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# GLIAL CELLS, BLOOD BRAIN BARRIER AND CYTOKINES IN SEIZURES: IMPLICATIONS FOR THERAPEUTIC MODALITIES

# ULOGA GLIJA ĆELIJA, KRVNO-MOŽDANE BARIJERE I CITOKINA U NASTANKU KONVULZIJA: IMPLIKACIJE ZA TERAPEUTSKE MODALITETE

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## Abstract

Epilepsy is a chronic, common, neurological disorder marked by transient, paroxysmal and hypersynchronous activity of the brain neurons, behaviorally manifested as seizures. It is developed through the process of epileptogenesis which alters neuronal excitability, establishes critical interconnections and develop neuronal hyperexcitability and degeneration, as well as the neuronal network reorganization as its main mechanisms.

There are a number of different mechanisms of epileptogenesis, including neuroinflammation as a recently highlighted important novel mechanism. In this review paper, our focus will be to light up the latest findings about neuroinflammation as a pathogenic factor in epileptogenesis.

Neuroinflammation is characterized by the structural and functional alteration of the CNS glial cells and peripherally derived immune cells with the presence of blood-brain barrier (BBB) dysfunction as main mechanisms. Disequilibrium in the CNS microenvironment is often followed by increased synthesis of proinflammatory cytokines (IL-6, IL-1β, TNF-α, IFN-γ) and chemokines. The interplay between glial alteration, BBB dysfunction, cytokines and chemokines establish a positive feedback cascade for further epileptogenesis.

It is still unclear if neuroinflammation is causing epileptogenesis or whether in a consequence of that, but, there are clear findings about positive feedback between these two processes. This interconnection could be a helpful key to better target therapeutic treatment of neuroinflammation for providing beneficial effects for patients with epilepsy.

**Keywords:**
epilepsy,
epileptogenesis,
neuroinflammation,
glial alteration,
BBB dysfunction,
cytokines,
chemokines



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## Sažetak

Epilepsija predstavlja hroničnu, učestalu neurološku bolest koja se karakteriše prolaznom, paroksizmalnom i hipersinhronom aktivnošću moždanih neurona, a manifestuje se u obliku konvulzivnih napada. Razvija se kroz proces epileptogeneze koji podrazumeva poremećaj ekcitabilnosti neurona, uspostavalje kritičnih međuveza između neurona, razvoj nervne hiperekscitabilnosti i degeneracije, kao i reorganizaciju neuronske mreže.

Postoje brojni različiti mehanizmi epileptogeneze koji uključuju neuroiflamacijju koja je u skorašnjem vremenu označena kao značajan i novi mehanizam. U ovom revijskom radu cilj će biti da se rasvetle najnovija činjenična saznanja o neuroiflamaciji kao patogeneteskom faktoru epileptogeneze.

Neuroinflamacija se karakteriše strukturnom i funkcionalnom alteracijom glijanih ćelija u CNS-u, nakupljenjem ćelija imuniteta poreklom izvan CNS-a uz disfunkciju krvno-moždane barijere (KMB). Poremećaj ekstracelularne mikrosredine u CNS-u je takođe često praćen povećanom sintezom proinflamatornih citokina (IL-6, IL-1β, TNF-α, IFN-γ) i hemokina u CNS-u. Uzajamno dejstvo između alteracije glijalnih ćelija, disfunkcije KMB, citokina i hemokina, dovodi do uspostavljanja pozitivne povratne sprege u kaskadnom procesu epileptogeneze.

Još u vek je nejasno da li neuroinflamacija dovodi do epileptogeneze ili je samo posledica iste, ali, postoje jasne činjenice koje ukazuju da postoji pozitivna povratna sprega između ova dva procesa. Činjenica da postoji povezanost ova dva procesa može biti korisno saznanje u smislu iznalaženja potentnijih terapeutskih tretmana neuroiflamacije koji bi doveli do pozitivnih efekata u lečenju pacijenata sa epilepsijom.

**Ključne reči:**
epilepsija,
epileptogneza,
neuroinflamacija,
alteracija glijanih ćelija,
disfunkcija KMB,
citokini,
hemokini

## Introduction

Epilepsy is a chronic neurological disorder marked by transient, paroxysmal and hypersynchronous activity of the brain neurons, behaviorally manifested as seizures and accompanied by abnormal electrical activity in the brain (1).

It is one of the most common serious neurological disorders affecting 3.3–7.8/1000 inhabitants in the general population and 3.4–5.8/1000 in pediatric population (2). It is more common in males than females and also in older than younger (3). These facts reflect impact of epilepsy on social and economic aspects of this disorder. Social isolation, stigmatization, disabilities and some psychiatric comorbid disorders are important life quality determinants in patients with epilepsy (4). Seizures can be effectively controlled by medication only in about 70% of cases (5). More than 80% of patients with generalized seizures can be pharmacologically treated, while this is the case in only 50% of patients with focal seizures (6).

The concept of epileptogenesis implies the state of central nervous system (CNS) in which previously healthy brain is functionally or morphologically altered and it is more prone to generate abnormal electric activity that provokes chronic seizures (7). Epileptogenesis is complex process which alters neuronal excitability, establishes critical interconnections and develop neuronal hyperexcitability, neuronal degeneration and the neuronal network reorganization as its main mechanisms (8), with the consequent predominance of excitatory over inhibitory phenomena in the CNS (9). It is often three-phase process which is firstly initiated by any precipitating factor, followed by second, "latent" period during precipitating factor transform previously healthy brain into an epileptic brain, with last phase which means presence of established epilepsy (7). The most common changes in epileptogenetic focus are: neurodegeneration, neurogenesis, neuroinflammation, gliosis, axonal damage, dendritic plasticity, blood–brain barrier (BBB) dysfunction, recruitment of inflammatory cells into brain tissue, and molecular changes in individual neuronal cells (10).

Neuroinflammation denotes inflammation of the CNS. It could be caused by numerous factors and consists in the activation of the cellular micro-environment, including microglia (resident immune cells of the brain), but also astrocytes, oligodendrocytes, and peripherally derived immune cells (11). Also, peripheral inflammation is potent to damage the CNS and impair neural homeostasis via disruption of the blood-brain barrier (BBB). BBB's role is to isolate the CNS, but when it loses its integrity in the presence of inflammatory process, CNS could be exposed to peripheral inflammatory cells (12) which can induce neuroinflammation (13). For details on interplay between peripheral inflammatory processes and seizure susceptibility see our recent review (14). Ongoing neuroinflammation in CNS is implicated in seizure induction and the development of epilepsy, because there is a positive feedback loop between brain inflammation and epileptogenesis (15).

Therefore, in this review paper, our focus will be to light up the latest findings about neuroinflammation as a pathogenic factor in epileptogenesis.

## Mechanisms of neuroinflammation: interplay between glial cells, BBB and cytokines

Neuroinflammation is a main pathological feature of a wide range of the CNS disorders, (16). As explained by Campbell et al. (11), similar cell types and inflammatory mediators are included across the range of these disorders, resulting in neurotoxic processes and release of proinflammatory cytokines or reactive oxygen species, activation of reparative processes and release of anti-inflammatory cytokines, neuroprotective and angiogenic factors.

### Changes in glial cells

Glial cells are highly represented in the CNS. Their ratio with neurons in the cerebral cortex is 3:1 (17). Percentages of the sub-populations of cortical glial cells are approximately 75% for oligodendrocytes, followed by astrocytes (~17%) and microglia (~6.5%) (18). Glial cells role is strongly connected with many neuronal functions: migration of neural stem cells during the CNS development; modulation of synaptic function and plasticity; regulation of the extracellular microenvironment in the CNS (buffering neurotransmitters, ions and water concentrations); isolation of axons; regulation of local blood flow and the delivery of energy substrates (19); regulation of the BBB permeability (20); and control of the cellular immunity in the restoration and healing of brain tissue (21). Therefore, physiological functions of unaltered glial cells guarantee tissue homeostasis in the CNS. Any disruption or disbalance of glial actions might trigger epileptogenesis or directly cause seizures (22). Glial cells can participate in the neuronal hyperexcitability and consequential epileptogenesis through two different processes: non-inflammatory or inflammatory (23).

Astrocytes are the main participants of the non-inflammatory glia-medited hyperexcitability. There are many different structural and biochemical alterations in astrocytes which could lead to hyperexcitability, and the most common are: under-expression of K+ ion channels on astrocytes membrane (24) and decreased number of intercellular gap junctions (25) can result in less extracellular potassium; aquaporin dysfunction can cause shrinkage of extracellular space due to decreased water delivery to extracellular space (26); under-expression of specific transporters causes extracellular glutamate increase (27); changes in adenosine kinase activity (28) and number of metabotropic glutamate receptors (mGluRs) results in disequilibrium between basal levels of two opposite types of neurotransmitters: excitatory glutamate, D-serine, and ATP and inhibitory GABA (29,30).

Inflammatory mechanisms of glia-mediated hyperexcitability are primary mediated by increased release of glia-derived proinflammatory molecules and augmented activity of IL-1R/TLR signaling pathway which causes higher DNA transcription of cytokine genes in glial cells and neurons (31). This alteration in levels of proinflammatory mediators has clear consequence: lower seizure threshold. Altered brain cytokines levels are responsible for higher $Ca^{2+}$ influx into astrocytes and lower reuptake of glutamate which will lead to higher extracellular levels of glutamate (32). Increased glial cells-derived proinflammatory molecules can also cause disturbances in multidrug transport proteins expression in endothelial and perivascular cells, which will bring to decreased antiepileptic drugs (AEDs) levels in brain, ending up in worse seizure control (33,34). Likewise, cytokines may play very important and direct influence on the BBB dysfunctions (20), which will be discussed in detail in the following paragraph.

Thus, although neurons are the only one cellular elements expressing seizure discharges, there are growing evidence about glial cells-mediated neuronal excitation and neuroinflammation. Moreover, glial cells could support the initiation, development, and establishment of epileptogenesis in situations when there is disrupted homeostasis of glial cells. The role of glial cells in excitation and neuroinflammation was traditionally considered trough independent pathways, but there is an overlap in these processes, because excitation can promote neuroinflammation, and opposite, neuroinflammation can promote neuronal excitation. In summary, understanding the roles of glial cells may provide insights into unanswered questions about epilepsy, including how epileptogenesis occurs and why some patients are resistant to medications. As the fundamental mechanisms of epileptogenesis and neuroinflammation come into better focus, strategic targets for new therapeutic interventions will emerge where neurons, glial cells, excitation, and inflammation converge (23).

### Blood - brain barrier dysfunctions

The BBB is a physical and metabolic barrier between the brain tissue and blood which is responsible for the homeostasis of brain microenvironment. It is composed of a monolayer of brain capillary endothelial cells with presence of tight junctions, thick basement membrane and astrocytes endfeet (35). It allows the passage of water, some gases, and lipid-soluble molecules by passive diffusion, as well as the selective transport of nutrients and potentially toxic molecules. It prevents the entry of potential lipophilic neurotoxins by way of an active transport mechanism mediated by P-glycoprotein (BBB efflux transporters). Astrocytes have been claimed to be necessary for creation and physiological function of BBB (36).

Considering specific mechanism of neuroinflammation, it is important to light up the role of astrocytes in the brain microvasculature and BBB function. Astrocytes endfeet, wrapped around endothelial cells, contribute to BBB function by releasing chemical signals that help to develop and maintain tight junctions between endothelial cells. They also regulate the movement of water and molecules between the blood and brain parenchyma (37). Histological examinations in temporal lobe epilepsy (TLE) proved that there is blood vessel proliferation and astrocytes endfeet alteration which positively correlates with seizure frequency and BBB permeability disorders (38). Also,

different experimental seizure models showed that astrocytes are able to release vascular endothelial growth factor (VEGF) which contributes to BBB damage and induces microvasculature proliferation (angiogenesis) by activating VEGF receptors on microvessels (39). Proinflammatory chemokines and cytokines, released by astrocytes, can interact with their receptors on brain microvessels, thus affecting BBB permeability at multiple levels. Result of higher BBB permeability is leukocyte transmigration and serum proteins and molecules leakage into brain microenvironment (40). Also, astrocytes-derived interleukin-1 beta (IL-1β) can compromise BBB integrity during seizures in the absence of circulating leukocytes (41). Brain extravasation of serum albumin due to BBB damage increases excitability and promotes epileptogenesis (42). Additionally, albumin is able to promote synthesis of inflammatory molecules in astrocytes, helping to perpetuate the proinflammatory milieu in the CNS (43).

The recent study from Johnson et al. (44) clarifies the important role of BBB efflux transporters in seizure prevention. BBB efflux transporters contribute to brain homeostasis by protecting the brain from potentially harmful endogenous and exogenous substances and they are recognized as important determinants of drug distribution to and elimination from the CNS (45). BBB efflux transporters inhibition, BBB tight junctions disruption, brain edema, elevated VEGF and tumor necrosis factor alpha (TNF-α) play an important role in neuroinflammation and consequently in epileptogenesis. Increased levels of astrocytes-derived inflammatory mediators or glutamate may increase BBB efflux transporters expression on endothelial cells (44). Importance of these transporters is reflected trough finding about their over - expression in resected brain tissue specimens taken from patients with drug-resistant epilepsy (45). In the one hand, BBB efflux transporters are useful for minimizing or avoiding neurotoxic adverse effects of drugs that otherwise would penetrate into the brain, but, in the other hand, BBB efflux transporters may also limit the central distribution of drugs that are beneficial to treat the CNS diseases and may result in pharmacoresistance to therapeutic medications (46). In particular, p-glycoprotein (type of BBB eflux transporter) is over expressed at the luminal side of endothelial cells, astrocytes endfeet and dysplastic neurons in the patients with glioneuronal lesions, causing uncontrolled epilepsy (34).

This set of evidences highlights important pathophysiological interfaces between glial cells-mediated inflammation, microvasculature, BBB integrity and excitability. Upcoming developments in methods of BBB stabilization and amelioration of BBB-related adverse mechanisms will be beneficial for treatment of patients with epilepsy.

## Cytokines and chemokines in seizures

It is concluded that hyperexcitability and hypersynchrony of brain neurons are well-known mechanisms producing seizures. That's the reason why, nowadays, most antiepileptic drugs target neuronal mechanisms (23). Recent studies which explore the immune or inflammatory mechanisms underlying epileptogenesis are useful in many ways. First, they can improve scientific knowledge of epileptogenesis. Additionally, results could provide insights into the development of more effective target-specific immunotherapies, better than general treatments. Inflammatory processes are implicated in the pathogenesis of seizures and their comorbidities. During neuroinflammation, as well as during systemic peripheral inflammation, release of mediators may have high negative impact on synaptic plasticity and neuronal networks functioning (47). Also, clinical data suggests that epilepsy development is associated with changes in immunological profile (48).

The recent view of immune-mediated neuroinflammation and epileptogenesis (15) includes both, brain resident cells which are capable for innate immune response and derived peripheral immune cells which are responsible for initiation of neuroinflammation. The variety of pathological triggering events, initiated in the brain or at the periphery may lead to an inflammatory cascade. One of the points of this cascade is cells activation in the CNS (glial, neural, or endothelial), which lead to release of proinflammatory cytokines, such as IL-1β and TNF-α. These factors activate signaling pathways in neurons which causes an intracellular calcium ion surge with modification of voltage-dependent ion channels (15). Dysregulated ion channels directly enhance the neuronal hyperexcitability and reduce seizure threshold. In addition, proinflammatory cytokines also stimulate chronic release of neuroexcitatory transmitters and decrease GABAergic neurotransmission (32,49).

The most recent study from Temp et al. (50) showed seizure-induced increase of the cyclooxygenase-2 (COX-2) derived metabolites in the brain and anticonvulsant property of COX-2 inhibitors. Increase IL-1β, interleukin-6 (IL-6), interferon-γ (IFN-γ), TNF-α and interleukin-10 (IL-10) levels in the hippocampus and cerebral cortex of mice was observed after seizure induction. In the other hand, COX-2 inhibitors, celecoxib and nimesulide, attenuated cytokines increase and seizure occurrence. COX-2 derived mediators and prostaglandins can also be involved in process of neuronal network remodeling by mobilization of intracellular calcium storage and an increase cAMP production. The established inflammatory milieu in the CNS is often accompanied by BBB leakage which introduces blood components, such as albumin and potassium ions, into the brain (35,51). Increased leukocyte adhesion to the endothelial cells additionally modifies the BBB through cytoskeletal organization, which results in enhanced leukocyte infiltration into the brain (52). Upon entering the brain, activated peripheral immune cells are capable of generating free oxygen radicals, releasing additional chemokines, cytokines, nitric oxide (NO) to establish a positive feedback cascade for further epileptogenesis (15). Indeed, modified NO levels could modulate seizure activity, as it has been recently reported that NO plays a role of endogenous convulsant in model of lindane convulsions in rats (53), as well as that NO acts as an anticonvulsant in homocysteine thiolactone-induced seizures (54).

Furthermore, considering that cytokine release is a key process of neuroinflammation and epileptogenesis, cytokines might be used as biomarkers for early detection of brain damage and consequent early intervention in order to prevent disease progression and further neurological complications (55).

### Cytokines

Cytokines are soluble molecules of intercellular communication and they have critical role in immune regulation. Recent studies showed that occurrence of epileptic seizures can induce increased levels of cytokines in serum and different brain regions, which may have influence in the neuroinflammation and consequential epileptogenesis. It has been demonstrated that concentration of several inflammatory cytokines, such as IL-1β, TNF-α and IL-6, is rapidly increased in patient or animal serum, immediately after epileptic seizure (56,57). It has been reported on both, proconvulsive and anticonvulsive effects of cytokines, probably due to their various roles through multiple signaling pathways (58). Nonetheless, these observations demonstrate the multifarious nature of cytokines and the complex relationship between the immune system and epileptogenesis.

Vezzani et al. (59) firstly noticed increased production of IL-1β in glial cells in hippocampus after applying of convulsant and/or excitotoxic stimuli to experimental animals. IL-1β enhances focal electrographic seizures induced by kainate through increased glutamatergic neurotransmission. Increased production of IL-1β is also observed in human temporal lobe epilepsy (60), thus suggesting that this cytokine may play a critical role in the neuroinflammation and epileptogenesis (59). Increased IL-1β levels may have neurotoxic effects or cause imbalanced neurotransmission leading to seizures (61). Other cytokines, such as IL-6 and TNFα, could be over expressed in patients with epilepsy, but their precise role in epilepsy is not clear yet. Levels of these cytokines increase quickly after generalized tonic–clonic or complex partial seizures and return to baseline after varying time intervals. The study of Uludag et al. (62) confirmed seizure-induced elevation in plasma concentrations of IL-6, interleukin-1 receptor antagonist (IL-1Ra) that peaked out at 12 h into the post-ictal period. Last experimental and clinical studies have demonstrated an upregulation of pro-inflammatory cytokines such as IL-1β and TNF-α, in Mesial Temporal Lobe Epilepsy with Hippocampal Sclerosis (MTLE-HS) (56,57).

There is a great need for further studies regarding the roles of cytokines in human epilepsy. A key point to be addressed in further studies is whether, and to what extent, endogeneous cytokine release is relevant for the process of epileptogenesis and if this process can be prevented by immunomodulatory treatment.

### Chemokines

Chemokines are a family of specific cytokines, or signaling proteins secreted by cells, which are produced as a "chemo-attractant molecules" with ability to induce directed chemotaxis in nearby responsive inflammatory cells to sites of infection/inflammation (63).

Recent study on chemkines by Tien et al. (64) showed that patients with temporal lobe epilepsy (TLE) express elevated levels of chemokine C-C motif ligand 2 (CCL2) and its receptor CCR2. The functional significance and molecular mechanism underlying to CCL2-CCR2 signalling pathway in epileptogenes remained still uninvestigated. The upregulation of CCL2 was mainly observed in hippocampal neurons and activated microglia in mice one day after seizures induced by kainic acid. Moreover, seizure-induced degeneration of neurons in the hippocampal region was attenuated in mice lacking CCL2 or CCR2. Increased CCR2 activation consists in increasing IL-1β production, causing neuronal cell death after status epilepticus (64).

Such investigations are the key to better understand of chemokines impairment in neuroinflammatory response, with a development of future potential therapeutic targets for the treatment of epilepsy.

## Conclusion

Neuroinflammation seems to be fundamental and crucial process in variety of neuropathological conditions and disorders including also epileptogenesis. The interplay between glial cells, BBB and cytokines are main feature of neuroinflammation responsible for its involvement in reduction of seizure threshold and epileptogenesis in general.

It is still unclear if neuroinflammation is causing epileptogenesis or whether in a consequence of that, but, there are clear findings about positive feedback between these two processes. This interconnection could be a helpful key to better target therapeutic treatment of neuroinflammation for providing beneficial effects for patients with epilepsy by reducing the seizures number.

There is a strong interconnection between astrocytes function, BBB dysfunction, cytokines and chemokines production in the occurrence of neuroinflammation. Every of these factors could be used as key point in next studies which should focus on targeting therapy for neuroinflammation-based epilepsy. Namely, having in mind previous considerations, we could underline once again that understanding the roles of glial cells may provide insights how epileptogenesis occurs and why some patients are resistant to medications. Also, upcoming developments in methods of BBB stabilization and amelioration of BBB-related adverse mechanisms will be beneficial for treatment of patients with epilepsy. On the other hand, cytokines might be used as biomarkers for early detection of brain damage and consequent early intervention. There is a great need for further studies regarding the roles of cytokines and chemokines in human epilepsy.

We do believe that further studies on interplay between glial cells, BBB and cytokines will provide novel therapeutic allies in our fight against epilepsy.

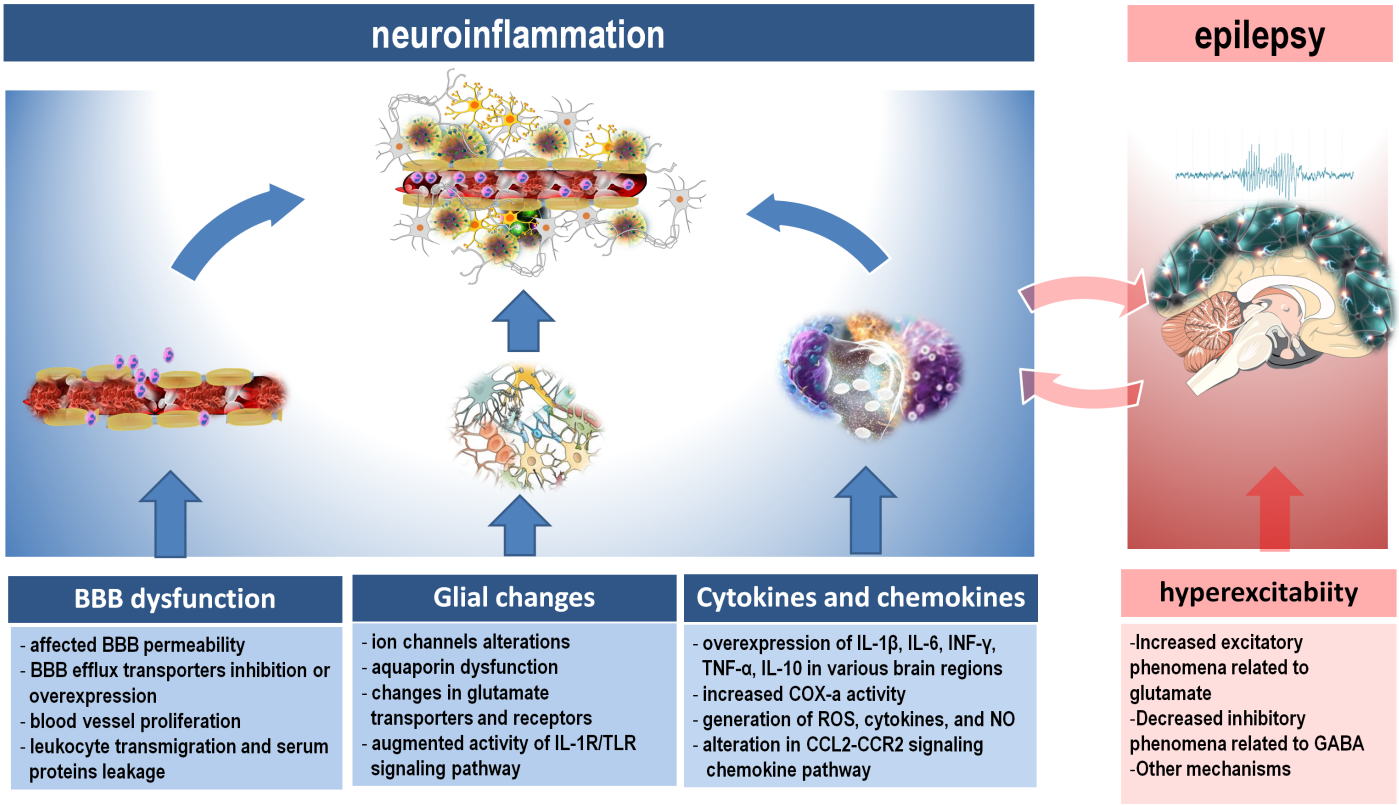


**Figure 1. Contributors of neuroinflammation and its relation with epileptogenesis:** Neuroinflammation is complex profess characterized by the BBB dysfunction, changes in glial cells and cytokines and chemokines production. Neuroinflammation is pathological substrate of epileptogenesis, but, there is feedback cycle between these two processes.

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