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Innate immune escape by Dengue and West Nile viruses

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Dengue (DENV) and West Nile (WNV) viruses are mosquito-transmitted flaviviruses that cause significant morbidity and mortality worldwide. Disease severity and pathogenesis of DENV and WNV infections in humans depend on many factors, including pre-existing immunity, strain virulence, host genetics and virus–host interactions. Among the flavivirus-host interactions, viral evasion of type I interferon (IFN)-mediated innate immunity has a critical role in modulating pathogenesis. DENV and WNV have evolved effective strategies to evade immune surveillance pathways that lead to IFN induction and to block signaling downstream of the IFN-α/β receptor. Here, we discuss recent advances in our understanding of the molecular mechanisms by which DENV and WNV antagonize the type I IFN response in human cells.

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Introduction

Dengue (DENV) and West Nile (WNV) viruses are arthropod-transmitted viruses of the genus Flavivirus (family *Flaviviridae*) that pose a significant global health concern. They are closely related to several other insect-transmitted viruses that cause disease worldwide including Zika (ZIKV), yellow fever (YFV), Japanese encephalitis (JEV), and tick-borne encephalitis viruses. DENV is transmitted to humans principally by two mosquito vectors, *Aedes aegypti* and *Aedes albopictus*, and causes a spectrum of disease ranging from dengue fever to severe dengue (previously called dengue hemorrhagic fever/dengue shock syndrome [DHF/DSS]), which is potentially lethal. Severe dengue is characterized by extravasation of fluid into peritoneal and pleural spaces, bleeding in the skin and gastrointestinal tract, thrombocytopenia and mild to moderate liver injury [1]. Four serotypes of DENV (DENV 1-4) exist and infection by one serotype confers durable protection against disease only to that particular serotype. Poorly neutralizing or low affinity cross-reactive antibodies generated during primary infection with one DENV serotype can facilitate severe disease during secondary infection with a heterologous serotype, a phenomenon attributed to 'antibody-dependent enhancement of infection' (ADE) [2]. DENV is the leading cause of mosquito-borne viral disease, with an estimated total of ~390 million infections globally each year, primarily in subtropical and tropical regions [3]. Although no specific therapies against DENV are available, this past year, the first tetravalent Dengue vaccine (Dengvaxia®) was approved for use in Brazil, Mexico, and the Philippines in individuals 9-45 years of age [4,5]. This tetravalent vaccine appears to reduce hospitalization in those with prior DENV immunity; however, concern has been raised as to whether it sensitizes naïve individuals to greater symptoms, disease, and hospitalization in the context of a subsequent naturally-acquired first DENV infection [6,7].

WNV is a neurotropic virus that circulates and amplifies in nature between several bird species and ornithophilic mosquitoes. Although WNV can be transmitted to humans by mosquitoes of the *Culex* species, humans are a dead-end host and do not participate in the enzootic cycle. WNV causes a self-limiting febrile illness in most individuals that occasionally can progress to severe neurological disease including encephalitis, meningitis, and acute flaccid paralysis, with a case mortality rate of up to 5–10%. Originally isolated in the West Nile region of Uganda in 1937, WNV is now endemic within areas of Asia, Europe, the Middle East, and also North America. Since its introduction into the United States in 1999, there have been more than 45 000 reported WNV cases and an estimated 3 million people infected in the US [8]. Currently there are no vaccines or specific antiviral agents licensed for use in humans to protect against WNV infection.

DENV and WNV are enveloped viruses that package a positive-sense single-stranded RNA genome encoding a single open reading frame. Translation results in a polyprotein that is cleaved by host and viral proteases and yields three structural (capsid [C], pre-membrane/membrane [prM/M] and envelope [E]) and seven non-structural

(NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) proteins. Upon attachment and entry into target cells — predominantly myeloid cells for DENV and multiple cell types for WNV, including skin-resident dendritic cells, keratinocytes, and neurons — these viruses replicate in endoplasmic reticulum (ER)-derived membrane vesicles in the host cytoplasm. The cytoplasmic replication strategy makes these viruses vulnerable to several innate immune sensors (also termed pattern recognition receptors [PRRs]), which detect viral RNA, or in some cases, host-derived molecules generated after virus infection. Upon their activation, PRRs initiate antiviral defense programs in infected and uninfected neighboring cells to impede viral replication and dissemination. However, DENV and WNV have evolved to evade or actively suppress innate immune responses through a variety of mechanisms. Many of the DENV and WNV non-structural proteins, which also are essential for viral RNA synthesis and assembly, have important functions in viral evasion of innate immunity. Recent discoveries have provided insights into the molecular mechanisms by which DENV, WNV, and other globally relevant flaviviruses (*e.g.*, ZIKV) escape innate immune defenses.

Herein, we summarize recent findings on innate immune detection of DENV and WNV and discuss in detail the strategies by which these viruses antagonize type I IFN-mediated immunity, with a focus on the RIG-I-MAVS, cGAS-STING, and IFN-α/β receptor (IFNAR) signaling pathways.

Innate immune sensing of DENV and WNV infection

Mammalian cells sense unique features of invading viral pathogens through a sophisticated innate immune surveillance network comprised of membrane-bound, cytoplasmic and nuclear PRRs (reviewed in [9,10]). Multiple PRRs work in concert to sense viral infections by recognizing viral nucleic acids, proteins and/or carbohydrates, commonly termed pathogen-associated molecular patterns (PAMPs). In addition, some PRRs sense host-derived 'danger signals' (also called damage-associated molecular patterns [DAMPs]) that are produced by the cell upon viral infection. At least four major classes of PRRs contribute to the efficient detection of WNV and DENV infection in human cells: the RIG-I-like receptors (RLRs), Toll-like receptors (TLR3 and 7), NOD-like receptors (NLRs), and the cGAS-STING-dependent sensing pathway (reviewed in detail in [11,12]). These sensors, many of which are upregulated upon WNV and DENV infection [13], activate signaling cascades that induce antiviral or proinflammatory genes, including cytokines such as type I (mainly IFN-α subtypes and IFN-β) and III (IFN-λ) IFNs, chemokines, and IFN-stimulated genes (ISGs). Proteins encoded by ISGs then interfere with specific steps in the viral lifecycle or regulate innate immune signaling, ultimately establishing an antiviral state. Furthermore, cytokines and chemokines produced upon PRR activation help shape the adaptive immune response as well as the infiltrating inflammatory cell response to infection.

RLRs detect cytoplasmic viral RNA species, such as viral replication products or RNA genomes, and are considered as key intracellular PRRs for the detection of DENV, WNV, and other flaviviruses. The RLR family members, RIG-I, MDA5, and LGP2 are expressed in most cell types and characterized by a DExD/H-box helicase domain and a C-terminal domain (CTD), which are both important for binding viral RNA. In addition, RIG-I and MDA5 have two N-terminal caspase activation and recruitment domains (CARDs), which are responsible for initiation of antiviral signaling *via* the adaptor protein MAVS, which is localized at the mitochondria, mitochondria-associated membranes (MAMs) and peroxisomes. In contrast, LGP2 lacks the CARD signaling module and has been suggested to function as a regulator of RIG-I and MDA5 signaling (reviewed in [9,14]).

RIG-I senses viral RNA species that are characterized by extensive secondary structures (*e.g.*, dsRNA loops or panhandle-like structures) with an adjacent 5' tri- or diphosphate moiety. RIG-I also recognizes poly-U/UC sequence motifs in the genome of the distantly related *Flaviviridae* family member, hepatitis C virus (HCV). MDA5 is thought to recognize long dsRNA or web-like RNA aggregates (reviewed in [15,16]); however, the physiological RNA ligands detected by MDA5 are largely unknown. In the context of WNV infection, RLRs are crucial for controlling pathogenesis in mice [17,18,19]. Furthermore, gene targeting of RIG-I and/or MDA5 in human and murine cells showed that both sensors contribute to efficient detection of DENV and WNV [18,20,21,22]. During WNV infection, RIG-I and MDA5 act in a temporally distinct manner: while RIG-I was activated early in infection (<12 h post-infection), MDA5 was activated later (>24 h post-infection) [18]. Whether RLRs act analogously during DENV infection remains to be determined. Furthermore, the RNA species that are recognized by RIG-I and MDA5 during DENV and WNV infection are unknown. As the RNA genomes of DENV and WNV contain a 5' type 1 cap structure and thus are expected to not stimulate RLR activity, it is likely that RNA replication intermediates (*e.g.*, negative strand RNA), which contain a 5' triphosphate group and may form dsRNA structures, or non-coding subgenomic flavivirus RNAs (sfRNA; reviewed in [23]) are sensed by RIG-I and MDA5.

Recently it has been shown that the cGAS-STING pathway, which is known to sense DNA viruses (reviewed in [24]), also restricts DENV and WNV infection. Gene silencing of the ER-resident adaptor protein STING (also called MITA, MPYS, or ERIS) enhanced DENV replication through reduction of proinflammatory cytokine