

Current Literature

In Basic Science



Going Viral: Modeling Limbic Infection and Seizure Susceptibility

Theiler's Virus Infection Chronically Alters Seizure Susceptibility.

Stewart KA, Wilcox KS, Fujinami RS, White HS. *Epilepsia* 2010;51(8):1418–1428.

PURPOSE: Central nervous system infections greatly increase the risk for the development of seizures and epilepsy (recurrent unprovoked seizures). We have previously shown that Theiler's murine encephalomyelitis virus (Theiler's virus or TMEV) infection causes acute symptomatic seizures in C57BL/6 (B6) mice. The objective of the present study was threefold: (1) to assess pathologic changes associated with acute TMEV infection and infection-induced seizures, (2) to determine whether Theiler's virus infection and associated acute seizures lead to chronically altered seizure susceptibility, and (3) to determine whether genetic background influences seizure susceptibility following Theiler's virus infection. **METHODS:** Immunohistochemical techniques were used to assess Theiler's virus antigen localization in the brain and associated neuronal cell death. A battery of electroconvulsive threshold (ECT) tests and corneal kindling studies were conducted to assess whether there were chronic alterations in seizure susceptibility and kindling development. Studies were conducted in both B6 and SJL/J mice to assess strain-dependent effects. **RESULTS:** Histopathologic analyses indicate that TMEV has specific tropism for limbic structures and causes widespread cell death in these regions. Results from ECT studies demonstrate that B6 mice that displayed acute symptomatic seizures have significantly reduced seizure thresholds and kindle faster than either control mice or infected mice without acute seizures. Furthermore, these effects were mouse-strain dependent, since SJL/J mice displayed a different seizure threshold spectrum. **DISCUSSION:** These findings indicate that Theiler's virus infection leads to chronically altered seizure susceptibility in mice. It is important to note that Theiler's virus infection of B6 mice represents a novel model to study postinfection hyperexcitability.

Commentary

Patients seen in the epilepsy clinic include those who have developed epilepsy after an episode of encephalitis, most typically viral or bacterial in developed countries, or parasitic in developing countries. Regional or diffuse brain injury may be demonstrated by imaging studies and EEG findings or simply assumed by the clinician when diagnostic test results are not available. Seizure control is variable, largely related to the type and severity of the encephalitis incurred, and antiepileptic drug (AED) therapy is chosen based on seizure types and patient-specific factors. Compared with the increasing knowledge base of pathologic mechanisms in other forms of brain injury, for example, hypoxia/ischemia, traumatic brain injury, and stroke, and the mechanisms of postinjury epileptogenesis, there is currently a more modest understanding of how brain infection results in the subsequent development of epilepsy. The reason for this is fairly clear—the lack of models of postinfection epilepsy.

Viral encephalitis can be caused by a variety of viruses. Infection by herpes simplex virus, type-1 (HSV-1) is most commonly associated with seizures and has both acute and chronic phases of infection. A review of rodent and murine models of HSV-1 infection and associated seizure activity indicated several limitations of the models, including high mortality with high seizure severity, shedding of the virus, a human pathogen, by the animals, and no evidence of the development of epileptic seizures (1). Early studies using HSV-1 and adult New Zealand white rabbits resulted in a high mortality rate without evidence of a dose-response relationship, severe involvement of temporal cortex (2), and EEG abnormalities of the posterolateral cerebral hemispheres, which worsened with reactivation of the virus (3). Strains of HSV-1 that resulted in high mortality produced various types of seizure activity, including both partial and severe motor seizures; all of the animals that demonstrated seizures became moribund or died (4, 5). Later studies using HSV-1 and the Lewis rat also demonstrated acute partial and generalized seizures (6). In BALB/c mice, HSV-1 dosage was directly related to the severity of observed acute abnormalities, including interictal spikes, bursting activity, and behavioral seizures; no subsequent spontaneous seizures were observed in animals that survived longer than one month after infection (7). Given the limitations of these studies using

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HSV-1 as the infectious agent, there remains a pressing need for the development of new, safe, and improved viral models of postinfection seizures and epilepsy.

In a previous publication, the authors of the current study reported that intracerebral inoculation of C57BL/6 (B6) mice with the Daniel's (DA) strain of Theiler's murine encephalomyelitis virus (Theiler's virus, or TMEV), a picornavirus, resulted in transient afebrile seizures in approximately 50% of the animals (8). Peak seizure activity occurred between 5 to 7 days after infection at which time the animals were sacrificed to assess for seizure-related histologic and immunohistochemical findings. Their results of significant hippocampal pyramidal neuron pyknosis and increased transforming growth factor (TGF)- β protein expression—a biomarker of seizure activity—in the same cells were interpreted to indicate the occurrence of hippocampal seizures. Importantly, the DA strain of TMEV is cleared from the CNS within 1 month of infection.

Building on the results of that study using the same strain and inoculation titer of TMEV in B6 mice 2 months after infection, the authors quantified transcorneal electrical stimulation seizure thresholds and the rate of corneal kindling in animals that demonstrated seizure activity shortly after inoculation and compared the results with those animals in which seizure activity was not observed. Although the animals were visually monitored for only 2 h/day during days 1 to 15 postinfection, this appeared to be an adequate period of observation to identify and separate *seized* from *nonseized* animal groups for subsequent testing (the authors noted the importance of continuous video-EEG monitoring for a more comprehensive characterization of the provoked seizures in the B6 mice). They found that seized animals demonstrated decreased seizure thresholds and an increased rate of corneal kindling compared with nonseized animals. These results indicated a persistent, increased hyperexcitability in the brains of animals that experience convulsive seizures shortly following TMEV infection and established important first steps to characterize, explore, and advance the epileptogenicity of the system to the development of a potential model of postinfection epilepsy.

A significant aspect of the study is the preferential tropism and infectivity of the DA strain of TMEV in B6 mice to limbic structures. Viral antigens were localized bilaterally, predominantly in hippocampal CA1 and CA2 pyramidal cells, periventricular thalamic and septal nuclei, as well as piriform, entorhinal, and parietal cortices, and were associated with extensive cell death and degeneration in infected areas. Interestingly, the CA3 region and dentate gyrus were relatively spared of viral infection and cellular injury. This described pattern of regional infectivity and cellular injury includes many elements of HSV-1 encephalitis in humans and both compares and contrasts with several common, chronic anatomical findings associated with models of chemically- or electrically-induced status epilepticus and the subsequent development of epileptic seizures.

A provocative element of the report is an open question of whether the seized B6 mice might eventually demonstrate spontaneous seizures beyond the survival time of the study. As previously described, animals underwent testing for seizure

thresholds and corneal kindling 2 months after infection and, apparently, had no further dedicated behavioral monitoring prior to sacrifice. This begs the issue of whether the DA strain of TMEV and B6 mouse experimental paradigm will be more thoroughly studied longitudinally with continuous video-EEG and additional anatomical studies or manipulated to determine whether it can produce unprovoked seizures and establish a relevant model of postinfection epilepsy. For example, assessment of residual or potentially new areas of secondary tissue injury or neuroplastic anatomical change, including the possibility of hippocampal sprouting, could be performed periodically over the natural lifespan of the animal in conjunction with video-EEG monitoring. Experimental manipulations could include varying the time of infection or the titer of plaque forming units (PFUs). The previous and current study injected PFUs within a unitary log range that may have been sufficient for neurovirulence and the production of acute seizures but not sufficient to result in injury that results in epileptic seizures.

The authors conclude their study by suggesting that TMEV infection of B6 mice might be a potential model for the study of postinfection hyperexcitability. Their results indicate that they have already accomplished this objective. However, the goal of these studies might reasonably be considered to be the development of a model of postinfection epilepsy. The authors appear to be in a favorable position to explore exactly this.

by Kevin M. Kelly, MD, PhD

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