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The enhancement of arbovirus transmission and disease by mosquito saliva is associated with modulation of the host immune response

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SUMMARY

Arthropod-borne viruses have emerged as a major human health concern. Viruses transmitted by mosquitoes are the cause of the most serious and widespread arbovirus diseases worldwide and are ubiquitous in both feral and urban settings. Arboviruses, including dengue and West Nile virus are injected into vertebrates within mosquito saliva during mosquito feeding. Mosquito saliva contains anti-haemostatic, anti-inflammatory and immunomodulatory molecules that facilitate the acquisition of a blood-meal. Collectively, studies investigating the effects of mosquito saliva on the vertebrate immune response suggest that at high concentrations salivary proteins are immmunosuppressive, whereas lower concentrations modulate the immune response; specifically, T_H1 and antiviral cytokines are down-regulated, while T_H2 cytokines are unaffected or amplified. As a consequence, mosquito saliva can impair the anti-viral immune response thus affecting viral infectiousness and host survival. Mounting evidence suggests that this is a mechanism whereby arbovirus pathogenicity is enhanced. In a range of disease models, including various hosts, mosquito species, and arthropod-borne viruses, mosquito saliva and/or feeding is associated with a potentiation of virus infection. Compared to arbovirus infection initiated in absence of the mosquito or its saliva, infection including mosquito saliva leads to an increase in virus transmission, host susceptibility, viraemia, disease progression, and mortality.

Keywords

arthropod-borne virus; mosquito saliva; immunomodulation; disease enhancement

Most arthropod-borne (arbo)viruses of public health significance are mosquito-borne, and thus transmitted to vertebrates via the feeding of an infected mosquito. For a mosquito to

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successfully obtain a blood meal from a vertebrate host it must overcome physical barriers and the complex and redundant physiological responses orchestrated by haemostatic and inflammatory systems that have evolved to prevent blood loss and to combat infection. Mosquitoes, like all haematophagous arthropods, have evolved mechanisms to effectively neutralize host haemostatic responses, based upon the release of saliva into the feeding site. Saliva is a pharmacologic cocktail of secreted molecules, principally proteins, that can affect vascular constriction, blood coagulation, platelet aggregation, inflammation, immunity, and angiogenesis (Arca et al. 1999, Arca et al. 2007, Arca et al. 2002, Arca et al. 2005, Billingsley et al. 2006, Calvo et al. 2004, Calvo et al. 2006a, Calvo et al. 2006b, Calvo and Ribeiro 2006, Champagne 2004, Champagne et al. 1995, Cross et al. 1994, James et al. 1991, Kerlin and Hughes 1992, Mellink and Vos 1977, Ribeiro 1987, 1989b, 1992, Ribeiro et al. 2007, Ribeiro et al. 2000, Ribeiro et al. 2001, Ribeiro and Francischetti 2001, 2003, Ribeiro and Nussenzveig 1993, Ribeiro et al. 1994, Ribeiro and Valenzuela 2003, Schneider et al. 2004, Stark and James 1998, Suwan et al. 2002, Valenzuela et al. 2003, Valenzuela et al. 2002, Wanasen et al. 2004, Wasserman et al. 2004, Zeidner et al. 1999). The saliva of all species of haematophagous arthropod analyzed to date contains at least one anticlotting, one anti-platelet, and one vasodilatory substance. Despite recent advances in our knowledge of these molecules and their role in blood-feeding, functions can be ascribed to less than half of the molecules present in mosquito saliva (Ribeiro et al. 2007, Valenzuela et al. 2002).

The enigma of immunomodulation in rapid feeding vectors

It is now clear that the feeding of mosquitoes has an immunomodulatory effect on their hosts (Billingsley et al. 2006, Cross et al. 1994, Depinay et al. 2006, Schneider et al. 2004, Wanasen et al. 2004, Wasserman et al. 2004, Zeidner et al. 1999). The presence of this activity in vector saliva is a reflection of the inherent overlapping and interconnected nature of the host haemostatic and inflammatory/immunological responses and the intrinsic need to prevent these host defences from disrupting successful feeding. Duration of feeding varies greatly among blood-feeders (minutes for fleas, sand flies, and mosquitoes; hours for soft ticks, and up to two weeks for hard ticks), but even the most rapid feeders must contend with the host haemostasis system, which activates within a matter of seconds (Champagne 2004). Key host targets for arthropod anticoagulation action, such as coagulation factor Xa, also serve to activate anti-microbial responses (Champagne 2004). Clearly, arthropods such as ticks that take a long time to engorge must additionally deal with host inflammatory and immune responses, thus necessitating vector anti-inflammatory/anti-immunological factors. It is less obvious why rapidly feeding dipterans, in particular mosquitoes and sand flies, have evolved salivary factors that directly modulate host immune defences that peak after completion of engorgement and after the arthropod has left the host. One possible explanation is that these molecules have evolved because they have long-term beneficial effects for the species/population rather than to the individual at the time of feeding. Since a host animal may be fed upon by large numbers of biting flies (Higgs et al. 2005), it is possible that immune reactions to arthropod salivary factors promoted by previous exposure, may be deleterious to subsequent arthropods feeding. Indeed, reduction of salivary protein levels in saliva causes an increase in intradermal probing time thus increasing the risk of vector detection by the host and deceasing the likelihood of a successful blood meal (Rossignol et al. 1984). New anti-tick vaccines designed to reduce the intensity and duration of tick infestations and thus the incidence of tick-borne disease are based upon antibody responses directed towards tick digestive tissue epitopes (Labuda et al. 2006). If vertebrates were to mount strong immune responses that reduced mosquito feeding efficiency a decrease in mosquito longevity and fecundity may occur. One can speculate that down regulation of these responses by mosquito salivary compounds may therefore have evolved to minimize such potential anti-vector effects and, as a consequence, would maintain the maximum reproductive capacity of the population as a whole.

Immunosuppression would reduce the possibility that a host could develop specific immune responses that impair the activities of anticlotting and vasodilatory molecules essential for blood acquisition by vectors. A second and, perhaps, more convincing reason for immunomodulatory activity in rapidly feeding arthropods is the high level of interaction between the host haemostatic, inflammatory, and immune responses. A mosquito by necessity must suppress the haemostasis system. The effect of saliva on vasodilation, coagulation, and platelet aggregation factors results in modulations of inflammation and immune responses. For example, indirect effects of mosquito salivary anticoagulant proteins, such as *Aedes* anticoagulant-factor Xa (Stark and James 1998) may suppress extravasation of some inflammatory cell types and suppress complement pathways. The *Aedes* anticoagulant inhibits fXa, which is required for activation of Hagemen factor, which in turn converts factor XI to its active form XIa and prekallikrein to kallikrein (Stark and James 1998). Kallikrein is responsible for generation of the anaphylatoxin C5a from compliment (Wiggins and Cochrane 1981). A reduction in C5a, as would be expected from inhibition of upstream effectors, could have significant effects at the site of mosquito feeding. Notably, C5a is important for recruitment of antibody, extravasation of complement, homing of polymorphonuclear leukocyte (PMN), and activation of macrophages and neutrophils. Therefore mosquito saliva, by inhibiting factor Xa, decreases the production of C5a, thereby suppressing these downstream effects that hasten the destruction of pathogens. Interestingly, a recent study confirmed that complement plays a critical role in controlling West Nile virus (WNV) infection, a mosquito-transmitted flavivirus (Mehlhop and Diamond 2006).

Modulation of the vertebrate immune response by mosquito saliva

Whilst the molecular mechanisms for mosquito saliva-induced alteration of the host immune response is unclear, the data clearly demonstrate that such an effect occurs. Studies evaluating the effect of mosquito saliva on the immune response began relatively recently (Bissonnette et al. 1993, Cross et al. 1994), following the discovery that sand fly saliva enhanced infections with *Leishmania major* (Ribeiro 1989b, Theodos and Titus 1993). Early work described a factor in *Ae. aegypti* saliva that directly suppresses tumour necrosis factor (TNF)-α release, but not antigen-induced histamine secretion, from activated mast cells (Bissonnette et al. 1993). This factor has a molecular weight greater than 10 kDa and is neutralized by boiling, suggestive of a protein. Several studies have focused on the effect of mosquito saliva on splenocytes in vitro. Experiments by Cross et al. (1994) demonstrated that the inclusion of *Ae. aegypti* salivary gland extract (SGE) into naïve cultures suppressed interleukin (IL)-2 and interferon (IFN)-γ production, but had no effect on the cytokines IL-4 and IL-5. Cellular proliferation promoted by IL-2 is clearly reduced by prior treatment of cells with SGE (Cross et al. 1994). Correspondingly, activated splenocytes isolated from mice fed upon by either *Ae. aegypti* or *Cx. pipiens* mosquitoes produce markedly higher levels of IL-4 and IL-10 concurrent with suppressed IFN-γ production (Zeidner et al. 1999). Unexpectedly, this T_H1 to T_H2 shift in cytokine expression from splenocytes is sustained for up to 10 d after mosquito exposure, suggesting that exposure to natural feeding of mosquitoes can have a profound, enduring, and systemic effect on T cell functions. Inoculation of the *Ae. aegypti* salivary vasodilator, sialokinin mimics this effect of mosquito SGE (Zeidner et al. 1999). Modulating these cytokines may have significant effects; IL-10 has pleiotropic effects in immunoregulation and inflammation (e.g. it inhibits pro-inflammatory and T_H1 cytokines, however, it stimulates certain T cells, mast cells and B cells), while IL-4 is the prototypical T_H2 cytokine (i.e. it differentiates $CD4^+$ T-cells into T_H2 cells and up-regulates major histocompatibility complex (MHC) class II production). Enhancement of IL-10 expression could account for reduction in secretion of other cytokines because it inhibits antigen presentation, IFN–γ expression, and macrophage activation (Thomson et al. 1998). IFN–γ is especially important in defence against RNA virus infections. This cytokine causes proliferation and differentiation of many cell types,

activates production of cellular proteins that prevent viral mRNA translation, and enhances macrophage nitric oxide production (Ribeiro and Nussenzveig 1993).

T cell populations are especially susceptible to the effects of mosquito saliva, showing increased mortality and decreased division rates (Wanasen et al. 2004). This immune suppression appears to correlate with the vector's host preferences. For example, the saliva of *Ae. aegypti* , a species that feeds on mammals and in particular humans, exerts an enhanced suppressive effect on mammalian splenocytes, whereas saliva of *Cx. pipiens*, an ornithophilic species (Wang 1975), has less immunomodulatory effect on mammals (Wanasen et al. 2004). Parallel work by Wasserman et al. (2004) demonstrated that T- and B-cell proliferation was inhibited in a dose dependent manner from concentrations as low as 0.15 salivary gland pairs (SGP)/ml. Each mosquito possesses a pair of salivary glands, and it has been estimated that mosquitoes inject about 50–60% of their active salivary factors during a feed and re-ingest approximately 20%, leaving roughly 30–40%, or the equivalent of 0.3–0.4 SGP, in the skin (Wasserman et al. 2004). Lower concentrations of SGE inhibited T_H1 cytokines (IL-2 and IFN-γ) and T cell proliferation, while higher concentrations suppressed T_H1, T_H2 (IL-4 and IL-10), and proinflammatory (granulocyte macrophage colony stimulating factor and TNF-α) cytokines and decreased T cell viability. This pattern suggests that at the immediate feeding site, an immunosuppressed environment is created, whereas more distal regions with decreasing saliva concentrations experience a dysregulation of the immune response. A 387 kDa protein was implicated in this observed activity (Wasserman et al. 2004). Depinay et al. (2006) observed a suppression of antibody-specific T cell responses mediated by *Anopheles stephensi* saliva and dependent on mast cells and IL-10 expression. The delayed-type hypersensitivity (DTH) response was reduced by 75% in mice that were exposed to mosquito saliva during the sensitization phase, and the number of leukocytes recruited to the draining lymph node (LN) were also reduced (50%) in this group as compared to controls (Depinay et al. 2006). This altered response corresponded with a suppression of IFN-γ and an up-regulation of IL-10.

Immunomodulation in the context of arbovirus infection

Recent in vivo data has established the relevance of these observations in the context of early arbovirus replication (Schneider et al. 2004). Following intradermal co-inoculation of the alphavirus Sindbis virus (SINV) and *Ae. aegypti* SGE intradermally, IL-4 and IL-10 expression levels in the skin were 3.3- and 7.6-fold higher, respectively, by 72 hpi as compared to mice injected with SINV alone. An increase in IL-4 could shift the T helper response towards T_H2, a response that is generally not favourable during viral infections. IL-10 may generate conditions favourable to viral replication by suppressing the innate and adaptive responses (Brooks et al. 2006). IL-10 down regulates MHC class II antigen expression by monocytes and inhibits antigen presentation by several types of antigen-presenting cells (APC) (Enk et al. 1993, Macatonia et al. 1993). The existence of viruses with IL-10 homologs reveals an evolutionary advantage to enhanced IL-10 levels for an invading virus (Griffiths 2002, Salek-Ardakani et al. 2002). Studies with peripheral blood leukocytes found that early after infection with dengue virus (DENV), a flavivirus, increased IL-10 production induces lasting T cell inactivation and decreases the control of virus infection (Ejrnaes et al. 2006). Consequently, the interaction of immunosuppressive IL-10-producing cells with T cells early during arbovirus infection may result in the loss of T-cell responsiveness, facilitate an enhancement of viral replication, and a impaired adaptive immune response. In addition to these cytokines, both type 1 and type 2 IFNs are suppressed at the site of virus inoculation when SGE is present (Schneider et al. 2004). This inhibition of IFN expression is supported by a complimentary in vitro study, which revealed by ribonuclease protection assay that IFN-α2 expression was depressed in vesicular stomatitis virus (VSV)-infected L929 cultures supplemented with SGE (Limesand et al. 2003). The contribution of type I IFN towards recovery from infection and defence against

arboviruses has been demonstrated in vivo by the therapeutic and prophylactic effects of administration of IFN-inducers or IFN (Haahr 1971, Taylor et al. 1980, Vargin et al. 1977). After a low-dose infection with the flavivirus Murray Valley encephalitis virus, mice deficient in IFN–α receptor succumb to infection, in contrast to a 70% survival rate in wild-type mice (Lobigs et al. 2003). Similar results are observed with IFN-α/β-deficient mice infected with SINV (Ryman et al. 2000), WNV, and DENV (Chambers and Diamond 2003). Additionally, administration of IFN-α prevents or suppresses flavivirus infections in vivo, and in vitro treatment of cells with type I IFNs 4 h prior to or shortly after infection with DENV or St. Louis encephalitis virus (SLEV) significantly inhibits viral replication (Crance et al. 2003). The abundance of research that demonstrates an effect of mosquito saliva on host immune mediators affirms a role for saliva in modulation of the host immune response pathways.

Mosquito associated potentiation of arbovirus transmission and infection

During blood-feeding an infected mosquito transmits virus in saliva secreted predominantly in the extravascular space of the skin (Amino et al. 2007, Hurlbut 1966, Ribeiro et al. 1984, Smith et al. 2005, Styer et al. 2007, Turell and Spielman 1992, Turell et al. 1995). Depending on the virus, mosquito species, and method of quantification employed, the estimated level of virus inoculated within mosquito saliva ranges from approximately 10^1 to 10^7 pfu (Colton et al. 2005, Colton and Nasci 2006, Gubler and Rosen 1976, Reisen et al. 2005, Smith et al. 2005, Styer et al. 2007, Vanlandingham et al. 2004). As described above, components of mosquito saliva have immunomodulatory activity, and thus, as a consequence, mosquito saliva can affect the anti-viral immune response and virus pathogenesis. Indeed, enhanced infection attributable to components of arthropod saliva is an accepted phenomenon, particularly with agents transmitted by ticks and sand flies (Cupp et al. 1998, Edwards et al. 1998, Limesand et al. 2000, Nuttall and Labuda 2004, Theodos and Titus 1993). For example, when *Leishmania major* is inoculated with salivary gland extracts of sand flies, parasite burden, lesion size, and disease outcome are all amplified (Titus and Ribeiro 1988). In this seminal research, Titus and Ribeiro (1988) found that co-inoculation of sand fly SGE lead to a parasite burden that was 5580-fold greater than in control groups inoculated with parasites alone. Increasingly, studies are additionally supporting the hypothesis that mosquito saliva influences the transmission and course of arbovirus diseases (Table 1).

Recently, the potential for mosquitoes to impact the course of WNV disease was investigated by assessing pathogenesis in the presence or absence of mosquito saliva (Schneider et al. 2006). Mice inoculated intradermally with 10^4 pfu of WNV subsequent to the feeding of mosquitoes (< 12/mouse) developed more progressive infection, higher viraemia, and accelerated neuroinvasion than the mice inoculated with WNV alone. At a lower dose (10^2 pfu), mice exposed to mosquitoes exhibited increased mortality. Mice that were co-inoculated with WNV and mosquito saliva experienced an accelerated and amplified WNV infection in the brain (Schneider et al. 2006). As the viral inoculum was held constant while the presence of mosquito saliva was varied, this study addresses the possibility that dissimilar level or source of virus is responsible for the different phenotypes between needle-infected and mosquito-infected mice. Similar studies with chickens and *Cx. pipiens* found that even compared to chicks that were needle-inoculated with high doses (10^7 pfu) of WNV, those chicks infected by mosquito feeding had significantly higher virus titres in the serum at the early time points of 8, 12, and 24 h post-infection (Styer et al. 2006).

Adult mice are typically resistant to La Crosse virus (LACV), a mosquito borne flavivirus, administered subcutaneously by needle, showing minimal signs of viral replication (Pekosz et al. 1995). However, when LACV-infected mosquitoes feed upon adult mice, the majority of mice die of encephalitis (Higgs et al. unpublished data). Fatal infection also results when adult mice are infected subcutaneously with LACV-infected salivary gland suspensions. In contrast

to mice inoculated with virus alone, mice infected in the presence of mosquito saliva developed a viraemia of 1 – 3 d duration and die of LACV encephalitis within 6 – 12 d following mosquito feeding. A dose response effect was noted; the more LACV-infected mosquitoes that fed, the earlier clinical symptoms appeared, the longer the duration and higher the viraemia. This differs substantially from the subcutaneous challenge of mice with LACV derived from brain suspensions from lethal infections or from *Ae. albopictus* cell culture, which resulted in the death of only a small fraction of mice (Higgs et al. unpublished data). Another study investigated the potential for mosquito feeding to influence the disease course and reservoir competence of wild mammals that naturally occur in regions where LACV is endemic (Osorio et al. 1996). Despite the recognition that white-tailed deer constitute 65% of *Ae. triseriatus* blood meals, needle-inoculation models of LACV had previously assumed that, due to low viraemias detected in deer, these animals did not play an important role in the maintenance of LACV in nature. However, subsequent experiments using infected mosquitoes to deliver LACV demonstrated that higher and longer lasting viremias are produced compared with infections initiated by needle inoculation (Osorio et al. 1996). A parallel study with chipmunks observed that chipmunks infected with LACV via mosquito as compared to needle-inoculation developed a viraemia that was 1000-fold higher (Osorio et al. 1996).

Mice generally do not become infected with Cache-Valley virus (CVV), an arthropod-borne bunyavirus, following needle-inoculation of the virus (Edwards et al. 1998). However, injection of CVV into sites of mosquito feeding results in production of viraemia and anti-CVV antibody within two weeks of exposure (Edwards et al. 1998). This observed enhancement of CVV infection resulted after feeding by either *Ae. triseriatus*, *Ae. aegypti*, or *Cx. pipiens* and occurred when virus injection was delayed up to 4 h after mosquito feeding, but it was not observed when virus inoculation was performed at a site distant from mosquito feeding. The results of this study suggest that arbovirus infection can be exacerbated as a consequence of the complex mosquito-vertebrate host interactions, and that enhancement is due to factors in arthropod saliva, rather than due to changes in viral characteristics during replication in the vector.

Furthermore, studies with vesicular stomatitis virus (VSV) further demonstrate the potential for mosquito feeding or mosquito saliva to potentiate viral disease (Limesand et al. 2000). Pathogenesis of VSV is age dependent: 3 day old mice infected with VSV by peripheral needle-inoculation develop encephalitis and die, while older mice similarly infected show almost no signs of viral replication with 13% and 11% of 3-week old and adult (>8 months) mice, respectively, producing neutralizing antibody after needle inoculation. Conversely, following mosquito inoculation of VSV, 94% of 3-week old mice and 73% of adult mice seroconvert to VSV. It is interesting to note that in a supplementary experiment where mice were continuously exposed to mosquito feeding, a lower rate of seroconversion was observed, suggesting that factors in mosquito saliva may have suppressed antibody production. Follow-up in vitro studies demonstrated a significant increase in viral growth in *Ae. triseriatus* SGE-treated mouse fibroblast cells as compared to untreated controls and suggested that the mechanism of VSV enhancement might be attributed to mosquito saliva-induced suppression of type I IFN expression (Limesand et al. 2003).

Contrary to these observations, a few studies have suggested that there is no effect of mosquito transmission on vertebrate infection, including studies on avian species infected with SLEV and western equine encephalitis virus (Reisen et al. 2000), and studies with hamsters infected with WNV (Sbrana et al. 2005), although the latter study compared mosquito transmission to intraperitoneal inoculations, a route that allows the virus to bypass peripheral immune responses that are robust in the dermis. These incongruous observations suggest that the effect of mosquito feeding/saliva is variable, and may be influenced by host species, viral dose, and saliva source as well as concentration.

Host immune response against mosquito saliva and implications for arbovirus pathogenesis

Direct immune modulation of the host response may be one mechanism whereby the mosquito alters the response to a virus, yet the immune response directed towards saliva may also be important factor to consider. The host reaction to salivary components following mosquito probing depends on host and mosquito species with overt cutaneous responses varying from small papules to large pruritic swellings. These responses are often mild in unsensitized hosts, and may become more pronounced if allergic sensitization occurs prior to exposure. The mechanisms include type I (immediate, IgE-dependent) and type-IV (DTH) cell-mediated reactions. Both hypersensitivity reactions cause a local increase in blood flow, vascular permeability, and cellular infiltration (Demeure et al. 2005). This reaction to mosquito feeding suggests that some components of mosquito saliva are allergenic. Mosquito saliva induces mast cell degranulation, leading to fluid extravasation and neutrophil influx (Demeure et al. 2005). Mast/dendritic cell (DC) interactions can enhance the immunomodulatory properties inherently endowing this cell type, which exert both stimulatory and inhibitory effects on the adaptive immune response (Hart et al. 1998, Villa et al. 2001). Following *Anopheles stephensi* feeding on mice MIP-2 and IL-10 are selectively increased while IL-13 and IL-4 are marginally increased in the skin and lymph nodes. This is relevant to mosquito-borne virus infection because MIP-2 controls migration and adhesion of monocytes, which are susceptible to a range of arboviruses, while the T_H2 cytokines IL-4, IL-10, and IL-13 have been associated with an ineffective immune response to viruses in the skin (Becker 2003).

Sensitization to mosquito feeding is associated with specific responses. In an animal model (BALB/c mice) that explored the natural sensitization leading to IgE- and lymphocyte-mediated hypersensitivities, mice were exposed twice a week for 4 weeks to at least 16 *Ae. aegypti* mosquitoes (Chen et al. 1998). Following mosquito exposure, sensitized mice develop a wheal 20 min after exposure and a delayed papule 24 h later. Mosquito saliva-specific IgG1 and IgE, but not IgG2a, is increased in sensitized mice, and comparisons show that most of the antigens also elicited human IgE responses. The degree of the host response to mosquito saliva is dependent on the duration and intensity of exposure to biting mosquitoes and the immunological profile of the host (Peng et al. 1996). Intriguingly, the immune response to mosquito saliva affects cytokine expression. IL-4 production is significantly increased while IFN-γ production is decreased in sensitized mice, suggesting that a T_H2 immune response predominates following sensitization (Chen et al. 1998). Mean lymphocyte proliferation after stimulation with mosquito antigens is higher in mice (Chen et al. 1998) and humans (Peng et al. 1996) previously exposed to mosquito bites, implying that lymphocytes are involved in the development of immunological reactions to mosquito saliva. A shift in the immune response such as this at the site of arbovirus delivery could affect the pathogenesis of the virus. Skewing the immune response, such as observed following mosquito feeding in sensitized hosts, towards a T_H2 response might be advantageous to a virus as outlined above. This and the inflammation-mediated enhanced recruitment of virus-susceptible cell types to the feeding site would suggest the possibility that immunological familiarity of the host to mosquito saliva may facilitate virus replication. Supporting this hypothesis, a recent study demonstrated that sensitization to mosquito feeding aggravated subsequent mosquito-transmitted WNV infection, leading to increased mortality (Schneider et al. 2007). Exacerbation of the disease is associated with more rapid viral replication, increased IL-10 expression, and enhanced influx of WNV-susceptible cell types to the inoculation site. These differences in the early response to arbovirus in mosquito-sensitized mice along with recent research that could not demonstrate a connection between probing time and WNV inoculation in *Ae. japonicus* or *Ae. triseriatus* (Styer et al. 2007) suggest that observed potentiation of disease may not be due to mosquito feeding efficacy, but rather a host response subsequent to probing. Nevertheless, the existence of a

positive association between probing time and virus inoculation in *Cx. tarsalis* (Styer et al. 2007), the intrinsic variability between species, and the possibility that the immune response in mosquito-sensitized hosts increases probing time by impairing salivary protein function suggest that further experiments are warranted to investigate whether mosquitoes feeding on pre-exposed hosts inoculate more virus. Given the observation that prior exposure to *An. stephensi* causes a dramatically different response in subsequent exposures (i.e. increased IFNg and decreased IL-4) (Donovan et al. 2007), additional research is necessary to elucidate whether the effect of immune familiarity to mosquito feeding varies between mosquito species.

Conclusion

This review has focussed on mosquitoes and has thus ignored ticks as vectors that attach to a host for relatively long periods and for which there are substantial data demonstrating immune suppression (Gillespie et al. 2000, Nuttall and Labuda 2004, Ribeiro 1989a, Ribeiro and Francischetti 2003, Wikel 1999). Despite the rapid feeding of mosquitoes, mosquito saliva clearly has immunomodulatory activity, and mounting data demonstrate that, as a consequence of this activity, mosquito-borne pathogens may be delivered to the vertebrate in an environment that is compromised in its ability to respond to and contain infection. As with other pathogens that have visibly co-evolved with the vector to take advantage of arthropod saliva and the unique immune environment it creates (Ramamoorthi et al. 2005), arboviruses also appear to exploit this niche to improve transmission and survival. Continuing research into the effect of mosquito saliva on host immune response and arbovirus infection will not only provide a progressively more accurate understanding of mosquito-transmitted virus pathogenesis, but may uncover the means to disrupt transmission or diminish disease by, in conjunction with viral prophylactic strategies, undermining the action of salivary proteins.

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Table 1

The enhancement of arbovirus transmission and infection associated with mosquito feeding/saliva

virus	host	mosquito	differential effect of mosquito on infection	reference
CVV	mouse	*Ae. triseriatus*, *Ae. aegypti*, *Cx. pipiens*	needle inoculated mice did not become infected, whereas mosquito inoculation led to production of viraemia and seroconversion	Edwards *et al.* 1998
LACV	deer, chipmunk	*Ae. triseriatus*	increased and extended viraemia	Osorio *et al.* 1996
LACV	mouse	*Ae. triseriatus*	mice develop viraemia and succumb to infection, needle inoculated mice do not	Higgs *et al.* unpublished data
VSV	mouse	*Ae. triseriatus*	~5-fold increase in seroconversion rate	Limesand *et al.* 2000
VSV	L929 cells	*Ae. triseriatus*	significant increase in viral growth	Limesand *et al.* 2003
WNV	mouse	*Ae. aegypti*	more progressive infection, higher viraemia, and accelerated neuroinvasion	Schneider *et al.* 2006
WNV	chicken	*Cx. pipiens*	elevated viraemia early in infection	Styer *et al.* 2006
SLEV, WEEV	chicken, finch	*Cx. tarsalis*	no difference in viraemia or seroconversion	Reisen *et al.*2000

Cache-Valley virus (CVV); La Crosse virus (LACV); vesicular stomatitis virus (VSV); West Nile virus (WNV); St. Louis encephalitis virus (SLEV); western equine encephalitis virus (WEEV).