Host inflammatory response to mosquito bites enhances the severity of arbovirus infection

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Mosquitoes can pass disease to humans when they bite, which includes infections caused by viruses, known as arboviruses. These viruses infect many millions of people each year and include viruses that cause diseases such as Zika, dengue, and chikungunya. Most such infections are usually found in the tropics, but a changing climate and globalization means their range has spread at an alarming rate. Cumulatively these viruses are highly diverse at the genetic level and include up to 90 known human pathogens. It is hard to predict the nature, timing and location of these outbreaks. Furthermore, these viruses replicate to exceptionally high levels in the blood of infected patients. Thus, arbovirus infection constitutes an increasing concern for blood transfusion services. We suggest that it is important to identify common aspects of these infections to aid diagnosis, patient stratification and develop medicines that are applicable to multiple arbovirus infections. An important stage, common to all such infections, is the inoculation of virus at mosquito bites. We have recently shown that mosquito bite inflammation, which is common to all such infections, inadvertently enhances infection. In this study, we identified key aspects of mosquito bite inflammation that are important determinants of the subsequent systemic course and clinical outcome of infection. As such, host responses to mosquito saliva may prove to be a predictor of subsequent disease severity and consequently could also be useful in risk-stratifying blood products.

Key words: cytokine, neutrophil immunobiology, transmissible infections, viral safety of plasma derivatives

Introduction

The number of emerging and re-emerging infectious disease agents has increased in recent years. A combination of several factors is responsible, including globalization, climate change, urbanization and our increasing exposure/interaction with new ecological systems [1–3]. In particular, the burden of mosquito-borne viral disease on human health is profound. There has been a rapid increase in both the incidence and geographical range of such diseases. As such this poses an increasing problem to the safe supply of blood for transfusion [4]. Medically important viruses spread by arthropods (arboviruses) infect hundreds of millions of people each year and include the Zika, chikungunya and dengue viruses [2, 5, 6]. The day-biting *Aedes aegypti* is the primary vector. The economic burden of these diseases to low- and middle-income countries is substantial, with the global annual cost of dengue alone estimated at US$8.9 billion [7], while sporadic outbreaks of, for example, chikungunya are associated with particularly detrimental effect on disability-adjusted life years [8]. The number of arboviruses worldwide may be as high as 600, of which at least 80 are known human pathogens [9, 10]. Arboviruses are mainly derived from three distinct family of viruses: Flaviviridae, which includes dengue (DENV), Zika (ZIKV), yellow fever (YFV) and West Nile (WNV) viruses; Togaviridae, which includes chikungunya (CHIKV), Semliki Forest (SFV) and Venezuelan equine encephalitis (VEEV) viruses; and Bunyaviridae, which includes La Crosse virus. These viruses are increasingly being...
imported to more temperature climates by infected travellers that transit from endemic regions [11]. As such, this poses an increasing problem for the safe supply of blood transfusion products, although the likelihood of imported arboviruses establishing autochthonous transmission in new geographic areas depends on the presence of suitable vectors are climatic conditions.

In summary, arboviruses are a large, diverse group of viruses [12–14] that cause a wide spectrum of diseases in humans [15–17]. 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 [1]. Studying common aspects of all these infections provides an opportunity to target mechanisms common to a large number of arboviruses. The unpredictability of these outbreaks also poses a challenge to those responsible for preventing transfusion-transmitted infections.

**Identifying common aspects to arbovirus infection**

All mosquito-borne viruses share a common attribute, their site of inoculation at mosquito bite sites. Infected mosquitoes transmit virus to the mammalian host as they probe the skin and deposit saliva [18, 19]. Establishment of viral replication in the skin represents a key stage of infection, during which virus replicates rapidly and then disseminates to the blood. Although it is known that the early stages of infection are important for dictating the systemic spread of infection and clinical outcome [9, 20, 21], little is known about what these early determinants are, or whether this knowledge can be used to prevent infection or aid patient care by clinicians [9]. In particular, despite substantial advances in our understanding of innate immunity [22, 23], the tissue-specific and system-wide level coordination of such immune responses remain poorly understood. This particularly applies to cutaneous responses to mosquito-borne virus infections. Importantly, the presence of a mosquito bite at the inoculation site enhances infection by arboviruses [21, 24–27]. We have shown that inflammatory responses to mosquito bites inadvertently enhance infection by virus [21]. As such, our seminal finding identifies an important and novel aspect of the host immune response to mosquito bites that paradoxically promotes arbovirus infection [9].

**Model systems for studying arbovirus infection**

To investigate the impact of mosquito bites, we allowed *Ae. aegypti* to bite a small piece of mouse skin. Bitten or resting skin was then injected with sub-μl volumes of mosquito-cell derived arbovirus (we use a model arbovirus called Semliki Forest Virus, SFV). This way, we mimic natural infection in a highly reproducible model. SFV is an ideal virus to use for studies with mice, as it is easy to genetically manipulate and disseminates effectively within immune-competent mice. Following infection in the skin, SFV spreads to the brain resulting in an encephalitis that is often fatal [28]. In comparison, studying host responses to human-specific pathogens, such as the Zika virus, is difficult. This is because Zika virus only replicates in mice that are immune-deficient (lacking type I interferon signalling) and therefore precludes study of host innate immune responses to infection [29].

**Inflammatory responses to bites aids virus infection**

Using SFV in mice, we found that the rapid, skin inflammatory response to the mosquito bites is highly beneficial for the virus. An oedematous bump that is well-described to occur after a mosquito bite retains much of the original virus inoculum in the skin. The host inflammatory responses to the bite then inadvertently augment virus infection, principally by recruiting leucocytes that are susceptible to infection. Mosquito saliva alone, when given experimentally by needle in the absence bite trauma, can also enhance infection with arboviruses [21].

Mosquito saliva is a complex mixture of biologically active factors, which have evolved to facilitate successful blood feeding and bacterial microbiota. To ascertain how bites and mosquito saliva enhance virus infection, we first defined the molecular and cellular responses of skin to mosquito bites and separately to virus infection. Transcriptional responses of the skin to bites and virus are quite distinct, with the former associated with rapid induction of neutrophil attracting chemokines, such as CXCL2 and IL-1β expression. The expression of these chemokines is associated with a rapid influx of neutrophils into the bite site, where these cells help to coordinate the skin inflammatory response to the bite. This includes the expression of additional monocyte-attracting chemokines, such as CCL2, that precipitates a later influx of virus-permissive monocyteic cells. Although the host response to bites has presumably evolved to initiate tissue repair and defend against opportunistic bacterial infection, arboviruses ‘make the most’ of this response by infecting and replicating within infiltrating and resident monocyteic cells. Because SFV, like all arboviruses, is most commonly cytolytic, infected myeloid cells are unlikely to disseminate the virus within the body. Instead, infected myeloid cells most likely become lyzed to release high titres of new virus that can then spread systemically by passive diffusion in the lymphatics to the blood [9, 21].
To provide further evidential support that inflammatory responses to bites inadvertently enhances infection with arboviruses, we experimentally manipulated key aspects of host responses to bites and determined what effect this has on virus infection. By suppressing the local skin responses to bites, virus was less able to replicate and disseminate within the mouse. For example, experimental depletion of neutrophils with an antibody or the temporary blockade of pro-inflammatory bite-associated IL-1β signalling suppressed host responses to bites, and importantly, this was associated with a better clinical outcome to infection. We also found that by preventing the migration of virus-permissive monocytes into the bite site, in this case using a chemokine receptor (CCR2)-deficient mouse, also blocked bite enhancement. Together this shows that host inflammatory responses to bites inadvertently enhances arbovirus infection. Indeed, other inflammatory agents unrelated to mosquito saliva that recruit similar cell types, such as a bacterial lipoprotein and the vaccine adjuvant alum, all had the ability to enhance infection with virus when administered at the site of infection [21]. In addition, others have importantly shown how mosquito saliva can induce an inflammatory oedema that similarly enhances infection with dengue virus [30]. Together these studies demonstrate that the early cutaneous responses to infection have an important role in dictating the systemic course and clinical outcome to infection [9].

**New therapeutic and patient management strategies**

Our current research focuses on improving our understanding of early events in the bitten skin during infection with arboviruses. A better understanding of this process could enable the design of new pan-viral medicines, or be used to aid in stratifying patient prognoses, based on their responses to mosquito bite allergens. We all react differently to mosquito bites, with some of us exhibiting quite pronounced hyper-sensitivity reactions. We predict that it may be possible to correlate patient responses to mosquito allergens with clinical outcome to infection, although further on-going studies are required to provide support for this theory. Conceivably, the degree of exposure to mosquito biting could also be used to as a basis to aid the screening of safe blood products for transfusion, especially when the supply of blood from conventional sources is low. In this scenario, blood that is positive for mosquito-specific antibodies, for which assays already exist, could be deemed as more at risk of containing arboviruses.

We are also investigating ways to modulate early skin immune responses to infection to improve clinical outcome. One approach is to reduce the bite enhancement effect by inhibiting host inflammatory responses to bites. A second approach is to promote the host innate immune responses to virus in the skin, in the hope that this will prevent virus replication before it can spread systemically. Our unpublished studies suggest these approaches are highly effective in suppressing viral replication in the skin, resulting in reduced systemic spread and increased survival to infection with virus. As the most common arboviruses, such as those that cause dengue and chikungunya, are transmitted by the day-biting Aedes mosquitoes, it is likely one could notice most bites within a few hours, providing a time window that is long enough to modulate the early immune response, for instance by applying a cream. This approach could be applicable to many arboviruses, as it targets an aspect common to them all; pathways activated at mosquito bites. This combined with other public health strategies to prevent exposure to biting mosquitoes, and the control of vector numbers could theoretically reduce the level of disease caused by arboviruses and as such warrants further research.

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