

Neuroinflammatory networks in epileptogenesis: microglia-astrocyte-neuron interactions

Epileptogenezde nöroinflamatuvar ağlar: mikroglia-astrosit-nöron etkileşimleri

Fümet Duygu Üstündağ^{1*}, Ünsal Veli Üstündağ²

¹Department of Biophysics, Faculty of Medicine, Ağrı İbrahim Çeçen University, Ağrı, Türkiye

²Department of Medical Biochemistry, Faculty of Medicine, Ağrı İbrahim Çeçen University, Ağrı, Türkiye

 0000-0001-5290-8924

Sorumlu Yazar: Fümet Duygu Üstündağ

e-mail: fdustundag@yahoo.com

fdustundag@agri.edu.tr

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Epilepsy is a chronic neurological disorder characterized by spontaneous and recurrent seizures; however, explaining its pathophysiology solely in terms of neuronal hyperexcitability and hypersynchronization remains inadequate. Clinical and experimental data indicate that epileptogenesis is a multistage transformation in which neuronal networks acquire the capacity to generate spontaneous seizures after traumatic brain injury, central nervous system infection or status epilepticus (1, 2). In addition to functional and structural reorganization, this process encompasses activation of the innate immune response, proinflammatory signalling and blood-brain barrier (BBB) dysfunction (3-5). Microglial activation, reactive astrogliosis, increased proinflammatory cytokine production, oxidative stress, disruption of extracellular ion homeostasis and aberrant synaptogenesis are the principal interacting components; microglia and astrocytes are not merely cells that respond secondarily, but components that actively contribute to the pathological remodelling of seizure networks through reciprocal signalling (3, 4). The temporal organization of this participation is one of the most critical mechanistic dimensions of epileptogenesis.

A sequential order of activation is observed in glial cells after status epilepticus: microglia respond considerably earlier than neurons, and this is followed by astrogliosis that begins with a delay and extends into the chronic phase; this sequential pattern has been proposed to set the stage for neuronal hyperexcitability (5). The same study reported that microglial inhibition was effective only in the acute phase (the first days after status epilepticus), whereas intervention at a later stage failed to suppress astrogliosis, astrocytic Ca²⁺ hyperreactivity and seizure susceptibility (5). This finding points to a time-restricted therapeutic window and to the decisive role of intervention timing in epileptogenesis. Adenosine triphosphate (ATP), high mobility group box 1 (HMGB1) and other damage-associated molecular patterns (DAMPs) released during neuronal injury and recurrent activity engage microglial pattern recognition receptors; with the recruitment of TLR/NF-κB and inflammasome pathways,

the production of IL-1β, TNF-α, IL-6, chemokines and reactive oxygen species increases (4-6).

Among these DAMPs, the signalling initiated by HMGB1 through toll-like receptor 4 (TLR4) and receptor for advanced glycation end products (RAGE) reinforces NF-κB-mediated proinflammatory transcription and lowers the seizure threshold by modulating glutamatergic transmission and N-methyl-D-aspartate (NMDA) receptor activity (6, 7). IL-1β/IL-1R1 signalling deepens the electrophysiological dimension of this picture: in hippocampal neurons, IL-1β activates the NR2B subunit of the NMDA receptor by tyrosine phosphorylation through Src family kinases, thereby increasing Ca²⁺ influx (8). In human temporal lobe epilepsy (TLE) tissue, it has been reported to suppress gamma-aminobutyric acid type A (GABA-A) receptor-mediated currents by up to 30%, an effect mediated by IL-1R1/PKC and detected only in epileptic tissue (9). TNF-α, in turn, increases extracellular glutamate levels by suppressing glutamate transporters, raises the membrane density of postsynaptic AMPA receptors and reduces the surface expression of inhibitory GABA-A receptors (10, 11). IL-1β and TNF-α are therefore not merely biomarkers of neuroinflammation, but direct regulators of the synaptic excitation-inhibition balance.

The NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome plays a central role in amplifying IL-1β and TNF-α-driven signalling. NLRP3 activation reinforces the neuroinflammatory loop by enabling the conversion of pro-IL-1β and pro-IL-18 into active cytokines through caspase-1. In experimental epilepsy models, pharmacological or genetic suppression of NLRP3 has been reported to reduce IL-1β and IL-18 levels and, consequently, to attenuate neuroinflammation, neuronal damage and seizure severity (12). While these findings highlight NLRP3 as a potential disease-modifying target, it should not be overlooked that preclinical anticonvulsant efficacy may not correspond directly to clinical antiepileptogenic efficacy.

The neuroinflammatory component of epileptogenesis is not limited to microglial activation; during

reactive astrogliosis, a substantial portion of astrocytic homeostatic functions becomes impaired. A reduction in EAAT2/GLT-1 expression or function has been shown to raise the extracellular glutamate concentration and to increase excitotoxic signalling (13). Astrocytic Kir4.1 channels are among the principal determinants of extracellular K⁺ buffering; under epileptogenic conditions, impairments in the membrane expression and function of Kir4.1 together with EAAT2, connexin 43 and aquaporin-4 set the stage for a simultaneous collapse of K⁺ and glutamate homeostasis and contribute to the maintenance of network hyperexcitability (13, 14). In addition, disruption of the integrity of the neurovascular unit is another critical component of epileptogenesis.

Following epileptogenic insults, disruption of the neurovascular unit and increased BBB permeability set the stage for the extravasation of serum proteins into the brain parenchyma. In this process, albumin binds to astrocytic TGF- β receptors and activates Smad2 phosphorylation and the TGF- β -dependent transcriptional programme. It has been demonstrated at the transcriptome level that this activation is associated with inflammatory gene expression, disruption of astrocytic intercellular junctions and reduced K⁺/glutamate buffering capacity (15). Subsequent studies have shown that this albumin-driven signalling triggers astrocyte-mediated excitatory synaptogenesis, and that the ALK5/TGF- β inhibitor SJN2511 and the angiotensin II receptor blocker losartan can prevent both synaptogenesis and the development of spontaneous recurrent seizures (16, 17). The TGF- β /ALK5 pathway is thus positioned as a mechanistic bridge between BBB injury and persistent network remodelling, and as a disease-modifying therapeutic target in acquired epilepsy.

This multilayered interaction also intersects with the mTOR pathway, the master regulator of cellular energy metabolism. mTOR signalling and neuroinflammation have been described as two critical pathways that mutually reinforce one another in structural epilepsies (18). In the pilocarpine-induced status epilepticus model, microglia specific mTOR deficiency has been shown to impair the phagocytosis of damaged neurons, to increase neuronal loss and to lead to more severe spontaneous seizures, indicating that microglial mTOR activation may serve a neuroprotective and antiepileptogenic function (19). In contrast to the proepileptogenic effects of neuronal mTOR activation, the fact that microglial mTOR constitutes a protective pathway provides a mechanistic framework explaining why mTOR inhibitors yield cell type- and model-dependent inconsistent results in epilepsy.

Microglia and astrocytes should not be considered independently of one another. A single-nucleus RNA sequencing study of human temporal lobe epilepsy tissue revealed an expansion of activated microglial and astrocytic subpopulations and the prominence of the secreted phosphoprotein 1 (SPP1)-CD44 (cluster of differentiation 44) axis in microglia-astrocyte communication, with reactive microglia reported as the principal source of SPP1 and reactive astrocytes as the target cells through CD44 (20). These findings show that glial phenotypes cannot be explained by the classical M1/M2 or A1/A2 binary

classifications, and that these cells display distinct transcriptional and functional states depending on disease stage, anatomical region and microenvironment.

The complement system is a component of the microglia-astrocyte-neuron interaction that extends to synaptic remodelling. C1q and C3, which regulate synaptic pruning during physiological development, have been shown to be reactivated in epileptic tissue in human drug-resistant epilepsy specimens (21). Following pilocarpine-induced status epilepticus, microglial C1q and astrocytic C3 expression increases; C1q neutralization applied in the subacute phase has been reported to reduce microglial synaptic phagocytosis, preserve dendritic spine density, and suppress interictal epileptiform discharges in the chronic phase (22). Complement activation is thus not merely a biomarker of neuroinflammation, but a modifiable mechanism directing the structural reorganization of epileptic networks. Although these mechanisms bring microglia and astrocytes to the fore, neurons are not passive targets of this network: recurrent depolarization and epileptiform activity re-trigger glial activation by increasing the release of ATP, glutamate, HMGB1, and other DAMPs, while glia-derived IL-1 β , TNF- α , and other mediators increase neuronal excitability by altering glutamatergic and GABAergic transmission, ion channels, and synaptic plasticity. Epileptogenesis therefore operates less as a linear cause and effect chain than as a pathophysiological network with positive feedback.

This framework calls for a re-evaluation of therapeutic targets in epilepsy. In the kainic acid model, blockade of the Kv1.3 potassium channel with PAP-1 has been shown to reduce seizure severity and neuronal loss and to prolong seizure latency, an effect achieved through suppression of the microglial Ca²⁺/NF- κ B pathway (23). Current antiseizure drugs mainly suppress seizure generation; no clinically proven treatment that prevents the inflammatory and structural remodelling underlying epileptogenesis is yet available. In contrast, the HMGB1/TLR4, IL-1 β /IL-1R1 and NLRP3 pathways are being investigated as potential disease modifying targets (3, 4, 24), and experimental evidence that interventions directed at TGF- β /ALK5 and complement signalling may prevent epileptogenesis is accumulating (15, 16, 22). The evidence regarding the predictive and diagnostic use of neuroinflammation biomarkers has been comprehensively reviewed (25).

In conclusion, epileptogenesis is a multicellular pathophysiological process in which neuronal hyperexcitability, microglial innate immune activation, astrocytic homeostatic dysfunction and neurovascular disruption mutually reinforce one another. Microglial HMGB1/TLR4 and NLRP3 signalling, astrocytic glutamate and K⁺ buffering impairment, the albumin-TGF- β /ALK5 axis, complement-mediated synaptic elimination and the SPP1-CD44 communication network are not independent mechanisms, but functionally connected components of the same epileptogenic network. The development of disease modifying treatments will require defining the pathological communication along the microglia-astrocyte-neuron axis at the temporal, regional and molecular levels in addition to

suppressing epileptic discharges, and modulating it selectively.

Ethical Approval

Ethics committee approval is not required for this article.

Conflict of Interest

The authors declare no conflict of interest.

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Authors' Contributions

FDÜ: Conceptualization, Literature review, Writing-original draft, Visualization, Writing-review & editing, ÜVÜ: Conceptualization, Literature review, Visualization, Writing-review & editing.

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Not available

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