DOI: 10.55278/OILB7950

Immune priming: An overview in insects

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Abstract

A characteristics feature of living systems is the ability to provide protection from different kinds of infection and various toxins. Importantly in insects, innate immune response is the most fascinating phenomenon. Though vertebrates' specific adaptive immune system is lacking in invertebrates including insects, defence priming is integral to many insect species. Immune priming is defined as the improved protection by the host to the same infectious agent upon a second encounter. This protection could be species/strain-specific, may be rendering lifetime protection or can be transgenerational. These attributes hence are effective protective system against a range of pathogens. Transgenerational immune priming can be attained primarily by two ways - direct parental transfer or elevated endogenous offspring immunity. The exact mechanisms for direct parental transfer is poorly understood though ways proposed involves direct transfer of antimicrobial peptides and mediators like lysozymes in the cytoplasm of the egg, epigenetic phenomenon like genomic imprinting or transfer of microbial fragments directly by the parents. Hence immune priming in insects are important line of defence and herein in this article an attempt on the elucidation of this phenomenon in the context of insects is done.

Keywords: Immune, Priming, Transgenerational

Introduction

Class Insecta (Latin *insectum*, “notched or divided body", that implies "cut into”) is the largest representative of Phylum Arthropoda, which in turn are the most predominant phyla (Snodgrass, 1960). They have 3 major divisions of the body – head comprising the mouthparts, eyes, and a pair of antennae; thorax (segmented) and abdomen that involves important systems like excretory and reproductive. They harbour very prominent and primitive innate immune system which exhibit both cellular and humoral immune responses. The insect immune system is known to possess fat bodies that secrete effector molecules into the circulating fluid (hemolymph) and diverse range of hemocytes. The fat body (a substitute of vertebrate liver and adipose tissue) is reported to secrete several soluble factors like antimicrobial peptides into the hemolymph (Lemaitre and Hoffmann, 2007). Hemocytes are versatile in forms and function.

Phagocytosis, encapsulation, nodule formation are some of the important roles worth mentioning. The most prevalent cell types are adipohemocytes, oenocytoids, prohemocytes, spherule cells, granulocytes, secretory, plasmatocytes, crystal cells and lamellocytes (Rosales, 2017).

Immune priming is another important immune mechanism shown by several insect species. It is a defence strategy in which a prior encounter to the pathogen or any of its derived material leads to profound immune response upon further encounter. Hence this strategy protects the insect by rendering it resistant to subsequent infections. This process involves an increase in the density of circulating hemocytes and subsequent enhanced production of antimicrobial peptides (Sheehan *et al*., 2020). Insects have always been an indispensable model to study microbial pathogen infection in human and hence detailed mechanisms of immune priming is integral to assess several parameters.

Immune priming – the detailed aspect

Immune priming is a fascinating aspect of invertebrate immunology which refers to swift, prompt and improved response of immune system to microbial population if they are exposed to the same pathogen previously at a relatively sub-lethal dose. Immune priming can result because of several factors like exposure to anti-microbial agents, different kinds of thermal and physical stress amongst others (Sheehan *et al*., 2020). Immune priming hence bestows insects to counteract potential infections with a history of previous exposure. The armamentarium involved in these process are different components of cellular and humoral immune system. Precisely immune priming is a complicated mechanisms and its effects may vary amongst insects of different species (Cooper and Eleftherianos, 2017).

Various experiments have been performed to demonstrate and understand immune priming. The classical biological model, *Drosophila melanogaster* have served as an integral model to study immune priming. The fruit flies were infected with sub-lethal dose of *Streptococcus pneumoniae* or its heat-killed form and a week later were re-exposed to the lethal dose of same pathogen (Pham *et al*., 2007). It was observed that the flies successfully withstood the infection. Similar observation were also noted with *Beauveria bassiana*, a natural fly pathogen. In another set of experiments with the bumblebee, *Bombus terrestris* were exposed to gram-negative *Pseudomonas fluorescence* or closely related gram-positive bacteria (*Paenibacillus alvei* and *P. larvae*) followed by repeat exposure with either the same bacteria (homologous) or one of the two bacteria (heterologous). The results showed that primed bees significantly withstand the homologous repeat infection than a heterologous repeat infection (Sadd & Schmid-Hempel, 2006).

These and several other infection studies in insects model have suggested and indicated the involvement of immune priming in insects. Further studies exploring the mechanism of priming have established the involvement of important signalling pathways like Toll and Imd in the phenomenon (Tanji *et al*., 2007). Both of these pathways are highly conserved signalling paths and follows similar patterns. The immune deficiency pathway or Imd primarily regulates antimicrobial peptides' expression. The Imd pathway is similar to TNFR and TLR signalling pathways. Precisely TNF-α requires *NF-κB* for the execution of functioning and Imd pathway relies on *Relish* signalling molecule. Relish is reported to contain *Rel* homology domain (that explains the nomenclature *Relish*) and an IkappaB-like domain that has ankyrin repeats (6 in numbers). Thus, Relish is a dual domain protein. This explains similarity of Relish to mammalian NF-kappaB precursors - p100 and p105 (Silverman & Maniatis, 2007). The Figure 1 explains the mechanism executed by Imd and TFNR pathways respectively. The conserved signalling molecules have been highlighted by similar colour and shapes (Myllmaki *et al*., 2014). It is worth mentioning here that *Relish* is typically involved in the humoral immune responses.

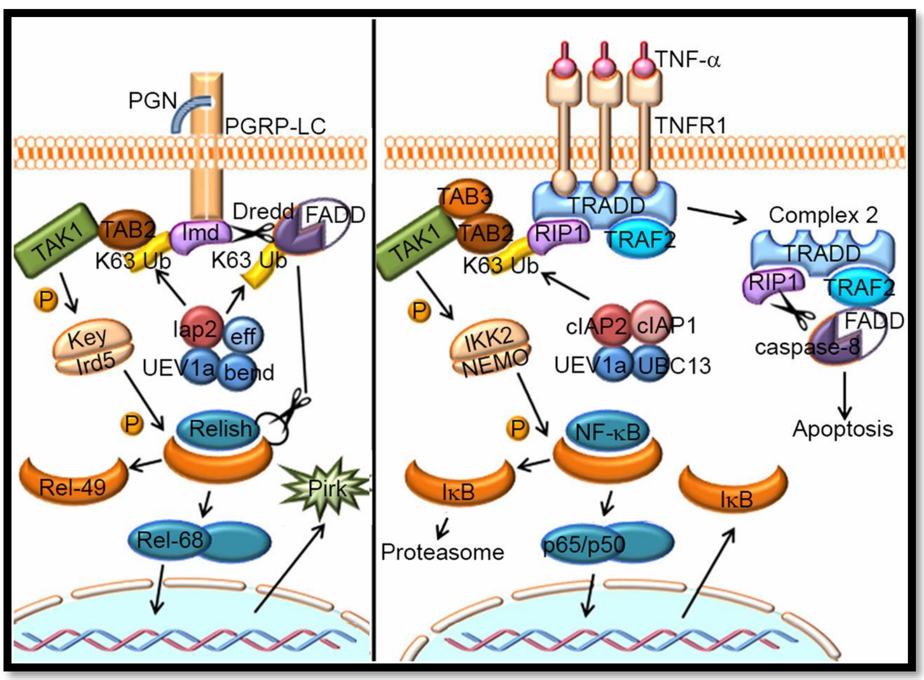

Figure 1: Schematic representation of the Drosophila Imd pathway and human TNFR signaling. Conserved components are indicated by similar shapes and colours (Myllmaki *et al*., 2014).

Drosophila Toll pathway is characterized by around 9 *toll* genes. They are primarily involved in cellular immune responses and act as important signalling molecules during phagocytosis, parasite encircling and nodule formation, encapsulation etc. The pathogen sensing is done by molecules extracellularly placed that triggers proteolytic cascade involving hydrolysis of the Toll receptor like prospatzle to spatzle (Lemaitre *et al*., 1996). The active spatzle binds to Toll receptor for the Toll pathway activation which in turn recruits a cascade of proteins consisting of MyD88, Tube and Pelle. As a consequence, there is a degradation of Cactus, eventually freeing Dorsal, and Dorsal-related immunity factor (DIF) facilitating their entry into nucleus to regulate the expression of AMPs such as drosomycin (Valanne *et al*., 2011).

Besides immune priming, both these pathways in downstream are severely implicated in disease pathogenesis and are the major determinants of immune evasion or protection against microbial infection. They are important initiators of several cascades like apoptosis, autophagy, necrosis to name a few. Howsoever, the Imd pathway is characterized for sensing diaminopimelic acid-containing peptidoglycan (DAP-type PGN) prevalent in Gram-negative bacteria. As mentioned earlier, they mainly regulate the expression of anti-microbial proteins like Diptericin involving Relish, an important transcription factor (Kleino and Silverman, 2014). Toll pathway operates in a different way by sensing lysine-containing peptidoglycan (Lys-type PGN) found in Gram-positive bacteria and by β-glucans characteristic of fungal cell walls. As a result they activate a different set of AMPs like Drosomycin that triggers downstream cascade *via* the involvement of dorsal-related immune factor (DIF), another important transcription factor.

Transgenerational immune priming

Transgenerational immune priming is an important aspect of insect immunology. Here the parental encounter to the pathogen provides protection to the progenies; hence suggesting immune protection persisting across generations. This dynamic phenomenon has been demonstrated by several representatives of different orders of class Insecta (Tetreau *et al*., 2019). A Transgenerational mechanism involves transfer of specific signalling molecules through developing eggs. These molecules could be some bacterial peptides translocated from mother's gut to the egg. Often this signal translocation involves enhanced expression of immune-related genes in the egg. Specifically in case of social insects *Apis mellifera*, vitellogenin is greatly implicated in the process. Vitellogenin is a lipoglycoprotein (egg-yolk precursor). They are synthesized and stored in the fat body but are secreted into the hemolymph. Though they are female specific proteins, in honeybees they are also expressed in the workers. Vitellogenin categorically recognises Pathogen associated molecular pattern like LPS and PGN. This can provide immunization to the progenies through many pathways including social immunization. In this later instance, bacterial fragments from the gut gets transferred to the worker bees' glands involved in producing the royal jelly that the queen bee feeds. This eventually reaches the eggs produced by the queen hence benefitting the colony (Salmella *et al*., 2015). In insect models like *Galleria mellonella* and *Tribolium castaneum*, transfers of specific microbial proteins occur by crossing the midgut epithelium. It is then entrapped into nodules in the haemocoel which is followed by deposition in the eggs (Freitak *et al*., 2014).

In insects there are different types of ovary reported - panoistic ovary, telotrophic ovary and polytrophic meroistic ovary. The later one is characterized by the arrangement of nurse cells and oocytes alternatively along the length of the ovariole whereas telotrophic ovary is characterized by placement of nurse cells at the apex of the ovariole. This facilitates feeding the oocyte through a nutritive cord as it descends down. In both these ovarioles, the trophocytes provides the oocyte with RNA, proteins, and ribosomes through much of their development that are otherwise provided only by the oocyte itself in panoistic ovarioles. Insects exhibiting telotrophic ovary (Orders - Hemiptera and Coleoptera) or polytrophic meroistic ovary (Orders - Hymenoptera, Lepidoptera, and Diptera) involve transferring maternal mRNAs in developing eggs during oogenesis. Many of these mRNAs apart from playing important role in development helps in the early immunological protection to the progenies (Johnstone and Lasko, 2001).

Another important transfer method involved is through epigenetic modifications of the parents like acetylation/deacetylation of histone proteins and/or by the methylation/demethylation of immune-related genes. Besides, females are capable of direct transfer of immune effector proteins to their eggs passively through the diffusion or sequestration into the proteins present in the mother's hemolymph. Also the transfer *via* active process takes place through specialized cells, such as nurse cells that mediates transfer to oocytes (Harwood *et al*., 2019).

Thus these are the different ways of how the developing embryo within the egg gets primed to different microbial patterns from exposure that the parents encountered during their life.

Conclusion and future prospects

Immune response against microbial pathogen is integral to survival of the host. In this respect immune priming is important as it primes the defence machinery and promotes increased protection to further challenges. In insects immune priming has been found as a widespread phenomenon owing to its prevalence in several insect species. This is further elaborated in insects as an event within generation and that persists across generations as well. Transgenerational immunity is a dynamic phenomenon in invertebrates and signifies the memory like response of vertebrates. However, monitoring several successive generations is crucial to establish the sustainability of transgenerational priming. Additionally, detailed mechanisms of immune priming in insects with special reference to transgenerational impact have to be studied. Further the studies has to be substantiated by molecular approaches. An elaborate transcriptomic and proteomic studies could decipher the intricate mechanisms. Several insect species shows metamorphosis of different extent. Hence it is crucial to investigate stage-wise analysis of transgenerational priming.

Equally important is the differentiation of hemocytes. The different sub-population of hemocytes keep circulating in the hemolymph that confers innate immune responses and as required differentiates to counteract the infection. Overall it can be concluded that immune priming is a conserved protective mechanisms though some more critical and elaborate studies may be required for more detailed understanding.

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MS Received 06 May 2022
MS Accepted 18 May 2022