The complex relationship between the emerging flaviviruses: dengue and Zika

Many flaviviruses cause important and serious human diseases, including yellow fever, West Nile, Japanese encephalitis and tick-borne encephalitis viruses. Two further flaviviruses, the closely related dengue and Zika virus, have emerged as significant threats to global health with their potential to inflict severe disease to millions of people. Here, we look at some of the molecular similarities and differences between these two emerging diseases, as this is key to the development of novel preventions and therapeutics.

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History and pathology

Flaviviruses are a group of enveloped viruses containing a positive stranded RNA genome of approximately 11 kilobases in length encoding three structural and seven non-structural proteins. The virion is comprised of the nucleocapsid, consisting of the RNA genome and encapsulating capsid (C) proteins, surrounded by a lipid membrane that integrates the other two structural proteins: the envelope (E) and membrane (M). There are some 70 different members within the Flaviviridae family, a major sub-group comprising those that are transmitted by mosquitoes (mosquito-borne flaviviruses; MBFVs). Dengue and Zika viruses belong to this group, with *Aedes aegypti* and *Aedes albopictus* mosquitoes as their primary vectors (Figure 1). Though suspected dengue virus outbreaks have been reported as early as the 17th century, changes in human travel, climate change and unsustainable vector control strategies have allowed the recent establishment or resurgence of its vectors in the tropics and sub-tropics around the globe. Each year, there are an estimated 390 million dengue virus infections with 96 million apparent manifestations around the world. In comparison, Zika virus is newer, having been first identified in Uganda approximately 70 years ago, and has caused few apparent clinical problems as it spread in Africa and Asia. However, it has become a pathogen of real concern in the past few years, as it spread with explosive outbreaks with severe sequela into naïve populations first in Oceania and then South and Central Americas. The requirement of a competent vector in principle defines the geographical spread of these viruses, but it is not the only factor in the case of Zika virus, since recent reports indicate direct human-to-human transmission of Zika virus via contact with bodily fluids of infected individuals.

Both dengue and Zika virus infection typically lead to the development of mild, febrile illness, but infection with these viruses can lead to vastly different severe clinical manifestations. While dengue virus infection can induce disabling disease by causing leakage from the circulatory system that leads to acute haemorrhagic fever and shock, Zika virus infection has been shown to cross the placenta and is implicated in the development of multiple neurological diseases during development involving congenital microcephaly and Guillain–Barré syndrome. Finally, Zika virus undergoes a prolonged infection associated with the testes that leads to sexual transmission, which is unique among the MBFVs. Whilst the presence of Zika in the blood is low in these cases, it does markedly separate it from other *Aedes* clade viruses.

Early phylogenetic studies of MBFVs divided them into epidemiologically and clinically distinct groups based on their vector, reservoir host and disease associations. Two principal clades emerged (see Figure 1): one transmitted by *Aedes* mosquitoes, the other by Culex mosquitoes. However, it is not just the transmission vectors that are different. The *Aedes* clade uses large terrestrial mammals as reservoir hosts and is associated with haemorrhagic disease, in contrast to the Culex clade that associates with rodents and birds, and causes encephalitic disease. Both Zika and dengue viruses are members of the *Aedes* clade, along with yellow fever virus, Spondweni virus and several veterinary viruses. Dengue and yellow fever viruses are permanently established in the New World, with the latter currently causing an outbreak in Brazil. The emergence of Zika virus in the New World is ominous, given the track record of uncontrolled spread of dengue and yellow fever viruses on this continent and especially
as Zika displays devastating consequences following maternal infection.

**Dengue and Zika sero-cross-reactivity: a blessing for the viruses but a curse for their human hosts**

Zika virus is serotypically similar to dengue virus, as demonstrated by strong cross-reactivity of the Zika virus E protein with monoclonal antibodies against the dengue virus E protein. Moreover, both viruses possess very similar epidemiological characteristics and their global expansions show remarkably similar rates of transmission in humans. Although phylogenetic analysis places Zika virus in the *Aedes* clade, some reports suggest it can be transmitted by *Culex* spp., but this association is not supported in all studies. Furthermore, Zika virus is not associated with haemorrhagic disease, and whilst it does not cause encephalitis infection, it is associated with neurological conditions.

There are four types of dengue viruses and they have co-existed in the face of strongly cross-reactive antibodies between them. Indeed, by exploiting antibodies from a primary infection, that are non-neutralizing to a secondary infection, subsequent infections often display increased viral replication in macrophages. This phenomenon is known as ‘antibody-dependent enhancement’ and is mediated by antibody interactions with the viral protein E (see Figure 2). While for the virus, increased viral titre in the blood increases the likely success of transmission; for humans, this can lead to increased risk of severe disease. While cross-reacting antibodies between Zika virus and dengue virus currently remain under-researched, such a phenomenon has also been proposed to lead to the development of severe Zika, as incomplete neutralization of the Zika virus by dengue virus antibodies may cause enhanced Zika virus replication and thus increased chances of fetus infection.

**Dengue and Zika: very similar and yet so different**

Despite their similarities in vector transmission and recognition by the human immune system, there are substantial differences between dengue and Zika viruses (see Table 1). This is most readily evident from the above-mentioned differences in severe clinical manifestations. These differences are probably due to distinct and complex molecular mechanisms of dengue and Zika virus infection, ranging from cell tropism to interactions of dengue and Zika viruses with the host during the course of virus infection.

| Table 1: Mosquito-borne flaviviruses have common and unique characteristics |
|-----------------------------|-----------------|-----------------|
| Clade | Aedes clade | Culex clade |
| Virus | Yellow fever | Dengue | Zika | West Nile |
| Vector | Aedes | Aedes | Aedes/ Culex(?) | Culex |
| Disease | Haemorrhagic | Haemorrhagic | Neurological | Encephalitic |
| NS1-induced vascular leakage | ? | Yes | ? | No |
The elucidation, followed by a comparison, of these interacting host proteins will be crucial to further our understanding of the dengue and Zika viruses.

The three structural proteins and seven non-structural (NS) proteins encoded by the flavivirus genome all play a wide variety of crucial roles during all phases of virus infection. These proteins exert their influence on the host cell processes by disrupting existing interactions or making new connections. Although each individual protein has high protein sequence similarity as well as enzymatic functional conservation to its homologous protein in other flaviviruses, they often interact with unique sets of human host proteins. Both the dengue and Zika virus NS5 proteins have methyltransferase and RNA-dependent RNA polymerase functions, and target the interferon-regulated transcriptional activator STAT2 for degradation. However, the molecular mechanism of degradation is different between the two NS5 proteins. While dengue virus NS5-mediated STAT2 degradation is dependent on the E3 ubiquitin ligase UBR4, Zika virus NS5-mediated STAT2 degradation does not require UBR4.

The unique interactomes of each individual flavivirus could account for the differences observed between the flaviviruses. Hence, a systematic elucidation and comparison of these flavivirus–host interactions would be integral to our understanding of flavivirus infection. Dengue–host interactions have been extensively studied for the past decade, and their elucidation has contributed significantly to our understanding of dengue infection and pathogenesis. In particular, the dengue NS1 protein has been recently shown to induce inflammation and vascular leakage associated with severe disease via binding to a host protein toll-like receptor 4 (TLR4). In addition, NS1 stimulates immune cells to release cytokines that fuel an over-active pro-inflammatory response, and hence essentially acts like a bacterial toxin. This discovery has opened a new avenue for the development of dengue antiviral drugs, as preliminary studies have begun to re-purpose existing drugs used in the treatment of sepsis that target TLR4 to treat dengue infection. These drugs could be highly specific for dengue virus treatment, as the NS1 protein of the closely related West Nile virus does not have the same effect as the dengue virus NS1 protein on vascular leakage. This finding is not particularly surprising considering West Nile virus infection does not induce vascular leakage, but it highlights how the different interactions of flavivirus proteins with host proteins can help account for the clinical outcomes of virus infection. Our knowledge of the clinical outcome of Zika virus infection would suggest that Zika NS1 would behave like West Nile virus NS1, but this remains to be demonstrated, as the Zika NS1 is largely uncharacterized. The fact that both Zika and West Nile virus can cross the blood–brain barrier to infect the brain makes it particularly interesting to uncover whether their NS1 proteins are involved in a common pathway for this process.

**Future directions**

The discovery and comparison of flavivirus-interacting host proteins, such as NS1 and NS5, will be crucial in both understanding the differences in pathology and targets for therapeutic intervention. In the Zika field, we are only beginning to uncover these Zika–host interactions and proteins, but a comparison of these interactions with those of dengue viruses will be particularly illuminating. The discovery of unique and common interactors of Zika and dengue will be instrumental in deepening our understanding of the infection and pathogenesis of these viruses, and bring hope by directing the development of antiviral drugs.

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*Figure 2.* The outer E and M proteins of the dengue 2 and Zika virus shown in cartoon. For comparison, the E proteins of dengue 2 virus (pink) and the Zika virus (light blue) have been superimposed. The E protein forms a dimer (in grey) forming a void into which the M protein (green) locates. Together these are embedded in a membrane via transmembrane helices forming a herringbone arrangement of E dimers at the surface of mature dengue and Zika viruses. A top and side view are displayed, respectively. Images were constructed using data reported under PDB accession numbers 3j27 and 5iz7. It is the variation in this protein, as the target for the human antibody response, which plays a crucial role in antibody-dependent enhancement.
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Further reading

- Grant, A., Ponia, S.S., Tripathi, S. et al. (2016) Zika virus targets human STAT2 to inhibit Type I interferon signaling. Cell Host Microbe 19, 882–890