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Review Article

Innate Immune Source and Functional Machinery in Decapods of Crustacea

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ABSTRACT
Invertebrate immune response mainly based on innate immune effector cells or hemocytes. Such immunocytes show diverse functional activities including aggregation, adhesion, coagulation, phagocytosis, degranulation, generation of cytotoxic agents to protect the invertebrates including decapods from pathogens and toxins of unknown chemicals within biounsafe environment. As a result in hostile environment a continuous source of fresh hemocyte production is necessary to overcome such effects. Hematopoietic tissue is the organ of production of new hemocytes which was reported by various scientists in different parts of decapods (like crabs, prawns, shrimps, crayfish etc). Present paper investigates and reviews the localization, structural and functional attribute of hematopoietic tissue and its cells in different decapods with respect to immunological consequence. Present study is aimed to provide a baseline information concerning the homeostasis of blood cell of order Decapoda under subphylum Crustacea.
Key Words: Decapoda, hematopoiesis, hematopoietic tissue, immune responses.

INTRODUCTION

Invertebrate immunity

The defence or immune system is commonly divided into two major categories named innate or nonspecific and acquired or specific. The innate immune system classified into humoral and cellular defence responses. This innate immunity is more ancient from of defence mechanisms than acquired immunity (Jiravanichpaisal *et al.*, 2006). The innate or nonspecific immune response depends mainly on recognition and killing of invading xenobiotics. However innate immunity is crutial for the first line of defence which include blood cells. Most of the invertebrates possess white blood cells or immunocytes that probably evolved from free living protozoa like ancestor (Roit *et al.*, 1996). Invertebrate host defense mechanisms do not show an advance degree of specificity of innate immune system (Galloway and Depledge, 2001).

Innate immunity consists of physico-chemical barrier to potential microorganisms are the cuticle and mucous layer. Rigid and wax covered cuticle serving as a mechanical barrier and they can also rapidly produce during infection (Lee, 2001). The materials in the crustacean cuticle are chitin which is the most abundant skeletal material in invertebrates and it is chemically similar to cellulose. It is a polysaccharide that is synthesized by the animal, and it can be degraded by extracellular enzymes chitinases released by bacteria and fungi. Among the 3 forms of chitin, crustacean cuticle is α–chitin which is most stable due to large number of hydrogen bonds present and the cuticle consists of 4-layers (Factor, 1995): the external layer epicuticle, exocuticle, endocuticle-I, last layer endocuticle-II. When the cuticle is damaged due to injury or infection, the wound is rapidly clotted and this prevents loss of hemolymph. Once a clot is formed, the area is melanized and wound appears dark black. Melanin in addition to sealing the wound and epidermis will form a new cuticle beneath the melanized layer (Wilt *et al.*, 2003).

Apart from these, other important innate defence is hemolymph and hemocytes (immunocytes) which are activated upon discrete immunological challenges (Holmblad and Soderhall, 1999). Hemocytes are the circulating immunoeffector blood cells of the invertebrates, which perform diverse immunological activities including aggregation, coagulation, adhesion, phagocytosis, generation of cytotoxic molecules, antioxidant enzymes and wound repairment (Saha *et al.*, 2008a; 2008b; 2009a; 2009b; 2010a; 2010b and 2010c; Ray *et al.*, 2011; Ray and Saha, 2011) (Figs. 2-4). In general function, cytochemistry and ultrastructure of hemocytes suggest there are three major type blood cells present in the invertebrate: hyalinocyte, semigranulocyte and granulocyte (Saha and Ray, 2006; Saha *et al.*, 2009a) (Table 1 and Fig. 1). Hyalinocytes are small, spherical and no or few granules and capable of phagocytosis (Smith and Soderhall, 1983). Semigranulocytes are generally oval which contain small granules and capable of encapsulation, phagocytosis and cytotoxic response (Saha and Ray, 2006; Jiravanichpaisal *et al.*, 2006; Ray and Saha, 2011). Granulocytes are round in shape and contain huge no of large eosinophilic granules that responsible for repairment of wound and cytotoxicity (Hose *et al*., 1990; Johansson *et al.*, 2000).

Hemocyte aggregation, adhesion and coagulation

Hemocyte aggregation is considered as an important cellular reaction involved in recognition of self and nonself foreign surfaces, clot formation at wound

site and encapsulation reaction. Cell-cell aggregation and adhesion are thought to be important metabolic behaviour of hemocytes (Kenney *et al.*, 1972; Takahashi *et al.*, 1994, 1995; Chen *et al.*, 1996) (Figure 2). In decapods, coagulation or clotting occurs through the process of polymerization of different coagulating protein in hemolymph and catalyzed by a calcium dependent transglutaminase that starts cross-linking plasma derived clotting protein and forms gel to prevent blood loss (Jiravanichpaisal *et al.*, 2006).

Surface adhesion of hemocytes to nonself particulates is regarded as an important immune response of metabolic significance (Chen and Bayne, 1995). Hemocytes are relatively adhesive and non-motile cellular components of blood which express adhesion behaviour and motility under specific immunological and toxicological conditions. During elicitation of cell mediated immune response, hemocytes exhibit cytoplasmic spreading and physically interact with foreign surface. Cellular attachment, cytoplasmic spreading and migration of hemocytes are the sequences of cellular adhesion (Armstrong, 1980). During the process of wound repairment, hemocytes undergo cell-cell attachment followed by adherence to wound site as reported in decapods (Johansson and Soderhall, 1988). Decapods immune system recognizes a wide variety of pathogens, represented by fixed common molecular patterns known as pathogen associated molecular patterns (PAMPs) presented on pathogenic microbes (Armstrong, 1980). Since these molecules are absent on host cells, they can serve as discriminators between self and non-self (Iwanaga and Lee, 2005). Phagocytosis of non-self particulates is a classical innate immune response in decapods by which cells engulf relatively smaller particulates (Saha *et al.*, 2008; Vijayavel *et al.*, 2009). This is an essential mechanism of host defense against infections caused by microorganisms at the wound site (Ratcliffe, 1985).

Hemocyte phagocytosis and cytotoxic agents

Phagocytosis is characterized and initiated by the recognition of target particle by a phagocyte followed by binding, ingestion, killing and clearance through hemocytes (Takahashi and Mori, 2000) (Figure 2 and 4). All phagocytic cells contain cytoplasmic organelles like lysosome which actively participates in intracellular digestion of material. The process of phagocytosis normally mobilizes lysosomes to move toward and fuse with the target. This fusion may begin before the particle is fully internalized, resulting in the leakage of lysosomal contents into the fluid medium and digestion of particles (Bayne, 1990). This process is characterized and initiated by recognition, followed by binding, internalization and destruction of target particle (Tyson, 1995). Recognition of foreign particles or damaged self-tissue by phagocytes may be achieved directly by means of membrane recognition molecules or may be an indirect process dependent on soluble factors that bind the foreign or damaged surface (Johansson and Soderhall, 1992). Phagocytic cells are highly conserved throughout evolution and by using phagocytic activity tests; it is possible to estimate the immuno-impairment effect of certain pathogens on the host immune system. Both the agranulocytes and granulocytes have a role in phagocytic response: granulocytes have distinct high phagocytic ability against foreign particles and on the other hand, agranulocytes exhibit a lower level of phagocytic response than granulocytes (Paterson *et al.*, 1976). Hemocytes are considered as immuno-active cells capable of eliciting effective immune response against toxins and foreign invaders (Figure 4). Decapods are distributed in polluted environment and depend on hemocytes which are potentially efficient in eliciting defense response. Once the pathogens are engulfed or the host is exposed to xenobiotics, phagocytes are subjected to cytotoxic response generated in the intracellular environment. Hemocytes are capable of generating various types of cytotoxic agents under the exposure of environmental toxins and parasites. These cytotoxic agents include superoxide anion, nitric oxide, and phenoloxidase (Anderson *et al.*, 1992; Nappi and Ottaviani, 2000; Nappi and Christensen, 2005; Saha *et al.*, 2008; Vijayavel *et al.*, 2009; Saha *et al.*, 2010b) (Figure 4). A low level of activity of cytotoxic agents in the normal hemocytes is indicative to a persistence of innate defense in crab distributed in its natural habitat. To eliminate the phagocytosed particulates, blood cells release cytotoxic chemical compounds in adverse physico-chemical environmental conditions (Anderson *et al.*, 1992). Hemocytotoxicity is often characterized by estimating the respiratory burst activity of hemocytes. Superoxide anions are the reactive oxygen intermediates generated during cytotoxic elicitation. Superoxide anions undergo reaction with hydrogen to produce hydrogen peroxide, hydroxyl radicals and finally water (Rodriguez and Moullac, 2000). Strategy of oxygen radical mediated killing is based on the premises of toxicity evolved due to high concentrations of molecular oxygen. Superoxide anions are extremely toxic, powerful and hyperactive killing agents which are capable of creating damage of cells and tissues. Generation of intrahemocyte superoxide anion is due to consumption of oxygen following the contact of phagocyte and micro-organisms involving membrane bound NADPH-oxidase complex (Munoz *et al.*, 2000).

Superoxide anion diffuses through mitochondrial membrane in a concentration dependent manner and its steady-state concentration is highly reduced by the action of superoxide dismutase (Lesser, 2006). Superoxide dismutase is considered as a potential scavenger of superoxide anions which is generated inside the cell as adaptive response against cellular damage. In decapods, cell types including hyalinocytes are capable of generating superoxide anions (Anderson *et al.*, 1992; Munoz *et al.*, 2000) (Figure 2).Nitric oxide is a major cytotoxic agent that elicits host defense reaction under the exposure of xenobiotics and parasites by its direct effect or intercellular signaling.

Nitric oxide acts as a mediator of killing of intracellular parasite. The mechanism involved synthesis of NO, NO_2^- , NO_3^- from arginine (Lesser, 2006). Interaction with nitric oxide causes iron loss from critical target enzymes resulting in metabolic failure of intracellular parasites. Nitric oxide can readily diffuse across biological membrane and reacts with free radicals, especially superoxide anion to form reactive peroxynitrite anions ($ONOO^-$). Peroxynitrite anion also expresses cytotoxic activity (Lesser, 2006). Nitric oxide is generated in hemocyte as cytotoxic agent for killing the invading pathogens by formation of peroxynitrite (Radomski *et al.*, 1991; Ottaviani *et al.*, 1993; Arumugan *et al.*, 2000).

Importance of phenoloxidase in decapods, as a defense enzyme has increased considerably since its localization was reported in hemocytes and hemolymph (Saha *et al.*, 2010b). The enzyme has been reported in various animal tissues and cell types including circulating blood cells, neurons and melanocytes. Phenoloxidase is a copper containing protein that is activated by limited proteolysis and it catalyzes both the O-hydroxylation of monophenols to diphenols and oxidizes diphenols to quinines, which can polymerize non-enzymatically to insoluble melanin (Sritunyalucksana and Soderhall, 2000). The enzyme is involved in the defense mechanism of many invertebrates by catalyzing the oxidation involved in sclerotisation and wound healing and participate in the process of encapsulation of foreign bodies and melanization (Nappi and Christensen, 2005; Tanner *et al.*, 2006). The enzyme usually exists in the cell as prophenoloxidase - an inactive precursor. It has been demonstrated that bacterial cell wall can activate prophenoloxidase in invertebrates (Soderhall and Cerenius, 1998). The reaction process is very specific and sensitive, involves the activation of enzyme and initiates a cascade of serine protease activation. Serine protease in turn, proteolytically cleaves prophenoloxidase and thus activates the precursor to an active phenoloxidase (Cerenius and Soderhall, 2004). Activation of prophenoloxidase by serine protease is similar to that of alternative pathway of complement activation in mammals (Soderhall, 1982; Cerenius and Soderhall, 2004). Physicochemical factors like pH and calcium ion concentration activate the prophenoloxidase cascade which in turn, initiate and augment other classical defense reactions namely; phagocytosis and exocytosis (Aspan *et al.*, 1990). Induction of phenoloxidase is established in crustaceans and was estimated in granular hemocytes by reacting with its substrate dihydroxyphenylalanine (L-DOPA) (Soderhall and Smith, 1983; Sung *et al.*, 1998; Sahoo *et al.*, 2005; Tanner *et al.*, 2006). Internal defense of crustaceans is dependent on circulating hemocytes capable of phagocyting pathogenic organisms. In performing the intracellular killing of ingested micro-organism, hemocytes are capable of generating reactive oxygen intermediates. In order to protect the self tissue from reactive oxygen metabolite produced during respiratory burst, various potentially active antioxidant agents are generated in the crustaceans (Reddy *et al.*, 1996; Reddy, 1997; Vijayavel *et al.*, 2005) as adaptive response.

Hemocytic enzymes and adaptive responses

Enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) are localized in the hemocytes as evident from biochemical and immunocytochemical analyses (Krishnan *et al.*, 2002; Saha *et al.*, 2007) (Figure 2 and 4). Antioxidation has been suggested as an useful tool in assessing the risk of oxidative damage and physiological stress due to the dynamic imbalance between the antioxidant defense and pro-oxidation conditions in animals exposed to xenobiotics (Gowlan *et al.*, 2002; Vijayavel *et al.*, 2004, 2005). Superoxide anions are scavenged by intrahemocytic superoxide dismutase (SOD) to form hydrogen peroxide (Holmblad and Soderhall, 1999; Lesser, 2006). SOD is an efficient catalyst that maintains the steady-state concentration of superoxide anion. In general, SOD assay systems involve two components: one is superoxide radical generating system and the second one is superoxide radical detecting system (Krishnan *et al.*, 2002). Heme-containing catalase enzyme localizes in peroxisomes that catalyses the conversion of hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2) (Holmblad and Soderhall, 1999). Catalase is the most efficient in scavenging high concentration of hydrogen peroxide and osmotic or thermal stress reduce the catalase activity (Lesser, 2006). Catalase activity was reported in the digestive gland and other tissues of invertebrates (Prakash and Rao, 1995). Glutathione S-transferase is an important

enzyme involved in Phase II reaction in drug metabolism in different species. Apart from drug detoxification, GST plays an important role in acquisition of drug resistance in different diseases (Pinho *et al.*, 2003; Funes *et al.*, 2006). Intracellular glutathione-S-transferase conjugates the free metals in glutathione (GSH) pool which is a protective mechanism against metals (Pinho *et al.*, 2003). GST is primarily involved in chemical disposition of toxic substances and catalyzes the conjugation of GSH to various electrophiles and inactive toxic compound by non-catalytic binding (Prakash and Rao, 1995; Gowland *et al.*, 2002).

Kinetics of wound repairment

The process of wound healing in invertebrates is a natural phenomenon that aid in stopping the fatal loss of hemolymph from body, maintenance of tissue architecture and minimization of opportunistic invasion of pathogens (Theopold *et al.*, 2004) (Figure 3). Wounds of decapods are caused by tissue injury (accidental and natural), parasitic invasion, food procurement, competition etc. Wounds are rapidly sealed by extrusion of fat body, muscular contraction, hemocyte aggregation, coagulation and deposition of melanin through activation of prophenoloxidase activating system (Soderhall and Cerenius, 1998; Holmblad and Soderhall, 1999). Chitinous exoskeleton or cuticle serves as the first line of defense in crab. This external physical barrier protects the animal from damage and infectious external agents. To prevent the loss of hemolymph and closure of wound, following physiological events are observed (Fontaine, 1975; Sritunyalucksana and Soderhall, 2000; Tanner *et al.*, 2006; Wood *et al.*, 2006) in invertebrates.

Hemocytes migration and aggregation

Hemocyte aggregation occurs at the site of accidental wounding for the purpose of bio-sealing. Situation leads to arrestation of loss of blood and subsequent recovery.

Lyses and degranulation of hemocytes: At the site of injury activated granular hemocytes get lysed and discharge their contents through degranulation. These cells get flattened after degranulation, stuck along the margins of the wound area and form a lamina. This result release of healing contents and activation of proclotting enzymes into active clotting enzymes and other factors and initiate coagulation.

Activation of phenoloxidase and melanin formation: The prophenoloxidase (proPO) activating system comprises of activation of enzyme cascade leading to activation of proPO and other compounds with related activities. The intrahemocytic proPO undergo zymosan mediated cleavage to form phenoloxidase. PO is a copper containing protein which is activated by limited proteolysis and it catalyzes both the O-hydroxylation of monophenols to diphenols and oxidizes diphenols to quinines, which can polymerize non-enzymatically to insoluble melanin. Melanin deposits around the damaged tissue and adhere to the surface of pathogens. Melanin bears the sealing ability of wound. Therefore, a constant supply of hemocytes is needed for protection of host in biounsafe environment as immunological machinery. The process of continuous fresh hemocyte production, maturation and release is regulated by hematopoiesis. This process involving the proliferation, differentiation and distribution from specialized hematopoietic tissues (HPT) (Chaga *et al.*, 1995; Van de Braak *et al.*, 2002; Johansson *et al.*, 2000; Zhang *et al.*, 2006).

Functional attribute of hemocyte and relation to hematopoiesis

Invertebrates including decapods lack of adaptive immune or non-lymphoid system and depend upon mainly on innate immune defenses. The innate immune system is phylogenetically more ancient defense mechanism than that of adaptive or lymphoid immunity (Figure 7). Decapods host defense mechanisms show a low degree of specificity and lack of adaptive component based on immunological memory, but they have complex and efficient host defense systems that can identify and eliminate potential pathogens very efficiently. Due to continuous fighting against aquatic environment and potential enemies within habitat of crustaceans become wounded. So rapid sealing of wound to the exoskeleton is required to prevent loss of hemolymph and minimize opportunistic invasion of pathogens through hemocytes. Hemocytes, the chief immunologically active cells of crustacean immunity, perform diverse physiological functions like aggregation, adhesion, phagocytosis, generation of cytotoxic agents and antioxidant enzymes in biounsafe aquatic environment (Saha *et al.*, 2008a; 2008b; 2009a; 2009b; 2010a and 2010b) (Figure 2 and 4). So functional attributes of hemocyte against infection or damage tissue directly related to the production and supply specific type of hematopoietic tissue cells (Van de Braak *et al.*, 2002). Therefore hemocyte deficiency is a serious threat to the animal health and rapid recovery of hemocytes is essential in order to survive an animal is stress condition (i.e. infection, toxic exposure). Hematopoietic tissue generally present in dorsal sides of stomach as oval or round lobular spot. Activation of Hematopoietic tissue cells release differential mature immunocytes or hemocytes according to necessity. Finally the hematopoietic process is a most important

for the animal to combat an infection in its natural habitat.

Hematopoiesis and hematopoietic organ

Hematopoiesis is the process of formation and development of blood cells from self renewal hematopoietic stem cell (Figure 6 and 7). Parentally hematopoiesis occurs in the yolk sac. Main characteristics of hematopoietic stem cell (HSC): Proliferation and differentiation; Regulation; Capable of self-renewal; Stable generation throughout adult life; Proliferation capacity increased by stimulation (xenobiotics). In general blood cells develop from pluripotential stem cells which differentiate into pluripotential cells that are committed to three, two or one hematopoietic differentiation pathway. The formation and development of mature blood cells involve proliferation, commitment and differentiation from undifferentiated hematopoietic cells (Figure 7). Hemocytes are constantly produced although the rate by which this process occurs can be altered under biounsafe hostile environment (Saha *et al.*, 2007; 2009a; 2009b and 2010c; Ray and Saha 2011).

The digestive tract of Decapoda is complex and acts as hematopoietic organ (Martin *et al.*, 1993; Johansson *et al.*, 2000; Van de Braak *et al.*, 2002). In adult decapods digestive tube is divided into 3 main parts - fore, mid and hindguts (Ceccaldi, 1989). Foregut is composed of a short esophagus, a stomach with two bags and internal wall with appendages specialized for grinding of foods. The midgut contains hepatopancreas (Figure 5). Digestive or midgut gland or hepatopancreas composed of different specialized cells that play crucial role in absorption and digestion. Hindgut is composed of rectum and anus. Foregut wall made of an epithelium covered by thick cuticle that is shed and replaced during molting. The cuticle is penetrated by ducts that run from the epithelium into lumen. These ducts connect with tegumental glands of the esophageal wall. In decapods the sheet like hematopoietic tissue is located on and covers the dorsal and dorsolateral sides of stomach chamber and surrounded by fibrous connective tissue cells layer, recognized to be hematopoietic cells of various morphology are densely packed in round or elongated small lobules (Johansson *et al.*, 2000). White round or elongated small spot areas are freely distributed as hematopoietic tissue lobules in shrimp or prawn (Van de Braak *et al.*, 2002).

Localization of hematopoietic tissues and cells

Hematopoiesis provided a mechanism by which hemocytes that are damaged or expired can be replaced by newly synthesized cells (Hose *et al.*, 1992; Jiravanichpaisal *et al.*, 2006). New hemocytes are required to be continuously arises from a separate organ called as hematopoietic tissue (Hpt) (Figure 7). Generally hematopoietic cells differentiate into other cell types and they are self-renewing - maintain their cell population level by cell division continuously. But the rate of hemocyte production can be altered rapidly under the influence of different biological conditions (repeated sampling, invading pathogens etc.) and environmental factors (xenobiotics, temperature, salinity etc.) (Lightner *et al.*, 1983; Jussila *et al.*, 1997). Such blood cells participate in cellular immune reactions such as phagocytosis, encapsulation, generation of cytotoxic molecules and antioxidant enzymes against invading pathogens. In most crustaceans the sheet like hematopoietic tissue is located on and the dorsal and dorsolateral sides of stomach or foregut (Johnson, 1980). Morphological analyses of hematopoietic tissue already recorded in marine crab by Ghiretti-Magaldi *et al.* (1977) and Badammer (1978); in shrimp Hose (1992); in lobster by Martin *et al.* (1993); in crayfish by Chaga *et al.* (1995); in black tiger shrimp by Van de Braak *et al.* (2002). Ghiretti-Magaldi *et al.* (1977) reported that in marine crab all hemocytes derive from a single cell line in the Hpt and released from Hpt into hemolymph as granular and hyaline form. Bazin (1979) studied cytological analyses of hematopoietic organ in the marine crab *Carcinus meanas*. Martin *et al.* (1993) reported that hepatopoitic tissue (Hpt) of American lobster is composed of loosely attached ovoid lobules containing the hematopoietic precursors and maturing hemocytes that release into the dorsal hemocel by rupturing connective tissue capsule. Hematopoietic tissue is situated on dorsolateral surface of the stomach in crayfish and a series of ovoid lobules on the dorsal surface of fore gut in crab that are surrounded by a thin sheath of spongy connective tissue and hematopoietic tissue cells of different morphology are organized and density packed in small lobules. In crayfish hematopoietic tissue five different morphological cell types are also found in the interlobular spaces under light and electron microscopy which correspond to different developmental stages of granulocytes and semigranulocytes (Chaga *et al.*, 1995). At the apical part of the lobules, type 1 cells are located and appearance of non-differentiated cells with semi lunar shape. Mitosis is observed in type 1 cells, have no or few granules, are closely attached to other cells or to the extracellular matrix (ECM) and are difficult to liberate from the tissue. Type 2-4 cells are more distally located. They have granules and are considered to be precursors of granular cells. These represent different stages in development of granule-containing hemocytes

with different cell shapes (multiangular, elongated and round). Type 5 cells have granules morphologically distinct (round shape) from the other types and were speculated to be precursors of semi granular cells. Van de Braak *et al.* (2002) examined that the hematopoietic tissue (Hpt) of black tiger shrimp is located in different areas in the cephalothorax, mainly at the dorsal side of the stomach and in the onset of the maxillipeds and to a lesser extent, towards in the antennal gland. They also identified four types of hematopoietic cells in Hpt by electron microscopy, viz. type 1, 2, 3 and 4. From Hpt lobules hemocytes can be released into the hemolymph of hemal sinus of the animals (Fig. 2). Soderhall *et al.* (2003) examined and recorded the proliferating mechanism of hematopoietic tissues in crayfish by injecting of β1,3-glucan and also detected that peroxinectin adhesive protein were present in the hematopoietic cells where as mRNA and for proPO was not detected. Zhang *et al.* (2005 and 2006) characterized three types of Hpt cells under TEM in Chinese prawn i.e. type 1 (high N/C ratio and no granules), type 2a and 2b (smaller size, cytoplasmic granules). Astakine, an endogenous cytokine like-factor containing a prokineticin domain from crayfish *P. leniusculus* (Soderhall *et al.*, 2005), has been shown to play a critical role in the differentiation and growth of hematopoietic stem cells *in vitro*. The proliferating cell nuclear antigen (PCNA), a unique marker for Hpt cells, was detected by searching the expressed sequence tags (EST)-library of Hpt cells. The newly described hemocyte lineage marker protein genes from crayfish *P. leniusculus* provide new clues for the differentiation of different stages of the crayfish blood cells. Present study suggestive a base line information about hematopoietic tissue and basic immunological response in Crustacea.

Immunological responses of hematopoietic tissue cells (HTCs)

In animals hematopoietic cellular immune response consists of the lymphoid and myeloid lineages. The lymphoid lineage is the main component of acquired immune response. In decapods there is no lymphoid lineage and cellular immune response consists of circulating immunocytes or hemocytes. Hemocytes play a key role in immune surveillance and are active against pathogen and environmental xenobiotics (Saha *et al.*, 2010a; 2010b and 2010c). When an invading particulate (living or nonliving) is recognized as nonself or foreign part, circulating hemocytes should release particulate through the process of phagocytosis or encapsulation (Saha *et al.*, 2008a) and generation of cytotoxic molecules (Saha *et al.*, 2008b, 2010b). Therefore a continuous supply of mature new hemocyte needs to be

Table 1: Comparative study of crustacean THC and hemocyte subpopulations

	Species	THC (cells/ml) Mean (± SD)	Hemocyte subpopulation	Reference
1.	*Carcinus granulatus* (shore crab)	$31.0x10^6$ ($18.1x10^6$)	Hyalinocyte, SG, G	Yeager, Tauber (1935)
2.	*Carcinus maenas* (shore crab)	$6.0x10^6$ ($5.4x10^6$)	Hyalinocyte, SG, G	Söderhäll, Smith (1983)
3.	*Ptamon fluviatilis* (fresh crab)	$1.11x10^6$ ($1.05x10^6$)	Hyalinocyte, SG, G	Yildiz, Atar (2002)
4	*Scylla serrata* (edible mud crab)	4.7×10^6 (3.4×10^6)	Hyalinocyte, SG, G	Saha and Ray (2006); Saha *et al.* (2007); Ray and Saha (2011)

produced and released from hematopoietic tissue (stem cell) against infection or damaged part of the animal for sealing and healing processes. Saha *et al.* (2009a) reported arsenic induced hyperproduction of hemocyte of *S. serrata*, which suggested a possible induction of hemocytopoietic organ of crab to release increased number of hemocytes in blood stream (Victor, 1993). Due to continuous struggle against environmental adversities and potential enemies, mudcrabs get physically wounded in their natural habitat frequently. Animals physiologically respond to external injury and rapid sealing of wound at the exoskeletal site is required to prevent the loss of hemolymph and minimization of opportunistic invasion of pathogens (Chen and Bayne, 1995; Tanner *et al.*, 2006). The wound is repaired by hemocytic function (Ottaviani, 2005, 2006). Tissue repairment involves coagulation of plasma associated with flattened blood cells which get adhered on the damage area to form a membrane like structure entrapping the surrounding cells (Lunetta, 2005). Migration and aggregation of semigranulocytes and granulocytes at the wound site exhibit degranulation and subsequent flattening which supported the observation of Theopold *et al.* (2004). Plasma gelation involves the activity of enzyme prophenoloxidase which upon conversion, transforms into phenoloxidase and activates the cascade of serine proteases and produces the reactive intermediates for killing the invading pathogens (Theopold *et al.*, 2004). Excess hemocyte production, aggregation and degranulation are reported to be the major mechanisms of clot formation at the site of injury arresting the loss of blood through the activation of hematopoietic tissue (Holmblad and Soderhall, 1999). The hematopoietic tissue expresses the cell adhesion protein (peroxinectin) which acts as opsonin and encapsulation promoting factor in crustacean immune defense (Soderhall *et al.*, 2003).

CONCLUSION

Decapods like crab, prawn, shrimp, lobster and crayfish are economically important species due to its palatability, nutritive value, large size, high unit price and great demand in the local and international markets (Saha and Ray, 2006; Saha 2010d; Ray and Saha, 2011). These species are mostly harvested indiscriminately from its natural habitat and selected areas of the estuarine for human consumption. The morphological characterization and classification of different types of hemocytes in crustaceans was achieved through phase contrast microscopy, light microscopy, scanning electron microscopy and transmission electron microscopy (Saha 2010d). The common criterion considered was the presence or absence of refractile granules of hemocytes (Soderhall and Smith, 1983; Hose *et al.*, 1990; Saha and Ray, 2006) as well as their size. For Decapoda blood cells, most scientists follow the classification of Bauchau (1981), who classified hemocytes as three main categories i.e. hyalinocyte, semigranulocyte and granulocyte. Discrimination of self and nonself surface adhesion, Phagocytosis, degranulation of chemical agents by hemocytes is regarded as classical immunological response both in decapods (Figure 4). Hemocyte deficiency during infection in crustacean refers a serious threat to the health status and a rapid supply of fresh hemocytes is essential in order to destroy invasive micro-organisms instantly and combat infection of invading microbes (Sequeira *et al.*, 1996) (Figure 7). So the hematopoietic process plays a crucial role in innate immunity (Soderhall *et al.*, 2003). It is commonly believed that crustacean hemocytes originate from a specialized haematopoietic tissue (Zhang *et al.*, 2006). The hematopoietic tissue has been identified in several decapod species including the crab *Carcinus maenas*, lobster *Homarus americanus*, crayfish *Pacifastacus leniusculus*, penaeid shrimp *Penaeus stylirostris* and *P. monodon* by various scientists using the phase contrast, light, scanning electron and transmission electron microscope (Zhang *et al.*, 2006). The haematopoietic tissue cells were classified morphologically into different types resembling those identified in circulating hemocytes (Zhang *et al.*, 2003). By combining morphological, cytochemical and functional features of circulating hemocytes, two classifications of hemocyte lineages were proposed in some crustaceans: hyaline and granular lineages in lobster *H. americanus* or large and small granular lineages in crayfish *P. leniusculus* and penaeid shrimp *P. monodon* (Zhang *et al.*, 2003). However, a progenitor stem cell that may give rise to hemocytes is still not clearly identified in Decapoda. In adult decapods the hematopoietic tissue cells are organized within many haematopoietic lobules and distinctly classified into two morphologically different types. Type 1 cells, with high N/C ratios, developed dispersed chromatins and absence of cytoplasmic granules, are considered as the undifferentiated precursor cells. The ultrastructural features of type 2 cells, such as smaller size, nuclei, developed condensed chromatin, appearance of cytoplasmic granules, more RER and mitochondria indicate that these cells are more mature than type I cells (Zhang *et al.*, 2006). Van de Braak *et al.* (2000) proposed four types of hematopoietic cells in prawn hematopoietic tissue (type 1 - 4). These four types generate two types of young

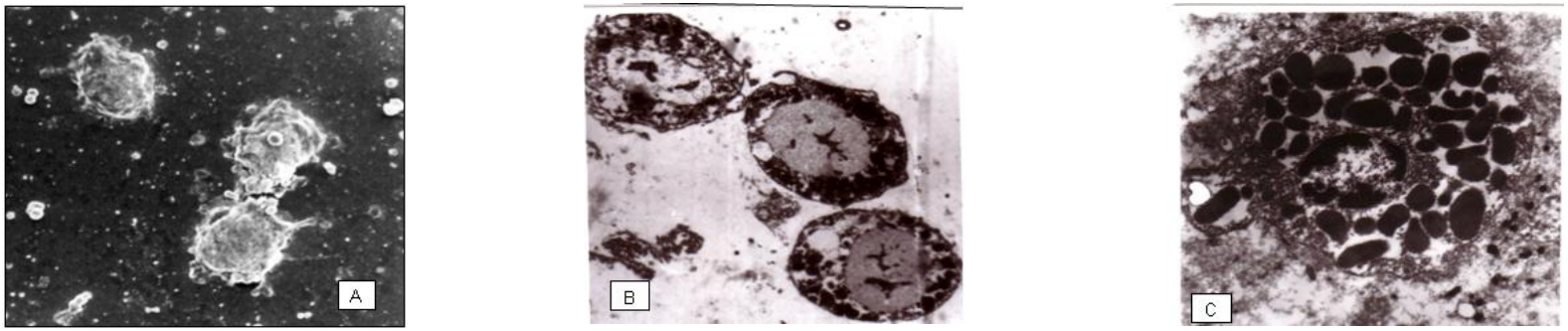

Figure 1: SEM and TEM images of hemocyte of Crustacea (mudcrab, *Scylla serrata*) after Ray and Saha (2011). A: Scanning electron microscopy exhibits cytoplasmic involutions in hemocytes and degree of cytoplasmic involutions and extensions are uniform. **B & C:** Transmission electron microscopy exhibit hemocytes are round or oval in shape with large nucleus and the cytoplasm bears rounded granules.

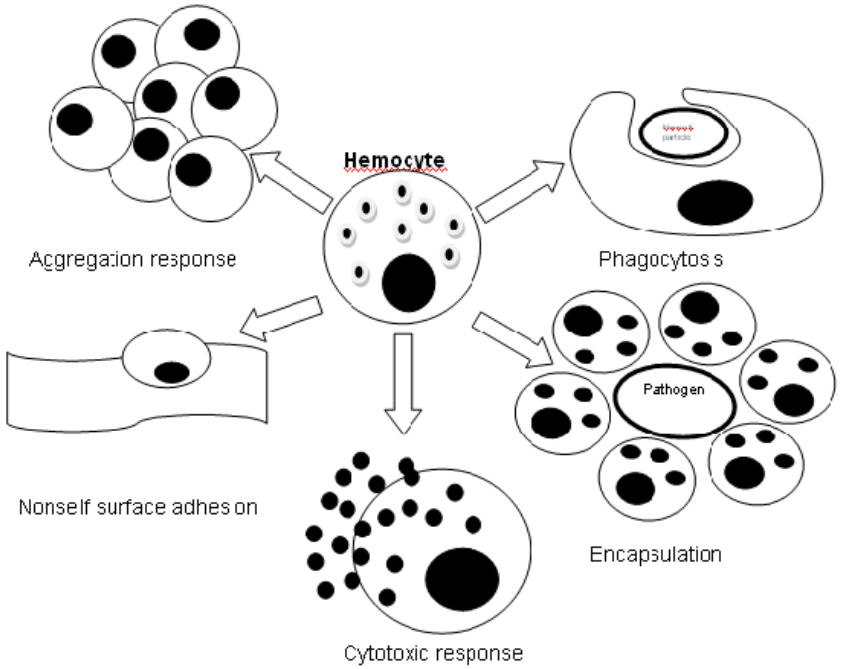

Figure 2: Functional attribute of hemocyte after Ray and Saha (2011).

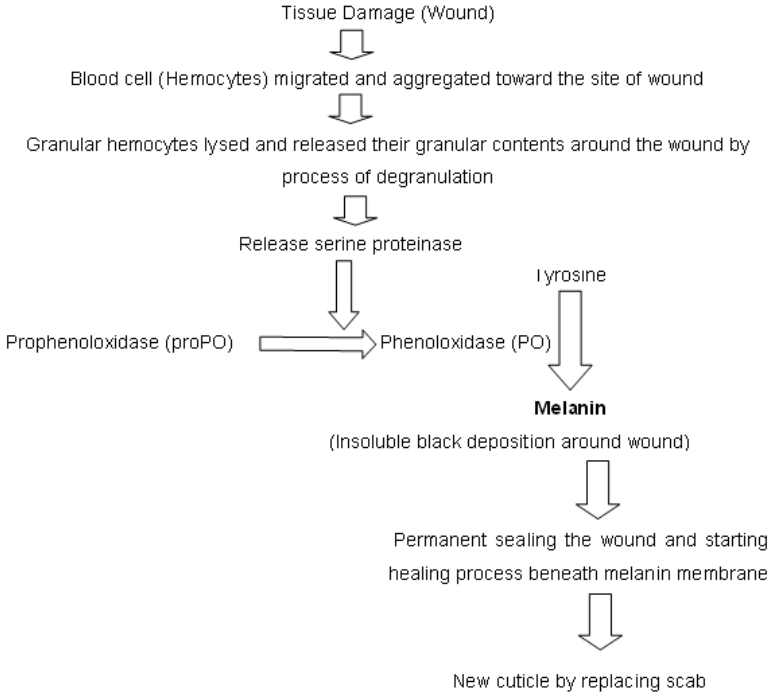

Figure 3: ProPO activating system and Process of wound healing in Decapoda after Ray and Saha (2011).

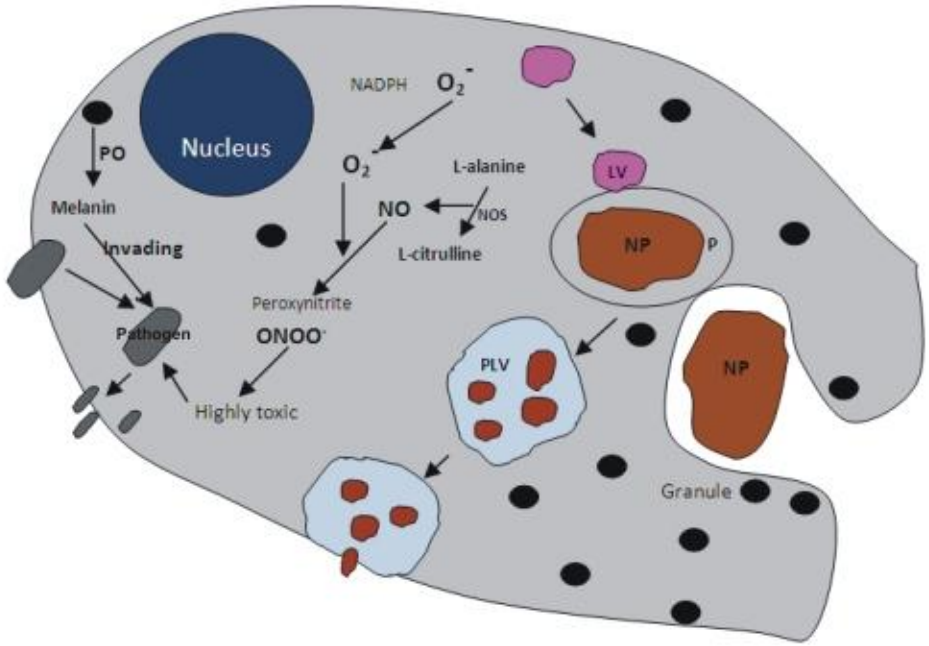

Figure 4: Functional attributes of the hemocytes of Crustacea involving phagocytosis and generation of cytotoxic agents - nitric oxide, superoxide anion and melanin. Phagocytosis is associated with endocytosis of nonself particle (NP), fusion of the phagosome (P) with lysosomal vesicle (LV), destruction and degradation of the nonself particulate within the phagolysosome vesicle (PLV), exocytosis of particulate residues. Cytoxic molecule nitric oxide (NO) is generated during conversion of L-arginine to L-citrulline by nitric oxide synthase (NOS); nitric oxide reacts with superoxide anion (O_2^-) generated by mitochondrial NADPH oxidase to form highly toxic molecule of peroxynitrite ($ONOO^-$). Melanin is synthesized with the help of phenoloxidase (PO) from proPO system of granules present within the hemocyte. Peroxynitrite and melanin destroy the invading pathogen through oxidation and released residues outside the immunocyte or hemocyte.

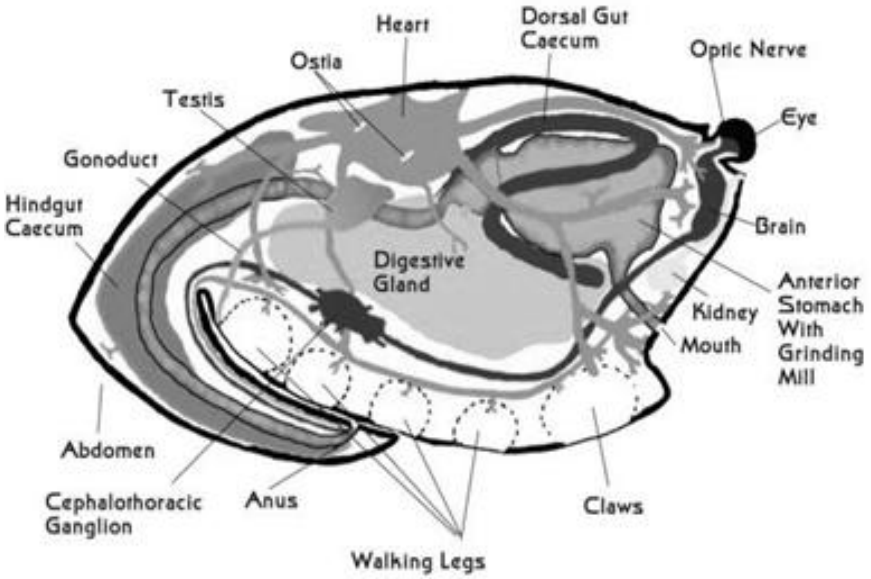

Figure 5: Diagrammatic view of internal body parts of crab (*Source*: http://reefkeeping.com/issues/2003-12/rs/index.php)

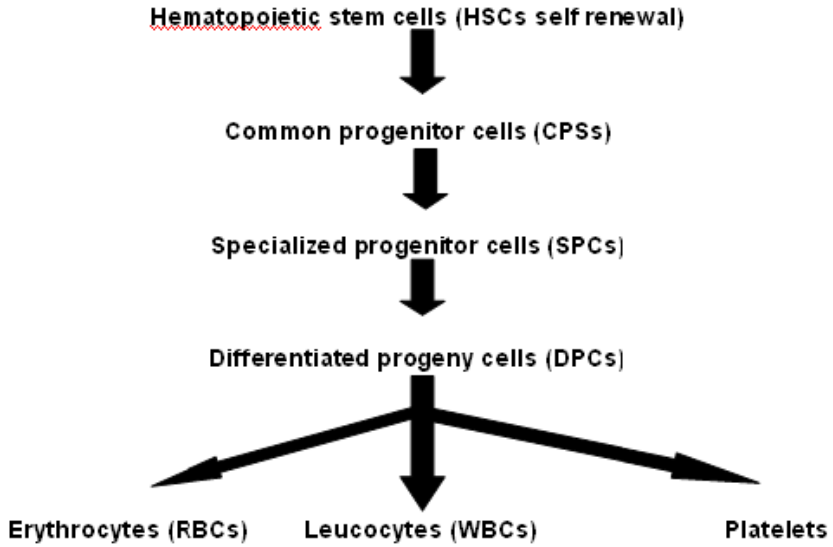

Figure 6: Common pathway of hematopoiesis in animal.

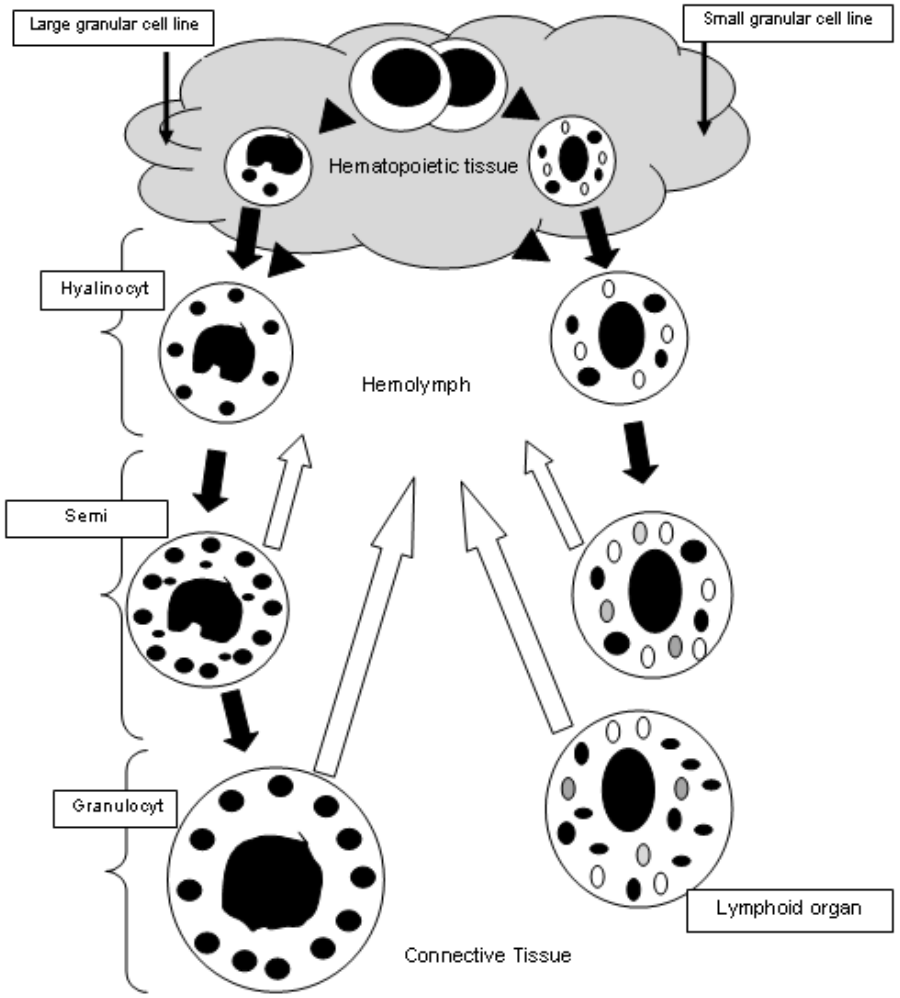

Figure 7: Proposed hemocyte production and maturation in Decapoda (*after* Van de Braak, 2002); ▲ = proliferation, ⬇ = Maturation, ⇧ = Released when needed

hemocytes: hyalinocyte and granulocyte. Transglutamase is important enzyme for maintaining the hematopoietic tissue cells in an undifferentiated stage inside the hematopoietic tissue and if expression of mRNA of transglutamase is prevented the hematopoietic tissue cells become migrate out of the tissue and it exhibits that transglutaminase enzyme prevent hematopoietic stem cells to release into the hemolymph but the function of proliferation is unchanged (Lin *et al*., 2008). Hematopoietic process is controlled by a large number of factors including hematopoiesis growth factors. Recently scientists purified cytokine like molecule (astakine) which plays a crucial role in differentiation of hematopoietic tissue cells (Soderhall *at al*., 2005). Present review proves that the hematopoiesis mechanism is a valuable tool for assessing innate immune status of Decapoda.

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