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CHANGES IN *PENAEUS CHINENSIS* HAEMOCYTES DURING WHITE SPOT BACULOVIRUS (WSBV)INFECTIONS

YEONG-JIN KIM[1] WON-CHUL CHOI[1], HEUNG-RAK KIM[3], SUNG-JU JUNG[2] AND MYUNG-JOO OH[2]
[1]Department of Biology, Pusan National University, Pusan 609-735, Korea
[2] Department of Fish Pathology, Yosu National University, Yosu 550-749, Korea
[3] Department of Food Science and Nutrition, Yosu National University, Yosu 550-749, Korea

Summary

Shrimp have efficient non-specific defence mechanisms based on activities of the haemocytes to pathogens. In this study, we found that one of the non-specific immune reactions was related to mutual association among types of haemocyte, granular cell, semi-granular cell, and hyaline cell. Haemocyte number decreased as time goes by WSBV infection. Haemocyte composition, especially granular cells that directly related to phagocytosis, was dramatically decreased after WSBV infection. Also, based on these results, we investigated to effect of immunostimulants for inhibition to viral infection in shrimp. At this time, increase of haemocyanin was observed by SDS-PAGE.

Introduction

Crustaceans have efficient means of defending themselves against most potential' pathogens in the surrounding environment and are not different from other animals in that the host defence is based on activities of the haemocytes (Soderhall *et al.,* 1992). Phagocytosis of infectious agents by haemocytes is a major non-specific defence mechanism in crustaceans such as shrimp (Bachere *et al.,* 1995). Three types of haemocytes are recognised in crustacean hemolymph. The first, hyaline cell is characterised by the absence of granules and are capable of phagocytosis. The second, semi-granular cell contains a variable number of small granules, the cell is responsible for recognising and responding to foreign molecules by degranulation and subsequently attaching or spreading on the foreign surface. The third type, the granular cell is filled with large granules and its main function seems to be related to the prophenoloxidase activating system (pro-PO system) Kondo *et al.*, 1992).

In this study, we investigated the non-specific immune reaction in shrimp in the form of haemocyte composition and haemocyanin analysis.

Materials and methods

Preparation of haemocytes: One ml of haemolymph was collected into a syringe via a 22 gauge needle inserted into the thorax. Haemocytes were recovered by centrifugation at 5,000 rpm and were mixed with 1 mg of M199 medium.

Counting of haemocytes: Twenty-five shrimps (average weight : 5 g) were used in both control and test groups. Numbers of haemocytes were calculated on a haemocytometer. Composition of the haemocytes were determined from smears on slide glass, stained with Giemsa, and then classified according to method of Kondo *et al.* (1992).

Observation of haemocytes after viral infection: Total twenty-five shrimps (average weight : 5 g) were used. Hemocyte number was counted at 0, 24, 48, 72, 96 hrs after inoculation with homogenised solution of lymphoid organ from white spot baculovirus (WSBV)-infected shrimp (Kim *et al.,* 1999). Hemocyte composition was investigated in both control and infected groups.

SDS-PAGE: Haemocytes were washed with PBS and then solubilised in SDS-Laemmli sample buffer. The proteins were analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described in method of Laemmli (1970).

Table 1. Changes of the total haemocyte number, after inoculation with WSBV

Time (hours after inoculation)	Average No. of haemocytes ($x10^5$ cells / ml)	
	Infected	Control
0	3.56	3.58
24	3.38	-
48	3.26	
72	3.18	-
96	3.11	3.54

Table 2. Haemocyte composition of shrimp, 96 hrs after infection with WSBV

Type of haemocyte	Percent of blood samples cells (%)	
	Control	Infected
Granular cell	49	27
Semi-granular cell	13	19
Hyaline cell	38	54

Table 3. Changes of haemocyte number and composition in shrimp by feeding of immunostimulants

			Feeding		96hrs after infection	
		Control	General	Immuno-stimulant	General	Immuno-stimulant
Number of haemocyte (x 10^6 cells/ml)		1.12	1.13	1.15	1.00	1.05
Haemocyte (%)	Granular	47.0	46.4	55.0	39.0	45.61
	Semi-granular	19.3	23.3	29.5	20.0	21.6
	Hyaline	33.7	30.3	15.5	41.0	34.0

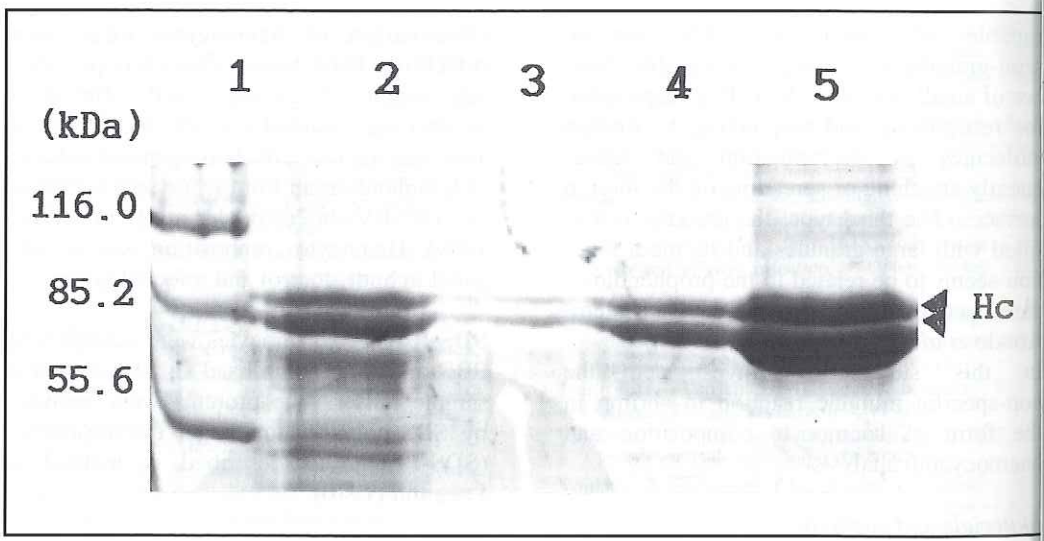

Figure 1. Identification of haemocyte proteins from experimental shrimp by SDS-PAGE. lane 1 : Molecular weight marker, lane 2 : Normal shrimp, lane 3 dead shrimp by viral infection, lane 4 : general feed group, lane 5 immunostimulant-contained feed group, Hc : haemocyanin

Result and Discussion

Change of haemocyte numbers: Before inoculation with WSBV infected shrimp lymphoid organ, the mean haemocyte numbers were $3.56x10^5$ cells/ml. However, the haemocyte number changed to $3.38x10^5$ cells/mg at 24 hrs after infection, $3.26x10^5$ cells/ml at 48 hrs after infection, $3.18x10^5$ cells/ml at 72h after infection, and $3.11x10^5$ cells/ml at 96 hrs after inoculation of viral infected samples (Table 1). The haemocyte number at 96hrs after inoculation was decreased to 87.4% compared with control groups Thus, the haemocyte number of shrimp was slightly decreased as over time during the viral infection

Change of haemocyte composition: The change of haemocyte composition was investigated at 96 hrs after inoculation with WSBV infected shrimp lymphoid organ (Table 2). The control group showed 49% of granular cells, 13% of semi-granular cells, and 38% of hyaline cells, but the virus infection group showed 27%, 19%, and 54%, respectively. From this result, we believe that shrimp immune reaction could be measured by changes in haemocyte cell type composition.

Effect of immunostimulants in feed: We studied on the effect of non-specific inhibition in shrimp, when treated by immunostimulant-contained feed. Shrimps treated for one month by feeding immunostimulant-containing feed compared to shrimps fed general commercial feed were similar to in the haemocyte numbers. However, in haemocyte composition, the immunostimulant contained feed treated group was higher than in normal commercial feed treated group in numbers of granular cells. Also, at 96 hrs after artificial infection, granular cells of immunostimulant feed treated group shrimp maintained normal numbers.

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