Developmental and Comparative Immunology xxx (2017) 1–10

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

The immune strategies of mosquito *Aedes aegypti* against microbial infection

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ARTICLE INFO

Article history:
Received 25 September 2017
Received in revised form
30 November 2017
Accepted 3 December 2017
Available online xxx

Keywords:
Aedes aegypti
Innate immunity
Arbovirus
Entomopathogenic fungus

ABSTRACT

Yellow fever mosquito *Aedes aegypti* transmits many devastating arthropod-borne viruses (arboviruses), such as dengue virus, yellow fever virus, Chikungunya virus, and Zika virus, which cause great concern to human health. Mosquito control is an effective method to block the spread of infectious diseases. *Ae. aegypti* uses its innate immune system to fight against arboviruses, parasites, and fungi. In this review, we briefly summarize the recent findings in the immune response of *Ae. aegypti* against arboviral and entomopathogenic infections. This review enriches our understanding of the mosquito immune system and provides evidence to support the development of novel mosquito control strategies.

1. Introduction

Mosquitoes require repeated blood feeding to achieve their nutritional requirements for reproduction, and this makes them effective vectors of many dangerous human diseases (Attardo et al., 2005; Schnitger et al., 2009). For example, malaria, transmitted by the *Anopheles* genus, resulted in almost half a million deaths in 2015 (WHO, 2016). Another serious epidemic, dengue fever, which is mainly transmitted by the yellow fever mosquito *Aedes aegypti*, causes over a hundred million cases annually. Zika virus (ZIKV), also transmitted by *Ae. aegypti*, has recently become a significant global health concern due to its rapid geographical expansion in 2015 (Rajah et al., 2016). A number of factors contribute to this serious situation, including the lack of effective vaccines, rapid development of drug resistance, and socio-economic problems in endemic countries. Therefore, it is necessary to explore effective strategies to control mosquito-borne diseases.

Vector insects control is the primary measure used to mitigate the spread of infectious diseases. However, insecticide resistance is one of the major obstacles to the control of insects pests (Wang et al., 2011, 2013). The long-term use of chemical insecticides in the past decades has increased resistance in vector insects. Additionally, heavy use of chemical insecticides has also seriously polluted the environment. A new approach for mosquito control is the use of bio-insecticides, such as entomopathogenic fungi, which are considered as safe and green alternatives to most chemical insecticides (Read et al., 2009). However, fungal pesticides require improvements due to their relatively low virulence and the immune defense reaction from the hosts (Fang et al., 2012). These require a deep understanding of the molecular interactions between pathogens and host defense.

Insects rely on their innate immune system to fight against invading bacteria, fungi, and parasites (Cirimotich et al., 2010; Hoffmann and Reichhart, 2002; Lemaitre and Hoffmann, 2007; Xing et al., 2017; Xiong et al., 2015). When microbes break through host's physical barriers (cuticle or epithelium of midgut) and reach the hemocoel, the host pattern recognition receptors (PRRs) identify pathogen-associated molecular patterns (PAMPs) located on the surface of microbes, which then trigger cellular and humoral responses (Kanost et al., 2004). The host's cellular responses are mediated by several types of immune cells-hemocytes (Hillyer et al., 2003a; Lavine and Strand, 2002). Humoral immunity mainly includes two major inducible responses, the Toll and immune deficiency (IMD) pathway, which produce antimicrobial peptides (AMPs) via a signal transduction cascade, melanin and reactive oxygen species (ROS) (Kanost et al., 2004; Kumar et al., 2010; Lemaitre and Hoffmann, 2007).

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https://doi.org/10.1016/j.dci.2017.12.001
0145-305X/

Mosquitoes also use innate immune system to fight against microbes during their life cycles. As vectors of many diseases, mosquitoes are susceptible to pathogen infection and the innate immune system, including the production of AMPs and lysozymes, phagocytosis, and melanization plays an important role in limiting the virus to a non-lethal level (Xi et al., 2008). Recently, molecular biology and genomics tools have provided a direction for the study of mosquito immunity.

As one of the best characterized mosquitoes, *Ae. aegypti* can be easily reared in the laboratory, and has provided much of the knowledge on physiology, reproductive biology, and vector competence (Severson et al., 2001). In addition, *Ae. aegypti* is considered as a model organism (Clemons et al., 2010) for studies on hormone regulated reproductive biology, carbohydrate and lipid metabolism, and to understand the genetic basis of vector competence particularly the interactions between filarial worms and mosquitoes (Attardo et al., 2005; Chen et al., 2008; Hou et al., 2015; Severson et al., 2004; Wang et al., 2017a). Here, we present a comprehensive review of the recent research advances on the immune responses of *Ae. aegypti* against viral and fungal pathogens. This review will improve our understanding of the interactions between mosquitoes and pathogens at the molecular level and will help develop highly effective and specific biopesticides.

2. The immune system of *Ae. aegypti*

2.1. Overview of the Ae. aegypti immune system

Although lacking of acquired immunity, mosquitoes can kill a variety of prokaryotic and eukaryotic pathogens using their innate immune system (Dimopoulos, 2003). Insects have a powerful innate immune system, and whole genome sequencing provides an opportunity to study its origin and diversity. Genome sequencing of the most important vectors, *Anopheles gambiae*, *Culex quinquefasciatus*, and *Ae. aegypti* has allowed the systematic study of mosquito innate immune system and also the interaction between mosquitoes and pathogens (Bartholomay et al., 2010; Christophides et al., 2002; Dudchenko et al., 2017; Holt et al., 2002; Nene et al., 2007).

Comparative analysis showed that there are 476 immune genes in *Ae. aegypti*, compared with 536, 400, and 345 immune genes in *An. gambiae*, *C. quinquefasciatus*, and *D. melanogaster*, respectively (Chen et al., 2015). Thus, the number of immune genes has undergone a major expansion in *Ae. aegypti* (Chen et al., 2015; Waterhouse et al., 2007). There is high conservation among the main immune pathways mosquitoes and other insects. However, due to their complex lifecycles, mosquitoes are exposed to a variety of pathogens and may have acquired certain features during evolution due to the stress of these pathogens. Among the immunity-related molecules, the pattern recognition molecules, genes involved in the melanization pathway and effector molecules exhibit a major expansion, indicating that *Ae. aegypti* has a complex innate immune system.

2.2. Pattern recognition receptors of Ae. aegypti

More than 100 immune recognition molecules have been identified in the *Ae aegypti* genome using bioinformatics analysis (Waterhouse et al., 2007). The main families of immune recognition molecules include C-type lectins (CTLs), peptidoglycan recognition proteins (PGRPs), and β-1,3- glucan binding proteins (β-1,3-GRPs). CTLs comprise a large family of PRRs and have the capability to bind carbohydrates in the presence of calcium, which help in the recognition of a broad spectrum of microbes (Fujita and Endo, 2004). An array of immune functions have been proposed for insect CTLs, including activation of the melanization cascade (Yu and Kanost, 2000), encapsulation (Ling and Yu, 2006), nodule formation (Koizumi et al., 1999), and opsonization (Wilson et al., 1999). CTLs are both negative and positive regulators in the immune response of mosquitoes. In *An. gambiae*, two CTLs (CTL4 and CTLMA2) were identified to have protective effects during the invasion and development of *Plasmodium* parasites in the midgut (Osta et al., 2004). However, these two CTLs are indispensable for the clearance of Gram-negative bacteria in the hemolymph of *An. gambiae* (Schnitger et al., 2009). In *Ae. aegypti*, mosGCTL-1 was found to assist the infection of West Nile virus (Cheng et al., 2010). Moreover, a C-type lectin with a serine protease domain, CLSP2, in *Ae. aegypti* was reported to be involved in modulating the antifungal immunity as a negative regulator (Wang et al., 2015).

PGRPs were first identified in silkworm *Bombyx mori* (Yoshida et al., 1996), followed by other insects. They can recognize peptidoglycan (PGN), a main molecule on the cell surface of pathogens (Royet and Dziarski, 2007). There are two types of PGN, Lys-type and DAP-type. Lys-type PGN is mainly located on the cell surface of Gram-positive bacteria, and DAP-type PGN is usually associated with both Gram-negative and -positive bacteria. PGRPs are classified into two forms, short (S) and long (L) form. In *D. melanogaster* and *Tribolium castaneum*, when PGRP-SA, -SC1, -SD recognize the Lys-type PGN, the Toll pathway and melanization pathway are activated (Michel et al., 2001; Zou et al., 2007). PGRP-LB and -LC are essential for the immune response against DAP-type PGN-containing bacteria through the IMD pathway (Zaidman-Remy et al., 2006). In *Ae. aegypti*, 9 PGRP genes have been identified, among which, PGRP-LC, -SC2 and -LB were thought to be involved in the immune response against *Escherichia coli* and *Micrococcus luteus* (Wang and Beerntsen, 2015). GNBPs were initially found in *An. gambiae*, and could mainly bind to β-1,3-glucan and lipopolysaccharide on the surface of pathogens (Dimopoulos et al., 1997). In *D. melanogaster*, GNBP3 binds to the cell components of fungi and activates the Toll pathway (Gottar et al., 2006). In *Ae. aegypti*, the expression of GNBP1 was up-regulated during fungal infection, indicating that GNBP1 may be involved in antifungal immune response (Wang et al., 2015). However, studies on the functions of *Ae. aegypti* PRRs is limited and more studies are needed in this area.

2.3. Immune signaling pathways deciphered in mosquito Ae. aegypti

Studies have shown that in *D. melanogaster* the Toll, IMD, and Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways constitute the major components of humoral immunity (Dostert et al., 2005; Ferrandon et al., 2007). When the Lys-type PGN from Gram-positive bacteria or β-1,3-glucan from fungi are recognized by host PRRs, the Toll pathway is triggered (Lemaitre and Hoffmann, 2007). Activation of an extracellular serine protease (SP) cascade cleaves Spätzle (Spz), a cysteine knot cytokine. Two clip domain SP (CLIPs)-Persephone and Spätzle-processing enzyme (SPE) participated in this process (Jang et al., 2006; Ligoxygakis et al., 2002). The cleaved Spz binds to and activates the Toll receptor, trigerring the kinase cascade mediated by MyD88, Tube and Pelle. Activation of the intracellular signal cascade results in the phosphorylation and proteasomal degradation of Cactus, a negative regulator of the NF-κB transcription factors—Dorsal and Dif. Then, degradation of Cactus allows Dif and Dorsal translocation to the nucleus and leads to the expression of AMPs and other immune effectors (Lemaitre et al., 1996). When the DAP-type PGN is recognized by PGRP-LC, the IMD adaptor is activated, and the signal is transmitted into the cell vis the intracellular signal cascade that includs FADD, Dredd and Relish. The release of Relish leads to the production of AMPs (Ferrandon et al., 2007).

Please cite this article in press as: Wang, Y.-H., et al., The immune strategies of mosquito *Aedes aegypti* against microbial infection, Developmental and Comparative Immunology (2017), https://doi.org/10.1016/j.dci.2017.12.001