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Morphological, structural, and functional characterization of the haemocytes of the scallop, *Argopecten irradians*

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Abstract

Argopecten irradians is one of the most important commercial species of Pectinidae family in China. The internal defense system of mollusks consists of circulating haemocytes. In order to characterize the haemocytes of the scallop *A. irradians*, light and electron microscopical studies were carried out. Four types of haemocytes were recognized: type I small hyalinocytes (2.38 ± 0.08 μm, 30–35%), type II large hyalinocytes (4.41 ± 0.33 μm, 15–20%), type III small granulocytes (4.15 ± 0.26 μm, 20–25%), and type IV large granulocytes (8.26 ± 0.52 μm, 25–30%). Granulocyte types showed smaller N/C ratios than hyalinocytes. The mean haemocyte concentration was about $(3.75 \pm 0.65) \times 10^7$ cells ml^{-1} of haemolymph. Among haemocytes, 44.7% are granular and 55.3% are agranular. These gave a relatively systematic classification scheme for haemocytes of *A. irradians*. Three types of granules were identified: type I, with high electron-density; type II, with low electron-density; and type III, with a middle level of electron-density, based on TEM studies. Different haemocyte types were not separated with DDGC of Percoll in this study. Both granulocytes and hyalinocytes showed a phagocytic response to the two strains of bacteria, *Escherichia coli* and RLOs. The phagocytic ability of granulocyte was significantly higher (41–48%) than that of hyalinocyte (9.2–11.2%).

Keywords: *Argopecten irradians*; Haemocyte; Hyalinocytes; Granulocyte; Morphology; Phagocytosis

1. Introduction

Internal defense in mollusk species utilizes an innate, non-lymphoid immune system, which consists of both cellular and humoral components of the circulatory system. These cellular and humoral components work together to eliminate potentially harmful

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microorganisms. Scallops, like all other bivalve mollusks, have an open circulatory system that does not confine haemolymph to traditional vessels. Instead, their open circulatory system circulates haemolymph through a number of cavities and sinuses in various parts of the organism. Bivalve haemocytes play an important and central role in the internal defense, and are known to be involved in other processes like wound and shell repair, nutrient digestion, transport, and excretion (Cheng, 1981). There have been many studies on the morphology, structure, function, and classification of haemocytes in bivalves. Foley and Cheng (1972) identified two haemocyte types in *Crassostrea virginica*: hyalinocytes and granulocytes. Several other authors have reported this classification scheme in bivalves based on morphological, cytochemical, and functional criteria (Cheng, 1975, 1981; Auffret, 1988; Moore and Eble, 1977; Suresh and Mohandas, 1990; Pipe, 1990; Kumazagua et al., 1991). It has been known that there are various types of haemocytes in bivalves, and there are some differences between different species.

Most of the studies on bivalve haemocytes have been carried out in oysters, mussels, and clams. Only a few were performed in species belonging to the Pectinidae family: *Patinopecten yessoensis* (Nakamura et al., 1985), *Pecten maximus* (Le Gall et al., 1991), and *Chlamys farreri* (Xing et al., 2002; Sun and Li, 2003). *Argopecten irradians* is one of the most important commercial species of this family in China. Since it was successfully introduced from America in 1983, its mass scale cultivation has been made for about 20 years along the coast of the Bohai Sea, the Yellow Sea, and the adjacent waters in China. In recent years, the mass mortality had occurred in the cultivated scallop with a great loss. Some studies revealed that this species were affected by several pathogens: Rickettsia-like organisms (RLOs), Mycoplasma-like organisms (MLOs), and virus-like particles (VLPs) (Zhang and Wu, 2003; Zhang et al., in press). However, no systematic studies have been carried out to investigate the morphology, structure, function, and classification of haemocytes of *A. irradians*. A better understanding of the defense mechanisms in this bivalve species may lead to practical approaches to control RLOs or other diseases and to avoid mass damage. Here we report our systematic morphological and structural characterization of haemocytes in the haemolymph of the scallop. Our study provides a morphological basis for the cellular defense mechanisms in this organism.

2. Materials and methods

2.1. Haemolymph collection

Scallops (shell length 4–6 cm) were collected from farms in Laizhou City, Shandong province, China. Approximately 0.2–0.3 ml of haemolymph was extracted from the posterior adductor muscle of each animal using a 25 gauge needle into an equal volume of either Baker's formol–calcium fixative (4% formaldehyde, 2% sodium chloride, 1% calcium acetate) or 0.05 M Tris–HCl buffer (TBS; pH 7.6, containing 2% sodium chloride), or into an equal volume of EM fixative (2% formaldehyde, 2.5% glutaraldehyde, 2%NaCl, 2 mM calcium chloride in 0.2 M cacodylate buffer, pH 7.4), as appropriate. A minimum of 20 samples were used for each immune parameter investigated.

2.2. Light Microscopy Observation (LMO)

In order to characterize the haemocytes, three staining techniques, Giemsa's, Wright's, and Hemacolor's stain on haemolymph smears were carried out. From which hyalinocytes, granulocytes, acidophilic and basophilic cells were distinguished. The percentages of hyalinocytes and granulocytes were calculated depending on Hemacolor's smears.

Total haemocyte counts were carried out with an improved Neubauer haemocytometer using Baker's fixed haemolymph samples. Mean cell diameters were calculated by measuring 25 of each cell type in Hemacolor's stained smears using Motic Images system. Statistical analysis is performed by using the SPSS software to determine whether there was a significant size difference between cell types. Values of $p < 0.05$ were considered significant.

2.3. Scanning Electron Microscopy Observation (SEMO)

Fresh haemolymph was fixed with glutaraldehyde at 2% (v/v) in Millonig 0.2 M; pH 7.3 buffer solution, washed in buffer, post-fixed with osmium tetraoxide