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Risk factors for West Nile virus Infection and Disease in Populations and Individuals

Ruth R. Montgomery^{1,*} and Kristy O. Murray²

¹Department of Internal Medicine, Yale School of Medicine, New Haven, Connecticut 06520

²Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX

Summary

West Nile virus (WNV) is a mosquito-borne enveloped positive-strand RNA virus that emerged in North America in 1999 in New York City. Over the past 15 years, WNV has become established throughout the USA and has spread into Canada, Mexico and the Caribbean. CDC reports indicate >41,000 clinical cases, including more than 1,700 fatalities. An estimated 3 million people in the USA may have been infected to date. Infection with WNV is dependent on many factors including climate, mosquito habitats and immunologically-naïve bird populations. In addition, variations within individuals contribute to the risk of severe disease, in particular, advanced age, hypertension, immunosuppression and critical elements of the immune response. Recent advances in technology now allow detailed analysis of complex immune interactions relevant to disease susceptibility.

Keywords

West Nile virus; Aging; mosquito vector; epizootic spread; avian reservoir; mouse model; immune response

I. Introduction

West Nile virus (WNV) is a mosquito-borne enveloped positive-strand RNA virus that emerged in North America in 1999 in New York City. Over the past 15 years, WNV has become established throughout the USA and has spread into Canada, Mexico, the Caribbean, and into South America. CDC reports indicate >41,000 clinical cases including more than 1,700 fatalities. An estimated 3 million people in the USA may have been infected to date (1). WNV has become an important public health concern in the USA due to its high prevalence, severe disease in humans, and the absence of effective treatments or vaccines (2-5). WNV patients exhibit considerable variation in clinical responses ranging

*For Correspondence: Ruth R. Montgomery, Ph.D., Department of Internal Medicine, Yale University School of Medicine, 300 Cedar Street/TAC S413, New Haven, CT 06520-8031, Tel. No: (203) 785 7039, FAX: (203) 785 7053, ruth.montgomery@yale.edu.

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from asymptomatic infection to severe neurological involvement and even fatal outcomes. There are currently no FDA-approved treatments available. Infants, the immunocompromised, and the elderly are more susceptible to neurological involvement that may result in death (6, 7). Many factors contribute to the risk of severe infection with WNV, including vector prevalence, exposure, and individual host factors and immune responses. Here we review recent studies that identify elements that contribute to infection with WNV and to divergent outcomes.

II. Emergence of WNV in North America

WNV was first recognized in the Western Hemisphere in the New York City area in 1999, with 62 human clinical cases reported (Fig. 1) (8). In addition to human cases of disease, avian and equine morbidity and mortality from WNV infection were also reported (9, 10). By 2001, WNV had disseminated as far south as Florida, marking an adaptation from the *Culex (Cx.) pipiens pipiens* species mosquitoes present in the northeastern region of the United States and Canada to the *Cx. pipiens quinquefasciatus* mosquito in the southern and mid-western regions of the US (11, 12). Between 1999 and 2001, a total of 149 human cases had been reported from 10 states (13). In 2002, an unprecedented epizootic of WNV occurred, with the virus reaching 39 states plus the District of Columbia, resulting in 4,156 reported clinical cases and 284 deaths. In 2002 the virus adapted to a new *Culex* species and also underwent several nucleotide changes, with the new WN2002 strain of virus sweeping across North America, ultimately displacing the NY99 strain cases of disease (11). Also during 2002, it became quickly evident that WNV could be transmitted through means other than from the bite of an infected mosquito, including blood transfusions, organ transplant, transplacental transfer from mother to fetus, and through infected breast milk (14-16). In 2003, blood banks across the US began to screen the blood supply for WNV using Nucleic Acid Amplification Testing (NAT), an amplification-based transcription technique, which identifies positive blood from WNV-infected individuals before they become symptomatic (17). Transfusion-transmitted infection is rare or absent since this testing despite persistence of WNV RNA for up to three months in red blood cells (18), however some cases of organ-transplant transmission have been reported (19).

Following the epizootic of 2002, WNV continued the wave of epizootic activity across the US, further adapting itself to the *Cx. tarsalis* mosquito and reaching the West Coast by 2003 (13, 20). By 2012, all lower 48 states plus the District of Columbia had reported locally acquired human WNV cases, and by 2014, more than 41,000 human cases of clinical WNV had been reported to CDC, including more than 1,700 fatal cases, and avian and equine morbidity and mortality from WNV infection were also reported (13). While the virus appeared to reach endemic levels between 2008 and 2011, with an average annual report of 908 cases, another unprecedented and unexpected epizootic occurred in 2012, with 5,674 cases (286 fatal) being reported to CDC, with Texas reporting 33% of cases (n=1,868) (21). With continued high levels of virus activity reported in both 2013 and 2014, WNV will remain a substantial public health threat in North America.

III. Risk Factors for Populations

There are numerous factors, both known and unknown, that contribute to viral amplification in the environment and subsequent epizootic WNV activity (22). Climate conditions, particularly ambient temperature and rainfall, are critical drivers of mosquito abundance and amplification of WNV (23-27). Studies on *Cx. pipiens* have found a direct correlation between increases in ambient temperature and increases in vector populations (28). Interestingly, elevated temperatures also affect the rate of virus replication, leading to more rapid infectivity of the mosquito (29). Precipitation is another key component to promoting vector abundance by providing ample breeding environments for mosquitoes (31), although heavy rains and floodwaters can also reduce mosquito breeding grounds by flushing out their preferred stagnant water sources (32). In contrast, drought conditions can promote WNV transmission by forcing birds and mosquitoes to share the same habitat through competition for scarce water sources; it was believed that the severe drought in Texas in 2011-2012 was the driver for the 2012 outbreak that resulted in 1,868 human cases (21, 33).

People living in close proximity to stagnant water sources where mosquitoes breed have been found to be at higher risk for infection. In Houston, Texas, where *Cx. quinquefasciatus* mosquitoes serve as the primary vector, a recent study found that WNV case patients were significantly more likely to reside near slow-moving/stagnant water sources with heavier vegetation (34). Similarly, in El Paso, Texas, where *Cx. tarsalis* is the primary vector, WNV case-patients were close to yards that were flooded regularly by irrigation canals (35). In northern Colorado, where *Cx. tarsalis* is also primary, irrigation and manual control of water on the landscape was also found to promote mosquito abundance (36). In addition to being in areas with high vector abundance, risk for becoming infected can be influenced by time spent outdoors and decisions on whether to adopt personal precautions against mosquito bites. In Houston, a serosurvey of homeless individuals found that time spent outdoors greatly influenced infectivity, with 12.5% of those who reported spending >12 hours outdoors being positive for WNV, compared to only 2% of those who reported spending 6 hours outdoors (37). Other studies have also found that increased time outdoors was associated with infection (38-40), in addition to inconsistent mosquito repellent use (38, 39) and younger age, with children being 5 times more likely to be infected with WNV when compared to adults (41). Vector control measures may have value in reducing infection exposure (30).

Finally, viral amplification and infectivity of mosquitoes relies completely upon the presence of an immunologically naïve avian host reservoir population (21, 42). Birds are responsible for maintenance of virus in the environment and are the critical link to the transmission cycle. As seen with the rapid expansion of WNV across North America, birds spread the virus into new geographic areas (43) and *Culex pipiens* species mosquitoes are predominately ornithophilic, in that they prefer feeding on avian hosts, particularly passeriformes (44, 45). Passerine birds, including Blue Jay (*Cyanocitta cristata*), Common Grackle (*Quiscalus quiscula*), House Finch (*Carpodacus mexicanus*), American Crow (*Corvus brachyrhynchos*), House Sparrow (*Passer domesticus*), cardinal (*Cardinalis cardinalis*), and American Robin (*Turdus migratorius*) have been found to contribute most to viral amplification and transmission of WNV to mosquitoes (46, 47). A study in Illinois

found that 80% of blood meals from field collected *Cx. pipiens* mosquitoes were from avian sources, with 25 different avian species identified; American Robins (*Turdus migratorius*) were the most overrepresented with 48% of avian blood meal sources (45). Interestingly, they also found in this study that of the mammalian blood meal sources, 83% were from humans. In California, *Cx. tarsalis* species mosquitoes were found to have a much higher diversity in its host seeking behaviors when compared to *Cx. pipiens* complex mosquitoes (48), though both species of mosquitoes were found again to be predominately ornithophilic. In contrast to the study in Illinois, the California mosquitoes rarely fed on humans, with human blood meal sources only found in 0.4% of *Cx. pipiens* and 0.2% of *Cx. tarsalis* mosquitoes, leading the authors to conclude that human cases only occur when there is substantial epizootic activity. In central and eastern Texas, the most common vector for WNV is *Cx. quinquefasciatus*, which has a remarkably different host seeking pattern. Blood meal analyses found that only 39% of blood meals came from an avian source (49). Canines were the most common blood meal identified (41%), while humans were the least common (0.4%). Continued research focused on modeling amplification and predicting epizootic activity would be of extreme value to protect public health.

IV. Risk Factors for Individuals

Clinical features and diagnosis

Human infection with WNV is often asymptomatic. WNV illness is characterized by fever and flu-like illness, which can include symptoms such as weakness, joint pain, chills. Rare cases (~1% of infected individuals) are diagnosed with severe WNV neuroinvasive disease characterized by meningitis, encephalitis, acute flaccid paralysis, and neurological sequelae may persist in some cases (50). Diagnosis of WNV is based on laboratory findings of specific IgM and IgG antibodies by ELISA or pleocytosis/PCR positive cerebrospinal fluid (50). Identification of encephalitis can be challenging although specific radiologic patterns resemble other related virus infections (St Louis encephalitis, Kunjin, Japanese encephalitis) (51) and initially negative serology or CSF testing may bear repeating at a later interval to clarify diagnosis.

Individual host risk factors including both innate and adaptive immune responses can contribute to severe neuroinvasive disease and death following infection with WNV. Risk for severe infection has been identified in particular with certain genomic determinants, advanced age, a history of cardiovascular disease, chronic renal disease, hepatitis C virus infection, and immunosuppression (50, 52, 53). Recent advances in high-throughput and bioinformatics technology now allow detailed analysis of complex interactions to generate a systems level understanding of disease susceptibility (54, 55).

Genomic determinants of severe infection

Markers associated with susceptibility to severe WNV infection include single nucleotide polymorphisms (SNPs) in several genes. Certain HLA types appear to be associated with risk of more severe outcome (56), and interferon response pathway elements such as OAS-1 (oligoadenylate synthetase 1b) -- involved in RNA degradation--, IRF3, MX-1, and a dominant negative splice variant of RNaseL, which functions in the anti-proliferative roles

of interferon (5, 57-59). Another genomic study investigated >1500 symptomatic subjects (severe vs mild), and showed more severe neurological disease associated with SNPs in RFC1, a replication factor; SCN1a, a sodium channel; and ANPEP, an aminopeptidase, although even more differences might have been revealed when comparing asymptomatic and symptomatic cases (60). And a deletion in CCR5, known to be protective in infection with HIV, was not associated with susceptibility to WNV, but did correspond to severity of infection, presumably due to reduced function of CCR5 pathways in infected hosts (61). As more host factors are identified, there are sure to be a number of new determinants of WNV infection. One area of active investigation is identification of polymorphisms relevant to successful aging, and while mechanistic studies are only now in progress, absence of these markers is likely to overlap with genes related to susceptibility to WNV (62).

Reduced response to WNV infection in elderly populations

Aging is associated with a progressive decline in immune function (63) and several anti-viral pathways in innate immunity show reduced efficiency in cells from older donors. Primary macrophages show age-dependent impairment in Toll-like receptor (TLR3)-mediated anti-WNV responses, leading to an early and sustained elevation of cytokines. In addition, WNV-induced type I IFN was significantly lower in dendritic cells (DCs) from older donors compared to younger donors. These deficits in regulatory pathways in anti-viral responses may contribute to the permeability of the blood brain barrier and enhanced susceptibility to WNV infections observed in aging (64, 65).

Innate Immune responses provide critical protection from severe disease

Protection from acute tissue injury is heavily reliant upon the innate immune system that functions early in the host response (66, 67). The first responding cells in infection, polymorphonuclear cells (PMNs), show a paradoxical role in human WNV infection, where infiltrating PMNs are permissive for WNV replication and may serve as an early reservoir of WNV replication, but after exposure to IFN, contribute to viral clearance (68). Macrophages are also critical: mice depleted of macrophages are more susceptible to WNV infection and have higher and extended viremia and higher mortality (67). The $\gamma\delta$ T cell population rapidly expands after WNV infection and promotes a protective adaptive immune response by producing IFN γ and facilitating dendritic cell maturation (69). Subjects with a history of severe disease had lower levels of plasmacytoid dendritic cells (pDCs), which are critical for anti-viral responses (70).

Human studies show a role for chemokines CXCL10 and CCL2 in control of early infection and an important role for IFN-mediated innate immunity in resolving acute WNV infection (71). The production of type I IFN and other cytokines that facilitate early control of viral replication follow recognition of the RNA of WNV through pathogen recognition receptors such as TLRs 3, 7, and 8 and cytoplasmic RNA helicases such as RIG-I and MDA5 that activate transcription factors IRF3, IRF5 and IRF7. IFN-inducible IFITM also inhibits the early replication of WNV (5, 72-75), and the RLR- and MAVS-induced type I IFN response is inhibited by UBXN1 (76). Relative deficiencies in expression or function of these pathways would reduce the efficiency of anti-viral responses.

Factors contributing to neurological involvement

Permeability of the blood brain barrier (BBB), which is enhanced by cytokine responses, has been shown to be critical to susceptibility of neuroinvasive WNV infection (77). Elements which decrease the integrity of the BBB contribute to susceptibility to infection with WNV (Table 1). Entry of WNV to the CNS may be afforded by cytokine responses, adhesion molecules or proteases (4, 78-82), trafficking of infected CD45+ leukocytes and CD11b+ macrophages (83), T cells (84), or neutrophils (85). TNF α may contribute to severe disease by promoting BBB permeability (78) or protect from severe WNV encephalitis by promoting monocyte entry into the brain (86). CXCL10 from the brain directs CD8 T cell recruitment and viral control specific regions of the brain T cell recruitment (87, 88). During infection with WNV, CD8+ T cells expand dramatically and migrate to the site of CNS infection (89).

In the murine model, the chemokine receptor ccr2 mediates monocyte accumulation that is critical to anti-viral activity in the brain (90). For control of virus in the brain, IL-1 and IL-1R1 signaling restricts WNV in the CNS (91, 92) as does ifit2 in a cell-type specific manner (93). Patients with severe WNV neurological involvement had elevated neutrophils in the cerebrospinal fluid that was only a modest predictor of disease outcome (94). CD8+ T cell clearance from the CNS is modulated by MDA5 (95). Advances in our understanding of these pathways at the Systems level will elucidate critical components of resistance to severe infection (70).

Adaptive immunity generates protective T cell responses and long-lasting antibody responses

The highest magnitude of specific T cell responses were CD8+ cells (96) which were not apparently related to disease severity (97). CD8+ T cells, through c-Myc induced transcription factor AP4 (98), express perforin and/or granzyme B, and in response to infection with WNV expand dramatically and migrate to the site of CNS infection (89, 99). WNV-specific murine CD4 T cells produced IFN γ and IL-2 and also showed direct anti-viral activity (100). More cytolytic memory T cells were found in patients with neurological disease (101). Tregs play an important role in protecting against severe disease and it has been shown in human patients (and animal models) that WNV subjects with severe infection have reduced numbers of Th1/Th17 and Tregs (102). Moreover, expression of T cell-inhibitory receptor TIM-3 during acute infection is associated with more severe disease (103).

Adaptive immunity provides protection against WNV via production of specific antibodies and both IgM and IgG antibodies are protective (104). IgM and IgG antibodies develop rapidly after viremia and before RNA levels become undetectable, which occurred a mean of 13.2 days (105). Anti-WNV envelope antibody serum levels were relatively constant for > 1 year (105, 106), or even up to 8 years after infection, but no significant difference in antibody levels was observed between subjects with a history of asymptomatic or severe WNV infection (107). This suggests that susceptibility to WNV was not a consequence of an inability to mount a humoral response, which has been noted for antibody responses to other viral antigens (108).

Biomarkers of susceptibility to severe WNV infection

Individual variations in serum cytokine levels were significantly different from WNV subjects with a history of severe infection (encephalitis) vs asymptomatic subjects. Subjects with a history of severe infection had significantly lower levels of IL-4 that were associated with altered gene expression patterns (107). An integrated systems-level study of transcriptional and functional datasets from WNV subjects identified a predictive signature of susceptibility (67% accuracy) that was detectable years after acute infection, with the most prominent alterations in severe susceptibility being decreased IL1B production by macrophages and decreased CXCL10 expression from myeloid dendritic cells (70). These results suggest that systems-level analysis of immune status can identify factors relevant for severe infections (70, 107).

Prospects for biomarkers and treatments

A novel positive regulator for IFN production, ELF4, may distinguish anti-WNV susceptibility. ELF4 is induced in primary human macrophages on infection with WNV and plays a role in signaling through the MAVS-TBK1 complex (109). WNV subjects with severe disease showed higher levels of ELF4 in macrophages after infection in vitro, which may lead to increases in multiple cytokine responses and more severe infection (70). Other anti-viral signaling components have been identified in vitro but have not yet been shown to define susceptibility in human cohorts.

Many potential avenues for treatment show promise in animal models including RNAi, antibody therapy, and IFN (5). Both passive and active immunization strategies directed against the envelope (E) proteins of WNV can protect mice from lethal infection (110-113). Single chain antibody (112, 114), targeted nanoparticles (115), attenuated virus vaccines (116), and plant-derived viral proteins have shown efficacy in animal models (117). Indeed, in equine models vaccination was both protective and cost-effective (118, 119). While phase I clinical trials showed promise for antibody therapy (50, 120), there are no FDA approved vaccines or therapeutics. Limited case reports of use of IFN α or ribavirin, valuable treatments for other viral infections, have been inconclusive for use with WNV (50). Future opportunities may benefit from advances in our understanding of individual immune susceptibility to target factors relevant for severe infections (70, 107).

Expert commentary

West Nile virus made a dramatic entrance to the US in 1999 and has rapidly spread throughout our region. Cases vary with climate conditions and are likely to increase. Prospects for a specific vaccine or therapeutic treatment are not immediately apparent but markers of immune susceptibility may provide directions for novel therapeutic interventions. Physical protective measures are valuable and our increased knowledge of immune determinants may provide clues to identify individuals at greater risk and to modulate severity.

Five year view: speculative viewpoint

The very dramatic introduction and rapid spread of WNV in the US and into neighboring countries has garnered considerable public attention and concern. As a result of investigations of the ecology and epidemiology of WNV reservoirs and cases, we have developed a detailed understanding of the method of infectivity, laboratory diagnostics, and measures to reduce exposure. However, specific therapeutic measures are needed and not as yet available. With continued high levels of virus activity reported in both 2013 and 2014, WNV will remain a substantial public health threat in North America. This will be a valuable frontier for progress in the near future. In addition, WNV may serve as a model for other emerging pathogens such as Chikungunya or Ebola viruses and highlight critical steps in identification, testing, and remedies needed for public health and safety.

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References

* indicates considerable interest

1. Petersen LR, Carson PJ, Biggerstaff BJ, Custer B, Borchardt SM, Busch MP. Estimated cumulative incidence of West Nile virus infection in US adults, 1999-2010. *Epidemiol Infect.* 2013; 141:591–595. [PubMed: 22640592]
2. Lindsey NP, Lehman JA, Hayes EB, Nasci RS, Komar N, Petersen LR. West Nile Virus Activity-United States, 2006. *JAMA.* 2007; 298:619–621.
3. Gubler DJ. The continuing spread of West Nile virus in the western hemisphere. *Clin Infect Dis.* 2007; 45:1039–1046. [PubMed: 17879923]
4. Arjona A, Wang P, Montgomery RR, Fikrig E. Innate Immune Control of West Nile Virus Infection. *Cellular Microbiology.* 2011; 13:1648–1658. [PubMed: 21790942]
5. Colpitts TM, Conway MJ, Montgomery RR, Fikrig E. West Nile Virus: Biology, Transmission and Human Infection. *Clinical Microbiology Reviews.* 2012; 25:635–648. [PubMed: 23034323]
6. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. *Ann Intern Med.* 2002; 137:173–179. [PubMed: 12160365]
7. Diamond MS. Development of effective therapies against West Nile virus infection. *Expert Rev Anti Infect Ther.* 2005; 3:931–944. [PubMed: 16307506]
8. Nash D, Mostashari F, Fine A, Miller J, O'Leary D, Murray K, Huang A, Rosenberg A, Greenberg A, Sherman M, Wong S, Layton M. The outbreak of West Nile virus infection in the New York City area in 1999. *N Engl J Med.* 2001; 344:1807–1814. [PubMed: 11407341]
9. Steele KE, Linn MJ, Schoepp RJ, Komar N, Geisbert TW, Manduca RM, Calle PP, Raphael BL, Clippinger TL, Larsen T, Smith J, Lanciotti RS, Panella NA, McNamara TS. Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City, New York. *Vet Pathol.* 2000; 37:208–224. [PubMed: 10810985]
10. Trock SC, Meade BJ, Glaser AL, Ostlund EN, Lanciotti RS, Cropp BC, Kulasekera V, Kramer LD, Komar N. West Nile virus outbreak among horses in New York State, 1999 and 2000. *Emerg Infect Dis.* 2001; 7:745–747. [PubMed: 11585543]
11. Davis CT, Ebel GD, Lanciotti RS, Brault AC, Guzman H, Siirin M, Lambert A, Parsons RE, Beasley DW, Novak RJ, Elizondo-Quiroga D, Green EN, Young DS, Stark LM, Drebot MA, Artsob H, Tesh RB, Kramer LD, Barrett AD. Phylogenetic analysis of North American West Nile

- virus isolates, 2001-2004: evidence for the emergence of a dominant genotype. *Virology*. 2005; 342:252–265. [PubMed: 16137736]
12. Brackney DE, Beane JE, Ebel GD. RNAi targeting of West Nile virus in mosquito midguts promotes virus diversification. *PLoS Pathog*. 2009; 5:e1000502. [PubMed: 19578437]
 13. ArboNET. West Nile Virus Statistics and Maps. C. f. D. C. a. Prevention, ed. National Center for Emerging and Zoonotic Infectious Diseases (NCEZID) Division of Vector-Borne Diseases (DVBD). 2014
 14. MMWR. Update: Investigations of West Nile virus infections in recipients of organ transplantation and blood transfusion--Michigan, 2002. *MMWR Morb Mortal Wkly Rep*. 2002; 51:879. [PubMed: 12375688]
 15. MMWR. Possible West Nile virus transmission to an infant through breast-feeding--Michigan, 2002. *MMWR Morb Mortal Wkly Rep*. 2002; 51:877–878. [PubMed: 12375687]
 16. MMWR. Intrauterine West Nile virus infection--New York, 2002. *MMWR Morb Mortal Wkly Rep*. 2002; 51:1135–1136. [PubMed: 12537289]
 17. Zou S, Foster GA, Dodd RY, Petersen LR, Stramer SL. West Nile fever characteristics among viremic persons identified through blood donor screening. *J Infect Dis*. 2010; 202:1354–1361. [PubMed: 20874087]
 18. Lanteri MC, Lee TH, Wen L, Kaidarova Z, Bravo MD, Kiely NE, Kamel HT, Tobler LH, Norris PJ, Busch MP. West Nile virus nucleic acid persistence in whole blood months after clearance in plasma: implication for transfusion and transplantation safety. *Transfusion*. 2014; 54:3232–3241. [PubMed: 24965017]
 19. Basavaraju SV, Kuehnert MJ, Zaki SR, Sejvar JJ. Encephalitis caused by pathogens transmitted through organ transplants, United States, 2002-2013. *Emerg Infect Dis*. 2014; 20:1443–1451. [PubMed: 25148201]
 20. Bradford CM, Nascarella MA, Burns TH, Montford JR, Marsland EJ, Pepper CB, Presley SM. First report of West Nile virus in mosquitoes from Lubbock County, Texas. *J Am Mosq Control Assoc*. 2005; 21:102–105. [PubMed: 15825772]
 21. Murray KO, Ruktanonchai D, Hesalroad D, Fonken E, Nolan MS. West Nile virus, Texas, USA, 2012. *Emerg Infect Dis*. 2013; 19:1836–1838. [PubMed: 24210089]
 22. Paz S, Semenza JC. Environmental drivers of West Nile fever epidemiology in Europe and Western Asia--a review. *Int J Environ Res Public Health*. 2013; 10:3543–3562. [PubMed: 23939389]
 23. Kunkel KE, Novak RJ, Lampman RL, Gu W. Modeling the impact of variable climatic factors on the crossover of *Culex restuans* and *Culex pipiens* (Diptera: culicidae), vectors of West Nile virus in Illinois. *Am J Trop Med Hyg*. 2006; 74:168–173. [PubMed: 16407364]
 24. Dohm DJ, O'Guinn ML, Turell MJ. Effect of environmental temperature on the ability of *Culex pipiens* (Diptera: Culicidae) to transmit West Nile virus. *J Med Entomol*. 2002; 39:221–225. [PubMed: 11931261]
 25. Reisen WK. Effect of temperature on *Culex tarsalis* (Diptera: Culicidae) from the Coachella and San Joaquin Valleys of California. *J Med Entomol*. 1995; 32:636–645. [PubMed: 7473618]
 26. Ruiz MO, Chaves LF, Hamer GL, Sun T, Brown WM, Walker ED, Haramis L, Goldberg TL, Kitron UD. Local impact of temperature and precipitation on West Nile virus infection in *Culex* species mosquitoes in northeast Illinois, USA. *Parasit Vectors*. 2010; 3:19. [PubMed: 20302617]
 - 27*. Manore CA, Davis J, Christofferson RC, Wesson D, Hyman JM, Mores CN. Towards an early warning system for forecasting human west nile virus incidence. *PLoS Curr*. 2014; 6 *Employs modeling of environmental and demographic variables including temperature, mosquito, bird, and human population variables to predict future WNV outbreaks*.
 28. Paz S, Albersheim I. Influence of warming tendency on *Culex pipiens* population abundance and on the probability of West Nile fever outbreaks (Israeli Case Study: 2001-2005). *Ecohealth*. 2008; 5:40–48. [PubMed: 18648796]
 29. Kilpatrick AM, Meola MA, Moudy RM, Kramer LD. Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. *PLoS Pathog*. 2008; 4:e1000092. [PubMed: 18584026]

30. Pawelek KA, Niehaus P, Salmeron C, Hager EJ, Hunt GJ. Modeling Dynamics of *Culex pipiens* Complex Populations and Assessing Abatement Strategies for West Nile Virus. *PLoS One*. 2014; 9:e108452. [PubMed: 25268229]
31. Takeda T, Whitehouse CA, Brewer M, Gettman AD, Mather TN. Arbovirus surveillance in Rhode Island: assessing potential ecologic and climatic correlates. *J Am Mosq Control Assoc*. 2003; 19:179–189. [PubMed: 14524538]
32. Koenraadt CJ, Harrington LC. Flushing effect of rain on container-inhabiting mosquitoes *Aedes aegypti* and *Culex pipiens* (Diptera: Culicidae). *J Med Entomol*. 2008; 45:28–35. [PubMed: 18283939]
33. Roehr B. US hit by massive West Nile virus outbreak centred around Texas. *BMJ*. 2012; 345:e5633. [PubMed: 22915691]
34. Nolan MS, Zangeneh A, Khuwaja SA, Martinez D, Rossmann SN, Cardenas V, Murray KO. Proximity of residence to bodies of water and risk for west nile virus infection: a case-control study in Houston, Texas. *J Biomed Biotechnol*. 2012; 2012:159578. [PubMed: 22315511]
35. Cardenas VM, Jaime J, Ford PB, Gonzalez FJ, Carrillo I, Gallegos JE, Watts DM. Yard flooding by irrigation canals increased the risk of West Nile disease in El Paso, Texas. *Ann Epidemiol*. 2011; 21:922–929. [PubMed: 21943648]
36. Schurich JA, Kumar S, Eisen L, Moore CG. Modeling *Culex tarsalis* abundance on the northern Colorado front range using a landscape-level approach. *J Am Mosq Control Assoc*. 2014; 30:7–20. [PubMed: 24772672]
37. Meyer TE, Bull LM, Cain Holmes K, Pascua RF, Travassos da Rosa A, Gutierrez CR, Corbin T, Woodward JL, Taylor JP, Tesh RB, Murray KO. West Nile virus infection among the homeless, Houston, Texas. *Emerg Infect Dis*. 2007; 13:1500–1503. [PubMed: 18257995]
38. Mostashari F, Bunning ML, Kitsutani PT, Singer DA, Nash D, Cooper MJ, Katz N, Liljebjelke KA, Biggerstaff BJ, Fine AD, Layton MC, Mullin SM, Johnson AJ, Martin DA, Hayes EB, Campbell GL. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. *Lancet*. 2001; 358:261–264. [PubMed: 11498211]
39. Murphy TD, Grandpre J, Novick SL, Seys SA, Harris RW, Musgrave K. West Nile virus infection among health-fair participants, Wyoming 2003: assessment of symptoms and risk factors. *Vector Borne Zoonotic Dis*. 2005; 5:246–251. [PubMed: 16187893]
40. Gujral IB, Zielinski-Gutierrez EC, LeBailly A, Nasci R. Behavioral risks for West Nile virus disease, northern Colorado, 2003. *Emerg Infect Dis*. 2007; 13:419–425. [PubMed: 17552095]
41. Mandalakas AM, Kippes C, Sedransk J, Kile JR, Garg A, McLeod J, Berry RL, Marfin AA. West Nile virus epidemic, northeast Ohio, 2002. *Emerg Infect Dis*. 2005; 11:1774–1777. [PubMed: 16318737]
42. Reisen WK, Fang Y, Martinez VM. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. *J Med Entomol*. 2005; 42:367–375. [PubMed: 15962789]
43. Rappole JH, Derrickson SR, Hubalek Z. Migratory birds and spread of West Nile virus in the Western Hemisphere. *Emerg Infect Dis*. 2000; 6:319–328. [PubMed: 10905964]
44. Gomes B, Sousa CA, Vicente JL, Pinho L, Calderon I, Arez E, Almeida AP, Donnelly MJ, Pinto J. Feeding patterns of molestus and pipiens forms of *Culex pipiens* (Diptera: Culicidae) in a region of high hybridization. *Parasit Vectors*. 2013; 6:93. [PubMed: 23578139]
45. Hamer GL, Kitron UD, Goldberg TL, Brawn JD, Loss SR, Ruiz MO, Hayes DB, Walker ED. Host selection by *Culex pipiens* mosquitoes and West Nile virus amplification. *Am J Trop Med Hyg*. 2009; 80:268–278. [PubMed: 19190226]
46. Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, Davis B, Bowen R, Bunning M. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. *Emerg Infect Dis*. 2003; 9:311–322. [PubMed: 12643825]
47. Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biol*. 2006; 4:e82. [PubMed: 16494532]

48. Thiemann TC, Lemenager DA, Kluh S, Carroll BD, Lothrop HD, Reisen WK. Spatial variation in host feeding patterns of *Culex tarsalis* and the *Culex pipiens* complex (Diptera: Culicidae) in California. *J Med Entomol.* 2012; 49:903–916. [PubMed: 22897051]
49. Molaei G, Andreadis TG, Armstrong PM, Bueno R Jr, Dennett JA, Real SV, Sargent C, Bala A, Randle Y, Guzman H, Travassos da Rosa A, Wuithiranyagool T, Tesh RB. Host feeding pattern of *Culex quinquefasciatus* (Diptera: Culicidae) and its role in transmission of West Nile virus in Harris County, Texas. *Am J Trop Med Hyg.* 2007; 77:73–81. [PubMed: 17620633]
50. Gray TJ, Webb CE. A review of the epidemiological and clinical aspects of West Nile virus. *Int J Gen Med.* 2014; 7:193–203. [PubMed: 24748813]
51. Guth JC, Futterer SA, Hijaz TA, Liotta EM, Rosenberg NF, Naidech AM, Maas MB. Pearls & oysters: bilateral thalamic involvement in West Nile virus encephalitis. *Neurology.* 2014; 83:e16–17. [PubMed: 25002571]
52. Murray K, Baraniuk S, Resnick M, Arafat R, Kilborn C, Cain K, Shallenberger R, York TL, Martinez D, Hellums JS, Hellums D, Malkoff M, Elgawley N, McNeely W, Khuwaja SA, Tesh RB. Risk factors for encephalitis and death from West Nile virus infection. *Epidemiol Infect.* 2006; 134:1325–1332. [PubMed: 16672108]
53. Sejvar JJ, Lindsey NP, Campbell GL. Primary causes of death in reported cases of fatal West Nile Fever, United States, 2002–2006. *Vector Borne Zoonotic Dis.* 2011; 11:161–164. [PubMed: 20687860]
54. Nakaya HI, Wrammert J, Lee EK, Racioppi L, Marie-Kunze S, Haining WN, Means AR, Kasturi SP, Khan N, Li GM, McCausland M, Kanchan V, Kokko KE, Li S, Elbein R, Mehta AK, Aderem A, Subbarao K, Ahmed R, Pulendran B. Systems biology of vaccination for seasonal influenza in humans. *Nat Immunol.* 2011; 12:786–795. [PubMed: 21743478]
55. Li S, Roupheal N, Duraisingham S, Romero-Steiner S, Presnell S, Davis C, Schmidt DS, Johnson SE, Milton A, Rajam G, Kasturi S, Carlone GM, Quinn C, Chaussabel D, Palucka AK, Mulligan MJ, Ahmed R, Stephens DS, Nakaya HI, Pulendran B. Molecular signatures of antibody responses derived from a systems biology study of five human vaccines. *Nat Immunol.* 2014; 15:195–204. [PubMed: 24336226]
56. Lanteri MC, Kaidarova Z, Peterson T, Cate S, Custer B, Wu S, Agapova M, Law JP, Bielawny T, Plummer F, Tobler LH, Loeb M, Busch MP, Bramson J, Luo M, Norris PJ. Association between HLA class I and class II alleles and the outcome of West Nile virus infection: an exploratory study. *PLoS One.* 2011; 6:e22948. [PubMed: 21829673]
- 57*. Lim JK, Lisco A, McDermott DH, Huynh L, Ward JM, Johnson B, Johnson H, Pape J, Foster GA, Krysztof D, Follmann D, Stramer SL, Margolis LB, Murphy PM. Genetic variation in OAS1 is a risk factor for initial infection with West Nile virus in man. *PLoS Pathog.* 2009; 5:e1000321. *Identifies genetic polymorphisms relevant to susceptibility in human populations.* [PubMed: 19247438]
- 58*. Bigham AW, Buckingham KJ, Husain S, Emond MJ, Bofferd KM, Gildersleeve H, Rutherford A, Astakhova NM, Perelygin AA, Busch MP, Murray KO, Sejvar JJ, Green S, Kriesel J, Brinton MA, Bamshad M. Host genetic risk factors for West Nile virus infection and disease progression. *PLoS One.* 2011; 6:e24745. *Identifies genetic polymorphisms relevant to susceptibility in human populations.* [PubMed: 21935451]
59. Yakub I, Lillibridge KM, Moran A, Gonzalez OY, Belmont J, Gibbs RA, Twardy DJ. Single nucleotide polymorphisms in genes for 2'-5'-oligoadenylate synthetase and RNase L in patients hospitalized with West Nile virus infection. *J Infect Dis.* 2005; 192:1741–1748. [PubMed: 16235172]
60. Loeb M, Eskandarian S, Rupp M. Genetic variants and susceptibility to neurological complications following West Nile virus infection. *J Infect Dis.* 2011; 204:1031–1037. [PubMed: 21881118]
61. Lim JK, McDermott DH, Lisco A, Foster GA, Krysztof D, Follmann D, Stramer SL, Murphy PM. CCR5 deficiency is a risk factor for early clinical manifestations of West Nile virus infection but not for viral transmission. *J Infect Dis.* 2010; 201:178–185. [PubMed: 20025530]
62. Ruan Q, Qian F, Yu Z. Effects of polymorphisms in immunity-related genes on the immune system and successful aging. *Curr Opin Immunol.* 2014; 29:49–55. [PubMed: 24780188]
63. Shaw AC, Goldstein DG, Montgomery RR. Dysregulated innate immune function in aging. *Nat Rev Immunol.* 2013; 13:875–887. [PubMed: 24157572]

- 64*. Kong KF, Delroux K, Wang X, Qian F, Arjona A, Malawista SE, Fikrig E, Montgomery RR. Dysregulation of TLR3 impairs the innate immune response to West Nile virus in the elderly. *J Virol*. 2008; 82:7613–7623. *Identifies age-related dysregulated immune pathways relevant to susceptibility to WNV*. [PubMed: 18508883]
- 65*. Qian F, Wang X, Zhang L, Lin A, Zhao H, Fikrig E, Montgomery RR. Impaired interferon signaling in dendritic cells from older donors infected in vitro with West Nile virus. *J Infect Dis*. 2011; 203:1415–1424. *Identifies age-related dysregulated immune pathways relevant to susceptibility to WNV*. [PubMed: 21398396]
66. Kramer LD, Bernard KA. West Nile virus infection in birds and mammals. *Ann N Y Acad Sci*. 2001; 951:84–93. [PubMed: 11797807]
67. Ben-Nathan D, Huitinga I, Lustig S, van Rooijen N, Kobiler D. West Nile virus neuroinvasion and encephalitis induced by macrophage depletion in mice. *Arch Virol*. 1996; 141:459–469. [PubMed: 8645088]
68. Bai F, Kong KF, Dai J, Qian F, Zhang L, Brown CR, Fikrig E, Montgomery RR. A paradoxical role for neutrophils in the pathogenesis of West Nile virus. *J Infect Dis*. 2010; 202:1804–1812. [PubMed: 21050124]
69. Wang T, Scully E, Yin Z, Kim JH, Wang S, Yan J, Mamula M, Anderson JF, Craft J, Fikrig E. IFN- γ -producing gd T cells help control murine West Nile virus infection. *J Immunol*. 2003; 171:2524–2531. [PubMed: 12928402]
- 70*. Qian F, Goel G, Meng H, Wang X, You F, Devine L, Raddassi K, Garcia MN, Murray KO, Bolen CR, Gaujoux R, Shen-Orr SS, Hafler D, Fikrig E, Xavier RJ, Kleinstein SH, Montgomery RR. Systems Immunology reveals markers of susceptibility to West Nile virus infection. *Clin Vacc Immunol*. 2015; 22:6–16. *Defines biomarkers of susceptibility from human subjects years after infection with WNV*.
- 71*. Tobler LH, Cameron MJ, Lanteri MC, Prince HE, Danesh A, Persad D, Lanciotti RS, Norris PJ, Kelvin DJ, Busch MP. Interferon and interferon-induced chemokine expression is associated with control of acute viremia in West Nile virus-infected blood donors. *J Infect Dis*. 2008; 198:979–983. *Identifies critical immune responses for control of acute WNV infection in humans*. [PubMed: 18729779]
72. Thackray LB, Shrestha B, Richner JM, Miner JJ, Pinto AK, Lazear HM, Gale M Jr, Diamond MS. Interferon Regulatory Factor 5-Dependent Immune Responses in the Draining Lymph Node Protect against West Nile Virus Infection. *J Virol*. 2014; 88:11007–11021. [PubMed: 25031348]
73. Errett JS, Suthar MS, McMillan A, Diamond MS, Gale M Jr. The essential, nonredundant roles of RIG-I and MDA5 in detecting and controlling West Nile virus infection. *J Virol*. 2013; 87:11416–11425. [PubMed: 23966395]
74. Suthar MS, Pulendran B. Systems analysis of West Nile virus infection. *Curr Opin Virol*. 2014; 6:70–75. [PubMed: 24851811]
75. Brass AL, Benita Y, John SP, Krishnan MN, Feeley EM, Ryan BJ, Weyer JL, van der Weyden L, Fikrig E, Adams DJ, Xavier RJ, Farzan M, Elledge SJ. The IFITM Proteins Mediate Cellular Resistance to Influenza A H1N1 Virus, West Nile Virus, and Dengue Virus. *Cell*. 2009; 139:1243–1254. [PubMed: 20064371]
76. Wang P, Yang L, Cheng G, Yang G, You F, Xu Z, Sun Q, Lin R, Fikrig E, Sutton RE. UBXN1 interferes with RLR-mediated antiviral immune response by targeting MAVS. *Cell Reports*. 2013; 4:1057–1070. [PubMed: 23545497]
77. Cho H, Diamond MS. Immune responses to West Nile virus infection in the central nervous system. *Viruses*. 2012; 4:3812–3830. [PubMed: 23247502]
78. Wang T, Town T, Alexoupoulou L, Anderson JF, Fikrig E, Flavell RA. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nat Med*. 2004; 10:1366–1373. [PubMed: 15558055]
79. Wang P, Dai J, Bai F, Kong KF, Wong SJ, Montgomery RR, Madri JA, Fikrig E. Matrix metalloproteinase 9 facilitates West Nile Virus entry into the brain. *J Virol*. 2008; 82:8978–8985. [PubMed: 18632868]

80. Wang P, Arjona A, Zhang Y, Dai J, LeBlanc P, Doiron K, Sultana H, Saleh M, Fikrig E. Caspase-12 controls West Nile virus infection via the viral RNA receptor RIG-I. *Nat Immunol.* 2010; 11:912–919. [PubMed: 20818395]
81. Arjona A, Foellmer H, Town T, Leng L, McDonald C, Wong S, Montgomery RR, Fikrig E, Bucala R. Abrogation of Macrophage Migration Inhibitory Factor Decreases West Nile Virus Lethality by Limiting Viral Neuroinvasion. *J Clin Invest.* 2007; 117:3059–3066. [PubMed: 17909632]
82. Dai J, Wang P, Bai F, Town T, Fikrig E. ICAM-1 participates in the entry of West Nile virus into the central nervous system. *J Virol.* 2008; 82:4164–4168. [PubMed: 18256150]
83. Town T, Bai F, Wang T, Kaplan AT, Qian F, Montgomery RR, Anderson JF, Flavell RA, Fikrig E. Tlr7 mitigates lethal West Nile encephalitis by affecting interleukin 23-dependent immune cell infiltration and homing. *Immunity.* 2009; 30:242–253. [PubMed: 19200759]
84. Wang S, Welte T, McGargill M, Town T, Thompson J, Anderson JF, Flavell RA, Fikrig E, Hedrick SM, Wang T. Drak2 contributes to West Nile virus entry into the brain and lethal encephalitis. *J Immunol.* 2008; 181:2084–2091. [PubMed: 18641347]
85. Wang P, Bai F, Zenewicz LA, Dai J, Gate D, Cheng G, Yang L, Qian F, Yuan X, Montgomery RR, Flavell RA, Town T, Fikrig E. IL-22 signaling contributes to West Nile virus encephalitis pathogenesis. *PLoS ONE.* 2012; 7:e44153. [PubMed: 22952908]
86. Shrestha B, Zhang B, Purtha WE, Klein RS, Diamond MS. Tumor necrosis factor alpha protects against lethal West Nile virus infection by promoting trafficking of mononuclear leukocytes into the central nervous system. *J Virol.* 2008; 82:8956–8964. [PubMed: 18632856]
87. Klein RS, Lin E, Zhang B, Luster AD, Tollett J, Samuel MA, Engle M, Diamond MS. Neuronal CXCL10 directs CD8+ T-cell recruitment and control of West Nile virus encephalitis. *J Virol.* 2005; 79:11457–11466. [PubMed: 16103196]
88. Zhang B, Chan YK, Lu B, Diamond MS, Klein RS. CXCR3 mediates region-specific antiviral T cell trafficking within the central nervous system during West Nile virus encephalitis. *J Immunol.* 2008; 180:2641–2649. [PubMed: 18250476]
89. King NJ, Getts DR, Getts MT, Rana S, Shrestha B, Kesson AM. Immunopathology of flavivirus infections. *Immunol Cell Biol.* 2007; 85:33–42. [PubMed: 17146465]
90. Lim JK, Obara CJ, Rivollier A, Pletnev AG, Kelsall BL, Murphy PM. Chemokine receptor Ccr2 is critical for monocyte accumulation and survival in West Nile virus encephalitis. *J Immunol.* 2011; 186:471–478. [PubMed: 21131425]
91. Ramos HJ, Lanteri MC, Blahnik G, Negash A, Suthar MS, Brassil MM, Sodhi K, Treuting PM, Busch MP, Norris PJ, Gale M Jr. IL-1beta signaling promotes CNS-intrinsic immune control of West Nile virus infection. *PLoS Pathog.* 2012; 8:e1003039. [PubMed: 23209411]
92. Durrant DM, Robinette ML, Klein RS. IL-1R1 is required for dendritic cell-mediated T cell reactivation within the CNS during West Nile virus encephalitis. *J Exp Med.* 2013; 210:503–516. [PubMed: 23460727]
93. Cho H, Shrestha B, Sen GC, Diamond MS. A role for Ifit2 in restricting West Nile virus infection in the brain. *J Virol.* 2013; 87:8363–8371. [PubMed: 23740986]
94. Tyler KL, Pape J, Goody RJ, Corkill M, Kleinschmidt-DeMasters BK. CSF findings in 250 patients with serologically confirmed West Nile virus meningitis and encephalitis. *Neurology.* 2006; 66:361–365. [PubMed: 16382032]
95. Lazear HM, Pinto AK, Ramos HJ, Vick SC, Shrestha B, Suthar MS, Gale M Jr, Diamond MS. Pattern recognition receptor MDA5 modulates CD8+ T cell-dependent clearance of West Nile virus from the central nervous system. *J Virol.* 2013; 87:11401–11415. [PubMed: 23966390]
- 96*. Lanteri MC, Heitman JW, Owen RE, Busch T, Geffer N, Kiely N, Kamel HT, Tobler LH, Busch MP, Norris PJ. Comprehensive analysis of west nile virus-specific T cell responses in humans. *J Infect Dis.* 2008; 197:1296–1306. *Defines T cell subsets involved in human response to WNV infection.* [PubMed: 18422442]
97. Parsons R, Lelic A, Hayes L, Carter A, Marshall L, Eveleigh C, Drebot M, Andonova M, McMurtrey C, Hildebrand W, Loeb MB, Bramson JL. The memory T cell response to West Nile virus in symptomatic humans following natural infection is not influenced by age and is dominated by a restricted set of CD8+ T cell epitopes. *J Immunol.* 2008; 181:1563–1572. [PubMed: 18606712]

98. Chou C, Pinto AK, Curtis JD, Persaud SP, Cella M, Lin CC, Edelson BT, Allen PM, Colonna M, Pearce EL, Diamond MS, Egawa T. c-Myc-induced transcription factor AP4 is required for host protection mediated by CD8(+) T cells. *Nat Immunol.* 2014; 15:884–893. [PubMed: 25029552]
99. Shrestha B, Diamond MS. Role of CD8+ T cells in control of West Nile virus infection. *J Virol.* 2004; 78:8312–8321. [PubMed: 15254203]
100. Brien JD, Uhrlaub JL, Nikolich-Zugich J. West Nile virus-specific CD4 T cells exhibit direct antiviral cytokine secretion and cytotoxicity and are sufficient for antiviral protection. *J Immunol.* 2008; 181:8568–8575. [PubMed: 19050276]
101. Piazza P, McMurtrey CP, Lelic A, Cook RL, Hess R, Yablonsky E, Borowski L, Loeb MB, Bramson JL, Hildebrand WH, Rinaldo CR. Surface phenotype and functionality of WNV specific T cells differ with age and disease severity. *PLoS One.* 2010; 5:e15343. [PubMed: 21179445]
102. Lanteri MC, O'Brien KM, Purtha WE, Cameron MJ, Lund JM, Owen RE, Heitman JW, Custer B, Hirschhorn DF, Tobler LH, Kiely N, Prince HE, Ndhlovu LC, Nixon DF, Kamel HT, Kelvin DJ, Busch MP, Rudensky AY, Diamond MS, Norris PJ. Tregs control the development of symptomatic West Nile virus infection in humans and mice. *J Clin Invest.* 2009; 119:3266–3277. [PubMed: 19855131]
103. Lanteri MC, Diamond MS, Law JP, Chew GM, Wu S, Inglis HC, Wong D, Busch MP, Norris PJ, Ndhlovu LC. Increased frequency of Tim-3 expressing T cells is associated with symptomatic West Nile virus infection. *PLoS One.* 2014; 9:e92134. [PubMed: 24642562]
104. Diamond MS, Shrestha B, Marri A, Mahan D, Engle M. B cells and antibody play critical roles in the immediate defense of disseminated infection by West Nile encephalitis virus. *J Virol.* 2003; 77:2578–2586. [PubMed: 12551996]
105. Busch MP, Kleinman SH, Tobler LH, Kamel HT, Norris PJ, Walsh I, Matud JL, Prince HE, Lanciotti RS, Wright DJ, Linnen JM, Caglioti S. Virus and antibody dynamics in acute west nile virus infection. *J Infect Dis.* 2008; 198:984–993. [PubMed: 18729783]
106. Prince HE, Lape-Nixon M, Yeh C, Tobler LH, Busch MP. Persistence of antibodies to West Nile virus nonstructural protein 5. *J Clin Virol.* 2008; 43:102–106. [PubMed: 18467165]
107. Qian F, Thakar J, Yuan X, Nolan M, Murray KO, Lee WT, Wong SJ, Meng H, Fikrig E, Kleinstein SH, Montgomery RR. Immune markers associated with host susceptibility to infection with West Nile virus. *Viral Immunology.* 2014; 27:39–47. [PubMed: 24605787]
108. Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. *N Engl J Med.* 2007; 357:1903–1915. [PubMed: 17989383]
109. You F, Wang P, Yang L, Yang G, Zhao YO, Qian F, Walker W, Sutton RE, Montgomery RR, Lin R, Iwasaki A, Fikrig E. ELF4 is critical for induction of type I interferon and the host antiviral response. *Nat Immunol.* 2013; 14:1237–1246. [PubMed: 24185615]
110. Wang T, Anderson JF, Magnarelli LA, Wong SJ, Koski RA, Fikrig E. Immunization of mice against West Nile virus with recombinant envelope protein. *J Immunol.* 2001; 167:5273–5277. [PubMed: 11673542]
111. Ben-Nathan D, Lustig S, Tam G, Robinson S, Segal S, Rager-Zisman B. Prophylactic and therapeutic efficacy of human intravenous immunoglobulin in treating West Nile virus infection in mice. *J Infect Dis.* 2003; 188:5–12. [PubMed: 12825165]
112. Gould LH, Sui J, Foellmer H, Oliphant T, Wang T, Ledizet M, Murakami A, Noonan K, Lambeth C, Kar K, Anderson JF, de Silva AM, Diamond MS, Koski RA, Marasco WA, Fikrig E. Protective and therapeutic capacity of human single-chain Fv-Fc fusion proteins against West Nile virus. *J Virol.* 2005; 79:14606–14613. [PubMed: 16282460]
- 113*. Oliphant T, Engle M, Nybakken GE, Doane C, Johnson S, Huang L, Gorlatov S, Mehlhop E, Marri A, Chung KM, Ebel GD, Kramer LD, Fremont DH, Diamond MS. Development of a humanized monoclonal antibody with therapeutic potential against West Nile virus. *Nat Med.* 2005; 11:522–530. *Development of an effective therapeutic antibody for WNV.* [PubMed: 15852016]
114. Sultana H, Foellmer HG, Neelakanta G, Oliphant T, Engle M, Ledizet M, Krishnan MN, Bonafe N, Anthony KG, Marasco WA, Kaplan P, Montgomery RR, Diamond MS, Koski RA, Fikrig E. Fusion loop peptide of the West Nile virus envelope protein is essential for pathogenesis and is

- recognized by a therapeutic cross-reactive human monoclonal antibody. *J Immunol.* 2009; 183:650–660. [PubMed: 19535627]
115. Demento SL, Bonafe N, Cui W, Kaech SM, Caplan MJ, Fikrig E, Ledizet M, Fahmy TM. TLR9-targeted biodegradable nanoparticles as immunization vectors protect against West Nile encephalitis. *J Immunol.* 2010; 185:2989–2997. [PubMed: 20660705]
116. Pinto AK, Richner JM, Poore EA, Patil PP, Amanna IJ, Slifka MK, Diamond MS. A hydrogen peroxide-inactivated virus vaccine elicits humoral and cellular immunity and protects against lethal West Nile virus infection in aged mice. *J Virol.* 2013; 87:1926–1936. [PubMed: 23221549]
117. Lai H, He J, Hurtado J, Stahnke J, Fuchs A, Mehlhop E, Gorlatov S, Loos A, Diamond MS, Chen Q. Structural and functional characterization of an anti-West Nile virus monoclonal antibody and its single-chain variant produced in glycoengineered plants. *Plant Biotechnol J.* 2014; 12:1098–1107. [PubMed: 24975464]
118. Gardner IA, Wong SJ, Ferraro GL, Balasuriya UB, Hullinger PJ, Wilson WD, Shi PY, MacLachlan NJ. Incidence and effects of West Nile virus infection in vaccinated and unvaccinated horses in California. *Vet Res.* 2007; 38:109–116. [PubMed: 17274156]
119. Seino KK, Long MT, Gibbs EP, Bowen RA, Beachboard SE, Humphrey PP, Dixon MA, Bourgeois MA. Comparative efficacies of three commercially available vaccines against West Nile Virus (WNV) in a short-duration challenge trial involving an equine WNV encephalitis model. *Clin Vaccine Immunol.* 2007; 14:1465–1471. [PubMed: 17687109]
120. Beigel JH, Nordstrom JL, Pillemer SR, Roncal C, Goldwater DR, Li H, Holland PC, Johnson S, Stein K, Koenig S. Safety and pharmacokinetics of single intravenous dose of MGAWN1, a novel monoclonal antibody to West Nile virus. *Antimicrob Agents Chemother.* 2010; 54:2431–2436. [PubMed: 20350945]
121. Daffis S, Suthar MS, Szretter KJ, Gale M Jr, Diamond MS. Induction of IFN-beta and the innate antiviral response in myeloid cells occurs through an IPS-1-dependent signal that does not require IRF-3 and IRF-7. *PLoS Pathog.* 2009; 5:e1000607. [PubMed: 19798431]

Key Issues

- People living in close proximity to stagnant water sources where mosquitoes breed have been found to be at higher risk for infection
- Increased time outdoors and absence of personal precautions against mosquitoes is associated with infection
- Physical controls to limit exposure to mosquitoes such as use of screens and insect repellent will limit infection
- Regional vector control programs with reducing standing water, or spraying larvicides may reduce the reservoir of infected mosquitoes and limit human exposure
- The elderly and immunocompromised individuals are particularly susceptible and should exercise heightened protection from mosquito exposure
- Advances in our understanding of immune markers of susceptibility hold promise for screening the highest risk individuals
- With continued high levels of virus activity reported in both 2013 and 2014, WNV will remain a substantial public health threat in North America.
- Developing therapeutic measures may include preventative vaccines as well as antibodies or other agents which act post-infection as therapeutics

Figure 1. Total number of human cases of West Nile virus in the USA, 1999-2014

*Number of WNV cases reported to CDC's Arbonet (13); 2014 cases are as of Oct. 14, 2014.

Table 1
Immune Components that protect from neurological infection with West Nile virus

Component	Human	Mouse	Notes
Cytokines	IFN α/β IL-1 IL-4 (64, 65, 70, 71, 91, 107)	IFN α/β IL-1 ifit2 (91-93, 121)	IFN controls early virus replication in the brain IL-1 elevated during infection; critical in clearance in the brain; decreased in macrophages of severe human subjects
Chemokines	CCL2 CXCL10 (70, 71)	CXCL10 Ccr2 CXCR3 (87, 88, 90)	CXCL10 elevated in myeloid DCs Neuronal CXCL10 directs CD8 T cells
Blood brain barrier (BBB) permeability		Caspase 12 ICAM-1 IL-22 MIF MMP9 TLR3 TLR7 TNF α (78-86)	Decreased integrity of the BBB promotes viral entry to the brain