Molecular Basis for Arbovirus Transmission by *Aedes aegypti* Mosquitoes

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Keywords

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Abstract

Mosquitoes are considered the most important vectors for the transmission of pathogens to humans. *Aedes aegypti* is a unique species, not only by its highly anthropophilic and peridomestic habits but also because it can transmit an important variety of pathogenic viruses. Examples are dengue, yellow fever, chikungunya, Zika, and Mayaro viruses. After ingesting viremic blood, a wide range of mechanisms are activated in the mosquito to counteract viral infection. Nevertheless, these arboviruses possess strategies to overcome barriers in the mosquito and eventually reach the salivary glands to continue the transmission cycle. However, the infection and eventual transmission of arbovirus depends on multiple factors. The current review focuses in detail on the anatomic, physiological, and molecular characteristics of the mosquito *A. aegypti* that participate in response to a viral infection. In the past decades, the awareness of the importance of this mosquito as a disease vector and its impact on human health was largely recognized. We need to improve

our comprehension of molecular mechanisms that determine the outcome of successful virus replication or control of infection for each arbovirus in the vector; this could lead to the design of effective control strategies in the future.

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Background

Arbovirosis refers to diseases caused by infections with “arthropod borne viruses.” Among the hematophagous arthropod vectors for viruses are mosquitoes, ticks, sand flies, and biting midges. Arboviruses cause diseases that have a great impact on public health and affect wild animals, livestock, and pets [1, 2].

These infectious agents predominantly contain RNA genomes, such as members of the Flaviviridae family (e.g., the *Flavivirus* genus), the Togaviridae family (e.g., the *Alphavirus* genus), and members of the Bunyavirales order, including: the La Crosse encephalitis (LACV) and the Rift Valley fever virus (RVFV) [3–5], and an *Asfivirus*, a DNA genome virus transmitted by an arthropod vector as well.

The high rate of mutation of RNA viruses enables their rapid evolution and probably host alternation. Selection processes in viral populations occur in both vertebrate and invertebrate hosts. The coevolution process involves these two types of organisms and definitely affects arbovirus fitness and transmission [6–9]. Vector control is a crucial part of the strategy to prevent transmission of these viruses, since approved vaccines exist to prevent only a small portion of these diseases [10–12].

Mosquitoes as Vectors

A considerable proportion of arboviruses are transmitted by mosquitoes. The respective diseases have increased in number in recent years and are gradually spreading towards previously unaffected areas. Among the factors that have favored the emergence of such diseases are climate change, globalization, lack of effective control over the vectors, resistance of the vectors to insecticides, and spreading of vector populations to new geographic regions. To date, over 500 arboviruses have been categorized, of which nearly 100 cause diseases in humans [3].

Mosquitoes of the *Culex* genus transmit West Nile virus (WNV), Japanese encephalitis virus (JEV), and Saint Louis encephalitis virus (SEV) [13]. However, *Aedes aegypti* mosquito alone is a vector for dengue virus (DENV) [14], the yellow fever virus (YFV), chikungunya virus (CHIKV), and Zika virus (ZIKV) [3, 5] (Fig. 1). Some arboviruses circulate among *Aedes* and *Culex* mosquitoes as well and affect non-human hosts, including horses, birds, rodents, and other small mammals. Some species could be “incidental hosts” or “dead ends,” meaning they do not amplify the virus sufficiently and therefore do not participate in transmission to a new mosquito vector, although viral infection in these species could be life threatening. This phenomenon is frequently observed with viruses like LACV, WNV, Venezuelan equine encephalitis virus, eastern equine encephalitis virus (EEEV), and JEV [4].

Another important factor to take into consideration is that some viruses are transmitted vertically, that is from infected female mosquitoes to their progeny (transovarially). Vertical transmission has been reported for Rift Valley fever virus [15], LACV, the Murray Valley encephalitis virus (MVEV), JEV, EEEV [4], ZIKV, and DENV [16–18]. Although vertical transmission in *A. aegypti* has been reported only for ZIKV [19], DENV [16, 20], and CHIKV [21–24].

Infection of *A. aegypti* Mosquitoes

A. aegypti mosquitoes participate in the transmission of YFV, DENV, CHIKV, and ZIKV, perhaps Mayaro virus (MAYV) [25]. The molecular interactions between a virus and mosquito cells start with the attachment of viral surface components to cellular receptor molecule. Receptors can either participate in the endocytosis of viruses, activate signaling pathways that facilitate entry, or induce changes in virus surface that finally drive to a penetration event [26].

For cell attachment, some viruses require more than one receptor to be taken up by the cell. In some cases, interactions with more than one receptor (assumed to act concertedly) are required for virus entry. It seems that the initial interaction between viral surface and cell occurs through attachment factors, and later becomes more specific [27].

For DENV, a number of putative cell receptors in mosquitoes have been reported [28–30]. Functional studies indicate that it is probable that several of these molecules, at some point, might be important for virus entry, but the identity of the specific receptor has remained elusive, partially due to lack of antibodies against mosquito proteins that could allow full characterization. Also, several studies on receptors have been carried out by overlay assays, which hinder the native state for the target protein, thus some interactions could not be genuine. But also, it is possible that some of these so-called receptors have lower affinity, while others exhibit higher affinity. The reasons underlying variability in specificity and affinity remain obscure, and a full portrait will probably require more studies on virus-receptors interactions in mosquitoes.

For CHIKV, two possible receptors have been identified in mosquito midgut, with a molecular weight of 60 and 38 kDa. [31]. For MAYV and ZIKV, no candidate receptors have yet been identified in the mosquito vector. The overall localization of the aforementioned potential receptors in the *A. aegypti* mosquito tissues and organs remains unknown.

During the extrinsic incubation period (EIP), a virus that enters the mosquito midgut and is able to pass into the hemolymph may infect fat body cells, trachea, hemocytes, ovaries, nerve tissue, and reach salivary glands [2, 18, 32, 33]. For an arbovirus to be transmitted to a new vertebrate host in a subsequent blood meal, it must first be secreted with the saliva by the acinar (epithelial) cells to the luminal side of the salivary glands.

Although there are reports on the specific quantity of virus ingested in infected blood, this is not necessar-

Fig. 1. Transmission of different arbovirus by mosquitoes. Different types of transmission cycles are shown with main host and incidental host. Also, animals participating in the zoonotic transmission are shown. VEEV, Venezuelan equine encephalitis virus;

WNV, West Nile virus; DENV, Dengue virus; CHIKV, chikungunya virus; ZIKV, Zika virus; LACV, La Crosse encephalitis; JEV, Japanese encephalitis virus.

ily a limiting factor for the infectious virus to reach the salivary glands [34]. Some researchers suggest that a minimum virus dosage will be necessary for *A. aegypti* to transmit viruses like DENV [35] or ZIKV [19] to a new host. Clearly, there is not a single determinant for pathogen transmission, but vector competence in each mosquito population must be one important factor [36].

Vector Competence and Vectorial Capacity

Vector competence is the intrinsic ability of the vector to successfully transmit a virus. Meanwhile vectorial capacity involves, in addition, some extrinsic factors such as temperature, availability of vertebrate hosts, vector's feeding behavior, population density, longevity, and predation [37, 38]. The interaction between *A. aegypti* and a virus is dynamic, related to the genetic background of each particular vector population and arbovirus variant.

Since temperature impacts virus replication rate, the EIP in the vector is affected by it. Thus, an increment in the temperature could shorten EIP enhancing vector ca-

pacity. For DENV-infected *A. aegypti* mosquitoes, a temperature of 35 °C results in a shorter EIP, while a drop to 21 °C causes a longer EIP [39–41].

Regarding time for transmission, experimental data show that CHIKV is found in the salivary glands as early as 2 days post-blood feeding (dpf) [42]. The time reported for DENV to reach this organ is 4 dpf [32], 10 dpf for YFV [43], and up to 14 dpf for ZIKV [44]. It is important to mention that these studies are hardly comparative, since studies using same *A. aegypti* mosquito population and equivalent viral titers must be necessary to draw final conclusions on arbovirus transmission times.

Infection with arbovirus might produce changes at the physiological, biological, and behavioral levels in the vector. *A. aegypti* mosquitoes infected with DENV-2 show an increase in the frequency and quantity of blood intake [45]. Changes with DENV clearly affect the physiology and impact behavior in *A. aegypti* due in part to the infection of olfactory organs that changes expression levels of OBP10 and OBP22 (odorant-binding proteins) [46]. Whether ZIKV affects fecundity or locomotor activity remains controversial [47, 48].

Fig. 2. Interplay among the different responses and barriers to infection. Mosquitoes possess different ways to respond to or counteract pathogens, mostly physical barriers and humoral and cellular responses with different effectors in each of these branches of the response.

Levels of Susceptibility to Viral Infection

Among the populations of *A. aegypti*, genetic variants might determine vector competence, resulting in mosquitoes that are more susceptible, partially susceptible and not susceptible to arboviral infections. Phylogenetic studies revealed a notable coevolutionary process occurring between circulating arboviruses and their coindigenous mosquitoes [49, 50].

Specific quantitative trait loci related to susceptibility to DENV infection have been identified in mosquito chromosomes. However, it remains unknown which genes located in these regions are responsible for these phenotypes [51]. Transcriptomic analysis has shown some differentially expressed genes in mosquitoes considered susceptible or resistant to DENV; these genes are either linked to metabolism (e.g., oxidative phosphorylation) or cellular processes. The latter include genes coding for proteins participating in endocytosis (V-ATPases), autophagy, and lysosomal activity [52, 53].

Another determining factor of susceptibility in the mosquito vector might be the microbiota in the midgut

[46], as reported also for various human pathogens [54, 55]. Mosquitoes with guts colonized by *Serratia odorifera* (a Gram-negative bacteria) exhibit an increased susceptibility to CHIKV [56]. In those infected by the fungus *Talaromyces*, a greater susceptibility for DENV is observed [57]. Another remarkable example of microbiota effect is *Wolbachia*. This Gram-negative obligated intracellular bacterium is maternally inherited and naturally infects *Culex quinquefasciatus* and *Aedes albopictus* mosquitoes, but not *A. aegypti*. However, evidence shows that infecting *A. aegypti* with *Wolbachia* drives resistance to DENV in the vector [58] and negatively impacts egg laying rates and lifespan [59].

In mosquitoes infected by DENV, other infection-limiting factors are related to gene expression of some cuticle proteins [52, 60], pro-apoptotic proteins (e.g., *Aedronc*, *Aedredd*, and *Casp-16*) [61], and molecules in the Delta/Notch pathway [62]. Cuticle protein function goes beyond structural; these do not only interact with chitin in the exoskeleton, but also some pupal cuticle proteins in adult *A. aegypti* mosquitoes seem to directly bind to envelope protein of WNV and inhibit infection in vitro [63].

Fig. 3. Signaling pathways participating in response to arboviruses at molecular levels. Response is orchestrated after recognition and response by different molecules. Main signaling pathways are Toll (a), IMD (b), JAK-STAT (c), and RNAi (d).

The Immune Response in *A. aegypti*

For mosquitoes, the first obstacles to pathogens are physical barriers, which seem to be anatomical, but more studies on them are still necessary. The first organ of contact for arboviruses is the mosquito midgut. A midgut infection barrier that impedes the establishment of infection in intestinal cells has been reported, as well as a midgut escape barrier that avoids the transfer of the arbovirus to the hemolymph and tissues [64]. There is also evidence of a barrier for infection in the salivary glands, and another against escape from this same organ [38, 65, 66] (Fig. 2).

When a mosquito confronts a viral infection, the cellular and humoral immune responses are regulated by the activation of four core signaling pathways, RNA interference (RNAi), Toll, IMD, and JAK-STAT [67] (Fig. 3). The principal effectors of the cellular response are hemocytes, which participate in phagocytosis, encapsulation, formation of nodules, melanization, and tissue repair. There are different types of hemocytes in *A. aegypti* [38, 68, 69], including granulocytes (phagocytes by nature), oenocytoids (involved in melanization), adipohemocytes, prohe-

mocyte, thrombocytoids, and plasmacytoids. Hemocytes are produced in the mosquito embryonic and larval stages and remain throughout adulthood [38, 70].

Regarding the humoral immune response, viral components interact with pattern recognition receptors and trigger a variety of processes such as the coagulation pathway in the hemolymph, melanization, and the production of various antimicrobial peptides [50, 71]. In *A. aegypti*, 18 genes have been characterized to codify for antimicrobial peptides, including attacin, cecropin (10 distinct types), defensin (A, C, D, and E), dipterecin, gambicin, and holotrocin [72, 73].

The RNAi Pathway

The RNAi pathway is central in the antiviral response in mosquitoes; thus, it is crucial for combatting viral infections [66, 74, 75]. This response involves the recognition and degradation of exogenous RNA; during replication of genomes for RNA virus, double-stranded RNA (dsRNA) intermediates are generated. The protein Dcr2, which contains RNase III domains, detects these dsRNA and cleaves them into small interfering RNAs (siRNAs) of 19–22 bp in length. These siRNAs bind to a multipro-

tein complex known as the RNA-induced silencing complex (or RISC), the siRNAs chains are separated, and the antisense sequence is used to match the exact complementary among all the mRNAs (which must correspond to viral RNAs) in the cell and degrade them.

Blocking the Dcr2 protein in *A. aegypti* resulted in an almost 10-fold increase in the viral titers of DENV-2 [76], indicating the importance of the RNAi pathway to limit infection. This pathway also takes part in limiting infections with CHIKV and the O'nyong'nyong virus (ONNV) [37]. RNAi pathway triggers production of Vago [66], which acts in the same way as vertebrate interferons.

Other RNA-targeting pathways that aid in controlling viral infections in mosquitoes are Piwi-interacting RNA (piRNA) [77] and micro-RNA (miRNA) [78]. Mosquitoes infected with CHIKV and DENV produce piRNAs, although the role of the latter in viral infections is not clear [37]. Among the responses triggered in *A. aegypti* by the *Wolbachia* symbiont is the production of diverse miRNAs, which might contribute to the inhibition of DENV replication observed in those *Wolbachia*-infected mosquitoes [79, 80].

The Toll Pathway

The Toll pathway is generally activated by peptidoglycan recognition proteins in response to fungi, Gram-positive bacteria, and virus. In *A. aegypti* mosquitoes, signaling of the Toll pathway triggers the activation of transcription factors, such as REL1 A/B, which are translocated to the nucleus and lead to the production of effector molecules, such as drosomycin [50], defensins, and cecropins [73]. Protein REL1 along with DIF (dorsal-related immunity factor) in *Drosophila* are orthologs of NF- κ B factor in mammals. Regulators for this pathway are Cactus (negative) and MyD88 (positive) proteins [71]. When MyD88 is silenced in *A. aegypti*, the infection by DENV is intensified in the midgut [81].

The IMD Pathway

Diptericin, attacin, and vago are effectors generated through the IMD pathway [82]. This response apparently limits the replication of the Sindbis virus (SINV) upon being activated by the microbiota of mosquitoes through the negative factor Caspar [83].

Vago protein seems to be activated by the IMD signaling pathway as well. Apparently, the resistance exhibited by the mosquitoes treated with the *Wolbachia* bacterium involves the secretion of this protein [84]. Subsequently, Vago binds to its receptor and triggers activation of the JAK-STAT signaling pathway [66, 85, 86].

The JAK-STAT Pathway

It has been demonstrated that the activation of the JAK-STAT pathway inhibits the replication of arbovirus like DENV and WNV [66]. A group of proteins denominated protein inhibitors of activated STAT are negative regulators of this pathway. Their activation leads to the generation of diverse effectors such as gambicin, cecropin, and defensin [87, 88]. It has also been reported that Aag-2 cells (a cell line of *A. aegypti*) increase quantitatively with the expression of the STAT transcription factor during infections by SINV [66]. The activation of the JAK-STAT pathway does not favor per se resistance to ZIKV or CHIKV but does indeed do so in relation to DENV [89]. When combined with the Toll pathway, the JAK-STAT pathway enhances resistance to ZIKV in *A. aegypti* [90].

Additional Factors Related to Infection in Mosquitoes

During the process of digesting a bloodmeal, pro-oxidant molecules are released, mosquitoes possesses activities to counteract this process and protect mosquito midgut. Nonetheless, protecting measures such as releasing catalase might facilitate infection of the epithelial cells in the midgut by viruses like DENV [91, 92]. Nevertheless, more studies will be necessary to establish the overall role of these events in arboviruses.

Surprisingly, infection of *A. aegypti* with arboviruses seems to give rise to the incorporation of DNA derived from the virus, which might cause tolerance in mosquitoes to infections with DENV and CHIKV [93] by a yet unknown mechanism.

Finally, there are other factors such as phagocytosis, autophagy, apoptosis, and conduits for infection (as trachea) that remain to be examined to determine their precise role in the vector infection by arboviruses (Fig. 4).

Discussion

A. aegypti is indisputably a vector of great importance for arboviruses, several of them of medical importance as mentioned before. This characteristic is due chiefly to its anthropophilic behavior [94]. In addition, intrinsic and extrinsic factors contribute to this trait.

In spite of numerous barriers that should be overcome for a successful infection in mosquito, some arboviruses reach salivary glands and accumulate in saliva to be trans-

Fig. 4. Effectors in the response to arboviruses. Among these participants are bacteria and several enzymatic activities, as well as conduits like trachea. AMP, antimicrobial peptides; ROS, reactive oxygen species; LMG, lumen of the midgut.

mitted in subsequent bloodmeals. To reach salivary glands, arboviruses might overcome natural barriers, infect various tissues, exploit physical conduits, and counteract innate and cellular defenses [32, 42–44].

The EIP may vary for each virus and mosquito population pair and the orchestration of transmission may depend upon genetically determined factors from both sides. A clear example for the mosquito determinants is a single mutant in CHIKV, E1 A266V, which confers a higher ability to infect *A. albopictus* [95]. Nevertheless, as mentioned before there are also determinants on the mosquito side determined by numerous genetic factors. Among the mosquito genes involved in infection with arbovirus are those encoding for immune response effectors, metabolism, proteolysis, etc. [46, 49, 96]. The intensity of mosquito immune response may affect the course of infection and transmission consequently.

The involvement of several immune response signaling pathways has been studied in detail [81, 97]. But in spite of these innate mechanisms, arboviruses make their

transit through the mosquito body and reach the salivary glands. SINV is one such case. It inhibits Toll-pathway signaling and antimicrobial peptide secretion in *A. aegypti* [50]. It is possible that the existence of mosquito genetic variants is related to these functions, which could affect the degree of susceptibility in mosquitoes. Another relevant factor for vector competence, as described in this review is microbiota [62].

The mosquito/virus interaction might have consequences not only in immune response but in the physiology of the vector; clearly, DENV-infected *A. aegypti* mosquitoes increased host seeking and feeding behaviors [46]. This event may have significant consequences on the epidemiology of the disease.

Moreover, the effect of coinfections on mosquito physiology has been addressed just recently [2, 98, 99]. Thus, so far we only have a partial view on the processes involved in vector susceptibility and transmission for arboviruses.

Conclusion

There is just a vague understanding of the mechanisms participating in the different levels of susceptibility to arboviruses in *A. aegypti* mosquitoes. Clearly, factors in *A. aegypti* are numerous and altogether determine vector infection. Although the entire picture of their impact on arbovirus transmission remains to be unveiled.

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The authors declare that they have no conflict of interest.

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