The Role of Cytokines in Seizures; IL-1 beta, IL-1Ra, IL-8 and IL-10

Short title: Significant Cytokines in Seizures

Youngah Youn, MD, PhD, In Kyung Sung, MD, PhD, In Goo Lee, MD, PhD
Department of Pediatrics, Seoul St. Mary’s Hospital, The Catholic University of Korea,
Seoul, Korea

Corresponding Author:
In Goo Lee, M.D., PhD
The Catholic University of Korea, College of Medicine
Seoul St. Mary’s Hospital
505 Banpo-Dong, Seocho-Gu, Seoul, Republic of Korea, Zip: 137-701
Telephone: 82-2-2258-6186
Fax: 82-2-537-4544
E-mail: iglee@catholic.ac.kr
Abstract

Brain insults such as neurotrauma, infection and perinatal injury like hypoxic ischemic encephalopathy generate inflammation in the brain. The inflammatory cascades induce storming of the whole spectrum of cytokines which can lead to degeneration of neurons, neurotoxic influence on brain tissue, and development to seizures, even if subclinical occurring at birth. Cytokines are secreted in the glial cells of central nervous system and play as mediators of immune system. Cytokines have two different roles, pro-inflammatory and anti-inflammatory. IL-1 beta and IL-8 are acting as pro-inflammatory cytokines activating further cytokine cascades, increase seizure susceptibility and organ damage, while IL-1Ra and IL-10 are acting as anti-inflammatory cytokines for their protective and anticonvulsant effect. As a result, the immune system and its associated inflammatory reactions seem to play an important role in brain damage. It is to be further addressed in clinical studies that cytokine release is relevant for the process of epileptogenesis and anti-epileptogenesis which may be prevented by immunomodulatory treatment in the future. Furthermore, early detection and intervention of brain damage are essential to prevent disease progression and further neurological complications. Cytokines can be used as biomarkers for the earlier detection of brain damage in high-risk infants.

Key words: Cytokines, seizures, immune system, biomarker
Introduction

A various kinds of brain insults such as neurotrauma, infection and perinatal injury can generate inflammation in the brain\(^1\). These injuries are risk factors for the development of seizures, even if subclinical, occurring at birth may initiate a cascade of chronic inflammatory process in the central nervous system (CNS)\(^2\). As a mediator of inflammatory processes, the cytokines in relation to immune system have been searched for their roles by molecular and pharmacological measures.

The immune system is a naturally occurring protective system designed to protect the host from both external (such as bacteria and viruses) and internal threats (such as malignant transformation)\(^3\). Generally, they are synthesized and secreted in response to an antigenic stimulus. Recently, the abnormalities in the expression of cytokines and immune cells have been observed in patients with seizures and in animal models. In many studies, the production and release of cytokines are regulated by immune system and aggravate brain damage when acting as mediators of seizures\(^4\).

Cytokines are soluble and potent glycoproteins involved in regulation of growth, activation of immune system cells and mediation of the inflammatory and immune response\(^5\). They are secreted in the glial cells of CNS. They signal between cells, bind to high affinity surface receptors and delivered by cells either to the systemic or to the local environment. In some cases, cytokines can travel to distant cells in other organs via the peripheral circulation. As an example, IL-6 produced at a local inflammatory site can enhance acute phase protein production in liver\(^3\). They are commonly measured in their soluble form, but they can be measured in the tissue. Many clinical and animal studies measure various body fluids in soluble form as a
way of measuring cytokines which provides a window into the pathogenesis of the inflammatory process\(^3\).

Elevated serum cytokines have also been documented in neurological disorders: cerebral ischemia, CNS trauma, multiple sclerosis, Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis, Alzheimer’s disease, and Parkinson’s disease\(^5\). In premature babies, pro-inflammatory cytokines have been associated with intraventricular hemorrhage and bronchopulmonary dysplasia and these conditions are negatively correlated to gestational age\(^6\). The storming of the whole spectrum of cytokines can lead to degeneration of neurons, neurotoxic influence on brain tissue, and induce seizures\(^5\). It has been postulated that an imbalance of pro- and anti-inflammatory cytokines aggravate organ damage. Interleukin (IL)-1, IL-3, IL-6, IL-8, interferon-gamma and tumor necrosis factor (TNF)-alpha are known to have pro-inflammatory roles while IL-1Ra and IL-10 are playing as anti-inflammatory roles for their anticonvulsant effect. Fibroblast growth factor (FGF)-2 is implicated to cause seizures\(^7\) in animal models and FGF-1 in kainic acid treated rats significantly decreased tonic-clonic convulsions and mortality\(^8\), thereby, acting as an anti-inflammatory role. Our previous clinical study on neonatal seizures induced by hypoxic–ischemic encephalopathy was to observe significant cytokine levels in relation to neonatal seizures and we noted a statistically significant difference in serum IL-8, IL-10 and IL-1Ra in the seizure group when compared to the control group. In this review, we’ll primarily focus on the roles of cytokines, IL-1 beta, IL-1Ra, IL-8 and IL-10 in brain injury associated to seizures.

**Mechanisms of neuronal injury**
1. *Induction of brain inflammation*

Brain injuries like hypoxia-ischemia and seizure initially causes energy failure and loss of mitochondrial function. This is accompanied by membrane depolarization and enhanced neurotransmitter release which, in turn, increases intracellular calcium that sets off additional pathologic cascades. These include oxidative stress, with the production of reactive oxygen species and interaction with nitric oxide pathway to produce reactive nitrogen species.

2. *Brain inflammation and epileptogenesis*

Brain injuries even subclinical seizures trigger brain inflammation which downstream inflammatory mediators. This event induces changes in brain parenchyma such as leakage of the brain blood barrier (BBB) which causes changes in functional properties of BBB. Several studies revealed BBB failure after administration of IL-1, IL-6, TNF-alpha, and interferon-gamma. Those changes cause cell damage which contributes to neuronal hyperexcitability which, in turn, lowers the threshold for seizure induction and trigger epileptogenesis, thus setting the basis for the onset of seizure. Activation of immune mechanisms can recruit more of inflammatory cells from the periphery, thus aggravating inflammation. Reperfusion also exacerbates the oxidative stress with a burst of reactive oxygen species.

**The roles of Cytokines in seizure activity**

1. *IL-1 beta and IL-1Ra*

The IL-1 family comprises three ligands: IL-1alpha, IL-1beta, and IL-1 receptor antagonist (Ra), all of which bind the IL-1 receptor (IL-1R). IL-1beta is mostly secreted whereas IL-1alpha is predominantly membrane-bound. IL-1Ra is a
naturally-occurring antagonist of IL-1 receptor type 1 (R1), which acts by limiting IL-1b-mediated actions\(^6\). It is capable of inhibiting receptor binding and biological activities of IL-1, especially IL-1 beta. IL-1 cytokines are constitutively expressed at very low levels in the human CNS. But when brain injuries like hypoxia or seizures occur, they enhance expression of IL-1 cytokines. Immunohistochemical analysis of cellular patterns of inflammatory changes in the rat forebrain detected a fast increase of IL-1 beta in activated microglia and astrocytes during the acute seizures which did not return to the basal level of expression after seizure subsided\(^17\). Meanwhile, TNF-alpha and IL-6 also increased in glial cells similarly to IL-1 beta, but their upregulation was only transient. The chronic expression of IL-1 beta during epileptogenesis highlights the possibility that this cytokine may contribute to the mechanisms underlying the onset of spontaneous seizures\(^2\). Contrary to previous animal studies, in our study on neonatal seizures, we observed no significant IL-1 beta plasma levels in the seizure group induced by hypoxic ischemic encephalopathy compared to control group\(^18\).

In genetic studies, homozygosity for the IL-1 beta-511 allele 2, which is suggested to be an inducer of IL-1 beta, was overrepresented in temporal lobe epilepsy (TLE) patients with hippocampal sclerosis (HS) when compared to control subjects\(^19\). Likewise, an association between the frequency of the IL-1 beta-511 allele 2 and an increased risk of febrile convulsions has been reported\(^{19-20}\), however, this association was refuted in another study. These contradicting results were presumably explained by different prevalence of the allele in different ethnicities. Several clinical studies addressed the change of IL-1 beta levels in blood and cerebrospinal fluid (CSF) of patients with focal epilepsy. There were no significant differences in the IL-1 beta concentration in blood and CSF within 24 h after tonic–
clonic seizures when compared to control subjects\textsuperscript{21}.

The response of the IL-1beta system to seizures is the induction of IL-1 Ra, which acts by limiting IL-1beta-mediated pro-inflammatory actions\textsuperscript{22}. IL-1 Ra is a powerful anticonvulsant in various models of seizures. IL-1Ra is induced by seizures several hours after IL-1beta to terminate rapidly the effects of IL-1beta upon its production. It is worth noting that the maximal expression of IL-1Ra in the rodent brain occurred later than that of the inflammatory cytokines (24 vs. 6 h)\textsuperscript{23}. In another study, a peak effect of pro-inflammatory cytokines like IL-1 beta, IL-6 and TNF-alpha occurs at 6hr after status epilepticus (SE) while the peak effect of anti-inflammatory cytokine, IL-1 Ra had a delayed effect at 24 hr after SE\textsuperscript{23}. Therefore, IL-1Ra is induced by seizures several hours after IL-1 beta\textsuperscript{24}.

Vezzani et al emphasizes the changes of IL-1Ra to IL-1 ratio as a mechanism to control seizures after seizure onset. He explains that IL-1Ra was usually produced in a molar ratio to IL-1 of 1:1, however, limbic seizures in rodents rapidly and reversibly induce changes in the IL-1Ra/IL-1 ratio in brain. During peripheral inflammatory reactions, IL-1Ra is generally produced together with IL-1 and 100-fold in excess to IL-1\textsuperscript{23}. This change may suggest an effective physiopathologic mechanism to control seizures. Different models of limbic seizures in rats and mice consistently showed that intrahippocampal application of IL-1 has proconvulsant actions\textsuperscript{2}, and the intravenous administration of IL-1Ra exhibited significant reduction of status epilepticus intensity in the rat\textsuperscript{24}. However, the brain is lacking an efficient mechanism to terminate rapidly the effects of IL-1beta upon its production\textsuperscript{2}.

In our study on neonatal seizures, IL-1Ra was continuously inactivated and dropped significantly lower in the seizure group within 72 h of seizure attack when compared to the control group. Because IL-1Ra has a neuroprotective and anticonvulsant
effect\(^5\), and closely correlated with the pro-convulsive cytokine IL-1beta, we assume that the lack of consistent induction of IL-1Ra in response to the epileptogenic environment may be characteristic of neonatal seizures, making the neonatal period more vulnerable to seizures\(^{18}\).

2. IL-8
IL-8 is known to be pro-inflammatory cytokine and reported to increase significantly in refractory epilepsy patients\(^4\). IL-8 concentration in serum and CSF of patients with refractory epilepsy was significantly increased after seizures, including focal, generalized tonic–clonic, myoclonic, atypical absence, and typical absence seizures\(^4\). IL-8 in CSF from patients with encephalopathy is considered to be originated from neural cells rather than leakage from serum, as CSF levels of IL-8 were significantly higher than those in serum\(^{25}\). Therefore, IL-8 plays an important role in the pathogenesis of traumatic brain injury. Interestingly, it is also reported to promote neuronal growth after neuronal injury. In cultured astrocytes, IL-8 stimulated production of nerve growth factor\(^{26}\). As a result, it is known to have both damaging and reparative mechanisms.

Whether measurement of IL-8 will serve as a useful prognostic indicator after traumatic brain injury remains to be established, although the study by Whalen and colleagues\(^{27}\) suggests that cerebrospinal fluid concentrations of IL-8 may serve as a useful prognostic indicator in head-injured children. In our study, the levels of IL-8 significantly increased both within 24 h and between 48 and 72 h in seizure patients suggesting that IL-8 may also serve as a biomarker for the earlier detection of neonatal seizures\(^{18}\).

3. IL-10
In addition to positive feedback loops involving cytokines, IL-10 is known to play negative feedback signals which subside the activated immune system after an inflammatory trigger\(^3\). IL-10 deactivates macrophages, which in turn decreases production of cytokines by T cells\(^4\). IL-10 also has broad anti-inflammatory properties by suppression of proinflammatory cytokines production\(^4\). The frequencies of the IL-10 592C allele and 1082A/-819C/-592C haplotype, which have been reportedly associated with increased production of IL-10, were significantly lower in patients suffering from focal seizure (FS) as compared to healthy controls\(^4\).

In our study with neonatal seizures, IL-10 was significantly elevated in the plasma after 48–72 h of seizure onset\(^18\). The surge of IL-10 levels after 24-72hr of seizure onset may indicate the enhanced protective role of IL-10, thereby, activating anticonvulsive effect in neonatal seizure patients by suppression of pro-inflammatory cytokine production.

**Conclusion**

Many clinical and animal studies have shown a complex relationship between seizure and the immune system mediated by cytokines. Abnormalities in expression of cytokines and immune cells have been observed in patients with seizures and in animal models. As a result, the immune system and its associated inflammatory reactions seem to play an important role in brain damage\(^4\). IL-1 beta and IL-8 are acting as pro-inflammatory cytokines activating further cytokine cascade, increase seizure susceptibility and organ damage, while IL-1Ra and IL-10 are acting as anti-inflammatory cytokines for their protective and anticonvulsant effect. But the brain is lacking an efficient mechanism to terminate rapidly the effects of pro-inflammatory cytokines such as IL-1beta upon its production. In addition to seizures, cytokines are
associated to brain ischemia, trauma, degenerative diseases and even contributed to intraventricular hemorrhage and bronchopulmonary dysplasia in premature babies. Many extrapolations and new notions about the roles of cytokines in seizure are still on going. Until now, reactive species were thought to cause harm only if antioxidant defenses were overwhelmed, however, newly interesting notion has proposed that the interaction itself between reactive species and antioxidant defenses ultimately causes cellular injury and death\textsuperscript{28}).

There is a definitely a limitation to study further cytokine detections and relevance in blood or CSF in human studies. However, it is to be further addressed in clinical studies that cytokine release is relevant for the process of epileptogenesis and anti-epileptogenesis which may be prevented by immunomodulatory treatment in the future\textsuperscript{(4)}. Early detection and intervention of brain damage are essential to prevent disease progression and further neurological complications. Cytokines can be used as biomarkers for the earlier detection of brain damage in high-risk infants.

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