embryo extracts were used to evaluate their effects and specificity on human peripheral blood mononuclear cells (PBMC) of chronic chagasic patients. It was previously observed that these extracts were able to promote and exacerbate the growth of the parasites when added to *Trypanosoma cruzi* culture medium[1].

A suspension of *D. maximus* four day old embryos was obtained from ground eggs in PBS (pH 7.2) and the extract filtered with 0.45µm millipore filter and stored at -20°C. The protein concentration was measured[2] and adjusted to 160µg/ml in the culture (final concentration).

The blood was collected from six non infected donors and from four patients with Chagas' disease, layed over a ficoll-diatrizoated mixture (LSM) (Litton Biometrics, Inc, Kensiton, Maryland) and centrifuged (40min/400 x g/20°C). The PBMC layer was collected, washed three times with Minimum Essential Medium (MEM) (Gibco, Grand Island, New York) and resuspended to a final concentration of 6×10^6 cells/ml of RPMI 1640 cell culture medium (Gibco). The PBMC was cultured in flat bottom tissue culture plate at a concentration of 150.000 cells per well in complete medium (91% RPMI; 1% L-glutamine stock of 2mM), 3% antibiotic-antimycotic (100x stock of 10.000U penicillin, 10.000µg streptomycin, 25µg fungizone per ml) and 5% heat inactivated, normal human, AB, Rh+ serum.

Cultures were also stimulated with 2.5µg/ml of PHA either in the presence or absence of triatomine's embryo extract and maintained at 37°C in 5% CO_2 in air for three days, after which each culture received 0.5uCi of tritiated thymidine (specific activity: 2.0Ci/mM). Cultured cells were collected 6 hr later on glass fiber paper using an automatic cell harvester and the retained radioactivity was determined by scintillation spectroscopy. Data are presented as mean CPM of triplicate cultures (CPM = E - C).

Surprisingly the results indicated that *D. maximus* embryo's extract is able to increase the proliferative response of peripheral blood mononuclear cells from patients with Chagas' disease and not of PBMC from non infected donors (Figure 1).

The data in these experiments lead us to conclude that triatomine's embryo extract is able to stimulate human PBMC when these cells are from patients with Chagas' disease, suggesting that there are antigenic epitopes in triatomine embryo cells that could be analogous to some of those found on the parasite, which are recognized by the immune system of infected human patients.

To further investigate the immune reactivity to *D. maximus* embryo extracts, sera from five chagasic patients and six non-infected donors

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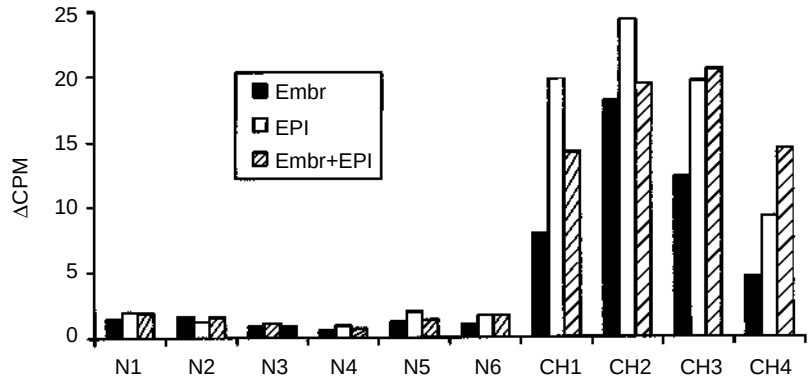

Figure 1- Human PBMC proliferation (ΔCPM) of non infected (N) and chagasic (CH), exposured to T. cruzi *antigen (EPI) and/or triatomine's embryo extract (Embr).*

were used in ELISA at dilutions starting at 1:40 to 1:160 with the *T. cruzi* antigen at the concentration of 40µg/ml.

Figure 1 shows increasing of proliferative response of PBMC as previously indicated.

The results have confirmed our previous observations in the proliferation assays showing that antibodies present in sera from chagasic patients react against triatomine's embryo extract with higher titers than those observed with sera from normal donors, confirming our previous suggestion that chagasic patients develop an immune response that cross-react with *D. maximus* embryo extract (Table 1).

Table 1- Sera titers of chagasic patients (CH) and non-infected donors (N) against antigens of D. maximus *embryo extracts and* T. cruzi.

Sera	*T. cruzi*	Embr.1:40	Embr. 1:80	Embr. 1:160
CH1	0,411	0,705	0,430	0,211
CH2	1,143	0,384	0,322	0,158
CH3	0,973	0,418	0,238	0,114
CH4	0,841	0,522	0,315	0,151
CH5	1,286	0,397	0,249	0,151
N1	0,139	0,156	0,043	0,024
N2	0,182	0,141	0,088	0,062
N3	0,194	0,198	0,070	0,041
N4	0,132	0,177	0,147	0,083
N5	0,052	0,241	0,108	0,045
N6	0,109	0,159	0,106	0,054

RESUMO

Extratos de embrião de Dipetalogaster maximus *foram usados para estimular a proliferação das células mononucleares do sangue periférico humano (PBMC) bem como em ELISA com soro de indivíduos infectados ou não pelo* Trypanosoma cruzi. *Os resultados mostraram significante proliferação das PBMC e títulos mais elevados nos soros de pacientes chagásicos.*

Palavras-chaves: Dipetalogaster maximus. Trypanosoma cruzi. *Extrato embrionário de triatomíneo. Resposta imune de chagásicos.*

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