XCL1 Expression in a Mouse Model of West Nile Virus Encephalitis

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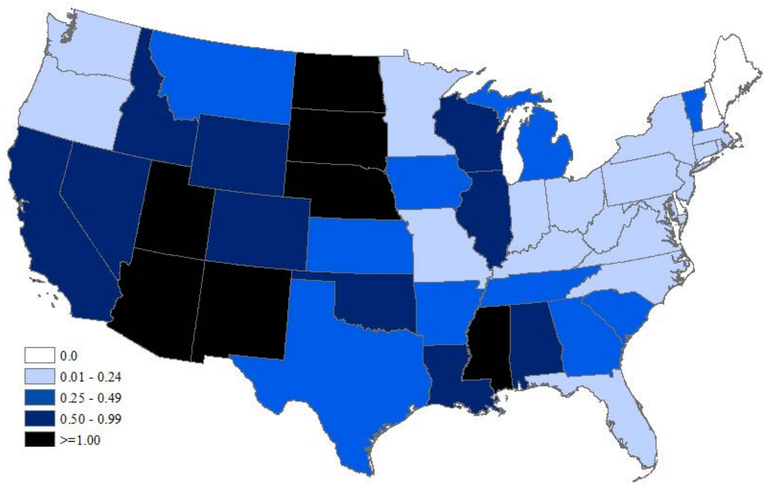
Abstract

West Nile virus (WNV) is a neurotropic flavivirus that can cause encephalitis and death. In order to limit viral infection and prevent neurological disease, it is crucial that antiviral CD8+ T cells be fully activated and recruited to the site of infection. Dendritic cells (DCs), specialized immune cells that initiate T cell activation, have been shown to be necessary for WNV clearance from the central nervous system (CNS). However, it is unknown how these cells regulate the antiviral immune response in the CNS. XCR1 is a chemokine receptor, that is exclusively expressed on a subset of DCs and is the sole receptor for XCL1, also known as lymphotactin. XCL1 is a potent and highly specific chemoattractant for this DC subset and is primarily secreted by CD8+ T cells. We hypothesize that following infection with WNV, antiviral CD8+ T cells express XCL1 in order to recruit DCs and ensure their own activation and proliferation. In order to examine the role of XCL1 during WNV infection, we utilized a mouse model of WNV encephalitis. In our model, mice were infected with WNV (i.e., Kunjin strain) and both peripheral (spleen, draining lymph nodes, and serum) as well as central nervous system tissues were collected at days 2, 4, 6, 8, and 10 post-infection. The expression of lymphotactin was shown to be increased in draining lymph nodes early in infection and was sustained in peripheral tissues throughout the infection. Within the CNS, lymphotactin expression increased and peaked at day 6 post-infection. However, immunohistochemical (IHC) staining of the CNS tissues failed to show any definitive detection of lymphotactin and the mice displayed no clinical signs of encephalitis based on body weight. These results suggest that either lymphotactin has no role or that our mouse model was not functioning correctly. Accordingly, RNA levels of our stock virus were measured and showed that our stock was no longer viable. Future directions for this project would be repeating with a viable culture of WNV.

Introduction

- West Nile virus is a mosquito-borne neurotropic flavivirus that emerged in North America and continues to spread



(CDC map of WNV Neuroinvasive Disease by State, 2017)

- WNV is capable of infecting the CNS potentially resulting in serious illness and/or death in infected individuals
- T cells are a major player in establishing antiviral immunity, specifically CD8+ T cells
- T cell activation and recruitment into the CNS is crucial to control viral infection of neurons and prevent WNV encephalitis
- Dendritic cells (DCs) are professional antigen presenting cells whose primary function is to activate T cells
- XCL1 is a chemotactic protein that is produced by T and NK cells during infectious and inflammatory responses
- XCR1, the receptor for XCL1, is predominantly expressed by a specific subset of DCs
- The XCL1-XCR1 axis plays an important role in DC-mediated CD8+ T cell immune responses
- The significance of our research is to clarify the role of XCL1 during WNV infection in the CNS
- This study will have clear implications for understanding WNV pathogenesis and potentially in the development of future therapies

Model

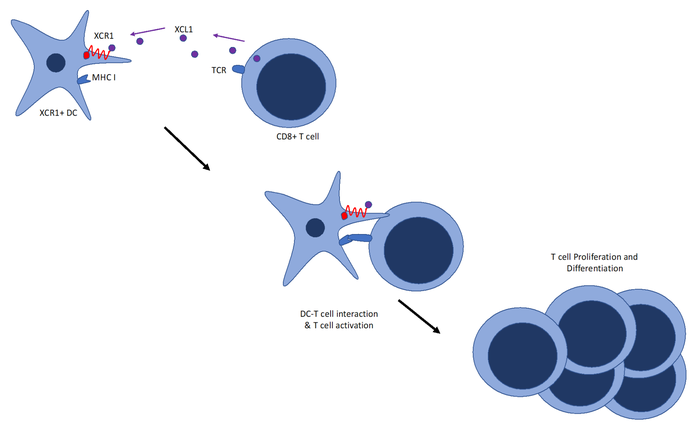


Figure 1: XCL1 attraction of XCR1-expressing DCs. XCL1 secreted by activated CD8+ T cells attracts XCR1-expressing DCs. The XCL1-XCR1-mediated chemoattraction facilitates antigen presentation from DCs to CD8+ T cells resulting in their activation and proliferation.

Materials and Methods

Infection and Tissue Collection:

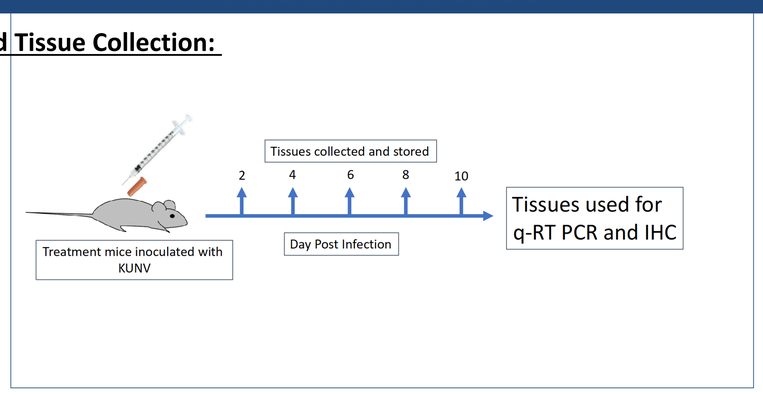


Figure 2: Mouse infection and tissue collection. WT mice were infected with 1,000 PFU of WNV (Kunjin Strain) via footpad injection. Mice were monitored daily for survival and disease severity. On days 2, 4, 6, 8, and 10 post-infection, peripheral and CNS tissue were isolated from WT mice. Peripheral and CNS tissues were homogenized and assayed for gene expression. Immunohistochemistry was performed on CNS tissues collected on day 8 and 10 post-infection.

Results

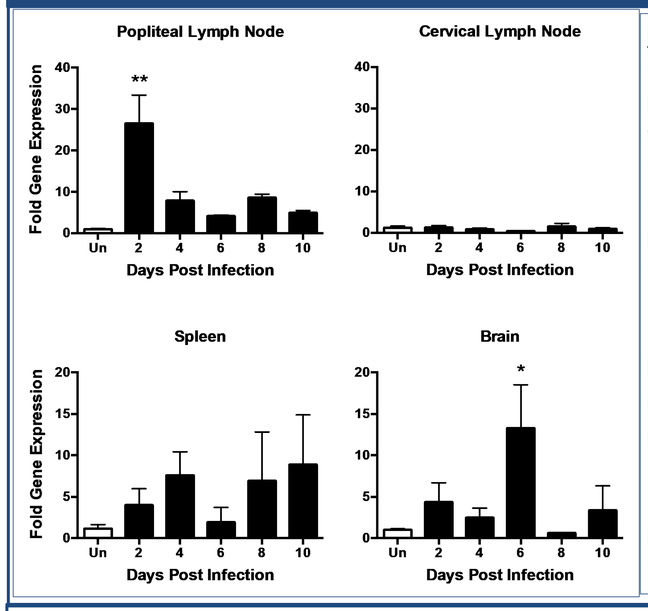


Figure 3: XCL1 levels during WNV encephalitis. 4-wk-old mice were inoculated with 10^3 PFU of WNV by footpad injection. Popliteal LNs, Cervical LNs, spleen, and brain tissues were collected at the indicated time points. mRNA levels of XCL1 were analyzed via qRT-PCR. Levels are shown as relative fold changes in gene expression compared to the housekeeping gene GAPDH. Data reflect the means from there to five mice per time point. Error bars are SEM. One-was ANOVA was used to determine statistical significance with respect to uninfected (Un) mice.
* $p < 0.05$

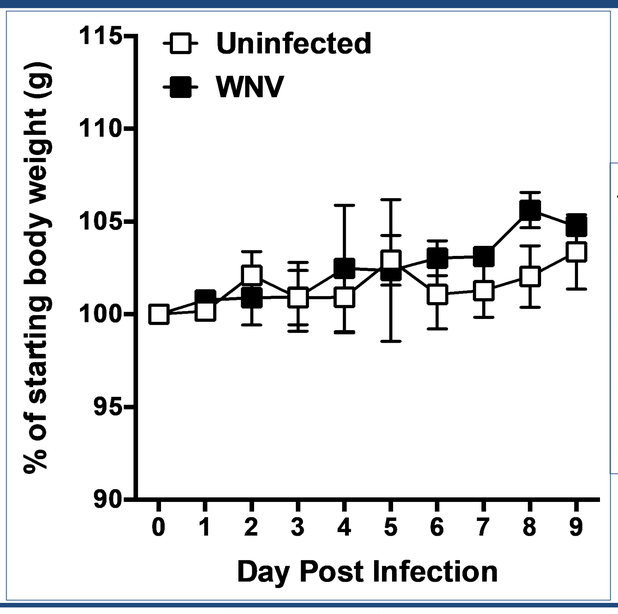


Figure 4: Weight change during WNV encephalitis. 4-wk-old mice were inoculated via footpad with WNV (10^3 PFU). Both uninfected and infected (WNV) mice were monitored daily for survival after infection as well as for changes in health (data not shown) and weight and were recorded on the indicated day post-infection.

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