# Chapter 6

# Lessons from the Murine Models of West Nile Virus Infection

**Brenna McGruder, Vandana Saxena, and Tian Wang**

## Abstract

West Nile virus (WNV), a mosquito-borne, single positive-stranded RNA virus, has been the leading cause of arboviral encephalitis in the U.S. and other parts of the world over the past decade. Up to 50% of WNV convalescent patients were reported to have long-term neurological sequelae or chronic kidney diseases. However, there are neither antiviral drugs nor vaccines available for humans. The underlying mechanism of the long-term sequelae is not clearly understood either. Animal models have been an effective tool to investigate viral pathogenesis and host immunity in humans. Here, we will review several commonly used murine models of WNV infection.

**Key words** West Nile virus, Mice, Immunity, Pathogenesis, Infection

## 1 Introduction

West Nile virus (WNV), a mosquito-borne neurotropic pathogen, belongs to the family of *Flaviviridae*, the genus Flavivirus, a group of plus-sense, single-stranded RNA viruses [1, 2]. It has become a global public health concern. The virus was originally isolated in Africa and later caused epidemics with mainly febrile illness in humans in Europe, Africa, the Middle East, and parts of Asia. In 1999, a more virulent WNV strain was introduced in North America and has since caused significant mortality in humans, horses, and birds. Additionally, it has been the cause of recent outbreaks of viral encephalitis in Europe and Australia [3, 4]. WNV infection of the central nervous system (CNS) commonly presents as encephalitis, meningitis, or acute flaccid paralysis. The overall mortality rate in persons who develop WNV neuroinvasive disease is about 10%, although it increases significantly in the elderly and immunocompromised. Up to 50% of WNV convalescent patients were reported to have long-term neurological

Tonya M. Colpitts (ed.), *West Nile Virus: Methods and Protocols*, Methods in Molecular Biology, vol. 1435,
DOI 10.1007/978-1-4939-3670-0_6, 

sequelae or chronic kidney diseases [5–8]. Currently, licensed vaccines are not yet ready for human use, and treatment is mostly nonspecific and supportive.

WNV has been studied in various animal models [9–12]. Among them, mice are generally susceptible to WNV infection, easiest to work with, economic, and are most amenable to immunological manipulation. Thus, several laboratory murine models of WNV infection have been utilized to investigate the host factors involved in protective immunity and viral pathogenesis in humans. Systemic WNV infection has been commonly performed in young adult mice (6–10 weeks old). Following the initial subcutaneous (s.c.) or intraperitoneal (i.p.) inoculation, WNV induces a systemic infection and eventually invades the CNS. The severity and symptoms of lethal infection observed in mice mimic severe cases observed in humans (*see* **Note 1**). The incidence of WNV neuroinvasive disease has been reported to increase with age, with the highest incidence among persons aged over 70 years [13–15]. Systemic WNV infection in old mice (18–22 months old) has been used to define aging as a risk factor in the murine model [16, 17] (*see* **Note 2**). There is increasing evidence that persistent WNV infection contributes to long-term morbidity. WNV antigen or RNA has been detected in the brain and/or urine of WNV patients ranging from a few months to several years after the initial acute illness [18, 19]. We have recently demonstrated that WNV H8912, an isolate previously cultured from the urine of a persistently infected hamster, induces a similar persistent infection phenotype in mice following an i.p. inoculation and preferentially persists in mouse kidneys [20]. Thus, WNV H8912 systemic infection in mice can serve as a tool to study persistent renal infection (*see* **Note 3**). Cutaneous WNV infection either by mosquito feeding or intradermal (i.d.) injection, which presumably mimics natural infection in humans, is an important model for examining host immunity to vector-borne pathogens [21, 22] (*see* **Note 4**). Finally, direct CNS infection by intracranial (i.c.) injection of mouse brain is used to study the neurovirulence of WNV (*see* **Note 5**). In summary, studies from the above well established murine models will provide critical insights into WNV pathogenesis and protective immunity in humans.

## 2 Materials

1. *Mice*: We purchased 6- to 10-week-old C57BL/6 (B6) mice and 18- to 22-month-old B6 mice from Jackson Laboratories (Bar Harbor, ME) and the National Institute of Aging (Bethesda, MD), respectively. All animal experiments were approved by the Institution Animal Care and Use Committee.

2. *WNV*: The parental strain WNV NY385-99 (WNV NY99, [12]), a kind gift from Dr. R Tesh (UTMB, Galveston, TX), was passaged once in Vero cells and twice in C6/36 cells to make a virus stock ($3.5 \times 10^7$ PFU/ml) for all acute infection studies. For persistent infection, WNV H8912, which was recovered from hamster urine 274 days post infection after three consecutive passages of a urine isolate from a chronically infected hamster, was used [23].
3. *Phosphate-buffered saline with 5% gelatin (PBSG)*: Dissolve 1.2 g of gelatin type B from bovine skin into 800 ml of PBS. Adjust the pH to 7.4. Autoclave the solution and cool the liquid to 50 °C. Aliquot to 10 ml aliquots until further use.
4. *Syringe needles*: A 1-cc tuberculin syringe with 26-G needle was used for systemic infection studies (acute or persistent, *see* **Notes 1–3**). A 0.5-ml, ultra-fine insulin syringe with 29-G needle was used in cutaneous and direct CNS injections (*see* **Notes 4** and **5**).
5. *Anesthetizing agents*: Isoflurane inhalation and Ketamine/xylazine injection were used for anesthesia of mice before systemic and direct CNS or cutaneous inoculations, respectively.
6. *Facilities*: Biosafety level 3 (BSL3) and animal BL3 (ABLS3) facilities were used for preparation of viral inocula and injection/housing of mice, respectively.

## 3 Methods

1. *Preparation of viral inocula*: WNV inocula were prepared by serial dilution of viral stocks in phosphate-buffered saline with 5% gelatin (PBSG) at BL3 facilities and were kept on ice before injections. A final volume of 200 and 50 µl of viral inocula was delivered to each mouse for systemic and cutaneous or direct CNS injections, respectively.
2. *Injection procedure*:
   (a) All animal inoculations were performed in ABL3 facilities in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.
   (b) For systemic infection studies, isoflurane inhalation was used first for anesthesia of mice in a small chamber. Next, the tuberculin 1 cc syringe was uncapped, the amount of viral inoculum needed was drawn up into the syringe and needle, and 200 µl of virus inoculum was injected in the left quadrant of the mouse abdomen towards the head at a 30–40° angle (*see* **Notes 1–3**).

(c) For i.d or i.c. inoculation, we first injected 200 μl of Ketamine/xylazine i.p. (administered at 70 mg/7 mg/kg mouse weight). Next, we used a 0.5-ml insulin syringe with a 29 G needle to inject anesthetized mice with WNV isolate in 50 μl PBSG.

(d) For i.d. injection, insert the needle into the dorsal side of the ear, make sure not to insert the needle too far and either go through the ear or into the skull (*see* **Note 4**).

(e) For i.c. injection, carefully insert the needle where the midline of the skull intersects with the midline between the eyes and make sure to use enough force to insert the needle into the skull, but not too deep into the brain to cause damage (*see* **Note 5**).

3. *Post-infection monitor*:

(a) Infected mice were housed in ABL3 facilities and monitored twice daily for 1 month.

(b) When mice showed any sign of morbidity (obvious severe illness, unable to right self when tipped on side or back, obvious extreme weight loss or signs of paralysis), they were immediately collected and euthanized by using $CO_2$.

4. *Conclusions*: Murine models of WNV infection have provided important insights into host immunity and viral pathogenesis in humans. Results from animal studies will help to identify therapeutic goals to modulate immune functions pharmacologically, as well as antiviral countermeasures, and to develop vaccines that can induce robust memory T cell responses.

## 4 Notes

1. *Acute systemic infection in young adult mice*: Following the initial i.p. infection, wild-type WNV NY99 strain was found to induce a systemic infection and eventually invaded the CNS [10, 24]. The virus quickly spread to the spleen, kidneys, and other peripheral tissues. Further, WNV crossed the blood brain barrier (BBB) after a brief viremia and entered the CNS (*see* Table 1). An increased viral burden in serum usually correlated with earlier viral entry into the brain [25]. Moreover, systemic WNV replication was reported to induce a pro-inflammatory cytokine response that modulates BBB permeability, which in turn, may enable viral entry into the brain and induce lethal encephalitis [26, 27]. Thus, it is critical to control virus dissemination in the periphery during early stages of WNV infection. WNV-infected mice developed symptoms starting on day 6, and succumbed to infection when encephalitis developed, usually within 1–2 weeks [10, 24, 28]. Similar outcomes have

**Table 1**
**WNV infection models in mice**

| WNV infection models | Routes of injection, virus dose | Phenotype | Significance |
|---|---|---|---|
| Systemic infection model | i.p. or s.c injection; wild-type or mutant WNV strains; 100 PFU and 1000 PFU are $LD_{50}$ and $LD_{100}$ respectively for wild-type WNV | Following wild-type WNV infection, virus spreads to spleen, kidneys, and other peripheral tissues. Virus enters the CNS following a period of viremia and encephalitis develops | Partially mimics symptoms observed in humans |
| Old mouse model | i.p. or s.c.; lower $LD_{50}$ compared to young adult mice | Higher viremia and viral loads in the brain and are more susceptible to WNV infection | Study aging as a risk factor for WNV encephalitis |
| Persistent infection model | i.p. or s.c.; wild-type WNV or WNV H8912 | WNV H8912 preferentially persists in the kidneys; the frequency of wild-type WNV persistence is tissue-dependent and was found in the skin, spinal cord, brain, lymphoid tissues, kidneys, and heart | Study persistent infection and possibly the long-term morbidity observed in WNV convalescent patients |
| Direct CNS infection model | i.c.; lower $LD_{50}$ compared to systemic infection | Infection in CNS tissues | Study neurovirulence of WNV |
| Cutaneous infection model | i.d. or mosquito feeding $10^5$ PFU as $LD_{50}$ | Virus disseminates from the skin to peripheral organs by migration of LCs | Mimic WNV natural infection |

been reported following s.c. infection in young adult mice [16, 25]. The $LD_{50}$ and $LD_{100}$ of wild-type WNV NY99 i.p. infections in young adult mice are 100 plaque forming unit (PFU) and 1000 PFU, respectively.

2. *Acute systemic infection in aged mice*: Following either i.p. or s.c. WNV infection, aged mice (18–22 months old) displayed persistently elevated viremia and higher viral loads in the brain (*see* Table 1). As a result, aged mice were much more susceptible to systemic WNV NY99 infection compared to young adult mice (6–10 weeks old). The decline in immunity seen in the elderly is a significant contributor to the increased risk of infection. Impaired innate and adaptive T cell functions in aged mice contribute to an enhanced host susceptibility to WNV encephalitis [16, 17]. Toll-like receptor (TLR)7 provides co-stimulatory signals for γδ T cell activation during WNV infection. An impaired TLR7 signaling led to dysfunction of these cells observed in WNV-infected aged mice [29].
3. *Persistent WNV infection in young adult mice*: WNV antigen or RNA has been detected in the brain or urine of WNV convalescent patients, ranging from a few months to several years after the initial acute illness [18, 19]. To define a murine model of persistent renal infection, we used WNV H8912, an isolate recovered from the urine of chronically infected hamsters. The virus showed a significantly reduced neuroinvasiveness in young adult mice. All mice survived i.p. inoculation of a dose up to $10^6$ PFU of WNV H8912, and only about 20% of infected mice developed mild disease symptoms. WNV H8912 induced constitutive interleukin (IL)-10 production and a delayed antiviral response. Viral RNA was detected quickly in blood and spleen but much later in kidneys (*see* Table 1). The virus persisted preferentially in the kidneys with mild renal inflammation, and less frequently in the spleen for up to 2.5 months post infection. This was concurrent with detectable serum WNV-specific IgM and IgG production [20]. In another study, Appler et al. [30] inoculated young adult mice by the s.c. route with a New York (2000) isolate (NY99 genotype) and for 16 months examined their tissues for infectious virus and WNV RNA. Infectious WNV persisted for 1 month in all mice and was found in 12% of mice at 4 months; WNV RNA persisted for up to 6 months in 12% of mice that had subclinical infections. The frequency of persistence was tissue dependent and found in the skin, spinal cord, brain, lymphoid tissues, kidneys, and heart, but the persistence less frequently in the kidney tissues [30, 31]. In summary, these two models share some similarity and discrepancy in symptoms and tissue tropism with the clinical findings in some WNV convalescent patients having long-term morbidity (either chronic kidney diseases or long-term neurological sequelae).

4. *Cutaneous infection model*: WNV infection in mice by i.d. challenge partially mimics natural infection in humans, though it is much less complicated due to a lack of concurrent exposure to components of mosquito saliva [32, 33]. One earlier study [34] revealed that the mean and median doses of WNV inoculated by *Culex tarsalis* mosquitoes as they probe and feed on peripheral tissues of a mouse were $10^{4.3}$ PFU and $10^5$ PFU. The same doses of WNV by i.d. infection in mice results in survival rates of 25–40 %. Cutaneous infection shares a disease outcome that is similar to those of systemic infection. However, its initial virus dissemination in mice differs from the latter. Following i.d. WNV challenge, the virus is first deposited in the skin cells and is further disseminated to lymph nodes and other peripheral tissues by the migration of the epidermal dendritic cells-Langerhans cells (LCs) to initiate systemic infection [35, 36]. TLR7 innate signaling contributes to host defense mechanisms, which leads to reduced viremia and lethality when WNV infection of mice was initiated by i.p. injection [37]. Nevertheless, the TLR7-induced immune response in mouse skin following cutaneous inoculation plays a role in viral pathogenesis by promoting WNV dissemination from the skin to initiate systemic infection. In particular, WNV infection in epidermal keratinocytes triggers a TLR7-dependent production of IL-1$\beta$, IL-6, and IL-12, which promotes LC migration from the skin to other peripheral organs. This process compromises the protective effects of TLR7 signaling during the systemic infection stage.
5. *Direct CNS infection*: It is often used to study the neurovirulence of WNV. The $LD_{50}$ of wild-type WNV NY99 following i.c. inoculation is usually lower than that of systemic infection. Wild-type WNV induces neuroinvasive disease within 2 weeks following i.c. challenge [38]. WNV H8912, which is highly attenuated in neuroinvasiveness, induces no lethality following systemic infection even with a dose up to $10^6$ PFU. Compared to wild-type WNV, WNV H8912 also displayed a reduced neurovirulence in mice by i.c. challenge. Mice inoculated with $10^4$ and $10^6$ PFU of WNV H8912 succumbed to infection; while 41 % of mice inoculated i.c. with $10^2$ PFU of WNV H8912 survived [20].

## Acknowledgements

This work was supported by NIH grants R01 AI072060 and R01 AI099123 (to T.W.).

## References


1. Anderson JF, Andreadis TG, Vossbrinck CR, Tirrell S, Wakem EM et al (1999) Isolation of West Nile virus from mosquitoes, crows, and a Cooper's Hawk in Connecticut. Science 286:2331–2333
2. Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M et al (1999) Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 286:2333–2337
3. Bakonyi T, Ferenczi E, Erdelyi K, Kutasi O, Csorgo T et al (2013) Explosive spread of a neuroinvasive lineage 2 West Nile virus in Central Europe, 2008/2009. Vet Microbiol 165:61–70
4. Frost MJ, Zhang J, Edmonds JH, Prow NA, Gu X et al (2012) Characterization of virulent West Nile virus Kunjin strain, Australia, 2011. Emerg Infect Dis 18:792–800
5. Carson PJ, Konewko P, Wold KS, Mariani P, Goli S et al (2006) Long-term clinical and neuropsychological outcomes of West Nile virus infection. Clin Infect Dis 43:723–730
6. Cook RL, Xu X, Yablonsky EJ, Sakata N, Tripp JH et al (2010) Demographic and clinical factors associated with persistent symptoms after West Nile virus infection. Am J Trop Med Hyg 83:1133–1136
7. Sadek JR, Pergam SA, Harrington JA, Echevarria LA, Davis LE et al (2010) Persistent neuropsychological impairment associated with West Nile virus infection. J Clin Exp Neuropsychol 32:81–87
8. Nolan MS, Podoll AS, Hause AM, Akers KM, Finkel KW et al (2012) Prevalence of chronic kidney disease and progression of disease over time among patients enrolled in the Houston West Nile virus cohort. PLoS One 7, e40374
9. Davis BS, Chang GJ, Cropp B, Roehrig JT, Martin DA et al (2001) West Nile virus recombinant DNA vaccine protects mouse and horse from virus challenge and expresses in vitro a noninfectious recombinant antigen that can be used in enzyme-linked immunosorbent assays. J Virol 75:4040–4047
10. Kramer LD, Bernard KA (2001) West Nile virus infection in birds and mammals. Ann N Y Acad Sci 951:84–93
11. Ratterree MS, Gutierrez RA, Travassos da Rosa AP, Dille BJ, Beasley DW et al (2004) Experimental infection of rhesus macaques with West Nile virus: level and duration of viremia and kinetics of the antibody response after infection. J Infect Dis 189:669–676
12. Xiao SY, Guzman H, Zhang H, Travassos da Rosa AP, Tesh RB (2001) West Nile virus infection in the golden hamster (Mesocricetus auratus): a model for West Nile encephalitis. Emerg Infect Dis 7:714–721
13. Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ (2002) West Nile virus. Lancet Infect Dis 2:519–529
14. Solomon T, Ooi MH, Beasley DW, Mallewa M (2003) West Nile encephalitis. BMJ 326:865–869
15. Wang T, Fikrig E (2004) Immunity to West Nile virus. Curr Opin Immunol 16:519–523
16. Brien JD, Uhrlaub JL, Hirsch A, Wiley CA, Nikolich-Zugich J (2009) Key role of T cell defects in age-related vulnerability to West Nile virus. J Exp Med 206:2735–2745
17. Welte T, Lamb J, Anderson JF, Born WK, O'Brien RL et al (2008) Role of two distinct gammadelta T cell subsets during West Nile virus infection. FEMS Immunol Med Microbiol 53:275–283
18. Penn RG, Guarner J, Sejvar JJ, Hartman H, McComb RD et al (2006) Persistent neuroinvasive West Nile virus infection in an immunocompromised patient. Clin Infect Dis 42: 680–683
19. Murray K, Walker C, Herrington E, Lewis JA, McCormick J et al (2010) Persistent infection with West Nile virus years after initial infection. J Infect Dis 201:2–4
20. Saxena V, Xie G, Li B, Farris T, Welte T et al (2013) A hamster-derived west nile virus isolate induces persistent renal infection in mice. PLoS Negl Trop Dis 7, e2275
21. Johnston LJ, Halliday GM, King NJ (1996) Phenotypic changes in Langerhans' cells after infection with arboviruses: a role in the immune response to epidermally acquired viral infection? J Virol 70:4761–4766
22. Welte T, Reagan K, Fang H, Machain-Williams C, Zheng X et al (2009) Toll-like receptor 7-induced immune response to cutaneous West Nile virus infection. J Gen Virol 90: 2660–2668
23. Wu X, Lu L, Guzman H, Tesh RB, Xiao SY (2008) Persistent infection and associated nucleotide changes of West Nile virus serially passaged in hamsters. J Gen Virol 89: 3073–3079
24. Wang T, Anderson JF, Magnarelli LA, Wong SJ, Koski RA et al (2001) Immunization of mice against West Nile virus with recombinant envelope protein. J Immunol 167:5273–5277

25. Diamond MS, Sitati EM, Friend LD, Higgs S, Shrestha B et al (2003) A critical role for induced IgM in the protection against West Nile virus infection. J Exp Med 198: 1853–1862
26. Arjona A, Foellmer HG, Town T, Leng L, McDonald C et al (2007) Abrogation of macrophage migration inhibitory factor decreases West Nile virus lethality by limiting viral neuroinvasion. J Clin Invest 117:3059–3066
27. Wang T, Town T, Alexopoulou L, Anderson JF, Fikrig E et al (2004) Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. Nat Med 10:1366–1373
28. Beasley DW, Li L, Suderman MT, Barrett AD (2002) Mouse neuroinvasive phenotype of West Nile virus strains varies depending upon virus genotype. Virology 296:17–23
29. Zhang J, Wang J, Pang L, Xie G, Welte T et al (2014) The co-stimulatory effects of MyD88-dependent Toll-like receptor signaling on activation of murine gammadelta T cells. PLoS One 9, e108156
30. Appler KK, Brown AN, Stewart BS, Behr MJ, Demarest VL et al (2010) Persistence of West Nile virus in the central nervous system and periphery of mice. PLoS One 5, e10649
31. Stewart BS, Demarest VL, Wong SJ, Green S, Bernard KA (2011) Persistence of virus-specific immune responses in the central nervous system of mice after West Nile virus infection. BMC Immunol 12:6
32. Skallova A, Iezzi G, Ampenberger F, Kopf M, Kopecky J (2008) Tick saliva inhibits dendritic cell migration, maturation, and function while promoting development of Th2 responses. J Immunol 180:6186–6192
33. Schneider BS, Soong L, Girard YA, Campbell G, Mason P et al (2006) Potentiation of West Nile encephalitis by mosquito feeding. Viral Immunol 19:74–82
34. Styer LM, Kent KA, Albright RG, Bennett CJ, Kramer LD et al (2007) Mosquitoes inoculate high doses of West Nile virus as they probe and feed on live hosts. PLoS Pathog 3:1262–1270
35. Byrne SN, Halliday GM, Johnston LJ, King NJ (2001) Interleukin-1beta but not tumor necrosis factor is involved in West Nile virus-induced Langerhans cell migration from the skin in C57BL/6 mice. J Invest Dermatol 117:702–709
36. Johnston LJ, Halliday GM, King NJ (2000) Langerhans cells migrate to local lymph nodes following cutaneous infection with an arbovirus. J Invest Dermatol 114:560–568
37. Town T, Bai F, Wang T, Kaplan AT, Qian F et al (2009) Toll-like receptor 7 mitigates lethal West Nile encephalitis via interleukin 23-dependent immune cell infiltration and homing. Immunity 30:242–253
38. Wang S, Welte T, McGargill M, Town T, Thompson J et al (2008) Drak2 contributes to West Nile virus entry into the brain and lethal encephalitis. J Immunol 181:2084–2091