CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO 
HAEMOCYTE TYPES OF THE SHRIMP, FENNEROPENAEUS CHINENSIS

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ABSTRACT

Five monoclonal antibodies (MAbs) (1A7, 1C6, 1E4, 1G8, 2C3) against haemocytes of Fen-
neropenaeus chinensis (Osbeck, 1765) were produced by immunizing Balb/c mice, followed by indi-
nected immunofluorescence assay tests (IIFAT). These showed specificity for more than one haemocyte type and for various haemocyte components of F. chinensis. Using Wright’s staining and IIFAT with haemocyte monolayers prepared by sucrose gradient centrifugation, MAb 1A7, 1C6 reacted with membranes of hyalinocytes and the cytoplasm of semi-granulocytes, respectively; MAb 1E4 reacted with membranes and the cytoplasm of hyalinocytes, and with membranes of semi-granulocytes; MAb 1G8 reacted with haemocyte granules; and MAb 2C3 reacted with surface membranes of all haemocyte types. Western-blotting of haemocytes and haemocyte membrane analysis demonstrated that MAb 1G8 recognized an antigen of 96 kDa in haemocyte; that MAb 1E4 reacted with antigens of 88 kDa and 79 kDa in the haemocyte membrane; and that MAb 2C3 reacted specifically to a single membrane protein band with an approximate molecular weight of 153 kDa; however, there was no protein band detected by either MAb 1A7, 1C6.

RÉSUMÉ

Cinq anticorps monoclonaux (MAbs) (1A7, 1C6, 1E4, 1G8, 2C3) contre les hémocytes de Fenneropenaeus chinensis (Osbeck, 1765) ont été produits par immunization de souris Balb/c, et contrôlés par un test en immunofluorescence indirecte (IIFAT). Ils ont présenté une spécificité pour plusieurs types d’hémocytes et pour divers composants d’hémocytes de F. chinensis. En utilisant la coloration de Wright et IIFAT avec une monocouche d’hémocytes préparée par centrifugation à gradient de concentration en glucose, MAb 1A7, 1C6 ont réagi respectivement avec les membranes des hémocytes hyalins et le cytoplasme des semi-granuleux; MAb 1E4 a réagi avec les membranes et le cytoplasme des hémocytes granuleux; et MAb 2C3 a réagi avec les membranes de tous les types d’hémocytes. Les résultats du Western-blot des hémocytes et de l’analyse de leur membrane ont démontré que MAb 1G8 reconnaît un antigène de 96 kDa dans les hémocytes; que MAb 1E4 a réagi avec des antigènes de 88 kDa et 79 kDa au niveau de la membrane des hémocytes; et que MAb 2C3 a réagi spécifiquement à une bande protéique, d’une poids moléculaire approximatif de 153 kDa; cependant aucune bande protéique n’a été détectée par MAb 1A7 ou 1C6.

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Shrimp immunity is mediated by haemocytes that participate in a variety of defense mechanisms, including phagocytosis, nodule formation, encapsulation, and cytotoxicity (Söderhäll & Cerenius, 1992). The haemocytes of the freshwater crayfish, Astacus leptodactylus (Eschscholtz, 1823) are identified by granule size morphometry as: hyaline haemocytes with no or only rare tiny granules; small-granule haemocytes; unimodal medium-diameter granule haemocytes; and both small and large granule-containing haemocytes (Giulianini et al., 2007). From microscopic and ultrastructural analyses, it is generally accepted that penaeid shrimp possess three main types of haemocyte: (1) hyalinocytes have no granules in the cytoplasm and a high nuclear-to-plasma ratio; (2) semi-granulocytes contain relatively few granules of small size in the cytoplasm; and (3) granulocytes contain a large number of relatively large granules in the cytoplasm. The latter two have a low nuclear-to-plasma ratio (Sung et al., 1999). The three haemocyte sub-populations have different functions in the shrimp’s defense reaction (Johansson et al., 2000; Evelyne, 2003).

Research about the immune system of penaeid shrimp is currently expanding, because of the economic importance of shrimp culture throughout the world. Due to the significance of infectious diseases, it is thus necessary to characterize shrimp immune cells, and to study their immune mechanisms at the cellular and the molecular level. The role of these mechanisms in cellular defenses relies mainly on non-specific immune responses, and this has been studied during the last two decades (Söderhäll et al., 1985; Hose et al., 1990; Söderhäll & Cerenius, 1992).

Monoclonal antibodies (MAbs) have long served as essential probes for the identification of cell types and their functions in mammalian immune cells, yet only recently they have begun to be used for crustacean haemocytes. However, MAb is a promising approach for the classification of haemocytes in penaeid shrimp (Reinherz et al., 1979; Noël et al., 1994). MAbs that could discriminate among different sub-populations of haemocytes would be helpful in studying their function in the immune process of penaeid shrimp.

The white spot syndrome virus (WSSV) is a pathogen that has caused severe mortality of cultivated shrimp in China (Zhan et al., 1998; Corbel et al., 2001). Since haemocytes are one of the most important target organs of WSSV, understanding the interaction between haemocytes and WSSV could lead to the discovery of novel prevention and control measures that utilize the shrimp’s own immune system.

Studies on shrimp haemolymph have revealed a significant decline in the number of free and circulating haemocytes after WSSV infection (Braak et al., 2002a). WSSV was shown to infect specific haemocytes of the shrimp (Wang et