

Defence mechanisms in crustaceans

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Aquatic animals are surrounded by microorganisms in aquatic environment that are capable of initiating infection in these animals. Unlike fish that possess both innate and adaptive immunity, the lower aquatic animals such as shrimps that are invertebrates lack true adaptive immune system and they depend upon their innate immune system for the defense mechanism against the invading pathogen. In normal conditions, crustaceans maintain a healthy state by mounting a defense reaction against potential pathogens. Among them, the external cuticle is a first line of defense to provide an effective physical and chemical barrier against the attachment and penetration of pathogens. The digestive tract, which is a main route of invasion of the pathogen, is partly lined with chitin layers and its hostile environment of acids and enzymes is able to inactivate and digest many bacteria and viruses.

Crustacean hemocytes (blood cells) play a central role in their immune reactions and are capable of phagocytosis, encapsulation, nodule formation and mediation of cytotoxicity. When the host defence encounters a pathogen, a series of pathways get activated to protect crustacean against infection by a variety of microorganism. The entry of a pathogen into the hemocoel of the host triggers a complex system of innate defense mechanisms involving cellular and humoral immune components.

Cellular immune components are :-

- Hemocytes
- Phagocytosis
- Encapsulation
- Clotting reaction
- Prophenoloxidase (proPO) system

Humoral immune components include:-

- Lysozymes,
- Anti-microbial peptides and
- Lectins

Cellular immune components

Hemocytes

Hemocytes present in the circulating hemolymph play a crucial role in invertebrate immunity providing protection against invading microorganisms. Three types of circulating hemocytes can be found in the crustaceans, they are hyaline (H) cells, semigranular (SG) cells and granular (G) cells.

Hyaline (H) cells

Hyaline cells are small, spherical and are least in numbers among all hemocytes. The number of hyaline cell population varies between different crustacean species. Hyaline cells are capable of phagocytosis and destruction of invading pathogens.

Semigranular cells

These semigranular cells have variable number of small eosinophilic granules that are mainly responsible for encapsulation and also contain the prophenoloxidase activating systems (proPO).

When pathogens or any foreign particles invade into the body these semigranular cells

activate and degranulate. After degranulation these cells attach and spread on the foreign particles.

Granular cells

These cells are filled with large number of granules in their cytoplasm. These cell do not participate in phagocytosis but involved in cytotoxicity activity, nodule formation, proPO system and sometimes involved in encapsulation process.

Phagocytosis

The process trapping, ingestion, destruction and elimination of pathogen or foreign body from host tissue by the phagocytic cells are called "Phagocytosis". The mechanism of intracellular destruction of microorganisms through phagocytosis in shellfish is similar to other animals.

Phagocytes are of two types

- Fixed phagocytes
- Mobile or circulating phagocytes

Fixed phagocytes

These fixed phagocytes are fixed in certain places in the tissue of the organs. These fixed phagocytes can be observed in the lacunae of gills, pericardial sinuses and base of appendages.

Mobile or circulating phagocytes

These phagocytes can be seen in haemolymph with continuous circulation. These phagocytes localize in the site of infection and destroy the pathogen by the process of phagocytosis.

Cellular immune components

Encapsulation

When the pathogen like parasites which are too big to engulf by phagocytes invade the crustaceans, the haemocytes present in haemolymph collaborate and deposit on the surface of the parasite and block off the parasite from circulation. Normally the cells involved in the encapsulation is semigranular cells.

Cytotoxicity

Haemocytes are capable of destroying tumor and non-tumor cells present in the host by secreting cytotoxic molecules. Cells that are mainly responsible for cytotoxicity is Granular and semigranular cells which are similar to mammalian natural killer cells (NK cells). Once these cytotoxic cells recognize the foreign particle or tissue, they attach to the foreign particle or tissue and release the cytotoxic chemicals, that kills the target.

Clotting reaction

Clotting reaction is the mechanism of formation of extracellular and intravascular clots for rapid sealing of wounds. Clotting prevents the loss of haemolymph and invading pathogens. Intravascular clots produce stasis by adhering to the walls of blood vessels and extracellular clots

Inhibit movement of pathogens and make them vulnerable to phagocytosis.

Two different coagulation mechanisms

- Haemocyte derived clotting cascade
- Transglutaminase dependant clotting reaction

Haemocyte derived clotting cascade

Haemocyte derived clotting cascade can be seen in horseshoe crab. This mechanism is activated by lipopolysaccharide (LPS) of bacteria leading to the cascade of reaction where the activated clotting enzyme catalyzes the change of a soluble protein (coagulogen) into an

insoluble clot (coagulin). That seals the wound and entraps parasites or pathogens that are trying to invade the host.

Transglutaminase dependant clotting reaction

Transglutaminase dependant clotting reaction can be seen in crustaceans like shrimp, lobster, crab, cray fish etc. Here clottable protein (CP) play a major role. TGase is an enzyme released from the haemocyte during the invasion of pathogen or injury. TGase after release activates by calcium ions (Ca^{2+}) then catalyze the linking of CP into large insoluble aggregates. These insoluble aggregates are responsible for clotting process.

Prophenoloxidase (proPO) system

The prophenoloxidase (proPO) system is an efficient innate immune response against foreign materials in the haemolymph of invertebrates. This system is involved in proPO mediated melanization process, in response to infection, invasion or wound healing.

It is initiated by the recognition of lipopolysaccharides and peptidoglycans from bacterial cell wall and β -1, 3 glucans from fungal cell wall, even when they are in minute amounts. This suggests that the system will be activated in the presence of pathogens. proPO system contains pattern-recognition proteins (PRPs), several zymogenic proteinases and prophenoloxidase (proPO) enzyme.

proPO is synthesized in the hemocytes and are localized in the granules of the hemocytes. When β -1, 3 binding protein (β -1, 3 BP) binds to β -1, 3 glucans, it becomes activated and binds specifically to a cell surface associated protein, a superoxide dismutase (SOD). The recognition of non-self materials triggers degranulation of semigranular and granular cells. Prophenoloxidase activating enzymes (proppA) is released during the process which is further activated to ppA by the pathogen associated molecular patterns (PAMPs). Active ppA converts the proPO to phenoloxidase. PO is a bifunctional copper containing enzyme, which is known as a tyrosinase and catalyzes the early steps in the pathway to melanin formation. Melanin is a pigment which is black in color and helps the host to neutralize the pathogen.

Humoral defence mechanism

The humoral defence mechanism is a defence mechanism in which the direct cellular involvement is absent. Instead the cellular products like Lysozymes, Anti-microbial peptides and Lectins can be seen involved.

Lectins

Lectins protect the animals against infection. Lectin, or agglutinin, is a carbohydrate binding protein that can bind to the carbohydrate on the cell wall of pathogens or foreign bodies, causing the reaction known as agglutination. Lectins are produced in hemocytes and some of them are transported on the membrane surface and here they participate in recognition of foreign molecules or participate in carbohydrate transportation. Lectins can induce agglutination that can lead to phagocytosis and lectins can also act as opsonins. Lectins are present in almost all crustaceans.

Antimicrobial peptides (AMPs)

The antimicrobial compound is a part of humoral defense system in crustaceans that plays an important role in innate immunity. Antimicrobial peptides (AMPs) which are 150-200 amino acids long are produced by different types of cells. A family of AMPs called penaeidins displaying both antifungal and antibacterial activities is present in hemolymph of the shrimp.

Anti-lipopolysaccharide factors (ALFs) have been recently identified from hemocytes of *P. monodon*, and this ALF has a broad spectrum of antifungal properties and antibacterial activities against both Gram-positive and Gram-negative bacteria. Another antimicrobial peptide named crustin a peptide present in *Carcinus maenas* of 11.5 kDa size has an antibacterial activity against Gram-positive bacteria.

Lysozymes

The haemocytes and haemolymph contain lysozymes that can act against the pathogen. Once the antigen or pathogen enters the body cavity, it will stimulate the haemocytes to synthesis the higher quantities of lysozyme followed by release of these enzymes into the haemolymph where these enzyme will act on pathogen or susceptible material and destroy them.