Brain Inflammation and Seizures: Evolving Concepts and New Findings in the Last 2 Decades

Commentary on: Vezzani A. Brain Inflammation and Seizures. *Epilepsy Curr.* 2004;4:73-75. doi:10.1111/j.1535-7597.2004.42012.x.

The concept of brain inflammation and its role in epilepsy has much evolved during the last 2 decades since my original Epilepsy Currents commentary,[1] and even more since its first description in autoptic tissue from Rasmussen's encephalitis or in epilepsies developing after central nervous system (CNS) infections. The new vision stems from in-depth investigations carried out since 1999 in preclinical models of provoked seizures and acquired epilepsies and in human focal epilepsies.[2] This commentary reports my subjective historical perspective of the progress in the field, also considering if predictions of the seminal findings were confirmed by the follow-up research, or if some original concepts were disproved. I will also discuss which aspects of this complex phenomenon need further investigations to help gather valuable insights for the clinical translation of the increasing experimental evidence.

The original studies reported histological and biochemical evidence for the induction of inflammatory cytokines, and related receptors, in rodent brain areas where seizures occurred,[3-5] as outlined in my original Epilepsy Currents commentary.[1] This phenomenon was confirmed by the induction of nuclear factor-κB transcriptional factor in astrocytes and neurons in human temporal lobe epilepsy (TLE).[1,6] Subsequently the presence of brain inflammation was extended to other common epilepsies, and specific inflammatory molecules, first discovered in animal models,[1] were described in surgical brain specimens from human drug-resistant structural epilepsies with acquired or genetic causes.[2,7,8] Notably, in tuberous sclerosis complex, inflammatory processes were observed in cortical tubers and subependymal giant cells in association with mammalian target of rapamycin activation, already in fetal brain lesions thus preceding epilepsy development.[9] This evidence supports the causal link between brain inflammation and epileptogenesis suggested by experimental studies (*see later*).

Growing evidence in both human epilepsy brain tissue and animal models of acute or chronic seizures reinforced over the years the critical involvement of *innate immunity* in initiation and maintenance of the inflammatory brain response,[2,10] as first described in kainate-injected rats.[1,4,5] In addition to the prototypical inflammatory cytokines, interleukin (IL)-1β, tumor necrosis factor (TNF), and IL-6,[1] *newly identified inflammatory molecules and pathways*, were incrementally found to be upregulated in microglia and astrocytes, neurons, and cell components of the blood–brain barrier (BBB) in seizure-generating areas.[11,12]

The well-established presence of inflammatory molecules in epilepsy brain begged the following questions: (1) what triggers this phenomenon, and (2) what is the pathophysiological role of the inflammatory mediators, and how do the receptor-activated pathways in targeted neurons, glia, and BBB impact neuronal network excitability? These questions have opened a new area of ongoing research focused on *sterile neuroinflammation*, that is, the brain innate immune response in absence of infections.

(1) *Recurrent seizures* and *cell death* were initially identified as main triggers of neuroinflammation in epilepsy.[1,4,5,8] This still stands true, and the subsequent clinical studies helped to confirm and refine the original findings. In particular, recent molecular imaging investigations by positron emission tomography (PET) in patients with TLE and focal cortical dysplasia (FCD) showed that the inflammatory brain response persists in the interictal phase and spreads beyond the epilepsy focus to regions of seizure generalization,[13-15] thus confirming data from the animal models.[1,8,16] Moreover, the type of neuropathology appears to play a role in determining the extent of brain inflammation, beyond recurrent seizures. In fact, brain inflammation is significantly more pronounced in FCD type 2 than type 1,[17] and there is recent description of gradual extent of neuroinflammation in various types of human malformations of cortical development with drug-resistant seizures.[18]

However, new knowledge in the field of neuropathic pain clearly showed that neuroinflammation may also arise during enhanced levels of neuronal activity in the absence of overt pathologic conditions, a phenomenon referred to as *neurogenic inflammation*.[19] Thus, the neuroinflammatory *milieu* may be induced by subclinical acquired or gene-related events associated with hyperexcitability, and not only by severe CNS insults as was initially conceptualized using animal models of status epilepticus.[1] Importantly, it was shown that long-lasting neuroinflammation can be induced in forebrain by a *systemic inflammatory challenge* in the absence of cell loss or seizures, and this was sufficient to reduce seizure threshold long-term and to promote neurological deficits, or epilepsy development following a second hit, particularly when the inflammatory challenge occurs in immature rodents.[20] Evidence of *the long-term effects of neuroinflammation on brain function* contributed to foster research on the role of inflammation in epileptogenesis and neurological comorbidities (*see later*).[2,21]

(2) Since the seminal identification of the ictogenic properties of the IL-1β-IL receptor type 1 axis in rodents injected with chemoconvulsive drugs[1,5,16] or exposed to hyperthermia mimicking febrile seizures,[22] flourishing studies showed the contribution to seizures of additional elements of neuroinflammation (eg, TNF-p55R, RAGE, ATP-P2X7R, IL-6, arachidonic acid cascade, oxidative stress, chemokines, complement).[12] This was demonstrated by inhibiting acute provoked seizures or chronic seizures,[2] and by reverting pharmacoresistance,[23] with specific anti-inflammatory interventions. Reduction of seizure-associated neurodegeneration, mortality, and neurological comorbidities were often observed, particularly with intervention upstream or downstream the arachidonic acid cascade.[12,24] A breakthrough was the discovery of the nucleus-to-cytoplasm translocation and release of the *danger signal* High Mobility Group Box1 (HMGB1) in neurons and glia following ictogenic or epileptogenic events. Released HMGB1 then acts on immune toll-like receptor 4, originally identified as first line of defense against infections, and promotes seizures[25] and epileptogenesis.[26,27] Overall, these interventional studies have been instrumental to ascribe pathophysiological consequences to the seminal description of neuroinflammatory molecules in epilepsy brain.[1]

Based on evidence that neuroinflammation contributes to set seizure threshold and occurs *before the onset of epilepsy* in animals exposed to status epilepticus, neurotrauma, or hyperthermia, pharmacological studies targeted potentially pathogenic inflammatory pathways during epileptogenesis. The field of research therefore extended the role of inflammation in sustaining acute or chronic seizures as addressed by the initial studies.[1] Specific anti-inflammatory drugs were found to provide disease-modifying effects, for example, delaying epilepsy onset, reducing spontaneous seizure, affording neuroprotection, and preventing memory deficits.[12,26,28,29] Growing evidence supports that neuroinflammation during epilepsy development can mediate the transition to pathology, and this process likely depends on lack of efficient homeostatic anti-inflammatory mechanisms and is reinforced by oxidative stress.[26] Boosting the endogenous anti-inflammatory response might be, therefore, a clinically feasible therapeutic option to improve the disease course by re-establishing the homeostatic function of inflammation.[2]

A concomitant question addressed during the interventional studies on seizures or epileptogenesis concerned the mechanisms underlying the effects of neuroinflammation. Direct neuromodulatory actions of cytokines, chemokines, and prostaglandins were well-known for a long time, but their involvement in epilepsy was a new discovery.[12,26,30] Initial evidence reported functional interactions between cytokines and neuronal ionotropic glutamate receptors, and cytokines's modulatory effects on gliotransmission,[1] then additional new mechanisms were described underlying the ictogenic cytokine's effects.[30] Worth mentioning is the inhibitory effect of neuroinflammation on dendritic Hyperpolarization-activated cyclic nucleotide–gated (HCN1) channel-mediated currents (I_h) in CA1 pyramidal cells,[31] and the decrease in γ-aminobutyric acid-evoked currents induced by IL-1β in human TLE tissue.[32] Notably, a fresh line of research pointed to the reciprocal interactions between neuroinflammation and BBB dysfunction, as underscored by the discovery of the crucial role of transforming growth factor (TGFβ)-SMAD2 signaling activation by extravasated serum albumin for inducing inflammation in astrocytes and its contribution to epileptogenesis.[11]

Finally, the increasingly detailed analysis of inflammation in human epilepsy brain tissue since its initial description in TLE[1] revealed that inflammation is not a stereotyped brain response but it occurs at different extents depending on etiologies and between patients and, in addition to the common presence of activated microglia and astrocytes, may include different cellular components such monocytes/macrophages and other peripheral immune cells.[10,17,18] This observation highlighted the need for discovering biomarkers of brain inflammation to stratify patients for the most appropriate therapeutic interventions. These research efforts are in progress, including validation of some promising blood molecules and in vivo neuroimaging of glia activation and BBB dysfunction by PET and magnetic resonance imaging/ magnetic resonance spectroscopy.[2,11]

In spite of the huge progress in the field, there are still many open questions to be addressed for improving the approach to therapy: (1) microglia and astrocytes are pivotal cells of the neuroinflammatory response,[1] but the role played by each cell population during disease development is still not fully clarified. Similarly, the dynamics of inflammatory pathways activation during epileptogenesis, and how to prevent the detrimental effects of some inflammatory mediators while preserving their homeostatic functions (*see for eg*, TNF[1] and

cyclooxygenase 2 signals) should be better defined. Animal models of epilepsies should help define the therapeutic outcomes of specific anti-inflammatory interventions at critical time points of disease development, since treatment at the wrong time may be ineffective or even harmful[24]; (2) presence and role of peripheral immune cells in epileptogenesis and chronic seizures in common epilepsies needs further studies[1,8,33-35]; (3) due to complexity of the inflammatory response, treatment combinations might be required to improve therapeutic success,[2] and nodal points of intervention should be defined with the help of systems biology[36]; (4) information about presence and role of neuroinflammation in genetic epilepsies remains scarce. Studies in rodent models of absence seizures and progressive myoclonus epilepsy have shown that neuroinflammation anticipates and contributes to *spike-and-wave* activity and precedes myoclonic seizures[2]; (5) optimal timing of intervention with anti-inflammatory drugs in each eligible clinical condition and patients selection with sensitive biomarkers are critical aspects of therapy which remain to be addressed.

In conclusion, the animal models and clinical findings gathered over the last 2 decades confirmed the presence of brain inflammation in common epilepsies and shed new light on the consequences of this phenomenon for seizure generation and the associated neuropathology and comorbidities, also discovering some of the mechanisms involved. Importantly, the newly acquired data are reinforcing the early prediction[1] that drugs that modulate specific inflammatory pathways could be a new therapeutic approach for pharmacoresistant focal epilepsies. Indeed, repurposed anti-inflammatory drugs (ie, anakinra and canakinumab against IL-1β, tocilizumab against IL-6, adalimumab against TNF), as suggested by early work in animal models,[1] are being used for rare epilepsy syndromes with encouraging results.[2] These treatments could be extended to epileptic encephalopathies,[37] refractory status epilepticus, or structural drug-resistant epilepsies either suspected or known to be associated with brain inflammation.[2] Early anti-inflammatory intervention after diagnosis of pharmacoresistance may provide improvement of the human disease course as suggested by evidence of disease modifications. Preventative approaches will require refinement of targets and timing of interventions and await for prognostic biomarkers.

Overall, this field of research has progressed incrementally forward with breakthroughs mostly related to discovery of new pathways, and molecular and cellular mechanisms, and the first attempts of clinical translation based on experimental findings. Perception of the field for providing new treatments and biomarkers has increased over time among clinicians, and the enthusiasm of basic science and preclinical research for exploring novel targets or validating existing ones in additional models has not declined. Therefore, this field of research has gained further attention since my original Epilepsy Currents commentary[1] which raises hopes for attaining a deeper understanding of this complex phenomenon and for new therapeutic interventions in eligible patients.

By Annamaria Vezzani 🆔

Dept of Neuroscience, Istituto di Ricerche Farmacologiche Mario Negri IRCCSVia Mario Negri 2, 20156 Milano, Italy

ORCID iD

Annamaria Vezzani 🆔 https://orcid.org/0000-0003-0573-6175

References

1. Vezzani A. Brain inflammation and seizures. *Epilepsy Curr*. 2004;4:73-75.
2. Vezzani A, Balosso S, Ravizza T. Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. *Nat Rev Neurol*. 2019;15(8):459-472.
3. Minami M, Kuraishi Y, Satoh M. Effects of kainic acid on messenger RNA levels of IL-1 beta, IL-6, TNF alpha and LIF in the rat brain. *Biochem Biophys Res Commun*. 1991;176(2):593-598.
4. Eriksson C, Winblad B, Schultzberg M. Kainic acid induced expression of interleukin-1 receptor antagonist mRNA in the rat brain. *Brain Res Mol Brain Res*. 1998;58(1-2):195-208.
5. Vezzani A, Conti M, De Luigi A, et al. Interleukin-1beta immunoreactivity and microglia are enhanced in the rat hippocampus by focal kainate application: functional evidence for enhancement of electrographic seizures. *J Neurosci*. 1999;19(12):5054-5065.
6. Crespel A, Coubes P, Rousset MC, et al. Inflammatory reactions in human medial temporal lobe epilepsy with hippocampal sclerosis. *Brain Res*. 2002;952(2):159-169.
7. Ravizza T, Boer K, Redeker S, et al. The IL-1beta system in epilepsy-associated malformations of cortical development. *Neurobiol Dis*. 2006;24(1):128-143.
8. Ravizza T, Gagliardi B, Noé F, Boer K, Aronica E, Vezzani A. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: evidence from experimental models and human temporal lobe epilepsy. *Neurobiol Dis*. 2008;29(1):142-160.
9. Prabowo AS, Anink JJ, Lammens M, et al. Fetal brain lesions in tuberous sclerosis complex: TORC1 activation and inflammation. *Brain Pathol*. 2012;23(1):45-59.
10. Vezzani A, Lang B, Aronica E. Immunity and inflammation in epilepsy. *Cold Spring Harb Perspect Med*. 2015;6(2):a022699.
11. Swissa E, Serlin Y, Vazana U, Prager O, Friedman A. Blood-brain barrier dysfunction in status epileptics: mechanisms and role in epileptogenesis. *Epilepsy Behav*. 2019;101(Pt B):106285.
12. van Vliet EA, Aronica E, Vezzani A, Ravizza T. Review: neuroinflammatory pathways as treatment targets and biomarker candidates in epilepsy: emerging evidence from preclinical and clinical studies. *Neuropathol Appl Neurobiol*. 2018;44(1):91-111.
13. Gershen LD, Zanotti-Fregonara P, Dustin IH, et al. Neuroinflammation in temporal lobe epilepsy measured using positron emission tomographic imaging of translocator protein. *JAMA Neurol*. 2015;72(2):882-888.
14. Butler T, Li Y, Tsui W, et al. Transient and chronic seizure-induced inflammation in human focal epilepsy. *Epilepsia*. 2016; 57(2):e191-e194.
15. Dickstein LP, Liow JS, Austermuehle A, et al. Neuroinflammation in neocortical epilepsy measured by PET imaging of translocator protein. *Epilepsia*. 2019;60(6):1248-1254.

16. Vezzani A, Moneta D, Conti M, et al. Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. *Proc Natl Acad Sci USA*. 2000; 97(21):11534-11539.

17. Iyer A, Zurolo E, Spliet WG, et al. Evaluation of the innate and adaptive immunity in type I and type II focal cortical dysplasias. *Epilepsia*. 2010;51(9):1736-1773.

18. Arena A, Zimmer TS, Van Scheppingen J, et al. Oxidative stress and inflammation in a spectrum of epileptogenic cortical malformations: molecular insights into their interdependence. *Brain Path*. 2019;29(3):351-365.

19. Xanthos DN, Sandkuhler J. Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity. *Nat Rev Neurosci*. 2014;15(1):43-53.

20. Pittman QJ, Gomez CD, Read J, Lewis ML, Acharjee S. Early life inflammation - it sticks to the brain. *Curr Opin Behav Sci*. 2019; 28(3):136-141.

21. Mazarati A, Vezzani A. Inflammation and immunomodulation in epilepsy and its comorbidities. In: Boison D, Masino SA, eds. *Homeostatic Control of Brain Function*. Oxford University Press; 2015.

22. Dubé CM, Ravizza T, Hamamura M, et al. Epileptogenesis provoked by prolonged experimental febrile seizures: mechanisms and biomarkers. *J Neurosci*. 2010;30(22):7484-7894.

23. Schlichtiger J, Pekcec A, Bartmann H, et al. Celecoxib treatment restores pharmacosensitivity in a rat model of pharmacoresistant epilepsy. *Br J Pharmacol*. 2010;160(5):1062-1071.

24. Rojas A, Chen D, Ganesh T, Varvel NH, Dingledine R. The COX-2/prostanoid signaling cascades in seizure disorders. *Expert Opin Ther Targets*. 2019;23(1):1-13.

25. Maroso M, Balosso S, Ravizza T, et al. Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures. *Nat Med*. 2010;16(4):413-419.

26. Terrone G, Balosso S, Pauletti A, Ravizza T, Vezzani A. Inflammation and reactive oxygen species as disease modifiers in epilepsy. *Neuropharmacology*. 2020;167:107742.

27. Zhao J, Wang Y, Xu C, et al. Therapeutic potential of an anti-high mobility group box-1 monoclonal antibody in epilepsy. *Brain Behav Immun*. 2017;64(1):308-319.

28. Semple BD, O'Brien TJ, Gimlin K, et al. Interleukin-1 receptor in seizure susceptibility after traumatic injury to the pediatric brain. *J Neurosci*. 2017;37(33):7864-7877.

29. Garcia-Curran MM, Hall AM, Patterson KP, et al. Dexamethasone attenuates hyperexcitability provoked by experimental febrile status epilepticus. *eNeuro*. 2019;6(6):ENEURO.0430–19. 2019.

30. Vezzani A, Viviani B. Neuro modulatory properties of inflammatory cytokines and their impact on neuronal excitability. *Neuropharmacology*. 2015;96(Pt A):70-82.

31. Frigerio F, Flynn C, Han Y, et al. Neuroinflammation alters integrative properties of rat hippocampal pyramidal cells. *Mol Neurobiol*. 2018;55(9):7500-7511.

32. Roseti C, van Vliet EA, Cifelli P, et al. GABA currents are decreased by IL-1beta in epileptogenic tissue of patients with temporal lobe epilepsy: implications for ictogenesis. *Neurobiol Dis*. 2015;82:311-320.

33. Fabene PF, Mora GN, Martinello M, et al. A role for leukocyte-endothelial adhesion mechanisms in epilepsy. *Nat Med*. 2008; 14(12):1377-1383.

34. Varvel NH, Neher JJ, Bosch A, et al. Infiltrating monocytes promote brain inflammation and exacerbate neuronal damage after status epilepticus. *Proc Natl Acad Sci USA*. 2016;113(38): E5665-E5674.

35. Xu D, Robinson AP, Ishii T, et al. Peripherally derived T regulatory and $\gamma\delta$ T cells have opposing roles in the pathogenesis of intractable pediatric epilepsy. *J Exp Med*. 2018;215(4):1169-1186.

36. Srivastava PK, van Eyll J, Godard P, et al. A systems-level framework for drug discovery identifies Csf1R as an anti-epileptic drug target. *Nat Commun*. 2018;9(1):3561.

37. Nabbout R, Vezzani A, Dulac O, Chiron C. Acute encephalopathy with inflammation-mediated status epilepticus. *Lancet Neurol*. 2011;10(1):99-108.