Review

Long-term, West Nile virus-induced neurological changes: A comparison of patients and rodent models

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ABSTRACT

West Nile virus (WNV) is a mosquito-borne virus that can cause severe neurological disease in those infected. Those surviving infection often present with long-lasting neurological changes that can severely impede their lives. The most common reported symptoms are depression, memory loss, and motor dysfunction. These sequelae can persist for the rest of the patients' lives. The pathogenesis behind these changes is still being determined. Here, we summarize current findings in human cases and rodent models, and discuss how these findings indicate that WNV induces a state in the brain similar neurodegenerative diseases. Rodent models have shown that infection leads to persistent virus and inflammation. Initial infection in the hippocampus leads to neuronal dysfunction, synapse elimination, and astrocytosis, all of which contribute to memory loss, mimicking findings in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). WNV infection acts on pathways, such as ubiquitin-signaled protein degradation, and induces the production of molecules, including IL-1β, IFN-γ, and α-synuclein, that are associated with neurodegenerative diseases. These findings indicate that WNV induces neurological damage through similar mechanisms as neurodegenerative diseases, and that pursuing research into the similarities will help advance our understanding of the pathogenesis of WNV-induced neurological sequelae.

1. Introduction

Viral infections of the central nervous system (CNS) can cause severe damage to the functional regions there, leading to dysfunction of the neural circuits that underlie proper neurological function. When this dysfunction continues past the acute phase of infection, these neurological deficits are termed "sequelae." These sequelae can have significant effects on patient quality of life and productivity.

West Nile virus (WNV) is the most widespread mosquito-borne virus in the United States. It is maintained in an enzootic cycle between birds and mosquitos, with spillover into humans and other animals. WNV was discovered in the West Nile region of Uganda in 1937, but neurological disease associated with infection was not reported until an outbreak in Israel in 1957 (Sejvar, 2003). It was first introduced to the US in 1999 and was detected when cases of unknown viral encephalitis in the state of

New York were diagnosed as cases of WNV infection (Nash et al., 2001). The virus has spread throughout the continental United States, Central and South America, and parts of Canada. It is also found in Europe, Australia, the Middle East, and Africa; cases have been reported throughout much of Asia and Europe (Donadieu et al., 2013a). Since monitoring for WNV began in the US, there has been a noted correlation between WNV infection and long-term neurological sequelae after recovery.

Screening of blood donations for viral RNA has been used to assess the rate of asymptomatic infections with WNV. The majority (estimated 70–75%) of human WNV infections are asymptomatic (Gea-Banacloche et al., 2004; Watson et al., 2004; Betsem et al., 2017). About one in four infections manifests as a non-specific, flu-like illness called West Nile fever (WNF). In roughly one in every 150 infections, the virus causes a severe neurological disease called West Nile neuroinvasive disease

(WNND) (Betsem et al., 2017; Sambri et al., 2013). Cases of WNND manifest as one or more of the following, based on the spread of the virus: West Nile encephalitis (WNE; infection of the brain parenchyma), West Nile meningitis (WNM; infection of the meninges), and West Nile paralysis (WNP; infection of the spinal cord leading to poliomyelitis) (Hart et al., 2014). These are diagnosed through a combination of clinical signs and laboratory confirmation of viral infection of the CNS, including analysis of cerebrospinal fluid for viral RNA or antibodies against the virus (Sambri et al., 2013; Kauffman et al., 2011). Based on US data, WNND has a 10% case fatality (CDC, 2018; McDonald et al., 2019). Treatment is generally limited to supportive care though there have been successful, small-scale trials using intravenous gamma globulin from survivors of WNV infection (Walid, 2009; Shimoni et al., 2012). Since 1999, there have been over 20,000 diagnosed cases of WNND in the US, with over 1000 cases per year each year since 2012 (McDonald et al., 2019).

The prevalence of sequelae among survivors of WNV infection varies between studies, but it is generally between 30% and 60% (Murray et al., 2014; Loeb et al., 2008; Hughes et al., 2007; Cook et al., 2010). Reported sequelae include a wide range of deficits such as paralysis, memory loss, depression, and fatigue (Patel et al., 2015). These may last for months or for the rest of the patient's life. The mechanisms that underlie these sequelae are poorly understood, and this review aims to catalog recent findings, both clinical and experimental, as well as provide guidance to advance our understanding of WNV-induced neurological sequelae.

2. Neuropathogenesis

Based on mouse models, WNV initially replicates in the skin, and then uses resident phagocytic cells to spread to the lymph nodes. The virus enters the bloodstream via the thoracic duct and enters the CNS around five days post-infection. The virus is cleared from the peripheral organs between six and eight days post-infection, though it may be replicating in the CNS at this time (Suthar et al., 2013).

WNV has shown been shown to enter the CNS through multiple pathways including transsynaptic spread, breakdown of the blood-brain barrier (BBB), transendothelial spread, and infection of immune cells that cross into the brain parenchyma. The roles that each of these forms of entry play in overall pathogenesis are still being studied and may not be common across all strains of WNV. Endothelial cell infection has been shown *in vitro* (Hasebe et al., 2010) but has not been recapitulated *in vivo*. WNV infection has been shown to decrease surface expression of BBB molecules important to maintaining cell-cell adhesion including claudin-1, occludin, JAM-A, and β-catenin, with an increase in matrix metalloproteases (Roe et al., 2012). This leads to increased permeability through the BBB and increased extravasation of immune cells. This has been attributed as a potential cause of direct viral crossover into the CNS and as a mechanism for infected peripheral cells to enter the CNS. Axonal transport of WNV has also been shown in hamsters, and WNV appears to be able to move both retrograde and anterograde (Samuel et al., 2007), indicating another mechanism for spread to the CNS.

The envelope protein of WNV mediates entry into cells through clathrin-mediated endocytosis (Chu and Ng, 2004), with the WNV envelope protein as the receptor-binding protein. The receptors for the virus have not yet been determined though there is evidence that DC-SIGNR (Davis et al., 2006), $\alpha_v\beta_3$ integrin (Lee et al., 2006), and laminin-binding protein (Chu and Ng, 2004) have been shown to facilitate entry into cells. Within the CNS, the virus primarily replicates in neurons (Hayes et al., 2005), but there is evidence that it can infect glial cells including astrocytes (van Marle et al., 2007).

The immune response to WNV in the CNS begins with recognition of the virus through pathogen recognition receptors including Toll-like receptors (TLRs). Studies using knockout mice have shown that signaling through the TLRs is necessary for survival of acute WNV infection in mice (Sabouri et al., 2014). TLR3 has been shown to be protective in infection (Daffis et al., 2008a) but also play a role in allowing viral entry into the

CNS (Wang et al., 2004). TLR7 is important for both innate and adaptive immune responses (Xie et al., 2016). The TLRs initially signal through microglia, which leads to the recruitment of peripheral immune cells including monocytes and T cells. The type-I interferon response is crucial to survival of acute infection including upregulation of Ifitm3 (Gorman et al., 2016; Daniels et al., 2017) and interferon-response factor-3 (Daffis et al., 2007) and −7 (Daffis et al., 2008b), all of which reduce viral load in the CNS.

WNV of neurons has been shown to induce the accumulation of misfolded, ubiquitinated proteins (Kobayashi et al., 2012) leading to neuronal malfunction and death. This has been linked specifically to activity of the capsid protein, which inhibits the autophagy pathway by inducing degradation of AMP-activated protein kinase (Kobayashi et al., 2020).

Molecular phylogenetic analyses of partial and complete genome sequences have defined multiple lineages of WNV, which is an additional consideration to risk of long-term neurological sequelae resulting from WNV infection. There are six defined lineages of WNV, with diversity up to 25% at the nucleotide level (Pesko and Ebel, 2012). The most widespread is lineage 1, which has been detected on all continents except Antarctica. Lineage 2 is the next most common and is primarily found in Africa and Europe (Petersen et al., 2013), and differs from lineage one by 17–20% at the nucleotide level (Kuno et al., 1998). Comparisons of virus pathogenicity in mice suggest that the neuropathogenicity varies from strain to strain within a lineage (Beasley et al., 2002). The outbreak in New York in 1999 involved introduction of a stain from lineage 1 and led to 63% of those affected showing ongoing clinical signs one-year post-infection (Klee et al., 2004). In addition, the strains of WNV present in the US have been shown to be mutating with potential changes to pathogenicity (Mann et al., 2013). Some North American variants show reduced virulence and pathogenicity in hamsters (Siddharthan et al., 2009a), mice (Davis et al., 2003, 2007), and birds (Davis et al., 2003; Brault et al., 2011), but the majority of circulating genotypes appear to retain a neuroinvasive phenotype. The Kunjin subtype from Australia is also grouped in lineage 1, and shows little to no neurovirulence (Donadieu et al., 2013a, 2013b; Frost et al., 2011). The Kunjin strain from Australia shows significant differences from the more pathogenic IS98 strain (Donadieu et al., 2013b). While the IS98 strain of WNV is more lethal in mice, the Kunjin virus induced neuronal apoptosis and inflammatory cell infiltration, indicating that. apoptosis and inflammatory cell infiltration are crucial for early control of the virus.

Lineage 2 WNV appears to have spread from Africa into Europe, Russia, and the Middle East some time in the late 19th or early 20th century and shows approximately 94% amino acid identity and 80% nucleotide identity with lineage 1 WNV. Some WNV strains from lineage 2 are neuroinvasive as seen in outbreaks in Russia, Greece, Italy, and Hungary (Bagnarelli et al., 2011; Magurano et al., 2012; Sitati and Diamond, 2006; Bakonyi et al., 2006). One follow-up study (Anastasiadou et al., 2013) in Greece showed similarly high levels of sequelae one year after infection with a lineage 2 strain of WNV. The reported symptoms were similar to those described after infection by lineage 1 strains of the virus. In experimental models, the lineage 2 strains of WNV are generally less neuroinvasive in hamsters and mice though this varies based on strain (Beasley et al., 2002). Whether infections with different strains of WNV lead to different risks of the development of sequelae is still being determined.

3. Risk factors for the development of neurological sequelae

Certain factors have been conclusively linked to an increased risk of developing WNND during WNV infection, including old age (Lindsey et al., 2010), immune dysfunction, concurrent chronic diseases such as diabetes or hypertension (Lindsey et al., 2012), and mutations in the *CCR5* gene (Glass et al., 2006). However, development of WNND does not always correlate with development of neurological sequelae. Some concurrent diseases such as diabetes and hypertension have been

associated with greater risk of sequelae (Cook et al., 2010). Old age appears to increase the risk (Murray et al., 2014; Patel et al., 2015; Balakrishnan et al., 2016), while younger age correlates with a greater chance of full recovery (DeBiasi and Tyler, 2006). Gender appears to play a role as well: men are less likely to develop depression (Nolan et al., 2012), and men tend to recover cognitive function faster than women (Loeb et al., 2008). This may be due to the difference in immune response to WNV between men and women. Men maintain a longer elevation of peripheral cytokines and reported fewer symptoms during the acute phase of illness (Hoffman et al., 2019), but this has not yet been correlated to incidence of sequelae.

Whether the initial clinical presentation reflects the risk of WNV-related neurological sequelae is controversial. It has been reported that sequelae occur more commonly in patients diagnosed with WNND (Ouhoumanne et al., 2018); however, it has also been reported that the severity of initial disease, including hospitalization, does not increase risk (Carson et al., 2006). Specific to WNND, those with WNM tend to show complete recovery, while those with WNE and WNP have greater risks of long-term sequelae (Hart et al., 2014). WNE tends to be associated with the highest risk of developing long-term neurological sequelae, with up to 86% of patients presenting with abnormal neurological exams up to three years post-infection (Weatherhead et al., 2015).

Initial CNS invasion does not appear to be necessary for development of sequelae, as patients with WNF have reported development of neurological sequelae. One study (Weatherhead et al., 2015) showed that 27% of patients diagnosed with WNF without diagnosed WNND had neurological abnormalities up to three years post-infection. When re-interviewed at 8–11 years post-infection, 57% of subjects reported new abnormalities. Neurocognitive sequelae appear to occur equally in patients diagnosed with WNND compared with patients diagnosed with WNF (Patel et al., 2015; Samaan et al., 2016) or at an increased rate (Ouhoumanne et al., 2018; Haaland et al., 2006; Sadek et al., 2010), depending on the study. Other studies have shown that patients diagnosed only with WNF have measurable deficits in one or more neuropsychological functions, including memory, executive function, depression, and motor coordination over one year post-infection (Carson et al., 2006; Sadek et al., 2010). One notable difference is that motor sequelae persist more in patients initially diagnosed with WNND (Hughes et al., 2007; Carson et al., 2006). However, most of the studies agree that neurological sequelae do occur in some patients after WNF. This could indicate either that the virus can enter the CNS without causing overt clinical signs of neurological disease, or that peripheral inflammatory responses lead to neurological malfunction. Further research will need to be done to determine the extent of CNS inflammation in patients with sequelae following WNF.

4. Types of neurological sequelae and insights into pathogenesis

Some viruses show tropism for specific brain areas, leading to changes in specific neurological functions. For example, respiratory syncytial virus has a specific tropism for olfactory sensory neurons (Bryche et al., 2019), while Zika virus has a tropism for neural stem cells in the brain, especially of the neocortex (Brault et al., 2016). WNV can infect many different regions of the CNS. Post-mortem, virus has been found in the hippocampus, cerebellum, basal ganglia, thalamus, midbrain, and pons (Armah et al., 2007; Penn et al., 2006). The cranial nerves and spinal nerves may be involved, as well. WNV infection causes inflammatory cell infiltration, neuronal cell death, gliosis, reactive astrocytosis, perivascular cuffing, and infiltration of monocytes and lymphocytes from the peripheral blood (Armah et al., 2007). The virus primarily replicates in neurons (Hayes et al., 2005), but there is evidence that it can infect glial cells including astrocytes (van Marle et al., 2007). Outside of the brain, WNV has been localized to the spinal cord, dorsal root ganglia, and peripheral motor neurons (Guarner et al., 2004). The variety of CNS regions that WNV can affect may explain the spectrum of sequelae that it can induce.

The most commonly reported sequelae are associated with motor functions, including fatigue, myalgia, and generalized or limb weakness (Patel et al., 2015). Motor issues are generally attributed to damage to peripheral motor neurons or the dorsal horn of the spinal cord from which sensory neurons emanate. In cases where the patient succumbed to infection, the virus has been found in the cerebellum and substantia nigra (Guarner et al., 2004). The cerebellum is largely responsible for fine motor coordination (Leiner, 2010) while the substantia nigra regulates the initiation of voluntary movements (Lanciego et al., 2012). Damage to either region could lead to motor incoordination or deficits. In cases of WNP, about one-third of those affected attain complete or near-complete recovery, one-third show partial recovery, and one-third show little to no improvement (Hughes et al., 2007). Weakness tends to persist even when other symptoms resolve (Hart et al., 2014). In those without complete recovery, the weakness is generally not associated with sensory loss. One study (Athar et al., 2018) indicated that, despite reporting weakness and numbness of the extremities, about one-fifth of the patients showed normal electromyelography (EMGs) results indicating that the peripheral weakness and numbness was likely of central origin. In the remaining 80% of patients, the abnormal EMG results were attributed to WNP, neuromuscular junction disorder, or sensory/sensorimotor polyneuropathy. Two of the patients showed signs of chronic and active denervation, indicating an ongoing inflammatory process. Patients with long-term paralysis or weakness often show decreased motor amplitudes on electrodiagnostics, indicating damage to motor neurons or cells of the anterior horn of the spinal cord (Sejvar et al., 2010). This indicates ongoing damage to the neurons of both the CNS and PNS, which could be due to excitotoxic mechanisms or persistent viral infection. As with other sequelae, the severity and persistence of motor deficits varies from patient to patient. These findings indicate that WNV can cause motor weakness through both central and peripheral mechanisms though the exact mechanisms need further study.

Memory loss is a commonly reported neurocognitive symptoms (Patel et al., 2015). One study (Murray et al., 2018) correlated neurological function (via neuropsychological testing) with the MRIs of patients who survived either WNF or WNND. Patients ranged from three to eight years post-infection. Almost half of the participants had abnormal neurological findings, including weakness, abnormal reflexes, tremors, and immediate or delayed memory loss. One-fifth showed neuropsychological impairments including short-term and long-term memory deficits. A subset of these patients received an MRI, which showed cortical thinning in multiple brain regions including the posterior cingulate cortex, superior frontal cortex, and the para-hippocampal region. Memory loss correlated with the thinning of the caudal middle frontal gyrus, rostral middle frontal gyrus, and supramarginal gyrus of the left hemisphere only. The middle frontal gyrus plays a role in maintaining attention (Corbetta et al., 2008) and the supramarginal gyrus is involved with multiple processes including cognitive functions (Quiñones-Hinojosa, 2012), both of which could account for changes in memory function. Although the functional changes only correlated with thinning of the cortices in the left side, it seems unlikely that laterality would always play a role. This study was based on 30 patients, so whether these regions are specifically affected or play a significant role in neurocognitive sequelae needs to be further studied.

Patients have reported developing depression after WNV infection to the level of major depressive disorder (MDD). The incidence of MDD among WNV survivors varies between different studies, but ranges between 21% and 56% of patients (Loeb et al., 2008; Cook et al., 2010; Nolan et al., 2012; Murray et al., 2007). In one study, 75% of patients with reported depression scored positively for mild to severe depression using the Center for Epidemiologic Studies Depression scale (Murray et al., 2007). However, one study reported that MDD occurred at higher rates in patients diagnosed with WNF compared to those diagnosed with WNND (Hart et al., 2014), though this has not been consistent across all studies. There have not been any studies investigating potential mechanisms for depression in patients surviving WNV infection. Many other

viruses have been associated with depression following infection, including influenza, varicella-zoster, human immunodeficiency virus (Coughlin, 2012), herpes simplex virus 2, and cytomegalovirus (Gale et al., 2018), and the mechanisms by which these viruses cause depression should serve as a starting point for WNV research.

Whether the virus persists in the human CNS after the acute neuro-invasive infection has not been fully demonstrated. In support that WNV may persist in the CNS, the virus has been shown to persist in the CNS of an immunocompromised patient for up to four months post-infection (Penn et al., 2006) and there is indirect evidence for persistent infection in that some patients maintain high levels of anti-WNV IgM in the cerebrospinal fluid and blood (Kapoor et al., 2004). It appears that WNV can also persist in the kidneys of some patients with other underlying chronic conditions for years after infection, and people with viral persistence in their kidneys tend to have higher rates of neurological sequelae (Murray et al., 2010). This may be due to consistent viral shedding or a more disseminated infection in these patients. Still, further research needs to be done to determine how often the virus persists in patients and how this impacts the development of sequelae. However, technical limitations to CNS virus detection are an impediment. Detection of live, replicating virus or viral nucleic acids indicating persistent infection requires invasive procedures such as biopsies that allow for histopathologic or molecular (RT-PCR) analyses targeting the virus. Biopsies of the brain or spinal cord require full anesthesia and present significant risk to patients. Pre-mortem, non-invasive methods to detect WNV are needed to determine the persistence of the virus and what role it plays in the development of sequelae.

5. Rodent models of WNND-Induced neurological sequelae

Rodent models provide an attractive option for testing potential mechanisms of WNV-induced CNS damage. WNV commonly invades the CNS of rodents including mice and hamsters resulting in disease outcomes that align well with human WNV pathology. In mice (Donadieu et al., 2013b) and hamsters (Xiao et al., 2001), it has been shown that WNV infects similar regions of the brain, spinal cord, and peripheral nerves as reported in humans. WNV infection also causes similar pathologic changes in rodents as those found in human cases: inflammatory peripheral immune cell infiltration, reactive astrocytosis, neuronal cell death, and gliosis (Zukor et al., 2017). Additionally, specific types of hamsters and mice show survival post-infection, allowing for the study of neurological sequelae. Established neurobehavioral tests can be used to probe WNV neurological sequelae in addition to the numerous genetically modified mouse strains that test specific immune, motor, emotional and cognitive mechanisms.

The mouse is a commonly used animal model of WNV infection, and the C57BL/6J strain is frequently employed. . Outcomes from multiple studies suggest that WNV can persist in the CNS of infected mice and that the host maintains an immune response against it. Live, replicating WNV has been recovered from the brains and spinal cords of mice up to four and six months, respectively, after peripheral inoculation with WNV (Appler et al., 2010). Similarly infected mice had increases in B cells and T cells in the brain up to 12 weeks post-infection. Antibody-secreting cells specific for WNV and virus-specific T-cells were found in the brain up to 16 weeks post-infection. This occurred in mice with and without clinical signs during the acute phase of infection (Stewart et al., 2011). These studies indicate that WNV can persist in the CNS and that the host maintains an immune response against it. These data suggest that our understanding of the pathophysiology of human WNV neurological sequelae could be advanced by investigating peripheral lymphocyte infiltration and activation as a hallmark of ongoing neuroinflammation.

There are difficulties in performing tests to assay neurological changes in rodent models that would allow for comparison to the long-term outcomes in humans. WNV requires biosafety level-3 (BSL-3) containment for work (Department of Health a, 2009). This limits the number of facilities, tests, and personnel that can work with the virus.

The virus can also be highly virulent in rodent models, making the study of long-term sequelae difficult. Wild-type WNV strains generally cause lethal disease when inoculated intracranially, even at very low doses, so ensuring neuroinvasion while still allowing for long-term studies is difficult. While there are numerous behavioral tests to study neurological changes in mice, many of these are not optimized for work in biocontainment, and those that are require training that is not common among virologists. The diagnostic methods most commonly used on patients-namely MRIs-are expensive, large, and would require a dedicated BSL-3 area for animal work, which further complicates the ability to compare clinical findings between humans and rodents. For these reasons, studies using animal models to assess behavioral and physical outcomes of nonlethal WNV infection have had to make concessions and try to provide comparisons to the limited behavioral and pathology data reported from clinical studies when available.

One mouse model that has provided unique insight into the pathogenesis of WNV-induced memory loss used a virus with a mutation in non-structural protein 5 (NS5). This attenuated the virus' ability to antagonize the interferon response. Following direct inoculation into the cerebral ventricles of C57Bl/6J mice (i.e. direct delivery of the virus to the brain), the NS5 mutant caused reduced mortality compared to wild-type WNV. When infected mice were tested for hippocampus-dependent memory function 46 days post-infection with the Barnes maze, they were found to perform worse than uninfected controls. Post-mortem analysis of these mice demonstrated that WNV-induced memory loss was caused by microglial phagocytosis of presynaptic termini in the CA3 region of the hippocampus and was confirmed when mice lacking microglia did not demonstrate memory loss. Mechanistically, it was demonstrated that the complement protein C1q bound the termini to promote phagocytosis (Vasek et al., 2016). The microglia were stimulated to perform phagocytosis through interferon-gamma (IFN-γ) that originated from CD8^{+} T-cells that had infiltrated the CNS (Garber et al., 2019). This indicates that peripheral immune cells crossed the blood-brain barrier, though whether this is a continuous process has not been determined. This infection model also exhibited astrocyte release of IL-1β, leading to diminished neurogenesis and increased astrocytosis in the hippocampus (Garber et al., 2018). The role of microglia, astrocytes, IFN-γ and IL-1β have been implicated in numerous neurodegenerative diseases (ND) such as AD (Smith et al., 2012).

The hamster model of WNV infection was initially used for study of the acute phase of disease, but hamsters have been used to study multiple other aspects of WNV infection. Hamsters can have persistent WNV infection in the CNS and in the kidneys, like humans. The virus has been found in the CNS up to three months post-infection and in the urine up to eight months post-infection (Tesh et al., 2005). Behavioral testing on hamsters after the acute phase has shown long-term neurological deficits in memory (Smeraski et al., 2011) and motor function (Siddharthan et al., 2009b). Memory function was found to improve with treatment using a WNV-neutralizing monoclonal antibody administered at four days post-infection. This would be after CNS invasion of the virus, indicating that initial viral invasion is not the only cause of memory loss. Motor weakness correlated with motor neuron death between 10- and 26-days post-infection. Persistent viral RNA and envelope protein were detected in the cortex, hippocampus, midbrain, cerebellum, and spinal cord of hamsters up to 90 days post-infection, indicating an active infection in the CNS. Detection of WNV envelope protein was primarily in regions of inflammation, indicating an association between ongoing infection and the potentially damaging inflammatory response (Siddharthan et al., 2009b).

There have not been any studies to develop an animal model of WNV-induced anxiety or depression-like behavior despite how commonly it occurs in human patients. However, there are studies indicating general pathogenesis that may be related. One study using vesicular stomatitis virus indicated that peripheral type-1 interferon signaling through endothelial cells of the brain causes depression-like behavior in mice during infection when tested using the force swim test (Blank et al.,

2016). This could correlate with the persistently elevated levels of pro-inflammatory cytokines found in some human patients. Further work needs to be done to determine if cytokines are persistently upregulated in rodent models of WNV infection.

6. Overlap between WNND and neurodegenerative diseases

One intriguing aspect of WNV infection is its overlap with ND such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and multiple sclerosis (MS). Animal models show that survival of the acute phase infection of the CNS with WNV requires an inflammatory response, including the production of cytokines (Shrestha et al., 2006; Ramos et al., 2012) and the infiltration of circulating monocytes and T-cells (Shrestha et al., 2008). Investigating the neuropathology of WNV infections as a form of ND and potentially treating it as one may be a good first step toward helping patients recover from the debilitating disease.

The microscopic pathology associated with ND and neuroinvasive WNV infection appears similar. Microglial nodules have been found in the brainstem, midbrain, medulla, and pons of WNV patients (Guarner et al., 2004) and in the hippocampus of mice post-infection (Vasek et al., 2016). Microglial nodules (Singh et al., 2013) and perivascular cuffing (Agrawal et al., 2013; Cuzner et al., 1988) are commonly found in cases of MS. WNND has been associated with PD-like signs, including damage to the substantia nigra (Schafernak and Bigio, 2006), similar to PD. Japanese encephalitis virus, which is a member of the same serogroup as WNV, shows tropism for the substantia nigra in patients and a rat model (Ogata et al., 1997). Inflammatory T cells infiltrate the brain in both WNV and in neurodegenerative diseases (Sommer et al., 2017). In ND, these T cells can induce neuronal cell dysfunction (Siffrin et al., 2010) and death (Liu et al., 2017), activation of inflammatory cells (Sommer et al., 2016), and pro-inflammatory cytokine release (Mietelska-Porowska and Wojda, 2017). Pathogenic T cells have been shown to induce pathologic synaptic pruning in a mouse model of WNV (Garber et al., 2019), and may contribute to long-term damage in other ways.

Permeability of the BBB is hallmark of ND, as BBB dysfunction allows for increased movement of immune cells and pro-inflammatory molecules into the CNS, which exacerbates neurological damage (Sweeney et al., 2018). However, none of the studies about long-term neurological sequelae in rodent models have investigated what role the BBB plays in these. Given that WNV infection has been shown to affect the BBB so dramatically, it seems likely that this plays a role in ongoing neurological damage.

WNV infection has been shown to disrupt normal protein turnover through inhibition of the autophagy pathway (Kobayashi et al., 2012, 2020). This mechanism has been shown to cause neurodegeneration in a model of HIV as well (Alirezaei et al., 2008). Neurodegenerative diseases are often associated with dysregulated protein turnover (Taylor et al., 2002). WNV has been shown to induce the production of neuronal proteins associated with ND. Alpha-synuclein (α-syn) is a presynaptic protein that is associated with PD. It forms into protofibrils that are toxic to neurons and its secretion can cause damage to neighboring cells (Stefanis, 2012). Accumulation of α-syn causes neuronal apoptosis and can decrease neurogenesis (Winner et al., 2012). In a mouse model, α-syn becomes elevated in the brain after infection, and is protective against WNV infection (Beatman et al., 2016). This may indicate that it has a role as an antiviral peptide, and that continued secretion of α-syn leads to pathogenic responses. AD is characterized by the formation of plaques made of amyloid beta peptide (Aβ) and neurofibrillary tangles of tau protein (Murphy and Levine, 2010). These proteins, when aggregated, can disrupt neuronal homeostasis and induce cell death. but is also associated with PD, where it forms neurofibrillary tangles responsible for neuronal degeneration and death *In vitro*, WNV has been shown to induce Aβ production in human neurons (Dhingra et al., 2005) indicating a possible connection. Tau neurofibrillary tangles were found in two patients who succumbed to WNV infection (Schafernak and Bigio, 2006; Briese et al., 2002), though whether this was linked to the infection has

not yet been determined.

The TLRs involved in the initial recognition and response to WNV in the brain have been linked to exacerbation of ND. Artificial activation of TLR3 increases Aβ levels in the hippocampus and causes cognitive deficits in mice (Weintraub et al., 2014) and is elevated in microglia near Aβ rich plaques in AD patients (Walker et al., 2018). TLR7 expression has been associated with regions of neurodegeneration in mouse models of AD (Yu Liu et al., 2017) and ALS (Letiembre et al., 2009). Activation of TLR7 can induce neuronal apoptosis and microglial activation in the brain (Rosenberger et al., 2014). In response to WNV infection, lipocalin-2 (Nocon et al., 2014) and α-synuclein (Beatman et al., 2016) are increased in the brains of mice. Lipocalin-2 is normally a bacteriostatic protein but in the brain it is released from astrocytes and acts as a neurotoxic molecule (Lee et al., 2015).

Many of the cytokines that are important for surviving acute WNV infection can have detrimental effects on normal CNS function. IL-1β (Ramos et al., 2012), IL-2 (Sitati and Diamond, 2006; Brien et al., 2008), IFN-γ (Shrestha et al., 2006), and tumor necrosis factor-alpha (TNF-α) (Shrestha et al., 2008) have been shown to be essential in early antiviral activities against WNV infection. These same cytokines have been associated with ND causing peripheral immune cell infiltration (Madrigal and Caso, 2014), glutamate toxicity (Clark and Vissel, 2016), and neuronal dysfunction and death in ND (Frankola K et al., 2011) including AD, PD, HD, and MS. IL-1 signaling has been shown to exacerbate dopaminergic neurodegeneration in mice (Stojakovic et al., 2017), and IL-1β induces excitotoxic neurodegeneration in MS patients (Rossi et al., 2014). IL-1 signaling recruits peripheral leukocytes to the CNS (Proescholdt et al., 2002). Despite IL-1β being protective during the acute phase of WNV infection, it reduces hippocampal neurogenesis and increases astrocytosis in the long-term, leading to memory loss (Garber et al., 2018). To be activated, pro-IL-1β needs to be cleaved by an activated inflammasome. Given that the inflammasome is activated in both ND and in acute cases of WNV infection, it is worth investigating if there is chronic inflammasome activation in any model of WNV-induced neurological sequelae. The formation of the inflammasome through NLRP3 activation is necessary for viral clearance (Ramos et al., 2012) but activation of the NLRP3 inflammasome has also been shown to exacerbate ND such as AD (Tan et al., 2013), PD (Mao et al., 2017), and MS (Inoue and Shinohara, 2013). TNF-α and IL-1β have also been shown to be released from pro-inflammatory, neurotoxic astrocytes in ND (Liddelow et al., 2017) with genes that mark these specific astrocytes upregulated in a model of WNV infection and that these astrocytes serve as the major source of IL-1β post-WNV infection (Garber et al., 2018). IL-2 appears to play a more beneficial role during AD in particular (Alves et al., 2017) through stimulation of immunomodulatory T-cells and reduction of amyloid plaques. IFN-γ is associated with increased neurodegeneration in a stroke model (Seifert et al., 2014) and increases microglial activation and peripheral monocyte infiltration (Kunis et al., 2013), which can be protective or detrimental, depending on the model (McManus et al., 2014). IFN-γ can also induce neuronal apoptosis via astrocyte production of neurotoxic molecules (Hashioka et al., 2011), and direct apoptosis in the neurons in response to amyloid-beta (Bate et al., 2006). This all indicates that neurons may serve as bystanders in the immune response to WNV infection, and that neurodegeneration occurs in response to the pro-inflammatory cytokine signals necessary for control of WNV. It may also indicate that persistent immune stimulation due to persistent viral infection or stimuli leads to a persistent inflammatory state in the CNS.

The elimination of synapses in the hippocampus via complement-mediated microglial phagocytosis has been found to occur in mouse models of AD (Hong et al., 2016) and hippocampal synapse loss correlates with early AD and cognitive impairment (Scheff et al., 2006). Reduced neurogenesis in the hippocampus has been found in AD (Vivar, 2015) and in the substantia nigra in PD (Mogi et al., 1999). Decreased neurogenesis in the hippocampus has been linked with major depressive disorder (Eisch and Petrik, 2012) and reduced neurogenesis is an early event in AD and PD in mouse models (Kohl et al., 2016; Demars et al.,

2010). This seems to be a common finding in neurodegenerative and indicates a connection between viral infection, inflammation, and neurological dysfunction.

Fatigue is a commonly reported sequelae in WNF/WNND patients (Patel et al., 2015). One reported mechanism is the persistently elevated levels of pro-inflammatory cytokines up to five years post-infection (Garcia et al., 2014). Some of the cytokines correlated with fatigue included IFN-γ, IL-2, and IL-6. Besides the roles these cytokines play in controlling acute WNV infection (Sitati and Diamond, 2006; Shrestha et al., 2006; Brien et al., 2008) and in ND, they have been implicated in chronic fatigue syndrome (CFS). CFS has previously been associated with viral infections (Yang et al., 2019), including Epstein-Barr virus and herpesvirus-6. Elevated levels of IFN-γ have been associated with CFS (Kerr and Tyrrell, 2003), and higher levels of the cytokine in serum correlates with increased severity of disease (Montoya et al., 2017). Plasma IL-6 is elevated in patients with CFS (Broderick et al., 2010), and has also been associated with MDD (Yoshimura et al., 2013) and fibromyalgia (Wallace et al., 2001). These studies indicate a common pathway leading to similar symptoms in patients suffering from CFS and those reporting fatigue after WNV infection.

A summary of the similarities between WNV infection and ND in rodent models is provided in Fig. 1. The animal models indicate that there are multiple mechanisms underlying WNV-induced neurological deficits and that these overlap in many cases with findings in ND. The rodent models show many of the same pathological hallmarks of WNV infection and some of the neurological deficits as are seen in human WNF/WNND patients. Memory loss seems to mimic other ND, by inducing synaptic loss, astrocytosis, and a decrease in neurogenesis. Motor deficits seem to correlate with inflammation and persistent viral infection. However, many of the reported neurological sequelae in humans have not been reported in rodent models yet. More animal studies using a variety of behavioral, neurological, and pathological techniques are needed to understand the mechanisms underlying the long-term changes WNV can cause.

7. Conclusions

Although there have been similar neurological findings between rodents and humans following WNV infection, direct comparisons are difficult to make due to the differences in testing that are typically employed clinically and in animal studies. This poses a challenge when determining how relevant the rodent models are to human infection, and whether the mechanisms found there also occur in human patients.

All of the pathological reports from WNV patients describe outcomes of acute, lethal infections and no studies of long-term pathological changes to the brain have been described. The accuracy of rodent models
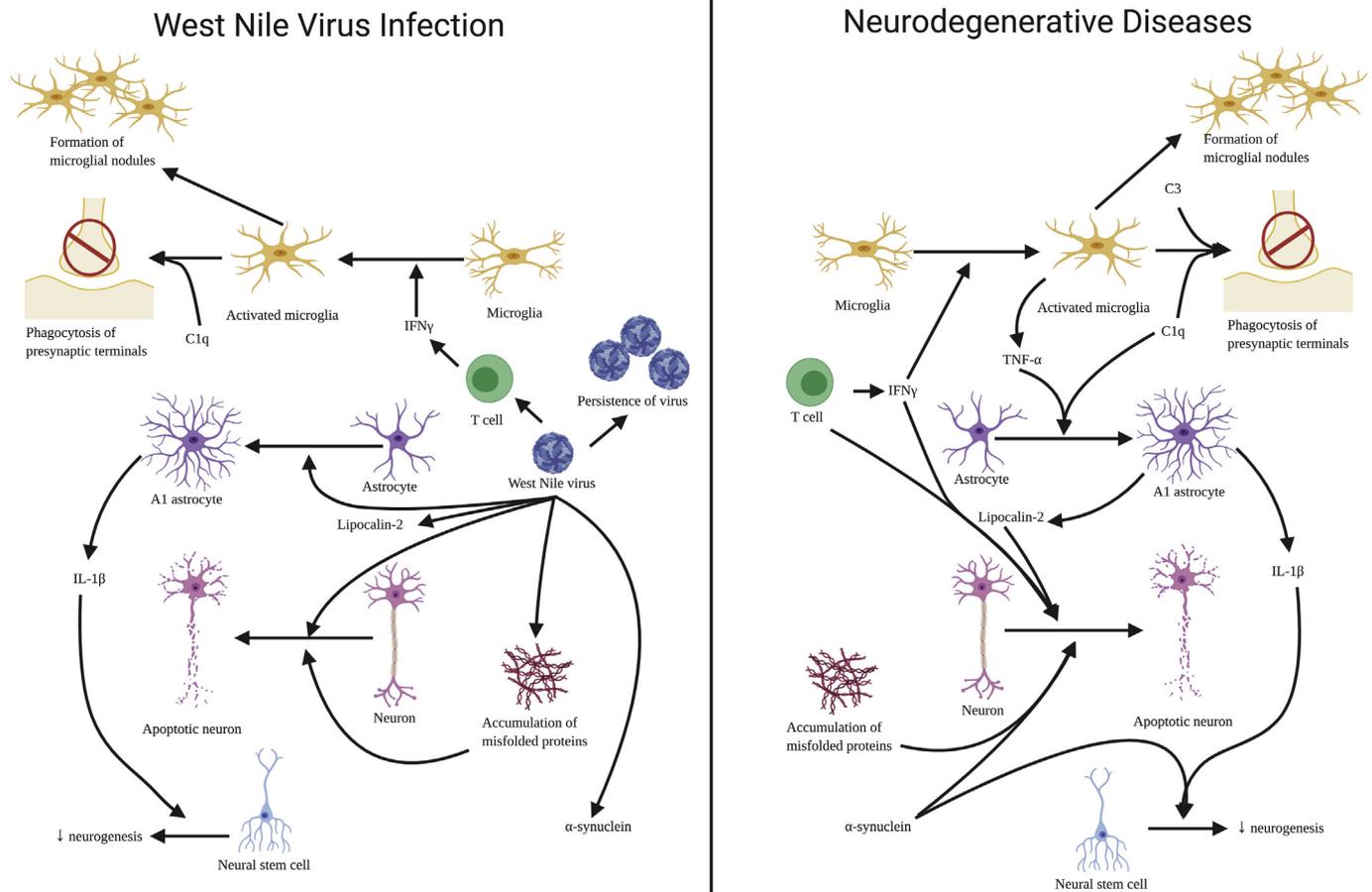

Fig. 1. Long-term, WNV-induced changes in the brain compared to ND. Both WNV infection (left) and neurodegenerative disease (right) are associated with long-term inflammation leading to neuronal damage and dysfunction. Infiltrating T cells activate microglia through IFN-γ signaling. In ND, T cells and IFN-γ have been shown to be directly neurotoxic. In both cases, activated microglia proliferate and form nodules. The activated microglia phagocytose presynaptic terminals that are marked with C1q, and C3 in the case of ND. This causes neurological deficits including memory loss. In ND, activated microglia produce pro-inflammatory cytokines including TNF-α, which activates astrocytes. In WNV and ND, astrocytes are signaled to become A1 astrocytes, which produce active IL-1β, stimulating astrocytosis and decreased neurogenesis in the hippocampus. Lipocalin-2 is upregulated in the brain in both cases, and this has been shown to come from A1 astrocytes in ND. Astrocyte-derived lipocalin-2 is neurotoxic in ND. Both WNV and ND induce the accumulation of misfolded proteins in neurons, inducing neuronal dysfunction and apoptosis. α-synuclein is upregulated in both ND and WNV infection. In ND this has been shown to cause neuronal apoptosis and decreased neurogenesis from neural stem cells.

for recapitulating human disease and pathology is currently determined primarily by comparing the findings with these cases, which may not accurately represent longer-term damage in survivors. The best way to rectify this would be to develop ongoing studies that track the long-term function of patients diagnosed with West Nile virus infection, regardless of initial clinical manifestation. Patients previously diagnosed with WNV who die of other causes should be examined for brain damage and be tested for persistent viral infection.

WNV can spread to many regions of the brain and can cause a wide spectrum of accompanying sequelae which makes development of animal models and studies of these models difficult. Future work should focus on studying the natural spread of the virus into different brain regions to determine if there is specific tropism for the virus in different regions, especially regarding long-term damage. More work should be done to correlate virus-induced damage in certain brain regions to specific neurobehavioral outcomes, and to assess if and how viral persistence plays a role in this. The intracranial infection models using attenuated WNV strains can mimic damage to specific regions, while models using wild-type WNV inoculated peripherally can explore the natural distribution of the virus.

Further work needs to be done to examine the apparent overlaps between WNND and ND in pathogenic mechanisms. Other viral CNS infections such as herpes simplex virus-1 and -2 have been suggested as inciting causes of ND, and WNV should be examined in the same way. The mechanisms found in these neurotropic viruses should be studied in the context of WNV infection. Further work to connect WNV infection with ND, such as the role of α-synuclein in long-term infections, the long-term effects of proteasome inhibition in the brain, and persistence of the virus in the brain should all be considered in this context as well.

WNV is not going to be eradicated from endemic areas and continues to cause thousands of infections per year. Given that even mild cases may lead to neurological damage, it should be of high importance to continue research to define and quantify the damage WNV causes in the CNS leading to long-term neurological deficits. Expanded research is needed to determine what causes the sequelae found after WNV infection.

Declaration of competing interest

None.

Acknowledgements

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