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Seminars in
Pediatric
Neurology

Experimental Studies in Epilepsy: Immunologic and Inflammatory Mechanisms

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In this article, we review the literature based on experimental studies lending credence to a relationship between epilepsy and immune-mediated mechanisms linked to central nervous system innate immunity. The brain innate immunity responses to neuronal injury or excessive neuronal activity are mediated by resident microglia and astroglia, but also neurons play an immunomodulatory role. Antigens or antibodies applied to the brain trigger an epileptogenic and inflammatory response. Furthermore, seizure activity and status epilepticus elicit the production and release of proinflammatory cytokines and chemokines. The immune pathogenesis of epilepsy involves complex cell-to-cell interactions including a cross talk between astrocytes and neurons, between astrocytes and brain microvascular endothelial cells, as well as reciprocal leukocyte-endothelial interactions in the context of disruption of the blood-brain barrier. There is a large body of literature from experimental studies showing that seizures can initiate a cascade of innate and adaptive immune responses from various cellular sources and perpetuate neuroinflammation through mechanisms involving transcription of inflammatory genes or posttranslational changes in cytokine release machinery. These inflammatory processes could also possibly contribute to the pathogenesis of comorbidities often associated with epilepsy. This opens exciting possibilities for the development of disease-modifying drugs aimed at mitigating neuroinflammation as a means of ameliorating epileptogenesis and lessening or preventing postictal brain injury.

Semin Pediatr Neurol 21:197-206

Introduction

The association between immune-mediated inflammation and excitatory activity in the brain was already suspected at the beginning of the 20th century with the experiments performed by the French physician and biologist Camille Delezenne.[1] He injected, into the anterior brain of dogs, serum containing antibodies obtained in rabbits and ducks by administering emulsions of canine liver and brain. The animals developed epileptic discharges, with most of them becoming paralyzed and some showing epileptic salivation and clonic-tonic convulsions.

Since these early discoveries, progress on basic research methodologies and molecular biology discoveries has advanced this area of neurology, and now the hypothesis of an association between epilepsy, immune system, and inflammation is a field of strong research and clinic interest.

In this article, we review the experimental studies ar basic science mechanisms of immune-mediated inflamm tion as they relate to epilepsy. We first describe th immunologic characteristics of the brain with the potenti of causing an inflammatory response. This review followed by a historical perspective of the scientific exper ments that have supported such pathogenic relationship.

Immunologic Characteristics of the Brain

Neuroinflammation

The central nervous system (CNS) was regarded as a immune-privileged site in the presence of an intact blood brain barrier (BBB). However, in recent years, this time honored concept has been the subject of critical reappraisa given the emerging role of CNS-resident cells as innat immune-competent cells.[2-6] A large body of literature ha come to light lending credence to the concept o

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1071-9091/14/$-see front matter
http://dx.doi.org/10.1016/j.spen.2014.10.001

neuroinflammation as an intricate and specialized immune response system triggered by a variety of pathologic factors, including infectious pathogens, toxins, traumatic brain injuries, hypoxic-ischemic and excitotoxic insults, as well as perturbed neuronal-glial interactions in the context of underlying degenerative and developmental abnormalities.[7] Altered inflammatory mediator profiles may have significant implications in a host of neurologic and neuropsychiatric disorders ranging from neuropathic pain and epilepsy to neurodegenerative diseases.[8,9]

Inflammation is a highly dynamic and complex adaptive process aimed at preserving tissue integrity, limiting cellular injury, and restoring tissue homeostasis.[10] Inflammatory responses in the CNS are governed by a constant interaction of the innate and the adaptive immune systems involving resident microglia and astrocytic glia as well as infiltrating immune cells (leukocytes), which are recruited from the periphery.[10] Common neurologic disorders, such as stroke, multiple sclerosis, and neurodegenerative diseases, elicit immune-mediated inflammatory responses aimed at curtailing the extent of tissue damage and facilitating tissue repair. Neuroinflammatory responses may also serve to maintain homeostasis, effectively enabling the CNS to deal with enhanced metabolic demands, and increase the computational power and plasticity of neural networks.[7] However, these proinflammatory responses may also have an adverse role on the disease process itself.[10] Neuroinflammation may exacerbate the neuropathologic process itself, and exaggerated neuroinflammatory responses may become maladaptive and ultimately worsen the outcomes of various neurologic disorders, including epilepsy.[7,11]

The cross talk between the immune and nervous systems is linked to the production of proinflammatory cytokines by microglia and astrocytic glia, which constitute major indigenous cellular sources of inflammatory mediators in the CNS.[8] Glial cells respond also to proinflammatory signals released by immune cells residing in the CNS, and in this regard, the relevance of mast cell-glial interactions is becoming increasingly recognized.[8,9,12] Such interactions may contribute to symptomatic exacerbation and acceleration of disease progression.[8,9,12] Targeting neuroinflammation opens exciting possibilities for the development of new innovative therapies in the context of diverse chronic neurologic diseases.[8,9,12]

Cell Types Involved in Neuroinflammation

Compared with the predominance of hematogenously derived immune cells (leukocytes), such as polymorphonuclear leukocytes, eosinophils, lymphocytes (B and T cells), plasma cells, and monocytes-macrophages, which are encountered in diverse inflammatory processes of the CNS, neuroinflammation involves to a significant extent indigenous cellular sources of inflammatory mediators, which may be expressed in a spectrum of neurologic diseases that are not conventionally regarded as "inflammatory." Such CNS-resident cells include mesenchymal cell types, such as microglia and microvascular endothelial cells of cerebral blood vessels, neuroepithelial cell types, such as astroglial cells (astrocytes), and inflammatory mast cells. When assessing the immune system of the CNS, one should always take into consideration the immune regulatory functions of the BBB, a dynamic cellular interface, the integrity of which is commonly disrupted and compromised in a host of neuropathologic processes.[8,13]

The major CNS cell types, that is, glia and neurons, have innate immune functions and are capable of expressing immunoregulatory receptors whose function is to recognize and clear apoptotic cells.[14] Phagocytosis of apoptotic cells is a noninflammatory process that provides immune regulation through anti-inflammatory cytokines and regulatory T cells. Neurons and glia produce cellular death signals, including CD95Fas or CD95L, FasL, tumor necrosis factor (TNF)-related apoptosis-inducing ligand, and TNF receptor 1, by which they trigger apoptosis in T cells and other infiltrating inflammatory cells.[14] Collectively, microglia, astrocytes, ependymal cells, and neurons express defense collagens and phagocytic receptors that recognize molecular profiles of apoptotic cells serving as markers of "altered self."[14]

The CNS is an immunologically specialized organ.[15] The BBB regulates the passage of molecules and cells into the CNS.[16] Robust immune responses occur in the CNS even though there is normally an absence of major histocompatibility complex (MHC) molecules, lack of normal lymphatic drainage, and reduced immune surveillance.[16] Astrocytes, endothelial cells or cerebral blood vessels, microglia, macrophages, and dendritic cells partake in the innate and adaptive immunity of the CNS and CD4-mediated T-cell autoimmune responses directed against neural antigens.[15]

The CNS encompasses both innate and adaptive immune responses. The former is a nonspecific response, which amounts to an acute defense against external noxious agents (pathogens or toxins) or focal brain damage.[3] The cell types predominantly involved in the innate immune response include monocytes or macrophages, microglia, and glial cells (astrocytes).[3] The adaptive immune response is antigen-specific and involves the recruitment of B and T lymphocytes. Communication between the innate and adaptive responses entails cell-to-cell interactions and the production of soluble signaling factors such as cytokines and chemokines.[3]

Microglia

Besides their phagocytic function, microglia have a key role in the regulation of inflammatory and adaptive immune responses through signals regulating microglial innate immune functions and also by virtue of their role in antigen presentation.[16,17] The immune surveillance of the CNS in most neurologic disorders involves activation and in some cases, dysregulation of microglia.[17] Accordingly, in a variety of CNS pathologic processes, including inflammatory, vascular, degenerative, developmental, and neoplastic diseases, microglia (and to a lesser degree, astrocytic glia) acquire antigen-presenting properties as evidenced by the expression of MHC molecules. Moreover, resident microglial and astroglial cells produce proinflammatory cytokines,

such as TNF-α, interleukin-1 beta (IL-1β), and interferon-gamma, as well as chemokines, eliciting the recruitment and infiltration of T lymphocytes into the pathologically damaged brain tissue.[17] Concomitantly, the proinflammatory cytokines stimulate microglial MHC expression in lesional CNS areas.[17] Conversely, the induction of brain immunity is counterregulated by neuronal signals in intact CNS areas.[18] In the developing human fetal CNS, microglial cells invade and migrate to the human cortical plate and telencephalic white matter according to a specific spatiotemporal gradient during the first 2 trimesters of gestation.[19] The spatiotemporal organization of microglia in the fetal brain suggests that these cells may play active roles in developmental processes and in prenatal brain injury.[19]

Resident macrophages and dendritic cells have also been demonstrated in the dura mater, pia mater, and choroid plexus of mice.[20] These cells participate in immune surveillance, phagocytosis of cellular debris, uptake of antigens from the surrounding cerebrospinal fluid (CSF), and immune regulation in various pathologic processes.[20]

As resident immune cells of the CNS, microglia have diverse and versatile functional roles centered on surveillance of the microenvironment for changes in signaling, pathogens, and injury.[21] In recent years, the functional repertoire of microglia has been expanded to include regulation of synapse formation, elimination, and function under physiologic conditions and pathologic states.[21] As exemplified in traumatic brain injury and epilepsy, microglia have a role in synaptic stripping and remodelization in the context of neuronal-glial interactions.[21] As such, microglia may function as mediators of the transition between injury-induced circuit dismantling and subsequent reorganization making them therapeutic targets.[21] A multitude of potential messengers have been postulated as substrates of the communication between microglia and neurons, including cytokines, purines, prostaglandins, nitric oxide, and the brain-derived neurotrophic factor.[22]

Astroglia (Astrocytes)

Traditionally, the microglia were generally considered to be the sole players of innate immune responses in the CNS.[17] However, over the past decade, the role of astrocytes has been also increasingly recognized in this regard.[3] Astrocytes, the main glial cell types, initiate, regulate, and amplify immune-mediated mechanisms involved in divergent neurologic diseases, including epilepsy.[3,23-27]

Astrocytes, particularly reactive astrocytes, represent an important source as well as a target of inflammatory molecules, including cytokines, such as IL-1β, IL-6, TNF-α, transforming growth factor beta, and chemokines, such as monocyte chemoattractant protein-1 (also known as chemokine, C-C motif, ligand 2), which are highly expressed in brain tissue from patients with seizures.[3,28,29] Astrocytes are also the target of inflammatory molecules, which can exacerbate reactive glial scarring (gliosis) and amplify the proepileptogenic inflammatory signaling through the activation of *pattern-recognition receptors* and related intracellular signaling pathways.[25,27,28] Special attention has been placed on the role of the IL-1 receptor or toll-like receptor superfamily (IL-1R/TLR) in epilepsy.[28,30-33] The IL-1β or toll-like receptor 4 (TLR4) pathway can be a potential therapeutic target for the amelioration of intractable epilepsy.[3] As part of the innate immune response, reactive astrocytes are also a source of complement-regulatory proteins and complement receptors.[3,25] Glial cells express pentraxins and complement proteins (C1q, C3b, and iC3b) that opsonize apoptotic cells, making them targets for the phagocytic receptors CR3 and CR4.[14] Immunoregulatory molecules such as the complement regulator CD46 are lost from apoptotic cells, stimulating phagocytosis; conversely, there is increased expression of CD47 and CD200 during apoptosis, exerting an inhibitory effect on proinflammatory microglial cytokine production thus mitigating the severity of inflammation.[14]

Unlike the well-established role of microglia as antigen-presenting cells (APCs), the ability of astrocytes to act as fully competent APCs remains controversial.[3,17] The detection of MHC-expressing astrocytes in human brain specimens is a subject of debate, even in *bona fide* inflammatory processes such as multiple sclerosis.[3]

Another aspect of astrocyte immune function relates to signaling via neurotransmitter receptors by which astrocytes can sense and respond to alterations in the extracellular microenvironment, modulating the immune response under pathologic conditions associated with astrocytic gliosis.[3,24] Reactive astrocytes are characterized by upregulation of groups I and II metabotropic glutamate receptor (mGluR) subtypes (mGluR5 and mGluR3).[3] Activation of mGluR3 in cultured human astrocytes modulates the release of IL-6 in the presence of IL-1β, lending support for a functional role of this receptor subtype in regulating the production of inflammatory cytokines by activated astrocytes.[3] There is also increasing evidence pointing toward a critical role of purinergic receptors in neuroinflammation (reviewed in Aronica et al[3]).

Blood Brain Barrier, Perivascular Cells, and Microvascular Endothelial Cells

The CNS is considered to be immunologically privileged because it lacks conventional lymphatic channels and is highly dependent on receptor-mediated entry of inflammatory cells through the BBB.[34,35] That being said, the CNS is endowed with perivascular pathways, which drain interstitial fluid (from the brain parenchyma) and CSF (from the subarachnoid space) into cervical lymph nodes.[34] This pathway does not allow trafficking of lymphocytes or APCs from the CNS to cervical lymph nodes.[34] A minor alternate route of CSF drainage from the subarachnoid space is through channels in the cribriform plate of the ethmoid bone into nasal lymphatics and from there on to cervical lymph nodes, which allows trafficking of lymphocytes or APCs from the brain to lymph nodes.[34]

Leukocyte migration into the brain parenchyma is a critical stage in the pathogenesis of immune-mediated inflammatory diseases of the CNS. Bidirectional cross talk between the immune cell and endothelium is essential in mediating diapedesis during both normal immune surveillance and under inflammatory conditions. The pattern and route of

leukocyte migration and entry into the CNS are modulated by reciprocal signals between cell surface molecules such as integrins on leukocytes and immunoglobulin superfamily cell adhesion molecules on vascular endothelial cells.[34,36]

Perivascular cells, also referred to as perivascular macrophages, perivascular microglia, fluorescent granular perithelial cells, or Mato cells, are a heterogeneous population of cells in the CNS.[16,37] Different terminology also points to the lack of clear consensus of what cells are perivascular cells in different disease states and models, especially after disruption of the BBB.[37] Perivascular cells, although a minor component of the CNS, are important immunoregulatory cells and potential sensors of pathologic signals.[37] They are thought to be bone marrow derived and are activated during CNS inflammation, autoimmune disease, and neuronal damage.[37] Perivascular cells are also primary targets of human immunodeficiency virus and simian immunodeficiency virus infection in the CNS of humans and primates.[37,38]

Immune cells enter the CNS from the circulation under normal conditions for immunosurveillance and in inflammatory neurologic diseases. Regarding the BBB, there are distinctions between diffusion or transport of solutes, which is regulated at the level of capillaries, and migration of cells from the blood circulation to CNS parenchyma, which takes place in parenchymal postcapillary venules.[39] Entry of immune cells into the CNS parenchyma in inflammatory conditions involves 2 differently regulated steps: transmigration of the vascular wall into the perivascular space and progression across the glia limitans into the parenchyma.[39]

Dendritic cells have been implicated in the pathogenesis of neuroinflammation, and there is evidence that they are recruited to the brain across the BBB highlighting the active role of microvascular endothelial cells of the human CNS in neuroinflammation.[40]

Myeloid cells are mediators of CNS damage and recovery in neuroinflammatory and neurodegenerative disorders. Besides endogenous myelomonocytic cell populations that reside in the brain already during development, newly migrated leukocytes are considered as important disease modulators in the adult brain. The presence of the chemokine receptors CCR2 and CX(3)CR(1) is considered to be critical for both myeloid cell trafficking along inflamed blood vessels and subsequent accumulation in the brain.[41]

Experimental Models of Epilepsy, Immune System Activation, and Inflammatory Response

Induction of Epileptic Seizures with Cerebral Application of Antigens or Antibodies

Following the pioneer experiments of Delezene[1] previously referred to, throughout the 20th century many investigators used different animal models to demonstrate the epileptogenic effect of antigens or antibodies applied to the brain. In 1947, Kopeloff et al[42] produced experimental epileptic seizures with the application of aluminum hydroxide and other foreign antigens to the cortex of monkeys.

In 1961, Mihailovic and Jankovic[43] administered to cats intraventricular anti–caudate nucleus gammaglobulin causing neuronal epileptic hyperexcitability.

In 1973, Karpiak et al[44] showed that intraventricular injection of antiserum to rat synaptosome membrane fraction in a chronic rabbit preparation produced recurrent epileptiform activity. They injected synaptic membrane fraction from rat brain into rabbits and collected antisera 4 weeks later. Multiple electrodes were placed in different areas of the cortex and basal ganglia, and a week later, 12 animals were injected with the antiserum. All of them showed hyperexcitable electroencephalographic (EEG) activity. A year later, the same group of authors[45] injected antiserum to rat synaptic membrane fraction in the ventricles of rats producing recurrent epileptiform activity bilaterally in the caudate nuclei, as well as behavioral alterations, on 2 caudate-mediated tasks involving body orientation. Similarly, in 1976, these investigators demonstrated that a single injection of antiserum to total brain ganglioside on the sensorimotor cortex of rats resulted in recurrent spiking lasting 7-17 days. When they removed the antiserum by applying an antibody with monosialoganglioside, the epileptogenic effect was abolished and the EEG returned to normal within 4 weeks.[46] In 1981, the group led by Karpiak[47] further demonstrated that antibodies against GM1 gangliosides purified by chromatography produced less epileptic discharges than native antibodies or immunoglobulin fractions. Antibodies to GM1 ganglioside injected into the sensorimotor cortex of the rat induced recurrent epileptiform activity. Their results supported the view that the binding of antibodies to ganglioside receptors in the synaptic membrane was sufficient to initiate membrane changes leading to epileptic discharges. In this model, seizure activity responded to phenytoin and ethosuximide but not to diazepam or aminooxyacetic acid.[48]

Further investigations tried to identify the underlying pathogenic mechanisms of the epilepsies induced with cerebral antibodies. Thus, in 1981, Frieder and Rapport[49] studied the effect of antibodies to GM1 ganglioside on the release of neurotransmitters from rat brain slices. They showed that such antibodies increased the release of γ-aminobutyric acid (GABA) induced by depolarization. In 1995, Takigawa et al[50] studied the role of anti-GM1 antibodies using the petroleum jelly-gap voltage clamp technique on isolated single myelinated rat nerve fibers. In the presence of active complement, such antibodies decreased the sodium current and caused a progressive increase of nonspecific leakage current. Their observations indicated that anti-GM1 antibodies can interact with Na^+ channels function.

Immunologic and Inflammatory Response in Animal Models of Epilepsy

In 1972, Harris[51] demonstrated that aluminum-induced epilepsy in monkeys causes infiltration of lymphocytes and antibody reaction.

During the past 5 years, multiple studies using different animal models of epilepsy have corroborated the findings of prior investigations previously summarized. Furthermore, they have demonstrated that seizures activate additional immune and inflammatory factors. Epileptic activity has been shown to increase the brain levels of inflammatory cytokines, including IL-1β, IL-1Ra, COX-2,[65-67] IL-1β and the inflammation-associated microRNA-146a,[65,68] TNF-α and microRNA-155,[23] platelet activating factor,[69] and chemokine C-X3-C motif ligand 1 (CX3CL1) also known as fractalkine.[70] Other inflammation-associated microRNAs that have been found to be upregulated in animal models of epilepsy are miR-124, miR-1334, miR-132, and miR21.[71] Similarly, the inflammatory response of microglia or macrophage is stimulated by seizures in animals and is enhanced by progranulin, a protein implicated in neuroinflammation.[72]

Recent investigations in experimental models using pharmacologic and genetic tools have identified a significant contribution of IL-1R/TLR signaling to seizure activity. This signaling can be activated by ligands associated with infections (pathogen-associated molecular patterns) or by endogenous molecules, such as proinflammatory cytokines (eg, IL-1β) or danger signals like damage-associated molecular patterns, for example, high-mobility group box 1 (HMGB1). IL-1β and HMGB1 are synthesized and released by astrocytes and microglia in the rodent brain during seizures. The activation of IL-1R or TLR signaling mediates rapid posttranslational changes in N-methyl-D-aspartate-fated ion channels in neurons, which increase excitability, and transcriptional changes in genes involved in neurotransmission and synaptic plasticity that contribute to lower seizure threshold chronically.[32,38,73] HMGB1 also activates the receptor for advanced glycation end products (RAGE), through the TLR4, present in neurons and astrocytes. RAGE contributes to hyperexcitability underlying acute and chronic seizures, as well as proictogenic effects of HMGB1.[74]

As research continues to be very active in the area of epilepsy and immune-mediated inflammation, a better, but more complicated, understanding of the underlying mechanisms is being acquired, as demonstrated by the following investigations.

In 2012, using a model of pilocarpine-induced status epilepticus, Gnatek et al[75] demonstrated a robust upregulation of acetylcholinesterase (AChE) as early as 48 hours following seizure induction. AChE was expressed in hippocampal neurons, microglia, and endothelial cells but rarely in astrocytes. Transgenic mice overexpressing the "synaptic" splice variant AChE-S (TgS) showed constitutive increased microglial activation, elevated levels of proinflammatory cytokines, and accelerated epileptogenesis compared with their wild-type counterparts. These results suggest that AChE directly suppresses brain innate immune response and that AChE upregulation after status epilepticus is associated with enhanced immune response, facilitating the epileptogenic process.

In 2013, Ndode-Ekane et al[76] studied the role of PLAUR gene encoding urokinase-type plasminogen activator receptor (uPAR) in epileptogenesis. In humans, a single nucleotide polymorphism in PLAUR gene represents a ri for autism spectrum disorders. Seizures were induced intrahippocampal injection of kainate in adult male wil type (Wt) or uPAR knockout (uPAR −/−) mice, a animals were monitored with continuous video-EEG f 30 days. The severity of status epilepticus did not dif between the genotypes. The spontaneous EEG seizures th developed were, however, longer, and their behavio manifestations were more severe in uPAR −/− than \ mice. The more severe the epilepsy phenotype associat with augmented inflammatory response, the more severe t neurodegeneration in the hippocampus.

In 2014, Nunan et al[77] investigated mechanisms regul ing neurogenesis, the production of new neurons fro neural stem or progenitor cells (NSPCs). The innate a adaptive immune systems are increasingly recognized important modulators of hippocampal neurogenesis unc both physiological and pathologic conditions, includi epilepsy. The authors demonstrated that depleting microg from hippocampal cultures reduced NSPC survival a proliferation. Furthermore, addition of purified hippoca pal microglia, or their conditioned media, showed to trophic and proliferative to NSPCs. Vasointestinal polype tide or VIP, released by dentate gyrus interneuro enhanced the proliferative and proneurogenic effect microglia via the VPAC1 receptor. This VIP-induc enhancement was mediated by IL-4 release, which direc targets NSPCs. These findings demonstrated a potent immune-neurogenic pathway, disruption of which may ha significant implications in conditions like epilepsy, whe cognitive impairments, interneuron loss, and immu system activation occur simultaneously.

Also, in 2014, Ganor et al[78] immunized DBA/2J mice wi the GluR3B peptide. Seizures were induced with pentyler tetrazol. GluR3B antibodies were produced only in GluR3 immunized mice and not in control animals. The antibodies facilitated seizures and induced behavioral or mo impairments. In summary, the authors developed an anim model to study autoimmune epilepsy and abnormal behav mediated by pathogenic anti-GluR3B antibodies.

Blood Brain Barrier Disruption and Leukocyte Migration

Nitsch and Klatzo[79] showed how electrographic seizu cause a regional change with breakdown of the BBB. Th investigated the permeability to macromolecules usi Evans Blue as indicator. Pentylenetetrazol-induced seizu caused bilateral leakage mainly in the hypothalamus, wi exception of the mammillary bodies, and the preoptic ar In contrast, seizures due to the GABA receptor block bicuculline brought about a penetration of the dye in t region of the pallidum, whereas the GABA synthe inhibitor methoxypyridoxine produced BBB breakdown the hippocampus. Seizures brought up by different age produced different patterns of BBB breakdown.

...long been held also that chronic seizures cause BBB ... Recent studies have furthermore demonstrated that ...uption triggers seizures.[80,81] In 2010, Marchi et al[81] ...e BBB osmotic damage procedure to examine the ...n between BBB opening, pattern of white blood ...BCs) entry into the brain, and seizure occurrence. A ...l BBB osmotic opening leads to the occurrence of ...leptiform discharges. At the time of such EEG ...es, WBCs populated the perivascular space of a ...B. Similar results were obtained at the time of ...ne-induced seizures. No frank WBCs extravasation ...im parenchyma was observed. In 2012, Frigerio ...easured albumin extravasation in the hippocampus ...bjected to status epilepticus induced by intra-...pal injection of kainic acid. Transient hippocam-...ure to albumin levels similar to those attained after ...n BBB breakdown resulted in increased seizure ...ity and long-term reduction in seizure threshold. ...ffects could be mediated by albumin-induced ...s dysfunction and the associated induction of ...matory molecules (eg, IL-1β).

...ognition of key astrocytic-neuronal communica-...the close interaction and cross talk between brain ...s and brain endothelial cells, has shifted attention ...BB and the "neurovascular unit." Leukocyte adhe-...ecules at the BBB have been proposed to play a role ...ating factor for pilocarpine-induced status epi-...and a viral infection model with a strong BBB ...has been used to study epileptogenesis.[82] Suidan ...ated a murine model of CD8 T-cell–mediated CNS ...ermeability using a variation of the Theiler murine ...myelitis virus model of multiple sclerosis. The ...ad previously found that CD8 T cells had the ...to initiate astrocytic activation, cerebral endothelial ...junction protein alterations, and CNS vascular ...ity through a perforin-dependent process. In a ...they demonstrated that neuronal expression of ...ndothelial growth factor was upregulated before or ...with CNS vascular permeability. Specific inhib-...neuropilin-1 significantly reduced CNS vascular ...ity.

...nimal model of pilocarpine-induced seizures, there ...n of circulating T lymphocytes and mononuclear ...eased levels of IL-1, and evidence of BBB damage. ...pretreated with IL-1 receptor antagonist (IL-1ra) ...significant reduction of status epilepticus and BBB ...n. These data supported the concept of targeting ...nflammation and BBB for the prevention of status ...s.[84]

...ly, it has been shown that leukocyte trafficking and ...-endothelial interactions play a key role in the ...esis of seizures and epilepsy.[85,86] Racusen et al[87] ...berg et al[88] demonstrated that even after 1 single ...D4(+) and CD8(+) T cells and CD45R (+) B cells ...m the brain in the neocortex and hippocampus. ...y6-G (+) neutrophils nor erythrocytes were ...n brains, and increased in BBB permeability was ...ed. The results indicate that lymphocyte entry into brain after a single brief seizure is due to a selective process of recruitment into cortical regions. This mechanism of intracerebral penetration of leukocytes may be different than the leukocyte trafficking that occurs in epilepsy.

In an animal model of pilocarpine-induced seizures, Marchi et al[84] showed activation of circulating T lymphocytes and mononuclear cells, increased levels of IL-1, and evidence of BBB damage. Animals pretreated with IL-1 receptor antagonist (IL-1ra) exhibited significant reduction of status epilepticus and BBB disruption. These data support the concept of targeting systemic inflammation and BBB for the prevention of status epilepticus.[84]

Conclusions

The information reviewed here demonstrates that since the beginning of the 20th century, experimental models of epilepsy have demonstrated that the brain is an immunologically active organ. The brain innate immunity responds to excessive neuronal injury or to excessive neuronal activity, which is mediated by its resident microglia and astroglia, but neurons also play a role. Thus, prostaglandins produced by neuronal COX-2 regulate signaling pathways normally involved in synaptic activity, but in response to seizures they affect the brain immune response.[89,90] Antigens or antibodies applied to the brain trigger an epileptogenic and inflammatory response. Furthermore, acute seizures or chronic models of epilepsy or status epilepticus induce the release of different types of classic cytokines (eg, IL-1, TNF-α, and platelet activating factor). The role of other proinflammatory factors in the pathogenesis of neuroinflammation in epilepsy is being actively investigated; these include fractalkine, microRNAs, IL-1R/TLR, HMGB1, RAGE, TLR4, AChE, PLAUR gene encoding uPAR, and VIP. A recent new concept, which is being studied within the context of inflammation and epilepsy, is the role that BBB plays in the pathogenesis of epilepsy. It has long been held that chronic seizures cause BBB damage, but recent studies have demonstrated that BBB disruption per se triggers seizures. Furthermore, the recognition of key astrocytic-neuronal communication and the close interaction and cross talk between brain astrocytes and brain endothelial cells have emphasized the need to investigate the BBB and the "neurovascular unit." Related to it, leukocyte trafficking and leukocyte-endothelial interactions may also play a key role in the pathogenesis of epilepsy, making it a topic of high research interest.

In summary, the results of experimental studies show that seizures can perpetuate inflammation in the brain via mechanisms that may involve transcription of inflammatory genes or posttranslational changes in the cytokine release machinery. Epileptogenesis may initiate a cascade of inflammatory processes, triggering the onset of epilepsy, and also possibly contributing to the pathogenesis of comorbidities often associated with epilepsy (Fig., Vezzani et al[91]). The knowledge of the mechanisms mediating immune-inflammatory reaction in epilepsy suggests the

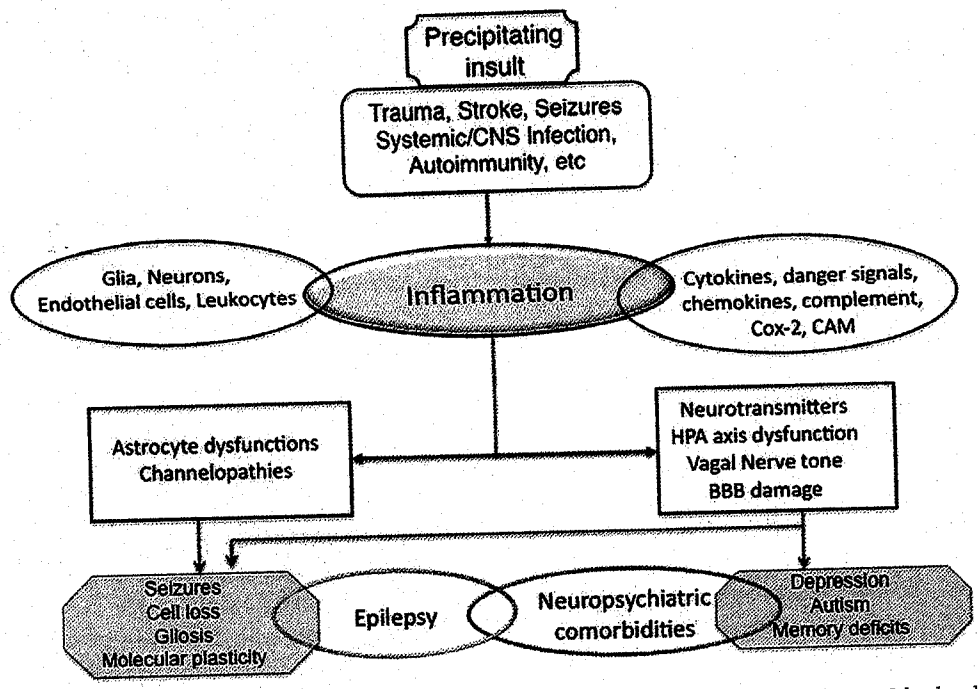

Figure The cascade of inflammatory events in epilepsy. Pathologic events initiated in either the CNS by local injuries or the periphery by infections, or in the context of autoimmune disorders, result in the activation of brain parenchymal cells (microglia, astrocytes, and neurons), endothelial cells of the BBB, and leukocytes. These cells produce and release inflammatory mediators in the brain, eliciting a cascade of downstream events causing a spectrum of physiopathologic effects. Specifically, cytokines and danger signals induce inflammatory molecules responsible for either direct activation of glial or neuronal signaling pathways or BBB breakdown. Inflammatory mediators can activate specific receptors expressed by glia and neurons, inducing rapid nontranscriptional effects on voltage-gated and receptor-gated ion channels, neurotransmitter release, and glutamate receptors leading to increased neuronal excitability. Transcriptional activation of genes can also be triggered by inflammatory molecules such as cytokines, which may perpetuate brain inflammation and contribute to long-term molecular plasticity involved in epileptogenesis. IgG or albumin extravasation in brain following BBB breakdown can promote activation of inflammatory signals and impair astrocyte functions. These effects contribute to the generation of individual seizures and cell death which, in turn, activate further inflammation, thus establishing a vicious cycle contributing to the development of epilepsy. Because brain inflammation has been implicated in the pathophysiology of several neuropsychiatric conditions, it is conceivable that inflammatory processes and mechanisms that are triggered in the brain by an epileptogenic insult may, concurrently with seizures, lead to the development of some neuropsychiatric abnormalities that are considered to be comorbidities of epilepsy, such as depression, memory impairments, and autism spectrum disorder. (Reproduced with permission from Vezzani et al.[91]) (Color version of figure is available online.)

possibility of targeting inflammation to develop new therapies for the treatment of epilepsy. Drugs that block specific inflammatory signals have entered clinical trials as potential therapeutics for autoimmune and inflammatory pathologies, and they may also have therapeutic potential in epilepsies with proinflammatory neurologic conditions.[91] The possible adverse effect of a prolonged anti-inflammatory treatment should be taken into consideration when investigating new anti-inflammatory drugs.[90] The development of disease-modifying anti-inflammatory drugs aimed at decreasing the frequency or severity of seizures is a rational therapeutic approach, backed by tangible experimental evidence.[11,24,89,91]

References

1. Myers RD: Handbook of Drug and Chemical Stimulation of the Brain: Behavioral, Pharmacological, and Physiological Aspects. New York, Van Nostrand Reinhold Co, 1974
2. Amor S, Puentes F, Baker D, et al: Inflammation in neurodegener diseases. Immunology 129:154-169, 2010
3. Aronica E, Ravizza T, Zurolo E, et al: Astrocyte immune respons epilepsy. Glia 60:1258-1268, 2012
4. Kramer U, Chi CS, Lin KL, et al: Febrile infection-related epil syndrome (FIRES): Pathogenesis, treatment, and outcome: A m center study on 77 children. Epilepsia 52:1956-1965, 2011
5. McNaull BB, Todd S, McGuinness B, et al: Inflammation and inflammatory strategies for Alzheimer's disease—A mini-review. ontology 56:3-14, 2010
6. Schwartz M, Kipnis J: A conceptual revolution in the relation between the brain and immunity. Brain Behav Immun 25:817- 2011
7. Xanthos DN, Sandkuhler J: Neurogenic neuroinflammation: In matory CNS reactions in response to neuronal activity. Nat Neurosci 15:43-53, 2014
8. Skaper SD, Facci L: Mast cell-glia axis in neuroinflammation therapeutic potential of the anandamide congener palmitoyletha mide. Philos Trans R Soc Lond B Biol Sci 367:3312-3325, 2012
9. Skaper SD, Giusti P, Facci L: Microglia and mast cells: Two trac the road to neuroinflammation. FASEB J 26:3103-3117, 2012