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Current developments in understanding of West Nile virus central nervous system disease

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Abstract

Purpose of review—West Nile virus (WNV) is the most important cause of epidemic encephalitis in the United States. We review articles published in the last 18 months related to the epidemiology, immunology, clinical features, and treatment of this disease.

Recent findings—There was a resurgence in WNV disease in the United States in 2012. The WNV strain now predominant in the United States (NA/WN02) differs from the initial emergent isolate in 1999 (NY99). However, differences in the genetics of currently circulating United States WNV strains do not explain variations in epidemic magnitude or disease severity. Innate and acquired immunity are critical in control of WNV, and in some cases pathways are central nervous system specific. The clinical features of infection are now well understood, although nonconfirmed observations of chronic viral excretion in urine remain controversial. There is no specific antiviral therapy for WNV, but studies of antivirals specific for other flaviviruses may identify agents with promise against WNV. Phase I and II human WNV vaccine clinical trials have established that well tolerated and immunogenic WNV vaccines can be developed.

Summary—WNV remains an important public health problem. Although recent studies have significantly increased our understanding of host immune and genetic factors involved in control of WNV infection, no specific therapy is yet available. Development of a well tolerated, immunogenic, and effective vaccine against WNV is almost certainly feasible, but economic factors and the lack of predictability of the magnitude and location of outbreaks are problematic for designing phase III trials and ultimate licensure.

Keywords

acute flaccid paralysis; antiviral therapy; encephalitis; meningitis; vaccine; West Nile virus

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INTRODUCTION

Since its emergence in the United States in 1999, West Nile virus (WNV) has become the dominant cause of arboviral central nervous system (CNS) disease, accounting for 98% of reportable cases [1]. The original source of virus emergence in the United States remains a mystery [2▪▪], with most theories favoring importation from either infected migrating or imported birds originating from Africa or the Middle East. In 2003, the United States experienced the largest WNV epidemic ever reported with 9862 cases including 2866 with neuroinvasive disease (meningitis, encephalitis, and acute flaccid paralysis) and 264 deaths. In subsequent years the incidence appeared to be waning, and in 2011 there were only 712 reported cases (486 neuroinvasive, 43 deaths). The year 2012 saw a major resurgence with case numbers rebounding to the second highest level on record (5674 cases, 2973 neuroinvasive, and 286 deaths). The year 2013, although quieter than 2012, saw 2318 cases (1171 neuroinvasive and 105 deaths). These statistics suggest that WNV will follow the pattern of Japanese encephalitis virus with periodic larger outbreaks occurring on a regular basis, rather than the more endemic pattern with much rarer large outbreaks seen with St Louis encephalitis [3]. Europe has also been increasingly affected by WNV, and a large outbreak of human disease occurred in north-eastern Italy in 2012 [4], and cases appeared in new regions including Croatia [5].

EPIDEMIOLOGY

Based on genetic analysis, at least eight lineages of WNV are now recognized; however, human pathogenic strains belong to lineages 1 and 2, with lineage 1a strains accounting for most recent epidemics associated with significant neuroinvasive disease [6]. Extensive studies of the molecular epidemiology of WNV strains have been conducted. By 2002, the original strain (NY99) that appeared in North America was almost completely displaced by a new strain designated North America/West Nile 2002 (NA/WN02) that currently cocirculates in the southwestern United States with a third strain designated NA/WN03 [7▪]. Despite this dynamic and ongoing evolution of strains, there is no clear evidence suggesting that differences in viral genetic factors between the isolated United States strains account for variation in outbreak magnitudes or alter disease pattern or severity [8,9].

The Kunjin strain of West Nile virus (WNVKUN) is endemic in parts of Australia and generally results in asymptomatic human infection. In 2011, there was a surprising and unprecedented outbreak of Kunjin virus-induced encephalitis in horses in southeast Australia. Sequencing of the responsible virus (designated WNVNS2011) revealed that it was closely related to WNVKUN as compared to other WNV strains; however, it contained two mutations resulting in amino acid substitutions that have been associated with virulence in WNVNY99 [10]. The availability of relatively attenuated WNV strains such as Kunjin and potentially more virulent related isolates such as WNVNSW2011 may help facilitate identification of molecular determinants of WNV neurovirulence.

In recent years, the majority of cases of WNV infection have been concentrated in the central United States. Since 1999, the states with the highest number of reported cases have been Colorado (4672), Texas (4070), California (3626), and Nebraska (3122). Predicting

where outbreaks will occur and their severity remains problematic. The most useful tool remains mosquito surveillance data, with the appearance of virus in trapped mosquito pools typically anticipating the appearance of human cases by several weeks [11].

The vast majority of WNV cases are acquired through the bite of an infected mosquito. Rare cases related to transplantation of infected organs [12] and through contaminated transfused blood products [13] continue to occur. For example, over 700 presumptive viremic blood donors were identified in 2012, only 14% of whom eventually developed symptomatic disease. The majority (80%) of WNV infected individuals remain asymptomatic, with less than 1% developing neuroinvasive disease. Age remains one of the most important risk factors for the development of neuroinvasive disease especially encephalitis, a feature confirmed again in the 2012 epidemic [14▪]. Patients who are immunocompromised also appear to be at increased risk of severe illness and death [15▪], with those receiving solid organ transplants from infected donors being at specially enhanced risk of developing severe disease. In this group of patients, the risk of infection is between 50 and 75% [16]. In addition to organs including liver and kidney, viral RNA has been detected in fat, muscle, tendon, and bone marrow, although not all these organs may have infectious virus [17]. Atypical disease manifestations may occur in immunocompromised patients [18].

Several genetic factors contributing to disease susceptibility have also been identified in genome-wide association studies including polymorphisms in the interferon (IFN)-induced oligoadenylate synthetase genes, and homozygosity for the delta 32 deletion in the gene encoding CCR5. Significant independent risk factors among patients with severe illness (e.g. hospitalized cases, encephalitis) include chronic renal disease (adjusted odds ratio (aOR): 4.1), history of cancer (aOR: 3.7), history of alcohol abuse (aOR: 3), diabetes (aOR: 2.2), and hypertension (aOR: 1.5) [15▪]. A retrospective chart review of hospitalized patients in Texas, the hardest hit state in the 2012 epidemic, confirmed the importance of age and preexisting conditions including hypertension and diabetes in patients with neuroinvasive disease [14▪]. Interestingly, spontaneously diabetic mice (db/db), like their human counterparts, are highly susceptible to WNV disease and have higher viral titers in brain and enhanced mortality that correlate with their delayed induction of innate and acquired immune responses including delayed induction of IFNα and reduced production of WNV-specific immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies [19].

IMMUNOLOGY

Immune responses against WNV involve a complex interplay of innate immunity, humoral antibody production, and effects of CD4, CD8, natural killer (NK), and gamma–delta T-cells [20,21▪▪,22,23]. Particular attention has recently focused on the critical role played by host innate immune responses in the initial defense against WNV infection. This is a process that involves sensing of pathogen-associated molecular patterns by antiviral defense sensors including RIG-I, MDA5, TLR3, and other Toll-like receptors and Nod-like receptors. The cytosolic pattern recognition receptors RIG-I and MDA5 are both activated following WNV infection and appear to each play specific and nonredundant roles in detecting and controlling WNV infection. These proteins detect WNV ssRNAs containing 5′-triphosphates. Mice lacking both receptors (RIG-I(−/−) × MDA5(−/−) double knockouts) are

extraordinarily susceptible to WNV infection [24]. Activation of these pathogen sensors ultimately leads to production of type 1 IFN, and other antiviral proinflammatory effectors including interleukin (IL)-1beta [25,26]. MDA5 also plays a role in facilitating optimal CD8 T-cell activation and the efficiency of viral clearance from the CNS, as MDA5(−/−) knockout mice have functional defects in their anti-WNV CD8 T-cell responses that result in impaired efficiency of viral clearance from the CNS [27]. MDA5 and RIG-I, and IFN-1 signaling also influence NK-cell effector activity, providing another linkage between innate and cellular immunity [28▪▪]. Other antiviral signaling pathways, including signaling through the IL-1 receptor (IL-1R), also appear to be critical for efficient T-cell responses [29].

As noted, type I IFN plays a critical role in host innate antiviral responses to WNV. It has recently been shown that the transcription factor ELF4 plays an important role in this process. Following IFN stimulation, ELF4 is translocated to the nucleus where it binds to IFN promoters and facilitates transcription induced by binding of the IFN-regulatory factors IRF3 and 7 [30]. Studies are also advancing understanding of the roles played by other specific interferon-stimulated genes (ISGs) in the response against WNV infection. Interestingly, some ISGs appear to have tissue-specific sites of action. For example, mice lacking Ifit2 show preferential enhanced replication of WNV in CNS but not in peripheral tissues, suggesting a special role for Ifit2 in restricting WNV replication in the brain [31]. Similarly, mice lacking the IL-1R(−/−) show enhanced susceptibility to WNV infection [32]. This may be due in part to deficient CD4 T-cell responses and $CD11c^+$ dendritic cell responses in the CNS despite intact adaptive immunity in peripheral tissues [29]. Related defects in CD8 T-cell function within the CNS have been found by some groups [32] but not others [29]. Tissue-specific differences in immune antiviral responses have also been found through transcription profiling of ‘permissive’ tissues such as spleen compared to ‘nonpermissive’ tissues such as liver in WNV-infected mice as well as in mice lacking IFN receptor or Rig-I-like receptor responses [MAV(−/−)]. These studies confirm the importance of NK cell responses and inflammatory cytokine production in these differences [28▪▪].

Not all chemokines and cytokines play antiviral or protective roles in WNV infection, and some chemokines may actually enhance viral infection. For example, mice lacking IL-22 show reduced viral loads in the CNS, less inflammatory infiltrate, and fewer apoptotic cells despite normal viral titers in the periphery. It was suggested that this effect may be in part due to the fact that IL-22R(−/−) mice have reduced expression of CxCr2, a chemokine involved in neutrophil migration into the CNS. This suggests that WNV-induced IL22 responses may actually contribute to viral CNS pathogenesis by promoting a neutrophil-associated pathway of WNV neuroinvasion [33]. Similarly, the protein Semaphorin 7a (Sem7a), which upregulates TGFbeta-1 and Smad6, seems to enhance WNV pathogenesis, perhaps through effects on the blood–brain barrier (BBB) that facilitate neuroinvasion. Mice lacking Sem7a have enhanced survival after WNV infection that is associated with reduced BBB permeability. However, anti-Sem7a antibody treatment of cultured neurons and macrophages can also block WNV infection, indicating that there are BBB-independent effects of this protein in facilitating WNV infection [34].

CNS tissue injury during WNV infection results from apoptosis induced both directly by the virus [35] and through virus-specific $CD8^+$ T cells employing the apoptosis-inducing ligand

TRAIL [36]. Mice lacking TRAIL show increased susceptibility to WNV infection, and CD8 T cells lacking TRAIL have impaired capacity to clear virus from the CNS. WNV infection can also directly activate the death-receptor-associated apoptotic pathway and the associated initiator caspase 8. This can occur following infection of ex-vivo brain slice cultures, which lack both peripheral infiltrating inflammatory cells and the influence of peripheral immune activity such as extra-CNS production of chemokines or cytokines [35]. A link between apoptosis and innate immune responses including activation of pathogen recognition receptors is provided by a protein called 'ASC' (apoptosis associated speck-like protein containing CARD), which appears to also play a key role in bridging signaling by pathogen recognition receptors and the subsequent activation of initiator caspase 1 in inflammasomes. ASC is also essential for the production of certain proinflammatory cytokines including IL-1beta [37].

Recent studies have identified several potential new candidate antiviral defense molecules including lipocalin 2 (lcn2) and viperin that may play a role in antiviral responses against WNV. In the case of lcn2, the mRNA and protein are clearly up-regulated in brains of infected mice although surprisingly mice lacking this protein (Lcn2 knockout) did not have an obvious phenotype in terms of mortality, increased viral load, changes in infiltrating inflammatory cells, or enhanced injury [38]. The protein viperin appears to also have a pleotropic role in antiviral defense against WNV, with studies suggesting that it can influence type 1 IFN production and alter cellular microenvironments in a way that is deleterious to viral replication [39].

CLINICAL FEATURES AND DIAGNOSIS

The basic clinical features of WNV infection have been previously reported in a number of reviews (see [40▪▪] for a recent example). As noted, neuroinvasive disease occurs predominantly in older individuals although isolated reports of severe neuroinvasive disease in children, including acute flaccid paralysis [41,42], continue to appear. Three recent case reports add to previous evidence suggesting that WNV infection during pregnancy is not typically associated with deleterious outcomes for either the mother or the baby [43].

The importance of WNV as a potential cause of acute ocular inflammation in patients with fever in endemic areas has received new emphasis. In one study of 52 such patients, a surprising 71% had laboratory evidence of WNV infection. The typical clinical pattern was of one of discrete superficial retinitis, arteritis, and phlebitis. Fluorescein angiography demonstrated areas of retinal inflammation with associated vascular and optic disc dye leakage. Optical coherence tomography (OCT) showed edema of the inner retinal layer during the acute phase and retinal atrophy at later stages. Many patients had persistent visual acuity loss [44]. Another interesting recent clinical observation is the possible association of antecedent WNV infection with subsequent development of myasthenia gravis. One report describes six such patients who developed acetylcholine receptor antibody-positive myasthenia gravis 3–7 months after WNV infection. The mechanism of this association is unclear and the authors speculated that it might involve molecular mimicry [45].

It has been recognized for some time that experimental WNV infection of golden hamsters and more recently of mice can result in chronic renal infection and persistent shedding of WNV in urine [46,47]. A study from Baylor in 2010 found evidence of WNV RNA in 20% of urine samples collected from patients 1.6 to 6.7 years after acute WNV infection [48], although a second study performed at the US Centers for Disease Control and Prevention failed to confirm these results [49]. A recent study [50] from Italy used real-time reverse-transcription PCR and found that 44% of acutely infected symptomatic patients had detectable WNV RNA in urine, suggesting that urinary excretion does occur acutely, but did not address the issue of chronic urinary shedding. The significance of WNV urinary excretion remains controversial. One recent opinion piece in *Nature* referred to the 'hidden threat' that WNV infection might be linked to subsequent development of chronic kidney disease [51]. The same group that initially reported chronic urinary excretion of WNV RNA recently conducted an uncontrolled retrospective study of patients with a prior history (4–9 years previously) of WNV infection and reported that approximately 10% had evidence of stage III or higher chronic kidney disease, and 30% had evidence of milder stage I or II disease, using the Kidney Disease Outcomes Quality Initiative criteria. Clinical and laboratory signs included proteinuria, hematuria, reduced estimated glomerular filtration rate, and elevated plasma neutrophil gelatinase associated lipocalin or monocyte chemotactic protein-1 [52]. The patients in this study were predominantly white males with a mean age of 57 and there were no controls. The role, if any, of WNV in chronic kidney disease remains extremely controversial and will remain speculative unless these results are confirmed by higher quality appropriately controlled studies.

Detection of WNV-specific antibody remains the mainstay of diagnosis and is significantly more sensitive than PCR. Acute infection is typically identified by the presence of IgM antibodies, and their detection in cerebrospinal fluid (CSF) is usually a reliable marker of neuroinvasive disease. It has been previously recognized that the mean time to seroreversion of IgM antibodies (from IgM+ to IgM−) was about 5 months postinfection, with approximately 17% of WNV-infected patients having persisting IgM antibody at 1 year postinfection [53]. A recent study of patients in Houston found a surprisingly higher prevalence and longer duration of IgM positivity; 42% were found to have IgM antibody at 1 year postinfection, with 34% still IgM+ at 6 years and 23% IgM+ at 8 years postinfection [54]. These results seem surprisingly high, and if confirmed in other studies may mean that detection of IgM antibody as a marker of acute infection will need to be more cautiously interpreted. It has generally been assumed that WNV-specific IgG, including neutralizing antibodies, persists indefinitely after infection and serves as a marker for protective immunity. Recurrent WNV infection has not been reported. In one recent study, a small group of 18 blood donors were re-tested for WNV antibodies at 5 years postinfection. All 18 donors (100%) remained seropositive, and there were minimal changes in the level of ELISA-detected IgG antibody or neutralizing antibody [55▪]. By contrast, the previously cited Houston study found that the presence of ELISA-detected IgG antibodies had declined to 46% by 8 years postinfection [54].

TREATMENT AND PREVENTION

There is no proven specific therapy for WNV infection. Efforts to find promising small molecule inhibitors of viral replication are ongoing [56,57]. One of the more promising approaches may be to test drugs known to be effective against other members of the flavivirus family including dengue and hepatitis C. The novel viral RNA polymerase inhibitor favipiravir (T-705, FujiFilm Pharmaceuticals) has also attracted interest. Favipiravir is a pyrazinecarboxamide derivative that in its active triphosphate forms mimics a purine nucleotide and thereby inhibits the RNA-dependent RNA polymerases of several families of RNA viruses including several species of alphavirus and flavivirus [58]. The drug has completed two phase II trials of safety and pharmacokinetics in patients with uncomplicated influenza (NCT01728753 and NCT01068912), and a phase III trial to determine safety and efficacy in uncomplicated influenza is currently underway (NCT02008344). If this drug is found to be well tolerated and efficacious in influenza, it will facilitate interest in testing it against WNV infection.

The identification of the importance of type 1 IFN in WNV control has led to use of IFN-alpha preparations in treatment of human WNV infection. No controlled trials of efficacy are available and reports remain essentially anecdotal descriptions of individual cases or small series. However, the strategy of enhancing type I IFN signaling as an antiviral treatment remains appealing based on current understanding of the key role of this signaling pathway in controlling infection both *in vitro* and in animal models *in vivo* (discussed above). An interesting approach to enhancing IFN responses in mice is to inoculate them with type I IFN inducing RNA transcripts such as those derived from the noncoding region of foot and mouth disease virus. These transcripts, which induce a potent IFN response, can protect both suckling and adult mice against lethal challenge with WNV [59]. These studies again suggest the potential for enhancing type 1 IFN responses as an antiviral strategy.

Several WNV vaccines are currently licensed for equine veterinary use and appear to be extremely effective. Effective human vaccines are available for several flaviviruses including yellow fever (17D) and Japanese encephalitis (SA14-14-2), suggesting that development of a well tolerated and effective human WNV vaccine is eminently feasible. The most advanced human WNV vaccine candidate is ChimeriVax-WN02. This vaccine is a live chimeric vaccine with the genes encoding the WNV NY99 pre-membrane (prM) and envelope glycoproteins inserted into the genetic backbone of the Yellow Fever virus 17D vaccine strain. The resulting chimeric virus protects monkeys against lethal intracerebral challenge with the WNV NY99 strain. The vaccine has been evaluated in two completed phase II human trials (NCT00442169 and NCT00746798) and has been shown to be both well tolerated and immunogenic. The vaccine elicits both WNV neutralizing antibody and cytotoxic T cells [60]. In the phase II trial in healthy individuals over the age of 50, over 92% of vacinees developed plaque-reduction neutralizing antibodies by day 28 postvaccination and had no increase in adverse effects compared to the placebo controls [61▪▪]. A live attenuated chimeric vaccine in which the WNV prM and E genes are inserted into the genetic backbone of an attenuated dengue virus vaccine strain (DENV-4delta30) has also been tested in two phase I human trials (NCT000537147 and NCT00094718) in healthy volunteers aged 18–50. The reported seroconversion rate against NY99 was approximately

75% after an initial dose and increased to 89% after a booster dose 6 months later [62▪]. Although not as advanced as the Chimerivax-WN02 trials, other strategies with promising early results in animals and *in vitro* involve using chimeric vaccines based on the Japanese encephalitis SA14-14-2 strain [63] and the live attenuated Schwarz measles virus vaccine strain [64▪].

CONCLUSION

WNV infection is clearly entrenched in North America and will likely continue its pattern of low-level endemicity with periodic major epidemics. The key risk factors for developing neuroinvasive disease are being recognized and include the age and immune status of the host as well as specific genetic factors often linked with innate and acquired immunity. The host immune response to WNV is being defined at an increasingly specific level and involves the participation of virtually every arm of the immune system including innate, cellular, and humoral immunity. Not surprisingly, innate immune responses are critical in initial viral control and in some cases show organ and CNS-specific patterns. The clinical and laboratory features of WNV are now fairly well characterized, but new reports continue to expand aspects as exemplified by new and still controversial and unconfirmed reports suggesting that WNV can produce chronic urinary tract infection and that this may predispose to the development of chronic kidney disease. No proven antiviral therapy for WNV currently exists but advances in antiviral therapy for other flaviviruses may provide novel drugs that will also have efficacy against WNV. Several WNV vaccines have completed phase I and II clinical trials and have been found to be both well tolerated and immunogenic. Establishing efficacy in phase III trials will be hampered by the unpredictability of the epidemiology of WNV infection (both geographically and in terms of number of cases).

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KEY POINTS

- WNV is the most important cause of epidemic encephalitis in the United States.
- Factors contributing to the development of WNV neuroinvasive disease include patient age and immune status as well as specific genetic determinants likely controlling innate and acquired immune responses to the virus.
- No specific therapy is currently available for WNV infection although several WNV vaccines have entered human phase I and II clinical trials and have been found to be well tolerated and immunogenic.