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The microbiome modulates arbovirus transmission in mosquitoes

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Abstract

Mosquito-transmitted arthropod-borne viruses (arboviruses) such as dengue virus, chikungunya virus, and West Nile virus constitute a major public health burden and are increasing in severity and frequency worldwide. The microbiota associated with mosquitoes (comprised of viruses, bacteria, fungi and protozoa) can profoundly influence many host phenotypes including vector competence, which can either be enhanced or suppressed. Thus, the tripartite interactions between the mosquito vector, its microbiota and the pathogens they transmit offer novel possibilities to control arthropod-borne diseases.

Introduction

It is becoming increasingly apparent that organisms do not function in isolation. A complex consortium of microbes resides within a host, which influences the individual's phenotype. This holds true for mosquitoes that transmit medically important arboviruses such as dengue virus (DENV), chikungunya virus (CHIKV), and West Nile virus (WNV). Interactions within the mosquito holobiome (host and associated microbes) can profoundly impact many phenotypes, including vector competence for pathogens. Here, we highlight recent advances in our understanding of how the microbial members of the mosquito holobiome influence arbovirus transmission. We focus our attention on bacteria with extracellular phases predominantly found within the mosquito gut, rather than obligate intracellular bacteria of mosquitoes that have been extensively reviewed elsewhere [1–3].

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Composition of the mosquito microbiome

Comprehending the diversity and dynamics of the microbial community is imperative in understanding the relationships within the mosquito holobiome. Studies exploiting high throughput sequencing have revealed that the bacterial microbiome of mosquito vectors has low diversity but is highly variable. Most studies thus far have focused on *Anopheles* mosquitoes [4•,5,6•,7••,8••,9], and while the number of studies examining the bacterial microbiomes from *Aedes* and *Culex* mosquitoes is growing [6•,8••,10], this is an area that needs further attention. Although not as comprehensive, culture dependent approaches have been used for characterization of the microbiome of important *Aedes* and *Culex* arboviral vectors [11,12]. Bacterial members that appear abundant across a range of mosquito species include aerobes and facultative anaerobes within the Gammaproteobacteria, Flavobacteria and Alphaproteobacteria [6•]. The variability within the mosquito microbial community appears to be influenced by environmental factors such as diet and host factors such as sex, species and developmental stage. While distinct differences are notable, there does appear to be some commonality, and in particular, *Aedes* and *Anopheles* vectors share common taxa including *Pseudomonas, Asaia*, *Serratia* and *Enterobacter* [6•,8••]. These bacteria are often located in the mosquito gut, meaning they are proximal to any ingested arboviruses, but they may also infect other tissues including the germline, salivary glands and malpighian tubules [13–15]. Intriguingly, the salivary glands of *Anopheles culicifacies* harbors a more diverse microbiota compared to the gut [15]. Whereas obligate intracellular symbionts such as *Wolbachia* are predominantly maternally transmitted, extracellular bacteria found in the gut likely have intracellular stages enabling their vertical transmission route (see [16] describing paternal transmission) and can also be horizontally acquired. In an elegant study, Coon *et al.* [8••] demonstrated that bacteria are transstadially transmitted in the important viral vector, *Aedes aegypti.*

While most studies have focused on bacteria, other organisms contribute to the species richness of the mosquito microbiome, including viruses, fungi and protozoans. Shotgun metagenomic sequencing offers a PCR-independent high throughput method to characterize these taxa and has been used to identify novel viruses within the *Bunyaviridae* and *Rhabdoviridae* in addition to many genera of fungi in the arboviral vector *Culex pipiens* [17]. Studies such as this fuel the growing appreciation that non-bacterial microbes contribute meaningfully to mosquito biology. To this end, insect-specific viruses have been discovered in several mosquito species [18–20], while culture dependent methods identified yeast species in *Aedes* and *Anopheles* mosquitoes [12,21]. Although the role of both insect-specific viruses and yeast in mosquito biology is unclear, there is evidence that these microbes can influence vector competence [22,23].

Influence on vector competence

The bacterial microbiome is a potent modulator of mosquito vector competence. While several studies have investigated the effect of the bacterial microbiome on *Plasmodium* infection in *Anopheles* mosquitoes [24,25], it is also evident the microbiome modulates arboviral vector competence. Most studies employ antibiotic treatment or reinfection of cultured microbes to perturb the microbiome. While antibiotic treated mosquitoes are far

from aseptic [7••], this dysbiosis is sufficient to influence viral pathogen dynamics. For example, DENV serotype 2 (DENV-2) titer decreased when *A. aegypti* were supplemented with a cocktail of antibiotics [26]. Similarly, when bacterial isolates were administered to mosquitoes, DENV-2 titers within *A. aegypti* were reduced. A *Chromobacterium* isolated from field caught *A. aegypti* colonized the midgut upon reinfection when fed to mosquitoes in a sugar meal and significantly reduced DENV-2 replication [27•]. Likewise, bacteria in the genus *Proteus* and *Paenibacillus* also inhibited DENV-2 density when administered to mosquitoes in the blood meal [28]. *Proteus* also inhibited DENV-2 titer when administered in a sugar meal [28]. In contrast to these findings, some microbes increase arboviruses in mosquitoes [29–31]. A *Serratia odorifera* isolate has been shown to increase both DENV and CHIKV replication [30,31]. Similarly, elegant work has shown that the resident microbiota are essential for O'nyong nyong virus (ONNV) to infect *Anopheles* [32••]. It was further demonstrated that infection of ONNV was rescued in antibiotic treated mosquitoes when supplementing the blood meal with a live culture of midgut bacteria, but not a heat killed culture [32••]. Using a comparative approach, the midgut bacterial composition was found to be different in three *A. aegypti* lines that vary in their susceptibility to DENV [33], although antibiotic treatment of these strains did not effect DENV vector competence [34]. These reinfection studies demonstrate the utility of culture-based approaches for dissecting the influence of microbes on arboviruses in mosquitoes and the mechanisms behind such traits.

Microbiota influence on vectorial capacity

In addition to direct and indirect effects on vector competence, the microbiota can also alter other mosquito traits that can influence vectorial capacity. These include various physiological traits like nutrition, reproduction and development. For example, antibiotic treatment of larvae results in aborted development, however this effect can be rescued by bacterial supplementation [8••,35]. In another case, bacteria isolated from the midgut of *A. aegypti* have been shown to influence blood digestion and egg development [36]. Parameters such as mosquito survival, development time, and reproductive capacity can have large influences on the population-level vectorial capacity for arboviruses that are equal or greater than the direct effects on vector competence.

Influence of virus infection on the microbiome

While it is evident that the microbiome affects arboviruses, there is evidence that the reciprocal interaction also occurs. In the Asian tiger mosquito *Aedes albopictus*, CHIKV infection increases the abundance of bacteria in the family *Enterobacteriaceae* and reduces *Wolbachia* and *Blattabacterium* [37]. Whether bacterial titers are responding directly to CHIKV or changing in response to other stimuli remains to be determined. For example, CHIKV is known to suppress the Toll pathway [38], and virus-mediated immune modulation inhibits the overall bacterial abundance in *A. aegypti* [28]. Alternatively, bacteria in the *Enterobacteriaceae* may be expanding in response to the reduction of *Wolbachia* and *Blattabacterium.*

Microbial interactions

While we recognize that important interactions occur between microbiota and arboviruses within mosquitoes, less is known regarding the interplay between the other members of the mosquito microbiome. However, some intriguing results are beginning to illuminate this field. This is important given the impact of the microbiome on many aspects of mosquito biology. The acetic acid bacterium *Asaia* interferes with *Wolbachia* transmission in *Anopheles* mosquitoes [7••], and recent work suggests this antagonism extends into *Culex* and *Aedes* species [39]. Culture-based experiments have also found inhibition between bacterial isolates from mosquitoes [27•,40], although this interplay needs to be confirmed *in vivo*. Contrary to these inhibitory interactions, a positive correlation was seen between *Asaia* and *Acinetobacter* in the midgut of *A. albopictus* [41].

Mode of action

Experimental evidence suggests there are several mechanisms by which bacteria affect arboviruses in mosquitoes (Figure 1). These fall into the categories of immunity [26,32••], production of metabolites [27•], resource competition [42] and regulating miRNAs [43,44], however it is likely that further mechanisms will be uncovered in the future.

Host immunity

There is a complex interplay between the resident microbiota and the insect immune system. Insights from *Drosophila* indicate the microbiome primes and matures host immunity while the immune system keeps microbial levels under tight control [45]. While less is known about the role of bidirectional cross talk in mosquito immune homeostasis, it is clear that immune pathways that ward off invading arboviruses also influence the microbiota. For example, overlap exists within the JAK-STAT pathway which is elicited by bacteria and fungi, but is also effective against DENV and WNV [46,47]. Similarly, the Toll pathway is activated by gram-positive bacteria and has anti-viral properties [26], while the IMD pathway has been shown to influence *Serratia* and ONNV densities in *An. gambiae* [32••, 48•]. Another important class of immune molecules, which also have complex interactions with pathogens and the microbiota, are reactive oxygen species (ROS). ROS has anti-pathogenic properties, regulates the microbiota density, and can activate the Toll pathway [49•,50]. RNAi is another major antiviral defense of mosquitoes [51], however the interplay between this pathway and the microbiota is yet to be determined. These complex interactions between host immunity, the microbiome and invading pathogens has led to the `holo-immunome' concept [52], which emphasizes the role of the microbial community's (and pathogenic microbes) influence on the immune status of an organism.

Metabolites

Bacteria produce secondary metabolites with anti-viral properties. Several bacterial isolates from the *A. albopictus* midgut produce bioactive compounds that inhibit La Crosse virus [53]. Additionally, a *Chromobacterium* isolated from field *A. aegypti* mosquitoes possesses anti-dengue and anti-*Plasmodium* activity [27•]. These compounds are promising candidates

for anti-viral drugs. However, further work is required to examine the biological relevance of these metabolites *in vivo* and their effect on arbovirus dissemination into the mosquito gut.

Resource competition

Both arboviruses and bacteria scavenge for resources in mosquitoes. Cholesterol and lipids are common molecules required by these microbes. In *Drosophila*, *Spiroplasma* utilizes lipids and vitamins from the host for replication [54], while *Wolbachia* sequesters cholesterol in mosquitoes [55]. Studies on the interplay between the gut microbiota and cholesterol in insects are limited, but evidence from a murine model system suggests microbes regulate cholesterol homeostasis [56]. DENV is known to perturb host lipid levels to facilitate replication, while cholesterol is essential for flavivirus replication [57,58]. Supplementation of exogenous cholesterol ablated the viral protection effect of *Wolbachia* in flies suggesting there is competition between these microbes for this molecule [42].

miRNA

The endosymbiotic bacterium *Wolbachia* modulates miRNA expression in *A. aegypti* [43]. *Wolbachia* has also been found to express miRNAs that manipulate mosquito gene expression [44]. Host derived miRNA can influence viral dynamics in *A. albopictus* [59]. However, it is unknown if gut bacteria influence mosquito miRNA expression, but insights from mammalian models suggest the microbiota has the capacity to modulate host miRNA levels [60].

Microbes for applied vector control

By far the most developed strategy for microbial control of arboviruses is the use of the intracellular bacterium *Wolbachia* (comprehensively reviewed [1–3]). However, other microbes of mosquitoes also have great potential for applied control strategies. These include not only harnessing their innate anti-viral abilities, but also to engineer microbes to interfere with pathogens; essentially using microbes as a delivery platform for the production of anti-viral molecules that either target the virus, mosquito pathways essential for virus replication, or to induce mosquito pathways antagonistic to arboviruses. This process, termed paratransgenesis, is being investigated in mosquitoes mainly for control of malaria parasites, but this application has the potential to be used for arbovirus control. In addition to bacteria, fungi and viruses are also promising candidates for use in paratransgenic control [61,62]. Excitingly, bacteria have been used to generate and transfer dsRNA to manipulate mosquito gene expression, offering new prospects for paratransgenesis strategies to reduce arboviruses [63•,64•]. The ability to deliver RNAi into field mosquito populations would be highly desirable and enable the development of a myriad of control approaches. The findings that close relatives of genetically tractable model bacteria are found in field mosquitoes [6•,7••,8••], that these model bacteria appear to be beneficial to the mosquito host [8••], and translocate into numerous mosquito tissues [65] will further enhance research in this field.

Future prospects

Studies perturbing the mosquito microbiome have elucidated its role in arboviral transmission. The next challenge in this area is to determine the biological relevance of these manipulations, and how this relates to natural variation of the mosquito microbiome in the field. Studies in an *Anopheles–Plasmodium* system suggest that the variability in the microbiome in field mosquitoes influences vector competence [9]. While we are beginning to characterize the bacterial microbiome in mosquitoes, we have a poor understanding of the viral and fungal communities, and this needs to be urgently addressed. Further to this, the role of bacteriophage in shaping the gut microbiome of mosquitoes or other insects is virtually unknown. This is an area of increasing interest in the vertebrate community [66], and insight from these systems may provide stimulus for studies in mosquitoes. Elucidating the core microbiome from transient microbes within mosquitoes and determining their influence upon the host, and whether bacterial members have functional redundancy are further challenges. While a mechanistic understanding of these tripartite interactions is desirable, the development of high throughput *in vitro* assays capable of assessing host–microbe–pathogen interactions will undoubtedly be useful in this regard [67,68]. Alarmingly, recent work suggests it is possible that the medicinal use of antibiotics and other products is also perturbing the microbiota within mosquitoes [69••,70]. Mosquitoes that fed upon humans treated with antibiotics were shown to have an altered microbiome compared to mosquitoes imbibing a blood meal from humans not using antibiotics [69••]. Similarly, the expulsion of antibiotics and other personal products from humans into the environment was shown in laboratory experiments to modify the larval microbiome of mosquitoes [70]. These findings suggest anthropogenic alteration of the environment by antibiotics and pharmaceutical products could manipulate the microbiota of mosquitoes, thus have wide-reaching implications for mosquito biology and pathogen transmission. This area of research warrants further investigation.

Conclusions

The influence of the microbiome on host biology is a burgeoning area of research. For mosquitoes that transmit arboviruses, this question is of great and timely importance, not only to increase our basic understanding of host–microbe interactions but also for its relevance for vector control. Given the increase in arboviral disease, novel microbial control approaches offer promising strategies to combat these pathogens.

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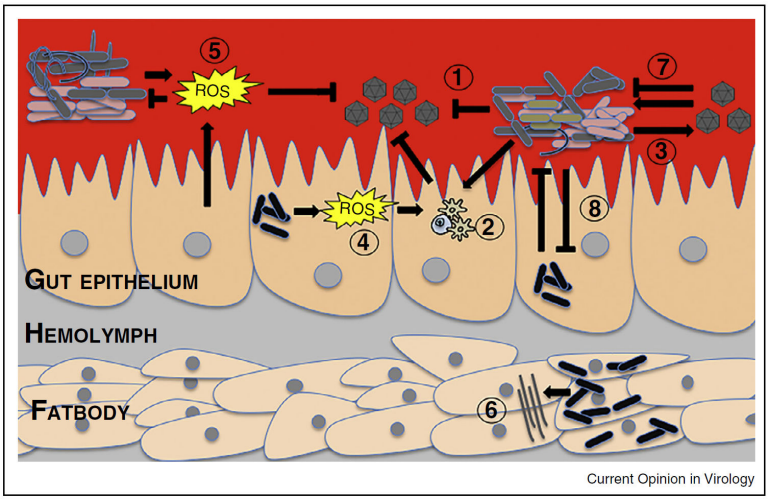

Figure 1.

Schematic illustrating the tripartite interactions between the mosquito host, the microbiome and arboviruses. Members of the microbiome can directly impede viruses (1), or can stimulate basal immunity leading to virus suppression (2). Conversely, some bacterial species can enhance viruses (3). Intracellular bacteria such as Wolbachia can also stimulate immunity by production of reactive oxygen species (ROS) (4). ROS can also be generated by the mosquito host and members of the microbiome and suppresses bacteria and pathogens (5). Intracellular bacteria can also manipulate host miRNA expression (6). Arboviruses can both suppress and enhance members of the microbiome (7) while bacterial interactions also influence the microbiome composition (8).