# Insects immunity: types and mechanisms

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**Abstract**

Insects, despite lacking an adaptive immune system, have powerful ways to fight infections. They can phagocytose bacteria and encapsulate parasites, and secrete antimicrobial peptides into the hemolymph. They recognize foreign pathogens using specific receptors like peptidoglycan recognition proteins (PGRPs) and β-glucan recognition proteins (βGRPs). These receptors activate signalling pathways that activate genes that encode antimicrobial peptides. This article discusses the innate immunity of insects, including both cellular and humoral responses to bacteria, fungi and parasites, and discusses recent advances in insect antivirus immune responses .

**Keywords:** Immunity, Phagocytsosis, Haemocytes, AMPs.

Immunity is a host defense system comprising many biological structures and processes within an organism that protects against disease. Insects have evolved cellular and molecular defense mechanisms against infections, primarily innate and adaptive immunity. Innate immunity is an immunological subsystem that comprises the cells and mechanisms that provide the first line of defence from infection in a non-specific manner. Adaptive immune system, also known as the acquired immune system or specific immune system, that is composed of highly specialized, systemic cells and processes that eliminate pathogens or prevent their growth. Insects does not have this immune system.

**The innate immune system of insects consists of**

1. Physical barriers
2. Cellular response
3. Humoral responses

**1. Physical barriers:** The integument and peritrophic membrane serve as physical barriers against abrasive food particles and digestive pathogens in insects. However, these membranes are semipermeable, making them inefficient for viruses. They protect the hemocoel and midgut epithelium against microorganisms, activating humoral and cellular immune responses when they enter these barriers.

Upon infection, haemocytes immediately engage in cellular immune responses (Hemocytes) upon hemocoel infection, followed by humoral response sseveral hours later. These defense mechanisms do not work independently from each other (Cao *et al.,* 2015).

**2. Cellular response:** In insects, phagocytosis, encapsulation, and nodulation are examples of cellular immune responses. Granular cells, crystal cells, oenocytoid cells, and plasmatocytes are examples of hemocytes, which are in charge of defense reactions. For example, three hemocyte types—crystal cells, plasmatocytes, and lamellocytes—have been more thoroughly defined

**Table 1: Difference between innate immunity & adaptive immunity (Fig.1).**

| Sl. No. | Characteristics | Innate immunity | Adaptive immunity |
|---|---|---|---|
| 1 | Presence | Innate immunity is something already present in the body | Adaptive immunity is created in response to exposure to a foreign substance |
| 2 | Specificity | Specific for pathogen associated molecular patterns | Highly specific even it can discriminate minor difference in molecular structure of microbial molecules |
| 3 | Response | Fights any foreign invader | Fights only specific infection |
| 4 | Inheritance | It's generally inherited from parents & passed to offspring | Adaptive immunity is not passed from the parents to offspring |
| 5 | Response time | Faster response<br>Minutes/hours | Slower response<br>Days |
| 6 | Major cell type | Plasamtocyte, granulocyte | T-cell, B-cell |
| 7. | Example | Insects | Mammals |

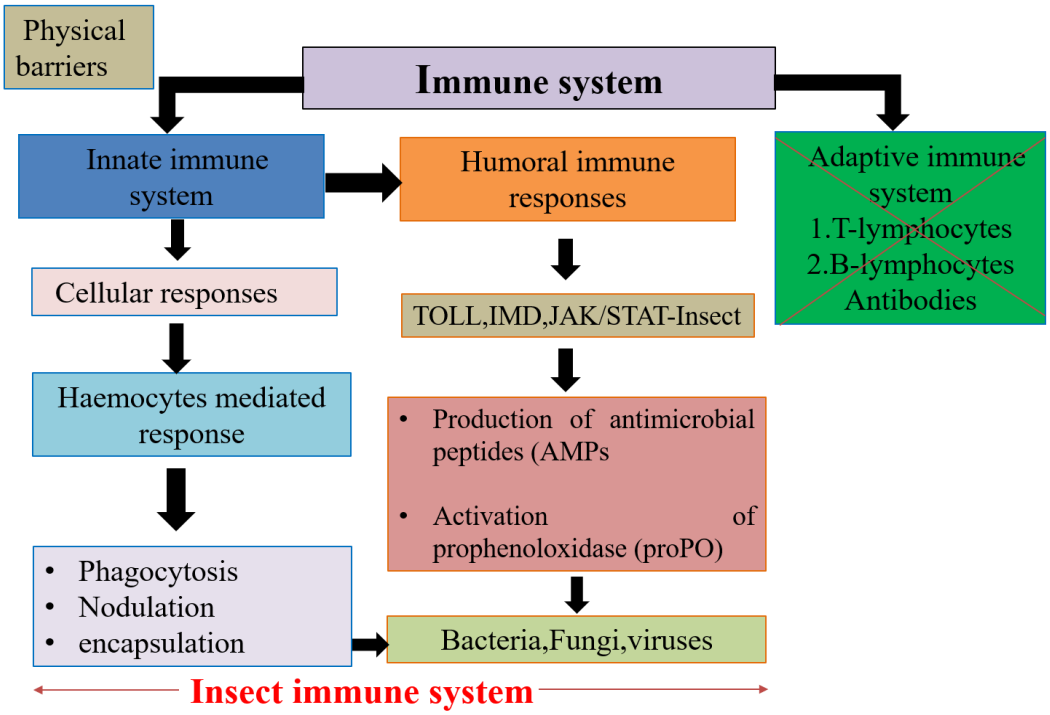


**Fig 1: General immune system classification in insects.**

in *D. melanogaster* (Parsons and Foley, 2016). Crystal cells have crystalline inclusions and are comparatively large cells. Prophenoloxidase, the zymogen they manufacture, is triggered during melanization (Fig. 2). Granular cells called plasmatocytes make up around 90% of all hemocytes. They have phagocytic receptors and use nodulation or phagocytosis to get rid of the majority of the invasive bacteria (Fig.2). Only when the larvae are infected by parasitic organisms can the flat cells known as lamellocytes be identified (Fig. 2). These hemocytes are mainly responsible for encapsulating the parasitoid wasp egg (Fig.2).

**2.1 Phagocytosis:** The process by which cells identify, attach to, and absorb big particles by tight contact with their plasma membrane, pinocytic vesicles, or pseudopodia is known as phagocytosis.

In Diptera and Lepidoptera, professional phagocytes are granular hemocytes and plasmatocytes, respectively. The primary phagocytic cells in the majority of insects are either granulocytes or plasmatocytes (Rosales, 2011).

**2.2 Nodulation:** When the initial phagocytic immune response is inadequate, hemocytes initiate methods to regulate infections, such as creating nodules to deal with high bacterial loads. Multicellular hemocyte aggregates are created during nodulation, trapping a lot of germs. Bacteria are encircled by hemocytes, which then develop into tiny clumps and eventually huge nodules. Bacteria are successfully isolated from the hemolymph by the melanized and flattened hemocytes covering these nodules (Fig. 3). For many insect species to develop nodules, certain chemicals like proPO, eicosanoids, and dopa decarboxylase (Ddc) are essential. Two proteins, Nodular and Reeler1, respectively, were shown to be crucial for mediating nodulation against *Escherichia col*i K12 and *Bacillus subtilis* bacterial challenges during screenings for novel immune genes from Indian saturniid silkmoth (*Antheraea mylitta*) larvae and *Bombyx mori* larvae. (Gandhe *et al.,* 2015).

**2.3 Encapsulation:** Hemocytes (lamellocytes) operate as effector cells, encasing bigger pathogens such as parasites, protozoa, and nematodes in a capsule. They create a melanized capsule by

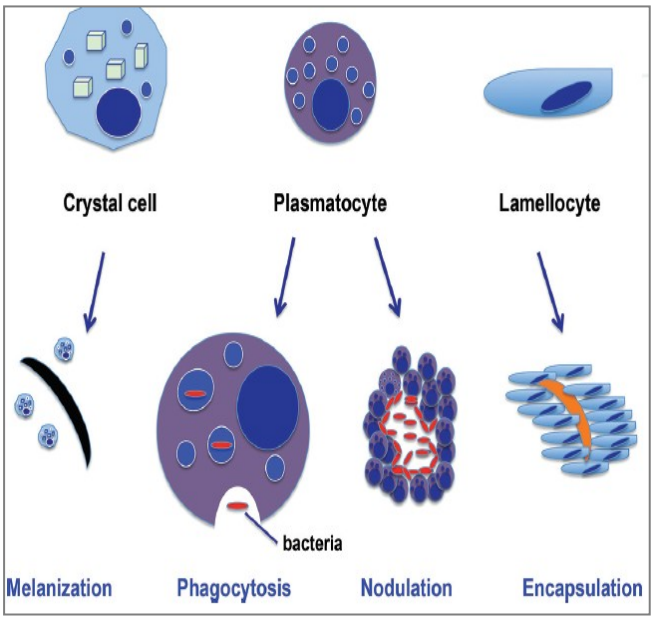


**Fig 2: General immune system classification in insects.**

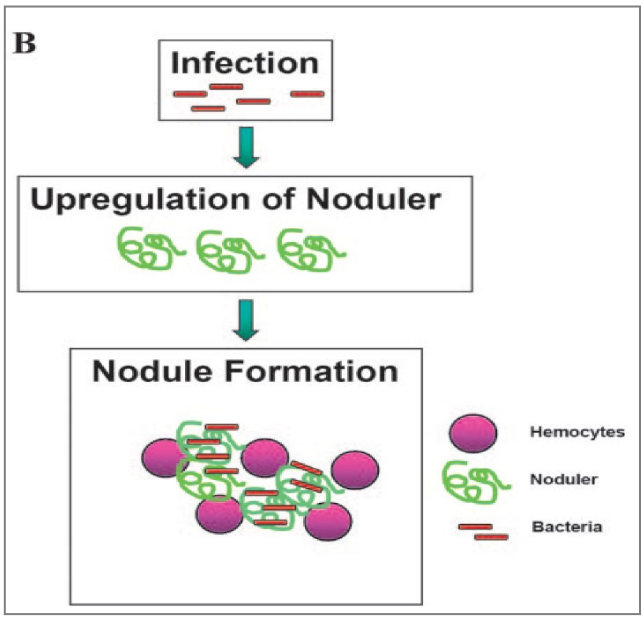


**Fig 3: General immune system classification in insects.**

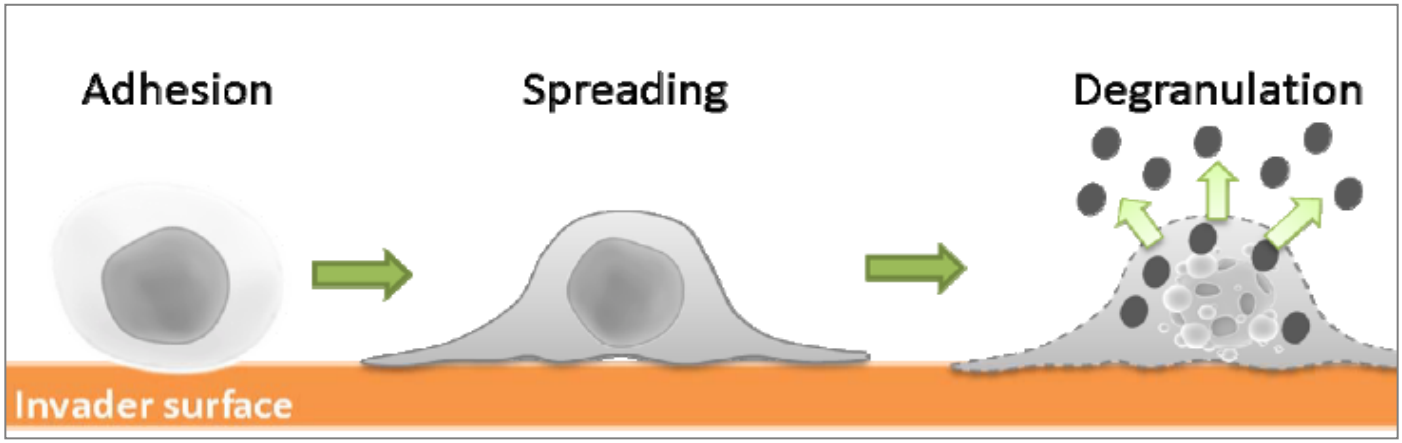


**Fig 4: Haemocytes adhesion, spreading and degranulation during encapsulation of parasites inside insects (Dubovskiy *et al.* 2016).**

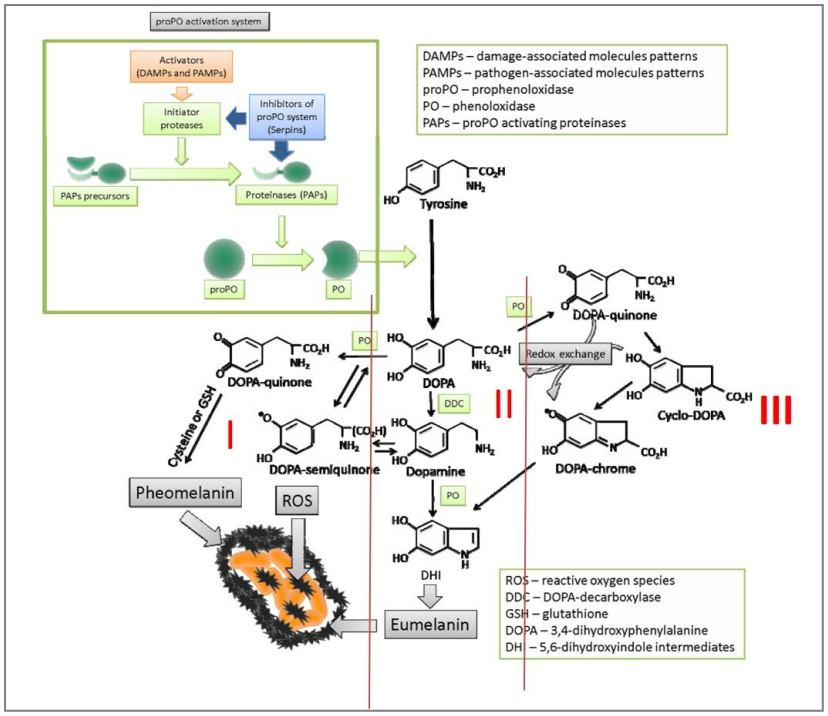


**Fig 5: Activation of proPO system and melanogenesis in insects (Dubovskiy et al., 2016).**

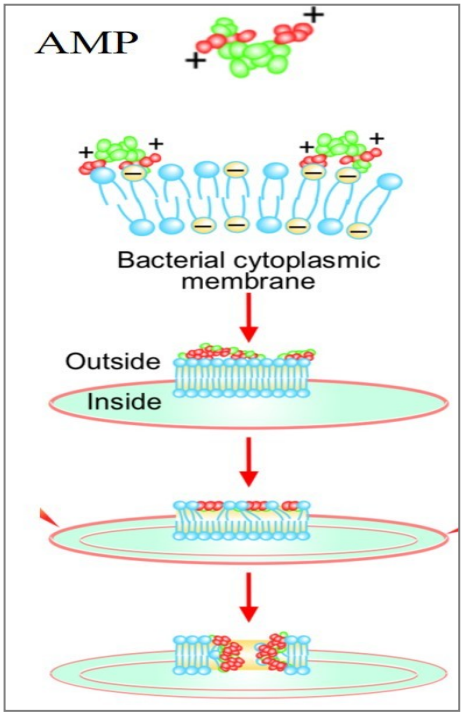


**Fig 6: Mode of action of antimicrobial peptides (Haine *et al.*, 2018)**

adhering to the target in several layers. After the hemocytes recognize the invader, they connect and begin to spread, killing the invading organism inside the capsule either by suffocation or reactive cytotoxic chemicals. (Fig. 3). Haemocyte destruction (degranulation), which releases effector molecules and immunomediators, is the next step in the cellular immune response (Fig. 3).

**2.4 Melanization:** Melanization is the process of melanin formation, activated during wound healing and nodule formation against pathogens or parasites in insects. The enzyme phenoloxidase (PO) is crucial in this process, which requires pattern-recognition proteins like PGRP or βGRP. PO binds to foreign surfaces, initiating melanin formation. PO converts tyrosine to dopa, which can be decarboxylated to dopamine or oxidized to dopaquinone and later may metabolized to eumelanin and melanin (Fig.5).

**3. Humoral responses:**

**3.1 Antimicrobial peptides**

Insects have a defense mechanism through the production of antimicrobial peptides (AMPs), which are released into the hemolymph after microbial infection. Mode of action of antimicrobial peptides: First they will attack the outer cell membrane of bacteria and it will cause ion imbalance and eventually cell wall lysis and death of bacteria will occur (Fig. 6).

These peptides are highly inducible and can vary in levels from undetectable in uninfected animals to micromolar concentrations in infected individuals' hemolymph. The first identified antimicrobial protein was the lysozyme from *Galleria mellonella*, which is structurally similar to chicken C-type lysozyme and can degrade bacterial cell wall including peptidoglycans of Gram-positive bacteria. It also has some activity against Gram-negative bacteria and some fungi. Biochemical analysis of hemolymph of the fruitfly *Drosophila melanogaster* and some other Dipterans has led to the discovery of seven groups of AMPs in insects (Haine *et al.,* 2018).

**a. Defensins:** Insect defensins, consisting of α-

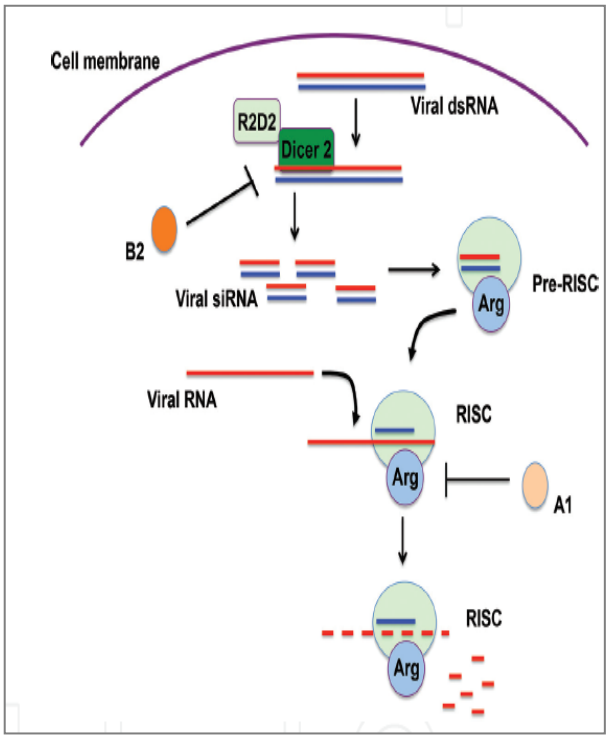


**Fig 7: Steps involved during the RNAi pathway (Rosales and Vonnie, 2017).**

helix/β-sheet mixed and triple-stranded antiparallel β-sheets, have been reported in many Lepidopteran species for their antibacterial and antifungal activity.

**b. Cecropins**: Cecropins are small, amphipathic peptides with 31-37 amino acid residues, found in the hemolymph of the silkworm *Hyalophoracecropia.* They act on antimicrobial activity by damaging pathogen cell membranes, inhibiting proline uptake, and causing leaky membranes.

**c. Drosocin:** Drosocin, a 19-mer cationic antimicrobial peptide from *D. melanogaster,* which has been found to possess an O-glycosylated threonine residue that is crucial for its antimicrobial activity.

**d. Attacins:** Attacins, 20 kDa AMPs, were isolated from *Hyalophora cecropia* hemolymph. Two isoforms, one acid and one basic, have been cloned and increase bacterial outer-membrane permeability, primarily binding to lipopolysaccharide.

**e. Diptericin:**Diptericin is an AMPs rich in glycine synthesized by insects in response to bacterial injections or injuries. It is active against a limited range of Gram-negative bacteria and disrupts their cytoplasmic membrane. Diptericin has been reported to inhibit bacterial growth and protect against oxidative stress, potentially trapping free radical anions and attenuating oxygen toxicity by increasing antioxidant enzyme activities in *D. melanogaster.*

**f. Drosomycin:** Drosomycin, an inducible antifungal peptide with 44 residues, was initially isolated from *D. melanogaster* but has shown potent antifungal activity against bacteria, with recent research showing it antiparasitic and anti-yeast properties.

**g. Metchnikowin:** Metchnikowin, a 26-residue proline-rich peptide in *Drosophila*, is an antimicrobial peptide that is active against both Gram-positive bacteria and fungi. It has been shown to protect transgenic plants from fungal pathogens, such as powdery mildew and *Fusarium* head blight, by expressing the metchnikowin gene in transgenic barley.

### 3.2 Antivirus insect response

Insects are infected by viruses, some of which are pathogenic to them and others are transmitted to mammals through biting. Understanding the insect innate immune response against viruses is crucial for medical and economic purposes. The RNA interference (RNAi) pathway, which produces tiny, interfering RNAs (siRNAs) in response to virus-derived double-stranded RNA (dsRNA), is the main antiviral defense mechanism. In turn, these siRNAs target viral RNA for destruction, so inhibiting the

spread of the virus. Dicer-2 and the protein R2D2 both detect double-stranded viral RNA. The ds RNA is subsequently broken down by Dicer-2 into tiny duplex DNA fragments of 21 nucleotides. The guide strand is chosen based on complementarity once the duplex is unwound. After that, the siRNA guide strand is inserted into the RNase Argonaute-containing RNA-induced silencing complex. When a target viral RNA pairs with the guide strand, Argonaut breaks it down (Fig.7).

Additionally, insect antiviral responses involve innate antimicrobial pathways like Imd, Toll and JAK-STAT. The JAK-STAT pathway functions similarly to the mammalian interferon system, activating in uninfected cells when a virus-infected cell sends a signal. Also, the autophagy pathway is suggested to be important in some viral infections (Rosales and Vonnie, 2017).

Emerging viral infections that pose a concern to public health include dengue and chikungunya fevers, as well as Zika virus infection. The bite of an *Aedes* mosquito is how their aetiologic agents are spread. Vector control is the primary method of stopping the spread of these diseases in the absence of viable treatments or vaccinations. the chitin necessary for *Aedes* mosquito larval survival structures. *E. coli* HT115 was used to express dsRNA molecules that target five distinct locations in the CHS A and B transcript sequences. These molecules were then created both in vitro and in vivo and tested by being added directly to larval breeding water. When exposed to dsRNA that targets the CHS catalytic sites, both immature and adult larvae exhibited markedly reduced viability, which was linked to a decrease in CHS transcript levels. In association with diflubenzuron, this bioinsecticide exhibited insecticidal adjuvant properties (Lopez *et al*., 2019).

## 4. Conclusion

Cellular and humoral responses to pathogens involve phagocytosis and encapsulation of bacteria, respectively, while humoral responses involve secretion of antimicrobial peptides into the hemolymph. Recognizing foreign pathogens involves specific receptors like PGRPs and βGRPs, which activate signaling pathways for gene expression of antimicrobial peptides and antiviral activity. However, the specific pathway activated by each pathogen and its outcome, especially for viral infections, is still unknown. Insects lack antibodies and their immune system is non-specific, making future research in insect immunity is promising.

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