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RESEARCH REPORT

Celomic cells of the marine fireworm *Hermodice carunculata* (Annelida, Polychaeta)

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Abstract

We have examined the morphological and functional characteristics of the celomic cells in the marine fireworm *Hermodice carunculata*. The cells differ in morphology, in relation to the presence of cytoplasmic granules, adhere to the glass, phagocytize zymosan A particles and contain ACTH-like molecules. We suggest the presence of only one cell type in the worm celomic fluid, *i.e.,* the immunocyte described in other invertebrates.

Key Words: polychete; *Hermodice carunculata*; celomic cells; immunocyte

Introduction

Dhainaut and Porchet-Hennerè (1988) described the presence of five main types of amebocytes in several species of polychetes. These defense cells were called granulocytes by Baskin (1974) and it is to be emphasized that not all the cell types were observed in every polychete species; for example three types of granulocytes, that can be separated by selective agglutination with lectins, were reported in *Nereis diversicolor* (M'Beri *et al*., 1988; Porchet-Henneré, 1990). Regarding the functions, it has been demonstrated that the cells play a role in cellular responses, including phagocytosis, cytotoxic reactions and encapsulation (Porchet-Henneré *et al*., 1987, 1992; Porchet-Henneré and Vernet, 1992; Maltseva *et al*., 2014). Other populations of free cells, such as eleocytes and erythrocytes, were described in the celom. They have almost no role in immune functions while are primarily involved in regeneration and respiration (Vetvicka and Sima, 2009). In *Perinereis cultrifera* the eleocytes can be easily separated by sedimentation or weak centrifugation (Bulet *et al*., 1983).

In this paper, we have studied the cell types present in the celomic fluid of the marine fireworm *Hermodice carunculata* and their possible involvement in immunity was examined.

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Materials and Methods

Animals

The bearded fireworm *Hermodice carunculata* is a large size amphinomid polychete (25 - 30 cm length) living in the coastal areas of the Central Atlantic Ocean and Central and Eastern Mediterranean Sea (Ahrens *et al.,* 2013). The specimens used in this study were collected on the cost of Porto Cesario (Lecce, Italy) and maintained in a recirculating aquaria system at 25 °C.

Celomic cells

The celomic fluid of *H. carunculata* (about 200 μl/animal) was collected by using a sterile 2 ml syringe and cytocentrifuged (400 rpm for 2 min) onto slides by Cytospin II (Shandon Instrument, UK) and air-dryed. Unfixed and fixed (4% p-formaldehyde in phosphate buffered solution) celomic cells were analyzed with morphological, cytochemical and immunocytochemical methods and for functional tests. A total of 20 animals were used.

Morphological stains

The celomic cells were stained with Diff-Quik kit (BioMap snc, Italy) and with May-Grünwald and Giemsa and then observed under an Olympus BH-2 light microscope (Olympus Corporation, Japan) equipped with a DS-5M-L1 Digital Sight Camera System (Nikon, Japan).

Cytochemical reactions

Fixed cells were stained with Periodic Acid-Schiff (PAS) reaction and Sudan black B method for fats and phospholipids (see Brancroft and Stevens,

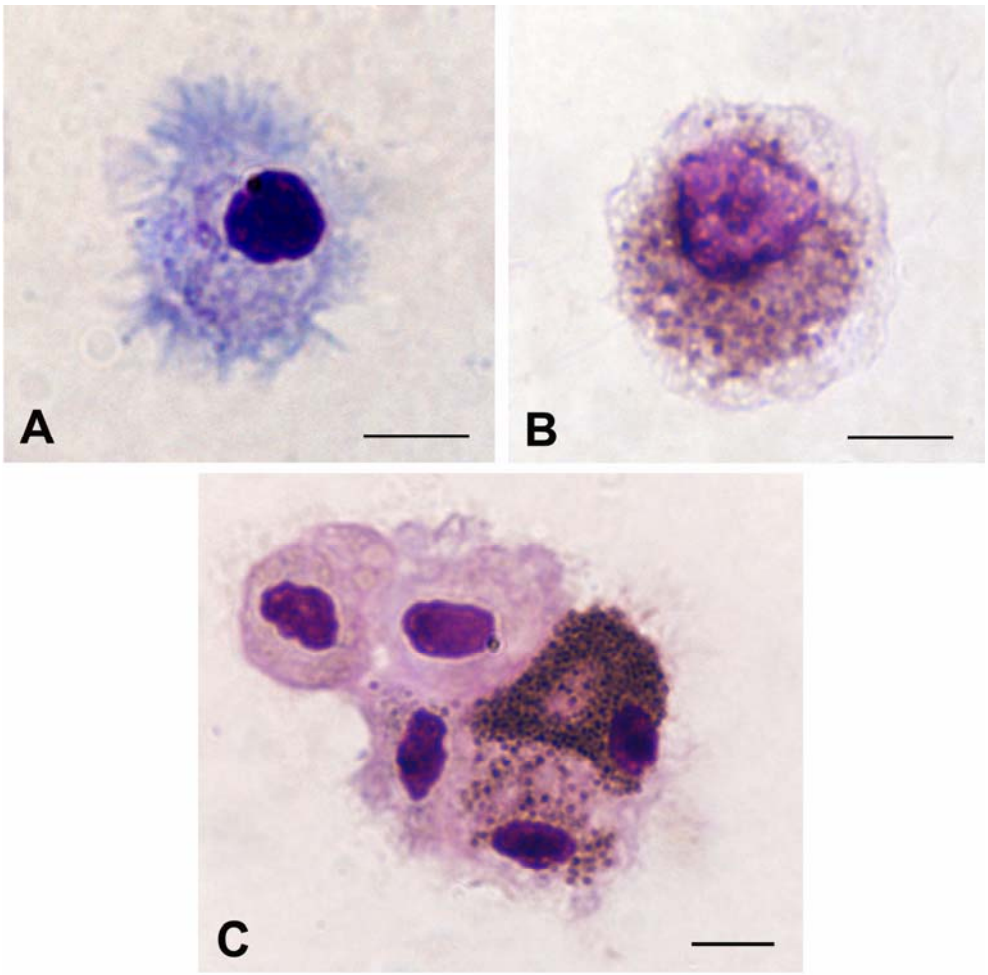

Fig. 1 Celomic cells of *Hermodice carunculata* stained with Diff-Quik kit and with May-Grünwald and Giemsa. Hyaline cells (A, C) and granular cells with variable numbers of cytoplasmic granules (B, C) are shown. Scale bar = 10 μm.

1996). Unfixed cells were also incubated in specific medium to localize acid phosphatase (Barka and Anderson, 1962) and β-glucuronidase (Watt, 1987) activities.

Immunocytochemical procedure

Unfixed cells were incubated with anti-ACTH (1-24) polyclonal antibody (Biogenesis, UK) (1:250) an overnight at 4 °C (for the detailed method see Ottaviani *et al.,* 1990). The reactivity was visualized by an immunoperoxidase technique using avidin-biotin-peroxidase complex (Hsu *et al*., 1981) and diaminobenzidine as substrate. Nuclei were counterstained with hematoxylin. Control of the immunocytochemical reaction was performed by substituting primary antibody with non-immune sera.

Cell adhesion and spreading

For the adhesion assay, a drop of celomic fluid was left to settle on a glass slide for 20 min in a humidified chamber. The celomic fluid was then carefully and gently removed with a micropipette and the slides underwent the morphological stains as previously described.

In vitro *phagocytosis*

For *in vitro* phagocytosis assay, the celomic fluid was mixed with zymosan A particles (Sigma, USA) (ratio 100 μg/ml celomic fluid), incubated for 30 min in a humidified chamber and subsequently stained with Giemsa's solution prior to observing cell phagocytic activity.

Results

The morphological observations revealed the presence of cells with two main different structures in the celomic fluid of *H. carunculata*. The first one appeared irregularly shaped with a round or oval eccentric nucleus that surrounded an abundant hyaline cytoplasm (Figs 1A, C). The other one differed for the presence of a variable number of intensely stained cytoplasmic granules (Figs 1B, C). The majority of these inclusions were positive to

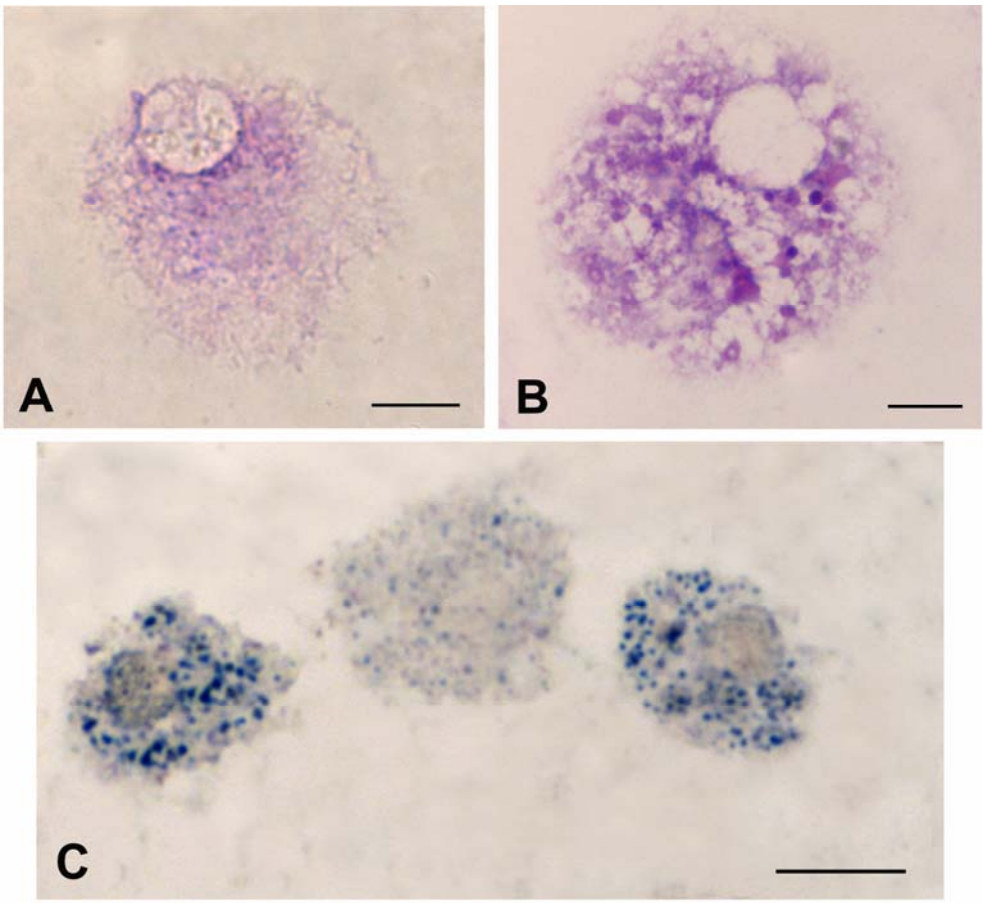

Fig. 2 Celomic cells stained with PAS (A, B) and Sudan black B (C) reactions. Scale bar = 10 μm.

PAS and Sudan black B reactions (Figs 2B, C). The functional studies showed that after 20 min the cells adhered strongly to glass microscope slides. Moreover, the cells actively phagocytized zymosan A after 30 min of incubation and single or grouped particles were seen inside different sized cytoplasmic vacuoles (Figs 3A, B). An higher phagocytic activity was observed in cells with hyaline cytoplasm. All cells were also positive to the cytoenzymatic reactions for the tested hydrolytic enzymes, *i.e.,* acid phosphatase (Fig. 3C) and β-glucuronidase. Regarding the immunocytochemical results, ACTH-like material was detected in the cytoplasm of hyaline and granular cells (Figs 4A - C).

Discussion

On the basis of our observations, we suggest that only one cell type is present in the celomic fluid of *H. carunculata*, although cells with hyaline or granular cytoplasm were detected. The conclusion is supported by the fact that, regardless different morphologies, the cells show the same behavior. Indeed, they are able to adhere to the substrate and to phagocytize foreign material. This cell is to be considered the immunocyte, the invertebrate defense cell previously demonstrated to have functional characteristics of vertebrate macrophages (Ottaviani, 2011; Malagoli *et al*., 2015).

Similarly, two main types of celomocytes, endowed of phagocytic capacity, were recognized in the celomic fluid of another polychete, *Arenicola marina* (Maltseva *et al.,* 2015). Moreover, sheep red blood cells (SRBC) injected in the celomic cavity of *Neoamphitrite regulus* and *A. marina* are phagocytized by celomocytes (immunocytes) and then transferred to the heart-body or extravasal tissue, thus indicating a role in the removal of foreign material (Braunbeck and Dales, 1984).

We also found that the phagocytic activity of *H. carunculata* cells is associated with the presence of ACTH-like molecules. An analogous correlation has been demonstrated for the phagocytic cells of several invertebrate species such as the annelid *Eisenia foetida* and the molluscs *Planorbarius corneus*, *Viviparus ater*, *Lymnaea stagnalis* and *Mytilus galloprovincialis* (Ottaviani *et al.,* 1990, 1991, 1995; Franchini *et al*., 1994; Cooper *et al*., 1995).

The present data are also in agreement with those emerged from comparative and morphofunctional studies performed on different aged *M. galloprovincialis* (Ottaviani *et al*., 1998). Two

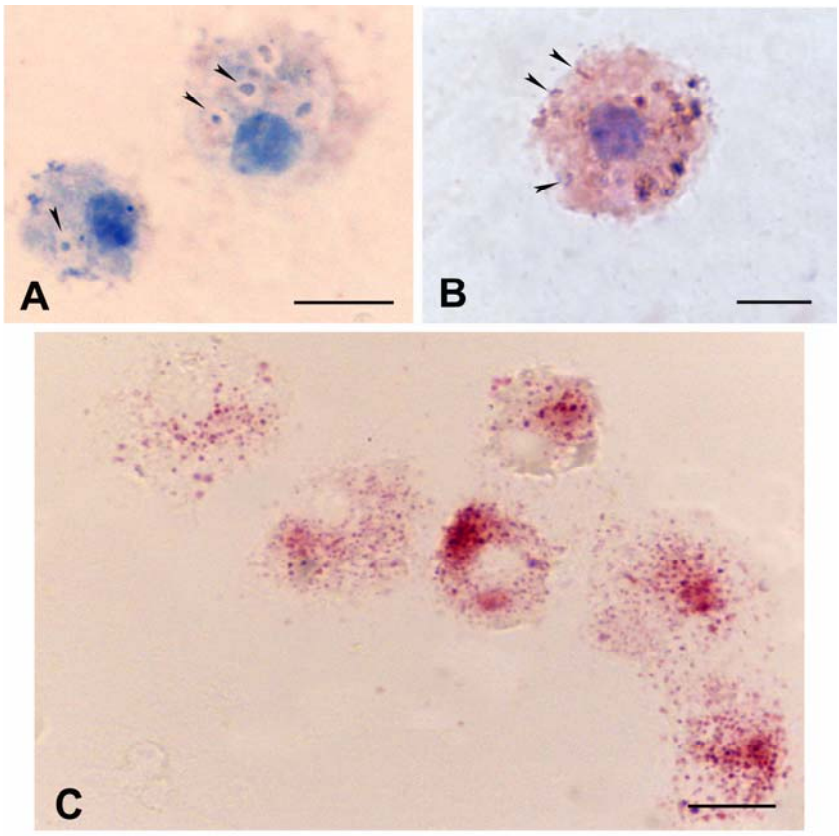

Fig. 3 Hyaline (A) and granular cells (B) phagocytize zymosan A particles (arrowheads) and are positive to the cytoenzymatic reaction for acid phosphatase (C). Scale bar = 10 μm.

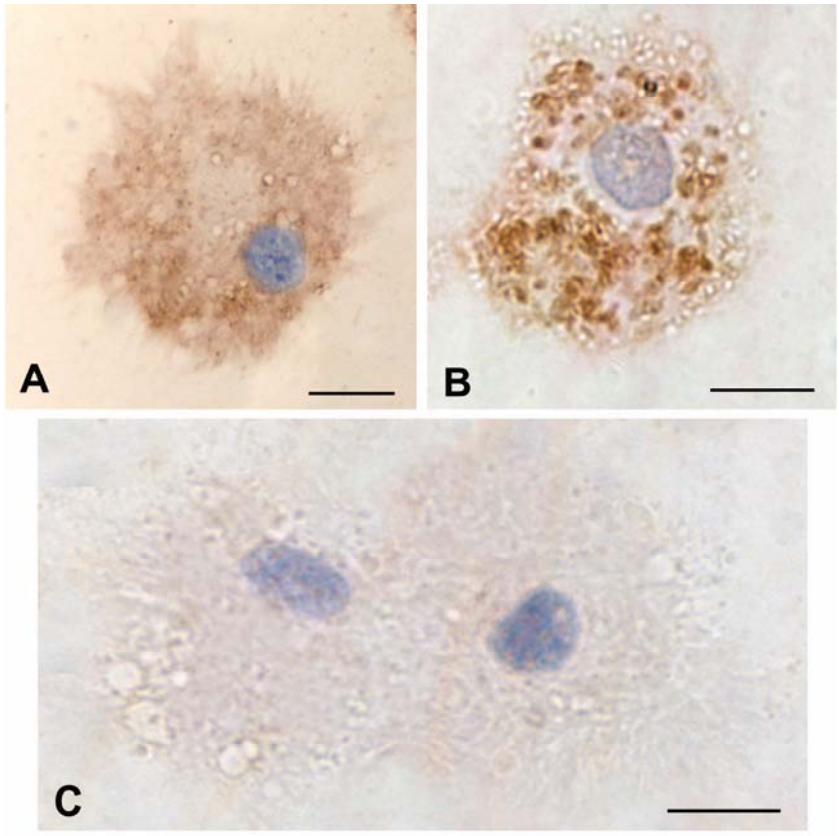

Fig. 4 Hyaline (A) and granular (B) celomic cells are immunoreactive to anti-ACTH antibody. Negative control of immunocytochemical reaction (C). Scale bar = 10 μm.

types of immunocytes, with hyaline cytoplasm or with cell inclusions containing lipofuscins, have been identified in the hemolymph. On the basis of the shared functions and the expression of common signal molecules, the cells are suggested to belong to a single cell type. The structural differences seem to be ascribed to cellular aging: the immunocytes with hyaline cytoplasm are the young stage, while cells containing lipofuscin inclusions are the adult one (Ottaviani *et al*., 1998).

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