REVIEW

West Nile Virus Infection in the Central Nervous System [version 1; peer review: 3 approved]

Evandro R. Winkelmann¹, Huanle Luo¹, Tian Wang¹,²

¹Department of Microbiology & Immunology, University of Texas Medical Branch, Galveston, Texas, 77555, USA
²Department of Pathology, University of Texas Medical Branch, Galveston, Texas, 77555, USA

Abstract

West Nile virus (WNV), a neurotropic single-stranded flavivirus has been the leading cause of arboviral encephalitis worldwide. Up to 50% of WNV convalescent patients in the United States were reported to have long-term neurological sequelae. Neither antiviral drugs nor vaccines are available for humans. Animal models have been used to investigate WNV pathogenesis and host immune response in humans. In this review, we will discuss recent findings from studies in animal models of WNV infection, and provide new insights on WNV pathogenesis and WNV-induced host immunity in the central nervous system.

Keywords

West Nile Virus , west nile virus infection in central nervous system , central nervous system infection , flavivirus , arboviral encephalitis

Open Peer Review

Reviewer Status ✓ ✓ ✓

Invited Reviewers

1. Sebastian Ulbert, Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany
2. Giorgio Palù, University of Padova, Padova, Italy
3. Anna Papa, Aristotle University of Thessaloniki, Thessaloniki, Greece

Any comments on the article can be found at the end of the article.
Corresponding author: Tian Wang (ti1wang@utmb.edu)
Competing interests: The authors declare that they have no competing interests.
Grant information: This work was supported in part by National Institutes of Health grant R01 AI099123 (TW). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Copyright: © 2016 Winkelmann ER et al. This is an open access article distributed under the terms of the Creative Commons Attribution Licence, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
How to cite this article: Winkelmann ER, Luo H and Wang T. West Nile Virus Infection in the Central Nervous System [version 1; peer review: 3 approved] F1000Research 2016, 5(F1000 Faculty Rev):105 (https://doi.org/10.12688/f1000research.7404.1)
First published: 26 Jan 2016, 5(F1000 Faculty Rev):105 (https://doi.org/10.12688/f1000research.7404.1)
Introduction

West Nile virus (WNV), a mosquito-borne, single-stranded, positive-sense flavivirus, has been the leading cause of viral encephalitis in the US for more than a decade. The virus was originally isolated from Uganda in 1937, later caused epidemic outbreaks in Asia, Europe, and Australia, and was introduced into the US in 1999. Human infection results mostly from mosquito bites, blood transfusion, organ transplantation, or occupational exposure. In addition, WNV transmission through breastfeeding and in utero exposure is possible, although recent evidence suggests that the risk is very low. About 80% of human infections with WNV are asymptomatic. The features of acute illness range from West Nile fever, to neuroinvasive conditions, including meningitis, encephalitis, acute flaccid paralysis, and death. Up to 50% of convalescent patients with WNV have been reported to have long-term neurological sequelae or develop chronic kidney diseases, or both. Although serologic and organ screening may reduce the risk of WNV infection through blood transfusion and organ transplantation, there is no specific therapeutic agent for treatment of WNV infection, and an approved vaccine is not currently available for humans.

Animal models, which recapitulate WNV-induced neurological diseases in humans, have been effective in vivo experimental models to investigate WNV pathogenesis and host immune response. In this review, we discuss recent findings from studies in animal models of WNV infection and provide new insights on WNV pathogenesis and virus-induced host immunity in the central nervous system (CNS).

West Nile virus entry into the central nervous system

The natural transmission of WNV in humans occurs through mosquito bites. Keratinocytes and Langerhans cells (LCs) are the initial target cells where the virus is naturally deposited. WNV infection in keratinocytes induces innate cytokine responses mediated by Toll-like receptor (TLR) 7, which further promotes LC migration from the epidermis and accumulation in the local draining lymph nodes, where the virus is amplified before dissemination to kidney, spleen, and other visceral organs. Following a systemic infection, WNV crosses the blood-brain barrier (BBB) after a brief viremia and ultimately invades the CNS.

The development of WNV encephalitis is correlated with the ability of the virus to gain access to the CNS (neuroinvasiveness). At present, the mechanisms by which WNV enters the brain are not well understood. As a higher viral burden in serum usually correlates with earlier viral entry into the brain, it has been suggested that WNV infects the CNS in part via hematogenous spread. The BBB is a complex structure that is composed of the tight endothelium formed by endothelial cells through tight junctions and smooth muscle cells surrounded by a layer of astrocytic foot processes. Systemic WNV replication-induced innate cytokine responses are known to control BBB integrity (Table 1). Pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and macrophage migration inhibitory factor (MIF), contribute to the disruption of the BBB. In addition, matrix metalloproteinase 9 (MMP9), which is upregulated upon WNV infection in both the periphery and mouse brain, facilitates WNV entry into the brain by enhancing BBB permeability. Semaphorin 7A (Sema7A), a potent stimulator of monocytes and neutrophils, acts upstream of the host inflammatory reaction during WNV infection. Following infection, Sema7A-deficient mice produced less TNF-α in the periphery and had a reduced BBB permeability compared with wild-type controls. In contrast to the effects of pro-inflammatory cytokines, both type I interferon (IFN-α and IFN-β) and type III (IFN-λ) are implicated in promoting BBB integrity. Daniels et al. have recently demonstrated that type I IFNs play a direct role in endothelial permeability and tight junction formation via balanced activation of the small guanosine triphosphatases (GTPases) Rac1 and RhoA interactions and indirect suppression of the effects of TNF-α and IL-1β. The TAM receptors Tyro3, Axl, and MerTK are receptor tyrosine kinases that dampen host innate immune responses upon interactions with their ligands.

<table>
<thead>
<tr>
<th>Host factors</th>
<th>Effects on BBB integrity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro-inflammatory cytokines, including TNF-α, IL-1β, and MIF</td>
<td>Disruption of BBB integrated</td>
<td>32–34</td>
</tr>
<tr>
<td>Matrix metalloproteinase 9</td>
<td>Enhancing BBB permeability</td>
<td>35</td>
</tr>
<tr>
<td>Semaphorin 7A</td>
<td>Enhancing BBB permeability by promoting TNF-α production</td>
<td>36</td>
</tr>
<tr>
<td>Type I IFNs (IFN-α and IFN-β)</td>
<td>Promoting BBB integrity via balanced activation of the small guanosine triphosphatase Rac1 and RhoA interactions and indirect suppression of the effects of TNF-α and IL-1β</td>
<td>33</td>
</tr>
<tr>
<td>TAM receptors Tyro3, Axl, and MerTK</td>
<td>Promote BBB integrity by synergizing type I IFNs</td>
<td>38</td>
</tr>
<tr>
<td>IFN-λ</td>
<td>Modulates tight junction protein localization in brain microvascular endothelial cells, which increases transendothelial electrical resistance and decreases virus movement across the BBB</td>
<td>39</td>
</tr>
<tr>
<td>CAMs, such as ICAM-1, VCAM-1, and E-selectin</td>
<td>Upregulation of CAM expression induces the adhesion and transendothelial migration of leukocytes across these cells</td>
<td>40, 41</td>
</tr>
</tbody>
</table>

BBB, blood-brain barrier; CAM, Cell adhesion molecules; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IL-1β, interleukin-1 beta; MIF, macrophage migration inhibitory factor; TNF-α, tumor necrosis factor-alpha; VCAM-1, vascular cell adhesion molecule-1.
Gas6 and Protein S, which recognize phosphatidylserine on apoptotic cells\(^7\). A recent study showed that activation of Merk synergized with IFN-β to tighten cell junctions and prevent virus transit across brain microvascular endothelial (BMVE) cells. As a consequence, mice lacking Merk or Axl (or both), but not Tyro3, exhibited greater vulnerability to infection with neuroinvasive WNV\(^6\). In another study\(^6\), mice lacking IFN-λ signaling were shown to have increased viral titers in the brain and spinal cord during WNV infection. This is not associated with a direct antiviral effect of IFN-λ in the CNS. Instead, IFN-λ signaling in BMVE cells modulates tight junction protein localization in a protein synthesis- and signal transducer and activator of transcription 1 (STAT1)-independent manner, which increases transendothelial electrical resistance and decreases virus movement across the BBB. Besides innate cytokines, upregulation of cell adhesion molecules (CAMs), such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and E-selectin expression on WNV-infected BMVEs induces the adhesion and transendothelial migration of leukocytes across these cells\(^4,11\) (Table 1). Moreover, direct infection of BMVE cells facilitates the entry of cell-free virus into the CNS without disturbing the BBB, and an increase on CAM further assists the trafficking of WNV-infected immune cells into the CNS via a “Trojan horse” mechanism, thereby contributing to WNV dissemination into the CNS and its associated pathology\(^3,4,11\). Finally, infection of the olfactory neurons and consequent dissemination to the olfactory bulb and direct axonal retrograde transport of virus that has infected peripheral neurons have been postulated as the other mechanisms of WNV entry into the CNS\(^5,6,11\).

The host has developed multiple strategies to limit virus dissemination in the periphery and prevent the trafficking of WNV across the BBB. First, WNV infection activates the signaling pathways of several pattern recognition receptors (PRRs), including TLRs 3 and 7 and RIG-I-like receptor (RLR), in order to boost innate immunity, culminating in the synthesis of antiviral cytokines, such as type 1 IFNs and pro-inflammatory cytokines\(^4,48-50\). Type I IFNs and IFN-stimulating genes (ISGs) both participate in the control of viral infections and prevent WNV from invading the CNS\(^3,4,11\). Next, innate immune cells such as γδ T cells (in particular, the Vγ1+ cell subpopulation) expand significantly during WNV infection and play a role in the early control of WNV dissemination mainly through the secretion of IFN-γ\(^3,5,6,11\). These cells are also involved in the regulation of adaptive immunity against WNV infection and persistence, presumably mediated by the crosstalk between γδ T cells and dendritic cells (DCs), either via direct contact or indirectly through secreting molecules, to promote DC maturation and activation and ultimately to help T cell priming during WNV infection\(^9\). TCRαβ- mice (γδ T cell-deficient mice) were shown to have a numeric and functional reduction in memory T cell responses to WNV infection compared with wild-type mice\(^9\). Furthermore, B cells and specific antibodies are critical in the control of disseminated WNV infection but are not sufficient to eliminate it from the host\(^9,40-42\). In particular, induction of a specific, neutralizing IgM response early during infection limits viremia and dissemination into the CNS and protects the host against lethal infection\(^9\). In addition, the complement system has been shown to control WNV infection, in part through its ability to induce a protective antibody response and by priming adaptive immune responses through distinct mechanisms\(^43,44\). αβ T cells\(^41\) provide long-lasting protective immunity and contribute to host survival following WNV infection. Among them, CD4+ T cells respond vigorously in the periphery\(^45\), whereas CD8+ T cell responses have been observed in both the spleen and brain following WNV infection\(^9\). CD4+ T cells provide help for antibody responses and sustain WNV-specific CD8+ T cell responses in the tissues that enable viral clearance\(^46\). CD8+ T cell responses are critical in clearing WNV infection from tissues and preventing viral persistence\(^9\). Furthermore, higher levels of peripheral regulatory T cells (Tregs) after infection are known to protect against severe WNV disease in immunocompetent animals and humans\(^47\). Tregs are also critical in generating a pool of WNV-specific memory T cells in the periphery\(^9\), RLR-mediated innate signaling is involved in regulating adaptive immunity against pathogenic WNV. Mice deficient in mitochondrial antiviral signaling protein (MAVS), the adaptor protein for RLR signaling, were reported to exhibit lower neutralization ability of WNV-specific antibodies and increased numbers of virus-specific CD8+ T cells, with non-specific immune cell proliferation in the periphery\(^9\).

Aging is a known risk factor for WNV-induced encephalitis\(^3,12,73\). The decline in immunity seen in the elderly is a significant contributor to the increased risk of infection. In an aged mouse model of WNV infection, Vγ1+ cells displayed a slower, reduced response to WNV infection compared with those of young adult mice\(^7\). Although the young adult CD4+ or CD8+ T cells readily protected immunodeficient mice upon WNV infection, T cells of either subset in aged mice were unable to provide WNV-specific protection\(^5\). Furthermore, a recent study\(^5\) reported that age-dependent cell-intrinsic and environmental defects in the draining lymph nodes result in delayed immune cell recruitment and antigen recognition by CD4+ T cells, leading to impaired IgM and IgG and increased susceptibility to WNV infection in old mice.

**West Nile virus infection and West Nile virus-induced immune responses in the central nervous system**

Once in the brain, WNV can infect and replicate in various types of CNS residential cells, including neurons, astrocytes, and microglial cells\(^76,77\). In a recent study, by using a spinal cord slice culture (SCSC) model, CNS-resident cells were demonstrated to have the capacity to initiate a robust innate immune response against WNV infection in the absence of infiltrating inflammatory cells and systemic immune responses\(^78\). CNS cells display differential susceptibility to WNV infection, dependent on their cell-intrinsic host defense programs and cellular activities (Table 2). The antiviral action of ISG-Ihh2 restricts WNV spread in the CNS, especially during the early stages of virus spread\(^9\). Granule cell neurons (GCNs) of the cerebellum, which comprise the largest population of neurons in the brain, express high levels of genes associated with the host defense pathway, including a STAT1- and IFN-dependent signaling signature both at the basal level and after IFN-β treatment. These cells are less susceptible than cortical neurons to WNV replication\(^9\). Multiple PRR pathways are involved in the induction of antiviral responses in neurons. IL-1β production triggered by non-obese diabetic (NOD)-like receptor family pyrin domain containing 3 (NLPR3)-inflammasome suppressed WNV replication in neurons\(^80\). Both myeloid differentiation primary response gene 88 (MyD88)- and TLR3-mediated innate signaling play protective
Under normal physiological conditions, the integrity of the BBB restricts the infiltration of leukocytes and maintains the CNS as an immune-specialized compartment. Following systemic WNV infection, immune responses in the CNS are induced by infiltrating inflammatory cells, including microglia/macrophages, neutrophils, and effector CD4+ and CD8+ T cells (Table 2), as well as further infiltration of avirulent WNV-MAD78 infection. However, inhibition of Ly6C+ monocyte trafficking into the brain by anti-very late antigen-4 (anti-VLA-4) integrin antibody blockade at the time of observation of the first weight loss and leukocyte influx resulted in long-term survival in mice infected with a neurotropic virus.

Table 2. Central nervous system cell types and West Nile virus-induced immune responses.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Immune induction or cellular activities, or both</th>
<th>Effects on host or cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurons</td>
<td>WNV infection triggers IL-1β, IFNs, IFN-stimulating genes, or chemokine production mediated via multiple pathogen recognition receptors, including non-obese diabetic-like receptor family pyrin domain containing 3 (NLRP3)-inflammasome, TLR3, myeloid differentiation primary response gene 88 (MyD88), and Caspase-12-dependent-RIG-I</td>
<td>Restrict WNV infection in the CNS or facilitate immune cells to cross blood-brain barrier and enter the CNS</td>
</tr>
<tr>
<td>Microglia</td>
<td>WNV infection triggers TLR3-mediated TNF-α, IL-6, and IFN-β responses</td>
<td>Restrict WNV infection in the CNS</td>
</tr>
<tr>
<td>Astrocytes</td>
<td>Inhibit high levels of furin-like protease activity</td>
<td>Restrict avirulent WNV infection in the CNS</td>
</tr>
<tr>
<td>Monocyte/Macrophage</td>
<td>Migration into the CNS via CCL2-, CCL7-, TLR7-, or IL-23-dependent pathways</td>
<td>Protective or detrimental?</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Recruitment into the CNS mediated by IL-22 and CXCR2</td>
<td>Control of viral infection at the late stage of infection</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>Recruitment into the CNS, producing IFN-γ and IL-2, and regulation by IL-1R-dependent CD11c+ cell response</td>
<td>Directly involved in viral clearance, or by sustaining WNV-specific CD8+ T-cell response in the CNS, or both</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>Recruitment into the CNS; producing IFN-γ, or direct killing of infected cells through secretion of perforin and Fas/Fas ligand, or TNF-related apoptosis-inducing ligand (TRAIL)-dependent pathways; dependent on RIG-I-like receptor (RLR) signaling, and mediated by CXCR4 and IL-1β</td>
<td>Control viral replication upon low-dose WNV challenge and induction of immunopathology in the CNS upon high-dose WNV infection</td>
</tr>
</tbody>
</table>

CNS, central nervous system; IFN, interferon; IL, interleukin; TLR, Toll-like receptor; TNF-α, tumor necrosis factor-alpha; WNV, West Nile virus.
cerebrospinal fluid from human cases of WNV meningitis and encephalitis showed high counts of neutrophils\textsuperscript{5,6}. Neutrophils were reported to display dual functions in WNV-infected mice. They could serve as reservoirs for virus replication in the periphery at early stage of infection, but are involved in viral clearance at the late stage of infection in the CNS\textsuperscript{11}. IL-22, a cytokine implicated in the modulation of the chemokine receptor CXCR2, is responsible for the recruitment of neutrophils\textsuperscript{12}. Finally, CD4\textsuperscript{+} T cells are required for the clearance of WNV from CNS and therefore mice survival, providing help for antibody responses, and sustaining WNV-specific CD8\textsuperscript{+} T cell responses in the CNS which enable viral clearance\textsuperscript{57}. They also have a direct antiviral role in the CNS by producing IFN-\gamma and IL-2 and have a potential for\textit{ in vivo} and\textit{ ex vivo} cytotoxicity\textsuperscript{10}. It has been demonstrated that IL-1R1 signaling modulates CD11c\textsuperscript{+} cell-mediated T cell reactivation and promotes virologic control during WNV infection within the CNS\textsuperscript{48,51,101}. CD8\textsuperscript{+} T cells have an important function in clearing infection from the CNS and preventing viral persistence upon a low-dose WNV challenge\textsuperscript{102,103,51}. Nevertheless, CD8\textsuperscript{+} T cells are also involved in promoting CNS pathology following high doses of WNV infection\textsuperscript{104,105}. Thus, depending on the viral dosage, CD8\textsuperscript{+} T cells could be involved in both recovery and immunopathology in WNV infection. The effector function of T cells to control virus replication and eliminate the infection is directed through the production of cytokines, including IFN-\gamma, or direct killing of infected cells through secretion of perforin and signaling through Fas to-Fas ligand, or TNF-related apoptosis-inducing ligand (TRAIL)-dependent pathways\textsuperscript{106}. RLR-mediated innate signaling was reported to shape optimal CD8\textsuperscript{+} T cell activation and subsequent clearance of WNV from the CNS\textsuperscript{10}. CD11b+CD45\textsuperscript{hi} infiltrating cells (macrophages) are the primary producers of IL-1\beta within the CNS. By using an\textit{ in vitro} BBB model, Durrant et al. have shown that IL-1\beta promotes CXCR4-mediated CD8\textsuperscript{+} T lymphocyte adhesion to BMVE cells. Furthermore, inhibition of CXCR4 promotes T lymphocyte entry into the CNS parenchyma, and this increases viral clearance and ultimately improves survival and reduces viral loads\textsuperscript{107}. Thus, perivascular localization might hinder antiviral immune responses during WNV encephalitis.

**Conclusions**

In summary, following systemic WNV infection, immune responses in the periphery help to control virus dissemination, whereas CNS immunity is critical for the clearance of the virus. WNV has been the leading cause of viral encephalitis in the US for more than a decade. Current efforts on drug development have been focused mostly on the inhibitors of virus replication. Results from animal studies will provide a better understanding of the mechanisms by which flaviviruses enter the brain and induce lethal encephalitis; they may also lead to the development of new strategies to prevent and treat WNV-induced encephalitis.

**Competing interests**

The authors declare that they have no competing interests.

**Grant information**

This work was supported in part by National Institutes of Health grant R01 AI099123 (TW).

I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

---

**References**

3. Charatan F: Organ transplants and blood transfusions may transmit West Nile virus. BMJ. 2002; 325(7364): 566. Published Abstract | Publisher Full Text | Free Full Text


Open Peer Review

Current Peer Review Status:  

Editorial Note on the Review Process

F1000 Faculty Reviews are commissioned from members of the prestigious F1000 Faculty and are edited as a service to readers. In order to make these reviews as comprehensive and accessible as possible, the reviewers provide input before publication and only the final, revised version is published. The reviewers who approved the final version are listed with their names and affiliations but without their reports on earlier versions (any comments will already have been addressed in the published version).

The reviewers who approved this article are:

| Version 1 |
|---|---|
| 1 Anna Papa  
Department of Microbiology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece  

**Competing Interests:** No competing interests were disclosed. |
| 2 Giorgio Palù  
Department of Molecular Medicine, University of Padova, Padova, Italy  

**Competing Interests:** No competing interests were disclosed. |
| 3 Sebastian Ulbert  
Department of Immunology, Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany  

**Competing Interests:** No competing interests were disclosed. |

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com