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Review Article

INNATE IMMUNE SYSTEM AND MOLECULAR ELEMENTS IN FRESHWATER CRAB: A MINI REVIEW

[1,2*]Mahadev Asaram Jadhav and [2]Encily Reymend Martin

[1*]Department of Biotechnology & Bioinformatics, Deogiri College (Autonomous), Chhatrapati Sambhajinagar- 431 005
[2]Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar-431 001

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ABSTRACT

The immune system of freshwater crabs is an intricate network essential for their survival in pathogen-rich environments. This review synthesizes current knowledge on the molecular mechanisms underlying immune responses in the hemolymph of freshwater crabs. Key components such as hemocytes, antimicrobial peptides, and the prophenoloxidase system are discussed alongside recent advancements in molecular techniques that have facilitated these discoveries. We review some basic aspects of crab effector defense processes, like agglutination, encapsulation, phagocytosis, clottable proteins, and bactericidal activity, induced by these carbohydrate-driven recognition patterns. Understanding these immune defense mechanisms provides insights into invertebrate immunity and has practical implications for aquaculture.

Keywords: Hemolymph, Immune defense, Hemocytes, Antimicrobial peptides, Prophenoloxidase system.

INTRODUCTION

Freshwater crabs inhabit environments with diverse pathogenic threats, relying primarily on their innate immune system for defense. Unlike vertebrates, crabs lack an adaptive immune system, making the study of their innate immune responses crucial for understanding their survival strategies. Immunity studies in vertebrate and invertebrate species suggest that the invertebrate defense mechanisms could be considered precursors of vertebrate immunity. These events require the participation of cellular groups and humoral factors generated against specific antigens, as has been shown in mammals (Hoffmam *et al.*, 1999; Iwanaga and Lee, 2005). In invertebrates, there is little evidence identifying whether any of these factors are antigen-specific. However, some cellular and cell-free hemolymph factors show high specificity for non-self or damaged cells, similarly as demonstrated for antibodies (Yeaton, 1981; Vasa *et al.*, 1999). Crustaceans represent a group of economic relevance, because of their adaptation to aquaculture; however, crustaceans are affected by diseases caused, mainly, by opportunistic pathogens causing huge economic losses. Studies on the immunity of invertebrates have focused on identifying defense mechanisms and biochemical pathways activated during an infection, and on identifying cell-free hemolymph and cellular factors involved in the destruction of pathogens, regulation, and damage repair. Crustaceans possess an open circulatory system, where nutrients, oxygen, hormones, and cells are distributed in the hemolymph. The hemocytes (circulating cells) could be functionally analogous to vertebrate leukocytes, because they are mainly involved in non-self-matter recognition and elimination (Sritunyalucksana and So¨derha¨ll, 2000), as well as in downstream coagulation (Cerenius *et al.*, 1994; Vázquez *et al.*, 1997).

Immune response in hemolymph of crabs is triggered by pathogen-associated molecular patterns (PAMPs) present on surface of microbes, such as lipopolysaccharides (LPS) of Gram-negative bacteria, β-1,3-glucans of fungi, and peptidoglycans of Gram-positive bacteria (Chen *et al.*, 2002). Generally, PAMPs are recognized via a set of pattern-recognition receptors (PRRs) that are germline-encoded receptors of the innate immune system (Ariki *et al.*, 2004). In horseshoe crabs, there are some special serine proteases in granular hemocytes, including Prochelicerase C, Prochelicerase B, and Prochelicerase G, which can directly recognize LPS and β-1,3-glucans and activate the innate immune response (Kobayashi *et al.*, 2015; Muta *et al.*, 1995). However, the cell-surface receptors in horseshoe

*Corresponding Author: Mahadev Asaram Jadhav, Department of Biotechnology, Deogiri College, Chhatrapati Sambhajinagar-431005. Email: majadhav22g@gmail.com

crabs have not been identified. In addition, a Toll-like protein named tToll has been identified in horseshoe crab hemocytes and shown to be expressed in multiple tissues (Inamori, 2004). Interestingly, there is no evidence to demonstrate that the tToll protein is involved in pathogens recognition. Obviously, the innate immune mechanism of horseshoe crabs is unique and complex, especially against Gram-negative bacteria containing LPS in the cell wall membrane. In previous studies, many innate immunity-related genes have been reported in Chinese horseshoe crab (Shibata *et al.*, 2018), but the immune response mechanism based on high-throughput analysis of pathogen challenge has been rarely reported.

Antimicrobial peptides or substances are the host defense compounds that have recently drawn attention, due to their properties and diversity. In crustaceans, these have substances are considered to be a main component of innate immunity (Smith and Chisholm, 2001). There are few reports evaluating the bioactivity of crustaceans, and many researchers have studied the antibacterial activity of marine crustaceans and prawns (Tonganunt *et al.*, 2008; Stewart and Zwicker, 1972; Noga *et al*., 1996; Khoo *et al*., 1999; Ravichandran *et al*., 2010). The crabs are the rich sources of bioactive compounds, but the researchers carried out the pharmacological properties of marine crabs (Veeruraj, *et al*., 2008; Anbuchezien and Ravichandran, 2009) but not in freshwater crabs. Hence, the present study was aimed to investigate the antimicrobial potency of haemolymph collected from freshwater crab, *Paratelphusa hydrodromous.*

Defense mechanisms in crustaceans

In invertebrates, the physical barriers are the first obstacle to detain pathogenic micro-organisms (Söderhäll, 1982). When there is damage and the micro-organisms invade the tissue, proteolytic pathways take place instantly, allowing elimination or diminution of microbes invading the organism (Ratcliffe *et al*., 1985). The effector mechanisms for invertebrate immune responses include the coagulation cascade, which avoids the loss of hemolymph and stimulates oxidative metabolites and production of melanin by activating the proPO system (Sritunyalucksana and Sodarhall, 2000; Vargas-Albores and Yepiz-Plascencia, 2000; Kawabata *et al*., 1996). Prophenoloxidase activation stimulates other important processes in the immune response, such as phagocytosis, encapsulation, and nodule formation (Söderhäll *et al*., 1990; Smith and Chisholm, 2001). Activation of such processes seems to be mediated through the specific recognition of glycosylated pathogen-associated molecular patterns (PAMPs) by crustacean proteins. This review focuses on the molecular characterization of immune defense mechanisms in the hemolymph of freshwater crabs, summarizing the roles of various immune components and highlighting recent research advancements.

Hemolymph composition and immune function

The hemolymph of freshwater crabs is analogous to the blood of vertebrates, containing immune cells (hemocytes) and soluble factors that play critical roles in pathogen defense.

Hemocyte types and functions

Hemocytes are categorized into three main types:

1. Granulocytes: Contain granules with antimicrobial substances and are involved in degranulation.
2. Hyalinocytes: Predominantly phagocytic cells that ingest and degrade pathogens.
3. Semigranulocytes: Participate in encapsulation and nodule formation, trapping larger pathogens (Johansson *et al*., 2000; Söderhäll *et al*.1983).

Soluble immune factors

The hemolymph also contains various soluble factors, including antimicrobial peptides (AMPs) and components of the prophenoloxidase (proPO) system, which are crucial for humoral immune responses (Cerenius and Söderhäll, 2004; Amparyup *et al*., 2008).

Pathogen recognition mechanisms

Pathogen recognition in freshwater crabs is mediated by pattern recognition receptors (PRRs) that detect pathogen-associated molecular patterns (PAMPs).

Pattern Recognition Receptors

Key PRRs include:

a. Toll-like Receptors (TLRs): Recognize a broad range of PAMPs and activate signaling pathways that induce immune responses.
b. Lectins: Bind to specific carbohydrate structures on the surfaces of pathogens, facilitating their recognition and clearance (Iwanaga and Lee, 2005; Rozen and Skaletsky, 2000) .

Cellular immune responses

Hemocytes execute various cellular immune responses, including:

1. Phagocytosis: Hyalinocytes engulf and digest pathogens.
2. Degranulation: Granulocytes release antimicrobial compounds from their granules.
3. Encapsulation: Semigranulocytes form capsules around larger pathogens to isolate and neutralize them (Shields, 2003; Cerenius *et al*., 2008).

Humoral immune responses

Humoral responses involve the production of AMPs and activation of the proPO system.

Antimicrobial peptides (AMPs)

AMPs, such as crustins and defensins, are produced in response to pathogen recognition and play a direct role in neutralizing pathogens by disrupting their cell membranes (Lee *et al.*, 2006).

Prophenoloxidase system

The proPO system is activated upon pathogen recognition, leading to melanization, which encapsulates and immobilizes pathogens, preventing their spread (Johansson *et al*., 2000; Cerenius and Söderhäll, 2004).

Signal transduction pathways

Activation of immune responses involves several signal transduction pathways.

Toll pathway

The Toll pathway is a central signaling cascade that regulates the expression of immune-related genes following pathogen recognition by TLRs.

Imd pathway

The Imd pathway mediates responses to Gram-negative bacteria, leading to the production of AMPs.

JAK/STAT pathway

This pathway is involved in antiviral responses and regulates the expression of genes associated with immune defense.

Advances in molecular techniques

Recent advancements in molecular biology have significantly enhanced our understanding of crab immunity.

RNA sequencing and proteomics

These techniques have facilitated the identification and characterization of numerous immune-related genes and proteins, providing a comprehensive understanding of the immune landscape in freshwater crabs.

Gene editing and rna interference

Emerging tools like CRISPR-Cas9 and RNA interference (RNAi) enable functional studies of immune genes, allowing researchers to dissect the roles of specific components in the immune response (Chomczynski and Sacchi, 1987; Livak and Schmittgen, 2001).

Implications for aquaculture

Understanding the immune mechanisms of freshwater crabs has practical implications for aquaculture. Enhancing disease resistance through selective breeding or immunostimulants can improve the sustainability and productivity of crab farming (Shields, 2003).

Future Prospective

Future research should focus on:

1. Investigating the effects of environmental stressors on immune competence.
2. Exploring the potential of immunomodulators in enhancing disease resistance.
3. Conducting comparative studies across different crab species to understand the evolution of immune strategies.

CONCLUSION

This review highlights the complexity and sophistication of the immune defense mechanisms in the hemolymph of freshwater crabs. Continued research in this field will not only advance our understanding of invertebrate immunity but also support the development of more resilient aquaculture practices as well as This comprehensive review provides an overview of the molecular immune mechanisms in freshwater crabs, with a focus on hemocyte functions, pathogen recognition, and the role of humoral responses. It also highlights recent advancements in molecular techniques that have enabled these discoveries and discusses their implications for aquaculture.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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