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# Inflammation Unleashed in Viral-Induced Epileptogenesis

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## Abstract

Viral infection of the central nervous system increasingly places people at risk of developing life-threatening and treatment-resistant acute and chronic seizures (epilepsy). The emergence of new human viruses due to ongoing social, political, and ecological changes places people at risk more than ever before. The development of new preventative or curative strategies is critical to address this burden. However, our understanding of the complex relationship between viruses and the brain has been hindered by the lack of animal models that survive the initial infection and are amenable for long-term mechanistic, behavioral, and pharmacological studies in the process of viral-induced epileptogenesis. In this review, we focus on the Theiler's murine encephalomyelitis virus (TMEV) mouse model of viral infection–induced epilepsy. The TMEV model has a number of important advantages to address the quintessential processes underlying the development of epilepsy following a viral infection, as well as fuel new therapeutic development. In this review, we highlight the contributions of the TMEV model to our current understanding of the relationship between viral infection, inflammation, and seizures.

## Keywords

Theiler's murine encephalomyelitis virus, temporal lobe epilepsy, mouse model, inflammation, macrophages, microglia, astrocyte, NG2-glia

Epilepsy is a devastating neurological disorder characterized by unprovoked recurrent seizures, affecting more the 70 million people worldwide and can result in comorbidities and disabilities. Furthermore, 30% of people with epilepsy are refractory to the currently available medications.[1] While greater than 50% of the epilepsies have an underlying genetic abnormality, epilepsy can also arise from central nervous system (CNS) insults, such as traumatic brain injury, brain tumors, and CNS infections. These CNS traumas set in motion both acute inflammation and long-term changes which are not well understood and thus hinder the development of treatment approaches to prevent long-term epilepsy. Specifically, brain infections are a significant risk factor for the development of seizures and epilepsy.[2,3] Along with bacteria, parasites, and fungi, more than 100 different viruses are capable of causing encephalitis in humans, such as herpes simplex virus type-1 (HSV-1), non-polio picornavirus, Zika virus (ZIKV), West Nile virus (WNV), Japanese encephalitis virus (JEV), cytomegalovirus (CMV),[4] and human herpes virus-6 (HHV-6).[5] Recent reports suggest that SARS-CoV2,[6] the causative agent of COVID-19, can also cause viral encephalitis.[7] Viral encephalitis can result in acute seizures, which develop up to 2 weeks after infection, and epilepsy that can manifest months to years following the initial infection. For example, in congenital CMV infection, 7 out or 19 infants developed epilepsy,[4] and HHV-6 is also associated with seizures and epilepsy development.[5]

To identify new disease-modifying therapies to prevent epilepsy from developing in high-risk groups and/or treat seizures/epilepsy following viral encephalitis, we need to understand the mechanisms underlying seizure development after viral infection. Determining mechanisms often requires the use of pre-clinical animal models. Some groups have used HSV-1 and WNV to study seizures and epilepsy in rodent models; however, a limitation in these studies is the high mortality rate observed in infected animals during the acute phase of the neurotropic infection.[8,9] Mouse Zika infections also have a high mortality, and only subcutaneous Zika infection of P3 Swiss mice resulted in a survivable infection with behavioral and motor seizures in juvenile mice.[10,11] Perhaps the most successfully utilized viral infection model of epilepsy is the Theiler's murine encephalomyelitis virus (TMEV) in C57BL/6J mice. Mice infected with TMEV develop seizures shortly after infection that resolve by 8–10 days post-infection. The mice survive the infection, and, following a latent period, many of the mice go on to develop epilepsy. The present review will focus on the TMEV mouse model of infection-induced epilepsy, but will also note how findings related to this model are relevant to consequences of other CNS infections.

## TMEV Infection as a Mouse Model of Viral-Induced Acute Seizures and Epilepsy

TMEV is a non-enveloped, single-stranded RNA virus from the cardiovirus genus, picornavirus family. TMEV causes enteric infection in rodents via fecal-oral route transmission. While these infections are usually asymptomatic or mild, virus can spread to the CNS and cause encephalitis and/or encephalomyelitis. The use of TMEV in experimental models of myocarditis, demyelination, and epilepsy is viral and mouse-strain dependent (reviewed elsewhere[12-14]). In this review, we only focus on the TMEV DA strain in C57BL/6J mice.

In this model (Figure 1A), intracerebral (IC) infection of C57BL/6J mice with TMEV results in acute behavioral seizures between 3 and 8 days post-infection (dpi), followed by a latent period, where no seizures are observed. Around 14 dpi, the virus is cleared from the CNS, presumably due to activation of the adaptive immune response. 30–90 dpi, 50–70% of the mice that experienced acute seizures show a significant decrease in seizure threshold and develop epilepsy.[15,16] The initial viral titer for the infection determines the percentage of mice that develop acute seizures. Convulsive seizures can be induced by handling during the acute infection period or observed to occur spontaneously via video-electroencephalogram monitoring (Figure 1B). A modified Racine scale is used to score seizure severity, as the seizures are limbic in origin.[17] Handling induced seizures no longer occur after the acute infection period, but spontaneous seizures can develop in 50–70% of the mice that experienced seizures in the acute phase of the infection, reflecting the development of epilepsy.

TMEV has a tropism for the pyramidal neurons of the CA1 and CA2 regions, resulting in extensive neuronal loss in this region of the hippocampus. This mouse model recapitulates

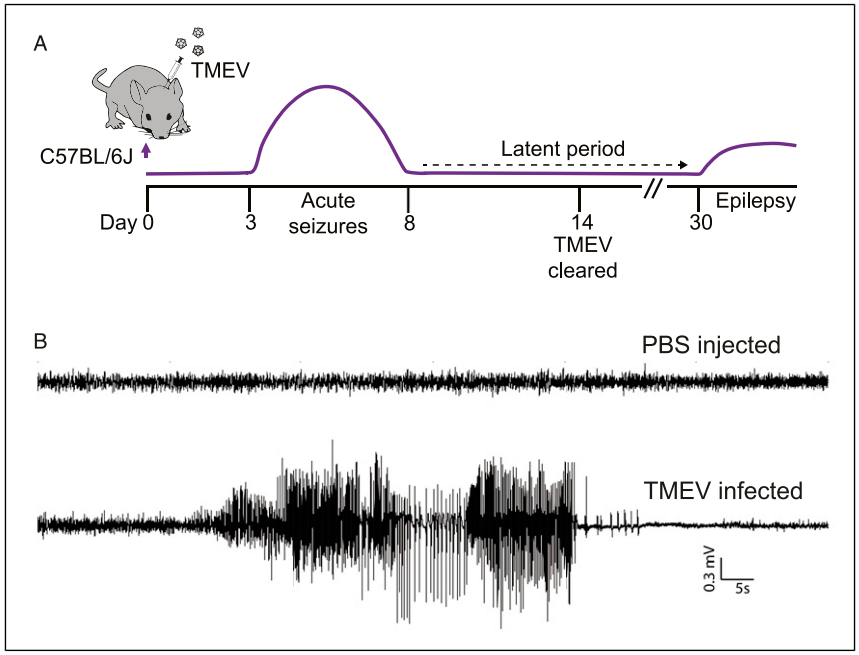


**Figure 1.** Mouse model of viral-induced epilepsy. (A) C57BL/6J mice are i.c. injected with Theiler's murine encephalomyelitis virus, a neurotropic virus member of the Picornaviridae family that can cause encephalitis in infected mice. Between 3 and 8 days post-infection (d.p.i.), mice develop acute seizures, followed by a latent period in which seizures are no longer observed. Between 30 and 100 d.p.i., some mice that experienced acute seizures develop spontaneous recurrent seizures (epilepsy). (B) Representative electroencephalogram from a phosphate-buffered saline-injected mouse, showing normal electroencephalogram recording, and Theiler's murine encephalomyelitis virus-injected mouse, 5 d.p.i., showing generalized convulsive seizures.

pathological and behavioral features observed in people with temporal lobe epilepsy (TLE),[18] including neuronal loss, astrogliosis, microgliosis, infiltration of peripheral macrophages, cognitive deficits, and anxiety-like behavior. Secretion of pro-inflammatory cytokines, especially by microglia and macrophages, amplify neuroinflammation and alter neuronal excitation leading to seizures during the acute infection period (Figure 2). Thus, this model offers an opportunity to understand how inflammation and infection lead to epilepsy. This review will focus on the acute phase of the infection, when seizures are quite prevalent.

## Innate Immune Response

Upon viral invasion of the CNS, activation of the innate and adaptive immune response is critical to control viral replication and spread. Following TMEV infection, the innate immune response is activated within hours, and both failure in controlling viral infection and persistent immune response activation can exacerbate CNS inflammation. When mice deficient in TNF-RI, TNF-α, or IL-6 are infected with TMEV, the incidence of seizures is significantly decreased.[17,19] In addition, increased levels of the pro-inflammatory cytokines IL-6 and TNF-α are also observed in the serum and in the brains of TMEV-infected mice and in the serum of TLE patients. Therefore, these 2 cytokines may play an integral role in contributing to seizure activity.

The 2 primary innate immune cells that participate in the immune response to TMEV infection of the CNS are microglia and macrophages. However, other glial cells such as astrocytes[20] and NG2 cells[21,22] can also influence the innate response. Looking at microglia and macrophages in TMEV-infected chimeric mice, Cusick et al.[23] demonstrated that while IL-6 is mainly produced by infiltrating macrophages, microglia produce high levels of TNF-α during the acute phase of the infection. Interestingly, inhibition of TNF-α during the acute phase of the ZIKV infection prevented hyperexcitation in mouse brains and decreased seizures,[10] thus elevated TNF-α is likely to be a common cytokine observed during encephalitis.

γ-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter, and decreased GABAergic inhibition in the CA3 region of the hippocampus is found in TMEV-infected mice during the acute infection period.[24] α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid type glutamate receptors

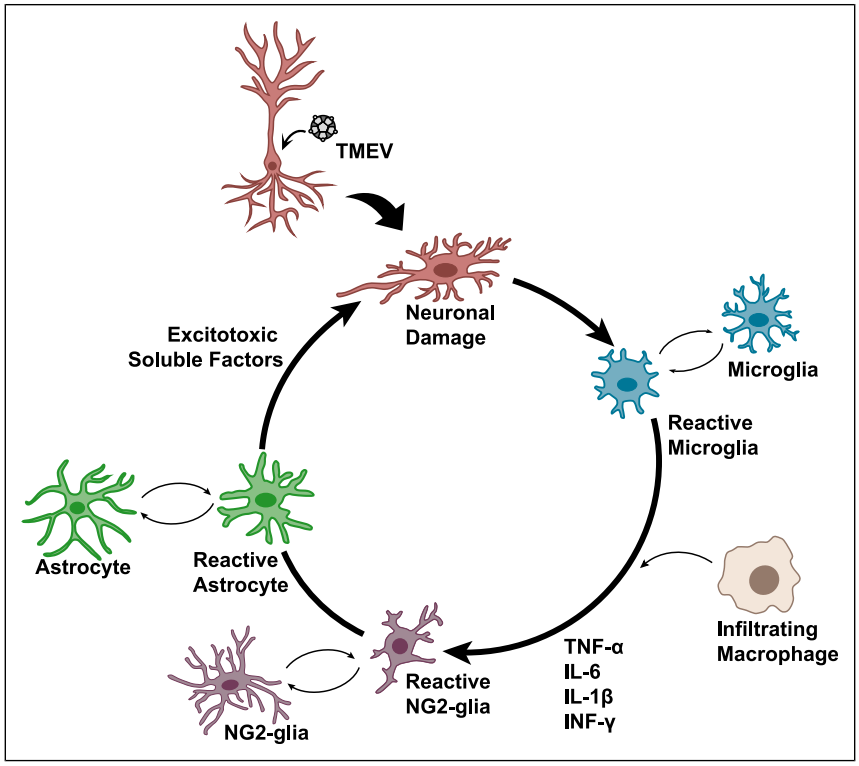


**Figure 2.** Neuroinflammation paradigm in the development of Theiler's murine encephalomyelitis virus-induced encephalitic seizures and epilepsy. In the Theiler's murine encephalomyelitis virus model of infection-induced epilepsy, Theiler's murine encephalomyelitis virus infects pyramidal neurons in the CA1 and CA2 region of the hippocampus. Viral replication causes large-scale neuronal death and damage. Microglia are the initial responders to infection in the brain and contribute to the infiltration of peripheral macrophages. Together, these immune cells release a combination of cytokine damage signals that initiate reactivity of other glial cells, such as NG2-glia, and astrocytes. Glial reactivity contributes to release or increased concentration of a variety of soluble factors that can cause overactivation of AMPA receptors on neurons, leading to increased neuronal damage, and sustained overproduction of cytokines. This cyclical pattern of neuron-glia-immune cell interactions initiates hyperexcitability and seizures in this model.

(AMPARs) are associated with excitatory neurotransmission, and CA3 neurons show increased excitation during the acute phase of TMEV infection. While evidence supports the idea that TNF-α increases AMPAR expression in the hippocampus of TMEV-infected mice,[17] IL-6 was shown to decrease cell surface expression of GABAR in a rat study.[25] Thus, the cytokines secreted during infection may contribute to enhanced excitation and decreased inhibition and lead to a more seizure prone state.

Peripheral macrophages are not found in the CNS parenchyma unless there is a CNS injury and the BBB is damaged.[26,27] While microglia are considered the "macrophages of the brain," these cells are transcriptionally and functionally distinct from peripheral macrophages.[28-30] Microglia and macrophages are heterogeneous and highly plastic cells, meaning they can rapidly change their phenotypic state based on microenvironment stimuli. Simplistically, these cells can acquire pro-inflammatory and anti-inflammatory phenotypes, characterized by the secretion of pro- and anti-inflammatory mediators, respectively. Activation of microglia and polarization toward an inflammatory state, together with infiltration of inflammatory macrophages into the CNS, are hallmarks of neuroinflammation. Their roles in viral encephalitis-induced epilepsy, along with that of NG2-glia and astrocytes, are discussed below.

## Microglia

In TMEV-infected mice, microglia acquire a reactive state and secrete pro-inflammatory cytokines that highly influence seizure development. Interestingly, seizure incidence and severity were not affected in TMEV-infected mice depleted of microglia; however, these mice could not control viral infection, developed paralysis, and had fatal encephalitis that was independent of the viral titer used in the initial infection, suggesting that microglia have a particular role in controlling viral infection and participating in the adaptive immune response.[31,32] TNF-α expression in the brain of TMEV-infected mice was not affected by microglia depletion, suggesting that CNS and/or infiltrating cells other than microglia are also secreting this cytokine.[32] Similarly, mice lacking microglia died during the acute phase of MHV infection.[33] Additional studies in animal models demonstrate that microglia exert an antiviral role during ZIKV, HSV, MHV, and VSV infections.[34] While microglia are key to resolution of infection, they also contribute to increased cytokine expression that can lead to increased hyperexcitability. Thus, therapies designed to reduce the impact of microglia cytokine production need to be carefully designed so as not to impair viral clearance.

## Macrophages

Macrophages are derived from monocytes originated from bone marrow hematopoietic stem cells. While they are primarily found in the periphery, CNS border-associated macrophages are found in the choroid plexus and meninges.[35-37] Under physiological conditions, the BBB limits the access of peripheral immune cells to the CNS; however, disruption of the BBB and secretion of chemokines allow these cells to migrate into the CNS, contributing to neuroinflammation.[38,39] The macrophage chemoattractant factor C-C motif ligand 2 (CCL2) is produced by several CNS cells such as microglia, neurons, astrocytes, and endothelial cells. CCL2 signals via the C-C motif chemokine receptor 2 (CCR2), expressed by circulating inflammatory monocytes, resulting in the recruitment of monocytes/macrophages into the brain. Notably, CCL2 is significantly upregulated in the brains of people with epilepsy,[40,41] and in the brains of animals with epilepsy[42,43]; similarly, a high number of infiltrating macrophages is found in the hippocampus of drug-resistant TLE patients and the hippocampus from patients who died after status epilepticus,[44] pointing to a substantive role for macrophages in neuroinflammation and seizure/epilepsy pathogenesis. Also, increased infiltration of peripheral macrophages into the brain is found during encephalitis in Simian immunodeficiency virus (SIV)-infected monkeys and WNV encephalitis.[45] Therefore, macrophage recruitment to the CNS appears to be a common outcome of CNS infection.

In the TMEV model, infiltration of macrophages from the periphery occurs as early as 3 days post-infection.[23,46] Once in the CNS, macrophages secrete high levels of IL-6[23] and seizure frequency is drastically reduced when macrophages are depleted from mice before TMEV infection.[46,47] These data support the role of macrophages in seizure generation. Similarly, reduced seizure incidence was observed by limiting CNS macrophage infiltration and inflammation followed by treatment with the anti-inflammatory drugs wagonin and minocycline.[23] Interestingly, knock out of CCR2, which inhibits macrophage migration to the CNS, although it did not prevent seizures from occurring in TMEV-infected mice, reduced seizure severity and inhibited hippocampal damage.[48] Comparing results from the depletion and migration studies may imply that (a) infiltration of macrophages into the CNS, alone, is not sufficient to drive seizures and (b) activation of macrophages and secretion of pro-inflammatory cytokines by these cells in the periphery could be sufficient to alter glial function, promote CNS inflammation, and decrease seizure threshold. However, these hypotheses remain to be tested. In addition, the mechanisms and signaling pathways activated in macrophages that contribute to its inflammatory phenotype and how it directly affects glial function and neuronal excitation is still unknown.

Using flow cytometry, microglia and macrophages can be discriminated by CD45 level of expression (microglia is $CD45^{low}Cd11b^{+}$, while macrophages as $CD45^{hi}CD11b^{+}$).[12,23,49] However, for a direct visualization method (e.g.,, IHC), distinct markers are required. While CCR2 can be utilized to label inflammatory infiltrating macrophages,[50,51] proteins found to be specifically expressed by microglia, such as P2RY12 and TMEM119, can be downregulated during inflammatory conditions,[52,53] and TMEM119 can also be expressed by macrophages.[52,54-56] Despite advancements in technology such as single-cell RNA sequencing, it remains difficult to differentiate between microglia and macrophages that have infiltrated the brain.[28,53,57-59]

### NG2

NG2-glia are a glial cell-type known to react to a variety of CNS infectious diseases. Although primarily known for their role as being oligodendrocyte precursor cells, adult animals maintain an abundant population of NG2-glia whose functions, aside from being progenitors of oligodendrocytes, remain largely unknown. NG2-glia are shown to react to CNS injury and infection, though participation within the inflammatory milieu can range from being the primary target of infection, such as during persistent infections causing chronic demyelinating disease such as TMEV infection of SJL mice[60,61] or Human herpes virus,[62,63] or as responders to infection and participants in the overall inflammatory responses and mechanisms of brain protection, such as in ZIKV infection[64] or TMEV infection of C57B/L6J mice in the viral infection–induced model of TLE.

Reactive NG2-glia are shown to undergo morphological and functional changes that may contribute to epileptogenesis following TMEV infection.[21] Recent studies indicate that NG2-glia are in part responsible for maintaining microglia homeostasis, and dysregulation of NG2-glia signaling to microglia during inflammation and disease heavily exacerbates the pro-inflammatory response,[22] a key factor driving seizure development. Additionally, NG2-glia are known to deposit the highly negatively charged NG2 protein, otherwise known as chondroitin sulfate proteoglycan 4 (CSPG4), which, when embedded in the dense extracellular matrix of the glial scar, likely contributes to long-term disruptions in intracellular and extracellular ion homeostasis.[65-67] Moreover, disruption in ion homeostasis can affect the transmembrane chloride gradient and the excitatory or inhibitory nature of GABAergic neurotransmission, which can, in turn, facilitate the development of seizure activity.[62,68,69] Because of their significant roles in contributing to network function and homeostasis, targeting physiological functions of NG2-glia following viral infection may be a way to reduce the sustained inflammatory changes that facilitate epileptogenesis. How and why NG2-glia react is important for considering how the nervous system protects us from pathogens and in considering how to prevent and protect brain structures that are sensitive to inflammatory damage and circuit rewiring that can lead to the development of epilepsy.

### Astrocytes

Since astrocytes play many critical roles in normal CNS function, alterations in these cells due to CNS infection can lead to serious neurological complications. Astrocytes support a functional BBB, regulate glutamate homeostasis, participate in innate and adaptive immune responses to viral infections, and express many pattern recognition molecules such as toll-like receptors, chemokines, and cytokines.[20] They can also be the target cell of many viruses such as JEV, WNV, and, HIV-1. Astrocyte reactivity to infection is linked to increased BBB permeability, peripheral immune cell infiltration, and CNS inflammation.[70] Also, glutamate excitotoxicity is associated with CNS viral infection. In TMEV-infected mice, persistent astrogliosis is observed and altered expression of ion channels and glutamate transporters may contribute to the development of long-term changes in network excitability that lead to chronic epilepsy.[71,72] However, whether reactive astrocytes have a causative role in the development of epilepsy remains uncertain. Interestingly, in a genetic mouse model of chronic astrogliosis, in which CNS inflammation and BBB dysfunction are not present, spontaneous seizures were observed,[73] suggesting astrocytes play a role in seizure development, but further studies are required.

## Conclusions and Future Directions

CNS infections are associated with seizures and epilepsy. Viral infections can alter brain homeostasis leading to glial activation, breakdown of the BBB, immune cell infiltration, and neuroinflammation resulting in neuronal excitation and seizure development. The use of animal models to study epilepsy has contributed extensively to the field of neuroscience, especially in regards to elucidating functional changes in neurons during epilepsy. The mouse model of TMEV-induced seizures/epilepsy has been critical in studying acquired epilepsy and elucidating roles for different brain resident and peripheral immune cells in both inflammation and seizure development. However, the mechanisms of how these cell types contribute to seizures/epilepsy are still not fully understood. Also, studies on microbiota-gut-brain axis suggest that the gut microbiota can also regulate CNS inflammation and function.[74-76] It is unknown whether and how changes in the microbiota can modulate seizures induced by TMEV infection. Further mechanistic investigation is therefore necessary and may set the stage for identifying novel disease-modifying targets.

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