

The role of cytokines in seizures: interleukin (IL)-1 β , IL-1Ra, IL-8, and IL-10

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Brain insults, including neurotrauma, infection, and perinatal injuries such as hypoxic ischemic encephalopathy, generate inflammation in the brain. These inflammatory cascades induce a wide spectrum of cytokines, which can cause neuron degeneration, have neurotoxic effects on brain tissue, and lead to the development of seizures, even if they are subclinical and occur at birth. Cytokines are secreted by the glial cells of the central nervous system and they function as immune system mediators. Cytokines can be proinflammatory or anti-inflammatory. Interleukin (IL)-1 β and IL-8 are proinflammatory cytokines that activate additional cytokine cascades and increase seizure susceptibility and organ damage, whereas IL-1 receptor antagonist and IL-10 act as anti-inflammatory cytokines that have protective and anticonvulsant effects. Therefore, the immune system and its associated inflammatory reactions appear to play an important role in brain damage. Whether cytokine release is relevant for the processes of epileptogenesis and antiepileptogenesis, and whether epileptogenesis could be prevented by immunomodulatory treatment should be addressed in future clinical studies. Furthermore, early detection of brain damage and early intervention are essential for the prevention of disease progression and further neurological complications. Therefore, cytokines might be useful as biomarkers for earlier detection of brain damage in high-risk infants.

Key words: Cytokines, Seizures, Immune system, Biological biomarker

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Introduction

Various brain insults, such as neurotrauma, infection, and perinatal injury, can generate inflammation in the brain¹. These injuries are risk factors for the development of seizures; even if they are subclinical, or occur at birth, they might initiate a cascade of chronic inflammatory processes in the central nervous system (CNS)². As mediators of inflammatory processes, cytokines have been studied through molecular and pharmacological methods to understand their role in the immune system.

The immune system is designed to protect the host from both external (such as bacteria and viruses) and internal (such as malignant transformation) threats³. Cytokines are generally synthesized and secreted in response to antigenic stimuli. Recently, abnormalities in the expression of cytokines and in immune cells have been observed in patients with seizures and in animal models of seizures. Many studies have shown that the production and release of cytokines are regulated by the immune system and that these cytokines can aggravate brain damage when acting as mediators of seizures⁴.

Cytokines are soluble, potent glycoproteins involved in the regulation of growth, immune cell activation, and the inflammatory and immune responses⁵. In the CNS, they are secreted by the glial cells. Cytokines mediate cell-cell signaling, bind to high affinity surface receptors, and are delivered by cells to either the local environment or system wide. In some

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cases, cytokines can travel to distant cells in other organs via the peripheral circulation. For example, interleukin (IL)-6 produced at a local inflammatory site can enhance acute phase protein production in the liver³. Although cytokines are commonly measured in their soluble form, they can be measured in tissues. In many clinical and animal studies, cytokines have been measured in various body fluids in soluble form to provide a window into the pathogenesis of the inflammatory process³.

Elevated serum cytokines have also been documented in a number of neurological disorders, including cerebral ischemia, CNS trauma, multiple sclerosis, Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis, Alzheimer disease, and Parkinson disease⁵. In premature babies, proinflammatory cytokines have been associated with intraventricular hemorrhage and bronchopulmonary dysplasia, which are negatively correlated with gestational age⁶. The storming of a wide spectrum of cytokines can lead to neuronal degeneration and neurotoxicity in brain tissue, and can induce seizures⁵. It has been postulated that an imbalance of proinflammatory and anti-inflammatory cytokines aggravates organ damage. IL-1, IL-3, IL-6, IL-8, interferon (IFN)- γ , and tumor necrosis factor (TNF)- α have proinflammatory roles, whereas IL-1 receptor antagonist (IL-1Ra) and IL-10 play anti-inflammatory roles and have anticonvulsant effects. Fibroblast growth factor (FGF)-2 has been implicated in seizures⁷ and in animal models, and FGF-1 significantly decreased tonic-clonic convulsions and mortality in kainic acid-treated rats⁸. Therefore, FGF-1 and FGF-2 play anti-inflammatory roles. In our previous clinical study of neonatal seizures induced by hypoxic-ischemic encephalopathy, we observed significant correlation between cytokine levels and neonatal seizures and noted a statistically significant difference in serum IL-8, IL-10, and IL-1Ra between the seizure group and the control group. In this review, we will primarily focus on the roles of IL-1 β , IL-1Ra, IL-8, and IL-10 in brain injury associated with seizures.

Mechanisms of neuronal injury

1. Induction of brain inflammation

Brain injuries such as hypoxia-ischemia and seizure initially cause energy failure and loss of mitochondrial function. This is accompanied by membrane depolarization and enhanced neurotransmitter release. These events increase intracellular calcium, which sets off additional pathologic cascades⁹, including oxidative stress, the production of reactive oxygen species, and the nitric oxide pathway, which produces reactive nitrogen species¹⁰.

2. Brain inflammation and epileptogenesis

Brain injuries, even subclinical seizures, trigger brain inflam-

mation and downstream inflammatory mediators. This inflammation induces changes in the brain parenchyma, such as leakage of the brain blood barrier (BBB), which changes the functional properties of the BBB². Several studies showed BBB failure after administration of IL-1, IL-6, TNF- α , and IFN- γ ¹¹⁻¹³. These changes cause cell damage that contributes to neuronal hyperexcitability, which lowers the threshold for seizure induction and triggers epileptogenesis. This sets the foundation for the onset of seizure. Immune activation can lead to the recruitment of more inflammatory cells from the periphery, which aggravates inflammation. Reperfusion also exacerbates the oxidative stress through a burst of reactive oxygen species¹⁴.

The roles of cytokines in seizure activity

1. IL-1 β and IL-1Ra

The IL-1 family is comprised of 3 ligands, IL-1 α , IL-1 β , and IL-1Ra, all of which bind the IL-1 receptor. IL-1 β is mostly secreted, whereas IL-1 α is predominantly membrane-bound. IL-1Ra is a naturally occurring antagonist of IL-1 receptor type 1, which acts by limiting IL-1 β -mediated actions¹⁵. It can inhibit receptor binding and the biological activities of IL-1, especially IL-1 β . IL-1 cytokines are constitutively expressed at very low levels in the human CNS. However, when brain injuries like hypoxia or seizures occur, the expression of IL-1 cytokines is enhanced. Immunohistochemical analysis of the cellular patterns of inflammatory changes in the rat forebrain showed a rapid increase of IL-1 β in activated microglia and astrocytes during acute seizures that did not return to the basal level after the seizure subsided¹⁶. Similarly, TNF- α and IL-6 also increased in glial cells; however, their upregulation was only transient. The chronic expression of IL-1 β during epileptogenesis highlights the possibility that this cytokine might be involved in the mechanisms underlying the onset of spontaneous seizures². Contrary to previous animal studies, in our study on neonatal seizures, we observed no significant difference in IL-1 β plasma levels between the seizure group (induced by hypoxic ischemic encephalopathy) and the control group¹⁷.

In genetic studies, homozygosity for the IL-1 β -511 allele 2, which is thought to be an inducer of IL-1 β , was overrepresented in temporal lobe epilepsy patients with hippocampal sclerosis when compared to control subjects¹⁸. Likewise, an association between IL-1 β -511 allele 2 and an increased risk of febrile convulsions has been reported^{18,19}. However, this association was refuted in another study^{18,19}. These contradicting results were explained by a difference in the prevalence of the allele in different ethnicities.

Several clinical studies have addressed the changes of IL-1 β levels in blood and cerebrospinal fluid (CSF) of patients with

focal epilepsy. There were no significant differences in the IL-1 β concentration in blood and CSF within 24 hours after tonic-clonic seizures compared to control subjects²⁰.

In response to seizures, the IL-1 β system induces IL-1Ra, which acts by limiting IL-1 β -mediated proinflammatory actions²¹. IL-1Ra has been shown to be a powerful anticonvulsant in various seizure models. IL-1Ra is induced by seizures several hours after IL-1 β to rapidly terminate the effects of IL-1 β . It is worth noting that maximal expression of IL-1Ra in the rodent brain occurred later than that of the inflammatory cytokines (24 hours vs. 6 hours)²². In another study, peak proinflammatory cytokine (IL-1 β , IL-6, and TNF- α) effects occur 6 hours after status epilepticus (SE), while the peak effect of the anti-inflammatory cytokine IL-1Ra was delayed, and occurred 24 hours after SE²². Therefore, IL-1Ra is induced by seizures several hours after IL-1 β ²³.

Vezzani et al.²² emphasized the changes in the IL-1Ra to IL-1 ratio as a mechanism of controlling seizures after onset. They explained that IL-1Ra and IL-1 were usually produced in a molar ratio of 1:1; however, limbic seizures in rodents rapidly and reversibly induce changes in the IL-1Ra/IL-1 ratio in the brain. During peripheral inflammatory reactions, IL-1Ra is generally produced at a 100-fold molar excess compared to IL-1². This change may be an effective physiopathologic mechanism to control seizures. Different models of limbic seizures in rats and mice have consistently shown that intrahippocampal application of IL-1 has proconvulsant actions², whereas intravenous administration of IL-1Ra significantly reduced SE intensity in the rat²³. However, the brain lacks an efficient mechanism to rapidly terminate the effects of IL-1 β ².

In our study on neonatal seizures, IL-1Ra was continuously inactivated and was significantly lower in the seizure group within 72 hours of seizure attack than in the control group. Because IL-1Ra has neuroprotective and anticonvulsant effects⁵, and was closely correlated with the proconvulsive cytokine IL-1 β , we assume that the lack of consistent IL-1Ra induction in response to the epileptogenic environment may be characteristic of neonatal seizures, making the neonatal period more vulnerable to seizures¹⁷.

2. IL-8

IL-8 is a proinflammatory cytokine and is significantly increased in refractory epilepsy patients⁴. The IL-8 concentration in the serum and CSF of patients with refractory epilepsy was significantly increased after seizures, including focal, generalized tonic-clonic, myoclonic, atypical absence, and typical absence seizures⁴. The IL-8 in the CSF of patients with encephalopathy is believed to originate from neural cells rather than from serum because CSF IL-8 levels were significantly higher than those in serum²⁴. Therefore, IL-8 plays an important role in the pathogenesis of traumatic brain injury. Interestingly, it has also been

reported to promote neuronal growth after injury. In cultured astrocytes, IL-8 stimulated the production of nerve growth factor²⁵. Therefore, IL-8 is known to have both damaging and reparative functions.

Whether measurement of IL-8 could serve as a useful prognostic indicator of traumatic brain injury remains to be established, although a study by Whalen et al.²⁶ suggests that the CSF concentration of IL-8 may serve as a useful prognostic indicator in head-injured children. In our study, IL-8 levels significantly increased both within 24 hours and between 48 and 72 hours in seizure patients, suggesting that IL-8 may also serve as a biomarker for earlier detection of neonatal seizures¹⁷.

3. IL-10

In addition to the role of IL-10 in positive feedback loops involving cytokines, IL-10 is known to relay negative feedback signals that dampen the activated immune system after an inflammatory trigger³. IL-10 deactivates macrophages, which in turn decreases the production of cytokines by T cells¹⁴. IL-10 also has broad anti-inflammatory effects, and acts through suppression of proinflammatory cytokine production⁴. The frequencies of the IL-10-592C allele and the -1082A/-819C/-592C haplotype, which are reportedly associated with increased IL-10 production, were significantly lower in patients suffering from focal seizure than in healthy controls⁴. In our study of neonatal seizures, IL-10 was significantly elevated in plasma 48–72 hours after seizure onset¹⁷. The surge in IL-10 levels 24–72 hours after seizure onset may indicate the enhanced protective role of IL-10, which has an anticonvulsive effect in neonatal seizure patients by suppressing proinflammatory cytokine production.

Conclusions

Many clinical and animal studies have shown a complex relationship between seizure and the immune system that is mediated by cytokines. Abnormalities in cytokine expression and immune cells have been observed in patients with seizures and in animal seizure models. Therefore, the immune system and its associated inflammatory reactions seem to play an important role in brain damage⁴. IL-1 β and IL-8 act as proinflammatory cytokines, further activating the cytokine cascade and increasing seizure susceptibility and organ damage, whereas IL-1Ra and IL-10 act as anti-inflammatory cytokines and have protective and anticonvulsant effects. However, the brain lacks an efficient mechanism to rapidly terminate the effects of proinflammatory cytokines such as IL-1 β . In addition to seizures, cytokines are associated with brain ischemia, trauma, and degenerative diseases, and even contribute to intraventricular hemorrhage and bron-

chopulmonary dysplasia in premature babies. Many studies of cytokines in seizure are still ongoing, and new hypotheses about their roles are being formed. Until now, reactive species were only thought to cause harm if the antioxidant defenses were overwhelmed; however, a new interesting idea has been proposed, that the interaction between reactive species and antioxidant defenses ultimately causes cellular injury and death²⁷.

There are definitely limitations to human studies of cytokines in the blood or CSF and its relevance. However, whether cytokine release is relevant to the process of epileptogenesis should be addressed in clinical studies as well as whether antiepileptogenesis could be prevented by immunomodulatory treatment⁴. Early detection of brain damage and early intervention are essential to prevent disease progression and further neurological complications. Therefore, cytokines might be useful as biomarkers for the earlier detection of brain damage in high-risk infants.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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