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SUPPLEMENT ARTICLE

Pharmacological targeting of brain inflammation in epilepsy: Therapeutic perspectives from experimental and clinical studies

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Summary

Increasing evidence supports a pathogenic role of unabated neuroinflammation in various central nervous system (CNS) diseases, including epilepsy. Neuroinflammation is not a bystander phenomenon of the diseased brain tissue, but it may contribute to neuronal hyperexcitability underlying seizure generation, cell loss, and neurologic comorbidities. Several molecules, which constitute the inflammatory *milieu* in the epileptogenic area, activate signaling pathways in neurons and glia resulting in pathologic modifications of cell function, which ultimately lead to alterations in synaptic transmission and plasticity. Herein we report the up-to-date experimental and clinical evidence that supports the neuromodulatory role of inflammatory mediators, their related signaling pathways, and involvement in epilepsy. We discuss how these mechanisms can be harnessed to discover and validate targets for novel therapeutics, which may prevent or control pharmacoresistant epilepsies.

KEY WORDS: Epileptogenesis, Glia, Cytokines, HMGB1, IL-1, COX-2, Anti-inflammatory drugs.

A dysregulated neuroinflammatory response has been suggested to play a pathogenic role in several central nervous system (CNS) diseases, including acute and chronic neurodegeneration,[1–3] and epilepsy.[4–7] Indeed, there are several examples in the literature showing that a persistent neuroinflammatory response in the injured brain may result in neuronal and glial cell dysfunction, alterations in blood–brain barrier (BBB) homeostasis, or neuronal cell death. Inefficient anti-inflammatory control by endogenous resolving mechanisms may play a pivotal role in igniting persisting neuroinflammation.[8,9]

There is evidence supporting a reciprocal causal link between neuroinflammation and epilepsy.[4,10–12] Experimental findings have highlighted that seizure activity is sufficient per se to trigger synthesis and release of pro-inflammatory molecules from brain resident cells. This event is part of a phenomenon defined "neurogenic inflammation."[13] However, in human epilepsy, seizures are unlikely to be the only factor triggering a persistent neuroinflammatory response. In fact, the inflammatory milieu detected in brain specimens from focal cortical dysplasia (FCD) type 1 v*s* type 2 is very different in the extent and type of inflammatory cells and their mediators despite similar seizure frequency.[14]

Increasing pre-clinical and clinical evidence points to the involvement of several inflammatory mediators in the pathogenesis of seizures, neuropathology, and neurologic comorbidities in epilepsy. The pathologic relevance of neuroinflammation is reinforced by the discovery that: (1) it is a common hallmark of various drug-resistant forms of

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Key points

- Inflammatory mediators may act as neuromodulators affecting neuronal function and excitability
- Targeting specific inflammatory signaling pathways in experimental epileptogenesis favorably modifies the disease course
- Proof-of-concept clinical trials and case report studies in patients show clinical efficacy of target-specific anti-inflammatory drugs

epilepsy with differing etiologies and is not only linked to autoimmune disorders or active CNS infections[15]; (2) inflammatory mediators are endowed with CNS-specific neuromodulatory roles that may contribute to hyperexcitability and excitotoxicity.[16]

In this review, we summarize and discuss the main evidence gathered in patients with epilepsy and the related experimental models that support the active involvement of specific inflammatory processes in brain hyperexcitability underlying onset and recurrence of seizures, as well as cognitive deficits. We focus on those inflammatory signaling pathways that have been also demonstrated to occur in brain specimens from patients with pharmacoresistant epilepsies.

Cytokines and Danger Signals

Interleukin-1 receptor/Toll-like receptor (IL-1R/TLR) signaling

The interleukin-1 receptor/Toll-like receptor (IL-1R/TLR) signaling is a key upstream generator of the neuroinflammatory response. Upon its activation by the endogenous ligands or their mimicry molecules, the IL-1/TLR signaling pathway leads to the transcriptional induction of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)–regulated inflammatory genes, and as a consequence, to the generation and rapid amplification of the neuroinflammatory cascade.[17,18] TLRs are pattern-recognition receptors (PRRs) sensing molecular patterns expressed by various pathogens during infection. PPRs can also be activated by damage-associated molecular patterns (DAMPs) expressed by endogenous molecules (i.e., danger signals) that are released by injured cells in the absence of infection, thereby eliciting sterile inflammation.[19]

IL-1R type 1 (IL-1R1) and TLR4, and their prototypical endogenous ligands (i.e., the pro-inflammatory cytokine IL-1β and the danger signal High Mobility Group Box 1 [HMGB1], respectively) are induced in neuronal and glial cells following various epileptogenic injuries in rodents—such as, status epilepticus (SE), stroke, neurotrauma, and CNS infection—as well as during seizures.[11,20,21] In particular, the IL-1R1-TLR4 axis is rapidly and persistently activated in rodent models of SE-induced epileptogenesis in brain areas involved in seizure generation and propagation.[20,22] Such changes do not solely reflect neuronal cell loss or ongoing seizure activity, since they also occur in nonlesional seizure models[21,23–25] and before the onset of spontaneous seizures, implying their potential involvement in epileptogenesis (i.e., the development and extension of brain tissue capable of generating spontaneous seizures).[20,22] The induction of this signaling pathway involves mostly activated microglia and astrocytes, as well as neurons and BBB cell components.[20,22]

The IL-1R/TLR4 signaling pathway is induced in surgically resected epileptogenic foci from patients with structural/lesional pharmacoresistant epilepsies, including malformations of cortical development (MCDs) such as low-grade epilepsy-associated glioneuronal tumors (gangliglioma, dysembryoplastic neuroepithelial tumors),[26,27] focal cortical dysplasia and tuberous sclerosis,[26,28,29] and temporal lobe epilepsy (TLE) with/without hippocampal sclerosis (HS),[21,22,30] as compared to control tissue. Control specimens included both human tissue obtained at autopsy from patients without history of seizures or other neurologic diseases, and surgical tissue from patients with a focal epileptogenic lesion not involving the hippocampus proper (for TLE) or perilesional tissue (normal-appearing cortex/white matter adjacent to the lesion) for MCDs. Similar to animal models, the clinical specimens showed that resident brain cells are major common contributors to the activation of this pathway.

Experimental models were crucial tools for understanding the pathophysiologic consequences of IL-1R/TLR signalling pathway activation. Pharmacologic studies and genetic interference with this signaling demonstrated that this pathway upon activation by IL-1β or HMGB1, respectively, promotes seizure generation in acute and chronic seizure models and favors epileptogenesis.[19,31] In particular, the activation of the IL-1R1/TLR4 pathway enhances seizure frequency. Accordingly, inhibitors of this signaling pathway (e.g., IL-1 receptor antagonist, IL-1Ra; caspase-1 inhibitors; TLR4 antagonists; and anti-HMGB1 monoclonal antibodies), and a negative regulator of this pathway (synthetic oligonucleotide analog of microRNA[miR]-146a), mediate significant anti-seizure effects also when seizures do not respond to clinical antiseizure drugs (ASDs).[21,23,32–41] These therapeutic effects were associated with reduced BBB dysfunction and neuroprotection.

In immature rodents, IL-1β influences the generation of febrile convulsions (FCs). In particular, the intracerebral injection of this cytokine in a postnatal (PN) day 14 mouse model of FCs, lowers the core temperature threshold that results in seizures, acting on IL-1R1.[42] Accordingly, in PN14 rats developing fever following systemic lipopolysaccharide (LPS), a TLR4 activator, there is evidence of increased IL-1β in hippocampus and cortex only in animals developing FCs following an acute injection of subconvulsive doses of kainic acid.[43]

Recent evidence demonstrated that simultaneous targeting of the IL-1R1 and TLR4 signaling pathway in rodents with

pharmacologic or epigenetic approaches (i.e., IL-1Ra + HMGB-1 antagonists; caspase-1 inhibitor + TLR4 antagonist; miR-146a mimic), either shortly after SE or at the time of epilepsy onset, prevented disease progression and significantly reduced chronic seizure recurrence by 70-90%.[44,45] Notably, these treatments mediated therapeutic effects outlasting drug withdrawal, and therefore supported disease-modification, rather than purely symptomatic, effects. Similarly, the transient administration of an anti-HMGB1 monoclonal antibody in mice during epileptogenesis resulted in disease-modifying effects consisting of a significant reduction in spontaneous seizure frequency and improvement of cognitive deficits.[39] Pharmacologic targeting of IL1-β using IL-1Ra in an experimental model of pediatric traumatic brain injury (TBI) in mice, reduced seizure susceptibility to pentylenetetrazol and the number of evoked seizures, and improved spatial memory.[46] This set of data suggests that the IL-1β signaling pathway is involved in the mechanisms underlying seizures and comorbidity development.

In accord with pharmacologic evidence, transgenic mice with an impaired IL-1R1/TLR4 signaling pathway showed significant reduction in seizure susceptibility,[21,32,33,35] or in spontaneous seizure recurrence,[35] thus supporting the involvement of this pathway in the mechanisms regulating neuronal excitability and epileptogenesis.

Tumor necrosis factor-alpha (TNF-α)

TNF-α is rapidly induced during seizures in glial and endothelial cells of the BBB in rodents, but its expression is more transient than IL-1β because it declines to basal levels after SE.[47–50] A concomitant reduction in neuronal tumor necrosis factor (TNF)-α receptor type 2 (TNFR2) and an increase of TNFR1 was found in neurons and astrocytes.[51,52]

TNF-α is induced in astrocytes and neurons also in TLE and tuberous sclerosis human brain specimens,[51,53] as compared to control tissue obtained at autopsy from subjects without a history of seizures or other neurologic diseases. Receptor analysis confirmed the changes observed in experimental models.[51]

TNF-α has either proconvulsant or anticonvulsant effects, which are dependent on its brain concentration (as for the other cytokines) and the receptor subtype predominantly activated in diseased tissue. Thus mouse recombinant TNF-α injected into the mouse hippocampus significantly reduced seizures by activating TNFR2, while it promoted seizures by activating TNFR1.[54] Accordingly, TNF-α significantly increased the susceptibility to seizures evoked in rats by intra-amygdala injection of kainic acid or by amygdala kindling acting via TNFR1, and attenuated the kindling rate via TNFR2.[52] In accordance, a protective role of TNF-α on seizures was reported in mice with a genetic deletion of the TNFR1.[55] Transgenic mice with low to moderate overexpression of TNF-α in astrocytes showed decreased susceptibility to seizure,[54] whereas mice with high expression of TNF-α develop signs of neurologic dysfunction, including seizures.[56]

Arachidonic Acid–related Pathways

In rodents, cyclooxygenase-2 (COX-2) is expressed in discrete populations of neurons and it is enriched in animal cortex and hippocampus under basal conditions.[57,58] COX-2 is induced in forebrain after various epileptogenic injuries.[59,60] Prominent changes in the expression of genes involved in prostaglandin synthesis and regulation, including COX-2, were observed in limbic rat areas after electrically induced SE.[61] Most genes had a biphasic pattern of expression, namely they were upregulated in the acute phase of SE while returning to baseline level before spontaneous seizures onset, and were again upregulated in the chronic epilepsy phase.[61] Immunohistochemistry confirmed that COX-2 has a biphasic pattern of expression following SE in rodents[62] showing an early upregulation in neurons followed by increased expression in astrocytes during epileptogenesis, which persisted in epileptic tissue.[62–64]

In hippocampal biopsies from patients with TLE, COX-2 immunoreactivity was increased in neurons.[65,66] Of interest, additional astrocytic expressions was observed but only in patients with HS.[65]

Transgenic mice with increased neuronal expression of COX-2 were more susceptible to kainic acid–induced SE,[67] thus highlighting a potential proconvulsive role of this enzyme. In accord, COX-2 deficient mice, or mice treated with the COX-2 inhibitor nimesulide, showed reduced kindling development.[68] However, detailed pharmacologic studies using selective COX-2 inhibitors, such as celecoxib, parecoxib, indomethacin, and SC58236, have shown dichotomous effects of COX-2 blockade on seizures.[5,63] If COX-2 inhibition is achieved before the induction of SE with pilocarpine or kainate, then proconvulsive effects are observed.[69–71] Conversely, if COX-2 inhibition is achieved post-SE by injecting selective antagonists during epileptogenesis, then either neuroprotection[72] or reduced spontaneous seizures severity,[73] or no effects,[64] were reported. Ictogenic or anticonvulsive effects are likely due to the generation of different sets of prostaglandins at the time of drug intervention in the various experimental models of seizures[70] and on the timing of pharmacologic intervention. More consistent results were obtained by targeting downstream effector molecules in the COX-2 signaling cascade, such as the EP2 receptor of prostaglandin (PG)E2. The administration of a selective EP2 antagonist after SE onset in mice reduced mortality, neuroinflammation, BBB dysfunction, and neuronal cell loss, and prevented memory impairment.[74–78] Further studies are required to determine the long-term effects of EP2 antagonism on epileptogenesis.

Recently, monoacyl-glycerol-lipase (MAGL) has been identified preclinically as a novel target for the treatment of drug-refractory SE. MAGL is the key enzyme responsible for the biosynthesis of arachidonic acid in the CNS from the

endocannabinoid 2-arachidonoylglycerol (2-AG). The early administration of a brain-penetrant, small molecule, potent, and selective irreversible MAGL inhibitor, namely CPD-4645, significantly reduced the severity and duration of benzodiazepine-refractory SE, and the consequent cell loss and cognitive deficits in mice.[79]

Transforming Growth Factor-β (TGF-β) Signaling

BBB regulates the reciprocal blood-to-brain exchange of molecules and immune cells, and its integrity is instrumental in protecting the brain from the entry of xenobiotics or potentially harmful molecules. BBB dysfunction occurs in epilepsy as in other neurologic conditions such as stroke and neurotrauma.

Inflammatory molecules released by perivascular glia play a prominent role in BBB dysfunction by several mechanisms, one example of which is the downregulation of tight junctions between endothelial cells.[37,80–83] As a consequence, blood-borne molecules and immune cells may enter the brain and induce, or perpetuate, an inflammatory response.[84,85] In particular, focal opening of the BBB can be recapitulated by exposing the rat neocortex to serum albumin. This procedure results in delayed development of paroxysmal hypersynchronous activity.[86,87] The presence of serum albumin in brain parenchyma activates TGF-β receptor type 2 (TGF-βR2) in astrocytes, thus leading to transcriptional activation of inflammatory genes, and concomitant downregulation of Kir4.1 potassium channels and the glutamate transporter.[84,86–89] The resulting neuroinflammation and high extracellular K^+ and glutamate, act in concert to decrease seizure threshold and induce excitatory synaptogenesis,[90] thus leading to hyperexcitability in surrounding tissue.[86,91] Accordingly, a transient post-injury treatment with losartan, an angiotensin II type 1 receptor antagonist that blocks TGF-β signaling, reduces incidence and severity of epilepsy as well as cell loss, BBB dysfunction, and neuroinflammation in rodent models of acquired vascular injury and SE.[92–94] Moreover, losartan attenuates spontaneous seizure frequency and neuronal cell loss in spontaneously hypertensive rats exposed to SE[95] and improved cognitive functions and neural damage after traumatic brain injury (TBI).[96]

Complement System

Although the synthesis of complement system components occurs predominantly in the liver, both glia and neurons can express these inflammatory mediators in pathologic conditions.[97,98] Various complement-related factors are induced in the brain during SE-induced epileptogenesis in rodents,[61,99,100] as well as in TLE patient with hippocampal sclerosis.[99,101,102] Immunohistochemistry identified glial cells (including the astrocytic end feet surrounding blood vessels) and in some neurons, as cellular source of complement factors.[99]

The permissive role of the complement system activation in seizures has been suggested by a seminal study showing that the sequential intrahippocampal injection of the complement factors C5b6, C7, C8, and C9, inducing the formation of the membrane attack complex, promotes seizures in rats and hippocampal neuronal loss.[103] Recently, the administration of PMX53, a C5ar1 antagonist, resulted in anticonvulsive effects in various murine models of seizures.[104] Moreover, blockade of C5ar1 during pilocarpine-induced SE reduced seizure power, SE-associated mortality, and neurodegeneration in the hippocampus.[104] The involvement of complement system in seizures has been also explored using transgenic mice lacking specific complement-related factors. Thus, C3-deficient mice develop significantly fewer behavioral seizures following Theiler's virus infection as compared to wild-type mice.[105] Kindling development was also delayed in C6-deficient rats.[106]

Chemokines

Various chemokines (CCL2 [MCP-1], CCL3 [MIP-1α], CCL4 [MIP-1β], and CCL5 [RANTES]) and their cognate receptors are elevated in brain tissue of patients with drug-resistant epilepsy and in experimental models, in neurons, glial and endothelial cells, as well as in infiltrating leukocytes.[107–110] In particular, CCR5 receptors activated by MIP-1α and RANTES contribute to neuroinflammation, cell loss, acute seizures, and BBB damage in experimental models.[111] Similarly, CCR2 activated by CCL2 contributes to spontaneous seizures in mice, and mediates seizure exacerbation by lipopolysaccharide (LPS), a TLR4 agonist that mimics bacterial infections.[109]

Oxidative Stress

Oxidative stress and brain inflammation are two phenomena that are intimately associated, since they are functionally interconnected and reinforce each other.[112] Moreover, like brain inflammation, oxidative stress is rapidly and persistently induced after epileptogenic brain injuries in animal models.[113,114] Notably, markers of oxidative stress are increased in blood and brain tissues in human epilepsy.[20,113,115,116]

Oxidative stress contributes to epilepsy-associated neuropathology and behavioral deficits, and plays a role in determining seizure threshold in animal models.[113,117–121] Recent evidence gives support for a disease-modification effect mediated by antioxidant treatments, namely the investigational compound 1400W[122] or the combination of clinically-used drugs (i.e., *N*-acetylcysteine [NAC] and sulforaphane [SFN]).[20] The therapeutic effects of the antioxidant drug combination were associated with the prevention of disulfide HMGB1 generation.[20] This is the oxidized

isoform of HMGB1 with ictogenic and inflammatory properties.[21,123] Disulfide HMGB1 therefore represents a point of intersection between oxidative stress and neuroinflammation and plays a role in seizure generation.[20]

Mechanisms of Hyperexcitability

Pro-inflammatory mediators may act as neuromodulators by activating nonconventional intracellular signaling pathways that affect neuronal function and excitability. For example, cytokines can modify the function of glutamate and γ-aminobutyric acid (GABA) receptors by altering receptor trafficking and their subunit assembly at neuronal membranes; cytokines can also modulate glutamate receptor–mediated calcium permeability by promoting AMPA-GluR2 and *N*-methyl-D-aspartate (NMDA)-NR2B receptor phosphorylation via PI3K or Src kinases, respectively.[16,21,30,124,125] The role of IL-1R1/TLR4 signaling pathway in seizure precipitation and recurrence involves the rapid activation of Src kinases and subsequent phosphorylation of NMDA-NR2B receptors.[21,126]

Additional mechanisms of hyperexcitability mediated by the IL-1R1/TLR4 signaling pathway include the downregulation of the HCN1 channel and the related Ih current on dendrites of hippocampal pyramidal neurons, as assessed in rats upon intraventricular LPS injection. Activation of the IL-1R/TLR4 signaling pathway reduces both levels and function of HCN1 channels.[127] These channels are important regulators of the filtering properties of hippocampal pyramidal cell dendrites, their responses to excitatory inputs, and they are involved in theta rhythms, which have been linked to cognitive functions. These channels are downregulated in experimental and human epilepsy tissue, and contribute to seizures.[128,129]

Cytokines and prostaglandins can also alter voltage-gated ion channel function.[16,124] In particular, somatic and dendritic membrane excitability was significantly reduced in CA1 pyramidal neurons using a selective COX-2 inhibitor, and this effect was mediated by cannabinoid receptor type 1 (CB1).[130] Moreover, PGE2 increases firing and excitatory postsynaptic potentials, most likely by reducing K^+ currents in CA1 neurons.[131] Thus the mechanisms underlying the effects of COX-2 inhibition may involve the reduction of prostaglandin levels or the modulation of CB1 receptors, being COX-2 at the interface between the eicosanoid and the endocannabinoid systems.[132]

In addition, cytokines inhibit glial glutamate reuptake[133,134] and enhance glutamate release by astrocytes,[135] thus resulting in increased extracellular glutamate concentration.

Activation of complement system in erythrocyte membrane leads to the formation of channel conductances, resulting in Ca^{2+} and Na^+ influx and K^+ efflux, with the net effect of depolarizing the membrane potential.[136] If this mechanism is operative in neurons, it may result in depolarization and increased neuronal excitability.

Chemokines modulate voltage-gated ion channels[137] and regulate neurotransmitter release.[137,138] For example, fractalkine (or CX3CL1) acts as a positive modulator of $GABA_A$ receptor in human TLE brain specimens by reducing the $GABA_A$ use-dependent desensitization (i.e., rundown).[108] This effect may be mediated by phosphorylation of one or more $GABA_A$ subunits, thus leading to a "stabilization" of the receptor. The upregulation of fractalkine receptor in epileptogenic tissue may therefore represent a homeostatic attempt to reduce hyperexcitability by promoting GABA receptor function.

Clinical Evidence: Target-specific Anti-inflammatory Drugs

Based on the pathologic involvement of specific inflammatory pathways in animal models of seizures and epilepsy, progress in clinical translation has been made. Notably, there are anti-inflammatory drugs in medical use for autoinflammatory or autoimmune diseases that target inflammatory pathways, which contribute to seizure mechanisms and neurologic comorbidities in animal models. Proof-of-concept clinical trials and case report studies have reported signs of clinical efficacy of target-specific anti-

Table 1. Examples of clinical studies in pharmacoresistant epilepsies using target-specific anti-inflammatory treatments

Target	Drug	Clinical study	References
Caspase-1 inhibitor (↓IL-1β, ↓HMGB1)	VX09-765-401	Phase IIA study (focal onset epilepsy, adult)	139
IL-1Ra (IL-1R1 antagonist)	Kineret	FIRES (case report, child)	140
		Drug-resistant epilepsy (case reports, adolescent)	141
		Systemic autoinflammatory disease with intractable epilepsy (case report, adolescent)	142
TNF-α (inactivating antibody)	Adalimumab	Rasmussen's encephalitis (open pilot study, adult)	143
Microglia (inhibitor)	Minocycline	Astrocytoma (case report, adult)	144

inflammatory drugs (Table 1). In particular, a 6-week phase IIA randomized, double-blind, placebo-controlled study was completed in patients with drug-resistant focal-onset epilepsy who were receiving the caspase-1 inhibitor (VX-765), a drug targeting IL-1R1/TLR4 signaling pathway, by reducing the biosynthesis and release of IL-1β and HMGB1. The administration of VX-765 resulted in seizure reduction in some patients that persisted for some time after drug discontinuation.[139] Kineret (i.e., anakinra, the human recombinant IL-1Ra) has been recently used in a child with super-refractory SE secondary to febrile infection-related epilepsy syndrome (FIRES),[140] in 4 adolescents with drug-resistant epilepsy,[141] and in 1 adolescent with systemic autoinflammatory disorder associated with intractable epilepsy.[142] In all clinical cases, the treatment significantly improved seizure control.[140–142]

An open-label study evaluating the efficacy and the safety of adalimumab, a monoclonal anti-TNF-α antibody, has been made in Rasmussen's encephalitis.[143] The treatment led to seizure improvement in some patients and was associated with a stabilization in functional deficits in a small cohort.[143]

Minocycline, an antibiotic with abroad spectrum of actions including inhibition of microglial activation and pro-inflammatory cytokine release in animal models, induced a marked reduction in seizure frequency in a patient with astrocytoma and drug-resistant epilepsy.[144]

No clinical trials using specific COX-2 inhibitors have been conducted so far in patients with epilepsy.[145] Indeed, due to their side effects, several international drug-control authorities have withdrawn these drugs from the market.[145]

Conclusions

Mechanistic studies and pharmacologic interventions on neuroinflammatory pathways activated in clinical and experimental epilepsy have increased our understanding of this complex response to brain injury and helped to identify anticonvulsive and/or antiepileptogenic properties of target-specific anti-inflammatory drugs. Experimental evidence suggests that a combination of anti-inflammatory treatments may be considered a therapeutic option due to the reverberant inflammatory cascade.

Treating therapy-resistant patients with specific anti-inflammatory drugs already approved for other indications may facilitate the clinical translation of experimental findings. It is also worth considering that there are therapeutic interventions in pharmacoresistant epilepsies, such as the use of steroids, the ketogenic diet, vagus nerve stimulation, and the cannabinoids, that display anti-inflammatory mechanisms of action which may mediate some of their therapeutic effects.

The encouraging initial clinical results support further investigations. Clinical studies would greatly benefit from the identification of noninvasive biomarker(s) reflecting the presence of neuroinflammation in patients. This would allow patient stratification to test the candidate anti-inflammatory drugs and for designing affordable and adequately powered antiepileptogenesis trials, and to monitor treatment response. In this context, much progress has been made and several noninvasive inflammation-related humoral[44,146–149] (blood/cerebrospinal fluid) and imaging measures[150–157] have been identified (Table 2). Such biomarkers could be used to increase the sensitivity and precision of the available clinical indicators.

For designing anti-inflammatory interventions in epilepsy, we need to gather deeper insights into the dynamic changes of neuroinflammation during disease development to determine the best therapeutic window. We also need to better distinguish homeostatic from pathologic inflammatory signaling pathways triggered by epileptogenic insults in order not to interfere with the repair mechanisms.

The progress in elucidating the mechanisms underlying the pathogenic effects of neuroinflammation in epilepsy is critical for developing safe and effective drugs with potential disease-modifying, and not purely symptomatic, therapeutic effects.

Table 2. Inflammation-related humoral and imaging measures identified in preclinical models and validated in clinical studies.

	Measure	Biomarker of	References
Blood/CSF	↑ HMGB1 and its isoforms	Drug-resistance & seizure relapse	44
	↑ TARC/siCAM5 ratio	Drug-resistance	146
	↑ CSF/serum IL-1β ratio	Post-traumatic epilepsy	147
	↓ IL-1Ra/IL-6 ratio	Hippocampal T2 hyperintensity after FSE	148
Imaging	↑ 11C-DPA-713 signal (PET) (microglia activation)	Epileptic focus and seizure generalization areas in TLE	150
	↑ 11C-PBR28 signal (PET) (microglia activation)	Epileptic focus in TLE	152,153
	↑ 11C-PK11195 signal (PET) (microglia activation)	Epileptic focus and interictal activity in FCD (case report)	154–156
	↑ 11C-deuterium-deprenyl signal (PET) (astrocyte activation)	Epileptic focus in TLE	157,158
	↑ mIns levels (^{1}H-MRS) (astrocyte activation)	Epileptic focus in TLE	159–161

TARC, thymus and activation-regulated chemokine; siCAM5, soluble intracellular adhesion molecule 5; FSE, febrile status epilepticus; TLE, temporal lobe epilepsy; FCD, focal cortical dysplasia; PET, positron emission tomography; mIns, myo-inositol; ^{1}H-MRS, proton magnetic resonance spectroscopy.

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