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Role of neuroinflammation factors as potential biomarkers of epilepsy: a narrative review

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ABSTRACT

Epilepsy is a common neurological condition with a significant socioeconomic impact. Approximately one in three patients is resistant to the available therapies, and the mechanisms of this resistance are often unclear. Neuroinflammation, recognised as a potential cause of drug-resistant epilepsy, plays a key role in modulating synaptic transmission and hyperexcitability.

In this narrative review, we explore the molecular basis of neuroinflammation in epilepsy and its potential as a source of biomarkers for diagnosis and treatment. Evidence from human and animal studies indicates a strong association between neuroinflammation and epilepsy, with significant involvement of pro-inflammatory molecules and blood-brain barrier dysfunction. We highlight the roles of microglia, astrocytes and inflammatory molecules in epilepsy, suggesting that targeted anti-inflammatory therapies could be promising for treatment. Further research is needed to fully understand the role of neuroinflammation in epilepsy and to develop new therapeutic approaches.

Keywords: neuroinflammation, blood-brain barrier, cytokine, epilepsy, seizure

Introduction

Epilepsy is one of the most widely prevalent neurological conditions affecting people of all ages. In most cases, the current treatment approach primarily focuses on termination of seizure activity, and does not address the underlying causes nor seek to alter the course of disease. Approximately one in three patients exhibits resistance to the available therapies, and the mechanisms of this resistance often remain unclear [1].

The International League Against Epilepsy defines drug resistant epilepsy as "failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" [2]. Drug resistance in epilepsy is most likely the result of multiple mechanisms that can occur simultaneously in the same patient [3]. Recent studies in preclinical models have led to a better understanding of the pathophysiological mechanisms underlying epilepsy and drug resistance [1]. These advances have led to revisions in drug development, including a variety of aetiology-specific potential drugs. It seems that in the era of personalised medicine, drug resistance, along with the aetiology of the disease, medical history, comorbidities, and previous responses to medications, should be considered when choosing antiepileptic treatment.

There are many hypotheses regarding drug resistance in epilepsy. Neuroinflammation, a key factor in modulating synaptic transmission (Fig. 1) has alongside hyperexcitability been identified as a possible common mechanism that links various theories of drug-resistant epilepsy. Persistent brain inflammation caused by any trigger and insufficient regulation of the body's own anti-inflammatory processes might be linked to the development of epilepsy. It should be emphasised that neuroinflammation is not the only mechanism of drug resistance, and its contribution to treatment failure may vary in different patients [4, 5]. Therefore, research on biomarkers of neuroinflammation, particularly the biomarkers of epilepsy with good response to anti-inflammatory treatment, is needed.

The aim of this narrative review was to show the molecular basis of neuroinflammation in epilepsy and evaluate its potential as a source of biomarkers for diagnosis and treatment.

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Moreover, the review sought to identify gaps in biomarker validation, highlight current challenges in their clinical application, and propose directions for future research to enhance their diagnostic and therapeutic utility.

Neuroinflammation in epilepsy

An association between neuroinflammation and epilepsy has been abundantly evidenced in human and animal studies [5–10]. Cerebral inflammation has been documented in humans with drug-resistant epilepsy of various aetiologies such as cortical dysplasia [7], hippocampal sclerosis [8], and tuberous sclerosis [9, 10]. Increased levels of pro-inflammatory molecules in epilepsy patients have been noted in many studies [5]. Interestingly, in patients with drug-resistant temporal lobe epilepsy, elevated levels of proinflammatory cytokines (interleukin (IL) 1β and interleukin 6) have been shown to decrease one year after surgical treatment, correlating with a reduction in the number of epileptic seizures. This supports the theory that seizures are the cause of the observed inflammatory changes, which subside after removal of the epileptogenic tissue [11].

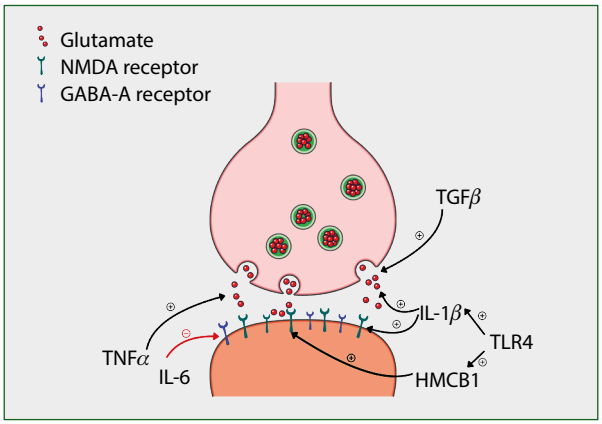

Figure 1. Effects of selected proinflammatory cytokines on neurotransmission

Neuroinflammation, triggered by a number of exogenous or endogenous conditions, is characterised by a series of inflammatory responses in the central nervous system (CNS; Fig. 2) [5]. This involves the activation of microglia, astrocytes, and endothelial cells in the blood-brain barrier (BBB), which in turn increase the expression of pro- and anti-inflammatory mediators. Not only is neuroinflammation associated with neuronal hyperexcitability and seizures, but prolonged or frequent seizures can also trigger a chain of neuroinflammatory reactions, leading to the so-called 'vicious circle' and epilepsy that is even more resistant [12]. Persistent stimulation from seizure activity and chronic neuroinflammation can result in BBB disruption, neuronal loss and sustained neuronal hyperexcitability. Inflammatory molecules that increase neuronal hyperexcitability lead to heightened seizure activity, resulting in excitotoxic damage to neurons and subsequent alterations in neuronal networks [5]. The increase in the level of inflammatory factors is accompanied by an induction of the expression of a wide range of surface receptors that accelerate the immune response. Activation of cytokine receptors on neurons changes their excitability via post-translational modifications of voltage-gated or receptor-coupled ion channel, and additionally induces presynaptic changes in neurotransmission. A special

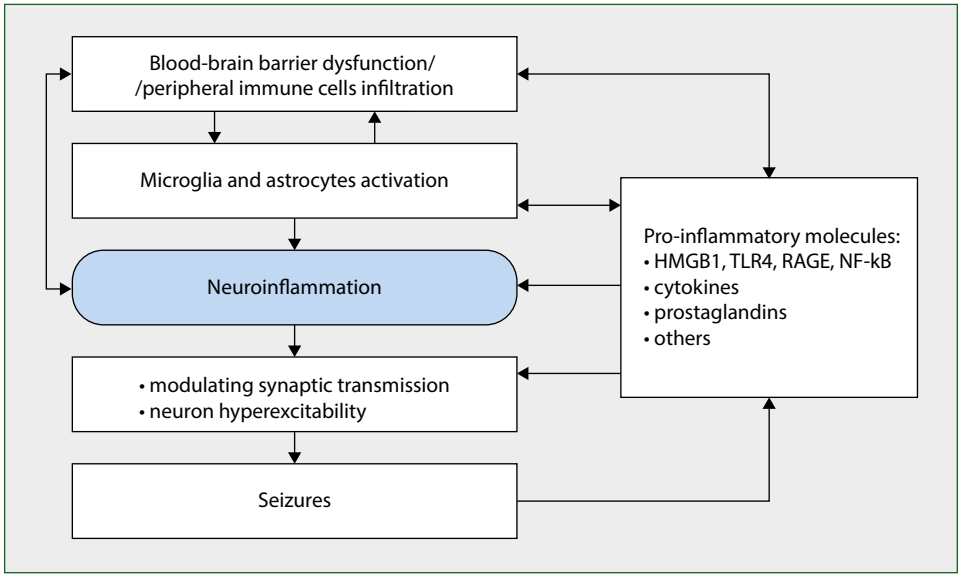

Figure 2. Role of neuroinflammation factors in epilepsy. HMGB1 — high mobility group box 1; TLR4 — toll-like receptor 4; RAGE — receptor for advanced glycation end products; NF-kB — nuclear factor kappa-light-chain-enhancer of activated B cells

feature of these changes is their rapid onset (seconds/minutes) and their long-term (hours/weeks) persistence *in vivo* [5]. Inflammatory mediators also induce transcriptional gene activation, leading to a prolonged inflammatory response and molecular plasticity changes that provoke epileptogenesis [13].

All molecules contributing to neuroinflammation can serve as potential biomarkers for the diagnosis and treatment of epilepsy. Identifying such biomarkers could play a pivotal role in selecting patients most likely to benefit from targeted therapies, while also providing pharmacodynamic indicators to evaluate therapeutic efficacy [14]. Recent studies have highlighted emerging molecular techniques, such as multi-omics approaches, as being pivotal in advancing biomarker discovery. These methodologies integrate diverse data types to unravel complex biological interactions, driving innovation in precision medicine [15]. Further research is necessary to build upon these findings.

Blood-brain barrier dysfunction and neuroinflammation

The blood-brain barrier plays a crucial role in maintaining the stability of the brain's environment. It is a system of capillaries functionally connected to the parenchymal cells of the brain which control the exchange of blood components from the blood to the brain. Brain endothelial cells are connected by tight junctions that prevent macromolecules (e.g. proteins and small lipid-insoluble molecules) from penetrating the brain. Under physiological conditions, molecular flow uses transcellular bidirectional transport pathways that can only pass through a series of membrane transporters, ATP-binding cassette proteins and vesicular transport systems [5].

BBB dysfunction is caused by multicellular failure, primarily of endothelial cells and astrocytes. This results in serum protein leakage and leukocyte infiltration into the CNS, which is a key process in neuroinflammation. In other words, BBB dysfunction is pathologically increased permeability. Dysfunction of the BBB not only contributes to the development of epilepsy, but is also influenced by seizures, indicating a bidirectional relationship. Impairment of the BBB contributes to the development of epileptic seizures, but is at the same time also a consequence of these seizures (a concept known as a positive feedback loop) [5, 16]. The feedback mechanism is unclear but probably involves the release of glutamate during seizures, elevating the expression and activity of matrix metalloproteinases (MMP-2 and MMP-9) at the BBB and leading to its dysfunction. The role of astrocytic and microglial IL-1β and vascular endothelial growth factor (VEGF) in increasing BBB permeability has also been considered [17–19]. In mouse models of epilepsy, provoked seizures have elevated leukocyte activity in cerebral vessels. Inhibition of their infiltration into the brain has mitigated seizures, BBB disruption, and neuronal damage [20, 21]. Ictal and interictal BBB dysfunction has been studied both in animal models and in humans with chronic epilepsy *in vivo* using quantitative magnetic resonance imaging [22–25]. Further studies on imaging biomarkers of BBB dysfunction should help to identify patients who might benefit from BBB-restorative approaches such as pharmacotherapy with losartan or new gene therapies targeting claudin-5 [22].

Roles of microglia and astrocytes in neuroinflammation

In response to neuronal damage, alongside functional changes in the endothelium of blood vessels and the recruitment of immune cells from the bloodstream to the affected brain tissue, there is also the expression of early genes responsible for the stimulation and activation of astrocytes and microglia.

Microglial cells are tissue-specific, resident macrophages of the CNS. They are classified as glial (non-neuronal) cells, and have the ability to transform from a resting (M0 subpopulation) to an activated form. Macrophages play a crucial role in maintaining metabolic homeostasis. There are two states of microglial cell activation: the classically activated macrophages (M1 subpopulation) with pro-inflammatory properties, and the alternatively activated macrophages (M2 subpopulation) with anti-inflammatory properties.

The balance between M1 and M2 varies and depends on external stimuli and regulatory factors. Abnormal regulation can result in a shift in the M1/M2 balance towards M1 or M2 phenotypes and contribute to the progression of inflammatory mediated-diseases [4, 26].

Studies on epileptic animal models have shown that microglia can lower the seizure threshold [27]. Activated microglia are capable of releasing cytokines, chemokines and complement proteins, contributing to the stimulation of a further inflammatory cascade [28]. Microglia activation has also been associated with increased levels of nitric oxide (NO), which activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, causing the release of proinflammatory mediators (e.g. tumour necrosis factor) [12]. Microglia secrete cytokines that in consequence trigger the activation, function, and proliferation of astrocytes. Astrocytes are a type of glial cell that play crucial roles in maintaining neuronal function and homeostasis. Astrocytes modulate neuronal excitability by controlling extracellular potassium levels and glutamate reuptake, two important processes in epileptogenesis [29]. Their role in the pathophysiology of epilepsy is complex, including neurotransmitter regulation, ion homeostasis, energy metabolism, inflammatory responses, and interactions with neurons and other glial cells. Activated astrocytes release

pro-inflammatory molecules, including high mobility group box 1 (HMGB1), nuclear factor Kappa-beta, and interleukin-1 beta [12, 30].

Inflammatory molecules in neuroinflammation

Roles of high mobility group box 1 (HMGB1), Toll-like receptor 4 (TLR4), receptor for advanced glycation end products (RAGE), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)

High mobility group box 1 is a chromatin-binding protein found intracellularly and extracellularly. The intracellular form is involved in the regulation of gene transcription, and the extracellular form acts as a 'danger signal' for the CNS, triggered by inflammatory processes. HMGB1 is actively released by neurons, microglia, and glia. In turn, Toll-like receptor 4 is highly expressed by microglia. HMGB1 activates TLR4 and the receptor for advanced glycation end products (RAGE), triggering the proinflammatory IL-1R/TLR4 cascade, which leads to the synthesis of proinflammatory cytokines and promotes neuronal hyperexcitability [5, 6, 28]. The proinflammatory IL-1R/TLR4 cascade also activates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Once activated, NF-κB translocates to the nucleus, where it promotes the transcription of genes involved in the immune response, such as those encoding cytokines, chemokines and adhesion molecules.

Because of the significant involvement of HMGB1, TLR receptor, RAGE and NF-kB in neuroinflammation, they are potential biomarkers of epilepsy, and therefore potential therapeutic targets, in drug-resistant epilepsy [28, 31]. Walker et al. demonstrated significantly higher levels of HMGB1 in the blood of patients with drug-resistant epilepsy than in patients with a good drug response and in healthy controls, suggesting that this protein may be useful as a biomarker for predicting response to therapy [32]. In mice, inhibiting HMGB1 using anti-HMGB1 antibodies resulted in a reduction of inflammation-related factors, reduced BBB permeability, and lowered the seizure threshold [33]. Upregulated expression of TLR4 has also been studied in many animal epilepsy models and in brain tissue from drug-resistant patients with mesial temporal lobe epilepsy, suggesting the involvement of TLR4 in ongoing inflammatory processes [34–37]. Injection of TLR4 antagonists reduced, but did not stop, seizures in mice induced by intrahippocampal injection of kainic acid and bicuculline, which suggests the process is multifactorial. These antagonists, including *Rhodobacter sphaeroides* lipopolysaccharide (LPS--RS) and cyanobacterial LPS (CYP-LPS), have decreased seizure frequency, duration, and onset delay, and reduced spontaneous seizures by c.75% in models of chronic epilepsy [30].

Role of cytokines

Inflammatory interleukines

Interleukin-1

The interleukin 1 family consists of 11 cytokines divided into three subgroups. The IL-1 subgroup contains IL-1α, IL-1β and IL-33, the IL-18 subgroup contains IL-18 and IL-37, and the IL-36 subgroup contains IL-36Ra, IL-36α, β, γ and IL-38 [36]. This family is mainly responsible for regulating innate immune responses. They bind to various IL-1R receptors which activate NF-kB and mitogen-activated protein kinase (MAPK) pathways, leading to cell apoptosis [39].

Among the interleukin 1 family of cytokines, the role of IL-1β in epileptogenesis has been the most extensively studied. IL-1β (beta) is one of the best known pro-inflammatory cytokines. It is not physiologically present within the CSF. Rather, during inflammation (due to e.g. brain insult resulting from an epileptic seizure), IL-1β is expressed in activated microglia and astrocytes. Along with IL-6 and tumour necrosis factor alpha (TNF-α), IL-1β is one of the most significant cytokines contributing to epileptogenesis. Increased levels of IL-1β have been found in brain specimens of patients with drug-resistant epilepsy and febrile infection-related epilepsy syndrome (FIRES) [39]. Elevation of IL-1β levels in CSF and serum have been confirmed in several studies, including one regarding a paediatric population [40].

On the other hand, several studies have shown an insignificant increase, and suggested that IL-1β cannot be used as an epileptogenesis marker [41]. Further research, focused on seizure type and age at epilepsy onset, is vital in order to establish the reliability of IL-1β as a biomarker of epileptogenesis.

IL-1β is a versatile particle acting via various pathways, and thus it plays many roles in epileptogenesis. For example, it promotes neuronal hyperexcitability by upregulating N-methyl-D-aspartic acid (NMDA) receptors and decreases γ-aminobutyric acid (GABA) neurotransmission by 30% [42]. IL-1β can also increase the Ca^{2+} influx via activating voltage-gated Ca^{2+} channels, and decrease K^{+} efflux. Moreover, it activates oxidative stress responses via nitrogen oxide as well as contributing to disruption of the BBB. In several case studies, the IL-1β antagonist anakinra has been used in patients with FIRES and drug-resistant epilepsy and has been considered to be a viable treatment option [39]. In addition, many studies have shown a link between IL-1β and mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE+HS) [43].

Overall, IL-1β is a cytokine that makes a significant contribution to the self-propelling vicious circle of epileptogenesis, i.e. seizures lead to IL-1β upregulation and this further promotes neuroinflammation as well as neuronal hyperexcitability. Interleukin 1α (alpha) activates the same intracellular pathways as IL-1β, although it is expressed not only in immune cells but also in the absence of inflammation, meaning that its role in the pathogenesis of epilepsy requires more research.

Interleukin-33

IL-33 is a relatively new member of the IL-1 family and belongs to the IL-1 subgroup. Its effects are similar to IL--1β. It also enhances oxidative stress responses. It has been demonstrated that IL-33 is present in higher concentrations in blood samples from patients with epilepsy, along with higher oxidative stress levels, compared to healthy controls [44].

Interleukin-6

IL-6 is one of the main pro-inflammatory cytokines and, similarly to IL-1β and IL-2, its concentration increases during a seizure, particularly in patients with new-onset refractory status epilepticus (NORSE) [45]. IL-6 increases glutamate concentration and GABA transporter (GAT-1 and GAT-3) levels, which leads to reduced extracellular GABA levels. These changes in neurotransmitter levels result in decreased inhibitory signalling and increased neural excitability. Elevated IL-6 levels are connected to enhanced BBB permeability. One study showed a strong correlation between IL-6 levels and recurrent epileptic seizures [46]. Tocilizumab (a recombinant humanised monoclonal antibody directed against IL-6 receptors) has been successfully used, along with anakinra, in a few patients as treatment in the chronic phase of febrile infection-related epilepsy syndrome (FIRES) [47, 48]. Interestingly, 4–6 months of treatment with valproic acid has been shown to reduce IL-6 serum levels, although its mechanism of immunomodulatory effect is still unknown [49, 50].

Interleukin-8

IL-8, also known as CXCL8, is a pro-inflammatory chemokine that can be produced by almost all nucleated cells in the body [51]. Its synthesis is stimulated by the NF--kB pathway, leading to further increase in concentrations of other pro-inflammatory cytokines and neuronal apoptosis. In paediatric patients with epilepsy, its level rises in the CSF as it is produced by glial cells or astrocytes [52].

Modulatory and anti-inflammatory interleukines

Interleukin-2

IL-2 is a modulatory cytokine expressed by activated CD4+ and CD8+ (effector) T cells. IL-2 is crucial for the differentiation of Treg cells, a subpopulation of CD4+ lymphocytes that regulate the immune system function. In most autoimmune diseases, Treg count and function is dysregulated. In one study, a low dose of IL-2 treatment that activates Treg, but not other T lymphocytes, was shown to be effective in the treatment of refractory autoimmune encephalitis by restoring the balance between regulatory and effector T cells [53]. On the other hand, IL-2 modulates the function of B cells and NK cells via IL-2R receptor. It has been reported that administration of IL-2 promotes the activation of microglia [54], and in some studies an increased serum level of IL-2 has been associated with epileptogenesis [55], although this requires further research because of the modulatory nature of this cytokine [39].

Interleukin-4

IL-4 is best known as a cytokine expressed by mast cells, Th2 cells, basophils and eosinophils [56]. In the CNS, it has an anti-inflammatory effect, as it promotes the differentiation of M2 macrophages from microglia. According to recent studies, IL-4 has a potentially anti-epileptogenic effect, not only by reducing inflammation, but also by reducing neuronal hyperexcitability [57]. Comparing epileptogenic lesions from paediatric patients after surgery to healthy tissue has revealed lower transcription levels of IL-4 in the patients suffering from epilepsy [39, 58].

Interleukin-10

IL-10 is an example of a classic anti-inflammatory cytokine. In the CNS, it is mainly expressed by astrocytes and microglia and acts as a protectant against apoptosis and inflammation [39, 59]. Low serum levels of IL-10 have been associated with drug-resistance in temporal lobe epilepsy with hippocampal sclerosis. IL-10 enhances GABAA receptor function, although this neuroprotective effect is blocked by higher concentrations of IL-1β [60].

Chemokines

Chemokines are small cytokines that attract specific leukocyte subgroups to the site of inflammation. Ongoing research is exploring their roles in epileptogenesis. It was recently found that pro-inflammatory cytokines such as CCL2, CCL3, CCL5, and CX3CL1 [61] are produced not only in blood, but also in brain cells. Elevated levels of particularly Th1-associated chemokines (e.g. CXCL9, CXCL10, CXCL11 as well as CCL2, CCL19 and CXCL1) have been identified in FIRES. In encephalitis, the profile of elevated chemokines and cytokines was wider [62]. Researchers measured the levels of CCL2, CXC3CL1 and IL-8 in plasma and CSF of patients with drug-resistant epilepsy, and they were all elevated. Particularly CCL2 is a potential epileptogenesis biomarker as it is well detected in blood plasma, which is much easier to collect than a CSF sample [51].

Tumour necrosis factor alpha

TNFα belongs to a superfamily of pro-inflammatory molecules. In the CNS, it is released by activated microglia and astrocytes, e.g. when glutamate levels are too low, thus leading to more pronounced neuronal excitability [63]. Higher serum and CSF concentrations of TNFα have been found in patients with epilepsy compared to healthy controls. Moreover, the levels were higher in patients with drug-resistant epilepsy than in patients with a less severe course of disease [64]. Adalimumab, an anti-TNFα monoclonal antibody, when administered to patients with Rasmussen's Disease, resulted in a reduced frequency of seizures [41, 65].

Interferons (IFNs)

Interferon gamma (IFN-γ) is a proinflammatory cytokine, which in binding to its heterodimeric receptor (IFN-γR) activates the Janus kinase-signal transducer and transcriptional activator (JAK/STAT) pathway [29, 41]. This pathway regulates the production of cytokines by T helper type 1. Activation of this pathway may reduce gamma-aminobutyric acid receptor subunit alpha-1 (GABAARα1) levels, which in turn can cause excessive hypersynchronous neuronal activity. Research has demonstrated that STAT1-deficient mice exhibit an impaired response to IFN, resulting in increased susceptibility to bacterial and viral infections [29]. Higher levels of IFN-γ have been detected in the serum and cerebrospinal fluid of patients with epilepsy, and in the hippocampus of rat models of epilepsy [64, 66].

In addition to the evidence for the active role of certain proinflammatory factors in epileptogenesis, researchers are still looking for ways to use them in the diagnosis of epilepsy. Gao et al. aimed to investigate the role of inflammatory cytokines, including IFN-γ, in active epilepsy by measuring their levels in both interictal and postictal states. Data from 48 epilepsy patients and 30 healthy controls revealed that IFN-γ levels were significantly higher in both states among patients with epilepsy compared to controls. Additionally, while no significant differences were found in cytokine levels based on seizure type, medication or epilepsy type, interictal IFN-γ levels positively correlated with seizure frequency. These findings suggest that elevated IFN-γ may serve as a biomarker for seizure severity and prognosis in epilepsy [67].

Transforming growth factor beta (TGF-β)

Transforming growth factor beta is a cytokine involved in numerous physiological processes including cell growth, differentiation and immune regulation. It functions through its receptors TGF-βR1 and TGF-βR2, activating intracellular signalling pathways. The key role of TGF-β in epilepsy lies in reducting glutamate reuptake by decreasing the activity of glutamate transporters in glial cells, which results in higher levels of extracellular glutamate, and enhanced excitatory signalling [13].

The role of TGF beta has been analysed in many animal models and in human studies [68]. Experimental models have demonstrated that TGF-β signalling exacerbates seizure activity by promoting neuronal hyperexcitability and inflammation. In turn, in patients with drug-resistant epilepsy, TGF-β signalling is notably upregulated, with elevated levels of TGF-β1 found in the cerebrospinal fluid [69]. The expression of cytokines including TGFs varies across patients, even when they share similar clinical features, which is probably due to their different genotypes. Genetic analyses have identified in a Chinese population single nucleotide polymorphisms in TGFβR1 that correlate with epilepsy risk, suggesting that certain genotypes may influence susceptibility to seizures [70].

TGF-β signalling facilitates the entry of albumin into astrocytes following direct brain exposure to serum albumin, which reduces the buffering capacity of extracellular potassium and leads to NMDA receptor-mediated neuronal hyperexcitability and seizure-like activity [71]. Additionally, activating TGF-β pathways generates epileptiform activity, marked by decreased expression of GABAergic-related genes and increased expression of glutamatergic-related genes [72]. Interestingly, inhibiting TGF-β signalling pathways by angiotensin II type 1 receptor antagonists successfully delayed the onset of recurrent spontaneous seizures in a rat model of vascular injury [73]. A similar mechanism was observed in a study of mice treated with intracerebroventricular albumin injections, inhibiting the TGF-β pathway and preventing seizures [74]. These findings underline the critical role of TGF-β in the pathophysiology of epilepsy, and highlight the potential for targeted therapies to manage or modify the disease [70, 71].

Prostaglandins

Prostaglandins (PG) play a significant role in the pathophysiology of epilepsy by modulating neuronal excitability and inflammation. Prostaglandin E2 (PGE2), produced primarily by astrocytes and microglia, interacts with its receptors EP1, EP2, EP3, and EP4 to influence neuronal activity. Stimulation of EP3 receptors on astrocytes by PGE2 increases glutamate release, contributing to neuronal hyperexcitability and cell death, while the inhibition of EP3 may delay seizure onset. Additionally, membrane-bound PGE2 synthase (mPGES) boosts the production of GFAP-positive astrocytes after seizure kindling, and PGE2 antagonists have been shown to reduce both seizure severity and neurological damage in experimental models. The role of cyclooxygenase-2 (an enzyme involved in PGE2 synthesis) as a therapeutic target remains controversial due to mixed results: its inhibition can sometimes exacerbate seizure susceptibility due to reduced production of anticonvulsant prostaglandins such as prostaglandin D2 (PGD-2). Ongoing research is exploring alternative targets within the prostaglandin synthesis pathways to develop more effective treatments for epilepsy [42, 75].

Complement

The complement system is a complex network of proteins that plays a pivotal role in the immune response. It enhances the ability of antibodies and phagocytic cells to eliminate pathogens and damaged cells [76].

Although an understanding of the role of individual components of the complement is crucial for exploring how complement dysregulation might contribute to diseases such as epilepsy, many of the mechanisms remain unclear [77]. Basaran et al. revealed that untreated patients with epilepsy

exhibit significantly elevated levels of serum C3 compared to healthy controls [78], suggesting that the complement system is more active in individuals with epilepsy, particularly those who are not treated. Further research has corroborated these findings, indicating that components of the classical complement pathway are also elevated in patients with epilepsy compared to healthy individuals. This elevation is not only observed in untreated epileptic patients but also shows a marked difference when compared to patients who are undergoing treatment for epilepsy.

These findings imply that successful reduction of seizures may influence the activity of the complement system [79–82]. In turn, a combined analysis of six different complement proteins (C3, C4, properdin, FH, C1Inh, and Clu) made it possible to distinguish between patients with epilepsy and a control group [68].

MicroRNA and neuroinflammation

MicroRNA (miRNA) is a non-coding single-stranded RNA molecule which inhibits mRNA expression by binding to complementary sequences in the 3′-untranslated region, thereby acting as negative regulators of gene expression. The regulatory effect of microRNA (miR) on neuroinflammation has been reported in multiple studies [83–85]. In drug-resistant epilepsy, downregulation of miR-34c-5p may upregulate HMGB1 and IL-1β, thus exacerbating neuroinflammation and neuron loss [84]. In turn, in epileptic rats, hippocampal miR-27a-3p expression is significantly elevated and administration of its inhibitor effectively reduces IL-1, IL-6 and TNF levels as well as neuronal apoptosis [85]. Significant upregulation of TNF-α and miR-155 has been studied in the hippocampi of an immature rat model of status epilepticus, suggesting that targeting the TNF-α/miR-155 axis could offer a previous biomarker of epilepsy and a new therapeutic approach [86].

The impact of microRNA on neuroinflammation has become a focal point of research, with recent findings indicating that miR-146a influences the TLR4 pathway by regulating the expression of NF-B, IL-1, and INF at the post-transcriptional level in an epilepsy model, and that its upregulation might mitigate neuroinflammation [87–89].

In a temporal lobe epilepsy mouse model, intranasal administration of miR-146a mimicked, prior to seizure induction, increased resistance to seizure development, delayed the onset of convulsions, reduced their severity, and alleviated hippocampal damage by decreasing inflammation via TLR pathway [88].

Dysregulation of miRNA expression may play an important role in the pathogenesis of epilepsy, for instance by regulating the expression of inflammatory factors. Therefore, it seems that miRNA could be a valuable biomarker in the diagnosis of epilepsy, or even an important therapeutic target [89].

Future directions in anti-inflammatory therapies

Given the pivotal role of inflammation in epileptogenesis, immune modulation and anti-inflammatory therapies are considered to be promising approaches for the treatment of epilepsy. To date, numerous preclinical studies have demonstrated that targeting neuroinflammation to prevent the development and progression of epilepsy may represent a promising therapeutic approach. Animal studies have provided evidence that anti-inflammatory treatments, particularly those targeting specific immune molecules, show efficacy and potential in models of epilepsy and seizures. In humans, broad-spectrum anti-inflammatory interventions, such as ketogenic diets or steroid therapy, have also demonstrated positive therapeutic outcomes [12].

In particular, antibody-based therapies are emerging as a promising strategy for managing neuroinflammation in epilepsy, particularly by targeting and neutralising specific inflammatory mediators or modulating immune responses. Research using anti-TNF-α antibodies (e.g. infliximab), anti-IL-1β antibodies (e.g. canakinumab), and anti-IL-6 receptor antibodies (e.g. tocilizumab) has shown the potential to alleviate neuroinflammation and to reduce seizure susceptibility in experimental models, as well as in human case reports and in small treated patient groups [90].

Further studies and clinical trials are needed to explore the potential of novel therapies targeting specific inflammatory pathways.

Conclusions

Clinical and experimental evidence has highlighted the role played by neuroinflammation in epilepsy, showing that specific inflammatory pathways remain chronically activated in epileptogenic tissue. Increasingly, research suggests that inflammatory and immune responses are pivotal in enhancing neuronal excitability, which lowers the seizure threshold and sustains a chronic inflammation in the brain. This state is consistently linked to the activation of multiple signalling pathways and primarily leads to damage to the BBB [3]. Further research is essential in order to move towards the identification and validation of biomarkers of neuroinflammation in epilepsy, particularly those predicting favourable responses to anti-inflammatory treatments [4]. Longitudinal studies are needed to track biomarker dynamics over the course of disease progression and treatment, offering deeper insights into their temporal relevance [91]. Advanced imaging techniques, such as functional MRI and PET, should be employed to correlate biomarker activity with neuroinflammatory processes [92]. Additionally, the application of machine learning approaches can enhance biomarker discovery and validation by identifying

complex patterns within large datasets, ultimately improving diagnostic precision and therapeutic targeting [93].

Better understanding the role of neuroinflammation in epilepsy could lead to the development of new therapeutic approaches as well as diagnostic and prognostic markers, thereby improving disease management and patient outcomes.

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