Molecular Neurobiology (2019) 56:1897–1907
https://doi.org/10.1007/s12035-018-1215-5

Viral Triggers and Inflammatory Mechanisms in Pediatric Epilepsy

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Received: 15 March 2018 / Accepted: 27 June 2018 / Published online: 5 July 2018

Abstract
Experimental and clinical findings suggest a crucial role for inflammation in the onset of pediatric seizures; this mechanism is not targeted by conventional antiepileptic drugs and may contribute to refractory epilepsy. Several triggers, including infection with neurotropic viruses such as human herpesvirus 6 (HHV-6), other herpesviruses, and picornaviruses, appear to induce activation of the innate and adaptive immune systems, which results in several neuroinflammatory responses, leading to enhanced neuronal excitability, and ultimately contributing to epileptogenesis. This review discusses the proposed mechanisms by which infection with herpesviruses, and particularly with HHV-6, and ensuing inflammation may lead to seizure generation, and later development of epilepsy. We also examine the evidence that links herpesvirus and picornavirus infections with acute seizures and chronic forms of epilepsy. Understanding the mechanisms by which specific viruses may trigger a cascade of alterations in the CNS ultimately leading to epilepsy appears critical for the development of therapeutic agents that may target the virus or inflammatory mechanisms early and prevent progression of epileptogenesis.

Keywords HHV-6 · Picornaviruses · Seizures · Inflammation · Theiler's murine encephalomyelitis virus

Introduction

Growing experimental and clinical evidence suggests an important role for viral infections as one of the triggers of an inflammatory cascade that may ultimately lead to seizure generation. While other pathogens, such as for example influenza virus [1], dengue virus [2], and Arboviruses [3], have been shown to cause systemic infections sometimes resulting in seizures, this review will focus on the role of ubiquitous neurotropic viruses belonging to the herpesviruses family, particularly human herpesvirus 6 (HHV-6), and picornaviruses. We will discuss clinical associations, potential pathogenic mechanisms of sterile inflammation, and viruses as trigger for inflammatory responses that may be associated with acute seizure generation and epileptogenesis. Finally, we will discuss a seizure model in mice induced by a picornavirus, Theiler's murine encephalomyelitis virus (TMEV), to better understand the role of the innate immune response in the development of acute seizures.

Infection in humans with picornaviruses, particularly severe forms of enteroviral infection [4] and infantile encephalitis with human parechovirus [5, 6], can manifest with acute seizures.

Primary infection with herpesviruses and reactivation have been associated with different forms of seizures in children, ranging from acute symptomatic seizures secondary to encephalitis [7], to febrile seizures [8], status epilepticus [9], and temporal lobe epilepsy (TLE) [10]. Early provoked seizures as the result of a viral infection, particularly encephalitis, are considered a risk factor for later development of chronic epilepsy [11], although the exact mechanism of epileptogenesis in these cases is still unclear [12]. Current hypotheses describe several contributing factors during the period between infection and onset of chronic epilepsy, that involve overexpression of inflammatory mediators, damage to the blood-brain barrier leading to neuronal hyperexcitability,

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neuronal cell loss, alteration of neuronal circuits, and modification of receptors and ion channels [13].

Viruses can enter the central nervous system (CNS) during initial viremia or retrograde neuronal spread and herpesviruses are also able to infect peripheral neurons. After the virus enters the CNS, direct infection of neurons can result in cell death and release of proinflammatory mediators which in turn may activate the innate first and then adaptive immune systems. Understanding the mechanisms by which specific viruses may trigger a cascade of alterations in the CNS ultimately leading to epilepsy appears critical for the development of therapeutic agents that may target the virus or inflammatory mechanisms early and prevent progression of epileptogenesis [12].

Role of Sterile Inflammation in Seizure Generation

Different mechanisms and triggers can be involved in an inflammatory response involving the nervous system which may contribute to seizure generation. Non-infectious brain damage, including enhanced neuronal activity, as it occurs during seizures, evokes neuroinflammation. This *sterile* form of neuroinflammation has been recently defined as "neurogenic inflammation" [14]. This process contributes to the pathogenesis of epilepsy, since it is not properly and timely resolved, and it has a widespread induction.

Notably, the large array of inflammatory molecules released by resident brain cells during epileptic activity (i.e., cytokines, chemokines, alarmins/danger signals, prostaglandins, complement factors, etc.) act as neuromodulators. They impact on neuronal function and excitability, thus having a CNS-specific role independent of their role in the classical immune/inflammatory responses. Specific inflammatory mediators were reported to significantly contribute to the mechanisms of seizure generation and epileptogenesis in preclinical models [15, 16] and in the clinical setting [17, 18].

Besides the classical induction of Nf-κB/activator protein (AP)1-mediated gene transcription, proinflammatory cytokines, prostaglandins, and danger signals act directly on neuronal function by altering voltage- and receptor-gated ion channels function in target neurons via post-translational modifications [16, 19–21]. IL-1β and TNF-α increase Ca^{2+} permeability of *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid (AMPA) receptor-gated ion channels respectively, by modifying receptor subunit composition at the membrane level [22, 23]. These cytokines also induce endocytosis of γ-aminobutyric acid $(GABA)_A$ receptors and inhibition of GABA-mediated Cl^- fluxes [23, 24]. All these changes contribute to the rapid increase in neuronal network excitability, and to the generation of seizures and seizure-related neuronal damage.

Indirect effects of inflammatory mediators on neuronal excitability have also been described, and they include changes in endothelial and astrocytic cell physiology. Among these mechanisms, the inflammation-mediated alteration of the blood-brain barrier permeability has a prominent role; these changes are one of the hallmarks of epileptogenic tissue. Inflammatory molecules released by perivascular glia promote the downregulation of tight junctions on microvasculature [25–27], thus favoring the extravasation of serum albumin into the brain parenchyma. This event activates transforming growth factor (TGF)-β signaling in astrocytes, thus inducing a plethora of phenomena such as (1) the transcriptional activation of inflammatory genes in astrocytes, and the concomitant downregulation of Kir4.1 potassium channels and glutamate transporter [28, 29]; and (2) the degradation of perineuronal nets—a protective structure of the extracellular matrix that provide synaptic stability and restrict reorganization of inhibitory interneurons [30]. These changes contribute to establish a hyperexcitable neuronal network in surrounding tissue [29], and long-lasting decrease in seizure threshold [31]. Accordingly, experimental findings suggest that blockade of TGF-β signaling impacts on epileptogenesis and reduces chronic seizures [32].

Reactive astrogliosis is a common feature observed in epilepsy-associated pathologies [33, 34]. In epileptogenic tissue, astrocytes acquire an inflammatory phenotype, and show reduced K^+, water, and glutamate buffering capacity [33, 34], thus promoting neuronal hyperexcitability.

Specific Viruses Associated with Seizures and Epilepsy

Human Herpesvirus 6

HHV-6 is an enveloped DNA virus that belongs to the 헥-herpesvirus family similar to cytomegalovirus (CMV). Primary infection with HHV-6 occurs in almost 80% of children by age 2 years with peak incidence of acquisition between 9 and 21 months and is therefore considered ubiquitous. The virus is usually transmitted through saliva and acute infection may result in one of the classic exanthematous diseases of childhood known as roseola or sixth disease. Common symptoms include fussiness (70%), rhinorrhea (66%), fever (58%), and rash (31%) [35], but infection may also result in more serious neurological manifestations such as seizures [36] and encephalitis.

There are two species of this virus, HHV-6A and B, which share approximately 90% homology. Although HHV-6A is thought to be more neurotropic, HHV-6B is the primary type causing roseola. A peculiar aspect of this virus is that it can integrate near telomeres of infected cells, a mechanism known as chromosomal integration; as a result, up to 2% of infected

individuals have the complete HHV-6 genome in every cell of their body and can transmit the virus vertically in Mendelian fashion. The mechanisms involved in HHV-6 integration are still largely unknown; it is hypothesized that through homologous recombination between the telomeric repeat sequence present within the HHV-6 genome and the telomeres, the HHV-6 genome gets integrated within human chromosomes [37]. Viral load, detected by PCR, in the case of chromosomal integration is much higher (1 copy/cell) than what is observed with viremia during primary infection [38], but primary infection can also result in high viral load and to discriminate between the two, in-situ hybridization is sometimes needed [39].

Detection of HHV-6 in Febrile Seizures and Status Epilepticus

Febrile seizures are associated with a variety of infections other than HHV-6, such as respiratory syncytial virus [40], influenza virus A [41, 42], and adenovirus [41]. Seizures may be the result of a complex interplay between exogenous pyrogens, endogenous pyrogens [43], and virus-mediated effects [44], which are often difficult to distinguish especially in a clinical setting.

Prior reviews of the literature between 1995 and 2004 identified 902 patients of age less than 3 years with primary infection with HHV-6 and fever and estimated that 16% of them had a seizure at presentation, including focal, generalized, and status epilepticus [45]. A prospective observational study that enrolled 1653 children under 3 years of age who presented to the emergency room with acute febrile illness detected primary HHV-6 infection (documented by viremia and seroconversion) in 160 children and 13% of them had seizures [46].

Other investigators came to different conclusions. A population-based study that followed 277 infants from birth until age 2 years analyzed serial saliva (and when available blood) samples by PCR, and showed a cumulative incidence of HHV-6 infection of 77% by 24 months of age but none of these children had seizures [35]. The Consequences of Prolonged Febrile Seizures in Childhood (FEBSTAT) study [9] was a large prospective multicenter study that aimed to evaluate the frequency of HHV-6 and HHV-7 infection using qPCR analysis of blood from children aged 1 month–5 years presenting with febrile status epilepticus. HHV-6B viremia was found in 54 of 169 children (32%), including 38 with primary infection and 16 with reactivated infection, defined as the presence of viral-specific antibodies at baseline in the presence of viremia. This frequency was higher than what had been reported in historical controls with acute non-febrile illness and in controls without acute illness [46].

An important consideration has to be made regarding significant genetic heterogeneity as highlighted by differences in both prevalence and clinical course/outcome between western studies and Asian and also among Asian studies themselves regarding HHV-6 and seizures; as an example, a geographic variability and lesser role for HHV-6 infection in the etiology is observed in Malaysia [47] and Thailand [48] compared with the USA and Canada [45].

HHV-6 Encephalitis

HHV-6 encephalitis is a rare occurrence, with a reported incidence of 0–12% after bone marrow or peripheral blood stem cell transplantation and 5–21% after cord blood transplantation [49]. Of 1000 patients enrolled in the California Encephalitis Project, 4 immunocompetent children tested positive for HHV-6 by means of PCR [50]. This form of encephalitis is frequently accompanied by seizures and it has also been described in immunocompetent children during primary infection in the context of roseola [51, 52]. A nationwide survey that included responses from 2293 hospitals in Japan between 2003 and 2004 reported 86 cases of exanthema subitum-associated encephalitis, primarily in children younger than 2 years, diagnosed by serology (53 patients) and PCR (33 patients). Full clinical data were available only for 60 patients, 72% of which had convulsions and altered mental status, and 28% of which had isolated seizures. HHV-6 DNA was detected in half of patients whose cerebrospinal fluid (CSF) was tested [53].

Role of HHV-6 in Mesial Temporal Lobe Epilepsy

HHV-6B was detected via rtPCR in brain specimens from four of eight patients with mesial temporal lobe epilepsy (MTLE) and from none of seven patients with neocortical epilepsy. In the subset of MTLE patients, the investigators localized viral antigen in glial fibrillary acidic protein (GFAP)-positive astrocytes [54]. A confirmatory study detected HHV-6B viral DNA by TaqMan PCR in surgical specimens from 11 of 16 additional patients with MTLE and from none of 7 additional patients without MTLE [10], with the highest viral load being found in the hippocampus. Other studies confirmed these findings in larger cohorts, suggesting a potential role for the virus in the development of MTLE [55, 56].

Several potential mechanisms have been hypothesized on how HHV-6B infection leads to glutamatergic excitotoxicity and neuronal damage in the mesial temporal lobe. It is possible that pathologic changes are the result of reactivation of latent virus due to an unknown trigger or are the result of damage from persistent subclinical active infection. Astrocyte cultures infected in vitro with HHV-6 had a significant decrease in expression of the glutamate transporter excitatory amino acid transporter 2 (EAAT2) [10]. Classic inflammatory changes such as cellular infiltrates are lacking in resected epileptogenic tissue where HHV-6 is detected [36]. Experimental evidence suggests other mechanisms are involved such as modulation of neuroinflammation mediated by chemokines/cytokines. Investigation of gene expression

revealed upregulation of GFAP and chemokine (C-C motif) ligand 2 (CCL2) in the amygdala of MTLE patients with HHV-6 infection and a positive correlation between expression level and viral load existed [55]. The increased expression of these proteins results in gliosis and neuronal loss, which may contribute to the development of MTLE [57, 58]. High expression of GFAP has been previously shown in resected epileptogenic tissue from the hippocampus [59]. CCL2 is an essential chemokine that regulates migration and infiltration of monocytes. HHV-6 can establish latent infection in these cells; therefore, increased expression of CCL2 can facilitate migration of infected cells into the amygdala and be conducive to chronic changes seen in MTLE. Some evidence suggests a potential role of upregulation of the transcription factor nuclear factor-κB (NF-κB) in patients with HHV-6 infection who develop MTLE [56].

Pathophysiology of Neuroinflammation Associated with HHV-6

HHV-6 exhibits neuroinvasive and proinflammatory properties. Studies suggested that this virus can invade the CNS and persist beyond primary infection [60]. Pathological analyses revealed that HHV-6 can infect astrocytes and oligodendrocytes [61] leading to upregulation of several proinflammatory cytokines, including interleukin (IL)-1β, interferon (IFN)-α, and tumor necrosis factor (TNF)-α, and can infect T cells leading to a reduction of IL-10 and IL-14 gene expression, suggesting that HHV-6 infection favors a T helper (Th) 1 type proinflammatory cytokine response [62]. Other hypotheses on how HHV-6 can induce neuroinflammation include cross-reaction via molecular mimicry of viral antigens, leukocyte chemoattraction mediated by U83, a chemokine-like protein encoded by the virus that promotes monocyte infiltration, infection of CNS endothelial cells with upregulation of proinflammatory chemokines resulting in increased permeability of the blood-brain barrier, and binding to CD46 with modulation of the adaptive immune response, with induction of IL-17 and inhibition of IL-10 production by T cells [63]. This latter mechanism involving complement activation has been suggested to contribute to neuroinflammation in patients affected by multiple sclerosis [64] and, as will be discussed later, complement also plays an important role in acute seizure generation in the TMEV-infected mouse model, and could, therefore, represent a common pathway for different forms of infection.

Epstein-Barr Virus, Herpes Simplex Virus, Varicella-Zoster Virus, Cytomegalovirus

Neurologic complications of Epstein-Barr virus (EBV) infection are not rare, accounting for up to 7% of EBV-related admissions in large historical series, and include encephalitis with seizures [65], status epilepticus [66], and even a case of infantile spasms [67]. EBV DNA was also detected in brain specimens from TLE patients [68]. Pathogenic mechanisms responsible for neurologic symptoms include a direct viral effect on neurons; indirect effect mediated by proinflammatory cytokines such as TNF-α, IL-6, and IL-1β, which are secreted from infected and immortalized B cells; and bystander damage caused by the interaction between infected, immortalized B cells and the T cell response against them [69].

Herpes simplex virus (HSV) is the pathogenic agent of approximately 10% of cases of encephalitis [70] and 50–100% of children with HSV-1 encephalitis develop seizures [71], particularly focal seizures. HSV, similar to HHV-6, most likely gains access to the CNS via the olfactory pathway and has particular tropism for the mesiotemporal lobe and orbitofrontal region of the brain. Several proinflammatory signaling pathways are implicated in the robust immune response that follows infection with HSV, and often leads to acute seizures. Dimerization of Toll-like receptors with subsequent production of several interleukins, interferons, and other inflammatory mediators appears to be an early mechanism [72]. Reduced dynorphin expression in dentate gyrus of the hippocampus due to HSV-1 infection was found in a mouse model and suggests an alteration in hippocampal excitability as a potential neurochemical basis for seizure generation [73].

Seizures are described among several major possible neurological complications of Varicella-Zoster virus (VZV) infection, either as isolated seizures or in the context of meningoencephalitis. In a pediatric study that analyzed neurological manifestations in patients that had a classic chickenpox rash or positive CSF PCR [74], 3 of 16 children who initially presented with isolated seizures developed epilepsy at the 1-year follow-up. Up to 40% of CNS VZV infections can occur without herpetic rash [75]; therefore, it is likely that several studies that utilized solely a clinical criterion for inclusion underestimated the real extent of VZV-related neurological manifestations, including seizures.

Asymptomatic or paucisymptomatic cytomegalovirus (CMV) infection is very common and the estimated seroprevalence worldwide is 60–100% [76]. CMV encephalitis is rare in immunocompetent hosts, and is usually seen as an acute monophasic infection, with seizures frequently described as part of the neurological manifestations [77].

Viruses in Brain Tissue of Subjects Without Epilepsy

PCR-based studies that analyzed control brain tissue found HHV-6 DNA in brain samples from individuals with neurological diseases other than epilepsy such as multiple sclerosis and brain tumors [78–80], but usually at lower viral load than samples obtained from resected epileptogenic foci. Similarly, viral RNA from other viruses such as EBV, CMV, HSV, and VZV was found in brain specimens from patients with schizophrenia and controls [81]. These findings suggest that

different viruses may establish a latent infection in the brain even under normal conditions, and highlight variability in detection rates, potentially owing to different selection criteria for controls and sensitivity of the assays across different laboratories. They also suggest that presence of the virus alone does not prove causation and that several other factors, including clinical context, specific type of epilepsy, and viral load, need to be taken into account when analyzing the role of viruses in epileptogenesis.

Picornaviruses and Clinical Associations with Seizures

Enteroviruses are among the most commonly identified pathogens in pediatric patients with aseptic meningitis, sometimes complicated by seizures [82].

Major pathogens of hand, foot, and mouth disease in children are enterovirus 71 (EV71) and coxsackievirus A16 (CA16), both of which are non-enveloped, single-stranded RNA viruses belonging to family Picornaviridae. Typically, this disease affects children younger than 5 years of age and is mild and self-limited, but severe cases with encephalitis with seizures are described [83]. In a series from Thailand including 156 children, 18/25 severe cases of enterovirus infection included seizures as initial manifestation [4]. EV71 was the major cause of severe disease, including four fatal cases.

Similarly, human parechoviruses, especially genotype 3, are an increasingly recognized cause of meningoencephalitis in young children, particularly ex-premature babies [5, 84]. Up to 90% of affected neonates can present with seizures and may go on to develop long-term neurodevelopmental disability [5]. Large prospective surveillance studies that analyzed stool samples of 284 children with suspected CNS infection/inflammation via PCR showed that 4% were positive for human parechovirus and 15% for adenovirus, after ruling out other etiologies. Human parechovirus-positive patients were more likely to present with seizures when compared to human parechovirus-negative patients [6].

Pathogenic Mechanisms Linked to Epilepsy Following Viral Infection of the CNS

Insights into triggers and the mechanisms by which inflammatory molecules can affect neuronal excitability and lead to seizure generation are summarized in Fig. 1.

Seizures have previously been induced through viral infection in rabbits, rats, and mice [85]. However, in most instances, acute encephalitis resulting from the viral infection causes the death of the animals [85]. Therefore, although multiple animal models exist of acute or chronic viral encephalitis, such as the persistent Borna disease virus (strain He/80-1) infection of Lewis rats [86], the West Nile virus (Sarafend strain) infection of C57BL/6 mice [87], the equid herpesvirus type-1 (Brazilian strains A4/72 and A9/92) infection of mice [88], and more recently the Zika virus (strain PRVABC59) infection of AG129 mice [89] which allow for the study of acute seizures, they are not suitable for the study of epileptogenesis and epilepsy. As it stands, only the TMEV-induced model exists to investigate viral and host immune contributions to the development of seizures and/or epilepsy during and following viral encephalitis. The TMEV-induced seizure model is unique in that epilepsy occurs late, after acute viral encephalitis with seizures, after the virus is cleared and the acute seizures resolve, and after a latent period [12, 90–95]. Close examination shows that this model recapitulates many of the clinical and pathological characteristics that are commonly seen in human TLE following encephalitis, such as CNS inflammation [92, 93, 96–98], hippocampal sclerosis with reactive gliosis [97–100], neuron loss [92, 96, 97, 99, 101–103], and anxiety-like and cognitive deficit behavioral comorbidities [96, 99, 101, 103, 104]. Thus, the TMEV-induced seizure model allows for the study of the mechanisms involved in the induction and progression of postinfection epileptogenesis in an animal model that closely reflects the prevalent form of human epilepsy, TLE, which has also been associated with herpesvirus encephalitis.

TMEV is a picornavirus which, upon intracerebral infection of C57BL/6J mice, causes acute behavioral seizures in a percentage of the mice infected, dependent on the viral titer in the inoculum [91, 93, 105]. The acute seizures are observable between days 3 and 10 post infection [92, 94]. A mechanistic role for viral encephalitis with acute seizures in the subsequent development of epilepsy was suggested by (1) the presence of significantly reduced limbic and forebrain seizure thresholds, equating with chronically increased seizure susceptibility; (2) increased hyperexcitability, detected through corneal kindling, at 2 months post infection [95]; and (3) the detection of spontaneous seizures, in a significant proportion (65%) of the mice that had experienced acute seizures, at 2–4 months post infection [95]. Therefore, the occurrence of acute seizures during acute viral encephalitis appears to set the stage for the subsequent development of epilepsy. Examination of the induction, development, and consequences of acute seizures should be informative as to the mechanisms underlying the later development of epilepsy.

Role of the Innate Immune System

Upon viral infection, the host mounts an immune response within hours that initially involves only the innate immune response [96, 101, 106–108]. The appearance of seizures as early as day 3 post infection, prior to the activation of the adaptive immune response (discussed below), suggests that the innate immune response to viral infection likely contributes to the development of acute seizures. Macrophages and

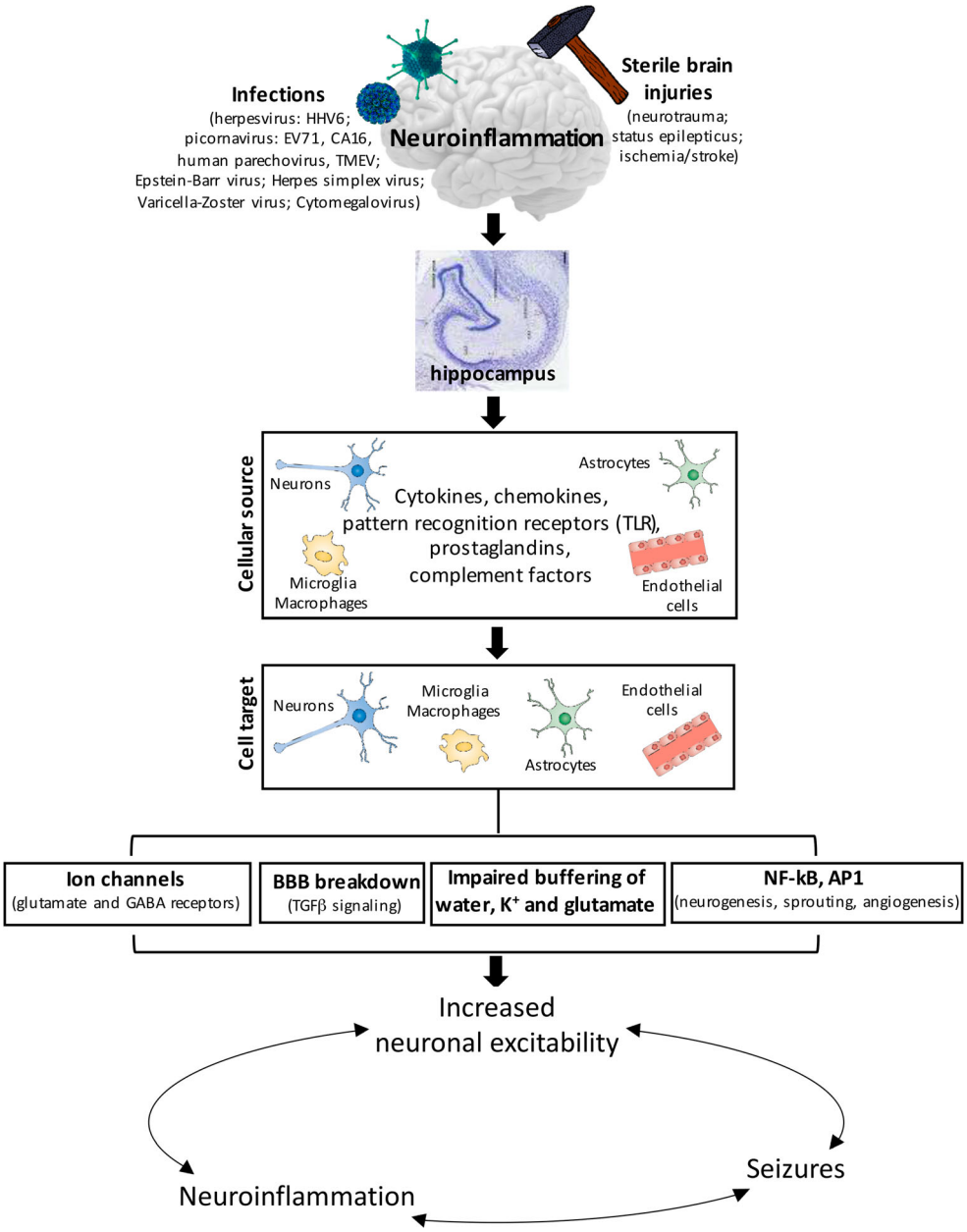

Fig. 1 Schematic representation of the chain of triggers and molecular events linking neuroinflammation to neuronal hyperexcitability and seizures

microglia, effector cells of the innate immune response, and the proinflammatory cytokines IL-6 and TNF-α produced by these effector cells have been shown to be instrumental in the development of acute seizures in the TMEV-induced seizure model [93, 94, 98, 109]. Infiltrating macrophages were found to be the major producer of IL-6, while microglia were found to be the major producer of TNF-α in the CNS. A twofold reduction in the number of macrophages infiltrating into the CNS was shown to be sufficient to significantly reduce the number of infected mice experiencing acute seizures [109]. Near complete in vivo depletion of macrophages from the periphery via intraperitoneal or intravenous injection of clodronate-containing liposomes resulted in a significant reduction in the number of infected mice experiencing acute seizures without altering the levels of TMEV antigen-positive cells in the brain [107, 110]. Conversely, adoptive transfer of bone marrow-derived monocytes (without in vitro differentiation into macrophages) directly into the brains of TMEV-infected mice significantly increased the number of mice experiencing acute seizures [91, 107].

Examination of TMEV-infected mouse brains prior to the day 3 time point demonstrated that hippocampal neuron loss occurred early post infection, was via apoptosis, and was dissociated from direct viral infection of the neurons [101, 111]. This hippocampal damage was instead caused by infiltrating inflammatory monocytes ($CD45^{hi}CD11b^{++}F4/80^{+}Gr1^{+}1A8^{-}$), not neutrophils ($CD45^{hi}CD11b^{+++}F4/80^{-}Gr1^{+}1A8^{+}$), although both cell types were present [96,

111]. Expression of the chemokine CCL2 by neurons drove the infiltration [112].

Examination of TMEV-infected mouse brains over the time course of seizures, days 3, 7, and 10 post infection, demonstrated that the peak of cellular infiltration of macrophages corresponded with the peak of seizures (day 7 post infection) and that these infiltrating macrophages were inflammatory macrophages ($CD45^{hi}$ $CD11b^{+}$ $Ly\text{-}6C^{+}$), not patrolling macrophages ($CD45^{hi}$ $CD11b^{+}$ $Ly\text{-}6C^{-}$) which increased over the time course and were highest at day 10 post infection [107].

Another component of the innate immune response, the complement system, has been shown to be involved in the development of acute seizures in this seizure model [100, 113]. More specifically, complement component 3 (C3) activation within the CNS, more specifically within inflammatory macrophages and activated microglia, contributes to the development of acute seizures, and its contribution may be through the IL-6 and TNF-α pathways [100, 113].

Other components of the innate immune response include neutrophils and natural killer cells. Although present in the CNS following infection with TMEV, both of these effector cell types have been discounted as contributing to the development of acute seizures in the TMEV-induced seizure model [114].

Role of the Adaptive Immune Response

The adaptive immune response develops days to weeks following the initial viral insult and is antigen specific [115]. The effector cells of the adaptive immune response include $CD4^{+}$ and $CD8^{+}$ T cells and B cells (lymphocytes), and these effector cells produce cytokines and antibodies. The numbers of lymphocytes in the brains of TMEV-infected mice were found to be elevated over the time course of seizures, days 3, 7, and 10 post infection, whether the mice were experiencing seizures or not [91], suggesting that these cells are not involved in the development of acute seizures. More specifically, $CD8^{+}$ T cells were shown not to be involved in the development of acute seizures through the use of OT-I mice with an ovalbumin-specific T cell receptor [98]. Additionally, TMEV infection of $RAG^{-/-}$ mice, which are deficient in mature T and B cells, resulted in a comparable number of mice experiencing acute seizures as control mice [107]. Finally, adoptive transfer of spleen-derived T cells directly into the brains of TMEV-infected mice did not lead to a significant increase in the numbers of mice experiencing acute seizures [107].

From Acute Seizures to Epilepsy

The immune response of the host to viral infection is important in order to effectively combat the infection and to prevent damage or repair tissues. TMEV infection of the CNS induces an immune response consisting of both inflammatory macrophages and lymphocytes. The rapid response made by the inflammatory macrophages likely serves to stem the replication of the virus but also, through their production of IL-6, induces acute seizures. The increasing and sustained response of the lymphocytes likely clears the virus from the CNS and late responding patrolling macrophages may function in the resolution of inflammation and repair of tissue damage.

Proinflammatory cytokines, such as IL-6 and TNF-α, may induce hyperexcitation leading to excitotoxicity and seizures. Additional support for a role for cytokines in seizure development comes through the study of a variant of TMEV named H101 [116, 117]. This viral variant does not replicate within the brain parenchyma [105, 118]; however, a significant proportion of C57BL/6J mice intracerebrally infected with this viral variant still develop seizures [105, 116]. Infiltration of macrophages into the CNS and activation of microglia was reduced, but IL-6 and TNF-α were found to be significantly higher in the serum in H101-infected animals [116]. Peripheral administration of recombinant IL-6 to these animals resulted in an increase in both macrophage infiltration/microglial activation and the number of mice experiencing seizures [116]. Therefore, when viral replication within the brain is limited, pathologic levels of IL-6 in the periphery may play a role in seizure development in this model. Serum levels of IL-6 have been found to be elevated in TLE patients as well, confirming the importance of this cytokine in the most common form of epilepsy [119].

IL-6 and TNF-α may induce seizures through modulation of glutamate signaling. IL-6 has been shown to disrupt the balance of neuronal excitation/inhibition by affecting glutamate clearance [120], the expression and/or function of glutamate receptors and receptor subunits [121], and by decreasing inhibitory tone [122]. TNF-α has been shown to contribute to hyperexcitability by affecting the expression of glutamate receptors and receptor subunits. Therefore, acute seizures during viral encephalitis likely result from disruption of the excitatory/inhibitory balance induced by proinflammatory cytokines acting through glutamate. The role of glutamate and glutamate signaling in the development of acute behavioral seizures is being actively investigated in the TMEV-induced seizure model [123].

Acute TMEV infection, the immune response to virus, and the presence of acute seizures leave lasting marks on the hippocampus. Mice which experienced acute seizures and then went on to develop epilepsy have extensive hippocampal sclerosis [95]. Neuronal loss, persistent activation of microglia and astrocytes, glial proliferation, and glial scarring likely contribute to a lasting neural network hyperexcitability that in turn likely contributes to epileptogenesis and seizure generation [97]. Although significant increases in the amplitude and frequency of spontaneous and miniature excitatory currents in CA3 pyramidal neurons of the hippocampus were recorded in brain slices during both the acute infection and

2 months post infection, the patterns of changes observed were different, suggesting pathological long-term changes in the network over time [124]. Analysis of inhibitory currents in CA3 pyramidal neurons demonstrated an initial decrease in inhibition during the acute infection, measured via amplitude of spontaneous and miniature inhibitory currents, which was lost at 2 months post infection [125]. Thus, two different mechanisms may be operating to cause an excitatory/inhibitory imbalance during the acute infection with seizures and during epilepsy in the TMEV-induced seizure model [124, 125].

Conclusions

Infection with herpesviruses, especially HHV-6, appears to be an important trigger for acute seizure generation and in certain cases later development of epilepsy. Growing preclinical data and observations in biological specimens from children with seizures have identified a complex cascade of specific neuroinflammatory mechanisms that may contribute significantly to the pathophysiology of epilepsy, and potentially represent an early therapeutic target.

Funding Dr. Bartolini reports receiving grant support from the American Epilepsy Society and Epilepsy Foundation of America. Dr. Ravizza reports receiving grant support from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n°602102 (EPITARGET), and Fondazione Italo Monzino. Dr. Gaillard reports receiving grant support from NIH, CDC, NSF, PCORI, American Epilepsy Society, Epilepsy Foundation, CURE, and the Pediatric Epilepsy Research Foundation. Dr. Fujinami reports receiving grant support from NIH (R01NS065714).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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