Chapter

The Innate and Adaptive Immune System of the Common Bed Bug, *Cimex lectularius*: Current Knowledge and Research Opportunities

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Abstract

The common bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), is a blood-feeding ectoparasite of vertebrates, primarily humans. In contrast to many other hematophagous arthropods, such as kissing bugs, mosquitoes, sandflies, and ticks that intermittently seek blood meals from vertebrate hosts, *C. lectularius* does not vector disease-causing pathogens and parasites to their human hosts. In this review, we summarize currently known immune responses by *C. lectularius,* and propose worthy research topics. Challenged by microbe ingestion or infection, *C. lectularius* mounts cellular immune responses such as phagocytosis of bacteria, as well as humoral responses such as secretions of antimicrobial peptides into the hemolymph. The functional immune system of the hemimetabolous *C. lectularius* resembles that of holometabolous insects but exhibits distinct deviations, including a sparser immune repertoire, the production of DNA nets by cells in response to pathogen invasions, and reproductive immune anticipation in the context of sexual reproduction (traumatic insemination). Many components of the *C. lectularius* immune system still await discovery, including the receptor molecules and immune pathways involved in antiparasitic and antiviral immune responses. Why *C. lectularius* does not vector pathogens to human hosts is hardly understood. Potential explanations include upregulated antimicrobial peptide activities that help eliminate invading pathogens.

Keywords: *Cimex lectularius*, bed bug, innate immunity, antimicrobial peptides, *Wolbachia*, traumatic insemination

1. Introduction

The insect immune system has two parts: the innate (general) immune system and the adaptive (specialized) immune system. The innate immune system is the first line of defense against invading pathogens and includes cellular and humoral responses that help detect and eliminate invasive pathogens without harming the

insects' own obligate microbiota [1, 2]. The adaptive immune system draws on immune memory from previous pathogenic attacks and prepares for more effective immune responses to subsequent infections [3]. The adaptive immune system enables pathogen-specific responses and relies on cells with specific receptors that recognize pathogens. The insects' true adaptive immune responses are not as elaborate as those of their mammalian counterparts, but in response to prior microbial insults insects display immunological priming which heightens humoral and cellular responses to subsequent insults and thus increases the likelihood of insect survival [3, 4].

Most of our knowledge about insect immunology stems from research on holometabolous model insects, such as the vinegar fly *Drosophila melanogaster* and the yellow fever mosquito, *Aedes aegypti*. Yet, the immunology of other insect taxa may be different [5–7]. For example, the immune pathways of hemimetabolous insects seem to have anomalies and to be sparser than those of holometabolous insects [8]. In this review, we focus on the immune system of the hemimetabolous common bed bug, *C. lectularius* (Hemiptera: Cimicidae), which is an obligate hematophagous ectoparasite, primarily of humans. We highlight immune responses of *C. lectularius* that deviate from those of the holometabolous immunological models.

The immune system of *C. lectularius* is thought to be unique and more efficient than that of most other insects. The extraordinary immune system of *C. lectularius* may have evolved in response to its peculiar mode of sperm transfer, known as hypodermic or traumatic insemination [9]. During traumatic insemination, the male pierces the female's abdomen with his aedeagus (penis), a sickle-shaped paramere, and injects his sperm and accessory gland fluids through the wound into her abdominal cavity (hemocoel). The sperm then diffuses through the female's hemolymph, eventually reaching her ovaries where fertilization takes place [9–11]. Traumatic insemination occurs more frequently than necessary for egg fertilization [12] and is detrimental to the female's health. It creates an open wound which is costly to repair and susceptible to infection. The injection of both sperm and ejaculatory fluids into the hemocoel may trigger immune responses by the female. As females commonly get mated after a blood meal, they anticipate traumatic insemination ('reproductive immune anticipation'), using blood feeding as a cue to 'prophylactically' produce lysozyme-like activity (LLA) in their spermalege [13], a pair of sperm-receptacles which may help provide hygienic protection against bacteria [11]. Copulatory wounding introduces pathogens to both male and female bed bugs [14].

Many hematophagous insects or arthropods, such as fleas, kissing bugs, mosquitoes, sandflies, tsetse flies, and ticks, that intermittently seek blood meals from vertebrate hosts, competently vector disease-causing pathogens and parasites, including bacteria, fungi, protozoa and viruses [15]. These pathogens or parasites cause debilitating diseases (e.g., Chagas, dengue, leishmaniasis, lymphatic filariasis, malaria) in humans [16] and millions of deaths each year [17]. In contrast, *C. lectularius* does not naturally (outside laboratory settings) transmit pathogens or parasites to vertebrate hosts [18–21], even though more than 40 disease-causing organisms have been recovered in or on tissue from *C. lectularius* [19]. There is no definitive knowledge as to why *C. lectularius* lacks vector competence, but potential explanations lie with the innate immune responses of *C. lectularius*. Here, we review aspects of immunity studied in *C. lectularius*, summarize findings about the *C. lectularius* immune system, and provide explanations as to why *C. lectularius* lacks vector competence outside controlled laboratory conditions.

2. The innate immune system of *C. lectularius*

As shown in other insects, the innate immune system of *C. lectularius* comprises multiple components that work in concert. These components are grouped in three broad categories (**Figure 1**): (1) physical barriers such as the cuticle and epithelia; (2) cellular responses including the elimination of pathogens through phagocytosis, nodulation, and encapsulation; and (3) humoral responses comprising the biosyntheses of antimicrobial peptides (AMPs) and lectins, and the activation of the prophenoloxidase (PPO) cascade. In all insects studied thus far, pathogen-associated molecular patterns (PAMPs) on the surface of pathogens are recognized by pattern receptor proteins (PRPs) as host sensors that initiate cellular and humoral responses.

2.1 Physical barriers

The insect integument (cuticle) serves as an exoskeleton, physical barrier, and first line of defense, preventing water loss, penetration, and invasion of chemicals and pathogens that interfere with the insect's homeostasis [22]. Prompted by surface injury, the outermost epicuticle produces AMPs and thus contributes to innate immune defense [23]. The cuticle is conserved across arthropods [24, 25]. It is made of chitin fibrils implanted in a matrix of proteins, lipids, and N-acylcatecholamines [24, 25]. Cuticular linings cover all external epithelial tissues, as well as the insects' foregut and hindgut.

The cuticle of *C. lectularius* differs from that of other insects in that it has two (instead of one) physical barriers with distinct regions of temperature-sensitive, lipid-based physico-chemical properties that protect against penetration of hydrophilic molecules [26]. The outer barrier constitutes cuticle protruding nano- and macrostructures [27], whereas the inner barrier consists of lipophilic waxes and free cuticular hydrocarbons [27–29]. These double barriers may provide fail-safe protection against penetration by external pathogens.

The peritrophic membrane covers and protects the midgut lumen of insects. It functions as a physical barrier against abrasive food particles and digestive pathogens [30], and it prevents various pathogens from readily passing into the hemolymph. In most hemipterans, the peritrophic membrane is absent and, instead, an extracellular lipoprotein membrane, the perimicrovillar membrane, protects the midgut epithelium. In *C. lectularius,* the functional role of this perimicrovillar membrane has not specifically been studied but its importance in hemipteran immunity has been discussed, and a protective function has been suggested for various hemipterans [31]. If microorganisms still pass through the midgut barrier into the hemolymph, humoral and cellular immune responses are activated [32].

2.2 Cellular immunity

2.2.1 Types of hemocytes in insects and C. lectularius

Hemocyte immune responses greatly vary among the many insect species, and we are just beginning to understand these variations [4]. However, in most species studied thus far, four frequent cellular immune responses have been described: nodulation, encapsulation, melanization, and phagocytosis. Cellular immune responses take effect immediately after pathogen invasions of the hemocoel, whereas humoral

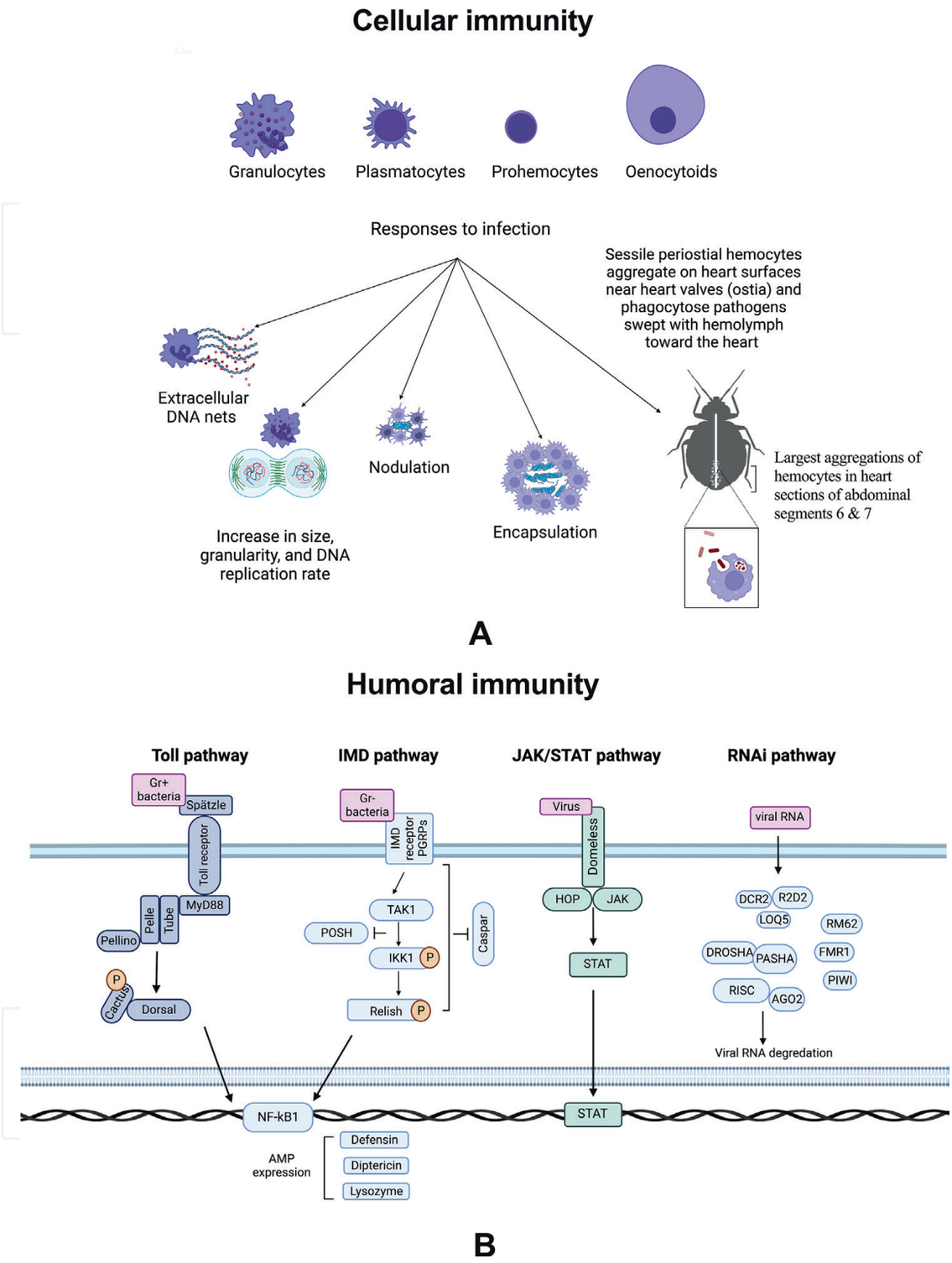

Figure 1.
Cellular and humoral immune responses of the common bed bug, Cimex lectalarius. **A.** *Cellular immune responses involve immune cells (hemocytes), such as granulocytes, plasmatocytes, prohemocytes and oenocytoids [21], that engage in phagocytosis, nodulation, and encapsulation of pathogens, and that form extracellular DNA traps in response to pathogen infections [21, 45].* **B.** *Illustration of the orthologs of the Toll, IMD, JAK/STAT, and RNAi pathways functional in C. lectalarius and portrayed based on general immune pathways in insects [5]. This figure was created with BioRender.com.*

responses appear several hours later [33]. Cellular immune responses are carried out by specialized immune cells, the sessile and circulating hemocytes. Sessile hemocytes are associated with specific tissue and adhere to internal organs such as the insect's

fat body and digestive tract, whereas circulating hemocytes are moving in the hemolymph [33]. The density of hemocytes in the hemolymph varies during the life of an insect and in response to pathogen invasions [33]. Like human immune cells, insect hemocytes recognize foreign particles and distinguish self from non-self during cellular immune responses [34].

Insect hemocytes are classed according to their form or function but classes are well characterized only in *Drosophila* by establishment of molecular markers [35]. Not all hemocyte types occur in all insects but at least eight classes of insect hemocytes are characterized to date: prohemocytes, plasmatocytes, granular cells (granulocytes), coagulocytes, crystal cells, spherulocytes, oenocytoids, and thrombocytoids. Prohemocytes are circular and contain a large nucleus. They are thought to be equivalent to stem cells and are the smallest of all hemocytes. In insects, plasmatocytes, along with granulocytes, are the most common cell type, performing distinct immune functions. Plasmatocytes contain lysosomal enzymes in their cytoplasm and their major function is phagocytosis [36]. Granulocytes are characterized by the presence of secretory vesicles (granules) in their cytoplasm and are involved mainly in phagocytosis in lepidopterans [36–38]. During the immune process of degranulation, granulocytes release antimicrobial, cytotoxic or other immune molecules from their granules. In lepidopterans, plasmatocytes and granulocytes are involved in most cellular defense responses [37], whereas in *Drosophila* plasmatocytes and lamellocytes mount equivalent responses [37]. In lepidopterans, oenocytoids are major producers of the enzymes that are required for melanization [37, 38]. Coagulocytes contribute to hemolymph coagulation (clotting) [39]. Spherulocytes are characterized by conspicuous spherical inclusions (spherules) in their cytoplasm. They have different shapes and sizes and perform different functions such as coagulation and transport of cuticular materials [39–41]. Finally, crystal cells are present in only some insects. In *Drosophila,* they contain the key enzyme PPO that mediates the biosynthesis of melanin (melanization; see below) [42].

There are only a few reports on hemocytes in the *Cimicidae*. *In vitro* studies of circulating hemocytes in nymphal *Cimex rotundatus* revealed six types of hemocytes: prohemocytes, plasmatocytes, granulocytes, spherulocytes, oenocytoids and thrombocytoids (a variant form of granulocytes and a functional analogue of coagulocytes) [43]. Plasmatocytes and granulocytes are considered the major circulating cells in *C. rotundatus* nymphs and adults, respectively [43]. In *C. lectularius,* circulating hemocytes were classed based on their size, morphology, and staining properties [21]. Granulocytes and plasmatocytes were the most abundant cells, and granulocytes, plasmatocytes, prohemocytes and oenocytoids – but not thrombocytes – were found in adult *C. lectularius* (**Figure 1A**) [21], with hemocyte granularity in females being significantly greater than in males [21]. Giant cells such as oenocytoids are present in *C. lectularius* but are rare in triatomine bugs [44]. Furthermore, experimental infection of *C. lectularius* and yellow fever mosquitoes, *A. aegypti,* with the Gram-negative bacterium *Escherichia coli* induced migration of hemocytes to periostial surfaces of the heart, thereby amplifying immune responses [45]. *Cimex lectularius* infected with *E. coli* had 2-times more heart-associated hemocytes than untreated control specimens [45]. In the innate immune system of *C. lectularius,* the role of each hemocyte type in response to different pathogens has not been specifically characterized. Determining the specific hemocytes associated with melanization, encapsulation, and nodulation would greatly enhance our understanding of the *C. lectularius* immune system.

2.2.2 Degranulation and size increase of C. lectularius hemocytes in response to infection

Degranulation, increase of hemocyte size, and greater DNA replication rates, are hallmarks of immune cell activation [32, 46–48], and were observed in male and female *C. lectularius* [21]. Sessile and circulating hemocytes of *C. lectularius* underwent cell divisions in response to bacterial exposure [49]. Moreover, hemocytes of male and female *C. lectularius* transitioned from the G0/G1 phase to the S- and G2-phase when exposed to heat-killed *E. coli* [21], thus confirming the activation of cell division as an immune response. All observations combined provide evidence for immune cell activation in *C. lectularius*.

2.2.3 Phagocytosis

During the process of phagocytosis, hemocytes engulf targets such as bacteria, yeast, and apoptotic bodies, and even small experimental artifacts such as synthetic beads or India ink particles [50, 51]. In insects, it is the plasmatocytes and granulocytes that serve a phagocytic function [4], but in mammals the phagocytic neutrophils serve that function [52]. In silkworms, *Bombyx mori*, activation of the PPO cascade is necessary for granulocytes to attach to pathogens and to initiate phagocytosis [53, 54]. After an immune challenge, only the plasmacytes of the triatomine bug *Rodnius prolixus* engaged in phagocytosis [55], whereas several (unspecified) types of *C. lectularius* hemocytes phagocytosed heat-killed *E. coli* [21]. Sperm of male *C. lectularius* that failed to travel to the periphery of the female's spermalege is phagocytosed by hemocytes or attacked by lysozyme [56]. Furthermore, experimental injection of *C. lectularius* with *E. coli* induced aggregation of hemocytes on the tubular heart and rapid phagocytosis of *E. coli* circulating with the hemolymph [45]. Consistent with hemocyte aggregation patterns reported in both *Rhodnius prolixus* [57] and the boxelder bug, *Leptocoris trivittatus* [58], *C. lectularius* hemocytes aggregate mainly on the section of the heart that resides in abdominal segments 6 and 7, near the mesospermalage in abdominal segments 5 and 6, where traumatic insemination takes place [12, 45]. The *C. lectularius* genome contains candid phagocytosis-related genes [5] that still need thorough exploration.

2.2.4 Melanization, nodulation and encapsulation

Melanization, the biosynthesis of melanin, is a prominent immune response in insects and arthropods [38, 40, 69] and serves multiple roles, including the encapsulation of pathogens and parasites, wound healing, and the production of cytotoxic chemicals that kill invading microorganisms [38, 45, 59–63]. Melanization contributes to the elimination or killing of bacteria, fungi, protozoan parasites, nematodes, and other organisms that have invaded an insect body. Melanization involves multiple components, including PRPs as host sensors that initiate cellular and humoral responses, serine protease cascades, and a phenoloxidase enzyme. Ultimately, melanin surrounds and sequesters an invading pathogen. The pathogen's death is thought to be caused by oxidative stress via reactive oxygen species or by starvation, achieved by isolating the pathogen from nutrient-rich hemolymph [60, 61]. In the spermalege and hemocoel of female *C. lectularius*, melanotic and cellular encapsulation in response to heat treatment was measured [64].

Nodulation (the aggregation of hemocytes around microorganisms) is the main insect defense response to eliminate large cohorts of bacteria that have invaded the hemolymph. In the process of nodulation, an overlapping sheet of hemocytes forms and surrounds pathogens [36]. Nodulation may further involve melanization and the activation of the enzyme PPO [38]. The end products of the phenoloxidase cascade are melanin and toxic byproducts such as free oxygen species, phenols and quinines, that may kill pathogens or prevent further growth [38, 63].

Encapsulation resembles nodulation but targets larger objects in the hemolymph, such as parasitoid eggs and larvae as well as nematodes [65]. Cellular encapsulation is common in dipterans, occurs with or without hemocyte assistance, and always involves PPO activation [59]. Humoral encapsulation, in contrast, occurs mainly in lepidopterans and can take place without melanization [65]. The volume of encapsulating cells and the degree of melanization in the spermalege and hemocoel of *C. lectularius* were measured in response to *Wolbachia* load [64].

Genes encoding encapsulation and nodulation processes as well as melanization/prophenoloxidase (PPO) pathways are conserved across many insect taxa and have also been identified in the genome of *C. lectularius* [5]. An increase in two PPO-like transcripts was shown in *C. lectularius* hemocytes exposed to heat-killed *E. coli* [21]. The activation of these genes should also be studied in response to other pathogens.

2.2.5 Extracellular DNA traps

As only recently shown, *C. lectularius* hemocytes form extracellular DNA traps in response to pathogenic infection [21]. These traps resemble the mammalian neutrophil extracellular traps (NETs) [66]. In mammals and some insects, the controlled release of chromatin (complex of DNA and protein) from the nucleus of hemocytes provides scaffolds, with hemocyte-mediated defense factors, that trap and eliminate microorganisms [67, 68]. Extracellular DNA traps have been found in only a few insect species. The effect of these DNA traps on specific pathogens, and the immune pathways that are activated in response to these pathogens need to be explored.

3. Humoral immune responses by *C. lectularius*

Insect humoral defenses include the biosynthesis of AMPs [69, 70] and reactive intermediates of oxygen or nitrogen [71, 72] as well as activation of complex enzymatic cascades that regulate coagulation or melanotic encapsulation of parasites and pathogens [73, 74]. PRPs of insect hosts detect PAMPs on the surface of pathogens and activate nuclear factor-κappa B (NF-κB) transcription factors that are involved in immune responses such as AMP expression. Pathways involved in this mechanism include the immune deficiency (IMD) pathway, the Toll pathway, and the JAK/STAT pathway [38].

The genome of *C. lectularius* contains strong candidate genes for recognition molecules, which are the key members of the Toll, IMD, Jak/STAT and RNA interference (RNAi) immune pathways that have been thoroughly characterized in model insects (**Figure 1B**) [5]. Compared to holometabolous insects, the immune repertoire of *C. lectularius* appears sparser for Toll, IMD, and Jak/STAT pathways [8], and only a few recognition molecules and AMPs have been identified. However, this paucity of immune components reported for *C. lectularius* may be attributed to inadequacies

of standard automated genome annotation programs. In analyses of hemipteran transcriptomes, many immunity-related genes may have been overlooked [75] due, in part, to the presence of multiple short introns and sequence divergence between holo- and hemimetabolous insects [75].

Furthermore, tissue- and pathogen-specific upregulation of immune pathways in *C. lectularius* has only recently received attention. In *C. lectularius,* upregulation of effector genes in Toll and IMD pathways has been studied only in hemocytes in response to *E. coli* infection, revealing no significant upregulation of most of the key components of these pathways [21]. In addition, NF-κB signaling inhibitors – that inhibit Toll and IMD pathways with high specificity in other organisms – were ineffective in *C. lectularius*, and only one inhibitor (IKK16) of NF-κB signaling significantly enhanced the rate of *C. lectularius* mortality from oral infections with the bacterium *Pseudomonas entomophila* [76]. Despite these anomalies, AMPs normally regulated by the IMD pathway are still expressed in *C. lectularius*, suggesting that the IMD and Toll pathways are functional [77, 78]. Further research on *C. lectularius* is needed to study pathogen-specific responses of multiple immune pathways and potential cross-talk between pathways, as shown in triatomine bugs and other insects [79].

3.1 Lysozyme-like activity (LLA), lysozymes, antimicrobial peptides (AMPs) and AMP activity in *C. lectularius*

Immune responses such as LLA and AMP activity have been studied in the midgut, hemolymph, fat body, and reproductive organs of female and male *C. lectularius* (**Table 1**).

Tissue	Key findings/topics studied	References
Entire body	Lysozyme-like genes, defensin-like peptides, diptericin-like peptides in males	[5]
Entire body	Antimicrobial peptide activity against Gram-positive and Gram-negative bacteria 24 h after blood ingestion or bacterial immune challenge in males and females	[81]
Saliva	Lysozyme peptides	[80]
Midgut	Antimicrobial peptide activity against Gram-positive and Gram-negative bacteria 24 h after blood ingestion or bacterial immune challenge in males and females	[81]
RoB[a]	Antimicrobial peptide activity against Gram-positive and Gram-negative bacteria 24 h after blood ingestion or bacterial immune challenge in males and females	[81]
Spermalege	Predictable infradian feeding cycles serve females as cues to impending immune insults by males during traumatic insemination	[13, 14]
Ejaculate & sperm	Lysozyme-like activity in males	[80]
Hemolymph	Lysozyme-like activity in males and females	[80]

[a]*RoB = Rest of Body (containing bodies minus the heads and midgut tissues).*

Table 1.
List of key findings, topics studied, and body tissues analyzed in immune response experiments with female and male bed bugs, ***Cimex lectularius****.*

3.1.1 Lysozyme-like activity and lysozymes

Immune response-induced LLA- and AMP-activities in the copulatory organ (spermalege) of female *C. lectularius* are well documented. With predictable infradian feeding cycles that serve as cues to imminent immune insults by males during traumatic insemination, females increase their LLA-activity in the spermalege [13]. Similarly, in response to bacterial transfers from males to females, females increase their LLA-activity [14]. While females receive bacteria from males, they also transfer bacteria to males [14]. There are at least three indications for mutual sexual transmission of bacteria between mating partners: (1) copulation increased the similarity of bacterial communities in male and female reproductive organs; (2) mated bed bugs harbored bacteria that were found in non-mated opposite-sex bed bugs but not in non-mated same-sex bed bugs; and (3) bacterial communities showed a high turnover between non-mated and mated bed bugs, suggesting a mating-induced replacement of bacteria [14]. Furthermore, the saliva of *C. lectularius* contains lysozyme and peptides with presumed antimicrobial activity [80]. LLA-activity was also shown in the sperm, seminal fluid and hemolymph of male *C. lectularius* [80]. Despite these reports of LLA activity in *C. lectularius*, and evidence for lysozyme-like genes in the genome of *C. lectularius* [5], the upregulation of transcripts in various tissues of *C. lectularius* has hardly been studied.

3.1.2 Antimicrobial peptide activity and defensins

There was time-dependent AMP activity in midgut and rest of body (RoB) samples (containing bodies *minus* the heads and midgut tissues) obtained from *C. lectularius* that were blood-fed or immune-challenged with Gram-positive and Gram-negative bacteria [81]. AMP activity was significantly upregulated in both midgut and RoB samples taken 24 h post infection with Gram-positive and Gram-negative bacteria, or 24 h post blood-feeding. The stronger immune response by females than males within 24 h of a blood meal [81] is likely an adaptation to battle immune insults caused by traumatic insemination. Cross-taxa studies revealed that blood-feeding induced stronger whole-body antimicrobial activity in female *C. lectularius* than in female *A. aegypti* [81], consistent with larger *E. coli*-induced aggregations of phagocytic hemocytes on the hearts of *C. lectularius* than on the hearts of *A. aegypti* [45].

In insects, AMPs are upregulated in response to bacterial exposure or blood feeding, and are produced in specific body tissues, including the intestinal tract, fat body cells, tracheae and hemocytes [82]. Of all identified AMP groups, only a few AMP-like genes/peptides and their variants have been identified from the genome or transcriptome of *C. lectularius* [5]. These include defensin-like peptides, diptericin-like peptides and lysozymes.

Defensins are small, variable cationic arginine-rich peptides [83]. They are ancient natural antibiotics with strong antimicrobial activity against a range of microorganisms [83]. More than 300 defensins have been identified but they are not specific to insects. The *C. lectularius* defensin (CL-defensin) is the only AMP that has been characterized for its effectiveness against human skin microbial flora including Gram-positive and Gram-negative bacteria [84]. Defensin-like transcripts increased in *C. lectularius* hemocytes in response to immune challenges with Gram-negative *E. coli* [21]. Diptericin-like peptides in the genome of *C. lectularius* [5] belong to a family of glycine-rich antibacterial peptides that are effective against Gram-negative bacteria [83]. The presence of diptericin-like transcripts has been illustrated in *E. coli*-infected

C. lectularius hemocytes [21]. Tissue- and bacteria-specific upregulation of defensin-like and diptericin-like genes needs to be studied further in *C. lectularius*.

4. Immunity-related effect of symbionts in *C. lectularius*

The microbiome is defined as the collection of microorganisms, their genomes, and the surrounding environment [85]. Within the last decade, the importance of the microbiome has become increasingly evident, both in humans [86] and in insects [87]. Insect immunity can no longer be considered in isolation of the insect microbiota and symbionts. The endosymbiotic relationship of microorganisms living inside a dissimilar organism (host) ranges from mutualism (both host and symbionts benefit from the relationship) to parasitism (symbionts benefit to the detriment of the host) [88]. Bacterial endosymbionts can aid the immune system of insects in response to invading pathogens and can increase survival and reproduction of their insect hosts. Endosymbionts induce immune priming for subsequent pathogen invasions [89–92], produce AMPs [90], and outcompete invading pathogens for resources [91].

The midgut microbiota of mosquitoes is a determinant factor for their vectoral capacity or susceptibility [92]. For example, mosquitoes harboring the Gram-negative bacterium *Serratia odorifera* in their midgut are more susceptible to infections with the Chikungunya virus that causes Chikungunya fever in vertebrates [93], and mosquitoes harboring the fungus *Talaromyces* (*Penicillium*) *marneffei* are more susceptible to the Dengue virus that causes Dengue fever in vertebrate hosts [94]. Compared to other indoor pest insects, the microbiome of *C. lectularius* is unusually sparse [95], and its contribution to host immunity is largely unknown. Proteobacteria, an unclassified γ-proteobacterium, intracellular *Wolbachia* bacteria, and a *Pectobacterium* accounted for 98–99% of all bacteria present in the midgut of *C. lectularius* [96, 97]. Comparing the microbiota of kissing bugs, mosquitoes and bed bugs would provide insight whether the *C. lectularius* microbiota can affect the immunity and vectorial capacity of its host. Thus far, the role only of *Wolbachia* in the immunity of *C. lectularius* has been investigated.

4.1 Wolbachia-induced immunity in *C. lectularius*

Wolbachia (order *Rickettsiales*) are Gram-negative, α-proteobacteria residing inside vacuoles of their hosts' cells [98]. *Wolbachia* symbionts cannot grow outside host cells but can be maintained in cell-free medium for short periods of time [99]. *Wolbachia* symbionts do not naturally occur in *A. aegypti* yellow fever mosquitoes which are major vectors of pathogens that cause debilitating and deadly diseases in humans (e.g., Dengue fever, Chikungunya, Zika [100, 101]. Experimental infections of *A. aegypti* with *Wolbachia* bacteria reduced virus loads and virus transmission rates [102, 103], and lowered loads of the protozoan parasite *Plasmodium falciparum* which causes malaria in humans [100, 104]. In combination, these data suggest that *Wolbachia* symbionts upregulate the expression of host immune genes, and increase host resistance to pathogens by priming host immunity [91]. The specific mechanisms by which *Wolbachia* symbionts enhance host immune responses are not known but may involve melanization (see above). Experimentally infected with *Wolbachia,* the vinegar flies *D. melanogaster* and *D. simulans* as well as *A. aegypti* had higher melanization rates, suggesting that melanization is one of the mechanisms by which *Wolbachia*

boost the immune response of their insect hosts [105]. In *A. aegypti, Wolbachia* symbionts upturned the production of AMPs, defensin and cecropin [102].

The essential nutrients riboflavin and biotin are absent from blood, and *C. lectularius* cannot produce them *de novo* [97]. To obtain these nutrients, *C. lectularius* relies on a mutualistic relationship with *Wolbachia* bacteria. In both field and laboratory settings, all instar nymphs and adult females and males of *C. lectularius* harbor *Wolbachia* symbionts in a specialized organ, the bacteriome, which is attached to the gonads [97]. *Wolbachia* symbionts are essential to the reproduction and survival of *C. lectularius.* In their absence, eliminated by antibiotic treatment, the reproductive fitness of adult *C. lectularius* was lower, and egg and nymphal development was hindered [106]. *Wolbachia* symbionts spread through body tissues and are transferred to offspring via maternal transmission [97].

There is also some degree of *Wolbachia*-mediated immunity in *C. lectularius.* With experimentally lowered *Wolbachia* loads, the survival of *C. lectularius* in response to microbial challenge decreased [64]. Heat-induced reduction of *Wolbachia* loads had no effect on LLA in the spermalege and hemocoel and the volume of encapsulating cells, but it significantly decreased melanization responses in the spermalege which – normally – mitigate wounding and infection costs associated with traumatic insemination [107]. Conversely, *Wolbachia* may not lessen the impact of viral infections in *C. lectularius*. The titer of the *Feline calicivirus* virus did not differ between *Wolbachia*-positive and *Wolbachia*-negative groups of *C. lectularius* [96]. The mechanisms by which *Wolbachia* symbionts confer at least some degree of immunity to their *C. lectularius* hosts warrant investigation.

5. Bactericidal activity of alarm pheromone components of *C. lectularius* and *C. hemipterus*

Under stress, nymphs, females and males of *C. lectularius* and of the tropical bed bug, *C. hemipterus,* all emit the alarm pheromone components (*E*)-2-hexenal and (*E*)-2-octenal from their metathoracic scent glands [108–110]. The ratio and amounts of these components differ between nymphs and adults of both species, and nymphs – unlike adults – also emit 4-oxo-(*E*)-2-hexenal and 4-oxo-(*E*)-2-octenal [109]. Emission of these compounds may not only signal distress but also contribute to the microbial defense of *C. lectularius* and *C. hemipterus.* Antimicrobial activity of these aldehydes has been demonstrated in two studies. (*E*)-2-Octenal suppressed growth of the Gram-positive bacterium *Staphylococcus albus,* but not of the Gram-negative *E. coli* [111]. Nymphs and adults of the pentatomid hairy shield bug, *Dolycoris baccarum*, emit a blend of (*E)*-2-hexenal, (*E*)-2-octenal and 4-oxo-(*E*)-2-hexenal, with each blend component expressing dose-dependent antibacterial activity against Gram-positive *Micrococcus luteus* and Gram-negative *Methylophilus* spp.*, Pseudomonas* spp. and *Spirosoma* spp. [111].

Chronic infestations of *C. lectularius* affect the microbial community of household dust [95]. The microbiome of homes infested with *C. lectularius* differs from that of uninfested homes, but microbiome characteristics of uninfested homes return following eradication of *C. lectularius* [95]. Whether the antimicrobial activity of *Cimex* alarm pheromone components affects the microbial community of households, and the transmission and impact of bacteria to human hosts warrants investigation.

6. Conclusion

In this review, we summarize currently known immune responses by *C. lectularius* and propose worthy research topics. Challenged by microbe ingestion or infection, *C. lectularius* mounts cellular immune responses such as phagocytosis of bacteria and encapsulation of parasites, and humoral responses such as AMP secretion into the hemolymph. Infections also activate signaling pathways such as the Toll, IMD, and JAK–STAT pathways but the particular pathway that is activated by specific pathogens is still largely unknown. The functional immune system of *C. lectularius* resembles that of holometabolous insects but exhibits distinct deviations. They include a sparser immune repertoire, the production of DNA nets by hemocytes in response to pathogen invasions, and reproductive immune anticipation in the context of traumatic insemination. Many components of the *C. lectularius* immune system still await discovery. For example, the receptor molecules and immune pathways involved in antiparasitic and antiviral immune responses are yet to be discovered. Findings made in immune studies with holometabolic insects may serve as a guide to compare and confirm the functional role of candid immune molecules suggested by genome and transcriptome studies of *C. lectularius* [1, 5]. The definitive mechanisms that render *C. lectularius* incompetent vectors of disease-causing pathogens are hardly understood but seem to comprise – among others – upregulated LLA and AMP activity that help eliminate pathogens.

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