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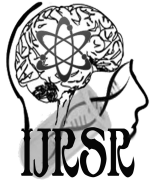
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## Research Article

### ANTIBODY DEPENDENT CYTOTOXICITY IN HEMOCYTES OF THE MUD CRAB *SCYLLA TRANQUEBARICA*

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#### ABSTRACT

Though phagocytosis is well known in hemocytes, the receptors in hemocytes which recognize the opsonising proteins are not yet investigated. Hence, in this present study an attempt was made to find clues on the similarities of the receptors with the Fc receptors in immune cells of mammals. Fixed hemocytes after treating with human IgG were injected into the animal's body and after incubation, the IgG sensitized cells were removed from the *in vivo* system which was evidenced by indirect agglutination test and microscopic observation. Hence, it is construed that hemocytes can recognize the human IgG bound hemocytes and phagocyte them.

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#### INTRODUCTION

Phagocytosis is well known in hemocytes [Rosales, 2011]. The role of humoral proteins in opsonisation for phagocytosis, its separation, purification and study on its mediation in enhancing cytotoxicity is also studied in recent research [Kim *et al.*, 2010]. However, the receptors in hemocytes which recognize the opsonising proteins are not yet investigated. Hence, in this present study an attempt was made to find clues on the similarities of the receptors with the Fc receptors in immune cells of mammals.

#### MATERIALS AND METHODS

##### Indirect agglutination test

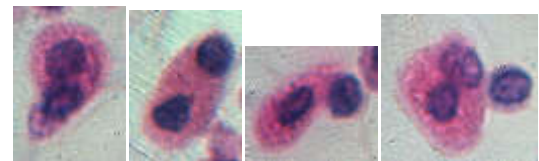
3ml of hemolymph from the mud crab *Scylla tranquebarica* was collected in a syringe containing 3ml of 10% formalin. After 1 hour of fixation, the cells were centrifuged at 1200 rpm for 10 min and washed once with 10ml of 0.9% saline. The washed cells were pelletized, resuspended in 3ml of human plasma and incubated for 10 minutes. Then the cells were washed once with saline and resuspended in 0.5ml of saline. A 20µl suspended cells was mixed with 20 µl of Anti-human globulin (Tulip Diagnostics). The remaining cells of 0.5ml suspension were injected back into the same animal at the base of the walking leg.

The same procedure was done in control animal except that the fixed cells were washed and injected in the animal without human plasma treatment.

##### Sectioning of hemocytes:

After 1 hour of *in vivo* injection, 6ml of hemolymph collected in 10% formalin in 1:1 ratio and washed with saline once. 20 µl of the sample was mixed with 20µl of anti-human globulin. The remaining cells were pelletized, 0.5ml of 2% agarose was added, solidified and added to 10% formalin. The fixed cells were taken for paraffin blocking and sectioning [Culling *et al.*, 1985].

#### RESULTS AND DISCUSSION



Figures 1a, 1b, 1c, 1d (1cm = 7.4µm)

When the fixed cells were mixed with human plasma, the cells agglutinated with anti-human globulin, whereas the hemocytes collected finally (ie., after 1 hour of *in vivo* injection) did not give any agglutination. This implies that the injected hemocytes sensitized with human IgG were removed from the circulating system. Moreover, the cells which were sectioned were observed under microscope showed phagocytosis of hemocytes (Figure 1 a,b,c & d). These results shows that the animal recognises its own cells as foreign cells after sensitizing with human IgG. On the contrary, the hemocytes in negative control didn't show any phagocytic activity in microscopic observation. This gives us the clue that the hemocytes can detect and phagocyte human IgG sensitized cells, which means that they may contain receptors for binding of human IgG.

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Many researchers have found humoral proteins mediate phagocytosis and cytotoxicity in hemocytes (Hoffmann, *et al.*, 1999; Stuart and Ezekowitz, 2008). They term such proteins as PRR (pattern recognition receptors). Kim *et al.*, (2010) have found out that these proteins which function as opsonins are of multiligands. As we see that the mammalian humoral proteins immunoglobulins are also multiligands which act as opsonins in mammalian immunity, future study on the purification of receptors present on hemocytes will give us clue on the evolution of the humoral immunity in vertebrates.

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