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Classification of haematopoietic cells and haemocytes in Chinese prawn *Fenneropenaeus chinensis*

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Abstract

It is commonly believed that crustacean haemocytes originate from a specialised haematopoietic tissue (HPT), whereas the differentiation relationship between HPT cells and circulating haemocytes is still not clearly understood. The HPT cells and haemocytes of *Fenneropenaeus chinensis* were characterised using morphological and histochemical methods. Three types of HPT cells were identified under the transmission electron microscope (TEM). Type 1 cells had high N/C ratios, developed dispersed chromatins and no cytoplasmic granules. Type 2 cells had smaller size, developed condensed chromatins and cytoplasmic granules, which were homogeneous or striated in type 2a cells, and homogeneous in type 2b cells. We deduce that type 1 cells may give rise to type 2 cells in terms of the presence of possible intermediates between type 1 and type 2 cells. The circulating haemocytes were divided into three populations, i.e. hyaline haemocytes (HH), small granular haemocytes (SHG) and large granular haemocytes (LGH), based on Wright–Giemsa staining and TEM observation. Comparing the HPT cells with the circulating haemocytes, type 2a cells of HPT may represent the HH due to similar granule types, cell size and N/C ratios, and type 2b cells may be the young and immature LGH. By Wright–Giemsa and acid α-naphthyl acetate esterase staining, the intermediates between the HH and SGH were observed, which indicates that the SGH may be derived from the HH in the circulatory system. Therefore, it is suggested that the *F. chinensis* haemocytes could be divided into two haemocyte lineages, i.e. the HH-SGH and LGH lineage.

Keywords: *Fenneropenaeus chinensis*; Haematopoietic tissue; Haemocyte; Haemocyte lineage

1. Introduction

Crustacean haemocytes play a central role in the host immune response, performing functions such as phagocytosis, encapsulation, nodule formation and mediation of cytotoxicity [1]. In contrast to the fairly uniform scheme of morphological and immuno-functional classification for vertebrate white blood cells, it is difficult to classify crustacean haemocytes in morphologically well-defined ontogenic classes due to the susceptibility and variability of cells, as well as the limited knowledge of their development and differentiation. Based on the presence of cytoplasmic

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granules and relative size of granules, crustacean haemocytes are usually divided into three main types, hyaline (agranular), semigranular (small granular) and granular (large granular) haemocytes, under the light microscope (LM) and transmission electron microscope (TEM) [2,3]. Cytochemical characterisations including lysosomes, cytoplasmic contents and granule enzymes were also attempted to provide information on the function of and relationship among different haemocyte types [4]. Monoclonal antibodies (mAbs) reacting with various proteins of haemoctyes have been obtained in *Penaeus monodon* [5,6], and more specific mAbs were produced to classify all three types of *P. monodon* haemocytes recently [7].

It is commonly believed from Cuénot's work in 1905 [8] that crustacean haemocytes originate from a specialised haematopoietic tissue (HPT). The HPT has been identified in several crustacean species including the crab *Carcinus maenas*, lobster *Homarus americanus*, crayfish *Pacifastacus leniusculus*, penaeid shrimp *Penaeus stylirostris* [9–12] and *P. monodon* [13]. The HPT cells were classified morphologically into different types resembling those identified in circulating haemocytes. By combining morphological, cytochemical and functional features of circulating haemocytes, two classifications of haemocyte lineages were proposed in some crustaceans: hyaline and granular lineages in lobster *H. americanus* [10] or large and small granular lineages in crayfish *P. leniusculus* [11] and penaeid shrimp *P. monodon* [13]. However, a progenitor stem cell that may give rise to haemocytes is still not clearly identified, and the distinction of one or several lineages is obviously open to further studies [14].

The Chinese prawn *Fenneropenaeus chinensis* is the most valuable species for shrimp aquaculture in China. The *F. chinensis* haemocytes were also divided into hyaline, small granular and large granular haemocytes using the phase contrast microscope and TEM [15], but few studies have focused on the HPT and haemocyte ontogenesis. In the present study, the *F. chinensis* HPT was observed under the LM and TEM, and the circulating haemocytes were classified based on Wright–Giemsa staining, TEM observation and the acid α-naphthyl acetate esterase (ANAE) activity determined by histochemical method. According to the morphological and histochemical features, the differentiation relationship between different types of cells observed in this study is discussed, and a classification pattern of two haemocyte lineages is proposed for *F. chinensis.*

2. Materials and methods

2.1. Chinese prawn

The postlarvae (PL_{1-5}) of *Fenneropenaeus chinensis* were collected from the prawn breeding farm of Rushan, China. The healthy intermolt prawns (15–17 cm body length) were obtained from the prawn farm of Qingdao, China, maintained in 1500-l tanks with running aerated seawater at 15 ± 1 °C, and fed by clamworm daily.

2.2. Localisation of HPT

The PL were fixed in Bouin's solution, and processed for routine histological examination using paraffin-embedded procedures after 24 h. The serial tissue sections were haematoxylin and eosin stained and observed by an Olympus BH-2 microscope.

2.3. Histology and cytology of HPT

After localisation, the dorsal HPT was obtained by dissection from the adult. The cephalothorax was cut apart, and then the stomach sheath was separated from surrounding tissues for histological observation as described above. For cytological investigation, the stomach sheath was immersed in cold 2.5% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4) and further dissected under a dissection microscope to separate the cell clusters from connective tissues, followed by a check for the presence of HPT under the LM. The HPT was composed of abundant lobules in which HPT cells with less than 10 μm in length and high nuclear to cytoplasmic ratios (N/C ratios) were densely packed (Fig. 1). Then the HPT was fixed in 2.5% glutaraldehyde overnight at 4 °C and post-fixed in 1% OsO_4 for 1 h, embedded in Epon 812. The ultrathin sections were made and mounted on copper grids, counterstained in uranyl acetate and lead citrate, and examined under a Hitachi-700 TEM.