Published in final edited form as:
Future Virol. 2011 November ; 6(11): 1339–1350. doi:10.2217/fvl.11.107.

Neurotropic viral infections leading to epilepsy: focus on Theiler's murine encephalomyelitis virus

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Abstract

Neurotropic viruses cause viral encephalitis and are associated with the development of seizures/epilepsy. The first infection-driven animal model for epilepsy, the Theiler's murine encephalomyelitis virus-induced seizure model is described herein. Intracerebral infection of C57BL/6 mice with Theiler's murine encephalomyelitis virus induces acute seizures from which the animals recover. However, once the virus is cleared, a significant portion of the animals that experienced acute seizures later develop epilepsy. Components of the innate immune response to viral infection, including IL-6 and complement component 3, have been implicated in the development of acute seizures. Multiple mechanisms, including neuronal cell destruction and cytokine activation, play a role in the development of acute seizures. Future studies targeting the innate immune response will lead to new therapies for seizures/epilepsy.

Keywords

animal model; epilepsy; inflammation; innate immune response; macrophages; microglia; picornavirus; seizures; Theiler's murine; encephalomyelitis virus; viral encephalitis

Seizures/epilepsy in humans

Epilepsy and seizures have been estimated to affect 3 million individuals in the USA [101]. An estimated 50 million people are affected by epilepsy worldwide, 90% of whom are found in developing countries [102]. All ages are affected; 1% of the population of the USA will develop epilepsy by the age of 20, and 3% by the age of 75 [101]. In the USA, 10% of the population will experience some type of seizure in their lifetime. It is estimated that 300,000 people have a first convulsion each year and that 200,000 new cases of epilepsy are diagnosed each year. The risk of developing epilepsy for the general population is 1%; however, there are certain populations that are at higher risk [101]. Epilepsy increases an individual's risk of premature death by two- to three-fold over the general population [101].

Viral encephalitis, inflammation within the brain due to direct viral infection, is often associated with seizures [1,2]. Viral encephalitis has recently been calculated to affect ~7.5 persons per 100,000 in the general population [1]. During the acute viral infection, many of these encephalitic patients will develop seizures [1,2]. In viral encephalitic patients, the risk

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Financial & competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

of seizures is increased more than 20% over the risk of seizures in the general population [1]. The appearance of seizures of any type (single generalized seizures, epilepsy and status epilepticus) during viral encephalitis was found to be a good indicator of disease severity and negatively influenced both the course and the outcome of the disease [3]. In addition, viral encephalitis increases the risk of developing late unprovoked seizures and epilepsy; 4–20% of viral encephalitis survivors go on to develop epilepsy [1,2].

Over 100 different neurotropic viruses cause encephalitis in humans, and of these, several different viruses have been suggested to play a role in the development of seizures and epilepsy [2,4]. Viruses from the herpes virus group are prominent among these. Human herpes virus type 6 (HHV-6) may cause seizures and epilepsy due to a primary infection, or due to reactivation of latent virus [5-7]. It has been estimated that 16.5% (149/902) of patients with a primary HHV-6 infection and fever experienced febrile seizures and 24% (101/416) of febrile seizure patients had a primary HHV-6 infection, for children under 3 years of age, reported between 1994 and 2005 [7]. Examination of hippocampal sections from patients with mesial temporal lobe epilepsy (TLE) demonstrated elevated levels of HHV-6 DNA, measured by real-time PCR, and HHV-6 protein, measured by western blot and immunohistochemistry, in what morphologically appeared to be astrocytes in four of eight brain samples (50%) [8]. In addition, HHV-6B DNA was detected in five of nine brain samples (55.6%) isolated from TLE patients with a history of encephalitis, but was absent from 26 other cases of TLE without a history of encephalitis and from ten autopsy controls [9]. These data support a potential pathogenic role for HHV-6 in at least a subset of patients with epilepsy.

Another herpes virus that has been implicated in the development of seizures and epilepsy is herpes simplex virus type-1 [2,4]. The presenting feature in 50% or more of patients with acute herpes simplex encephalitis (HSE) may be seizures [2,4,10]. Late, unprovoked seizures and epilepsy develop in 42–60% of HSE survivors [4]. More specifically, a follow-up study of 16 children with HSE demonstrated that 44% (7/16) of these HSE survivors experienced seizures anywhere between 3 and 10 years after discharge [10].

Several viruses other than herpes viruses have also been suggested to play a role in the development of seizures and epilepsy. Encephalitis caused by the Japanese encephalitis virus (JEV), prevalent in Asia and Australia, is commonly accompanied by acute symptomatic seizures in children (61%), although the reported frequency of seizures in JEV encephalitis is variable (7–67%) [2]. Encephalitis caused by the Nipah virus, also prevalent in Asia, is accompanied by acute symptomatic seizures in 24% (22/91) of acute encephalitis cases and 50% (12/24) of relapsed or late-onset encephalitis cases [11]. Both JEV and Nipah virus are emerging viruses. Other emerging viruses that have been associated with encephalitis and seizures include West Nile virus, enterovirus 71, Toscana virus and chikungunya virus [4].

Infection with HIV has become an important cause of acute symptomatic seizures, which can result from primary HIV infection (50%) or secondary causes such as opportunistic infections (toxoplasmosis, tuberculosis, progressive multifocal leucoencephalopathy, cryptococcal meningitis and polymicrobial infections), lympho mas, drugs and electrolyte and metabolic disturbances [4,12]. The occurrence of seizures in HIV-seropositive individuals has been estimated to be between 2 and 20%; status epilepticus has been reported to occur in between 8 and 18% of HIV-seropositive individuals [12]. However, it was found that 40% (19/48) of HIV-infected children with neurological involvement (ranging in age from 2 months to 15 years at time of presentation) had seizures [13]. Seizures may be the presenting manifestation of the infection; however, seizures more

commonly occur late in the course of the infection in HIV-seropositive individuals and seizure recurrence is frequent in these individuals [12].

Still other viruses implicated in the development of febrile seizures include influenza A and B viruses, parainfluenza, rotavirus, HHV-7, adenovirus, respiratory syncytial virus and cytomegalovirus [6,7,14-17]. The observed association between influenza virus and febrile seizures is prevalent in Asia, seasonal (winter months) and related to epidemics [7,18]. However, infection with several of these viruses (rotavirus, respiratory syncytial virus) can also often lead to seizures without fever (afebrile) [6,17].

Certain nonpolio picornaviruses within the *Picornaviridae* Family and *Enterovirus* and *Parechovirus* Genera have also been implicated in the development of febrile seizures in infants and children. These viruses include entero viruses, coxsackie A and B viruses, echoviruses and parechoviruses [18-20]. In one study, enterovirus and parechovirus infection was found in 39% (20/51) of infants with a laboratory-confirmed viral infection, and 45% (9/20) of those infants presented with severe prolonged seizures [19]. In another study by the same group, 73% (8/11) of infants with parechovirus infection presented with seizures while 42% (9/21) of infants with enterovirus infection presented with seizures [20]. Direct picornaviral infection of the CNS as the causative agent of the seizures was suspected in yet another study, as PCR ana lysis demonstrated the presence of picornaviral genome sequences in the cerebrospinal fluid in approximately 21% (14/67) of subjects with febrile seizures and in approximately 62% (13/21) of subjects with febrile seizures during the summer months [18].

As infection with many different neurotropic viruses can result in acute symptomatic seizures and epilepsy, the specific mechanisms of seizure generation most likely vary with the type of virus and are most likely multifactorial [4]. However, in general, a common underlying factor in viral encephalitis is the induction of the inflammatory cascade with the resultant release of inflammatory cytokines. These cytokines in turn can induce neuronal hyperexcitability through excess activation of glutamate receptors, such as the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors, thus resulting in the development of acute symptomatic seizures. The development of epilepsy following viral encephalitis is most likely due to structural damage, such as neuronal cell loss, and/or persistent neuronal hyperexcitability [4].

Animal models

There are many different kinds of seizures and epilepsies in humans ranging from febrile seizures to temporal lobe epilepsy to status epilepticus [21]. Epilepsies of known cause (acquired epilepsies) can be described in three phases: the inciting event, the silent or latent period (during which epileptogenesis occurs) and the onset of recurrent spontaneous seizures/epilepsy [22,23]. Epidemiologic data suggest that the leading inciting events of acquired epilepsies in humans are trauma, stroke and brain infections [24,25]. Current animal models available to investigate the mechanisms of seizure development in acquired epilepsies typically use status epilepticus, trauma or stroke as the inciting event [22,24,26]. In turn, status epilepticus can be induced through electrical stimulation, such as electrical kindling, or chemical stimulation with neurotoxins, such as kainic acid or pilocarpine [22-24,26]. Direct comparison of an electrically induced status epilepticus model, a single type of trauma model and two different stroke models demonstrated that there were many differences between the models, including the length of the latency period, the prevalence of epilepsy, the epilepsy phenotype (seizure frequency, type and duration) and the distribution, type and severity of cellular alterations to the brain [24]. This suggests that these models,

status epilepticus-, trauma- and stroke-induced epilepsy models, are not likely to be satisfactory models for brain infection-induced epilepsy.

Viral infection has been used to induce seizures in rabbits, rats and mice [27]. However, in most instances, these animals succumb to the acute encephalitis caused by the viral infection [27]. Therefore, these animal models may or may not be used to study seizures during the acute infection, depending on how rapidly the animals succumb, but are useless for the study of epileptogenesis and epilepsy. As it stands, no good animal model exists to investigate viral and host immune contributions to the development of seizures and/or epilepsy during and following viral encephalitis.

The remainder of this article will describe the recent discovery [28] and characterization [29-35] of a virus-induced mouse model for the development of acute seizures and epilepsy: the Theiler's murine encephalomyelitis virus (TMEV)-induced seizure model. A brief discussion of the current avenues of research on this animal model is included, followed by a discussion of where this research may lead and how research on this animal model may benefit human patients in the future.

The TMEV-induced seizure model

A description of the virus

Theiler's murine encephalomyelitis virus, a non-enveloped, positive-sense, ssRNA virus of the *Picornaviridae* family and *Cardiovirus* genus, is a naturally occurring enteric pathogen of the mouse [36,37]. The strains of TMEV are divided into two groups based on their neurovirulence following intracerebral (i.c.) inoculation of mice [38]. The less neurovirulent group is the Theiler's original (TO) group and includes the TO, Daniels (DA), BeAn 8386 (BeAn) and WW strains of TMEV. The highly neurovirulent group of TMEV is the GDVII group, which includes the GDVII and FA strains. Infection (i.c.) of the susceptible SJL/J strain of mouse with the DA strain of TMEV results in an acute disease (encephalitis) occurring at 8–10 days postinfection. The mice survive the acute disease; however, virus persists and the mice go on to develop a chronic inflammatory demyelinating disease at around 1 month postinfection [38]. Infection (i.c.) of C57BL/6 mice with the DA strain of TMEV causes an acute disease but, unlike SJL/J mice, C57BL/6 mice have the ability to clear the virus during the first month following infection. Therefore, the virus does not persist and these animals do not go on to develop the late disease and are considered to be resistant to the TMEV-induced demyelinating disease [39-42]. Infection (i.c.) of either susceptible SJL/J mice or resistant C57BL/6 mice with the GDVII strain of TMEV causes an acute polioencephalomyelitis that is fatal within 2 weeks [28,38].

Due to the development of the chronic inflammatory demyelinating disease, infection of susceptible mice with strains of the TO group of TMEV has been used as an animal model for multiple sclerosis for approximately the past 40 years [43]. The C57BL/6 strain of mouse has been used as the classic resistant mouse strain, which does not develop the demyelinating disease. In all of the years that TMEV infection of C57BL/6 mice has been used as an example of mice resistant to TMEV-induced demyelination, our group was the first to recognize the importance of these mice in that they experienced acute behavioral seizures [28].

A description of the model

Initially, approximately 50% of C57BL/6 mice (male and female) infected i.c. with the DA strain of TMEV developed acute behavioral seizures [28,32]. This percentage increased to 75% if continuous video-electroencephalography (EEG) was used to monitor the mice [33]. Subsequently, it was found that the percentage of C57BL/6 mice (male) that developed

seizures correlated to the initial viral dose of the DA strain: 30% at 3×10^3 plaque forming units (PFU), 40% at 3×10^4 PFU, 65% at 3×10^5 PFU and 80% at 3×10^6 PFU (visual observation) [34]. Typically, seizures were first observed on day 3 postinfection, the peak of seizure activity was day 6 postinfection and no seizures were observed after day 10 postinfection [28]. The seizures occurred at a frequency of one per mouse per 2 h observation period and typically lasted for 1–2 min [28,32]. The seizures were afebrile and limbic in nature [28]. The majority of seizures had a Racine Racine scale seizure score of three (forelimb clonus) and above (score of four, rearing; score of five, rearing and falling) [28,44]. There was mouse-to-mouse variation in both seizure score for any given day and the pattern of days on which seizures were observed. C57BL/6 mice experiencing seizures were impaired in both motor function and coordination as evaluated by means of the righting reflex test and a rotorod [28].

The development of seizures appeared to be specific for the C57BL/6 strain of mouse as no SJL/J (male and female), BALB/c (male) or FVB/N (male) mice developed seizures following i.c. infection with the DA strain of TMEV [28]. The BALB/c strain was chosen based on its similarity to the C57BL/6 strain in resistance to the TMEV-induced demyelinating disease [42] and in susceptibility to kainic acid-induced seizures [45]. The FVB/N strain was chosen based on its similarity to the C57BL/6 strain in susceptibility to both kainic acid-induced seizures and pilocarpine-induced seizures [46-48]. However, 6.7% of the C57 × SJL (male) F1 generation developed seizures following i.c. infection with the DA strain of TMEV, suggesting that resistance to seizures involves at least one gene locus with a dominant effect [28].

The development of seizures was not specific to the DA strain of TMEV as other TMEV strains and mutants were able to induce seizures to various degrees: 25% for the WW strain, 40% for both the BeAn strain and H101 mutant, 55% for the GDVII strain, 57.5% for the DA strain and 65% for the DApBL2M mutant [34]. The H101 and DApBL2M mutants of TMEV were generated previously. The H101 mutant was inadvertently created as a result of transcription error by the T7 polymerase while using a modified full-length infectious cDNA clone of the DA strain as a template [49]. The H101 mutant encodes a point mutation (T101I) in VP1 loop II (Figure 1) , as was expected from the modified full-length infectious cDNA clone. However, there are also several nucleotide substitutions in the 5′ untranslated region as well as additional amino acid substitutions in the capsid protein coding region [49]. The DApBL2M mutant of the DA strain was created through standard molecular biological manipulations of the full-length infectious cDNA clone of the DA strain [50,51]. The DApBL2M mutant encodes the VP1 loop II of the GDVII strain plus a point mutation (S171R) in VP2 puff B on the background of the DA strain [51]. VP1 loop II and VP2 puff B are critical components of the receptor binding site of the virus (Figure 1) [51]. The cell surface receptor(s) utilized by the various TMEV strains and mutants within the CNS is currently unknown.

One prominent cell tropism of the DA strain of TMEV in the brains of C57BL/6 mice with seizures at day 7 postinfection (the peak of seizure activity) was shown, through immunohistochemistry, to be the pyramidal neurons of the hippocampus [29]. However, viral antigen was not restricted to the hippocampus, but was also observed in the cortex, frontal lobe, cau-doputamen, septum, thalamus and hypothalamus [34]. Those mice that developed seizures survived the acute viral encephalitis and cleared the virus by day 14 postinfection [29,31,34]. The survival of these animals and the clearance of the virus make this virus-induced seizure model an excellent model for the study of postinfection epileptogenesis and epilepsy.

The potential correlation between viral infection with acute seizures and the subsequent development of epilepsy was examined in this model by following C57BL/6 mice infected i.c. with the DA strain of TMEV past both the resolution of the acute seizures (day 10 postinfection) and the clearance of the virus (day 14 postinfection) through 2, 4 and 7 months postinfection [32,33]. Chronic seizure susceptibility was tested at 2 months postinfection using transcorneal electrical stimulation and three different stimulation protocols to test limbic, forebrain and hindbrain seizure thresholds [32]. The limbic and forebrain seizure thresholds were found to be significantly lower in TMEV-infected C57BL/6 mice that had experienced acute seizures. No difference was found in the hindbrain seizure threshold. Corneal kindling was also performed at 2 months postinfection to detect increased hyperexcitability. The TMEV-infected C57BL/6 mice that had experienced acute seizures required significantly fewer stimulations to reach either a partial seizure (Racine scale seizure score of 1 or 2), a fully generalized seizure (Racine scale seizure score of 4 or 5) or a stable kindled state, indicative of hyperexcitable neuronal circuits. Taken together, these data demonstrate that TMEV-induced acute seizures lead to chronically increased seizure susceptibility and hyperexcitability in limbic structures [32]. Monitoring, with long-term video-EEG at 2, 4 and 7 months postinfection, of the TMEV-infected C57BL/6 mice that had experienced acute seizures demonstrated that a significant proportion (65%) of the mice developed profound, spontaneous epileptic seizures following a distinct latent period in which no behavioral seizures were visually observed [33]. In addition, those animals with epilepsy had hippocampal sclerosis characterized by neuronal cell loss in the pyramidal cell layer and gliosis (activated astrocytes). Thus the TMEV-induced seizure model is a novel animal model for the study of postinfection epilepsy [33].

Pathological effects of the acute viral infection and/or seizures

As an initial means of comparing the pathological effects of the acute seizures in the TMEV-induced seizure model to other seizure models, immunohistochemical ana lysis was used to examine the expression of TGF-β in the TMEV-induced seizure model [28]. Studies have shown that members of the TGF-β cytokine family are expressed in reactive microglia in the brain following kainic acid-induced epileptic seizures [52,53]. In the TMEV-induced seizure model, TGF-β expression was found to occur in cells with neuronal morphology and to correlate with seizure activity in the hippocampus [28]. The similar presence yet different cellular localization of TGF-β expression in the brain in the TMEV-induced seizure model is suggestive of the novel nature of this model.

Further histological examination of the brains of C57BL/6 mice infected with the DA strain of TMEV during the peak of seizure activity demon strated many pathological features within the hippocampus. These will be described in detail, along with some discussion as to whether the pathologic features are likely to result from direct viral infection, the immunological response to the infection and/or the occurrence of seizures.

Neuronal cell death was noted within the hippocampus of TMEV-infected C57BL/6 mice during the peak of seizure activity. Significant pyknosis (condensation and reduction in the size of the cell body) of neurons was observed in the pyramidal layer of the hippocampus and correlated with the occurrence of seizures [28]. FluoroJade-B staining consistently identified nonviable neurons specifically within the CA1 and CA2 regions of the pyramidal cell layer of the hippocampus of TMEV-infected C57BL/6 mice [32]. Others have shown that CA1 pyramidal neurons that were not infected with TMEV were undergoing apoptosis early after infection (days 2–4 postinfection) prior to the development of the adaptive immune response [54]. Based on these observations, early neuronal cell death appears to be caused by the innate immune response to the viral infection and may lead to the development of seizures. However, the presence of seizures may contribute to continued neuronal cell loss, as the extent of neuronal cell loss within the hippocampus was

significantly greater in those mice with seizures at day 7 postinfection [31], days 14 and 21 postinfection [29,31] and day 35 postinfection [29] than in those mice without seizures.

The extent of inflammation in the brain has been determined in this model through examination of perivascular cuffs (PVCs), gliosis and activated microglia and macrophages. PVCs composed of infiltrating $CD3^+$ T cells and other mononuclear cells, were seen within the hippocampus of TMEV-infected C57BL/6 mice, both with and without seizures, during the peak of seizure activity [28,29]. By day 14 postinfection, a significantly greater number of PVCs could be seen in TMEV-infected C57BL/6 mice with seizures than in TMEV-infected C57BL/6 mice without seizures [34].

Gliosis, detected by scoring glial fibrillary acidic protein (GFAP)-positive activated astrocytes, was present within the hippocampus of the brains of TMEV-infected C57BL/6 mice, both with and without seizures [29,31]. Gliosis was significantly greater in TMEV-infected C57BL/6 mice with seizures than in TMEV-infected C57BL/6 mice without seizures on day 14 post-infection [29,31], similar to the PVC, but also on day 7 postinfection [31]. The continued presence of PVC and gliosis, within the hippocampus of mice with seizures, beyond both the resolution of the seizures (day 10 postinfection) and the clearance of the virus (day 14 postinfection) suggest that both virus and seizures contribute to the occurrence of PVC and gliosis.

Activated microglia and macrophages, detected through *Ricinus communis* agglutinin (RCA)-I lectin histochemistry, were also present within the hippocampus of TMEV-infected C57BL/6 mice; however, unlike PVC and gliosis, the numbers of these cells were significantly greater in those mice with seizures compared with those mice without seizures for days 5, 7, 14, 21 and 35 postinfection [29,31]. The early and continued presence of activated microglia and macrophages within the hippocampus of mice with seizures is suggestive of a role for the innate immune system in the development of acute seizures.

Immunological contributions to acute seizures

As neuronal apoptosis occurs early following infection (days 2 to 4 postinfection) [54] and acute seizures develop by day 3 postinfection [28], prior to the development of the adaptive immune response, and microglia and macrophages are highly activated in TMEV-infected C57BL/6 mice with seizures [29,31], it is likely that the innate immune response to viral infection contributes to the development of acute seizures. The innate immune system is composed of a variety of effector cells, of which microglia and macrophages are examples, and proteins, such as proinflammatory cytokines produced by both effector cells and CNS resident cells, that participate in the inflammatory response to infection and have antiviral activity (reviewed in [55]). In other seizure models (chemical, electrical and audiogenic), the production of the proinflammatory cytokines IL-1β, IL-6 and TNF-α has been shown to be rapidly increased in microglia and astrocytes within the hippocampus (reviewed in [56-58]). In addition, IL-1β, IL-6 and TNF-α are produced within the CNS early following viral infection (reviewed in [55]). We have previously shown increased mRNA expression of IL-6 and/or TNF-α in the brains of TMEV-infected (DA and GDVII strains and H101 mutant) SJL/J mice sacrificed 1 week after infection [59]. Therefore, these three proinflammatory cytokines, which are all also produced by macrophages, were chosen for examination in the TMEV-induced seizure model.

The involvement of these proinflammatory cytokines in the development of acute seizures was initially assessed through TMEV infection of C57BL/6 mice deficient in the individual cytokine, the receptor for the cytokine or another downstream member of the cytokine signaling pathway [29]. IL-1, assessed through the use of IL-1 receptor I-deficient mice and myeloid differentiation primary response gene 88 (MyD88; down-stream IL-1 signaling

pathway member)-deficient mice, was found not to contribute to the development of seizures as the numbers of mice experiencing acute seizures were comparable to wild-type C57BL/6 mice. TNF-α, assessed through the use of TNF receptor I-deficient mice, and IL-6, assessed through the use of IL-6-deficient mice, were both found to contribute to the development of acute seizures as the numbers of mice experiencing seizures were significantly less than that of wild-type C57BL/6 mice. Analysis of the mRNA expression of the cytokines IL-1α, IL-1β, TNF-α and IL-6 in the brains of TMEV-infected C57BL/6 mice with and without seizures confirmed the involvement of only TNF-α and IL-6 in the development of acute seizures [29].

Another component of the innate immune system that functions to recognize and eliminate pathogens and can contribute to the release of cytokines is the complement system, in which complement component 3 (C3) plays an essential role [60]. C3 activation has been shown to result in the release of TNF-α and IL-6 [61]. Importantly, complement proteins are produced in the CNS by neurons, microglia, astrocytes and oligodendrocytes ([62-64]; reviewed in [60,65]), and the expression of complement proteins increases in the CNS following viral infection and is localized to microglia and macrophages [66,67]. The involvement of complement in the development of acute seizures in the TMEV-induced seizure model was assessed through the use of C57BL/6 mice deficient in C3 and through the depletion of C3 from the periphery via cobra venom factor treatment of C57BL/6 mice [31]. Complement activation within the CNS was found to play an important role in the development of acute seizures as significantly fewer C57BL/6 mice deficient in C3 experienced seizures whereas the number of mice experiencing seizures following depletion of C3 from the periphery was comparable to wild-type C57BL/6 mice. The contribution that C3 makes to the development of acute seizures could be through the TNF-α and IL-6 pathways. The C57BL/6 mice deficient in C3 that experienced seizures were found to have a delay of a few days in the occurrence of pathological effects (neuronal cell loss, gliosis, activation of micoglia and macrophages), suggesting that the pathological effects were due to the seizures. In addition, these mice were unable to clear the virus, suggesting that the persistence of virus does not play a role in seizures and that complement does play a role in viral clearance [31].

The initial host immune response to virus involves the innate immune response, but after a period of days to weeks, the adaptive immune response, which is an antigen-specific response, develops [68,69]. Naive B cells and naive $CD4^+$ and $CD8^+$ T cells are activated and induced to proliferate and differentiate through cell surface antibody binding of viral antigen (B cells) and presentation of viral antigen in the context of the MHC molecules on antigen presenting cells (T cells) (reviewed in [70]). B cells and $CD4^+$ T cells will not be discussed further. $CD8^+$ T cells are activated by the interaction of the T-cell receptor with viral antigenic peptides complexed to MHC class I molecules on antigen presenting cells. Activated $CD8^+$ T cells, termed $CD8^+$ cytotoxic T lymphocytes, kill virus-infected cells and, therefore, play a significant role in viral clearance from the host (reviewed in [70]). The involvement of $CD8^+$ cytotoxic T lymphocytes and viral clearance in the development of acute seizures in the TMEV-induced seizure model was assessed through the use of OT-I transgenic mice (C57BL/6 background), in which the majority of the $CD8^+$ T cells carry an ovalbumin-specific T-cell receptor [29]. The number of TMEV-infected OT-I mice experiencing acute seizures was comparable to wild-type C57BL/6 mice, suggesting that the seizures were not influenced by TMEV-specific $CD8^+$ T cells. Also, the seizures resolved by day 10 postinfection in TMEV-infected OT-I mice, similar to wild-type C57BL/6 mice. However, both viral RNA and antigen were found to be present in the brains of TMEV-infected OT-I mice with and without seizures through day 17 postinfection, long after the point at which the wild-type C57BL/6 mice clear the virus (day 14 postinfection). This resolution of seizures in the presence of viral persistence showed that the cessation of seizures was not due to the clearance of virus by the $CD8^+$ T-cell response [29]. Therefore,

studies with both OT-I mice [29] and C3-deficient mice [31] lead to the conclusion that persistence of virus does not play a role in seizures.

Resident CNS cells versus infiltrating cells

The cytokines TNF-α and IL-6 and C3 of the complement system are all effectors of the innate immune response and have all been shown to be important in the CNS for the development of acute seizures in the TMEV-induced seizure model [29,31]. As C3 may contribute through the TNF-α and IL-6 pathways [61] and TNF-α induces the production of IL-6 [71,72], focusing on the production of IL-6 and determining whether it is the resident CNS cells or the infiltrating cells that produce the IL-6 would elucidate the contribution of the resident CNS cells versus the infiltrating cells to the development of acute seizures in the TMEV-induced seizure model.

As a first step in determining the role of infiltrating cells versus resident CNS cells in the development of acute seizures, treatment with minocycline and various antibodies was explored [30,35]. Minocycline treatment blocks recruitment of polymorphonuclear leukocytes (PMNs; neutrophils, basophils and eosinophils; infiltrating cells), activation of microglia (resident CNS cells) and activation and recruitment of monocytes/macrophages (infiltrating cells) [73-76]. Mice treated with minocycline had significantly fewer seizures than wild-type C57BL/6 mice, thus implicating both resident CNS cells (microglia) and infiltrating cells (PMNs and monocytes/macrophages) in the development of seizures [30]. However, treatment of mice with anti-Gr-1 antibody, which depletes peripheral blood and splenic neutrophils, anti-CXCR2 antibody, which blocks entry of PMNs into the CNS, or anti-NK1.1 antibody, which depletes splenic natural killer cells, did not alter the number of TMEV-infected C57BL/6 mice experiencing acute seizures. Therefore, PMNs, specifically neutrophils, and natural killer cells are not the infiltrating cells that contribute to the development of seizures. The infiltrating cells that most likely contribute to the development of seizures are monocytes/macrophages. The resident CNS cells that contribute to the development of seizures are most likely microglia and/or astrocytes [30]. Pathologic examination of minocycline-treated mice that developed seizures demonstrated that the extent of neuronal cell loss and inflammation (perivascular cuffing, gliosis and activated microglia/macrophages) and the numbers of virus-infected cells were very similar to what was seen in TMEV-infected wild-type C57BL/6 mice that developed seizures [35]. Therefore, it appears that once seizures develop, the pathological changes are consistent independent of the treatment [35].

Irradiation bone marrow chimeric mice, generated from IL-6 deficient mice, were infected with TMEV in an attempt to determine which cell population, resident CNS cells or infiltrating cells, was the source of the IL-6, within the CNS, which was important in the development of seizures [30]. Irradiation bone marrow chimeric mice that were either IL-6-deficient in the CNS and IL-6 normal in the periphery or IL-6 normal in the CNS and IL-6-deficient in the periphery developed significantly fewer seizures following TMEV infection than wild-type mice, thus implicating IL-6 production by both resident CNS cells and infiltrating cells in the development of seizures [30]. As with the minocycline-treated mice, pathologic examination of irradiation bone marrow chimeric mice that developed seizures demonstrated that the pathological changes are consistent once seizures develop, irrespective of the genetic background [35].

Cytokine activity versus neuronal cell destruction

Although all of the different TMEV strains (DA, BeAn, GDVII and WW) and mutants (H101 and DApBL2M) tested in this model induced acute seizures (albeit to varying degrees), pathological examination of the infected mice displaying seizures demonstrated

many differences [34]. The DA and BeAn strains and DApBL2M mutant were very similar in numbers of mice displaying seizures, extent of neuronal cell loss, extent of inflammation (perivascular cuffing) and numbers of virus-infected cells. The WW and GDVII strains both caused 100% mortality early following infection with extensive neuronal cell loss, perivascular cuffing and virus-infected cells. The H101 mutant also caused 100% mortality early following infection; however, this occurred in the absence of neuronal cell loss, perivascular cuffing and virus-infected cells. This suggests that multiple pathways, including neuronal cell destruction (GDVII) and cytokine activity (H101), may lead to the development of seizures following TMEV infection [34].

Our studies to date using the TMEV-induced seizure model suggest that IL-6 is an important cytokine [29,30,35]. In support of this, it has previously been shown that overexpression of IL-6 within the CNS, specifically targeted to astrocytes due to regulatory control by the GFAP gene promoter, resulted in spontaneous seizures in (C57BL/6J × SJL)F_1 hybrid mice that were high expressor GFAP-IL-6 mice [77], and increased the sensitivity of low expressor GFAP-IL-6 mice (which did not spontaneously develop seizures) to both kainic acid- and NMDA-induced seizures [78]. In addition, GFAP-IL-6 mice demonstrated hippocampal electrophysiological hyperexcitability, possibly due to a loss of inhibitory control through the loss of inhibitory GABAergic neurons ([79], reviewed in [80]).

Cytokine activity and neuronal cell destruction, the two pathways elucidated, can be represented most simply as two opposing and complementary gradients (Figure 2) . The various viral strains and mutants could then be positioned across the gradient, based on both the cytokine contribution and the extent of neuronal cell destruction. The H101 mutant would represent the virus requiring the greatest cytokine contribution to the development of seizures, while the GDVII strain would represent the virus requiring the greatest neuronal cell destruction contribution to the development of seizures (Figure 2) . Other viruses, such as the DApBL2M mutant, would be placed somewhere in-between depending on the relative requirements for cytokines and/or neuronal cell destruction in the development of seizures (Figure 2).

Future perspective

Epilepsy is a significant burden on the individuals affected, their families and society as a whole. Seizures are often associated with viral encephalitis and it has been shown that the occurrence of any type of seizure (single generalized seizures, epilepsy, or status epilepticus) during viral encephalitis is a good indicator of disease severity and negatively influences both the course and outcome of the disease [3]. Infection-initiated seizure disorders are often refractory to many established antiepileptic drugs. Approximately 30% of individuals with epilepsy are refractory to currently existing antiseizure medications [102]. Therefore, finding new biological models for epilepsy and potentially new therapeutics is important for public health.

Herein is described the first infection-driven animal model for epilepsy: the TMEV-induced seizure model. Examination of this model has led to the implication of the innate immune response to viral infection in the development of acute seizures. Several components of the innate immune response shown to play a role include the proinflammatory cytokine IL-6 and C3 of the complement system. The innate immune system and complement in particular have not previously been considered as targets for drug therapy in the prevention and/or treatmentof epilepsy.

Over the next 5–10 years, the mechanism(s) of seizure induction in this animal model will be extensively dissected. Multiple mechanisms involving both cytokine activation (H101 infection) and/or neuronal cell destruction (GDVII infection) are likely to be elucidated.

Once the mechanism(s) is fully understood, steps can be taken towards modulation and treatment of both acute seizures and epilepsy. Treatments found to be effective in the animal model can then be translated over to human patients. If a new drug treatment regimen can be developed that benefits a third of all patients diagnosed with epilepsy, then the impact of this research on the epilepsy community would be major. The remaining two-thirds of all patients diagnosed with epilepsy can adequately control their seizures with currently available therapies; however, side-effects and comorbid conditions still affect their quality of life. If a new drug treatment regimen, using existing antiseizure medications in combination with drugs targeting the innate immune response, can be developed that decreases the side-effects and comorbid conditions seen with existing antiseizure medications alone, then the impact of this research on the epilepsy community would be even broader.

Acknowledgments

The authors would like to thank MF Cusick, KS Wilcox and HS White for many helpful discussions. The authors acknowledge K Borick for manuscript preparation.

This work was supported by NIH 1R01NS065714.

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Executive summary

Background

- Epilepsy affects 50 million people worldwide.
- Viral encephalitis (inflammation due to direct viral infection of the brain) increases the risk of developing epilepsy.
- Viruses implicated in the development of seizures and epilepsy include: herpes viruses, Japanese encephalitis virus, Nipah virus, HIV, influenza viruses, parainfluenza virus, rotavirus, adenovirus, respiratory syncytial virus, cytomegalovirus and nonpolio picornavirus.
- Previously, no suitable virus-induced animal models for seizures and epilepsy existed.

Theiler's murine encephalomyelitis virus-induced seizure model

- Theiler's murine encephalomyelitis virus (TMEV) is a naturally occurring enteric picornavirus of mice.
- Intracerebral infection of C57BL/6 mice with the various TMEV strains and mutants results in acute behavioral seizures, to various degrees, between days 3 and 10 following infection.
- Mice experiencing seizures survive the acute viral encephalitis and clear the viral antigen by day 14 following infection.
- A significant proportion of TMEV-infected C57BL/6 mice that experienced acute seizures later developed spontaneous epileptic seizures.
- During the peak of seizure activity, neuronal cell loss and inflammation, in the form of perivascular cuffing (PVC), gliosis and activated microglia/macrophages, were noted within the hippocampus.
- The innate immune response to viral infection likely contributes to the development of acute seizures.
- The cytokines TNF-α and IL-6 and complement component 3, all of the innate immune response, have been found to be involved in the development of acute seizures.
- IL-1, viral persistence and TMEV-specific $CD8^+$ T cells, of the adaptive immune response, have been found not to influence seizures.
- Both resident CNS cells, microglia and/or astrocytes, and infiltrating cells, monocytes/macrophages, contribute to the development of seizures.
- The GDVII strain of TMEV induced seizures in the presence of extensive neuronal cell loss, PVC and virus-infected cells, whereas the H101 strain of TMEV induced seizures in the absence of neuronal cell loss, PVC and virus-infected cells, suggesting two different pathways lead to the development of seizures.

Future perspective

- Elucidate mechanism(s) involved in acute seizures and epilepsy in the TMEV-induced seizure model.

- Find drug therapies able to modulate and treat both acute seizures and epilepsy in this animal model.
- Translate these therapies to human patients.

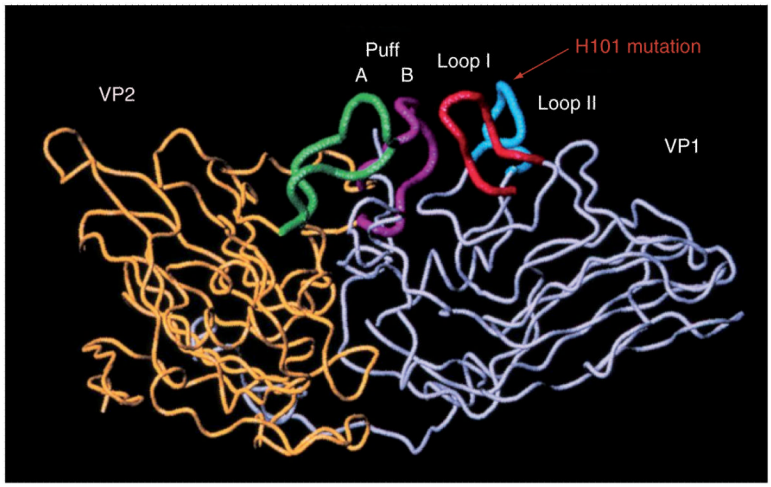

Figure 1. Predicted structure of VP1 and VP2

VP1 is shown in gray; VP2 is shown in yellow. Loop I of VP1 is shown in red and loop II of VP1 is in blue. Puff A and B found in VP2 are green and purple, respectively. The location of the T101I mutation in VP1 loop II of the H101 mutant virus is shown by the arrow. Reproduced with permission from [81]© American Society of Microbiology.

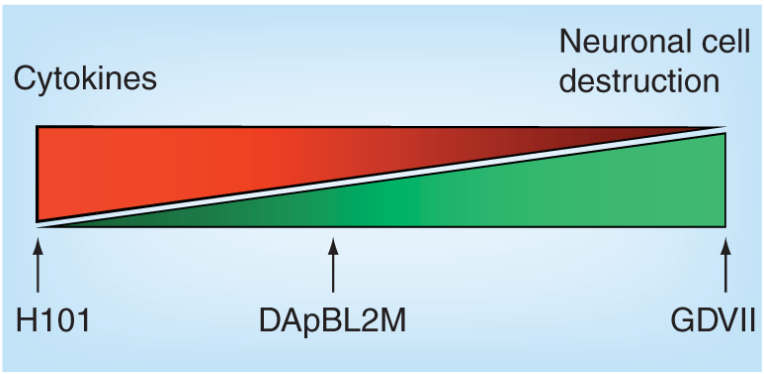

Figure 2. Contributions of cytokines and neuronal cell destruction to the development of seizures

Two opposing and complementary gradients represent the contributions of cytokines and neuronal cell destruction within the CNS to the development of seizures following viral infection. Seizures in H101-infected mice likely develop as a result of cytokines within the CNS, while seizures in GDVII-infected mice likely develop as a result of neuronal cell destruction. The other virus strains and mutants, such as the DApBL2M mutant, fall somewhere inbetween.