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Research Note

BINDING SITES FOR IgG-Fc IN HEMOCYTE ADHERENT CELLS OF HEMATOPHAGOUS BUGS *(RHODNIUS PROLIXUS)*

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The cellular defense reactions of invertebrate against foreing agents which is mediated by the hemocytes may be important for deeper understanding of the interaction between parasites and insect vectors. Insect hemocytes are able, for example, of phagocytosing several microorganisms *in vivo* and *in vitro* (J. C. Jones, 1962, *Amer. Zool., 2:* 209-246; N. A. Ratcliffe & A. F. Rowley, 1974, *Nature, 252:* 391-392). However, it is still not fully understanding the phagocytic mechanisms of defenses in insects against invading agents (N. A. Ratcliffe, 1982, *Zbl. Bakt.*, Suppl. *12:* 223-244). In vertebrate the imunoglobulins G possess an opsonic activity towards sheep erythrocytes and trigger the ingestion of these cells (B. Mantovani et al., 1972, *J. Exp. Med., 135:* 780-792). On the other hand, it is well known that vertebrate phagocytosis involves at least two phases: attachment and ingestion. Phagocytosis in insects, as in vertebrates, also presents both biological processes (N. A. Ratcliffe, 1982, *loc. cit.*). The attachment phase of phagocytosis which is mediated by serum opsonins in many vertebrates and invertebrates, in insects seems to be non-specific (N. A. Ratcliffe & A. F. Rowley, 1979, p. 331-414. In A. P. Gupta, *Insect haemocytes, development, forms, functions and techniques.* Cambridge University Press). Whether in insects, in general, very few informations on the phagocytic activity of hemocytes are available, in hematophagous insects, in particular, nothing is known on the factors involved in phagocytosis.

These facts previously described led us to give attention to the phagocytic activity of hemocytes of the bloodsucking bug, *Rhodnius prolixus*. Our experiments show that *Rhodnius* adherent hemocytes have binding sites for IgG-Fc which enhances the attachment and, consequently, the ingestion of sheep erythrocytes by these cells.

Hemocytes were taken from hemolymph of one hundred fifth-instar larvae of *R. prolixus* four days after feeding on citrated human blood. Hemocytes were isolated on 16 mm glass print-slides due to their exclusive adherence property on glass. Adherent cells were washed in 0.15M phosphate buffered saline (PBS), pH 7.2. A preliminary study showed that with this procedure predominates plasmatocytes and granulocytes and that mainly plasmatocytes had phagocytic property. Sheep red blood cells (SRBC) were washed three times in PBS and then sensitized or not with purified IgG anti-sheep erythrocytes (IgG purified by G.200 colum chromatography). In all experiments the phagocytic activity was measured one hour after the incubation (28 °C) of adherent hemocytes with previously SRBC sensitized or not with IgG. The slides were washed with PBS, fixed in 2.5% glutaraldehyde. Phagocytosis was observed with a phase contrast microscopy.

Preliminary observations revealed that adherent cells presenting a maximal phagocytic activity after one hour of incubation with IgG sensitized SRBC. Non-specific attachment of SRBC with adherent hemocytes were considered which presented until three erythrocytes on their membrane surfaces. Figure 1 shows that more then 50% of hemocytes incubated with SRBC previously coated with IgG antisheep erythrocyte presented more than three sensitized erythrocytes. In contrast, when adherent cells were incubated with non-sensitized SRBC the percentage of attached erythrocytes was significantly decreased (10%). Figure 1 also shows that the ingestion of sheep erythrocytes were significantly different if compared the

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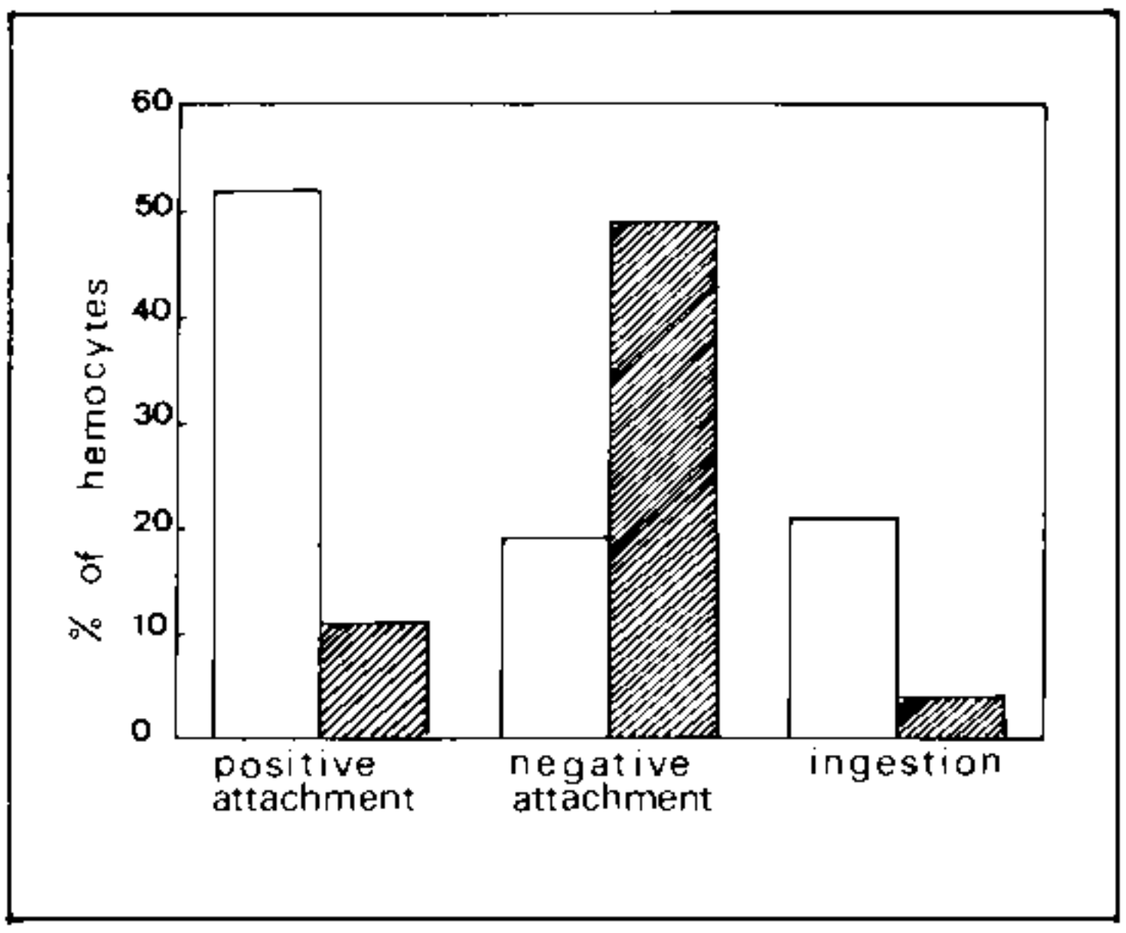

Fig. 1: percentage of attachment and ingestion of sheep red blood cells (SRBC) sensitized (□) or not (□) with IgG antisheep erythrocytes by adherent hemocytes (n = 1,200 hemocytes).

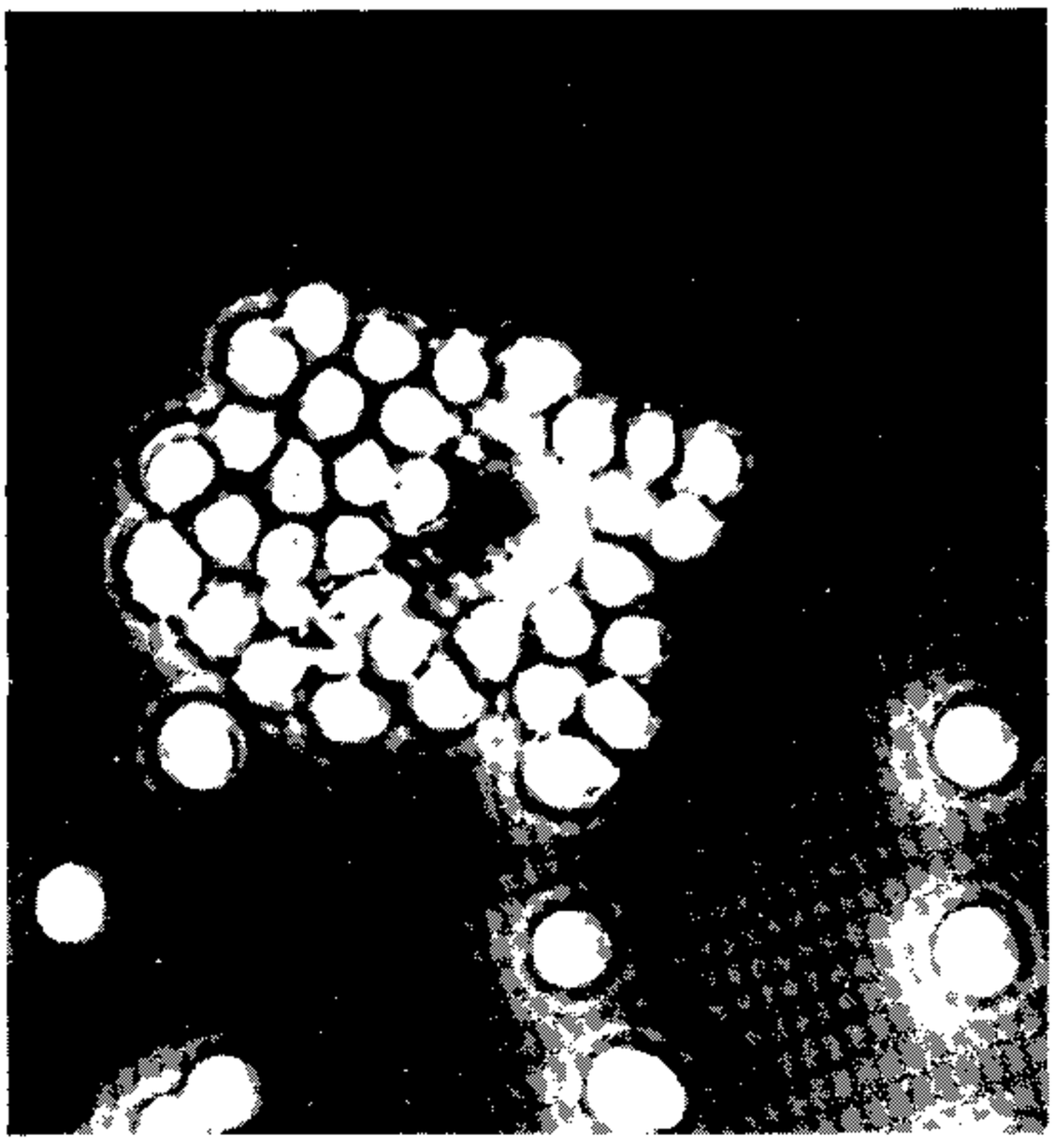

Fig. 2: adherent hemocytes of *Rhodnius prolixus*, presenting a maximal attachment of sheep red blood cells coated with IgG anti sheep erythrocytes (600x).

group with SRBC coated with IgG (22%) and the group incubated with non-sensitized SRBC (only 4%). Finally, the Figure 1 also demonstrates that only 19% of adherent hemocytes did not have attached SRBC IgG sensitized while 47% of adherent hemocytes did not present non-sensitized sheep erythrocytes surround them. Figure 2 shows adherent hemocytes with a high number of attached SRBC IgG sensitized. Taken together all these results we concluded that vertebrate IgG are able to enhance the phagocytosis in *R. prolixus*.

Phagocytosis is a complex and sophisticated biological process. Most work on phagocytosis in insects has concentrated on the micro-organism/hemocytic interactions showing that plasmatocytes are the predominant cell type involved in this process (N. A. Ratcliffe, 1982 *(loc. cit.)*). There is, for example, no data demonstrating the existence of well defined receptors on insect phagocytic cells capable to enhance the phagocytic activity. As far as we are aware, our results represent the first clear demonstration of IgG-Fc binding sites on adherent hemocytes of an hematophagous insect. In our experimental conditions, both attachment and ingestion of SRBC were drastically increased with the sensitization of the erythrocytes by IgG antisheep erythrocytes. Since phagocytosis in vertebrate is related to the presence of Fc IgG receptors on the surface of phagocyte membrane, we suggest that similar receptors may be found on the surface membrane of adherent hemocytes in hematophagous bugs. However, preliminary studies indicate that similar binding sites in adherent hemocytes derived from a phytophagous bugs, *Oncopeltus fasciatus* could not be found. This fact suggests that IgG-Fc binding sites are not detected in all species of Hemiptera. The question thus arises if the hematophagy is related to these binding sites and what is the function of such biological process for the insect. We are therefore investigating now whether IgG-Fc binding sites found in adherent hemocytes are related to the blood meal and whether IgG from the blood meal are involved in the increase in the hemocyte phagocytosis in *R. prolixus*. A more complete description of these results will appear elsewhere.

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