Trends in Parasitology

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Review

Mosquito Defense Strategies against Viral Infection

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Mosquito-borne viral diseases are a major concern of global health and result in significant economic losses in many countries. As natural vectors, mosquitoes are very permissive to and allow systemic and persistent arbovirus infection. Intriguingly, persistent viral propagation in mosquito tissues neither results in dramatic pathological sequelae nor impairs the vectorial behavior or lifespan, indicating that mosquitoes have evolved mechanisms to tolerate persistent infection and developed efficient antiviral strategies to restrict viral replication to nonpathogenic levels. Here we provide an overview of recent progress in understanding mosquito antiviral immunity and advances in the strategies by which mosquitoes control viral infection in specific tissues.

Trends

Mosquitoes are natural vectors that allow systematic and persistent arbovirus infection. The infection in mosquitoes is usually associated with few fitness costs, allowing the mosquitoes to transmit the virus efficiently. Mosquitoes have evolved systemic and tissue-specific antiviral mechanisms to limit viral propagation to nonpathogenic levels.

Mosquitoes ingest an arbovirus-infected blood meal into the midgut. The virus subsequently infects the midgut epithelial cells and spreads systematically through the hemolymph to other tissues.

RNAi and several conserved innate immune pathways play systemic roles against arbovirus infection of mosquitoes.

Specific antiviral strategies are armed in the mosquito midgut, hemolymph, salivary glands, and neural tissues for the control of arboviral propagation.

Mosquito-Borne Viral Disease: A Threat to Global Health

Mosquitoes are primary vectors for hundreds of human pathogens throughout the world. Mosquito-borne viruses are etiological agents of severe human diseases including hemorrhagic fever, biphasic fever, encephalitis, and meningitis. These viruses infect hundreds of millions of people each year and cause a large number of deaths [1,2]. Human viruses transmitted by mosquitoes are generally categorized into five genera: *Flavivirus* (*Flaviviridae* family), *Alphavirus* (*Togaviridae* family), *Orthobunyavirus* and *Phlebovirus* (*Bunyaviridae* family), and *Seadornavirus* (*Reoviridae* family) [3,4]. Dengue virus (DENV), Chikungunya virus (CHIKV), Japanese encephalitis virus (JEV), and West Nile virus (WNV) are the most prevalent arboviruses throughout the world [2,5]. For example, DENV transmitted by *Aedes aegypti* and *Aedes albopictus* is estimated to result in 390 million infections per year worldwide, 96 million of which manifest with apparent clinical symptoms [1]. In 2013, CHIKV, a member of the *Alphavirus* genus, emerged in 43 countries and territories in the Americas causing acute fever and arthralgia in more than 1000 000 suspected cases [5]. While the majority of mosquito-borne diseases occur in endemic tropical and subtropical regions, burgeoning international travel and expanded urbanization have increased their prevalence in new territories [6]. Unfortunately, there are no vaccines or therapeutics available for most mosquito-borne diseases. Together, these factors have led to rapid increases in endemics and epidemics over the past decade. A better understanding of mosquito–virus interactions may provide novel strategies to target virus transmission in nature.

Mosquito-borne viruses are generally maintained in a cycle between mosquitoes and vertebrate animals. After transmission to the hosts through mosquito bites, the viruses can rapidly replicate to a high level of viremia in the blood circulation system that is sufficient for acquisition by other mosquitoes taking a blood meal. The viruses subsequently infect the midgut epithelial cells and spread systematically through the hemocoel to other tissues, such as the salivary glands and neural system. Then, the infected mosquitoes are ready to transmit the virus to other hosts through bites [7–9]. In contrast to the severe diseases observed in vertebrates, persistent viral

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replication in mosquitoes neither results in dramatic pathological sequelae nor influences mosquito behavior or lifespan [10]. The infection in mosquitoes is usually associated with few fitness costs, thereby allowing the mosquitoes to transmit the virus efficiently [11]. Our knowledge of the mosquito immune system has advanced rapidly in the past decade and was aided by complete genome sequencing and annotation (https://www.vectorbase.org/). The advances in mosquito genomics and molecular biology have significantly facilitated the study of virus–mosquito interactions and antiviral mechanisms at the molecular level [12–16]. Mosquitoes have evolved efficient antiviral strategies to restrict viral replication to nonpathogenic levels. The mosquito antiviral mechanisms are very different from those of mammals. Unlike mammals, which have both innate and adaptive immune systems, mosquitoes lack immunoglobulin-based humoral responses and instead rely heavily on intrinsic intracellular antiviral mechanisms such as RNAi and analogous innate immune responses to limit viral propagation [17,18]. Knowledge gained over the past decade suggests that RNAi is an essential and systemic antiviral response in mosquitoes and other insects [17,19]. In addition to RNAi, mosquitoes also possess several immune machineries and organ-specific antiviral effectors against arbovirus infections (Figure 1, Key Figure). Dissection of antiviral immunity in mosquito tissues permissive for arbovirus infection will provide insights into the sophisticated interactions between mosquitoes and their transmitted arboviruses.

The Dissemination of Arboviruses in Mosquitoes

Mosquitoes ingest an arbovirus-infected blood meal into the midgut. After replication in midgut epithelial cells, the tracheal system or muscle may act as a conduit for viral escape into the hemolymph [8,20,21]. Subsequently, the virus spreads via the hemolymph circulation to the fat body, muscles, salivary glands, and neural tissue (Figure 1), while the amount of viral antigen and viral titers in the midgut may decline over time [8,21]. Once a mosquito is infected with an arbovirus, the infection can be persistent in its tissues and the infected mosquito can transmit the virus throughout its whole lifespan. The extrinsic incubation period (defined as 'the interval between the acquisition of an infectious agent by a vector and the vector's ability to transmit the agent to other susceptible vertebrate hosts' [22]) is an index that is representative of the kinetics and tropism of virus dissemination in its vector. The length of the extrinsic incubation period varies significantly with different arboviruses, mosquito species, and their combinations [23–25]. Mosquito antiviral immunity modulates the dynamics of viral dissemination and replication in tissues, thereby acting as a key determinant in the regulation of the extrinsic incubation period during arboviral infection.

Systemic Antiviral Strategies in Mosquitoes

Multiple studies have reported that RNAi and several other conserved innate immune responses such as the Toll, immune deficiency factor (Imd), and Janus kinase (JAK)–signal transduction and activators of transcription (STAT) pathways play systemic roles against arbovirus infection in mosquitoes (Figure 1A). The mechanisms regulating these immune pathways have been elucidated in *Drosophila*. There are orthologs of the core components of these pathways present in the genomes of major vector mosquitoes. Therefore, we hypothesize that the immune signaling pathways might be highly conserved between *Drosophila* and mosquitoes [12,14,26]. However, it is important to note that *Drosophila* is not a vector for arboviruses. Thus, the antiviral responses triggered by *Drosophila* viruses such as *Drosophila* C virus (DCV) may be different from the responses induced by arboviruses in mosquitoes.

The RNAi mechanism is an important antiviral response in invertebrates that comprises three independent pathways: siRNA, miRNA, and piwi-interacting RNA (piRNA) [27]. The antiviral role of the siRNA pathway is the most thoroughly studied because virus-derived siRNA is a potent and common antiviral immune response in mosquitoes and *Drosophila* [17,19]. The antiviral mechanism of the intracellular siRNA pathway is depicted in Figure 1 and systemically reviewed elsewhere [27,28]. Several recent studies have characterized the antiviral siRNA response to diverse arboviral infections in mosquitoes. Knockdown of the core components of the siRNA