



Review

Role of inflammation and its miRNA based regulation in epilepsy: Implications for therapy



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ABSTRACT

There is a need to develop innovative therapeutic strategies to counteract epilepsy, a common disabling neurological disorder. Despite the recent advent of additional antiepileptic drugs and respective surgery, the treatment of epilepsy remains a major challenge. The available therapies are largely based on symptoms, and these approaches do not affect the underlying disease processes and are also associated frequently with severe side effects. This is mainly because of the lack of well-defined targets in epilepsy. The discovery that inflammatory mediators significantly contribute to the onset and recurrence of seizures in experimental seizure models, as well as the presence of inflammatory molecules in human epileptogenic tissue, highlights the possibility of targeting specific inflammation related pathways to control seizures that are otherwise resistant to the available AEDs. Emerging studies suggest that miRNAs have a significant role in regulating inflammatory pathways shown to be involved in epilepsy. These miRNAs can possibly be used as novel therapeutic targets in the treatment of epilepsy as well as serve as diagnostic biomarkers of epileptogenesis. This review highlights the immunological features underlying the pathogenesis of epileptic seizures and the possible miRNA mediated approaches for drug resistant epilepsies that modulate the immune-mediated pathogenesis.

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1. General introduction

Epilepsy is a brain disorder, which is characterized by a predisposition to generate seizures that are associated with neurobiological, psychological, cognitive and linguistic problems. Approximately 0.5–1% of the world's population has active epilepsy. Current treatments of epilepsy remain difficult, and antiepileptic drugs (AEDs) fail for some patients.

Despite the introduction of ~15 new AEDs over the past two decades, about one third of epilepsy patients do not become seizure free. It is these patients who carry the greatest burden of the disease. Most of these AEDs are primarily targeted against neuronal ion channels and both gamma-aminobutyric acid (GABA) and glutamate receptors [1,2].

Because seizures are the result of uncontrollable neural excitation in the brain, epilepsy has been considered to primarily be a neuronal disease. However, studies that have focused exclusively on neurons fail to address the questions that arise from more complex models of epileptogenesis. To date, many studies using animal models and

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human patients with epilepsy have shown that the pathogenesis of epilepsy may be associated with both neuronal and non-neuronal components such as glial cells [3], brain vasculature [4], and leucocytes from the periphery [5].

The aberrant regulation of glial functions can elicit seizures and promote epileptogenesis. Glial abnormalities, including chronically activated astrocytes and microglia, glial scars, and various gliomas, are likely to form epileptic foci in the brain [3]. The mechanisms through which glial cells can promote epileptogenesis include increased neuronal excitability and inflammatory processes. The key roles of inflammatory processes in relation to epilepsy have been clarified over the last decade.

The possibility that inflammatory processes in the brain contribute to the etiopathogenesis of seizures and the establishment of a chronic epileptic focus is increasingly recognized as a result of supportive evidence in experimental models and in the clinical setting.

Studies of the mechanisms of AEDs have focused on ion channels, transporters, and excitatory/inhibitory neurotransmission [6]. However, the anti-inflammatory effects of AEDs have received recent attention due to their relevance to antiepileptic properties. For example, carbamazepine and levetiracetam can reduce the expression of inflammatory mediators in glial cell cultures [7,8]. Levetiracetam can also normalize the resting membrane potential of astrocytes increased by inflammatory mediators. One of the anticonvulsive effects of levetiracetam could be mediated through suppression of astroglial activation by inflammatory mediators [7].

2. Mechanism of inflammation involved in epilepsy

In the last decade, evidence from clinical and experimental studies indicates that brain inflammation is an intrinsic feature of the hyperexcitable pathologic brain tissue in pharmacoresistant epilepsies of differing etiology [9]. Brain inflammation contributes significantly to determine seizure threshold in susceptible brain regions, thus playing a role in seizure precipitation and their recurrence [9–13].

Experimental and clinical evidence have demonstrated the increased synthesis of specific inflammatory mediators, and the upregulation of their cognate receptors in the chronic epileptic brain, indicating that some proinflammatory pathways are activated in seizure foci. Inhibition of experimental seizures by pharmacological interference with specific proinflammatory signaling, together with evidence of changes in intrinsic susceptibility to seizures in transgenic mice with perturbed inflammatory pathways, was instrumental to establish the concept that brain inflammation has a role in the etiopathogenesis of seizures [14,15]. Increasing evidence also highlights the possible involvement of inflammatory processes arising in the injured brain in the development of epilepsy (i.e., in epileptogenesis) [16]. Since brain inflammation in epilepsy is not a mere epiphenomenon of the pathology but is likely involved in the mechanisms underlying neuronal hyperexcitability, the onset of seizures and their recurrence, it might be considered as a biomarker of disease development and severity, and as such could be used for diagnostic, prognostic or therapeutic purposes, provided that adequate noninvasive methodologies are developed to detect and quantify brain inflammation in humans.

Experimental studies show that once seizures develop, they can contribute to perpetuate inflammation in the brain via mechanisms which may involve transcription of inflammatory genes or post-translational changes in cytokine release machinery. Specific inflammatory mediators contribute to decrease seizure threshold in animal models either by direct effects on neuronal excitability or by activating transcription of genes involved in synaptic and molecular plasticity. Thus, the available evidence suggests that an epileptogenic event, even if subclinical, occurring at birth or during the lifetime may initiate a cascade of inflammatory processes contributing to the onset of epilepsy and to seizure recurrence. The presence of activated inflammatory pathways in epileptic brain may also contribute to comorbidities often associated with epilepsy [17].

The activation of inflammatory pathways, involving both reactive astrocytes and microglia in human temporal lobe epilepsy (TLE), is supported by gene expression profile analysis [18–21]. An inciting event, such as trauma, stroke, febrile seizures, status epilepticus (SE), infection or gene mutation, may lead to activation of microglia, astrocytes and neurons, and/or to blood–brain barrier dysfunction, and release of proinflammatory cytokines, such as interleukin-1 beta (IL-1 β), IL-6, IL-10 and tumor necrosis factor-alpha (TNF- α), complement system, acute phase proteins, chemokines [22]. As demonstrated by Silveira et al. [23] and Vezzani et al. [24], these elicit proinflammatory events in the target cells (i.e., neurons and glia) via activation of interleukin-1/Toll-like receptor (IL-1/TLR) signaling pathways. Downstream of this signaling lead to IRAK-4, TRAF6 mediated activation of the MAPK or NF κ B [25]. It also increases calcium influx into neurons by N-methyl-D-aspartate (NMDA) receptors, contributing to COX-2 overexpression [26]. The rapid release of high-mobility-group box 1 (HMGB1) from neurons, microglia, and astrocytes following proconvulsant injuries, and its activation of Toll-like receptor signaling in astrocytes and neurons has been proposed as a crucial event for initiating brain inflammation and decreasing seizure threshold [27].

All of these events give rise to neuronal network hyperexcitability, cell injury and network reorganization, which are responsible for the onset of seizures and the development of epilepsy. The major inflammatory pathways studied so far in patients for their possible contribution to epileptogenesis are the activation of the interleukin-1/Toll-like receptor (IL-1/TLR) signaling pathways, BBB breakdown, TGF-beta signaling pathway and COX-2 (Table 1).

3. Interleukin-1/Toll-like receptor (IL-1/TLR) signaling pathway

The IL-1 cytokine family consists of IL-1 alpha (IL-1 α), IL-1 beta (IL-1 β) and IL-1 receptor antagonist (IL-1Ra). This signaling is pivotally involved in the initiation of tissue inflammation since it is the upstream activator of innate immunity, the first immune system to be induced by tissue injury or following a large variety of biological stressors. The activity of the IL-1 system depends on the balance between agonist/antagonist ligands; IL-1 β and IL-1Ra. Disequilibrium in the IL-1 β /IL-1Ra ratio may increase the inflammatory and neurotoxic potential of IL-1 β and lead to brain damage and epilepsy [28].

IL-1R/TLR signaling can regulate neuronal excitability, including the alteration of synaptic transmission, the reduction in GABA production, and the inhibition of outward current of Ca $^{2+}$ channels [29–32]. IL1R/TLR signaling may activate the Src kinase-mediated phosphorylation of NMDA receptor subunit 2B (NR2B). This phosphorylation can increase Ca $^{2+}$ permeability of NMDA receptor [26]. Consequently, this NMDA mediated Ca $^{2+}$ influx could be enhanced in neurons, leading to increased neuronal excitability and excitotoxicity [10,27]. Recent evidence demonstrates a functional link between the activation of IL-1R1, TLR4 and TLR3 and rapid changes in neuronal excitability: in the hippocampus, IL-1 β and lipopolysaccharide (LPS), a component of Gram negative bacterial wall and prototypical activator of TLR4, can both induce rapid changes in synaptic transmission, and affect LTP via activation of a specific set of kinases (e.g. JNK, P38 MAPK and PKC) [33–36]. Excitatory effects of IL-1 β have been reported in various brain regions [10,29, 37]. In particular, IL-1 β reduces synaptically-mediated GABA inhibition in area CA3 of the hippocampus via still unidentified kinases [38–39] and increases CA1 neuron excitability by reducing NMDA channel- and voltage-gated calcium channel-induced outward current via phosphorylation of large-conductance Ca $^{2+}$ -dependent K $^{+}$ channels [40].

Among the TLRs, the TLR4 subtype is the most extensively studied for its involvement in brain excitability. In rat cortical application of LPS induces rapid-onset increases in neuron excitability, and these effects were prevented by IL-1Ra implicating a role of released IL-1 β . At higher concentration, LPS could evoke neocortical epileptiform discharges [41]. Notably, TLR4 can also be stimulated by “danger signals” such as HMGB1, which are endogenous molecules released by damaged

Table 1

Summary of potential pro-and anti-epileptogenic properties of inflammatory mediators in epilepsy patients.

Inflammatory mediators	Potential function	References
IL-1Ra	Neuroprotective and anticonvulsant effects. Levels found to be lower in cases of neonatal seizures. Upregulated in patients with MTLE-HS, upregulation may be a response of the seizure activity.	[88–89,92,97–98]
IL-1 α	Changes in the levels are associated with MTLE. Polymorphisms that lead to increased transcription are associated with TLE and febrile seizures.	[89,98]
IL-1 β	Increases NMDAR subunit NR2B phosphorylation leading to increased Ca $^{2+}$ influx. Increased levels have been found in MTLE epilepsy patients. Polymorphism is associated with increased risk of febrile seizures. Several clinical studies have addressed the changes of IL-1 β levels in blood and cerebrospinal fluid (CSF) of patients with focal epilepsy also.	[88–89,92,97–98]
IL-10	Inhibits development of seizures in febrile seizures and a hypoxia model for epilepsy. SNPs that result in increased IL-10 are decreased in febrile seizure patients. Changes in the levels have been found in cortex and hippocampus of MTLE patients.	[88–89,92,97–98]
IL-6	The upregulation of IL-6 is found to be correlated with the severity of the seizure	[88–89,98]
TNF- α	No significant changes of TNF α have been found in plasma or CSF in patients within 24 h after acute tonic–clonic seizures or partial secondarily generalized seizures. No changes have been observed in febrile seizures and MTLE patients.	[89,98]
TGF- β	Transcriptomic profiling reveals TGF- β signaling in epileptogenesis, no studies reported on patients	[60]
COX-2	Induction of astrocytic cyclooxygenase-2 in epileptic patients with hippocampal sclerosis has been reported.	[75]
ICAM-1	Implicated in BBB disruption, found to be highly upregulated in patients with MTLE	[89]
CCL-2	Proepileptogenic, changes have been seen in MTLE patients, contributes to immune-cell recruitment across the BBB	[89]
CCL-3	Proepileptogenic, changes have been seen in the MTLE patients, Capable of inducing Ca $^{2+}$ transients in neuronal and microglial cultures.	[89]
CCL-4	Proepileptogenic, changes have been seen in the MTLE patients, capable of inducing Ca $^{2+}$ transients in neuronal and microglial cultures	[89]
CCL5	Inhibition of systemic receptor leads to decrease in seizure activity Proepileptogenic, changes have been seen in the MTLE patients.	[89]

or stressed neurons and by activated astrocytes and microglia, thus generating the hypothesis that activation of TLR4 by endogenous molecules may contribute to seizure activity.

Activation of the IL-1R/TLR pro-inflammatory pathway may promote neuronal hyperexcitability not only by inducing rapid and direct post-translational changes in neuronal channels and neurotransmitter receptors [29], but also indirectly by affecting target systems involved in neuronal network hyperexcitability, e.g. inhibition of glutamate reuptake and promotion of its release by astrocytes, changes in glutamate receptor subunit expression thus leading to increased glutamatergic neurotransmission [42–44], and by affecting BBB permeability [3,9,45]. A reduction in GABA-mediated inhibition in inflamed brain tissue can be anticipated by the ability of IL-1 β and TNF- α to reduce GABA-mediated chloride currents or GABA-A receptor expression at neuronal membranes [9,17,29].

Conceptually, IL-1R/TLR signaling may have an impact on epileptogenesis also via its demonstrated effects on neuronal stem cell proliferation and differentiation, by promoting cell death, and by contributing to synaptic molecular reorganization and neuronal plasticity [46–48].

The activation of the IL-1R1/TLR4 signaling may also trigger transcriptional changes which could promote chronic inflammation via IRAK-4, TRAF6 mediated activation of the NF κ B-dependent transcription of inflammatory genes. Moreover, transcriptional events induced by inflammatory mediators may contribute to a lasting decrease in seizure threshold by inducing the expression of genes involved in neurogenesis, cell death, and molecular and synaptic plasticity [3,17,49], which are processes develop during epileptogenesis, and possibly contribute to it [50].

4. Blood–brain barrier and the TGF- β signaling

In addition, dysfunction of the blood–brain barrier (BBB) may be responsible for abnormal neuronal firing. Disruption of the BBB causes the leakage of serum protein and leucocyte invasion into the brain. These exogenous inflammatory mediators have the potential to decrease seizure thresholds, which could alter channel sensitivity, neurotransmitter uptake or release, and glia-associated regulation of extracellular environments, such as potassium concentration [3,9,29,45]. Accordingly, brain inflammation is one of the etiological factors that promote epileptogenesis and ictogenesis. Indeed, BBB opening per se may lead to the induction of epileptogenesis [45] and promote the generation of seizures [51–52], and thus may serve as a potential surrogate biomarker for the brain inflammatory response and epileptogenesis. These findings indicate that a breakdown in the BBB can increase neuronal excitability by enhancing inflammatory responses in the brain.

TGF- β s are pleiotropic cytokines that play a pivotal role in intercellular communication [53], and their signaling pathways are frequently involved in cell growth, embryogenesis, differentiation, morphogenesis, wound healing, immune response, and apoptosis in a wide variety of cells [54]. TGF- β signaling is mediated mainly by two serine threonine kinase receptors, TGF- β RI and TGF- β RII, which activate an intracellular signaling system, such as phosphorylation of the Smad protein complex and the p38 mitogen-activated protein kinase (MAPK) pathway. The canonical TGF- β signaling activation is followed by translocation of the phosphorylated Smad2/3 complex to the nucleus regulating transcriptional responses. TGF- β is upregulated in many epileptogenic conditions [55], including AIDS, Alzheimer's disease, stroke, tumors, or trauma. TGF- β has also been shown to be involved in pericyte induced BBB functions [56] and in microglial activation [57].

The potential involvement of TGF- β in epileptogenesis is supported by animal experiments showing TGF- β up-regulation as part of the inflammatory response in the brains of amygdala-kindled rats [58] and the hippocampus of rats exposed to status epilepticus [59]. Recent studies in rats demonstrated that serum albumin extravasated through a dysfunctional BBB binds to TGF- β R and activates TGF- β signaling [60–61]. Accordingly, transcriptome analysis revealed a strikingly similar transcription modulation pattern in animals exposed to BBB dysfunction, serum-derived albumin or following direct brain exposure to physiological levels of TGF- β 1. Importantly, blocking TGF- β signaling in the albumin model of epileptogenesis reversed inflammation and transcriptional patterns associated with activated glia and prevented the development of epileptiform activity. The extent of BBB leakage positively correlates with the frequency of spontaneous seizures in rats suggesting a reciprocal cause-effect relationship [62]. However, the detailed mechanisms and cellular pathways bridging TGF- β signaling to seizures in different cell types within the neurovascular network are still a matter of investigation.

5. COX-2 and prostanoids

COX-2 is constitutively expressed at low to moderate levels in both neuronal cell bodies and dendritic spines in hippocampal neurons, is regulated by synaptic activity [63], and is markedly induced in neurons within an hour after a seizure [64], partly via a pathway involving NMDA receptors [65]. Interestingly, each COX-2 molecule undergoes suicide inactivation after converting about 400 arachidonate molecules [66], which renders the COX-2 system very dynamic in response to changing levels of neuronal activity. COX-2 and mPGES-1 are known to play key roles in the inflammatory responses to insults and consequently increase post seizure inflammation and the resulting hyperexcitability of brain neurons. In addition to inflammatory cytokines, prostaglandins (PGs) are major factors that stimulate inflammation processes. PGs are known to markedly increase following seizures and may contribute to epileptogenesis and reduction in seizure threshold [29, 67]. Because PGs play an important role in inflammatory responses, the functions of PGs in epileptogenesis have been studied for a considerable amount of time [68,69]; however, data on the roles of COX-2 in epilepsy appear to be bidirectional. Several lines of evidences suggest that PGE2 could have an important role in epileptic neuronal cell death and the reduction in seizure threshold. Consequently, more precise molecular roles of PGs in epileptogenesis will need to be clarified.

6. Other inflammatory molecules

HMGB1 is considered to be a danger signal released from injured or stressed cells to alert the microenvironment of an immediate or ongoing injury. Its interaction with cognate TLR4 triggers innate immune mechanisms in tissue and activates the related inflammatory events [70]. The activation of both innate and adaptive immune systems has been described in human epilepsy. The analysis of brain specimens from drug-refractory epileptic patients showed upregulation of IL-1 β and HMGB1 and their receptors IL-1R1 and TLR4, in glia and neurons in epileptogenic tissue. This suggests that the activation of these signaling pathways occurs in human epilepsy [27,67,71–74]. Moreover, upregulation of complement system and COX-2 were also shown in parenchymal brain cells [11,19,75]. Noteworthy, in epilepsy associated with malformations of cortical development, a positive correlation was found between the percentage of IL-1 β -positive brain cells and the frequency of seizures prior to surgical resection [74]. Cells of adaptive immunity were detected in some but not all types of epilepsy; for example, a notable absence of lymphocytes was described in temporal lobe epilepsy specimens [67], and this is clearly different from Rasmussen's encephalitis or from epilepsies associated with malformations of cortical development where these cells were found often in close apposition with degenerating or dysmorphic neurons [76]. Inflammatory cytokines, including IL-1 β and HMGB1, are released from astrocytes and microglia after seizures [76–79]. IL-1 β and HMGB1 activate IL-1R type I [80] and Toll-like receptor 4, respectively [81]. Up regulation of protein levels of several inflammatory mediators (IL1 β , CCL2, CCL3, CCL4, VCAM1, ICAM1, and VEGF), have been found to be up-regulated in human mTLE tissue and animal models for TLE having pro-epileptogenic properties. Particularly, IL-1 β has frequently been associated with pro-epileptogenic properties, a protein which affects neuronal Ca²⁺ influx through NMDA-dependent signaling. The endogenous antagonist of IL-1 β , IL-1ra, has been attributed with seizure-inhibiting properties [82–92].

TNF- α is another inflammatory factor, and its expression is also up-regulated following seizures [93,94]. TNF- α is mainly released by microglia in the brain [95], and it can stimulate astrocytes to release glutamate [96]. Mice overexpressing cytokines, such as TNF- α or IL-6, within astrocytes developed age dependent neurological dysfunctions including a reduction in seizure threshold, spontaneous seizure frequency, and neuronal cell loss [94,95]. Basal IL-6 levels may be increased in patients with chronic epilepsy when compared with healthy controls. In some studies, basal IL-6 has been reported to be more affected in

patients with TLE than in patients with extra-temporal lobe epilepsy [87,88].

In neonatal seizures, IL-10 was significantly elevated in plasma 48–72 h after seizure onset [97,98]. The surge in IL-10 levels 24–72 h after seizure onset may indicate the enhanced protective role of IL-10, which has an anticonvulsive effect in neonatal seizure patients by suppressing proinflammatory cytokine production. IL-8 act as proinflammatory cytokines, further activating the cytokine cascade and increasing seizure susceptibility and organ damage. IL-8 is a proinflammatory cytokine and is significantly increased in refractory epilepsy patients. The IL-8 concentration in the serum and CSF of patients with refractory epilepsy was significantly increased after seizures, including focal, generalized tonic-clonic, myoclonic, atypical absence, and typical absence seizures [98–99].

Moreover, in rats with TLE, there is a prolonged increase in rat hippocampal chemokine signaling (an early and persistent upregulation of CCL4/CCR5 signaling) during epileptogenic process [100].

We have also studied the cytokine levels (IL-10, IL-Ra, IL-1 β , IL-6, CCL3, CCL4 and TNF α) in surgically resected tissues of MTLE and focal cortical dysplasia (FCD) patients. Levels of IL-Ra, IL-1 β , CCL3 and CCL4 have been found to be increased in MTLE and FCD patients as compared to controls, whereas IL-10 levels was decreased in patients as compared to controls. Levels of IL-6 and TNF- α did not show variation among groups (Unpublished data).

Consequently, epileptic seizures and inflammatory mediators can form a positive feedback loop, reinforcing each other. Taken together, epileptic seizures can provoke inflammatory responses, which enhance calcium influx into neurons, activate glial cells to increase extracellular potassium and glutamate, and induce further inflammatory response via BBB break down. These inflammatory responses may promote neural hyperexcitability and decrease the seizure threshold. In summary, this set of evidence highlights the possibility that the activation of IL-1R/TLR signaling may decrease the seizure threshold, a phenomenon that also develops during epileptogenesis.

7. Therapeutic approaches to target inflammation

These studies suggest the idea that brain inflammation could promote seizures and epileptogenesis. These findings highlight the possibility to block the activation of inflammatory signaling as potential targets for therapeutic intervention with disease-modifying effects, particularly for epileptic patients not responding to conventional antiepileptic drugs.

This feature of brain inflammation could be exploited for therapeutic purposes, for example, to identify the patient population with more significant brain inflammation since these patients might benefit from specific anti-inflammatory treatments adjunctive to anticonvulsant drugs. Moreover, brain inflammation could be used as a biochemical marker of the therapeutic success of a treatment with disease-modifying properties. Thus, seizure relapse might be predicted in patients using anticonvulsant drugs, which may transiently decrease seizures without provoking a concomitant significant reduction of brain inflammation in the seizure focus. Finally, inflammation may be used to identify seizure foci with the highest degree of epileptogenicity for surgical or alternative therapeutic interventions.

Biochemical measurements of inflammatory mediators in blood and serum are, not mutually exclusive, approach [101]. The drawback of these types of measurements is the difficulty in demonstrating that peripheral biomarkers meaningfully reflect the degree and extent of brain inflammation. This is due to interference of peripheral sources, such as the liver, the lymphoid organs or even the muscles, which can release cytokines during intensive activity. Antiepileptic drugs may also increase blood proinflammatory cytokines [102]; therefore, caution should be taken when considering blood cytokines as biomarkers in epilepsy.

The rapid half-life of many inflammatory cytokines makes it also difficult to accurately detect their levels in peripheral fluids. Cerebrospinal fluid measurements should give a more direct measure of the inflammatory mediators released from an epileptic tissue. However, these samples are not routinely available, and cytokine levels may differ dramatically owing to the size of brain tissue involved and not only because of the inflammatory load. Moreover, dilution effects along the ventricles and spinal CSF may render the levels of relevant cytokines undetectable or may not readily reflect the extent of inflammation. In addition, blood and CSF measurements lack critical information on the spatial characteristics of the brain's inflammatory response and may vary significantly depending on the extent of the lesion. These aspects are likely to underlie the variability of data reporting on changes in peripheral blood or CSF levels of several cytokines in human epilepsy, either after seizures or interictally.

Neuroimaging approaches (MRI, PET) have been also investigated to detect the inflammatory processes in epilepsy. Several magnetic resonance imaging (MRI) techniques, such as magnetization transfer, gadolinium enhancement, diffusion-weighted imaging, and magnetic resonance spectroscopy may be able to show aspects of active inflammatory processes as has been suggested in multiple sclerosis, although the relation of scan findings to pathologic processes and clinical correlation may be indirect [103]. There are limited data for MRI detection of inflammation in epilepsy.

One disadvantage of PET tracers is that about 10% of people fail to show a high enough level of specific central nervous system binding to allow adequate imaging [104]. Variation in binding across a population would complicate comparisons of patients with controls, although a not side-to-side within-patient comparison, when binding is high enough for adequate PET imaging. It would be valuable to have PET tracers with higher specific binding, and to understand the mechanism behind nonspecific binding. In addition, it will be important to confirm the implications of binding detected by PET with post hoc studies of inflammatory markers in resected tissue from patients with intractable epilepsy.

Therefore, the future challenge is to characterize reliable and stable markers of inflammation in peripheral fluids specifically reflecting the brain phenomenon is utmost important. Regulatory molecules that reflect inflammation or neurodegeneration in an epileptic lesion should also be considered as potential molecular biomarker underlying this pathogenic mechanism. In this context, miRNAs are coming to light as crucial regulators of innate and adaptive immune responses, and their abnormal expression and/or function in the immune system have been linked to multiple human diseases including epilepsy. The possible use of miRNAs as biomarker, as well as miRNA-related novel treatment modalities, might open a new future for the diagnosis and treatment of epileptic conditions. miRNAs are attractive alternative for several reasons. Individual miRNAs can have several targets within the same cell and impact more than one pathway. Second, the processes under miRNA control include several central to epileptogenesis, including neuronal death, gliosis, inflammation and neuronal microstructure [105–106]. Third, the field of RNA based therapeutics has advanced dramatically in recent years with many innovative medicines now in clinical trials. And the most important is that miRNAs have tremendous potential as non-invasive biomarkers for the diagnosis and prognosis of disease, monitoring of treatment, and patient stratification, because of their stability and ease of detection in most tissues, especially blood.

8. Regulation of inflammatory pathways by microRNA in epilepsy

MiRNAs are a class of gene regulators that have recently emerged as key players in the innate and adaptive immune system. miRNAs are short (20–23 nucleotides), non-coding RNAs that recognize partially complementary target sequences in select mRNAs and predominantly inhibit protein expression by either destabilizing their mRNA targets or by inhibiting protein translation [107–111].

Mi RNAs have a significant role in inflammatory pathways which have been shown to be involved in epilepsy (Table 2). MiRNA is a key regulator of the innate immune response in the modulation of astrocyte-mediated inflammation. These miRNAs can possibly be used as a novel therapeutic target in the treatment of epilepsy. In addition to the brain, miRNAs are also reported to be regulated in blood, suggesting the possible use of blood miRNAs as biomarkers for brain injury and other neuronal diseases [112].

A study by Asirvatham et al. [113] utilized a computational approach to identify miRNA targets of 613 immune genes that predicted ~275 immune gene-miRNA interactions including transcription factors, chromatin modifiers and genes involved in immune signaling pathways and inflammation including cytokines. In silico analyses suggest that the TGF- β pathway is highly regulated by miRNAs. However TGF- β 1 and the receptors TGF- β RII, Endoglin, and Cripto-1 do not have any predicted miRNA target sites whereas multiple miRNA binding sites are predicted in the downstream signaling components including all 7 mad genes and the co-repressor TGIF. Seventeen of the 24 components in the TGF- β pathway are likely to have 3'UTR binding sites for 64 different miRNAs. Moreover the 17 target genes are all hubs with 8 or more different miRNA sites in their 3'UTR making them high probability sites of miRNA control [114]. The observation that miRNAs are likely regulators of cytokine mRNAs suggests that they might also be involved in diseases related to abnormal immune responses including certain inflammatory disorders.

Over the past 5 years, several target studies and genome-wide miRNA expression profiling studies have identified changes to over 100 different miRNAs in epilepsy patients and animal models, and provided compelling evidence that epilepsy is associated with widespread changes to miRNA expression. Several studies support the hypothesis that miRNAs may contribute to the pathogenesis of epilepsy [115–120]. Analysis of miRNA signatures in epilepsy patients revealed different levels of miRNA deregulation (changes in expression and subcellular

Table 2
MicroRNAs potentially involved in regulating inflammatory processes in epilepsy.

MicroRNA	Potential role/target	References
miR-146a	IL-1 α , IRAK1, IRAK2, IL-8, CXCR4, TRAF6, TLR2, IFNG, CD40LG, TLR-4, IFI27, CCR9	[118,119,123,126,128,154]
let-7f	IL-6, IL-10, IL13	[118,154]
miR-203	ICAM-1, IL-6, TNF, IL24, MYD88, SMAD4, LEF1	[118,154]
miR-221	ICAM-1, TRAF4, FN1	[118,131,133,154]
miR-222	ICAM-1, IRAK1,	[118,131,133,154]
miR-155	TNF- α , ICAM1, IL8, IFNGR1, IRAK3, CCR9, IL13RA1, IL17RB, CCL-2, IL-6, NFKB1, TLR6	[130,154]
miR-27a	IL-10, MMP-13	[118,154]
miR-16	MCL-1, TNFSF9, VEGFA, FGFR1, IFNG, TNFRSF12A, IL36RN,	[118,154]
miR-17	ICAM1, VEGFA, CCL1, TGFBR2, TNFSF12, SMAD4, TNFRSF21, FN1, IL8, MMP-2, TNFSF13, IRAK1	[118,154]
miR-20a	VEGFA, TGFBR2, IL8,	[118,154]
miR-106a-5p	IL-10, VEGFA,	[118,154]
miR-204-5p	IL-1 β , IL-11, IL-8, TGFBR1, TGFBR2, MMP-3, MMP-9, IL1RAP,	[120,154]
miR-21-5p	IL-1 β , ICAM, TRAF-6, NFKB1	[119,154]
miR-125a	CCL5, VEGFA,	[119,154]
miR-146b	IRAK1, TRAF-6, NFKB1	[118,119,154]
miR-144	TGF- β 1, ILB1,	[119,154]
miR-98	IL-13, IL-6, IL-6R, TRAF1, TNFRSF9, SMAD7, TNFSF9, IL32, ICAM1, NFKB2, IFNAR1, IFNGR1, CXCL2	[119,154]
let-7d-5p	PDGFA, IL-13, TGFBR1,	[119,154]
miR-106b-5p	VEGFA, CD34, TLR2	[119,154]
miR-130a-3p	SMAD5, SMAD 4	[119,154]
miR-15a-5p	NFKB1, IFNG, IKBKKG	[119,154]
miR-301a-3p	NKRF, SMAD4	[120,154]

distribution) and led to identify astrocytes and the immune response as a target of deregulated miRNAs in epilepsy. Inflammatory mediators were most prominently targeted by deregulated miRNAs [118]. This is in line with the idea that inflammation may play a central role in epilepsy [24]. Of several cellular processes implicated in epilepsy, the immune response was most prominently targeted by deregulated miRNAs. Enhanced expression of inflammatory mediators was paralleled by a reduction in miRNAs that were found to target the untranslated regions of these genes.

Emerging evidences highlight that miR-146a may be involved in epileptogenesis through regulating the inflammatory response. MiR-146a has been identified as a key regulator in a feedback system whereby induction of nuclear factor kappa-B (NF- κ B) through a myeloid differentiation factor 88 (MyD88)-dependent pathway may up-regulate the miR-146a, which in turn could down-regulate the levels of two key adapter molecules, IL-1RI-associated protein kinases – 1 (IRAK1) and – 2, and TNF receptor-associated factor 6 (TRAF6) downstream of TLR and cytokine receptors, reducing the activity of this inflammatory pathway [121–122]. In Wang's study (2015), they also found that IRAK1 is dysregulated in the opposite direction to miR-146a, and associated with inflammation in the KEGG analysis [119]. Moreover, both miR-146a and IL-1 β were demonstrated to be up-regulated in astrocytes in epilepsy models [123,124], and IL-1 β represents a major pro-inflammatory cytokine involved in the induction of miR-146a [123,125], thus it is possible that expression of miR-146a in astrocytes may represent an attempt to modulate the inflammatory response triggered by IL-1 β [123].

MiR-146a is significantly up-regulated in tissues obtained from patients with MTLE [116,123]. MiR-146a has been implicated in regulation of astrocyte-mediated inflammatory response [126]. In addition, *in vitro* experiments showed a significant up-regulation of miR-146a in astrocytes when exposed to IL-1 β stimulation, which is known to be up-regulated in the acute phase of some animal models of MTLE. MiR-146a is significantly upregulated both in lithium-pilocarpine-induced SE rat model and also in human with MTLE [115,116,127]. Highest miR-146a expression is found in the latent stage of epilepsy, whereas its expression is lowest at acute stage. In response to inflammatory cues, miR-146a acts as a negative-feedback regulator of the astrocyte-mediated inflammatory response [128]. Modulation of miR-146a expression by transfection of astrocytes with anti-miR146a regulates the mRNA expression levels of downstream targets of miR-146a (IRAK-1, IRAK-2 and TRAF-6) and the expression of IRAK-1 protein. In addition, the expression of IL-6 and COX-2 upon IL-1 β stimulation was suppressed by increased levels of miR-146a and increased by the reduction of miR-146a [121,128,129].

Thus it has been concluded that miR-146a represents a negative regulator of TLR signaling-highlighting new roles for miRNAs in signaling pathways.

Another miRNA that has been associated with inflammatory pathways in MTLE is miR-155. It has been demonstrated an increase in the expression of miR-155 in hippocampal tissue from children with MTLE, as well as in an immature rat epilepsy model. Moreover, the observed increase in miR-155 expression correlates with an increase in TNF- α and ICAM-1 in the nervous tissue [130].

In vitro experiments revealed that miR-221 and miR-222 target the 3'UTR of ICAM1 (CD54). ICAM1 mediates interactions with other (immune) cells to influence processes such as inflammation [131]. Although the function of astrocyte-associated ICAM1 remains poorly understood, it has been proposed to mediate leukocyte accumulation, microglia recruitment and cytokine production (e.g. IL-1 β , IL-6) [132–134]. Kan et al. (2012) [118] proposed that the down-regulation of miR-221 and miR-222 in mTLE + HS is linked to a local up-regulation of ICAM1 in astrocytes. Interestingly, recent work has shown that miR-222 can regulate ICAM1 in glioma cells [135]. Enhanced ICAM1 expression may then contribute to the release of other inflammatory mediators and the recruitment of immune cells, thereby augmenting and/

or sustaining the immune response. Previous work also indicates that the expression of ICAM1 is regulated posttranscriptionally in mTLE. Kan et al.'s study [118] provide support to this idea by revealing a down-regulation of miRNAs that target ICAM1.

Wang's recent studies [119–120] to identify novel miRNAs in serum for diagnosis and drug resistance has also identified some of the dysregulated miRNAs associated with inflammation. With respect to let-7d-5p, it has been found dysregulated in alzheimer's disease (AD) [136] and multiple sclerosis (MS) [137]; and in MS, let-7d-5p showed a positive correlation with the pro-inflammatory cytokine IL-1 β . AD, MS and epilepsy are all belong to neural diseases, thus it is possible that let-7d-5p may participate the pathogenesis of these three diseases in some common pathways. For miR-106b-5p, – 130a-3p, – 15a-5p and – 194-5p, it has been indicated that these miRNAs were involved in inflammation, apoptosis and cell proliferation in cancers [138–142]. Expression of miR-194-5p, – 301a-3p, – 30b-5p, – 342-5p and – 4446-3p were found to be significantly decreased in drug-resistant patients compared to drug-responsive patients and healthy controls [120]. Targets of these miRNase include many genes related to inflammation and apoptosis, such as MAPK1, ATM, MYD88, RBL1, TRAF6, PIK3CD, IFNAR2, etc., indicating that these miRNAs may play a role in drug-resistant epilepsy through inflammation and apoptosis. MiR-301a-3p was involved in inflammatory response through impacting NF- κ B signaling pathway in cancer [143].

The functions of miR-23a, which was upregulated in all profiling studies on experimental epilepsy, include control of apoptosis, inflammation and transcription factors involved in differentiation [144].

In the future, manipulation of the identified deregulated miRNAs related to neuroinflammation in animal models of epilepsy will help to design some novel therapeutic strategies for epilepsy as well as explore the potential to serve as biomarker.

9. Future perspectives

More recently, with the remarkable progress of immunology, immunity and inflammation are considered to be key elements of the pathobiology of epilepsy. The possibility that inflammatory processes in the brain contribute to the etiopathogenesis of seizures and the establishment of a chronic epileptic focus is increasingly recognized as a result of supportive evidence in experimental models and in the clinical setting. Immunity and inflammation are an integral part of epileptogenesis and their central role in the pathogenesis of epilepsy has raised the prospect of new therapeutic approaches to counteract epilepsy.

Targeting inflammation to treat epileptic seizures rather than targeting neural function has the advantage of potentially less side effects than traditional anticonvulsant therapies, and further this allows action on a pathogenic cause of epilepsy rather than modifying the disease. Another advantage would be the potential prophylactic aspect, which could help prevent the development of seizures in patients at risk of epilepsy, including victims of traumatic brain injury, stroke, and cerebral infection. Although the potential mechanisms of immunotherapeutic strategies in drug-resistant seizures have been extensively discussed, evidence on the efficacy of such therapy is limited. However, treatment strategies employing general or specific anti-inflammatory agents in animal models for MTLE have not been fully effective thus far [15,27,145–148].

MiRNAs are implicated in establishing and maintaining the cell fate of immune cells (e.g. miR-181a and miR-223), and they are involved in innate immunity by regulating Toll-like receptor signaling and ensuing cytokine response (e.g. miR-146a). Moreover, miRNAs regulate central elements of the adaptive immune response such as antigen presentation (e.g. miR-155) and T cell receptor signaling (miR-181a). Expressions of different miRNAs in different brain regions are implicated in epileptogenic activity. MiRNAs are regulators of the innate immune response in the modulation of astrocyte-mediated inflammation. These miRNAs can possibly be used as a novel

therapeutic target in the treatment of epilepsy. Targeting miRNA in epilepsy supports it as a feasible strategy for the treatment of epilepsy. In this context, miRNA 146a, miRNA 155, let-7d-5p, miRNA 221 and miRNA222 which have been found to be involved in inflammatory signaling pathways in different epilepsy models need to be extensively studied. In the future, manipulation of these miRNAs related to neuroinflammation in animal models of epilepsy will help to design some novel therapeutic strategies for epilepsy as well as explore the potential to serve as biomarker. These discoveries, if validated in humans, could lead the way to simple diagnostic tests that could support patient treatment decisions and prognosis.

There is growing interest in targeting miRNAs in a range of diseases, including CNS disorders. Blocking liver-expressed miR-122 is effective in combating hepatitis C infection, and miravirsin, an antagomir targeting miR-122 and the first to enter clinical trials, was found to be well tolerated and effective in humans. There will of course be major challenges with targeting and delivery of miRNAs for CNS disorders. Foremost, antagomirs are too large to cross an intact blood–brain barrier. We need more data on the effects they produce in animals, and we may need a means to target miRNA manipulations to specific cell types to avoid off-target effects. If these problems can be overcome, miRNA-based treatments could be deployed as antiepileptogenic or disease-modifying treatments [149–153].

A second potential clinical application of miRNA research is to use biofluid profiles as molecular diagnostics which will allow monitoring of the dynamics of pathologic processes and to combine the detection of these biomarkers with outcome data to improve the diagnostic/prognostic accuracy. Because of the chemistry of miRNAs and their manner of transport in biofluids enclosed in microparticles and complexed to Ago2 — they are stable and can be reliably detected in serum or plasma. Data show that blood levels of certain miRNAs are altered following epilepsy-precipitating injuries, including status epilepticus, as well stroke, intracerebral hemorrhage and trauma.

Certainly miRNAs are changing the way we think about the development of the immune system and regulation of immune functions in epilepsy and represent an entirely new potential target for the treatment and prevention of epilepsy. Drug like molecules targeting miRNAs (antagomirs) are now in clinical trials for other diseases. Further studies are necessary to develop strategies to target inflammatory factors in order to inhibit seizures or prevent epileptogenesis particularly in the clinical setting.

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