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Research Note

TRIATOMINE'S HEMOCYTES AND GRANULOMA FORMATION AROUND BIOLOGICAL AND NON-BIOLOGICAL MATERIAL

N. J. ALVARENGA; E. BRONFEN; R. J. ALVARENGA*& M. A. BARRACCO**

Centro de Pesquisas René Rachou – FIOCRUZ, Caixa Postal 1743, 30190 Belo Horizonte, MG, Brasil
*Dept. Patologia Geral, ICB/UFMG, Belo Horizonte, MG **Dept. Biologia, UFSC, Florianópolis, SC, Brasil

The mechanism of cellular defense in insects is basically mediated by hemocytes and is known as an encapsulation process. The reaction is characterized by the superposition of several layers of hemocytes surrounding a foreing agent giving rise to what is called hemocytic capsule (A. F. Rowley & N. A. Ratcliffe, 1981, in N. A. Ratcliffe & A. F. Rowley, ed. *Invertebrate Blood Cells*, Academic Press, London). An important aspect often noted with capsule formation is the occurence of melanization as a result of profenoloxidase activation in presence of substances probably supplyed by the same hemocytes (G. A. Almirante, 1986, in A. P. Gupta, ed. *Hemocytic and Humoral Immunity in Arthropods*, John Willey & Sons, NY).

We have injected into the hemocoel of thirty (3rd, 4th and 5th stage) larvae of *Dipetalogaster maximus* pollen grains of *Hibiscus rosasinensis*, sterile polyacrylamide and polymethacrylate beads, coated and/or uncoated with different antigens. Pollen grains were previously fixed in a 3% aqueous formaldehyde solution, three times washed and suspended in PBS (pH 7.2) before its injection. Polyacrylamide beads (Bio-gel P-4, 200-400 mesh, BIORAD, Richmond, California, USA) and Polymethacrylate beads (Degalan V26, 10-600 μm, Duke Scientific Corp., USA) were sterilized by gama radiation and hydrated with distilled water for 48 h before use. Beads were then washed in 0.5 M bicarbonate buffer by gentle centrifugation. 200 mg of beads were incubated for 4 h in a 63 ^{O}C water bath with gentle agitation and then washed for three times with sterile distilled water. Polyacrylamide beads were then mixed with 20 mgs of PPD soluble antigen and the polimethacrylate beads with *Trypanosoma cruzi* antigen. The mixtures were gently agitated for 18 h at 4 ^{O}C and then washed with sterile PBS and stored at 4 ^{O}C.

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Hemolymph from triatomines 72 h previously fed were collected from exiced leg with Pasteur pipette and added to a final concentration of 1:2 (v/v) in Minimum Essential Medium (MEM) (Gibco, Grand Island, New York) and distributed in Eppendorf micro tubes.

Each tube received 0.5 ml of the solution and 0.015 ml of pollen grains, polyacrylamide and polymethacrylate beads suspention and were maintained in gentle mixing action in a test tube rocker for four hours. One drop of each suspention was then examined under phase-microscope and photographed.

Pollen grains (0.015 ml), polyacrylamide and polymethacrylate beads (coated and uncoated with antigens) solution were inoculated in the base of the third basal coxa of each insect with an 1 ml sering provided of 0.45 x 13 mm needle. The triatomines were dissected at 6 h and 1, 2, 7, 15 and 30 days after under a stereoscopic microscope and the material colected from the hemocoel examined under phase contrast microscope and photographed.

Results showed the occurrence of encapsulation over injected pollen grains and antigen coated beads in the hemocoel of triatomines since the first 6 h after injection. In different periods of examinations it was possible to visualise the cellular arrangement in a "granuloma like" figure when grains and beads showed to be surrounded by multiple layers of cells (Figs 1a, b). Cellular reaction over polyacrylamide and polymethacrylate uncoated beads were not observed. 15 and 30 days after pollen grains injection it was observed a cellular reaction in three triatomines involving the external "stomach" cell wall resembling a cyst body differently from the capsules found freely in the hemocoel of those insects. Histological sections showed a granulomatic cell distribution around these pollen grains (Fig 1c) appatently with the involvement of plasmatocytes and coagulocytes.

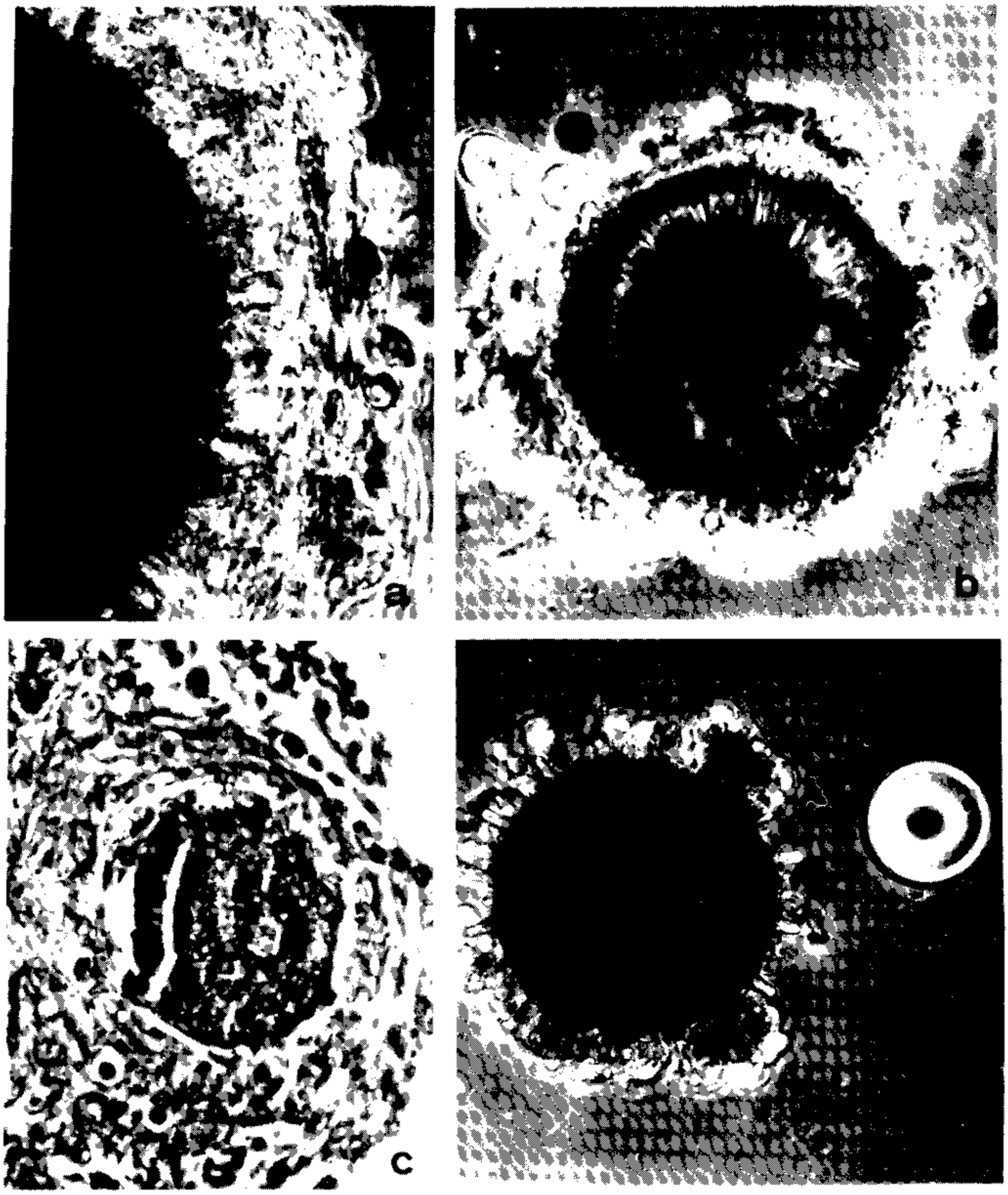

Fig. 1a: detail of a pollen grain surrounded by hemocytes 30 days after its injection in the hemocoel, 400 x. Fig. 1b: antigen coated polymethacrylate bead surrounded by hemocytes 24 h after its injection in the hemocoel, 400 x. Fig. 1c: section of a pollen grain surrounded by hemocytes 15 days after its injection in the hemocoel, H. & E. 160 x. Fig. 1d: 4 h *in vitro* reaction of hemocytes around pollen grain and presence of one polyacrylamide bead not coated with antigen, 160 x.

The process of melanization was observed over pollen grains and antigen coated beads since the first 6 h after injection but sometimes it was faint and even impossible to be detected.

In vitro observations showed pollen grains and antigen coated beads surrounded by hemocytes resembling the same observed pictures of the material collected from hemolymph of the first 6 h injected triatomines (Fig. 1d).

The results were similar as those described as occuring also in *Panstrongylus megistus* (N. J. Alvarenga et al., 1989, *Mem. Inst. Oswaldo Cruz, 84,* Suppl. II: 127) and others insects (G. Salt, 1970, *The Cellular defense reaction in insects,* Cambridge Monographs In Experi-

mental Biology no. 16, Cambridge University Press, London; A. M. Lackie, 1970, *Immunology, 36:* 909-914; A. F. Rowley & N. A. Ratcliffe, 1981, *loc cit.*) It was not possible to visualise the same reaction when an steril non-biological body was introduced in the hemocoel. These findings make us to speculate about the presence of hemocytes membrane receptors that should be able to recognize only biological materials, reinforcing the idea of the existence of an immune system in insects. We also speculate if the so called hemocytic capsules could be called "granuloma" considering that the observed reactions are similar to those found by M. C. B. F. Melro & M. Mariano (1987, *Mem. Inst. Oswaldo Cruz, 82,* Suppl. IV: 245-252) when describing extra tissular *Shistosoma mansoni* egg granuloma in the peritoneal cavity of mice and also considering an hemocytic reaction with the possibility of involvement of other tissues.

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