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Persistence of West Nile virus

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Abstract

West Nile virus (WNV) is a widespread global pathogen that results in significant morbidity and mortality. Data from animal models provide evidence of persistent renal and neurological infection from WNV; however, the possibility of persistent infection in humans and long-term neurological and renal outcomes related to viral persistence remain largely unknown. In this paper, we provide a review of the literature related to persistent infection in parallel with the findings from cohorts of patients with a history of WNV infection. The next steps for enhancing our understanding of WNV as a persistent pathogen are discussed.

Keywords: West Nile virus; Persistence; Renal outcomes; Neurologic outcomes; Morbidity; Mortality

1. West Nile viral characteristics, acute disease presentation, and epidemiology

West Nile virus (WNV) is a positive-sense single stranded RNA virus from the Japanese encephalitis complex of the Flaviviridae family [34]. Other medically important viruses from the Flaviviridae family include Japanese encephalitis, dengue, yellow fever, and hepatitis C [34]. WNV is a 50 nm spherical enveloped particle containing a 10.8 kb genome. The 3433 amino acid polyprotein codes for viral capsid, envelope, membrane, and nonstructural proteins [10,15]. Virus replication occurs in host cellular cytoplasm [15]. Virions are then assembled in the endoplasmic reticulum and transported in vesicles to the cell surface for exocytosis [10,8,76].

WNV is an avian zoonosis maintained in nature through an enzootic cycle between mosquitoes and avian species. *Culex* species, particularly *Cx. pipiens, Cx. tarsalsis,* and *Cx. quinquefasciatus*, are important mosquito vectors for disease transmission in North America [64]. Avian amplifying hosts vary by geographic location, and over 330 avian species have tested positive for WNV in North America alone [64,17]. However, migratory birds are suspected to be the principal vehicle for the geographic spread of WNV [21,62]. Birds become infected from the bite of an infected mosquito via transmission of virus in the salivary fluid, and immunologically naïve birds can develop viremia for up to 100 days, allowing for a long period of time to infect mosquitoes seeking out a blood meal [17,33]. Horses and humans are considered dead-end hosts, as they are unable to generate a sufficient viral titer for infection of naïve mosquitoes [80]. However, horses and humans are capable of developing an immune response and clinically-apparent disease as a result of infection.

Humans are traditionally infected by the bite of an infected mosquito via transmission of virus in the salivary fluid. Other less common transmission sources include blood transfusion, organ transplantation, congenital, and possibly through ingestion of infectious breast milk [30,52,29,56]. The majority of infected persons do not develop symptoms, while 20% have self-limiting febrile illness, and less than 1% develop acute neuroinvasive disease that can manifest as encephalitis, meningoencephalitis, meningitis, or acute flaccid paralysis

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[71,70]. There are no specific treatments for symptomatic disease other than supportive care.

The first reported case of WNV infection was described in a febrile woman from the West Nile district of Uganda in 1937 [73]. Sporadic epidemics occurred over the subsequent 60 years, particularly in the Mediterranean basin [41,42]. In 1999, the first outbreak of WNV was described in New York City [48]. Following this initial outbreak, WNV quickly spread across the North American continent resulting in continuous epidemics in 47 contiguous states of the United States, Canada, and Mexico [37,11,65,19,40]. Human cases began to taper off over the subsequent years coinciding with establishment of the disease in an endemic cycle with modest epizootics every 3–4 years [51,37,28,63].

In the summer of 2012, an unprecedented large outbreak occurred in the United States, with Texas being in the center of the majority of virus activity with more than 1800 human cases reported [16]. This outbreak served as a reminder that WNV would continue to cause substantial morbidity and mortality in the United States. To date, more than 41,000 human cases of disease, including 1700 deaths, have been reported to CDC's arbonet surveillance system since WNV was first recognized in New York in 1999 (http://www.cdc.gov/westnile/statsmaps/). Based on the number of neuroinvasive disease cases reported, an estimated 3 million adults have been infected in the United States, with geographic pockets of higher seroprevalence seen in the central plains region [57]. The highest seroprevalence has been reported from North Dakota where 1 per 12 residents is believed to be infected based on blood donor studies [13]. Despite the high disease burden, there are no effective prevention or treatment strategies clinically available. Additionally, the long-term morbidity, including neurological outcomes, is still relatively unknown. Having established a prospective cohort of patients with a history of WNV infection starting in 2002 in Houston, Texas, we have been granted the opportunity to study disease outcomes first hand. Here, we review the evidence of persistence of WNV infection in animal models and published human observational studies, and parallel these studies with our own cohort findings.

2. Molecular determinants of acute and persistent infection

Host genetics, host immune responses, and co-morbidities have been implicated in the underlying pathogenesis of acute disease severity. Single-nucleotide polymorphisms in the interferon response pathway, particularly the OAS gene, were found to be associated with symptomatic and neuroinvasive WNV disease [9]. Interleukin-4, a key regulator in adaptive immunity as a B and T cell stimulator, has been demonstrated as being particularly important in distinguishing between asymptomatic and severe human disease [61]. Co-morbidities associated with increased risk for development of neuroinvasive disease include hypertension, diabetes, chronic renal disease, immunosuppressing conditions, cardiovascular disease, history of alcohol abuse, and history of cancer [46,36]. While these factors' influence on acute infection have been well documented, their role in persistent neurologic and renal infection have not been studied.

During acute infection, WNV replicates in dermal cells at the site of inoculation and in draining lymph nodes resulting in a systemic viremia [74]. The exact mechanisms of neuroinvasion are less known but a few hypotheses exist. The blood brain barrier is suspected to be the primary route of invasion due to it being the interface between viremic blood and the brain, and the temporal kinetics of pathogenesis. The blood brain barrier is a highly selective barrier comprised of four main components: endothelial cells, astrocytes, microglial cells and pericytes [1]. Two hypotheses for WNV neuroinvasion mechanisms include transendothelial viral entry of epithelial cells and/or permeability of the blood brain barrier. Transendothelial viral entry has been demonstrated *in vitro* with WNV and Japanese Encephalitis virus, a close relative to WNV, where transcellular vesicle transportation was noted in cerebral endothelial cells [81,38,27]. Unfortunately, the few *in vivo* studies looking at neuroinvasion across these cerebral endothelial cells have not been able to reproduce the same results [12,23].

The second hypothesis of WNV neuroinvasion involves an increased permeability of the blood brain barrier resulting in paracellular migration [66,83]. Tight junctions between cerebral endothelial cells are important in maintaining structural integrity of the blood brain barrier. Two important tight junction proteins responsible for maintaining cellular contact, claudins and occludins, have been seen at reduced levels in WNV post-infection particularly in the presence of pro-inflammatory cytokines [18]. Their reduced levels could be reflective of a disruption of the barrier possibly allowing paracellular migration [74]. With elevated pro-inflammatory cytokines seen years post-infection correlating with neuropsychological symptoms in persons, this neuroinvasive mechanism should be further investigated, as no human studies have been performed [22]. Blood brain barrier leakage post-infection could be measured in neurologically symptomatic WNV patients using contrast magnetic resonance imaging techniques [75].

Immunoglubin M (IgM) antibody is an indicator of active infection that presents within the first 3 days of WNV infection [59]. IgM titers should decline approximately 2–3 months following acute infection, but extended IgM levels could indicate continued activation of the humoral immune response. Multiple studies have shown persistent IgM antibodies at 6 months to 1 year post-infection in both serum and cerebrospinal fluid of WNV infected patients [59,67,53,60,31,14]. With our cohort in Houston, we had the opportunity to longitudinally evaluate serial IgM antibodies over an extended time period (up to 8 years). Unexpectedly, we found a fifth of our patients had detectable IgM antibody levels at both 6 and 8 years post-infection [45]. While IgM levels fluctuated over time, they demonstrated an overall decline. In our cohort, IgM persistence was associated with potentially immunosuppressing social behaviors, such as chronic alcohol abuse and tobacco smoking [45]. Interestingly, we did not find history of