Trends in Parasitology

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Review

Mosquito Biting Modulates Skin Response to Virus Infection

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Mosquito-borne infections are increasing in number and are spreading to new regions at an unprecedented rate. In particular, mosquito-transmitted viruses, such as those that cause Zika, dengue, West Nile encephalitis, and chikungunya, have become endemic or have caused dramatic epidemics in many parts of the world. *Aedes* and *Culex* mosquitoes are the main culprits, spreading infection when they bite. Importantly, mosquitoes do not act as simple conduits that passively transfer virus from one individual to another. Instead, host responses to mosquito-derived factors have an important influence on infection and disease, aiding replication and dissemination within the host. Here, we discuss the latest research developments regarding this fascinating interplay between mosquito, virus, and the mammalian host.

Trends

Mosquitoes inoculate both saliva and virus into the dermis when probing for blood vessels.

Mosquito saliva has multiple potent biological effects on skin, including inflammation and edema.

Host inflammatory responses to mosquito bites and saliva drives the recruitment of leukocytes that then become infected and replicate virus.

The local response to mosquito bites inadvertently enhances arbovirus replication, dissemination and morbidity.

Targeting common mosquito-dependent factors could be a new strategy for preventing transmission or decreasing susceptibility to onset of disease.

Mosquito-Borne Viruses Constitute an Increasing Threat to Human and Animal Health

Pathogens transmitted by vectors such as flies, snails, ticks, and mosquitoes constitute a profound and growing health burden, causing more than 1 billion cases and 1 million deaths annually, according to the World Health Organisation (http://www.who.int/mediacentre/factsheets/fs387/en/). Increasing globalisation, migration, and changing land use are allowing more opportunities for the spread of infections. In addition, a warming planet is enlarging the geographic range of endemic viruses and their vectors – including arboviruses, which are spread by arthropod vectors. Of concern, the frequency and magnitude of arboviral epidemics has increased in both established and new geographic areas. Globally, up to 400 million people are infected each year by dengue virus, and many millions more by arboviruses that cause epidemics of, for example, Zika, yellow fever, and chikungunya [1–4], of which the day-biting *Aedes aegypti* mosquito is the primary vector. The economic burden of these diseases is enormous, with the global annual cost of dengue alone estimated at US$8.9 billion [5], while chikungunya is commonly associated with long-term detrimental sequelae, as reflected in disability-adjusted life years [6]. The recent and continuing pandemic of Zika is particularly concerning due to its association with severe congenital birth defects following infection of pregnant women [7] and Guillain–Barré syndrome in adults [8]. No effective antiviral treatments are available for arbovirus-associated diseases and only a few effective vaccines exist.

Arboviruses are genetically highly diverse and represent one of the largest virus groups, with more than 600 members, of which at least 80 are known human pathogens [9]. Most medically important arboviruses transmitted by mosquitoes are found in three distinct families: Flaviviridae, which includes dengue (DENV), Zika (ZIKV), yellow fever (YFV), and West Nile (WNV) viruses; Togaviridae, which includes chikungunya (CHIKV), Semliki Forest (SFV) and

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Box 1. Commonalities between Mosquito-Transmitted Viruses and Arthropod-Transmitted Parasites

Extensive literature on the effects of sandfly saliva on the transmission of *Leishmania* parasites pioneered the field of vector-derived factors in human infectious diseases [78]. Similar to *Aedes aegypti* mosquitoes [16], bites from sandflies enhance recruitment of neutrophils to the site of *Leishmania* infection [79]. Here, neutrophils serve as a 'Trojan horse' reservoir for *Leishmania* replication and enhanced infection [79,80]. Vaccination with specific sandfly-derived components can either protect rodents [81–83] or make them more susceptible to subsequent *Leishmania major* challenge in the presence of salivary gland extract [84]. Interestingly, injection with autoclaved *L. major* parasites protected against challenge with *L. major* via needle inoculation, but not against challenge with sandfly-transmitted parasite, due to the recruitment of neutrophils by the sandfly bite [85]. *L. major*-infected monocytes at sand-fly bites can instead differentiate into DCs to support protective Th1-type $CD4^+$ T cell responses [86].

Studies that examined whether mosquito salivary components directly modify *Plasmodium* infection in malaria are more controversial. Some have demonstrated that prior sensitization to uninfected mosquitoes or their saliva confers protection against infection [87], while other data suggest transmission via infected mosquitoes is more efficient than needle inoculation [88]. More recently, mosquito saliva was shown to have no detectable effect on *Plasmodium* infection in mice [89]. Perhaps more important is the observation that passage of the malarial parasite through mosquitoes appears to attenuate virulence in mice. In this study, mosquitoes were shown to modify the biology of the parasite, resulting in altered mammalian host immune responses to infection that rendered the infection less virulent [90].

Venezuelan equine encephalitis (VEEV) viruses; and Bunyaviridae, which includes La Crosse virus. Depending on the virus, infection can result in a diverse range of severe manifestations that include arthritis, encephalitis, or vascular leakage leading to shock [10–12]. This heterogeneity, combined with our inability to accurately predict the nature and timing of future epidemics, makes developing and stockpiling virus-specific drugs and vaccines very challenging [13].

Despite their considerable diversity, arboviruses share a common attribute: transmission via the skin at the site of the arthropod bite. In the case of infected mosquitoes, virus is transmitted to the mammalian host as they probe the skin for a blood meal and deposit saliva [14,15]. Local virus replication in the skin represents a key stage of infection, which is followed by rapid dissemination to the blood and tissues remote from the bite. Importantly, mosquito-derived factors deposited at the bite site, and the resulting local host immune response, play an important role in determining the severity of viral infection [16–21].

This review describes the current state of knowledge regarding early cutaneous events during arbovirus transmission and discusses how localized immune responses to vector-derived components influence infection outcome. Modulation of parasite transmission by host responses to mosquito bites is also briefly discussed (Box 1).

Mosquito-Derived Factors Augment Systemic Arbovirus Pathogenesis

The ability of mosquito-sourced factors to augment arbovirus infection has been established in a variety of experimental systems [9,15]. Together, these data show that arboviruses inoculated via a mosquito bite, or accompanied experimentally by mosquito saliva or salivary gland extracts (SGEs) (Box 2), induce more rapid viraemia, higher pathogen load, and greater morbidity compared to needle inoculation in the absence of mosquito-derived factors (Table 1). Although different models for delivery of vector-derived salivary factors may yield similar results, care needs to be taken when comparing these approaches (discussed in Box 2). Thus, mosquito-derived factors appear to influence infection by modulating events at the inoculation site, as delivery of saliva via a mosquito probing for blood vessels or via needle inoculation at sites distal from the site of virus infection do not augment infection [18,22,23].

Mosquito bite enhancement of WNV infection and mortality has been studied in mice. Following transmission of WNV via infected *Culex* mosquitoes, needle inoculation of WNV mixed with SGE, or needle inoculation of WNV alongside bites by uninfected mosquitoes ('spot feeding'),