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Plasma Cytokines Associated with Febrile Status Epilepticus in Children: A Potential Biomarker for Acute Hippocampal Injury

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Summary

Objective—Our aim was to explore the association between plasma cytokines and febrile status epilepticus (FSE) in children, as well as their potential as biomarkers of acute hippocampal injury.

Methods—Analysis was performed on residual samples of children with FSE (n=33) as part of the FEBSTAT study and compared to children with fever (n=17). MRI was obtained as part of FEBSTAT within 72 hours of FSE. Cytokine levels and ratios of anti-inflammatory vs. pro-inflammatory cytokines in children with and without hippocampal T2 hyperintensity were assessed as biomarkers of acute hippocampal injury after FSE.

Results—IL-8 and EGF were significantly elevated after FSE in comparison to controls. IL-1 β levels trended higher and IL-1RA trended lower following FSE, but did not reach statistical significance. Children with FSE were found to have significantly lower ratios of IL-1RA/IL-1 β and IL-1RA/IL-8. Specific levels of any one individual cytokines were not associated with FSE. However, lower ratios of IL-1RA/IL-1 β , IL-1RA/IL-6, and IL-1RA/IL-8 were all associated with

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FSE. IL-6 and IL-8 levels were significantly higher and ratios of IL-1RA/IL-6 and IL-1RA/IL-8 were significantly lower in children with T2 hippocampal hyperintensity on MRI after FSE in comparison to those without hippocampal signal abnormalities. Individual cytokine levels were not predictive of MRI changes, nor were ratios of IL-1RA/IL-1 β or IL-1RA/IL-8. However, a lower ratio of IL-1RA/IL-6 was strongly predictive (OR 21.5, 95% CI: 1.17–393) of hippocampal T2 hyperintensity after FSE.

Significance—Our data support involvement of the IL-1 cytokine system, IL-6, and IL-8 in FSE in children. The identification of the IL-1RA/IL-6 ratio as a potential biomarker of acute hippocampal injury following FSE is the most significant finding. If replicated in another study, the IL-1RA/IL-6 ratio could represent a serologic biomarker which offers rapid identification of patients at risk for ultimately developing mesial temporal lobe epilepsy (MTLE).

Keywords

cytokine; interleukin; febrile status epilepticus; mesial temporal lobe epilepsy; hippocampal injury

Introduction

Retrospective studies have suggested an association between febrile status epilepticus (FSE) and mesial temporal lobe epilepsy (MTLE) ¹. The mechanisms by which this association occurs are not fully understood though there is now increasing evidence that acute hippocampal injury occurs in some cases ². Why some children with FSE sustain acute hippocampal injury evident on MRI while others do not also remains unknown. Although genetics clearly has a substantial influence ³, animal models have suggested inflammatory processes may also play an important role in the development of FSE and subsequent epileptogenesis⁴.

It has been proposed that the interleukin (IL)-1 cytokine system may play a pivotal role in the development of FSE and MTLE^{4–6}. IL-1 β is the primary cytokine responsible for mediating febrile responses in humans and a powerful pro-convulsant implicated in epileptogenesis^{5,7}. Elevation of IL-1 β induces a robust release of other pro-inflammatory cytokines including IL-6 and IL-8, as well as its competitive antagonist IL-1RA (interleukin-1 receptor antagonist) a potent anticonvulsant^{6,8}. IL-1RA induction in response to IL-1 β is an important component of anti-inflammatory autoregulation ⁹. It has been proposed that the ratio of IL-1 β and other pro-inflammatory cytokines to IL-1RA plays a key role in the development of febrile seizures and the mediation of neuronal responses within the brain following injury ^{6,10,11}. Animal models have demonstrated elevations of pro-inflammatory cytokines (including IL-1 β and IL-6) to be associated with acute hippocampal injury and epileptogenesis following FSE^{5,12}.

Other pro-inflammatory cytokines including tumor necrosis factor- α (TNF- α), interferon- α (IFN- α), interferon- γ (IFN- γ), fibroblast growth factor (FGF), IL-2, IL-10, IL-17, vascular endothelial growth factor (VEGF), as well as chemokines monocyte chemoattractant protein-1 (MCP-1 or CCL-2), macrophage inflammatory protein-1 α (MIP-1 α or CCL-3), macrophage inflammatory protein-1 β (MIP-1 β or CCL-4), regulated on activation, normal T expressed and secreted (RANTES or CCL-5), monokine induced by gamma interferon (MIG

or CXCL-9) and interferon gamma-induced protein 10 (IP-10 or CXCL-10) and danger signal HMGB1 have been implicated in playing some role in the pathogenesis of either status epilepticus or epilepsy¹³. Comprehensive analysis of plasma cytokines following FSE in humans to our knowledge has not been reported.

The Consequences of Prolonged Febrile Seizures in Childhood study (FEBSTAT) is a prospective multi-center study designed to assess the potential relationship between FSE and subsequent development of MTLE¹⁴. We reported hippocampal T2 hyperintensity following FSE may occur and likely represents acute injury which may evolve into hippocampal sclerosis². Whether these patients eventually go on to develop MTLE is still under investigation. Using a sub-cohort of the FEBSTAT study, our aim was to perform a pilot study to explore the potential association between plasma cytokines and FSE in children. Our primary hypothesis was that FSE would be associated with higher plasma IL-1 β levels in comparison to febrile controls. We also sought to explore the potential of plasma cytokines as a biomarker of acute brain injury following FSE. As such, we evaluated plasma cytokines as predictors of acute hippocampal changes on MRI following FSE in a subset of the FEBSTAT cohort.

Methods

All FSE study subjects were consented and enrolled into the FEBSTAT study between June 2003 and January 2010. The FEBSTAT study, as well as the secondary study described here, was approved by the Institutional Review Board. All subjects met FEBSTAT inclusion criteria: six years of age or younger with a febrile convulsion (temperature $\geq 38.4^{\circ}\text{C}$) lasting 30 minutes or two or more convulsions without return to baseline over a 30 minute period without evidence of central nervous system (CNS) infection. As part of the FEBSTAT study, MRI of the brain and EEG were obtained within 72 hours of FSE. Detailed description of the FEBSTAT study design has been previously published^{14,15}.

Plasma cytokine analysis was not part of the initial FEBSTAT study design. As part of the FEBSTAT protocol plasma samples were obtained for the purpose of viral testing within 72 hours of FSE, and collected in EDTA vacuum tubes¹⁶. Importantly, timing from FSE to specimen acquisition was not standardized and varied from subject to subject. Specimens were stored at room temperature after acquisition and shipped to Northwestern University. Upon arrival plasma was separated via centrifuge and stored at -80°C for future testing. Cytokine profiling was subsequently performed on a sub-cohort of these repository samples. Only those subjects with concomitant cerebrospinal fluid (CSF) samples were used for this pilot study, as CSF samples were also to be analyzed for cytokine levels. As lumbar puncture was not performed as part of the FEBSTAT protocol, only those patients with CSF obtained clinically with residual samples following clinical testing and study viral analysis were available for cytokine testing (n=33). CSF cytokines did not reveal recordable levels of most cytokines tested (likely a result of sample degradation), and thus did not provide any additional information. Plasma controls were obtained separately from the FEBSTAT study from children six years of age and younger with fever (temp $>38.4^{\circ}\text{C}$) alone without seizure or evidence of CNS infection. Samples were processed within 24 hours of acquisition and handled in the same manner as the FEBSTAT cohort.

Cytokine profiling was performed using a multi-plex protein array assay (Invitrogen Human Cytokine 30-Plex Luminex Assay Cat #LHC6003M; see Table 1). For statistical purposes, cytokine levels (pg/mL) below the lower limit of quantification (LLQ) were reported as the midpoint between the zero and the LLQ per analyte. Cytokine levels above the upper limit of quantification (ULQ) were reported as the upper limit per analyte. Ratios of the following cytokines were calculated as markers of anti-inflammation versus pro-inflammation (lower ratio implies greater inflammation, higher ratio implies less inflammation): IL-1RA/IL-1 β , IL-1RA/IL-6, and IL-1RA/IL-8. To assess for association between cytokines and hippocampal T2 abnormality on MRI, the following cytokines, commonly implicated to have a role in epileptogenesis, were included in the analysis: IL-1 β , IL-1RA, IL-6, IL-8, MCP-1, MIP-1 α , and MIP-1 β , as well as ratios of IL-1RA/IL-1 β , IL-1RA/IL-6, and IL-1RA/IL-8^{6,10–13}.

Statistical methods

Data were summarized using mean $\pm$ standard deviation for each cytokine and cytokine ratio. Nonparametric statistics using the Wilcoxon rank sum test was performed for all comparisons. Level of significance was set at $p < 0.05$. This was applied to the primary hypothesis of IL-1 β is higher in children with FSE. When a higher number of statistical comparisons was made (e.g., FEBSTAT plasma cytokines vs. control), a Bonferroni correction was performed to account for multiple comparisons (critical p value < 0.0024). Only cytokines with detectable levels in at least one of the two test groups were included in calculation of the Bonferroni correction. Odds ratios assessed the risk for FSE associated with cytokine ratios in 10 unit intervals. Within the FSE group, odds ratios assessed the risk for increased hippocampal T2 signal using cytokine ratios in 10 unit intervals.

Results

Cytokine analysis was performed on plasma samples obtained from children with FSE ($n=33$) and controls ($n=17$; Table 1). Profiles for each cytokine/chemokine of interest are displayed in Figure 1. Considerable inter-subject variability was seen. Although IL-1 β plasma levels trended higher in children with FSE, results were not statistically significant. IL-1RA trended lower in children with FSE, although after correction for multiple comparisons, results were not statistically significant. IL-8 and EGF (epidermal growth factor) had significantly higher plasma levels in children with FSE, whereas VEGF, G-CSF (granulocyte colony stimulating factor), and IL-2R (soluble interleukin-2 receptor) were significantly lower. No other individual cytokines reached statistically significant difference between FSE and control groups. However, children with FSE had a significantly lower IL-1RA/IL-1 β and IL-1RA/IL-8 ratios, and the IL-1RA/IL-6 ratio trended lower, but did not reach statistical significance (Table 1/Figure 1). Although, none of the individual absolute values for any one particular cytokine was associated with FSE, lower ratios of IL-1RA/IL-1 β , IL-1RA/IL-6, and IL-1RA/IL-8 were all associated (Table 2, Figure 2). There were no associations between cytokine levels or cytokine ratios and seizure duration or peak temperature of fever in our cohort.

We further assessed the potential association between cytokines and the development of hippocampal T2 hyperintensity on MRI within 72 hours of FSE (Table 3). In our cohort, 5 children had acute T2 abnormalities, and 27 did not. Children with acute T2 signal had significantly higher IL-6 and IL-8 plasma levels, and lower IL-1RA/IL-6, and IL-RA/IL-8 ratios (Table 3). On logistic regression, IL-1RA/IL-6 ratio strongly increased the risk of acute T2 hippocampal signal abnormality (Table 2). There were no significant differences in plasma cytokines in subjects with or without focal slowing or attenuation on EEG.

Discussion

Peripheral Cytokines and FSE

Peripheral cytokines can impact brain function as evidenced by central nervous system (CNS) symptoms (fever, anorexia, lethargy, lower seizure threshold) during times of heightened systemic inflammation (febrile illness, sepsis). Animal models have shown that systemic inflammation may increase seizure susceptibility by provoking inflammatory cytokine (TNF- α and IL-1 β) induction within the CNS (hippocampus and cortex).¹⁷ Blockade of this process with neutralizing antibodies against these cytokines as well as inhibition of microglial activation, has prevented the subsequent development of long-term seizure susceptibility later in the life.¹⁷ Furthermore, peripheral inflammation has been shown to synergistically potentiate seizures and enhance seizure-induced pro-inflammatory cytokine production and microglial activation in a recently described animal model of febrile seizures¹⁸.

Although peripheral cytokines levels may not be an accurate reflection of CNS cytokine activity, there are mechanisms by which their presence may induce a reciprocal inflammatory response within the brain. Direct binding of peripheral cytokines to receptors located within the circumventricular organs and indirect activation through afferent vagal nerve pathways, can activate CNS cytokine signaling pathways¹⁹. In the CNS, cytokines may be released by neurons, astrocytes, microglia, and infiltrating macrophages. The presence of cytokines in the periphery has been associated with breakdown of the blood brain barrier (BBB) resulting in increased permeability to ions and serum albumin which may result in acute and chronic neuronal hyperexcitability⁹. Furthermore, these changes in BBB permeability may favor entry of peripheral cytokines, as well as cells of the innate and adaptive immune system into the CNS resulting in further activation of the inflammatory cascade within the brain⁹. Similarly, breakdown of the BBB may also allow for leakage of cytokines released by cells in the CNS to cross over into the blood and contribute to changes in peripheral cytokine levels. Induction of inflammatory cytokines within the brain following FSE is well documented in animal models in support of this possible process.^{4,12}

Our data support an association between the IL-1 system, IL-6, IL-8, and FSE in children. Although implicated in animal models of FSE, several studies in humans, (including ours) have found no difference in plasma levels of IL-1 β in children with or without febrile seizures^{20–22}. There are several explanations for these findings. A recent study using an animal model of FSE reported increased hippocampal levels of IL-1 β occurring 1–3 hours after FSE, with rapid decline over 24 hours and return to baseline by 96 hours. IL-6 peaked later at 24 hours¹². As such, timing of specimen acquisition from FSE is important as levels

may change over time. Enrollment into FEBSTAT occurred within 72 hours of FSE. Due to the consenting process, it is unlikely that any of the FEBSTAT study blood specimens were acquired in the 1–6 hour window. One may assume the majority of the specimens were acquired 24–48 hour post-FSE. Based upon the animal data, we may predict IL-1 β levels to be declining over this time while IL-6 levels would be rising. This is consistent with a substantial number of our patients having low IL-1 β . Interestingly in this same model of FSE, investigators reported a significant degree of inter-subject variability of cytokines, similar to our findings (Figure 1)¹². They reported only a subset of rats had elevated hippocampal inflammatory markers (IL-1 β and IL-6) where as others had no significant changes. These findings suggest that certain populations may be predisposed to heightened inflammatory responses. Importantly, in this same model, this response was associated with hippocampal T2 changes on MRI and predictive of the development of epilepsy¹². These findings are consistent with our report of higher IL-6 and IL-8 levels in our patients with MRI changes.

There are other further possible explanations for variability of IL-1 β in the periphery following FSE. Extracellular IL-1 β has a higher affinity for binding to large plasma proteins such as α -2-macroglobulin and complement, and perhaps more importantly, soluble type II IL-1 receptor (IL-1sRII) circulating in the extracellular fluid²³. Elevated levels of IL-1sRII have been found in conditions known for excessive inflammation such as sepsis²⁴. The presence of IL-1sRII may reduce detection of IL-1 β in clinical samples by 50%²⁵. Also, minimal concentrations of IL-1 β are required to induce a robust inflammatory response²⁶. Following initial IL-1R binding, significant amplification of the IL-1 β signal occurs through multiple and sequential phosphorylation of protein kinases which activate numerous other signal transduction pathways. For these reasons, plasma IL-1 β is not likely best marker of IL-1 system activation. Conversely, based upon their robust release following IL-1 β induction, IL-1RA, IL-6, and IL-8 have been implicated as excellent surrogate markers of IL-1 β activity^{23,27,28}.

In our study, children with FSE trended towards lower absolute levels of IL-1RA. In animal models of SE, a substantial rise in IL-1RA occurs peaking at 24 hours in response to the IL-1 β surge.^{29,30} As such, we would expect our FSE group to have higher IL-1RA levels, corresponding to a predicted increase in IL-1 β signal transduction. However, this was not seen in most of our subjects. In fact, the FSE group overall had lower ratios of IL-1RA/IL-1 β and IL-1RA/IL-8 ratios in comparison to the febrile controls (IL-1RA/IL-6 trended lower as well). These findings might suggest that in some children with FSE the IL-1RA autoregulatory response to IL-1 β induction may be inadequate to suppress the pro-convulsant effects of IL-1 β , thus creating the propensity for FSE (Figure 2). A few patients in the FSE group had quite high IL-1RA levels. This could suggest that in this group, the IL-1RA being produced may not be effective. Polymorphisms in the IL-1 β , IL-1RA, or IL-1R genes (as well as IL-6) have been reported in children with FS³¹ and could result in the following pro-convulsant circumstances: excessive IL-1 β release with saturation of IL-1Rs, defective IL-1RA production, inadequate IL-1RA release, defective IL-1RA binding to IL-1R. These genetic variations could provide yet another explanation for some of the extreme variability in the absolute cytokine levels in seen in our subjects.

Importantly, although several cytokines had statistical differences between the plasma levels in the FEBSTAT cohort in comparison to controls (high IL-8 and EGF, low VEGF, G-CSF, and IL-2R), the absolute values of each individual cytokine were not associated with FSE. Our results suggest that assessing the ratios of anti-inflammatory (IL-1RA) to pro-inflammatory (IL-1 β , IL-6, IL-8) to be much more relevant, with lower IL-1RA/IL-1 β , IL-1RA/IL-6 and IL-1RA/IL-8 ratios all being associated with FSE. These ratios more appropriately describe the intensity of inflammation versus the adequacy of the anti-inflammatory response. As such, one possible mechanism for the heightened pro-convulsant state leading to the development of FSE may involve imbalance between the pro-inflammatory and anti-inflammatory cytokines involved in the IL-1 system.

We also found higher plasma EGF in our cohort which further supports the involvement of the IL-1 system in FSE. EGF has been established as a sensitive assessment of IL-1 β induced signal transduction³². IL-1 β binds to the EGF receptor resulting in phosphorylation which impacts the receptors affinity for binding EGF³². This results in an overall increase in the unbound EGF which is detected in the plasma. EGF has been reported to induce posttranscriptional production of IL-1 β ³³ which would then further perpetuate the inflammatory cycle. This makes EGF another potential surrogate marker of IL-1 β activity and provides one explanation of the EGF elevation seen in the FSE cohort, aside from being induced by seizure activity alone²³. Furthermore, activation EGF-RAS signal transduction pathways by EGF or IL-1 β may be proposed to play a role in FSE and epileptogenesis. Animal models have demonstrated that through activation of the MEK-ERK signal transduction pathway (induced by EGF-RAS) NMDA receptor subunits can be augmented and is associated with spontaneous epileptic seizures³⁴. Furthermore blockade of this pathway had a strong antiepileptic effect³⁴. To our knowledge, this is the first description of EGF associated with FSE in the literature.

Interestingly, IL-8 was also elevated in our cohort. IL-8 strongly promotes leukocyte migration into the CNS by inducing neutrophil-endothelial adhesions and contributes to blood brain barrier (BBB) breakdown in acute brain injury^{35,36}. Blockade of peripheral leukocytes infiltration into the CNS, as well as peripheral depletion, has been shown to prevent acute seizure induction and epileptogenesis in animal models³⁶. Through these mechanisms IL-8 may contribute to the pro-convulsant environment created by the IL-1 system in FSE and possibly promote epileptogenesis.

It is important to acknowledge that our cytokine levels were obtained after FSE. Cytokine levels have been reported to be elevated following seizures alone^{37,38}. As such the differences seen in our 2 groups may be secondary to the FSE itself, and may not imply causality. That being said, the association seen between FSE and an imbalanced anti-inflammatory to pro-inflammatory cytokine state in our cohort remains an interesting one. The potential cascade by which the IL-system could play a role in the pathogenesis of FSE as well as epileptogenesis is shown in Figure 3.³⁹

Cytokines as potential biomarkers for the development of MTLE

The most important finding in our study is the identification of IL-1RA/IL-6 ratio as a potential biomarker for the development of acute brain injury following FSE. The FEBSTAT

study was designed to assess the observed relationship between FSE and subsequent MTLE, with the aim of assessing potential biomarkers (MRI, EEG, neuropsychological testing) which may predict MTLE in children following FSE. We previously reported that following FSE, hippocampal T2 hyperintensity may occur and evolve into a radiographic hippocampal sclerosis at 1 year ². In an animal model of FSE, neither MRI hippocampal T2 signal nor interictal EEG activity predicted epileptogenesis ⁵. However, hippocampal levels of IL-1 β were significantly elevated for 24 hours after FSE, and remained chronically elevated in rats that went onto to develop spontaneous seizures, suggesting a role of IL-1 β in epileptogenesis ⁵. We found that children who develop hippocampal T2 signal abnormality (representative of acute brain injury) following FSE, have a greater degree of systemic inflammation (elevated IL-6 and IL-8 plasma levels, as well as lower mean IL-1RA/IL-6 and IL-1RA/IL-8 ratios). Although plasma IL-1 β levels and IL-RA/IL-1 β ratio were not associated with MRI changes, these findings are consistent with the previous discussions regarding IL-1RA, IL-6, and IL-8 as better surrogate markers of IL-1 β activation rather than IL-1 β itself. Only lower IL-1RA/IL-6 ratio was predictive of increased risk of acute T2 hippocampal signal abnormality (Table 2) and carried a strong association (OR 21.5, CI 1.17, 393). Given the small sample size of MRIs with abnormal hippocampal T2 the large confidence intervals are expected. Despite this, the odds ratio is very high and remains statistically significant.

Role of IL-1RA/IL-6 ratio as a biomarker of MTLE

Albeit pilot data, the strong association between low IL-1RA/IL-6 ratio and T2 hippocampal signal abnormality carries substantial implications. If T2 hippocampal hyperintensity within 72 hours of FSE ultimately proves to be a biomarker for the eventual development of MTLE, then the IL-1RA/IL-6 ratio may also be a sensitive biomarker as well. To our knowledge the IL-RA/IL-6 ratio has not been assessed as a biomarker of brain injury in FSE previously, but it has been reported as a marker of outcome in patients with TBI. Bartfai et al reported a positive correlation between good clinical outcomes (Glasgow Outcome Scale) and rising ratios of CSF IL-1RA/IL-6 over 7 days after TBI ¹⁰. Similar to our study, absolute values of cytokines varied considerably, and did not correlate with outcome.

If replicated in another study designed specifically to evaluate cytokines, the IL-1RA/IL-6 ratio could represent a serologic biomarker which may offer rapid identification of patients at risk for ultimately developing MTLE, without the need of neuroimaging and the logistic issues that often accompany. Once identified early, patients at risk for MTLE could then be candidates for anti-epileptogenic interventions. Our data support previously raised questions as to whether these cytokine pathways should be considered as targets to potentially abort epileptogenesis. There are a number of potential therapeutic agents currently available which target IL-1 signal transduction system and IL-6 which could be investigated including corticosteroids, anakinra, and tocilizumab.^{40,41}

Limitations

Our study has significant potential limitations. Optimally, studies designed to evaluate cytokines have samples drawn into EDTA tubes, processed (centrifuged at 2000g for 10 min at room temperature and aliquoted), and frozen at -80°C within 90 minutes of

acquisition⁴². Studies have shown that delays in processing can result in significant changes in cytokine levels within a given sample⁴³. Depending upon the cytokine, both increases and decreases may be seen. Cytokine testing was not part of the initial FEBSTAT protocol. As such following acquisition, samples were not handled in a standardized manner. Samples were stored at room temperature for 24–48 hours prior to being processed. Jackman et al performed detailed cytokine analysis to assess the effect of blood sample age on measured endogenous cytokine concentrations in human plasma⁴³. Importantly, IL-8, EGF, IL-1 β , IL-1RA, and TNF- α all elevate over time, whereas IL-6 decreases. These findings, could explain our elevations of IL-8 and EGF, as well as the trend of IL-1 β being higher following FSE. However, we would also expect to see lower IL-6 and higher IL-1RA and TNF- α in the FEBSTAT cohort as the controls were processed within 24 hours of acquisition and would have less anticipated changes to cytokine levels. We reported the opposite findings with IL-6 trending higher and IL-1RA levels trending lower, and TNF- α below the lower limits of quantification for both groups. This, along with our findings being consistent with those seen in animal models, would suggest our results are likely valid. We also do not know the exact timing of specimen acquisition in relation to the FSE. As previously discussed, some cytokine levels could vary (either higher or lower) depending upon the proximity to the FSE¹². However if we assume the majority of samples were likely obtained in the 24–48 hours window following FSE, our findings are consistent with that seen in the animal models.

Conclusion

In conclusion, our pilot data supports involvement of the IL-1 cytokine system, IL-6, and IL-8 in association with FSE in children, as well as a potential role in the development of MTLE. We hypothesize that in some children, the IL-1RA autoregulatory response may be inadequate to suppress the pro-convulsant effects of IL-1 β , IL-6, and IL-8, thus creating the propensity for FSE. This hypothesis is supported by our findings of low anti-inflammatory (IL-1RA) to pro-inflammatory cytokines (IL-1 β , IL-6, and IL-8) ratios in association with FSE. Extreme imbalance of IL-1RA to IL-6 appears to be a possible biomarker for acute hippocampal injury, and may serve as a potential biomarker of eventual development of MTLE. Further studies are needed to further delineate the role IL-1 system and related cytokines in the development of FSE and TLE in humans.

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Key Point Box

- Following FSE there is an imbalance between pro-inflammatory cytokines (IL-1 β , IL-6, and IL-8) and the anti-inflammatory cytokine IL-1RA.
- Elevated systemic inflammation as indicated by a lower IL-1RA/IL-6 ratio is a strong predictor of hippocampal T2 hyperintensity on MRI after FSE.
- IL-1RA/IL-6 ratio is a potential biomarker of acute hippocampal injury following FSE, which may rapidly identify those at substantial risk for developing MTLE.
- The IL-1 system, including those induced by IL-1 β (IL-6, IL-8), may serve as targets for anti-epileptogenesis interventions in the future.

Figure 1.

Plasma cytokine profiles of children within 72 hour of febrile status epilepticus (FSE) and children with fever alone (control). Plasma levels of IL-8, EGF, and IL-2R were all statistically higher in children with FSE in comparison to controls. The ratios of IL-1RA/IL-1 β and IL-1RA/IL-8 were significantly lower in the FSE group. (* = statistically significant, p < 0.0024).

Figure 2.

Relationship between IL-1 β and IL-1RA in the development of febrile status epilepticus (FSE). Patients with FSE (blue diamonds) favored and imbalance between IL-1 β (higher) and IL-1RA (lower) as opposed to control patients with fever only (red boxes). As the balance shifts toward higher IL-1 β and lower IL-1RA (orange) seizure threshold goes down and the likelihood of FSE increases (orange). As the balance shifts towards lower IL-1 β and higher IL-1RA (purple) seizure threshold is higher and the likelihood of FSE decreases.

Figure 3.

Potential role of the IL-1 system in the development of FSE and epilepsy. Precipitating events result in activation of cytokine secreting cells (orange boxes). Neurons are represented by the light blue boxes, and all other cytokine secreting cells (glia in the CNS, peripheral leukocytes, and endothelial cells of the blood brain barrier) by the light green boxes. Activated cells release IL-1 β which induces a robust reciprocal production and release of IL-1RA at significantly higher concentrations of IL-1RA to IL-1 β (A). Normally, IL-1RA competitively inhibits the effects of IL-1 β on the IL-1 receptor 1. As such, signal transduction pathways are minimally activated, ion channels continue to function normally, there is no excessive release of other inflammatory cytokines, and the blood brain barrier remains intact without brain hyperexcitability, and no seizure activity occurs (B and C). In children with abnormal IL-1 systems, activation results in a lower ratio of IL-1RA to IL-1 β (D). This may occur through excessive IL-1 β release, inadequate release of IL-1RA, or inadequate IL-1RA binding to the IL-1R1, all of which may be driven by underlying genetic predisposition. Increased IL-1 β results in upregulation of IL-1 β receptors. The process further perpetuates itself by IL-1 β competing with EGF for binding to the EGF receptor, with resultant increase in free EGF. EGF increases the posttranslational production of IL-1 β . The end result is excessive IL-1 β binding to the IL-1R. In neurons, excessive IL-1 β activation results in neuronal hyperexcitability through 1) activation of Src-mediated phosphorylation of the NR2B subunit of the NMDA receptor resulting in rapid influx of Ca²⁺, 2) augmentation of Ca²⁺ influx through voltage-gated dependent Ca²⁺ channels (VGC C) 3) inhibition of K⁺ efflux via Ca²⁺ dependent K⁺ channels (KCaC) 4) and inhibition of Cl⁻ influx through GABA channels (E). In other cytokine producing cells (including neurons), signal transduction pathways activated by IL-1 β induce gene transcription (F) resulting in production and release of more inflammatory cytokines (IL-6 and IL-8) from cells within the CNS (may occur independent of the presence of systemic

inflammation) or if in the periphery cross over the BBB at the circumventricular organs, as well as contribute to blood brain barrier breakdown themselves. These cytokines also induce intracellular signal transduction pathways regulating gene transcription. This perpetuates the inflammatory cascade within the CNS and along with their impact on ion channels, contributes to the cycle of increased excitability, lower seizure threshold, and ultimately development of status epilepticus. Long term, gene transcription produces cellular changes, which along with chronic inflammation, leads to the eventual development epilepsy.³⁹

Table 1

Plasma cytokines of children obtained within 72 hours febrile status epilepticus versus children with fever alone.

Cytokine	FSE (N=33)		Controls (N=17)		P-value
	Mean (pg/mL)	Std Dev	Mean (pg/mL)	Std Dev	
VEGF	4.1	5.3	24.3	14.4	< .0001*
IL-1 β	270.8	1171.7	25.2	29.2	0.0633
G-CSF	69.0	172.4	480.5	662.7	0.0004*
EGF	98.1	68.9	28.3	67.5	< .0001*
HGF	398.9	361.5	326.7	246.1	0.7755
FGF	31.0	24.3	32.6	18.8	0.3612
IFN- α	147.7	136.0	71.9	25.3	0.0261
IL-6	476.7	1345.8	106.1	154.1	0.2918
IL-12	354.9	194.9	348.6	234.3	0.6989
Rantes	1994.6	162.6	5393.2	10430.8	0.896
Eotaxin	87.5	72.8	43.4	35.8	0.0116
IL-15	153.6	176.5	94.8	130.7	0.0734
MIP-1 α	181.1	312.2	47.0	46.6	0.0193
MIP-1 β	522.6	1104.3	143.6	132.7	0.0348
MCP-1	1398.3	2269.2	1036.4	983.7	0.4895
IL-1RA	710.1	870.0	1257.2	927.4	0.0031
IL-7	23.7	18.0	34.5	47.9	0.4017
IP10	80.2	69.9	291.5	513.9	0.0046
IL-2R	690.8	449.5	1522.5	566.4	< .0001*
MIG	40.9	36.4	106.4	164.4	0.0143
IL-8	3332.5	4234.6	80.5	82.7	0.0012*
Cytokine Ratios					
IL-1RA/IL-1β	30.2	25.4	119.9	107.2	0.004*
IL-1RA/IL-6	23.1	38.7	128.9	166.0	0.06
IL-1RA/IL-8	4.5	8.6	43.5	50.7	< 0.001*

The following cytokines did not have plasma cytokine values above the LLQ for either the FSE or controls group: IL-2, IL-4, IL-5, IL-10, IL-13, IL-17, TNF- α , IFN- γ , GM-CSF

Non-parametric Wilcoxon Test was performed to calculate p-values. Bonferroni was performed for multiple comparison adjustment ($\alpha=0.05/21=0.0024$), any comparisons with $p \leq \alpha$ were claimed as significant (*). For the ratios, critical p value <0.05 was considered significant. FSE, febrile status epilepticus; LLQ, lower limits of quantification

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Table 2

Plasma cytokine ratios in association with FSE and as predictors of increased hippocampal T2 after FSE.

Cytokine ratios	Odds Ratio (95% Confidence Interval) for FSE ¹
IL-1RA/IL-1 β *	1.27 (1.08, 1.50) *
IL-1RA/IL-6 *	1.14 (1.02, 1.26) *
IL-1RA/IL-8 *	2.77 (1.35, 5.70) *
Cytokine ratios	Odds Ratio (95% Confidence Interval) for Increased Hippocampal T2 Signal in MRI 72 hours after FSE ¹
IL-1RA/IL-1 β	1.51 (0.83, 2.74)
IL-1RA/IL-6 *	21.5 (1.17, 393) *
IL-1RA/IL-8	Not Applicable

* Statistically significant

- No absolute values of individual cytokines were associated with FSE or increased hippocampal T2 signal in FSE
- Cytokines obtained within 72 hours of FSE
- ¹Cytokine ratio was categorized in 10 unit intervals

Plasma cytokines of children obtained within 72 hours of FSE in children with and without hippocampal T2 hyperintensity.

Table 3

Hippocampal T2 abn (N=5)		No Hippocampal T2 abn (N=27)	
Cytokine	Mean (pg/mL)	Std Dev	P-value
IL-1 β	1406.86	2986.86	0.14
IL-1RA	615.65	449.39	0.88
IL-6	1447.83	2320.22	0.02 *
IL-8	7575.98	4099.27	0.04 *
MCP-1	3173.86	3851.39	0.36
MIP-1 α	305.18	351.12	0.38
MIP-1 β	757.03	1272.71	0.50
Cytokine Ratios			
IL-1RA/IL-1 β	15.44	25.40	0.06
IL-1RA/IL-6	2.45	3.38	0.01 *
IL-1RA/IL-8	0.34	0.60	0.02 *

Non-parametric Wilcoxon Test was performed to calculate p-values. Critical p value <0.05 was considered significant.

* Statistically significant values.

FSE, febrile status epilepticus;