RESEARCH NOTE

Cell Growth Inhibitor Factor in Hemolymph of *Dipetalogaster maximus*

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In the present study we have investigated the presence of the already described cell inhibitor factor (NJ Alvarenga & MJF Morato 1988 *Mem Inst Oswaldo Cruz 83*: 531-532) in the hemolymph of all developmental stages of *Dipetalogaster maximus* comparing it with the data obtained with the hemolymph from 5th larval stage of *Rhodnius prolixus*. We also investigated the effects of this cell inhibitory factor in the presence of different protease inhibitors. The presence of *Trypanosoma cruzi*-derived exoantigens in the hemolymph of infected *D. maximus* was also investigated by ELISA and eletrophoretic analysis of the hemolymph from different larval and adult stages.

One hundred *D. maximus* nymphs of the 2nd and 3rd stage, 50 of the 4th and 5th stages, 30 males and females and 200 *R. prolixus* triatomines of the 5th stage were used in the experiments. The insects were fed 72 hr before collecting its hemolymph by excising one of the metathoraxic legs and aspirating it with a Pasteur pipette. The hemolymph was collected in 3 ml Eppendorf tubes in ice-bath, centrifuged (300 g), filtered (0.45µm Millipore filter) and stored at -20^{o}C before use. A pool of 2 ml hemolymph from *D. maximus* 4th stage nymphs and an equal volume of 5th stage *R. prolixus* nymphs were collected in tubes containing 10 ml of absolute ethanol solution of the following protease inhibitors: PMSF (142 mg), Pepstatin (686 µg), TPCK (20 mg), TLCK (5 mg) (all from SIGMA Chemical Company), diluted to a final concentration of 1:100. After centrifugation and filtration the material was aliquoted and dialysed (against PBS), before addition to PBMC cultures. All experiments were performed in triplicate.

The results showed that haemolymph from every developmental stage of *D. maximus* inhibited the growth of the Yp_3 clone of *T. cruzi* and of human peripheral blood mononuclear cells (PBMC). The growth inhibition was greater when higher concentrations of hemolymph were used. Hemolymph from adult females was able to promote greater inhibition than hemolymph from adult males as were those collected from the nymphal stages (Fig. 1). PBMC culture media containing dialyzed hemolymph from *D. maximus* and *R. prolixus* collected with or without protease inhibitors maintained the cell inhibitory activity. Higher titers of inhibition were constantly observed with the addition of *R. prolixus* hemolymph. These findings lead us to conclude that the inhibitory factor must be a large protein without enzymatic activity and probably found in greater concentrations in the hemolymph of *R. prolixus* than in *D. maximus*.

In ELISA, the pool of chagasic sera as well as of normal donors reacted to the same extent with infected and non-infected triatomines hemolymph (Fig. 2). Electrophoretic analysis of hemolymphs from *D. maximus* showed striking differences in the profiles of adult male and female. Statistical analysis using one-way analysis of variance and Student-Newman-Keuls Multiple Comparisons Test demonstrated that all experimental groups had statitically significant ($p < 0.001$) differences when compared to the control values. Bands of apparent molecular weights of 51.7, 115 and 147kDa were absent from male hemolymph, while bands of 18.7 and 25kDa were not found in hemolymph collected from female insects (data not shown). Electrophoretic analysis for the presence of *T. cruzi* antigens in triatomines hemolymph, performed by Western immunoblots showed no differences in the profile of infected and non-infected *D. maximus* hemolymph, evidencing the absence of *T. cruzi* antigens in the hemolymph of triatomines. The observed ELISA reactivity may be explained by the possibility that among the antigens in the hemolymph of *D. maximus* there may also be a number of antigens present in the saliva of common anthropophilic hematophagous mosquitoes, that naturally feed on man.

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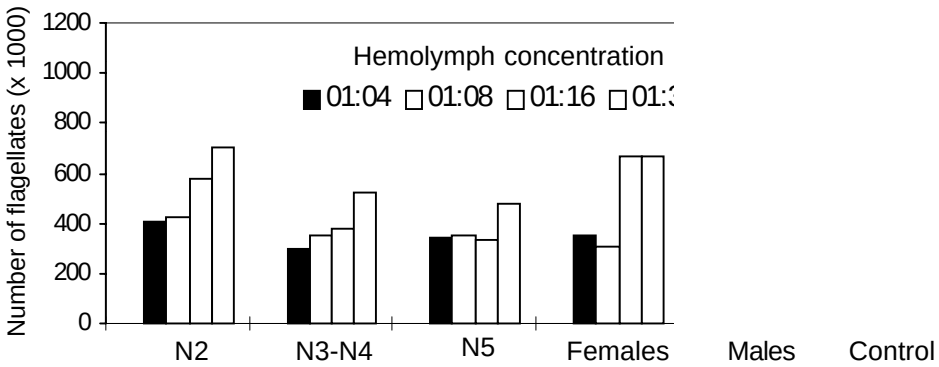

Fig. 1: number of *Trypanosoma cruzi*, in LIT culture in presence of different concentrations of hemolymph from various nymphal stages (N2-N5), females and males of *Dipetalogaster maximus*.

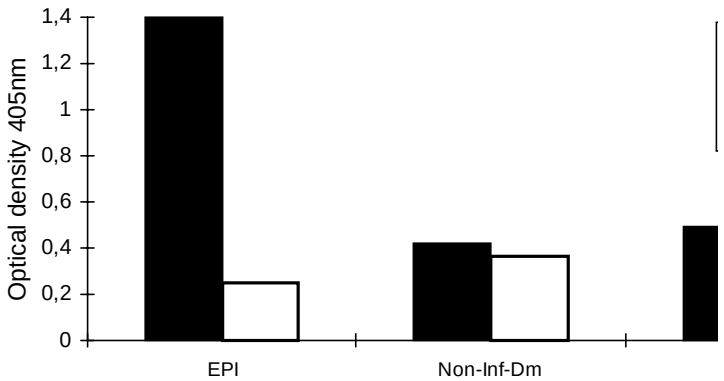

Fig. 2: reaction of pooled chagasic sera (Chg) and normal human sera (Norm), measured by ELISA to *Trypanosoma cruzi* epimastigote soluble antigen (Epi), non-infected *Dipetalogaster maximus* hemolymph (Non-Inf-Dm) and hemolymph from infected *D. maximus* (Inf-Dm).