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Brain inflammation as a biomarker in epilepsy

Annamaria Vezzani[†] and **Alon Friedman**[1]

[1]Departments of Physiology & Neurobiology & Biomedical Engineering, Faculty of Health Sciences & Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, Israel

Abstract

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. Increasing evidence also highlights the possible involvement of inflammatory processes arising in the injured brain in the development of epilepsy (i.e., in epileptogenesis). 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.

Keywords

blood–brain barrier; brain imaging; cytokines; glia; leukocytes; proinflammatory molecules

Inflammation as a biomarker of epileptogenesis

The first insights into the role of brain inflammation in epileptogenesis originate from studies in transgenic mice overexpressing cytokines, such as TNF-α and IL-6, in astrocytes [1,2]. These mice develop age-dependent neurological dysfunctions including cell loss, decreased seizure threshold and spontaneous seizures. A second set of evidence is provided by immunohistochemical and biochemical studies demonstrating that pro-epileptogenic brain injuries, such as trauma, infection, and febrile and nonfebrile status epilepticus, are followed by a rapid rise in specific inflammatory mediators in brain regions affected by the injury [3–5]. In some instances, the kinetics and extent of the inflammatory response appear to be developmentally regulated [6–8]. The induction of the inflammatory response to a

[†]Author for correspondence: Department of Neuroscience, Mario Negri Institute for Pharmacological Research, Via G. La Masa 19, 20156 Milano, Italy, Tel.: +39 023 901 4410, Fax +39 023 546 277, annamaria.vezzani@marionegri.it.

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given experimental brain injury can last several days or weeks and often precedes the development of epilepsy [9]. This circumstantial evidence highlights the possibility that the onset of spontaneous seizures arising after the original precipitating event might be determined also by the extent and/or duration of the inflammatory response in susceptible brain regions.

Since inflammation is a homeostatic mechanism of defense against infection or other pathological threats, its involvement in epileptogenesis should be considered at the light of its aberrant induction, and consequent uncontrolled signaling in the target cells. There are at least two notable examples of uncontrolled brain inflammation during the latent phase, which follows status epilepticus. The first example relates to the delayed peak induction and limited biosynthesis of IL-1 receptor antagonist as compared with IL-1β [10–12]. IL-1 receptor antagonist is an anti-inflammatory peptide that competitively blocks IL-1 receptor type 1 and it is instrumental to rapidly terminate IL-1β signaling to avoid detrimental effects in tissues [13]. The second example concerns the limited induction of complement inhibitors in glia and neurons after brain injury, predicting that the activation of the complement cascade is not properly controlled [14,15]. Therefore, it appears that the mechanisms for rapidly resolving inflammation upon its induction, following tissue damage or during recurrent seizures, are not very efficient in the brain.

What determines the extent of induced inflammation after a pro-epileptogenic injury, or for how long inflammation develops, is currently unknown. However, it is evident that apparently similar brain injuries in rodents provoke brain inflammation that may differ in extent and duration, suggesting that the degree of brain inflammation could contribute either to determine whether or not epilepsy will develop, or to tissue epileptogenicity after epilepsy is established (as discussed later). In addition, the genetic background of an individual could significantly contribute to determine the development of the inflammatory response following an injurious pro-epileptogenic event.

Compelling pharmacological studies, demonstrating in experimental models that inflammation is indeed involved in epileptogenesis, are still lacking. The major inflammatory pathways studied so far for their possible contribution to epileptogenesis are the activation of the IL-1β system, COX-2 and mTOR [16]. The IL-1β system has been studied only using the surrogate kindling model of epileptogenesis, both in adult and immature rats: blockade of IL-1β signaling leads to prevention of seizure generalization or delays stage 5 seizure occurrence, respectively, and increases the threshold for afterdischarge induction [17,18]. However, studies on the involvement of IL-1β in epileptogenesis induced by status epilepticus or brain insults (e.g., traumatic brain injury) are still lacking.

Inhibition of COX-2 during the latent phase, which follows status epilepticus, and is prodromal to spontaneous seizures onset, has given variable results. Either neuroprotection and decreased spontaneous seizures [19], or no major effects on neuropathology and various functional outcomes have been reported [20,21], depending on the anti-COX-2 treatment schedule and the severity of chemically or electrically induced status epilepticus in each of these studies. mTOR inhibition by rapamycin has also given contrasting results since anti-epileptogenesis actions consisting of inhibition of sprouting and decreased chronic seizure frequency have been reported in kainate-treated rodents [22], while chronic epilepsy onset or severity was not affected in pilocarpine-treated animals [23]. Recently, mTOR signaling has also been implicated in the recurrence of seizures in chronic epileptic tissue [24].

These apparent discrepancies are likely due to the different role that the activation of specific proinflammatory pathways may have on neuronal excitability, cell survival, and

cellular and synaptic plasticity, depending on the intrinsic properties of the first damaging event used to trigger epileptogenesis. Thus, this critical feature would determine the tissue context in which inflammation develops, that is, the type of mediators released and which receptors are induced, the type of effector and target cells, which will, in turn, affect the outcome features of tissue inflammation.

The role of brain inflammation in mood disorders and behavioral deficits, such as autism and cognitive impairments, has also been substantiated by increasing evidence in the experimental and clinical setting; interestingly, these pathological aspects often present as comorbidities of epilepsy [25] and are now conceptually included as outcomes of the epileptogenic process [26].

In this view, it is worth mentioning the studies demonstrating that a systemic pro-inflammatory challenge inducing a mirror focus of brain inflammation, even in the absence of brain damage or seizures, when imposed on rats during their infancy, induces a long-term decrease in seizure threshold, as well as learning and memory deficits, and anxiety-like behaviors in adulthood, accompanied by long-lasting changes in the forebrain expression of glutamate receptor. Therefore, inflammation during brain development can set permanent changes in brain functions, likely by modifying genomic programs, thus representing a convincing evidence of long-term modifications induced by inflammation with an impact on brain excitability associated with neurological dysfunctions.

The concept that inflammation may induce chronic tissue dysfunction applies not only to epilepsy, but also to several CNS disorders, including neurodegenerative diseases [27,28] where inflammation appears to contribute to disease progression.

Inflammation as a biomarker of epileptogenicity

Immunohistochemical and biochemical studies in human chronic epileptic tissue, resected at surgery from drug-resistant patients, demonstrated increased levels of specific inflammatory mediators and their receptors in activated glial cells, in neurons and in endothelial cells of the blood–brain barrier (BBB) [9]. Although this inflammatory trait is common to epilepsies of differing etiologies, there are notable differences in the type of cells contributing to inflammation, as well as in the extent of inflammation in the specimens analyzed. In particular, brain tissue from temporal lobe epilepsy (TLE) with hippocampal sclerosis is mainly characterized by intrinsic inflammation involving activated microglia, parenchymal and perivascular astrocytes, scattered neurons, and endothelial cells of microvessels. Macrophages are also found, both surrounding vessels and in parenchyma, while cells of adaptive immunity, such as T cells and B lymphocytes, are scarce or absent, and predominantly found within vessels [29,30].

Analysis of focal cortical dysplasia tissue highlighted differences with TLE – although glia and neurons, as well as vessels, are sources of inflammatory molecules similar to TLE, the contribution of peripheral immune cells, such as T cells and dendritic cells, is more significant than in TLE [30]. Moreover, focal cortical dysplasia type 2 has more pronounced brain inflammation than focal cortical dysplasia type 1 as far as the extent and number of inflammatory cells involved are concerned [31]. Finally, brain inflammation in Rasmussen's encephalitis demonstrates a peculiar involvement of cytotoxic T lymphocytes contributing to tissue pathology in concert with intrinsic brain cells [32,33]. Another notable evidence is that the number of microglia cells in the epileptic foci, and the level of expression of IL-1β and its receptor, IL-1R1, in glia and neurons, correlate positively with the frequency of seizures in the surgically treated patients [11].

Therefore, we can envisage that there is a spectrum of different degrees of brain inflammation and cell components in epilepsy, either intrinsically contributing to this phenomenon or imported from the blood; this spectrum is determined both by the frequency of seizures and the underlying neuropathology.

Based on these findings, one can envisage that brain inflammation may be considered a biomarker of tissue epileptogenicity, as supported by data in experimental models of epilepsy where pharmacological interventions to resolve brain inflammation dramatically reduce chronic seizure recurrence [34–36], most likely by raising the threshold of neuronal excitability or reducing network pathological plasticity.

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.

BBB dysfunction: a surrogate marker of brain inflammation & a biomarker of epileptogenesis

Dysfunction of the BBB has been described by many authors as a frequent result of injuries to the brain [37,38] as well as in the chronic epileptic tissue [39]. BBB dysfunction may be a result of the primary injury to blood vessels but may also result from secondary mechanisms including metabolic compromise, prolonged seizures and inflammation [40]. In fact, inflammatory mediators have been reported to modulate both the paracellular tight junctional pathway and vesicular mechanisms [41]. Thus, it seems that any significant inflammatory response within the brain tissue will be associated with BBB dysfunction, raising the potential use of BBB permeability measure as a surrogate marker for a local inflammatory response. Furthermore, recent evidence indicates that BBB opening and consequent exposure of brain tissue to serum proteins (specifically albumin) induce a robust astrocytic response resulting in upregulation of proinflammatory cytokines and activation of the complement system. This brain response to serum albumin is mediated by TGF-β signaling [42], and suggests a positive feedback between increased permeability of the brain endothelium, the local immune/inflammatory response and neuronal hypersynchronicity.

Status epilepticus induced by kainate or pilocarpine in mice has been demonstrated to upregulate adhesion molecules, such as ICAM-1, VCAM-1 and E- and P-selectin, on endothelial cells of brain microvasculature [43,44]. This phenomenon was also described following epileptifom activity induced by bicuculline in an *in vitro* guinea pig preparation [45]. In this *in vitro* model, the absence of circulating blood cells or blood-derived large molecules allowed the establishment of a strict relationship between seizure-associated inflammation in parenchymal and perivascular astrocytes, and BBB dysfunction [Librizzi L, Noé F, Vezzani A, de Curtis M, Ravizza T, Manuscript in Preparation]. Leukocyte adhesion on inflamed brain endothelium was implicated in the vascular leakage during seizure activity *in vivo*, and the interference with this phenomenon after pilocarpine-induced status epilepticus reduced the frequency of spontaneous seizures in epileptic mice [44].

Indeed, BBB opening *per se* may lead to the induction of epileptogenesis [46] and promote the generation of seizures [47,48], and thus may serve as a potential surrogate biomarker for the brain inflammatory response and a biomarker of epileptogenesis.

How to measure brain inflammation with noninvasive techniques

Imaging techniques could be advocated and developed to detect and possibly quantify inflammation in the brain of epileptic patients, or those individuals at risk of developing epilepsy. Initial studies have been developed using PET ligands to detect activated microglia in seizure foci [49–53]; magnetic resonance spectroscopy could also be a promising way to go since it allows one to monitor and quantify the degree of astrocytic activation in specific brain regions [54–56] as these cells are pivotally involved in the production and release of inflammatory molecules. Changes in T_2 signals in experimental models of febrile status, which may reflect edema associated with BBB breakdown, have been described as being possibly predictive of the subsequent development of epilepsy [6].

More direct methods for the detection and quantification of BBB permeability changes are being developed; while preliminary reports suggest a significant number of injury-related epileptic patients showing BBB damage [57], future studies are awaited to clarify to what extent vascular permeability reflects brain inflammatory response or may predict seizures. Further development of more sensitive and specific tools is mandatory, to devise methods for detecting specific inflammatory molecules in the brain or to visualize the brain vessels' upregulation of inflammatory mediators or for measuring the extent of BBB breakdown.

Biochemical measurements of inflammatory mediators in blood and serum are another, not mutually exclusive, approach [53]. 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 [58]; therefore, caution should be taken when considering blood cytokines as biomarkers in epilepsy.

Moreover, the rapid half-life of many inflammatory cytokines makes it difficult to accurately detect their levels in peripheral fluids. Cerebrospinal fluid (CSF) 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.

As described in the previous section, soluble vascular adhesion molecules may serve as a marker of vascular inflammation in epilepsy, thus mirroring parenchymal inflammation. Indeed, several studies have demonstrated the presence of elevated soluble vascular adhesion molecules in the serum and CSF of patients with stroke, and elevated levels of soluble endothelial adhesion molecules have been associated with disease severity in multiple sclerosis patients [59–61]. In addition, soluble endothelial adhesion molecules have been proposed as biomarkers in Alzheimer's disease and aging [62].

Because of the reciprocal brain-to-blood communication mediated by the BBB, and the known interactions between the brain and the peripheral immune system, attempts have been made to measure the *in vitro* responsiveness of leukocytes isolated from the blood of patients with epilepsy to proinflammatory challenges [63–65]. This was taken as an index of the individual propensity to develop an inflammatory response, which could mirror a similar response in the brain parenchyma. However, the results are not very encouraging so far since the majority of these studies were unable to demonstrate a clear-cut difference with the control nonepileptic population.

Cell sorting measurements of leukocytes in epilepsy patients have shown differences in the profile of peripheral immune cells in some cohorts [66,67] but more information is still required to understand whether these changes could be used as reliable and meaningful biomarkers of inflammatory traits in epileptic syndromes.

Therefore, the future challenge is to characterize markers of inflammation in peripheral fluids specifically reflecting the brain phenomenon, either by direct leakage out of the brain and into the CSF and blood, or released from inflamed brain vessels, or inducing a secondary peripheral response that specifically reflects the primary CNS signal.

Finally, a growing number of specific auto-antibodies are being detected in patients with new-onset epilepsy and immuno-mediated seizure disorders [68,69]. These antibodies are directed to intracellular targets (i.e., glutamic acid decarboxylase), or to cell-surface membrane proteins, such as voltage-gated potassium channels (voltage-gated potassium channel-complex proteins) or NMDA receptors [68,70]. Increasing evidence shows that these antibodies may serve as biomarkers for underlying immunopathology of limbic encephalitis which represents a precipitating event in adult-onset TLE with hippocampal sclerosis [69,71]. Whether these antibodies could be of value as biomarkers in seizure disorders without infectious or immune-mediated etiology remains to be established.

Conclusion & future perspective

The increased knowledge of the role played by brain inflammation and BBB breakdown in seizure recurrence supports the concept that these phenomena may represent biomarkers of epileptogenicity in chronic epileptic tissue. Their putative contribution to the induction of epileptogenesis underlines they could be used as putative predictors of epilepsy development in the injured brain.

This new notion highlights the need of developing adequate noninvasive brain imaging methods, or CSF/blood biomarkers, for detecting and quantifying brain inflammation and BBB damage (Figure 1 & Box 1). This effort might provide powerful tools for diagnostic, prognostic and therapeutic purposes.

Box 1

Potential biomarkers of brain inflammation in epilepsy

- Brain imaging (cell types or macromolecules)
  - PET (microglia/macrophages, endothelial cell adhesion molecules)
  - Magnetic resonance spectroscopy (astrocytes)
  - Molecular MRI (endothelial dysfunction; VCAM)
  - Contrast-enhanced MRI (endothelial dysfunction; increased permeability)
- Soluble inflammatory mediators in cerebrospinal fluid/blood
  - #x02022; – Cytokines/chemokines/danger signals [†]

- Cell adhesion molecules
- Autoantibodies

- Leukocytes
  - Cell sorting profile
  - *In vitro* responsiveness to proinflammatory challenges
  - Pro- or anti-inflammatory gene polymorphisms‡

See main text for details.

†Danger signals are endogenous molecules released from cells exposed to stressful events. For example, high-mobility group box 1 is a danger signal released from glia and neurons in epileptic tissue [34]; increased high-mobility group box 1 blood levels have been measured in neurological disorders [72].

‡A modest association between the IL-1β gene and epileptic disorders has been reported [73,74].

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Executive summary

- Brain inflammation in epilepsy is not a mere epiphenomenon but is likely to directly contribute to neuronal hyperexcitability, network plasticity, the onset of seizures and their recurrence.
- The onset of spontaneous seizures arising after the original precipitating event might be determined also by the extent and/or duration of blood–brain communication, vessel permeability and the inflammatory response in susceptible brain regions.
- Long-term modifications induced by inflammation have an impact on brain excitability associated with neurological dysfunctions.
- ; In epilepsy there is a spectrum of different degrees of brain inflammation and inflammatory cell types; this spectrum appears to be determined both by the frequency of seizures and the underlying neuropathology.
- Brain inflammation, including vascular inflammation, may be considered as a potential biomarker of epileptogenicity and be exploited for diagnostic and therapeutic purposes.
- Blood–brain barrier dysfunction has been described as a frequent result of injuries to the brain as well as in the chronic epileptic tissue.
- A positive feedback appears to occur between increased permeability of the brain endothelium, the local immune/inflammatory response and neuronal hypersynchronicity.
- Blood–brain barrier opening *per se* may lead to the induction of epileptogenesis and promote the generation of seizures; it may serve as a surrogate biomarker for brain inflammatory response and a biomarker of epileptogenesis.
- Imaging techniques are under development to detect and quantify inflammation in the brain of epileptic patients, or those individuals at risk of developing epilepsy.
- Biochemical measurements of inflammatory mediators/cells in blood and serum should meaningfully reflect the degree and extent of brain inflammation.

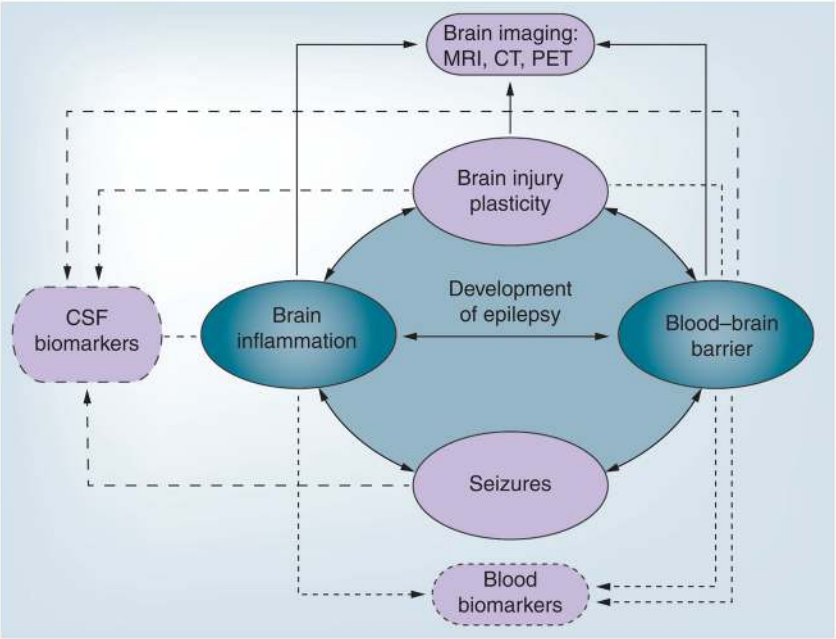

Figure 1. Strategies to monitor crucial events putatively contributing to epileptogenicity or the development of epilepsy

Brain imaging techniques will serve for detection and quantification of brain inflammation and the associated blood–brain barrier breakdown in established epilepsy, or to predict the development of epileptogenesis after brain injury. CSF biomarkers could reflect pro-epileptogenic brain injury/plasticity as well as the severity of seizures. CSF-born molecules may also drain out of the brain into the systemic circulation via the arachnoid villi/venus sinus or via the nasal lymphatics (not shown). Blood biomarkers are soluble molecules that could mirror brain inflammation, either indirectly by leakage into the circulation via the compromised blood–brain barrier, or directly by activation of brain efferent vagal nerve pathways projecting to the reticulo–endothelial system. Brain injury may also give rise to blood biomarkers of pro-epileptogenic type of damage. Leukocytes and autoantibodies could be considered as surrogate markers of brain inflammation, to be included as putative blood biomarkers. Seizure activity *per se* can alter each one of the mentioned biomarkers by contributing both to brain inflammation and blood–brain barrier damage, as well as to brain injury and plasticity.

CSF: Cerebrospinal fluid; CT: Computed tomography.