Inflammatory Processes, Febrile Seizures, and Subsequent Epileptogenesis

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Febrile seizures (FS) are the most common type of seizures in infants and preschool children. Inflammatory mediators, which are known triggers of fever, have also been implicated as contributors to the onset of these seizures. Evidence that inflammation is present following FS and during established epilepsy suggests that it could also influence epileptogenesis. However, the potential involvement of inflammatory mediators to the epileptogenic process that may follow prolonged FS has yet to be fully determined. This article reviews the current state of our knowledge and major gaps that remain by focusing on four questions: Does inflammation contribute to the generation of FS? Does prolonged FS or febrile status epilepticus (SE) cause temporal lobe epilepsy in the absence of predisposing factors? Does inflammation contribute to the process by which febrile SE causes limbic epilepsy? And finally, can inflammation be a foundation for biomarkers and therapy for FS-induced epileptogenesis?

Febrile seizures (FS) are the most common type of convulsions in infants and young children, with a worldwide incidence of one in 20–50. They are clinically defined as seizures that arise during fever in the absence of central nervous system infection (1, 2), although it is not well understood how fever generates FS. There is little evidence for adverse outcomes of simple FS on the immature brain. However, complex FS—particularly prolonged FS or febrile status epilepticus (SE; defined as seizures lasting more than 30 minutes)—has been associated with subsequent limbic epilepsy in both prospective and retrospective clinical studies (1, 3, 4). Still, it is difficult to determine whether this relationship between prolonged FS and epilepsy is causal, in part, because in these studies the definition of FS does not exclude children with preexisting neurologic deficits (1–7). Our understanding of the basic mechanisms of FS, especially of the role of inflammatory mediators, has benefited from work on animal models (8–14). These have enabled the direct examination of the consequences of FS, the mechanisms by which these seizures are generated, and whether the seizures themselves cause epilepsy.

The following paragraphs describe clinical and animal studies that investigate the role of inflammation in fever, how inflammation leads to FS, and how it may contribute to the subsequent development of epilepsy (Figure 1).

Does Inflammation Contribute to the Generation of FS?

Fever increases brain temperature, at least in part, via the release of inflammatory mediators such as cytokines (15, 16) in the brain. Less is known, however, about whether inflammatory mediators induce seizures. Clinical studies have attempted to address this question by comparing levels of the cytokines interleukin-1 β (IL-1 β), interleukin-6

FIGURE 1. The possible role of inflammatory mediators in the progression from fever to FS to epileptogenesis. Inflammatory mediators such as IL-1 and TNF are potent pyrogens, and there is evidence for their involvement in seizure generation. In addition, they are present following FS, raising the possibility that they may influence epileptogenesis. However, the precise role of inflammatory mediators remains to be clarified.

TABLE 1. Clinical Studies Comparing the CSF and/or Blood Levels of Cytokines in Children Who Had FS With Those Who Had Fever Only

Increased Cytokine Detected in FS Compared With Fever		n (FS, fever)	Cytokines Measured	Study
CSF	Blood			
ND	ND	10, 10	IL-1 β	Lahat et al. 1997 (17)
TNF- α	IL-1 β	15, 20	IL-1 β , TNF- α	Tutuncuoglu et al. 2001 (18)
	ND	11, 9	IL-1 β	Miceli Sopo et al. 2001 (19)
IL-1 β	ND	29, 15	IL-1 β , TNF- α	Haspolat et al. 2002 (20)
IL-ra, IL-6, IL-10	IL-ra/IL-1 β	55, 20	IL-1 β , IL-ra, IL-6, IL-10, TNF- α	Virta et al. 2002 (21)
n/a	ND	33, 38	IL-1 β	Tomoum et al. 2007 (22)
ND	n/a	16, 8	IL-1 β , IL-2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IFN- γ , TNF- α , G-CSF, GM-CSF	Asano et al. 2010 (23)

Abbreviations: CSF, colony-stimulating factor; FS, febrile seizures; *n*, number of patients; ND, no difference; IL, interleukin; TNF, tumor necrosis factor; n/a, not applicable; IFN, interferon; G-CSF, granulocyte–colony-stimulating factor; GM-CSF, granulocyte macrophage–colony-stimulating factor.

(IL-6), and tumor necrosis factor alpha (TNF- α) in the CSF and/or blood of febrile children with and without seizures, but their results have been contradictory (Table 1) (17–23). However, even if these studies did show increased cytokine levels associated with seizure, they would not have been able to indicate causality. Animal studies have helped to determine the nature of this interaction. In one study, mice lacking IL-1 β receptor type 1 were shown to be significantly more resistant to the generation of FS, strongly suggesting the involvement of IL-1 β in the seizure generation (Figure 2) (24). A potential role of IL-1 β in FS was also shown in postnatal day-14 rat pups injected with low-dose kainic acid (KA) and lipopolysaccharide (LPS) to model FS. In this model, administration of the cytokine into the lateral ventricles enhanced seizure frequency, whereas an antagonist for IL-1 β receptor type I (IL-1ra) reduced or abolished the seizures (11). These findings are not surprising as there is evidence for the active role of inflammatory mediators including IL-1 β , TNF, IL-6, prostaglandin E2, and the complement cascade in the generation and exacerbation of nonfebrile seizures (25). IL-1 β promotes neuronal hyperexcitability via several mechanisms, including by activating Src-family tyrosine kinases, which increase intracellular calcium flow through glutamate receptors (26, 27). IL-1 β has also been shown to interact with other convulsants to exacerbate seizures (28, 29).

Clinical studies suggest that fever generated by certain infections, particularly human herpes virus 6, might increase the risk of FS (30, 31). However, it is unknown whether this virus induces higher levels of cytokines in the brain than do other pathogens. In addition, genetic analyses of individuals with FS

have identified a mutation in the IL-1 β gene promoter (32) that leads to increased expression of this cytokine (for an opposing view, see Tan et al. [33]). Taken together, this body of clinical and experimental work suggests that inflammation, which is intrinsic to the fever response, is involved in the generation of febrile seizures.

FIGURE 2. Mice lacking the interleukin-1 receptor (IL-1 R1^{-/-}) are more resistant to the induction of experimental febrile seizures than wild-type controls. A reliable measure of seizure susceptibility in the experimental FS model is the temperature at which the seizures begin. Seizures began in IL-1 R1^{-/-} animals at significantly higher temperatures than in control animals, indicating that the IL-1 R1 is important for the generation of these seizures. The asterisk indicates a statistical difference between the controls and IL-1 R1^{-/-} mice of $p < 0.05$. Data are given as mean $\pm$ standard error. Adapted with permission from Dubé et al. (24).

Do Prolonged FS or Febrile SE Cause Temporal Lobe Epilepsy in Non-Predisposed Children?

The majority of clinical studies suggest that there is little enduring adverse impact of short FS on the developing brain (4, 7, 34, 35). By contrast, prolonged or focal FS has been associated with a significantly increased risk for temporal lobe epilepsy (TLE; 7, 36–38). This statistical correlation could be a result of one of four potential scenarios: 1) prolonged FS or febrile SE might directly cause temporal lobe epilepsy; 2) the seizures could be merely a marker of emerging TLE resulting from preexisting pathology; 3) they could be the result of a common cause that independently provokes FS and TLE; or 4) TLE could be induced by the combination of FS and a preexisting condition.

As mentioned above, because of the diverse genetic makeup of children and the presence of other uncontrolled potential preexisting factors, it is difficult to demonstrate a causal relationship between prolonged FS and the development of TLE in clinical studies. While single gene mutations or a combination of multiple susceptibility genes might predispose some children to FS or to the emergence of epilepsy following FS (39–42), a child's prenatal, perinatal, or postnatal experiences may also contribute to an increased susceptibility to FS or to FS-related epilepsy. Controlling for all of these genetic and environmental factors is a challenging proposition.

Here again, animal models of prolonged FS (11–14, 43–48) have proven useful in advancing our understanding of the effects of these seizures (9, 12, 49–53). Because fever cannot be reliably induced in neonatal and infant rodents (54), several groups have employed several means to simulate fever-like conditions. By elevating core and brain temperatures to between 39.5°C and 41.0°C (hyperthermia), seizures can be elicited in 10- to 11-day-old rats (13, 45). At this age, rodents have reached a stage of hippocampal development similar to that in human infants (55). In this animal model, like in those based on combining LPS with KA, the augmented temperature leads to the release of fever-involved cytokines that contribute to seizure initiation (11, 24).

In their model of prolonged FS in rats, Dubé et al. found that FS of about 20 minutes resulted in spontaneous recurrent seizures in 35% of the rats (51). No such seizures were observed either in normothermic control rats or in those exposed to hyperthermia alone (hyperthermic control). The resulting spontaneous seizures were short and included freezing and orofacial (“limbic”) automatisms (51). Associated EEGs also revealed poly-spike/sharp-wave trains with increased amplitude and decreased frequency. All epileptic animals developed interictal epileptiform discharges; furthermore, such discharges were found in 88% of the FS rats but were never observed in normothermic or hyperthermic controls (51). Because both genetic and acquired predisposing factors are largely excluded in this model, this study directly supports a causal relationship between prolonged FS and the development of TLE, and sets the stage for investigation of the mechanisms by which these seizures promote epileptogenesis.

In clinical studies, about 15% of FS cases are prolonged or progress on to febrile SE (1, 56–58). Of importance, it is these longer seizures that are statistically associated with subsequent TLE. Dubé et al. investigated whether the duration of

experimental FS influenced the incidence of limbic epilepsy by comparing outcomes of experimental FS durations of 20 minutes with those of 60 minutes (13). They found that the former resulted in limbic epilepsy in 35% of their FS rats, whereas the latter yielded spontaneous seizures in 45% of the rats (13). Of importance, while the epileptic seizures resulting from the 20-minute FS were mild (stage 1, Racine 1972; average duration approximately 8 seconds), the spontaneous seizures in rats that experienced “febrile SE” (60-minute FS) lasted more than 2 minutes on average and were more severe (stages 2–5 with head bobbing, alternating or bilateral clonus, rearing, and falling; 13). These experimental data support the hypothesis that the duration of FS is an important predictor of the severity of the TLE that develops after FS (59–62).

Does Inflammation Contribute to the Process by Which Febrile SE Causes Epilepsy?

Epilepsy, including the catastrophic epilepsy associated with Rasmussen encephalitis, can result from inflammatory brain processes (25). In addition, resected hippocampal material and cortex from patients with intractable TLE often harbor reactive astrocytes, activated microglia, proinflammatory cytokines, and other measures of inflammation (63–66). Many studies using animal models have focused on uncovering how inflammation is generated in the brain in the context of epilepsy, and how it might cause or modulate epilepsy. It is now clear that seizures, including those that provoke epileptogenesis, can generate several waves of inflammation (see Vezzani et al. [25] for review). There is also significant evidence that these inflammatory cells and molecules can contribute to neuronal hyperexcitability and seizures (11, 24). However, establishing a direct causal role for inflammation in the epileptogenic process would require additional findings showing that modulation of inflammatory mediator function affects epileptogenesis.

If inflammation is involved in seizure-induced epileptogenesis, then what are the mechanisms that underlie this transition to epilepsy? A number of studies suggest that seizure-induced or injury-related inflammation might contribute to loss of neurons and to synaptic reorganization, which are important factors for the development of hyperexcitable circuits after insults to the adult brain (67–69, for an opposing view see Buckmaster and Lew [70]). Inflammation is rapidly induced following such insults preceding neurodegeneration (71–73), supporting the notion that inflammation promotes cell death (74). However, there is little evidence for cell death after prolonged experimental FS, febrile SE (8, 13, 45, 51), and other developmental seizures that may promote epilepsy (see Baram et al. [75] for review). Similarly, activation of astrocytes and microglia and production of cytokines and prostaglandins are found in some adult rat seizure models where cell loss is not detected (21, 22, 76–78). These findings exclude the possibility that the inflammation is merely a by-product of dying cells. They also support the idea that inflammation contributes to epileptogenesis via mechanisms that are independent of cell death (“damage”).

Indeed, existing and recent experiments have uncovered a number of mechanisms for inflammation-mediated epilepsy that may be important after prolonged FS and febrile SE. These constitute potential molecular targets for pharmacologic

FIGURE 3. Long FS promoted prominent activation of astrocytes and mild activation of microglia within 24 hours after the seizures. Left panels, astrogliosis denoted by the hypertrophic cell body and long and thick processes of the astrocytes was evoked 6 and 24 hours after the seizures (**C–F** and their insets) compared with the control (**A, B**, and their insets) in CA3 hippocampal area (**C, E**, versus **A**) and in the hilus of the dentate gyrus (**D, F**, versus **B**). Middle panels, OX-42 immunoreactivity was detected in resting microglia with small cell bodies and ramifications in control rats (**A', B'**, and their insets). Scattered, mildly activated microglial cells (moderate hypertrophic processes) were evident at 6 hours (**C', D'**, and their insets) and 24 hours (**E', F'**, and their insets) after the onset of FS. Right panels, IL-1 β immunoreactivity was undetectable in control rats (**A'', B''**) and was robust in glial cells at 6 hours (**C'', D''**) and 24 hours (**E'', F''**) after seizures. Double immunostaining (yellow in insets) demonstrated that IL-1 β (green) protein expression was localized to astrocytes (GFAP; red). Scale bars: **A–F, A'–F', B', D', F'**, 25 μ m; **A'', C'', E''**, 50 μ m; **A–F, A'–F'** insets, 12 μ m; **C'–F'** insets, 10 μ m. Adapted with permission from Dubé et al. (13).

drug design. For example, one novel inflammatory mediator that is released from injured cells is high mobility group box-1 (HMGB1), which activates the Toll-like receptor 4 (TLR4). Mice lacking TLR4 were resistant to seizures, and antagonists of either TLR4 or of HMGB1 reduced chronic seizures (79). Of interest, expression of both TLR4 and HMGB1 was enhanced in tissue from patients with TLE and from mouse epilepsy models (79). Perhaps the initial neuronal injury provoked by febrile SE (45) causes an HMGB1 release, which in turn leads to constitutive upregulation and activation of TLR4. This suggests that TLR4 could be a potential target for pharmacologic intervention of epileptogenesis. In addition, the potential etiologic role of human herpes virus 6B (HHV6B), implicated in a significant proportion of FS patients, has been raised (80). Active HHV6B has been found in hippocampal astrocytes of temporal lobectomy specimens from about two-thirds of patients with mesial temporal sclerosis-associated TLE (81). If a relationship between this viral infection and FS and epileptogenesis can be established, antiviral therapies could be developed targeting HHV6B.

Which Inflammatory Mediators Might Contribute to FS-Induced Epilepsy?

IL-1 β and TNF- α are both potent pyrogens and are released under certain circumstances in developing and adult brains (82), influencing neuronal excitability both acutely and chronically.

A potential role for IL-1 β in the epileptogenesis that follows prolonged FS and febrile SE has recently been identified (13). As mentioned above, endogenous IL-1 β is released in the hippocampus during fever as well as during experimental FS and contributes to seizure generation (24). In addition, these

seizures also increase the synthesis of IL-1 β so that the levels of the cytokine remain elevated for 24 to 48 hours (Figure 3). Of interest, IL-1 β levels were significantly higher in hippocampi of rats that became epileptic, as compared with those of rats that experienced febrile SE but did not develop epilepsy (13); however, it is unclear whether this is a cause or a consequence of the spontaneous recurrent seizures. Taken together with evidence that systemic administration of LPS, which leads to release of endogenous IL-1 β (11), enhances epileptogenesis of pilocarpine-induced SE in P14 rats and after rapid kindling (83), these findings support the hypothesis that IL-1 β is involved in the epileptogenic process (13). The mechanisms underlying this process still need to be fully determined, but protection from cell death does not seem to be an important factor (13). Studies that block IL-1 β production and receptor signaling after the inciting FS would be required to resolve a causal epileptogenic role for IL-1 β in FS-induced epilepsy.

TNF- α might also be released in the developing brain during fever-related seizures (18). Although direct evidence for its role in FS and FS-related epileptogenesis is still lacking, its ability to alter neuronal excitability suggests that it would be a good candidate for further investigation. Notably, in a neonatal rat with pneumococcal meningitis, suppressing the levels of TNF- α significantly reduced the incidence of spontaneous seizures without affecting the CSF levels of bacteria (84). Further evidence for a proconvulsive effect of TNF- α comes from work in an adult mouse model of systemic infection (*Shigella dysenteriae*) that revealed an increased sensitivity to pentyleneetetrazol-induced seizures due to systemic TNF- α levels (85). However, when the levels of TNF- α were increased, an anticonvulsant effect was found (86). Such a bidirectional

effect may be attributed to the affinity of TNF- α for its receptors, the high-affinity proconvulsant p55 and the low-affinity anticonvulsant p75 (87, 88). TNF- α has been found to increase surface expression of AMPA receptors and decreasing GABA receptors (89), thus leading to long-term changes in neuronal excitability (90). These data suggest that this cytokine could contribute to both seizures and the development of epilepsy.

Can Inflammation Be a Foundation for Biomarkers and Therapy for FS-Induced Epileptogenesis?

Because only a minority of children sustaining FS and febrile SE eventually develop TLE, identifying this at-risk population is crucial for prevention and intervention strategies. CSF sampling for the presence of inflammatory mediators has not proven helpful to date, and new approaches are required. MRI within the first few days after FS has identified hippocampal abnormalities in a subset of such children (59, 61, 91, 92), as well as in animal models (93, 94), raising the possibility that these augmented hippocampal T_2 signals may be a marker of epileptogenesis. The origin of T_2 signal changes is unclear, but such changes often indicate increased tissue water content, as found with inflammation. Nevertheless, whether these acute MRI changes can predict epileptogenesis in rodent models or children and with sufficient sensitivity and selectivity remains unresolved.

Longitudinal studies using techniques that directly scrutinize key components of inflammation may establish a correlation between early inflammation following FS and later epilepsy and determine its potential as a marker of epileptogenesis. Molecular MRI contrast agents (95, 96) and PET markers (for review see Banati 2002 [97]) for inflammation have been developed; however, studies that use these to investigate seizure outcome have yet to be conducted. Another potential marker is the increased permeability of the blood brain barrier that has been associated with seizures, a mechanism through which inflammatory mediators can increase brain excitability (98). Studies to evaluate the integrity of the blood brain barrier in FS models are ongoing.

If the mechanisms by which inflammation might contribute to the development of TLE following febrile SE could be understood, then the “molecular signature” of these particular mechanisms might provide a diagnostic tool. In addition, blocking selective inflammatory mediators (e.g., the production and/or receptor binding of IL-1 β) might be useful in ameliorating the pro-epileptogenic consequences of febrile SE in at-risk populations. It is clear that uncovering the role of inflammation not only in the FS themselves but also in their aftermath is a challenge of major clinical significance.

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