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Review

# Fundaments of the honey bee (*Apis mellifera*) immune system. Review

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**Abstract:**

Honey bees (*Apis mellifera*) pollinate plants in both natural and managed ecosystems, contributing to food production and sustaining and increasing biodiversity. Unfortunately bee depopulation and colony losses are becoming increasingly common worldwide. Several factors contribute to the decline of bee populations, including pathogens (parasites, fungi, bacteria and viruses), ecosystem alteration or loss, and/or agrochemical use. All of these factors alter the defense mechanisms of the bee immune system. Honey bees have an innate immune system that includes physical barriers and generalized cellular and humoral responses to defend themselves against infectious and parasitic organisms. Pathogens, acaricides, fungicides, herbicides and other pesticides affect the bee immune system and consequently bee health. The defense mechanisms of the bee immune system include

signaling pathways, pathogen recognition receptors and innate immune system effectors. Although *A. mellifera*'s immune system is very similar to that of *Drosophila* flies and *Anopheles* mosquitoes, they possess only about a third of the immune system genes identified in these genera. This relatively low number of genes is probably a consequence that *A. mellifera* has developed social immunity. This defense strategy lowers pressure on the individual immune system of bees. This review article summarizes and discusses the bases of the honey bee immune system.

**Key words:** Immunity, defense mechanisms, immune system regulation, pathogens, *Apis mellifera*.

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# Introduction

Along with other wild pollinators, honey bees (*Apis mellifera*) contribute to pollinating plants in both natural and managed agriculture systems. In these ecosystems, pollination constitutes an environmental service, which contributes to increasing natural biodiversity, as well as the production of food and fibers for human consumption[1,2]. Unfortunately, bee depopulation events and loss of honey bee colonies have occurred worldwide during the last decade, particularly during late winter[3-6]. Various factors apparently lead to declines in bee populations, including pathogens (parasites, fungi, bacteria and viruses), ecosystem alteration or loss, and/or the use of agrochemicals. Since all these potential factors can alter defense mechanisms of the bees' immune system, it is necessary to first understand how it functions to be able to analyze its response to the different infectious or non-infectious conditions that affect bees.

Immune systems in plants and animals involve organs and defense mechanisms that protect them against foreign substances and pathogenic organisms by recognizing them as threats and responding against them. Much of current knowledge on immune systems and their responses has been generated using insects as research subjects; as a result, immunity in insects is very well studied. Many insects are vectors of animal and human diseases, and others cause major damage to agricultural crops. Most insect species live relatively short lives, but they have complex and efficient immune systems. For example, insects' immune systems are more efficient at detecting pathogens and responding to them than are those of vertebrates[7].

The fruit fly *Drosophila melanogaster* is the most studied insect species and, in addition to many other research areas, studies on this fly have helped to better understand innate immunity in other organisms. Research with fruit flies has generated knowledge on pathogen recognition mechanisms, immune signaling and effector responses against pathogens. Completion of the *Drosophila* genome sequence in the year 2000 has allowed more potent and specific analyses of immune responses, substantially increasing knowledge of the molecular foundations of immune systems. Not only these studies showed how insect immune systems work, but how the innate immune system of humans function, because many of the basic immune mechanisms are shared by *Drosophila* and humans. Studies of other insects whose genomes have been sequenced such as *A. mellifera*, can also contribute to exploration of immune responses at the molecular level. Because their natural pathogens and genetic structure are well known, the honey bee can join several species of flies and moths as important models for researching the genetic mechanisms of immunity and diseases.

The honey bee immune system is very similar to that of *Drosophila* flies and *Anopheles* mosquitoes, except that honey bees have approximately one third of the immune genes shared by *Drosophila* and *Anopheles*, which are grouped into 17 families[8,9]. Honey bees however, have more genes for odor receptors, as well as specific genes that regulate pollen and nectar collection, which is consistent with their behavior and social organization[10]. The implicit reduction in the number of immune genes in bees may reflect the importance of social defenses (i.e. based on social behavior) and/or their tendency to be attacked by a limited set of pathogens which are highly co-evolved with them[11]. Among the similarities of the innate immune systems of honey bees, fruit flies and *Anopheles* mosquitoes, is that all of them posses the same signaling pathways. Therefore, much of the knowledge that we have about the immune system of *A. mellifera* has been deduced from the knowledge of dipteran immune systems.

Advances in genomics allow study of both the evolution of biological systems and immune systems. The resulting deeper knowledge has proved valuable in understanding, treating and preventing disease in species of social or economic importance. Indeed, the sequencing of the *A. mellifera* genome has led to prediction of their immune system components, such as the recognition receptors, effectors and pathways involved in host defense[8].

The present review of the honey bee immune system covers both general and specific aspects of current knowledge on the innate immune system, its components and regulation, immune responses and social immunity.

## Types of immune systems

Of the two types of immune systems, innate and adaptive, higher vertebrates have both to fight against pathogens, while insects have the innate immune system as their sole line of defense (Table 1).

Innate immunity responds to exposure to pathogens or toxic substances with acquired (pre-existing) mechanisms, such as physical barriers (e.g. cuticle, mucous membranes, etc.), and cells and chemicals that neutralize toxins and pathogens. The innate immune system in higher vertebrates uses cellular effectors including phagocytes, dendritic cells, natural killer cells and mast cells, among others[7]. Humoral effectors consist of supplement system fractions, acute phase proteins, antimicrobial peptides (AMPs), natural antibodies, and the various cytokines that modulate immune response[7]. Innate immune system specificity is in part inherited, resulting from coevolution of individual immune systems with myriad pathogens[12].

Adaptive, or acquired, immunity refers to specific immune reactions tailored to particular toxins or pathogens. These toxins or pathogens are known as antigens (antibody generators) or immunogens. Adaptive immunity in vertebrates implies the ability to remember specific pathogens and react with production of antibodies specific to each pathogen when an organism is exposed to the same pathogen more than once.

One way to differentiate between innate and adaptive immune systems is based on the way an organism encodes the molecules with which it recognizes pathogens. Innate immunity involves encoding these recognition receptors directly into the germline, which is then inherited by offspring. In this sense, the repertoire of receptors identified in studied species is limited and promiscuous. Adaptive immunity requires far more receptors than innate immunity, with a repertoire of adaptive immunity receptors that is broad enough to potentially recognize an infinite number of pathogens[7].

**Table 1:** Characteristics of innate and adaptive immunity systems.

| | Insects | Higher Vertebrates | |
|---|---|---|---|
| | Innate | Innate | Adaptive |
| | CHARACTERISTICS | | |
| Specificity | Against structures shared by related microbial groups. | Against structures shared by related microbial groups. | Against microbial and non-microbial antigens. |
| Receptor diversity | Limited | Limited | Very broad |
| Memory | Null | Null | Yes |
| Self reactivity | Yes, non-specific collateral damage. | Yes, non-specific collateral damage. | Yes, specific autoimmunity. |
| | COMPONENTS | | |
| Humoral effectors | Antimicrobial peptides, thioester linkage proteins, melanization and coagulation proteins. | Complement system. Cytokines.<br>Interferon system. Chemokines.<br>Acute phase proteins. Coagulation system. | Antibodies. Cytokines. |
| Cellular effectors | Phagocytes. Hemocytes. | Macrophages, dendritic cells, neutrophils, innate immunity lymphocytes, mastocytes. | Lymphocytes |

## The innate immune system and its components

Physical barriers, coupled with humoral defense mechanisms and different cellular processes, acting in synergy, are powerful tools for neutralizing parasites, pathogens and xenobiotics.

### *Physical barriers*

The pathogens and xenobiotics that affect insects must first cross the physical barriers of the innate immunity system, such as the exoskeleton, tracheal tubes, and intestinal mucosa. Viruses in particular, often are able to penetrate these barriers with the aid of a vector; for instance, many viruses are transmitted to *A. mellifera* by the mite *Varroa destructor*, which pierces these physical barriers, thus facilitating viral infection.

### *Cellular immunity*

Cellular immunity is provided by hemocytes, cells transported by the hemolymph, which perform processes such as phagocytosis, encapsulation and melanization[13]. In insects, hemocytes also synthesize and store humoral effectors such as antimicrobial peptides[14], in association with other sources of immune system soluble effectors such as the salivary glands[15] and the fat body. The latter is the functional analogue of the liver in higher vertebrates since it produces proteins to fight pathogens[16,17]. Cellular mechanisms contribute to elimination of foreign agents; in the face of an infectious or external particle, hemocytes can respond by phagocytizing or lysing it, or by engulfing it to neutralize it[13,18].

Small foreign agents can be phagocytized by hemocytes for removal. Larger ones (or aggregates of small ones), however, can trigger nodulation or encapsulation, which involves cooperative action among several hemocytes[19]. This process requires aggregation and partial disruption of hemocytes on the surface of the agent to be removed[20]. Oxygen and nitrogen mediators that affect microorganisms are then released, and process-regulating substances which act as antioxidants are simultaneously generated, minimizing any potential damage from foreign agents.

For hemocytes to fulfill their phagocytic and restorative functions, they may have some kind of adhesion molecules that allow them to bind to different surfaces, other cells or each other, which is what happens in nodulation or encapsulation[21,22].

Although the number of hemocytes varies in the different stages of bee development, this encapsulation function is unaffected[23]. This is notable since in adult bees, including workers, queens and drones, the number of blood cells decreases as they get older[24].

Insect hemocytes have been identified and classified by their morphological, histochemical and functional characteristics. In bees particularly, hemolymph cytology has been characterized using different methods. Initial studies identified five main hemocyte types[25], 90% of which are represented by plasmatocytes. These in turn have been classified into four subtypes: prohemocytes, clot hemocytes, granular cells and oenocytoids; the latter two related to melanization during and after the encapsulation process[20]. Flow cytometry analyses have not found significant morphological differences between hemocytes[26], but have identified two types of plasmatocytes. In another study hemolymph cell groups were classified as proleukocytes, eosinophils, basophils, neutrophils, picnonucleocytes, adipoleukocytes, spherukocytes, granulocytes, macronucleocytes, microleukocytes, and spindle-type cells[27]. Still others propose functional classification of hemolymph cells (e.g. adhesion to glass), thus avoiding any possible confusions from morphological classification[21].

Melanization is a combination of humoral and cellular processes that occurs during encapsulation or nodulation and healing, and is aimed at dealing with injuries, be they pathogen-mediated or otherwise. This cellular reaction in the insect defense system eliminates large numbers of bacterial cells, parasites and xenobiotics[19]. Its main function is to limit agent propagation and retain it for elimination[13]. This central and very effective defense strategy is the focus of evasion mechanisms employed by many entomopathogenic microorganisms, confirming its importance as a defense mechanism[19,28].

Prophenoloxidase (proPO) is a hemolymph protein that mediates melanization. Activation of proPO in insects occurs through an activation cascade beginning with recognition of pathogen-associated molecular patterns (PAMPs) by pathogen-recognition receptors (PRRs) deployed by hemocytes. These begin an adhesion process on the invading agents, generating an overlapping sheath, and producing and releasing proPO to degranulate or lyse the agents. In conjunction with formation of melanin and its polymerization (along with other proteins) to encapsulate the invading agent, reactive intermediaries of oxygen and nitrogen are produced, such as superoxide anion, hydrogen peroxide[20], and nitric oxide[21,29]. These collaborate in agent destruction and induction of melanization. This process has been demonstrated in *A. mellifera*[29]. Bees have but a single proPO gene, whereas *Drosophila* sp. have three and *Anopheles* sp. have nine. This proPO-encoded gene is expressed more strongly in adult bees than in larvae or pupae[9].

### *Humoral and chemical immunity*

Humoral response is a second category of innate immunity, and the most important defense system of insects, including honey bees. It is mediated by chemicals and antimicrobial peptides (AMPs). These are small, highly conserved proteins, generally between 12 and 50

amino acids in size, which are produced and released into the insect hemolymph in response to bacterial and fungal infections, but can also be synthesized during viral infections[14]. These humoral effectors are fundamental to innate immunity in insects. In some pollinating insects, such as *Bombus pascuorum*, the humoral response is detected within 24 to 48 h post-infection. Humoral effectors can be produced in hemocytes, epithelial cells and salivary glands, but the fat body of the dorsal cavity is the main organ of effectors synthesis[30,31].

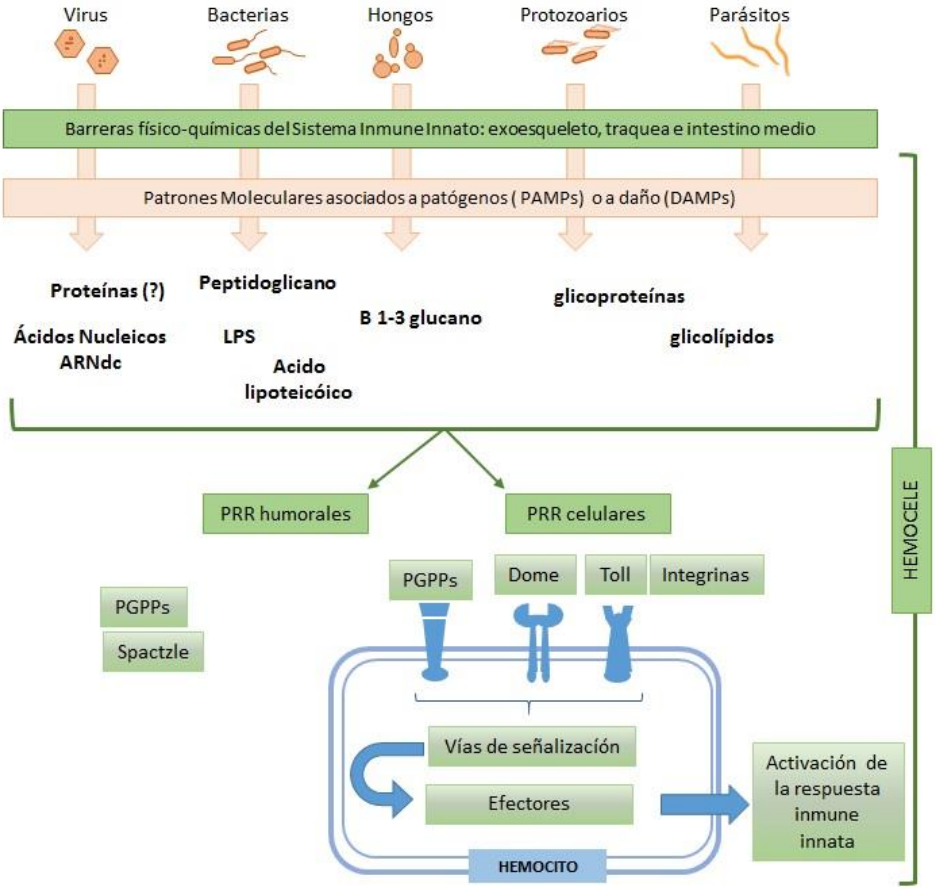
Over 170 AMPs have been described in insects, although honey bees produce fewer humoral effectors than other insects such as *Drosophila* and *Anopheles*[32]. Honey bees have four AMP families with broad hemolymph activity: apidaecin, abaecin, hymenoptaecin and defensin. Defensins are small AMPs that act mainly against Gram-negative bacteria such as *E. coli*, although they do effect Gram-positives and fungi[33]. There are 29 different cDNA sequences for defensins, numbered Defensin1 to Defensin 29. Eleven cDNA sequences exist for abaecin, encoding for two different abaecin peptides called AcAb1 and AcAb2. Apidaecin has thirteen cDNA sequences encoding for four peptides: AcAp1 to AcAp4. Finally, there are 34 different cDNA sequences for hymenoptaecin encoding for 13 different peptides[34].

In *B. pascuorum* and *B. terrestris*, AMPs have been shown to act in synergy to provide greater antimicrobial additive effects; this can involve potentiation in that one AMP can improve another's activity. The combination of AMPs increases the spectrum of responses, as well as their specificity, effectiveness and robustness, thus allowing a reduction in the resources allocated the immune system by augmenting the antimicrobial activity of AMPs at low concentrations[35].

## Regulation of the immune response

All immune responses involve a sequence of events that can be generally grouped into three stages: 1) recognition, 2) activation of signaling pathways and 3) cellular and humoral effector mechanisms aimed at eliminating pathogens (Figure 1)[36]. The immune response is triggered by the recognition process in which PAMPs are identified by PRRs in immune system cells. In response, different signaling pathways are activated, promoting synthesis of the effectors and receptors involved in the humoral and cellular immune response, as well as peptidoglycan recognition proteins (PGRP)[20].

**Figure 1:** Immune system regulation




## Pathogen recognition

Microorganisms are antigenic mosaics that can be recognized differentially by the innate and adaptive immune systems. The innate immune system recognizes PAMPs, which are preserved and vital protein structures present in defined germ groups; for example, lipoparasaccharides (LPS), lipotheicoic acid, zymosan, glycolipids, glycoproteins or double-stranded RNA[7]. The innate immune system also recognizes damage-associated molecular patterns (DAMPs), which are molecules expressed in cells that have suffered infectious or non-infectious damage, such as thermal shock protein. However, in insects, it is more common to refer to microbe-associated molecular patterns (MAMPs), which include so-called virus-associated molecular patterns (VAMPs)[32]. These structures act as exogenous

ligands and are recognized by proteins or PRRs, which are present in soluble form or in immune system cells[12].

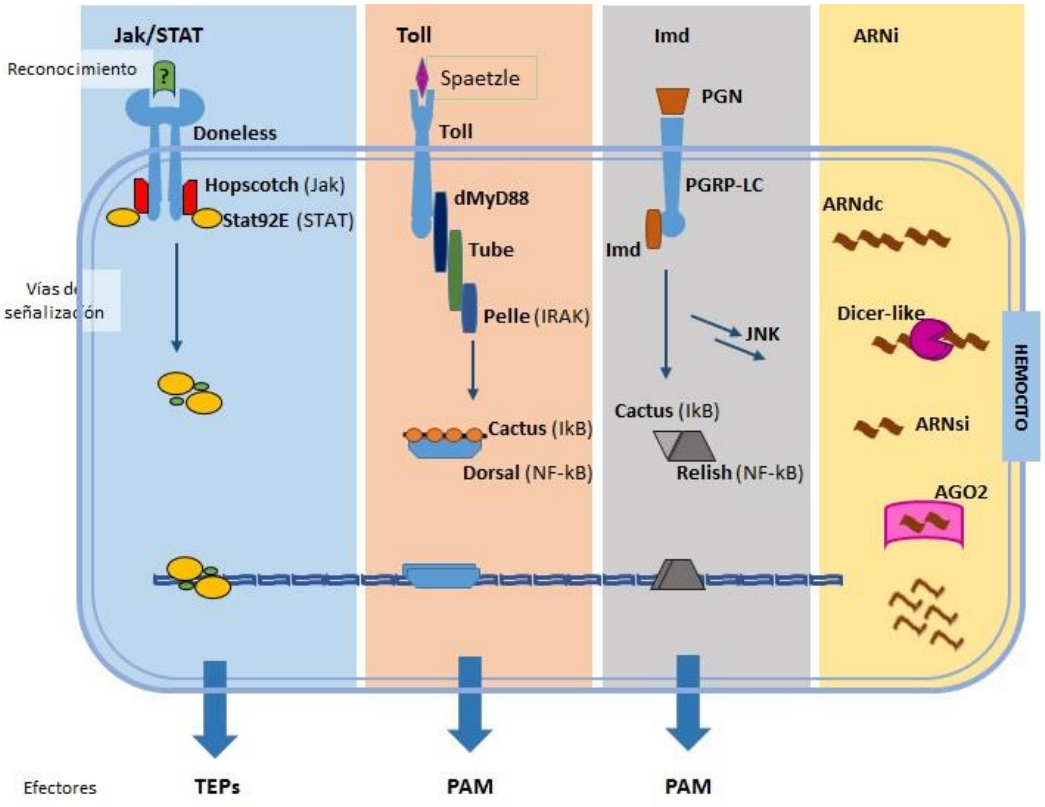
Multiple PRRs occur in *Drosophila*; for example, some members of the PGRP family. Of the 13 PRRs in *Drosophila*, honey bees share four, two of which are synthesized in response to infections (PGRP-S2 for the Toll pathway, and PGRP-LC for the Imd pathway). Other proteins recognize Gram-negative bacteria, such as GNBP1, which recognizes 1,3 glucans, but can also recognize fungi and are involved in recognition of certain Gram-positive bacteria[37,38]. These pattern recognition proteins may be involved with serinproteases, which initiate division of Spaetzle and Toll's endogenous ligand in *Drosophila*; both of these are activated in embryogenesis and immune response[39]. Two orthologous genes of the Spaetzle family have been identified in the bee genome[8,32,40,41,42].

Recognition of microbial structures triggers two main events: 1) signaling events, which occur when Toll and/or IMD receptors are stimulated, and 2) phagocytosis events. The genes *DSCAM* and *Eater* are two examples of genes related to endocytosis in bees. In *Drosophila* *DSCAM* is known to be involved in bacteria recognition by hemocytes[42,43]. Peptidoglycans, LPS and zymosan also recognize MAMPs. Vitellogenin are carrier proteins of bacterial fragments; they are acquired transgenerationally, producing a kind of sensitization or "priming" of the innate immune system in progeny[44,45].

## Signaling pathways

Intracellular signaling pathways translate external signals or stimuli into actions within cells, inducing immune response; for example, by activating a series of genes encoding proteins related to host defense systems (e.g. thioester linkage proteins - TLPs). Signaling pathways depend on large multiprotein complexes that trigger stimuli of cell surface receptors by a specific ligand, and emit an intracellular signal initiating a cascade of enzymatic activity. Receptors made up of transmembrane proteins are associated with enzymes such as protein kinases. These normally phosphorylate the amino acid tyrosine, and are thus called tyrosinases. Onset of this intracellular signaling cascade directs the various biochemical responses that characterize a specific cellular response. Bees have orthologous genes for the central members or components of the four intracellular signaling pathways involved in activating innate immunity effectors (Figure 2), with the Toll and Imd pathways being the most important in insects, including bees.

**Figure 2:** Signaling pathways, molecular detail




(Modified from Brutscher *et al.*, 2015)[32].

### *Toll signaling pathway*

Toll receptors across the membrane of cells play a critical role in both ontogenic development and the immune system. Only five Toll-related genes have been identified in bees (Toll-1, -6, -2/7, -8, -10); these are also present in the genome of other insects belonging to the orders Diptera, Lepidoptera and Coleoptera, with a few exceptions. The combination of Toll genes present and absent in these insects suggests that these five genes encode the basic set of Toll receptors present in their common ancestor[8,32].

Activation pathways involve recruitment of cytoplasmic adapter proteins, which activate kinases that lead to activation of nuclear factors and deregulation of genes that encode immune system effectors, such as AMP growth factors. Detachment of Spaetzle stimulates Toll receptors, which recruit death-domain proteins (DD-death) to assemble a receptor complex. In this process, the adapter protein MyD88 recruits TUBE and activates the protein kinase PELLE (IRAK counterpart) which then recruits the adapter dTRAF0. This complex induces degradation of CACTUS (counterpart of the NF-κB inhibitor protein, IκB) allowing the DORSAL transcription factor (NF-κB's counterpart) to be transported to the nucleus to

link to regions promoting immune effector genes, inducing their expression. The effectors synthesized when this pathway is activated are mainly AMPs and lysozymes[8,46].

### *Imd signaling pathway*

In bees and flies, the immune-deficiency signaling pathway (Imd) activates the RELISH transcription factor (homologue to NF-κB transcription factor). In flies, it controls expression of most AMPs, making this pathway indispensable for immune response against microorganisms. Presence of CACTUS as a transcription factor inhibitor has also been demonstrated. This pathway is highly preserved in bees with possible orthologues for all components. Although this strongly implies that signaling pathways in flies and bees are similar, it does not necessarily mean that they share exactly the same biological functions[8]. Microorganism recognition via peptide-glucan recognition protein (PGRP-LC) is the first step in immune response onset via Imd signaling[47]. Activation of the Imd pathway also leads to activation of components of the JNK signaling pathway, and there is evidence that the latter controls expression of AMP synthesis through both positive and negative feedback. Possible orthologues of this pathway, such as Basket, JNK and JNK-protein 1 interaction, among others, are known to be present in bees[48].

### *JAK/STAT signaling pathway*

The JAK/STAT (Janus-family tyrosinkinases [JAK]/transcription activator proteins [STAT]) signaling pathway in insects is involved in synthesis of effectors similar to the complement system, as well as in proliferation and induction of phagocytosis by blood cells, and antiviral responses[8]. In higher vertebrates, this signaling pathway is essential for the synthesis of many cytokines. It is a relatively fast signaling pathway since it directly phosphorylates STATs, which are dimerized transcription factors. These are transported to the nucleus where they stimulate expression of genes that can be induced by the receptor ligand. The only protein that seems to be completely absent in the bee is the JAK /STAT signaling pathway ligand.

In bees, there are five *Drosophila* homologue genes for JAK/STAT pathway components: 1) DOMELESS cytokine receptor (*dom*), 2) JAK tyrosine kinase (*hopscotch*), 3) STAT92E transcription factor, 4) negative pathway regulatory proteins such as suppressors of cytokine signaling (SOCS), and 5) protein inhibitor of activated STAT (PIAS). This pathway ends with deregulation of the genes encoding for immune system humoral effectors; for example, the various thioester-carrying proteins (TEPs) in bees. However, no *tot* genes have been identified, which in *Drosophila* encode for humoral effectors as a result of severe stress and are produced by activation of this pathway[49,50]. In bees, there are also two component orthologues of this pathway: the tyrosine phosphatase Ptp61F and WD40[8]. Although the

key ligand for JAK/STAT is unknown, the presence of the cytokine receptor *Domeless*' counterpart, in addition to the presence of other JAK/STAT components, indicate it to be a common mechanism in insects, appearing intact in bees and fruit flies.

***RNAi signaling pathway***

Recognition of VAMPs in bees has been linked to the RNA interference system (RNAi), a physiological mechanism for gene silencing that also functions as a defense mechanism against viral infections by silencing the virus replication cycle. The main RNAi pathway components exist in viral infections in bees; during this process, double-stranded RNAs (dsRNA) are recognized by a dsRNA sensor produced by the *dicer-like* gene in bees[51]. This sensor is related to the PRRs family or RIG-1 cytosolic sensors in mammals (*dicer*). Once DICER cuts the dsRNA, the resulting small dsRNA fragments, known as small interfering RNAs (siRNA) and microRNA (miRNA), are recognized by the RNA-induced silencing complex (RISC). The latter contains proteins of the AGO2 family (*argonaute-2*)[51], which it transforms into small single-stranded RNAs (ssRNA). These small ssRNA bind to mRNA transcripts, which contain complementary sequences, thus preventing protein synthesis. Activation of this pathway in bees results in increased expression of the *vago* gene, an orthologue found in *Drosophila*, resulting in suppression of viral replication[47,52]. Another epigenetic mechanism in bees with antiviral function is DNA methylation, which is part of the antiviral response[52].

## Immune response effectors

Recognition of pathogen PAMPs or MAMPs by PRRs, which activates the different signaling pathways, ends with the synthesis or activation of cellular and/or humoral effectors of the immune system. While AMPs are the main post-infection induced effectors, transferrin has been identified in bees and other insects. In higher vertebrates, transferrin is part of the acute phase proteins group, whose immune function is to sequester iron and thus limit bacterial infection[53,54]. Like *Drosophila* and *B. mori*, honey bees have three members of the transferrin family[55], and their expression pathways would be Imd and Toll[9]. Activation of the JAK-STAT signaling pathway results in synthesis of other innate immune system effectors, such as TEPs, which have the C3 fraction of the complement system, a characteristic thioester bond of their counterpart in higher vertebrates. This characteristic bond allows activated proteins to covalently bind to the surface of microorganisms and trigger an immune response[12].

In *Drosophila*, these proteins are synthesized by the fat body, while in *Anopheles*, they are produced by hemocytes. In the latter, direct evidence has shown the relationship of TEPs to

the protein recognition function in microorganisms and their participation in phagocytosis of Gram-negative bacteria; they are consequently equated with opsonins. Only four C3 counterpart genes encoding for TEPs have been found in the bee genome, compared to 15 in the *Anopheles* genome and six in *Drosophila*[8,56,57].

Serin proteases (SP) are enzymes involved in various physiological processes such as digestion, development and immune response. Synthesized as zymogens, they participate in activation cascades that result in synthesis of effectors. In mammals the best known representatives of this protein family with immune function are those involved in the coagulation cascade and complement system; in invertebrates, they participate in the acute phase response[8,58]. Of the 57 SP-related genomic sequences in the bee genome, 44 correspond to SP and 13 to SP homologues. As is the case with many other genes[8], the 57 SP-related sequences in bees pale before the 204 sequences of *Drosophila*[59], and the 305 of *Anopheles*[60].

In bees, the Toll signaling pathway recognizes putative *snake* and *eater* orthologues related to Spaetzle splitting and pathway activation, which results in the synthesis of effectors such as DROSOMICINE, as occurs in the fruit fly. Bees also have SP genomic sequences similar to other insects, which are related to the prophenoloxidase activation cascade[58].

The last regulatory mechanism is that of the SERPINES, which are highly conserved proteins present in the insect hemolymph. These proteins are responsible for eliminating excess protease, maintaining homeostasis, and preventing unregulated activation of immune responses such as melanization or synthesis of the Toll-mediated antimicrobial proteins[61]. Seven orthologues have been identified in honey bees, five of which encode SERPINES, the remaining two coding for SERPINE-type proteins[58].

## Social immunity

One characteristic of social insects in general, and of bees in particular, is their social life, sharing a nest. Nests usually contain food stores and a high density of individuals living in relative homeostasis. The nests of social insects are therefore attractive sites for the development of various infectious agents[62]. However, social insects have developed social immunity[11], which is characterized by cooperative behavior within a colony through different mechanisms, such as the following:

1) Social fever. Social fever results from bees generating additional heat in the nest. This mechanism is costly for healthy individuals but allows pathogen control in infected hosts. Raising the nest temperature favors the control of the pathogenic fungus *Ascosphaera apis*[63].

2) Grooming. Grooming is the ability of bees to remove external parasites from their bodies by using their mandibles and legs[36,64]. There are two types of grooming behavior, self grooming and social grooming. Social grooming, involves the collaboration of several individuals[65], but self grooming is more common than social grooming. Colonies in which a high proportion of workers express this trait are more resistant to infestations by the mite *Varroa destructor* than colonies in which fewer members express it. Moreover, the vigor with which a colony's workers carry out grooming is directly related to the number of mites they remove from their bodies[66,67]. Grooming behavior is influenced by genetic factors for which the degree of expression varies between honey bee colonies of different races and stocks[68,69]. In several studies, a gene (*Neurexin*) has been mapped and associated with this behavior[70,71].

3) Hygienic behavior. Hygienic behavior is the ability of worker bees to detect and remove diseased or parasitized brood (larvae and pupae) from comb cells[36]. This is a two-step defense mechanism. First, workers uncap cells containing diseased or parasitized larvae or pupae, and then remove them from the nest[36]. This social behavior is a defense mechanism that helps to control the fungus *A. apis* (causal agent of chalkbrood)[72], the bacterium *Paenibacillus larvae*[73](etiological agent of American foulbrood), and the mite *V. destructor*[68]. Bees of different genotypes vary in the level of expression of this behavior [73,74,75]. Hygienic behavior is influenced by a group of at least seven genes, meaning it has a more complex genetic coding than previously thought[74], and also appears to be inherited maternally[75].

4) Gathering and use of propolis. Bees collect propolis, resins of trees (mainly from conifers) that have antiseptic and antimicrobial properties. They use them essentially as a prophylactic measure. Propolis is used to coat the interior of brood cells or to mummify any invertebrates or small vertebrates that enter and die inside the colony, preventing or minimizing the development of pathogenic bacteria and fungi[64]. In addition, the presence of certain types of propolis inside the colony can promote the expression of genes of the bee immune system[3,76].

5) Decreased contact between congeners. Individuals express this type of altruistic behavior when sick by moving away from the colony to die outside the brood nest[77].

6) Offspring cannibalism. In stressful situations that can cause brood death (e.g. lack of food, extreme temperatures), nurse bees usually cannibalize dead brood to prevent the development

of pathogenic microorganisms such as *A. apis*. This mechanism also prevents loss of nutrients from the colony.

As a defense strategy, social immunity substantially lowers pressure on the immune system of individual bees, thus reducing the number of genes required for defense against infection when compared to the Diptera. This may explain why *A. mellifera* possesses just one-third of the recognition and immune effector signaling genes of *Anopheles* and *Drosophila*[8, 9,11].

# Conclusions

Honey bees possess an innate immune system, also known as individual immunity. This system includes physical barriers, as well as cellular and humoral responses, which are generalist in nature and allow them to defend themselves against a wide variety of infectious and parasitic organisms. In addition to the various pathogens affecting bees and activating their immune system, xenobiotics such as acaricides, fungicides, herbicides and pesticides, may also exercise effects on bee health and the immune system. Defense mechanisms involve signaling pathways, pathogen recognition receptors and innate immune system effectors.

The high-density conditions of honey bee nests, coupled with the presence of food stores, makes them attractive for different pathogens. However, these conditions also promote social immunity, characterized by cooperative behavior within the colony by means of different mechanisms such as social fever, grooming behavior, hygienic behavior, and collection and use of propolis, among others. Social immunity is a defense strategy that greatly diminishes pressure on the immune system of individual bees, resulting in fewer genes related to defense. This may explain why *A. mellifera* has one-third of the genes linked to recognition and immune effector signaling compared to *Anopheles* or *Drosophila*. The immune system of *Apis mellifera* is influenced by multiple factors, such as pathogens and pesticides, highlighting the importance of continued study of the effects these factors have on immune responses. Future research should focus on studying immune system molecular mechanisms, as well as on the potential application of certain effectors for treatment and/or prevention of pathologies and diseases.

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