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*Review Articles*

# Penaeid Shrimp Immune System

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## Abstract

**Research on an innate immune system of penaeid shrimp is greatly motivated by economical requirements, because their culture is limited by the development of infectious diseases. As invertebrates, shrimp's natural immunity acts as a fast and efficient defence mechanism against the pathogens. Their immune system involve hemocytes (for encapsulation, nodule formation and phagocytosis), several plasma components (antimicrobial peptides, histones, lysosomal enzymes, lipopolysaccharide, β-1,3-glucan binding proteins, and recognition molecules), and multimeric systems (clotting protein cascade, prophenoloxidase system). When these defense mechanisms fail to protect the shrimp against bacteria, viruses, fungi, protozoa and their products, disease develops and a negative impact takes place in the shrimp culture system. Studying the shrimp immune system is attractive for the advancement of a basic knowledge on invertebrate and vertebrate general immunity, because it offers various possible alternatives for disease management in shrimp aquaculture. The aim of this document is to present the general status of the shrimp defense system, to help in the development of strategies that favour the control and prevention of disease.**

**Keywords :** immune system, innate immune system, shrimp

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## บทคัดย่อ

# ระบบภูมิคุ้มกันของกุ้ง

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**งานวิจัยทางระบบภูมิคุ้มกันสืบทอดของกุ้งมีความสำคัญอย่างยิ่งต่อระบบการผลิตกุ้งในด้านการลดความสูญเสียทางเศรษฐกิจ เนื่องจากการเพาะเลี้ยงกุ้งมักพบปัญหาโรคติดเชื้อ ในกลุ่มสัตว์ที่ไม่มีกระดูกสันหลัง ระบบภูมิคุ้มกันทางธรรมชาติจะทำหน้าที่อย่างรวดเร็วและมีประสิทธิภาพในกลไกต่อต้านการรุกรานของจุลชีพต่างๆ ระบบภูมิคุ้มกันประกอบด้วยเซลล์ ฮีโมไซต์ (ทำหน้าที่ในการสร้างถุงหุ้มและการเก็บกิน) สารน้ำ (เปปไทด์ต่อต้านเชื้อแบคทีเรีย ฮิสโตน เอนไซม์ไลโซโซม ไลโปโพลีแซคคาไรด์ โปรตีนที่จับกับ β-1,3-กลูแคน และโมเลกุลที่จำเพาะ) และระบบการทำงานร่วมกัน (ขบวณการแข็งตัวของโปรตีน ระบบโพรฟีโนโลออกซิเดส) เมื่อระบบภูมิคุ้มกันดังกล่าวล้มเหลวในการคุกคาม ของเชื้อ แบคทีเรีย ไวรัส เชื้อรา โปรโตซัว หรือ จุลชีพอื่นๆ ก็จะก่อให้เกิดโรคต่างๆ ในการเพาะเลี้ยงกุ้ง ดังนั้นการศึกษาทางด้านระบบภูมคุ้มกันของกุ้ง นับว่ามีประโยชน์ด้านเป็นข้อมูลพื้นฐานของระบบภูมิคุ้มกันในสัตว์ทั้งกลุ่มที่มีและไม่มีกระดูกสันหลัง และเพื่อที่จะนำไปเป็นแนวทางเลือกในการป้องกันโรคในอุตสาหกรรมเพาะเลี้ยงกุ้ง จุดประสงค์ของการทบทวนวรรณกรรมบทนี้ คือ ให้ข้อมูลพื้นฐานของระบบภูมิคุ้มกันของกุ้ง เพื่อนำไปเป็นแนวนโยบายในการควบคุมและป้องกันโรค**

**คำสำคัญ :** ระบบภูมิคุ้มกัน ระบบภูมิคุ้มกันสืบทอด กุ้ง

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## Introduction

Total world fisheries production has decreased while human consumption of aquatic organisms has increased (FAO, 2006). This reduction in capture fisheries has been partly compensated by the fast growth of the aquaculture industry, where the commercial culture of penaeid shrimp is an important sector in tropical and subtropical countries (FAO, 2006). However, shrimp diseases have been responsible for the collapse of aquaculture in a number of countries, and are considered as a limiting factor for its development (Flegel, 2006). Epizootics are attributed to the inadvertent transmission of pathogens due to biosecurity problems and poor management practices, affecting the growth of the cultured organisms and generating important economic losses (FAO, 2006). The implementation of biosecurity methods and the timely diagnosis during culture may avoid pathogen's access and propagation, helping in the reduction of loses due to mortalities and treatment costs (Flegel, 2006). Furthermore, the importance of understanding shrimp physiology and immunology is important for the control and prevention of disease (Raico et al., 2003). Therefore the purpose of this minireview is to present the general status of the shrimp defense system, to help in the development of strategies that favour the control and prevention of the disease. For practical purposes this minireview will address first the physical defence barriers, then the cellular and humoral defence systems, shrimp immunostimulation and finally future research perspectives.

### I. Physical barriers

Physical barriers are the first line of defense on shrimp and consist of a rigid exoskeleton, which protects

from injury and microbial attacks. The exoskeleton is composed of calcium carbonate, carbohydrates and proteins, and contributes to different physiological processes associated with the immune response (Mylonakis and Aballay, 2005). There are reports of diffuse distribution of hemocyanin and catalytic phenol oxidation over the exocuticle and endocuticle of crustaceans; both are important immune response against microbes (Adachi et al., 2005). However, the mechanisms involved in crustaceans' cuticle hardening and the role of phenoloxidase are poorly understood. For practical purposes this mini-review will address first the physical defence barriers, then the cellular and humoral defence systems, shrimp immunostimulation and finally future research perspectives.

**II.- Cell mediated immune defense**

Crustaceans have an open circulatory system with blue-green hemolymph, which circulates through the hemocele and irrigates the crustacean tissues. Hemocytes and humoral components are transported by the hemolymph favouring their encounter with foreign particles (Rendón and Balcázar, 2003).

Hematopoiesis is the source for mature effectors cells for the innate immune system, which show roles on host defense and homeostasis. Blood cell formation is regulated from extra and intracellular signs that result in the activation of specific downstream signalling cascades. The hematopoietic tissue (HPT) in crustaceans is an extensive network of packed lobules located at the dorsal and dorsolateral sides of the stomach, close to the antennal artery and at the base of the maxillipedes. Hemocytes are produced within the walls of these tubules and released into the vessel lumens (Söderhäll et al., 2003). The HPT of *Penaeus monodon* and other penaeid shrimp is located in different areas stomach, maxillipeds and antennal gland (Van de Braak et al., 2002).

Penaeid shrimp hemocytes have the same biological properties and functions with vertebrate macrophages, granulocytes and natural killer cells (Van de Braak, 2002). These cells participate in phagocytosis, encapsulation, nodule formation, wound repair, clotting, and proPO activation. They also help the production of adhesion molecules, agglutinins and antimicrobial peptides (AMP) (Destoumieux et al., 1997; Bachère et al., 2000). Hemocytes also have inhibitory enzymes needed for regulating the proteolytic cascade, preventing its over stimulation and the resultant tissue damage, while also producing cytotoxic molecules such as lysozyme, phosphatase, esterase, phospholipase, peroxidase, protease, etc. (Van de Braak, 2002; Johansson et al., 2000).

There are three classes of hemocytes, hyalinocytes, granulocytes and semi-granulocytes. Hyalinocytes (5-15% of circulating hemocytes or CE) are small non-refractive cells, with a small nucleus relative to their cytoplasm, which have few or no cytoplasmic granules. Hyalinocytes have no phagocytic activity and easily adhere to glass surfaces, like fish and mammals macrophages. The primary role of these cells is related to clotting and phagocytosis (Zhang et al., 2006). Granulocytes (10-20% of CE) have the smallest nucleus and a high number of cytoplasmic granules (0.8 μm width). Granulocytes display phagocytic activity and store the enzyme prophenoloxidase (proPo). These cells may be stimulated by β-1,3-glucans, peptidoglycans (PG) and lipopolysaccharides (LPS) to provoke exocytosis and enzyme release. Their function is encapsulation, initiating the proPO cascade and phagocytosis (Zhang et al., 2006). Semi-granulocytes (75% CE) have a large numbers of small granules (0.4 μm width) similar to vertebrate granulocytes. These cells posses β-1,3-glucans receptors and their principal function involves phagocytosis, encapsulation and clotting (Martin and Graves, 2005; Zhang et al., 2006).

***Phagocytosis***

Phagocytosis involves the internalization of foreign material. This is the main cellular defense mechanism in invertebrates, and is carried out by the semi- and granulocytes; it consists of chemotaxis, adherence, ingestion, pathogen destruction and exocytosis (Kondo

et al., 1998; Vargas-Albores and Yepiz-Plascencia 1998). Phagocytic cells destroy the internalized organisms by two routes, an aerobic process which uses NADPH or NADH as an electron donor, and reduces an oxygen electron to form the superoxide ion. This radical in turn changes to hydrogen peroxide ($H_2O_2$) spontaneously or by the action of the superoxide dismutase (SOD), producing a new oxygen molecule. In penaeid hemocytes, the activation of the aerobic process has been demonstrated by the use of bacteria *(Vibrio parahaemolyticus and V. vulnificus)* and surface microbial antigens (β-1,3 glucan, PG, LPS, and zymosan), as both increase the phagocytic capacity of hemocytes to destroy pathogens (Itami et al., 1998; Song and Huang, 2000; Campa-Córdova et al., 2002). The second, anaerobic process is attribute to the action of diverse microbicidal enzymes, such as lysozyme and low molecular weight AMP (Nappi and Ottaviani, 2000).

***Encapsulation and nodule formation***

Semi granulocytes are responsible for the recognition of the invading agents and their encapsulation with proteins (76 kD) that work as an opsonins associated to the proPO activation system. These proteins act as a degranulation and adhesion factor for semi and granulocytes, and as an encapsulation promoter (Vargas-Albores and Yepiz-Plascencia, 1998; Wang et al., 2001[a]; Van de Braak, 2002). The hemolytical nodules, detected in gill and hepatopancreas, are formed by numerous hemocytes acting synergistically to trap microorganisms or big antigens that cannot be removed by phagocytosis. These nodules undergo the subsequent activation of the proPO system, melanisation and destruction of microbes (Wang et al., 2001[a]; Van de Braak, 2002).

***Antioxidant system***

Antioxidant factors protect the shrimp from the cytotoxic effects caused by the cellular metabolism and oxidative stress generated by the disequilibrium of the reactive oxygen intermediates (ROIs), stress tests had been done in marine organisms by Downs et al.(2001). The study detected an increase in the levels of the antioxidant enzymes and immune system molecules pointing out the important role of the antioxidant enzymes as the immune response modulators.

ROIs and reactive nitrogen intermediates (RNIs) are generated in phagocytic vacuoles. These molecules are capable of crossing the cell barrier and damaging the neighbouring cells (Nathan and Shiloh, 2000). To prevent this damage, antioxidant defense strategies have been developed including enzymatic substance (catalase, glutathione peroxidase (GPx), and SOD) and non-enzymatic components (ascorbate, β-carotene, flavonoids, α-tocopherol and vitamin E), which may neutralize the ROIs or repair the molecular damage done to the cell (Nathan and Shiloh, 2000).

SODs are one of the main defense mechanisms against oxidative stress caused by pollution, infections, hypoxia, hyperoxia, temperature and immunostimulants (Neves et al., 2000). SODs have been classified in manganese SOD (mitochondria and prokaryotes), iron SOD (bacteria and plants) and copper-zinc SOD (eukaryotic cytosol). An extracellular SOD (EC-SOD) has been reported in lobster, associated with phagocytosis, opsonization, encapsulation and generation of microbicidal compounds (Homblad and Söderhäll, 1999).

***Oxyradical scavenging capacity***

The production of oxidative compounds with antimicrobial effects has been studied in hemocytes from invertebrates (Van de Braak, 2002; Buggé et al., 2007). This cellular response is rapid and transient, and is produced during microbe phagocytosis. These compounds include superoxide anions ($O_2^-$), hydroxyl radical ($OH^-$), $H_2O_2$, ROIs, and RNIs such as nitric oxide and peroxynitrite (Roch, 1999). The ROIs and RNIs are an innate immune responses present in echinoderms, nematodes, annelids, insects, crustacean and molluscs (Nappi and Ottaviani, 2000). Their production is mediated by the enzymes NADPH oxidase and nitric oxide synthetase (NOS) respectively. Other enzymes involved in ROI production

are xanthine oxidase and glucose oxidase (Nappi and Ottaviani, 2000). The RNI are nitric oxide derivatives, which are synthesized from L-arginin by NOS.

*Prophenoloxydase system*

Granulocytes are responsible for the synthesis, storage and secretion of the proPO system, which is activated by fungal β-glucans, PG and LPS. These molecules induce the granulocyte secretion of inactive proPO granules and their transformation (cascade reaction) to proPO enzyme. This oxidizes phenols into quinones, which may help to kill pathogens and are used for melanin production (Lee et al., 2004; Hellio et al., 2007). In addition, the proteins interacting in the proPO cascade process are associated with cell recognition and hemocyte communication.

Peroxynectin is a proPO system associated factor that creates cellular adhesion and acts as a peroxidase. This molecule is synthesized and stored by the granulocytes and activated upon cell secretion. Hemocyte's transmembrane receptors are responsible for the peroxynectin cell adhesion, hemocyte dispersion, phagocytosis, encapsulation, nodule formation and agglutination which resulted in peroxide activation and the invading agent destruction (Söderhäll and Cerenius, 1998; Söderhäll et al., 2003).

***Melanization***

This is an interesting, little known and complex biochemical process associated to different proteases (trypsine-like serine, serine protease) mediated by the phenoloxidase (PO) system (Robalino et al., 2007; Pais et al., 2008). Melanization plays an important role in the invertebrate defense mechanisms wherein a thick acellular capsule of melanin is generated around foreign objects (Barillas-Mury, 2007). Melanin, a product of the proPO system, is a dark brown pigment with antibacterial properties that inhibits antigens (Holmblad and Söderhäll, 1999). Although microbicidal properties have been attributed to melanin and the other agents such as $O_2^-$ and hydroxyl radicals which are generated during quinones formation (Vargas-Albores et al., 1998; Hellio et al., 2007).

***Cytokines***

The activation of antimicrobial responses in invertebrates is mediated by cytokines which are produced by hemocytes. Some analogues of vertebrate cytokines such as interleukins (IL-1, IL-2, IL-6) and tumour necrosis factor alpha (TNF-α) have been identified in invertebrates (annelids, echinoderms, mollusc and tunicates). These cytokines analogues have biological functions similar to their corresponding vertebrate molecules (Nappi and Ottaviani, 2000).

Heat shock proteins (HSP) or chaperonins are invertebrate cytokines which are capable of protecting and restoring proteins damaged by stress factors, such as high temperatures, etc. (Frankenberg et al., 2000). The first study that shows association between the heat shock proteins and the stress or immune responses of shrimp was done by Wan-Yu et al. (2004), who cloned and characterized the cDNA of the heat shock cognate 70 gene (652 amino acid sequence, 7.14 kDa) of *P. monodon*. This protein shows a phylogenetic relationship with invertebrate and vertebrate hsc70 proteins, and possibly functions as a chaperone. The expression of hsc70 mRNA, in shrimp hemocytes, increased 2 to 3 fold on one hour post heat shock and had a 30 min recovery time, until these molecules decreased gradually to 2baseline levels.

***Clotting protein cascade***

Coagulation is used to prevent the loss of hemolymph through cuts and wounds in the exoskeleton, and to immobilization of invading pathogens (Meng-Yi et al., 2005). Three types of hemolymph clotting systems (cascade) are known in crustaceans. Type A consists of a rapid hemocyte agglutination without plasma coagulation; type B consists of cellular aggregation with limited plasma coagulation; and type C is a limited

cellular aggregation and lysis followed by plasma coagulation. Type C hemolymph coagulation is present in shrimp and other decapods (Yeh et al., 1999; Van de Braak, 2002). In crustaceans, the coagulation process is regulated by clotting proteins (coagulogens) and compartmentalized cellular factors within circulating cells. Clotting proteins in plasma are converted to covalently joined polymers by a $Ca^{++}$ dependent transglutaminase secreted by the hemocytes (Wang et al., 2001[b]). The cellular clotting proteins can be activated by LPS or β-1,3-glucan, and are related to the proPO activation system (Roux et al., 2002).

## III Humoral immune defense

### *Recognition molecules*

There is little knowledge about the cell to cell communication system during the immune response in invertebrates (Nappi and Ottaviani, 2000). Lectins are non-enzymatic proteins or glycoproteins that act in opsonization, agglutination, phagocytosis and pathogen encapsulation. Invertebrate lectins are considered primitive recognition molecules capable of detecting carbohydrates (Nappi and Ottaviani, 2000) which promote proPO system activation (Wang et al., 2001[a]).

The pattern recognition proteins (PRP) are lectins that detected the molecules like LPS, PG, bacterial lipoteichoic acid, fungal β-1,3-glucans and viral RNA (Song and Huang, 2000; Lee and Söderhäll, 2002), and which favour the activation of specific defense mechanisms by the host. The biological functions of PRPs are the initiation of a protein cascade and/or defense mechanisms' signalization routes and elimination of blood system invaders. When PRPs detect the antigens, the hemocytes are migrating to their location by chemotaxis, generating an inflammatory response. The crustacean open circulatory system favours this phenomenon, resulting in a fast and efficient defense mechanism against pathogens. Examples of PRPs present in crustacean plasma are β-1,3-glucan binding protein which induces degranulation and proPO system activation, and LPS binding protein, which helps in bacterial agglutination and removal by phagocytosis (Vargas-Albores and Yepiz-Plascencia, 1998; Sritunyalucksana et al., 2002).

The innate immune system identifies pathogens through PRP and their corresponding pattern recognition receptors (PRR), which also are proteins. Toll-like receptors (TLRs) are an evolutionarily ancient family of PRRs presented in animals ranging from cnidarians to mammals, which can detect all kinds of pathogens (Janeway and Medzhitov, 2000; Robalino et al., 2004). TLRs are activated by bacterial and virus infection and have been reported in *Fenneropenaeus chinensis* and *Litopenaeus vannamei* (Li-Shi et al., 2007; Changjian et al., 2008). In mammals, TLRs on specialized antigen-presenting cells function as signal transducers by the way of nuclear factor κB, leading to the production of pro-inflammatory cytokines and the expression of costimulatory molecules on the cell surface (Inamori et al., 2004).

### *Antimicrobial peptides*

An important element against invertebrate's pathogens are the AMP. These are cationic and amphipathic proteins of low molecular weight (<10 kDa), essential in organisms that lack of adaptive immunity (Marshall and Arenas, 2003). AMPs have wide spectrum of activity, low specificity and are weakly cytotoxic to animal cells. These peptides make pores in the cell membranes of bacteria, fungi, parasites, enveloped viruses and even cancer cells, provoking an instability of ions and energy (Hancock, 1998; Bulet et al., 1999; Lehrer and Ganz, 1999). Based on their amino acid sequence, secondary structure and functional similarities, AMPs have been classified as 1) peptides stabilized by intermolecular disulphide bonds, 2) peptides and linear polypeptides with α-helicoidal structures, and 3) peptides and linear polypeptides with a high content of proline residues and/or glycine (Shai, 1998; Bulet et al., 1999). AMP activity can be reduced by a variety of *in vivo*

factors, including high concentrations of mono and divalent cations, polyanions, apolypoprotein A-1, etc. However, many peptides seem to be relatively resistant to several of these agents (Hancock, 1998).

Penaeidins, a family of AMPs, are initially characterized from *L. vannamei*, and their sequences have been described in *L. setiferus, L. stylirostris, Penaeus semisulcatus, Marsupenaeus japonicus, P. monodon* and *F. chinensis* (Destoumieux et al., 1997; Gross et al., 2001; Rojtinnakorn et al., 2002; Supungul et al., 2002). Penaeidins are synthesized and stored in the granulocyte, and present Gram (+) antibacterial and antifungal activities (Destoumieux et al., 1997; Destomieux et al., 1999; Bachère et al., 2000).

Other AMP found in the shrimp, is hemocyanin-derived peptides, whose C-terminal fragments have antifungal activity; however, the mechanism by which hemocyanin is cleaved and activated are still unclear (Destoumieux-Garzon et al., 2001). Histone proteins; $H_2A$, $H_2B$, $H_3$ and $H_4$ found in hemocytes from *L. vannamei* show antimicrobial activity against Gram (+) bacteria and related to vertebrate histones, they may be a component of innate immunity more widely conserved, and of earlier origin, than previously thought (Patat et al., 2004).

***Lysosomal enzymes***

Lysozyme degrades the mucopolysaccharides of Gram (-) bacterial cell walls, and modifies the molecular conformation of the cell surface, allowing their recognition by phagocytic cells. Lysozymes take part in the degradation of microbes within and outside hemocytes, and some play a role of sterases and chitinases (de-la-Re-Vega et al., 2004).

**IV. Peneaid shrimp immunostimulation**

Vaccination is used to stimulates the innate and acquired immune response in vertebrates. It involves the administration of specific and non-specific compounds that induce an organismís response against pathogen agents (Song and Huang, 2000). Unfortunately, only an innate immune system has been found in invertebrates. Several antigens (vibrio cells, yeast glucans or their derivatives) have been experimentally tested to elucidate the innate immune mechanisms in shrimp (Böhnel et al., 1999; Sakai, 1999; Vici et al., 2000). Astanxhantins, chitosan, fucoidan, β1-3 glucan, herbal extracts, laminaria, LPS, PG, saponins, and vitamin C are the main antigens experimentally tested in shrimp (Newman, 1999). These substances can be administered by injection, immersion, bioencapsulation, per os intubation, and in the feed in marine organisms (Robles et al., 1998). The results suggested that they can be an important element in the control of disease.

***Glucans:*** These molecules are non-specific immuno-stimulants in crustaceans, inducing resistance against bacterial pathogens (Vargas-Albores et al., 1998). However, crustaceans can digest glucans and use them as sources of energy, losing their function in the animal immune system. Interestingly, the use of LPS together with yeast glucan acts synergistically inducing a better stimulation of the crustacean immune system than when they are used separately (Newman, 1999).

***Peptidoglycans:*** PGs are a mix of amino acids and carbohydrates from the cell wall of many bacteria and have been deemed as potent immunostimulants for the immune system (Lee et al., 2004). These molecules are recognized as immunogen by the shrimp immune system, and *Bifidobacterium thermophilum, Brevibacterium lactofermentum* and *Bacillus* sp. PGs have been tested against yellow head virus (YHV) and white spot syndrome virus (WSSV). The results showed that the PG exposed shrimp exhibit a higher survival rate than non exposed shrimp (Itami et al., 1998; Lee et al., 2004).

***Fucoidan:*** These molecules are sulphated polysaccharides form microalgae cell walls which have been used as immunostimulants for shrimp. These products have been somewhat successful against pathogens like WSSV, *Vibrio* sp. and other bacterial species. Experimental

administration of fucoidan mixed in the diet has resulted in a 93% increase in survival compared to controls (Chotigeat et al., 2004). However, the action mechanisms are not well understood and more research with these products is necessary.

***Lipopolysaccharides:*** LPS affect the specific and non-specific immune system of many animals, including crustaceans (Vargas-Albores et al., 1998; Newman, 1999). LPS are part of the cell wall of Gram (-) bacteria and are the first molecules recognized by the host's immune system, thus they have been used in shrimp as a potential tool to prevent diseases. However, most of the work has been done under controlled laboratory conditions and results may vary when performed in the field.

**V. Future Perspectives**

Understanding the role of the invertebrate immune system against pathogens is growing steadily; however, knowledge about the molecular and cellular mechanisms of immune system associated in the recognition and elimination is scarce. New research areas are including genes, their products, and activation of genetic mechanisms associated to the shrimp immune system. Other research areas where further studies are warranted, including the proPO system, cell adhesion proteins, peroxinectins, pattern recognition proteins, 1,3-glucan binding protein (GBP), and hemocyte formation/activation and their protein synthesis.

Many of the previous studies on shrimp immunology have been done with proteins, immune products, or preparations of limited purity, which made difficultly the understanding of their effects on the penaeid immune system. Future studies will have to focus on (i) the use of highly purified proteins or immune products, (ii) the use of assays with the ability of quantitative detection (real-time polymerase chain reaction, enzyme-linked immunosorbent assay) of individual mRNAs or proteins during immunostimulation or disease, (iii) the characterization of the specific microbial or viral structures, or their products associated with infectius diseases, and target organs during the course of infections, (iv) the formation and activation of hemocytes in response to pathogens. New research in those areas will involve the cooperation of biochemists, immunologists, bacteriologists, virologists, molecular biologists and infectious disease experts to discern new immune system models on invertebrates, which will possibly have a significant impact on the understanding of the immune system in general.

## References

Adachi, K., Endo, H., Watanabe, T., Nishioka, T. and Hirata, T. 2005. Hemocyanin in the exoskeleton of crustaceans: enzymatic properties and immunolocalization. Pigment Cell Res. 18: 136-143

Bachère, E., Destoumieux, D. and Bulet, P. 2000. Penaeidins, antimicrobial peptides of shrimp, a comparison with other effectors of innate immune. Aquaculture. 191: 71-88.

Barillas-Mury, C. 2007. CLIP proteases and *Plasmodium* melanization in *Anopheles gambiae*. Trends Parasitol. 23: 297-299.

Böhnel, H., Lohavanijaya, P., Rungin, S., Schnug, C. and Seifert, H.S.H. 1999. Active immunisation of black tiger prawn *(Penaeus monodon)* against vibriosis in Thailand. Berl. Munch. Tierarztl. Wochenschr. 112: 289-295.

Buggé, D.M., Hégaret, H., Wikfors, G.H. and Allam, B. 2007. Oxidative burst in hard clam *(Mercenaria mercenaria)* haemocytes. Fish Shellfish Immunol. 23: 188-196.

Bulet, P., Hetru, C., Dimarcq, J.L. and Hoffmann, D. 1999. Antimicrobial peptides in insects; structure and function. Dev. Comp. Immunol. 23: 329-344.

Campa-Córdova, A.I., Hernández-Saavedra, N.Y., Philippis, R. De and Ascencio, F. 2002. Generation of superoxide anion and SOD activity in haemocytes and muscle of American white shrimp *(Litopenaeus vannamei)* as a response to β-glucan and sulphated polysaccharide. Fish Shellfish Immunol. 12: 353-366.

Changjian, Y., Jiquan, Z., Fuhua, L., Hongming, M., Qingli, Z., Jose Priya, T.A., Xiaojun, Z. and Jianhai, X. 2008. A Toll receptor from Chinese shrimp *Fenneropenaeus chinensis* is responsive to *Vibrio anguillarum* infection. Fish Shellfish Immunol. 24: 564-574.

Chotigeat, W., Tongsupa, S., Supamataya, K. and Phongdara, A. 2004. Effect of fucoidan on disease resistance of black tiger shrimp. Aquaculture. 233: 23-30

de-la-Re-Vega, E., García-Orozco, K.D., Calderón-Arredondo, S.A., Romo-Figueroa, M.A, Islas-Osuna, M.A., Yepiz-Plascencia, G.M. and Sotelo-Mundo, R.R. 2004. Recombinant expression of marine shrimp lysozyme in *Escherichia coli*. J. Biotechnol. 7: 298-304.

Destoumieux, D., Bulet, P., Loew, D., Van Dorsselaer, A., Rodríguez, J. and Bachère, E. 1997. Penaeidins, a new family of antimicrobial peptides isolated from penaeids shrimp (Decapoda). J. Biol. Chem. 272: 28398-28406.

Destomieux, D., Bulet, P., Strub, J.M. and Bachère, E. 1999. Recombinant expression and range of activity of penaeidins, antimicrobial peptides from penaeid shrimp. Eur. J. Biochem. 266: 335-346.

Destoumieux-Garzon, D., Saulnier, D., Garnier, J., Jouffrey, C., Bulet, P. and Bachere, E. 2001. Crustacean immunity. Antifungal peptides are generated from the C terminus of shrimp hemocyanin in response to microbial challenge. J. Biol. Chem. 276: 47070-47077.

Downs, C., Fauth, J.E. and Woodley, C.M. 2001. Assessing the health of grass shrimp *(Palaeomonetes pugio)* exposed to natural and anthropogenic stressors: a molecular biomarker system. Mar. Biotechnol. 3: 380-397.

FAO (Food and Agriculture Organization of the United Nations), 2006. The state of world fisheries and aquaculture (SOFIA).

Flegel, T.W. 2006. The special danger of viral pathogens in shrimp translocated for aquaculture. Sci. Asia. 32: 215-221

Frankenberg, M.M., Jackson, J.S. and Clegg, J.S. 2000. The heat shock response of adult *Artemia franciscana*. J. Therm. Biol. 25: 481-490.

Gross, P.S., Bartlett, T.C., Browdy, C.I., Chapman, R.W. and Warr, G.W. 2001. Immune gene discovery by expressed sequence tag analysis of hemocytes and hepatopancreas in the pacific white shrimp, *Litopenaeus vannamei,* and the Atlantic white shrimp, *L. setiferus*. Dev. Comp. Immunol. 25: 565-577.

Hancock, R.E.W. 1998. The therapeutic potential of cationic peptides. Expert Opin. Investig. Drugs. 7: 167-174.

Hellio, C., Bado-Nilles, A., Gagnaire, B., Renault, T. and Thomas-Guyon, H. 2007. Demonstration of a true phenoloxidase activity and activation of a ProPO cascade in Pacific oyster, *Crassostrea gigas* (Thunberg) *in vitro*. Fish Shellfish Immunol. 22: 433-440.

Holmblad, T. and Soderhäll, K. 1999. Cell adhesion molecules and antioxidative enzyme in a crustacean, possible role in immunology. Aquaculture. 172: 111-123.

Inamori, K., Ariki, S. and Tawabata, S. 2004. A Toll-like receptor in horseshoe crabs. Immunol. Rev. 198: 106-115.

Itami, T., Asano, M., Tokushige, K., Kubono, K., Nakagawa, A., Takeno, N., Nishimura, H., Maeda, M., Kondo, M. and Takahashi, Y. 1998. Enhancement of disease resistance of kuruma shrimp, Penaeus *japonicus* after oral administration of peptidoglycan derived from *Bifidobacterium* thermophilum. Aquaculture. 164: 277-288.

Janeway, C.A. and Medzhitov, R. 2000. Viral interference with IL-1 and Toll signaling. Proc. Natl. Acad. Sci. U.S.A. 97: 10682-10683.

Johansson, M., Keyser, P., Sritunyalucksana, K. and Söderhäll, K. 2000. Crustacean haemocytes and haematopoiesis. Aquaculture. 191: 45-52.

Kondo, M., Itami, T., Takahashi, Y., Fujii, R. and Tomonaga, S. 1998. Ultrastructural and cyto-chemical characteristics of phagocytes in kuruma prawn. Fish Pathol. 33: 421-427.

Lee, M.H., Osaki, T., Lee, J.Y., Baek, M.J., Zhang, R., Park, J.W., Kawabata, S., Soderhäll , K. and Lee, B.L. 2004. Peptidoglycan recognition proteins involved in 1,3-β-D-glucan-dependent prophenoloxidase activation system of insect. J. Biol. Chem. 279: 3218-3227.

Lee, S.Y. and Söderhäll, K. 2002. Early events in crustacean innate immunity. Fish Shellfish Immunol. 12: 421-437.

Lehrer, R.I. and Ganz, T. 1999. Antimicrobial peptides in mammalian and insect host defense. Curr. Opin. Immunol. 11: 23-37.

Li-Shi, Y., Zhi-Xin, Y., Ji-Xiang, L., Xian-De, H., Chang-Jun, G., Shao-Ping, W., Siu-Ming, C., Xiao-Qiang, Y. and Jian-Guo, H. 2007. A toll receptor in shrimp. Mol. Immunol. 44: 1999-2008.

Marshall, S.H. and Arenas, G. 2003. Antimicrobial peptides: A natural alternative to chemical antibiotics and a potential for applied biotechnology. Electron. J. Biotechnol. 6: 271-284.

Martin, G.G. and Graves, B. 2005. Fine structure and classification of shrimp haemocytes. J. Morphol. 185: 339-348.

Meng-Yi, C., Kuang-Yu, H.U.B, Chih-Cheng, H. and Yen-Ling, S. 2005. More than one type of transglutaminase in invertebrates? A second type of transglutaminase is involved in shrimp coagulation. Dev. Comp. Immunol. 29: 1003-1016.

Mylonakis, E. and Aballay, A. 2005. Worms and flies as genetically tractable animal models to study host-pathogen interactions. Infect. Immun. 73: 3833-3841.

Nappi, A.J. and Ottaviani, E. 2000. Cytotoxicity and cytotoxic molecules in invertebrates. BioEssays. 22: 469-480.

Nathan, C. and Shiloh, M.U. 2000. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc. Natl. Acad. Sci. U.S.A. 97: 8841-8848.

Neves, C.A., Santos, E.A. and Bainy, A.C.D. 2000. Reduced superoxide dismutase activity in *Palaemonetes argentinus* (Decapoda, Paleminedae), infected by *Probopyrus ringueleti* (Isopoda, Bopyridae). Dis. Aquat. Org. 39: 155-158.

Newman, S.G. 1999. A review of the use of non specific immune-stimulants to reduce the impact of the WSSV. 5th Ecuadorian Aquaculture Conference. October 28-30, Ecuador.

Pais, R., Khushiramani, R., Karunasagar, I. and Karunasagar, I. 2008. Effect of immunostimulants on the haemolymph haemagglutinins of tiger shrimp *Penaeus monodon*. Aquac. Res. 39: 1339-1345.

Patat, S.A., Carnegie, R.B., Kingsbury, C., Gross, P.S., Chapman, R. and Schey, K.L. 2004. Antimicrobial activity of histones from hemocytes of the Pacific white shrimp. Eur. J. Biochem. 271: 4825-4833.

Raico, E., Lamela, L., Silveira, R. and Mart?nez, M. 2003. Actividad peroxidasa (POD) en juveniles del camarón *Litopenaeus schmitti*. II Congreso Iberoamericano Virtual de Acuicultura (CIVA). p.99-104.

Rendón, L. and Balcázar, J.L. 2003. Inmunología de camarones: Conceptos básicos y recientes avances. Revista AquaTIC. 19: 27-33.

Robalino, J., Browdy, C.L., Prior, S., Metz, A., Parnell, P., Gross, P. and Warr, G. 2004. Induction of Antiviral Immunity by Double-Stranded RNA in a Marine Invertebrate. J. Virol. 78: 10442-10448.

Robalino, J., Almeida, J.S., McKillen, D., Colglazier, J., Trent, H.F., Chen, Y.A., Peck, M.E.T., Browdy, C.L., Chapman, R.W., Warr, G.W. and Gross, Pí.S., 2007. Insights into the immune transcriptome of the shrimp *Litopenaeus vannamei:* tissue-specific expression profiles and transcriptomic responses to immune challenge. Physiol. Genomics 29: 44-56, 2007

Robles, R., Sorgeloos, P., Van Duffel, H. and Nelis, H. 1998. Progress in biomedication using live foods. J. Appl. Ichthyol. 14: 207-212.

Roch, P. 1999. Defense mechanisms and disease prevention in farmed marine invertebrates. Aquaculture. 172: 125-145.

Rojtinnakorn, J., Hirono, I., Itami, T., Takahashi, Y. and Aoki, T. 2002. Gene expression in haemocytes of kuruma prawn, *Penaeus japonicus,* in response to infection with WSSV by EST approach. Fish Shellfish Immunol. 13: 69-83.

Roux, M.M., Pain, A., Klimpel, K.R. and Dhar, A.K. 2002. The lipopolysaccharide and β-1,3-glucan binding protein gene is upregulated in white spot virus-infected shrimp *(Penaeus stylirostris).* J. Virol. 76: 7140-7149.

Sakai, M. 1999. Current research status of fish immunostimulants. Aquaculture. 172:63-92.

Shai, Y. 1998. Mode of action of antibacterial peptides. In: Molecular mechanisms of immune responses

in insects. 1st ed. P.T. Brey and D. Hultmark (eds). London: Chapman and Hall, 111-134.

Söderhäll, K. and Cerenius, L. 1998. The prophenoloxidase activating system in invertebrate immunity. Curr. Opin. Immunol. 10, 23-28.

Söderhäll, I., Bangyeekhun, E., Mayo, S. and Söderhäll, K. 2003. Hemocyte production and maturation in an invertebrate animal; proliferation and gene expression in hematopoietic stem cells of *Pacifastacus leniusculus*. Dev. Comp. Immunol. 27: 661-672.

Song, Y.L. and Huang, C.C. 2000. Aplications of immunostimulant to prevent shrimp diseases. In: Resent advances in marine biotechnology. 1st ed. M. Fingerman and R. Negabhusanam (eds). Playmouth: Science Publishers Inc.: 173-187.

Sritunyalucksana, K., Lee, S.Y. and Söderhäll, K. 2002. A β-1,3-glucan binding protein from the black tiger shrimp, Penaeus monodon. Dev. Comp. Immunol. 26: 237-245.

Supungul, P., Klinbunga, S., Pichyangkura, R., Jitrapakdee, S., Hirono, I., Aoki, T. and Tassanakajon, A. 2002. Identification of immune-related genes in hemocytes of black tiger shrimp *(Penaeus monodon)*. Mar. Biotechnol. 4: 487-494.

Van de Braak, K. 2002 Hemocytic defense in black tiger shrimp *(Penaeus monodon)*. Doctor degree thesis. Wageningen Institute of Animal Science. Wageningen University. The Netherlands.

Van de Braak, C.B.T., Botterblom, M.H.A., Liu, W., Taverne, N., Van der Knaap, W.P.W. and Rombout, J.H.W.M. 2002. The role of the haematopoietic tissue in haemocyte production and maturation of the black tiger shrimp *(Penaeus monodon)*. Fish Shellfish Immunol. 12: 253-272.

Vargas-Albores, F., Hernández-López, J., Gollas-Galván, T., Montaño-Pérez, K., Jiménez-Vega, F. and Yepiz-Plascencia, G. 1998. Activation of shrimp cellular defense functions by microbial products. In: Advances in shrimp biotechnology. T.W. Flegel (ed). National center for genetic engineering and biotechnology, Bangkok, 161-166.

Vargas-Albores, F. and Yepiz-Plascencia, G. 1998. Shrimp immunity: A review. Trends Comp. Biochem. Physiol. 5: 195-210.

Vici, V., Bright Sing, I.S. and Bhat, S.G. 2000. Application of bacterins and yeast *Acremonium dyosporii* to protect the larvae of *Macrobrachium rosenbergii* from vibriosis. Fish Shellfish Immunol. 10: 559-563.

Wang, R., Lee, S.Y., Cerenius, L. and Söderhäll, K. 2001[a]. Propertis of the prophenoloxidase activating enzyme of the freshwater crayfish, *Pacifastacus leniuscalus*. Eur. J. Biochem. 268: 895-902.

Wang, R., Liang, Z., Hal, M. and Söderhäll, K. 2001[b]. A transglutaminase involved in the coagulation system of the freshwater crayfish, *Pacifastacus leniusculus*. Tissue localization and cDNA cloning. Fish Shellfish Immunol. 11: 623-637.

Wan-Yu, L., Kuan-Fu, L., I-Chiu, L. and Yen-Ling, S. 2004. Cloning and molecular characterization of heat shock cognate 70 from tiger shrimp *(Penaeus monodon)*. Cell Stress Chaperones. 9: 332-343.

Yeh, M.S., Huang, C.J., Leu, J.H., Lee, Y.C. and Tsai, I.H. 1999. Molecular cloning and characterization of a hemolymph clottable protein from tiger shrimp *(Penaeus monodon)*. Eur. J. Biochem. 266: 624-633.

Zhang, Z.F., Shao, M. and Ho Kang, K. 2006. Classification of haematopoietic cells and haemocytes in Chinese prawn *Fenneropenaeus chinensis*. Fish Shellfish Immunol. 21: 159-169.