"*For Whom the Bell Tolls*": Blockade of Toll-Like Receptors May Regulate Seizure Occurrence

Toll-Like Receptor 4 and High-Mobility Group Box-1 Are Involved in Ictogenesis and Can Be Targeted to Reduce Seizures. Maroso M, Balosso S, Ravizza T, Liu J, Aronica E, Iyer AM, Rossetti C, Molteni M, Casalgrandi M, Manfredi AA, Bianchi ME, Vezzani A. *Nat Med* 2010;16(4):413–419. Brain inflammation is a major factor in epilepsy, but the impact of specific inflammatory mediators on neuronal excitability is incompletely understood. Using models of acute and chronic seizures in C57BL/6 mice, we discovered a proconvulsant pathway involving high-mobility group box-1 (HMGB1) release from neurons and glia and its interaction with Toll-like receptor 4 (TLR4), a key receptor of innate immunity. Antagonists of HMGB1 and TLR4 retard seizure precipitation and decrease acute and chronic seizure recurrence. TLR4-defective C3H/HeJ mice are resistant to kainate-induced seizures. The proconvulsant effects of HMGB1, like those of interleukin-1β (IL-1β), are partly mediated by ifenprodil-sensitive *N*-methyl-D-aspartate (NMDA) receptors. Increased expression of HMGB1 and TLR4 in human epileptogenic tissue, like that observed in the mouse model of chronic seizures, suggests a role for the HMGB1-TLR4 axis in human epilepsy. Thus, HMGB1-TLR4 signaling may contribute to generating and perpetuating seizures in humans and might be targeted to attain anticonvulsant effects in epilepsies that are currently resistant to drugs.

COMMENTARY

Inflammation plays a prominent role in the etiology of symptomatic epilepsies that result from brain injuries, including those from stroke and status epilepticus. An emerging literature suggests inflammation and immune processes contribute to both drug-resistant temporal lobe epilepsy and epilepsies associated with cortical malformations. The expression of inflammatory mediators from glia and neurons in epileptic tissue indicates that the contribution to the pathology may be essential to the ictogenic or epileptogenic process, rather than merely epiphenomena. Insight into the key role of cytokines in epilepsies, especially that of interleukin-1β (IL-1β), has occurred via novel molecular and pharmacological studies, using in vivo models and genetically engineered mice with altered cytokine signaling (1).

The chronic upregulation of IL-1β expression during epileptogenesis in activated astrocytes suggests a predominant role for astrocytes in sustaining activation of inflammatory cascades before the appearance of spontaneous seizures (1). IL-1β triggers a signaling pathway involving the IL-1 receptor 1, IL-1 receptor accessory protein complex, and myeloid differentiation primary response protein (MyD88) complex. The activation of the adaptor protein MyD88 by IL-1β simulates the Src family kinases, leading to NMDA receptor-2B phosphorylation and subsequent enhancement of NMDA-dependent Ca^{+2} influx. The NMDA-dependent Ca^{+2} influx then facilitates ictogenesis (2). IL-1β signaling and this intracellular cascade may alter the excitability of a neural network without cell loss, per se. In a study by Dubé and colleagues, hippocampal IL-1β levels are elevated chronically in activated astrocytes in rats with spontaneous seizures after febrile status epilepticus at postnatal day 11 (3). The hippocampal IL-1β levels and spontaneous seizures were not dependent on cell loss, T2 MRI abnormalities in the hippocampus, or interictal EEG activity. Experimental febrile seizure duration in these rats influenced both the probability of developing limbic epilepsy and the severity and duration of the spontaneous seizures. However, the relationship between hippocampal IL-1β levels and the duration of initial neural activity (i.e., the duration of febrile status epilepticus) remains to be defined.

The pathways activated by IL-1β depend on the intracellular protein MyD88, which is central to signaling of other cell surface receptors, notably the Toll-like receptor 4 (TLR4) during pathogen recognition. However, in the absence of pathogen recognition, TLR-signaling pathways recognize molecules released from injured tissue, named damage-associated molecular patterns/proteins (DAMPs). High-mobility group box-1 (HMGB1) is a DAMP component of chromatin that is passively released from necrotic cells and actively secreted by cells in profound distress. In the Maroso et al. study, presented here, bicuculline and kainic acid models of epileptogenesis were used to understand the nature of HMGB1–TLR4 interactions, its intracellular signaling, and the role of HMGB1–TLR4 signaling in ictogenesis and seizure recurrence.

Using rats and human autopsy tissue, Maroso and colleagues observed increased TLR4 and HMGB1 expression in neurons, astrocytes, and microglia in the rat hippocampus (after intrahippocampal injections of kainic acid or bicuculline) as well as in human hippocampus from intractable temporal lobe epilepsy patients. The addition of recombinant HMGB1 to hippocampi treated with kainic acid had a proconvulsant effect, which was abolished in mice defective in TLR4 signaling.

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These data suggest that HMGB1–TLR4 signaling is intimately involved in seizure generation (i.e., ictogenesis). However, both the origin of HMGB1 release and the triggers for HMGB1 or TLR4 expression in glia or neurons during seizures in these models are far from clear. Maroso et al. demonstrated that 250 μM of glutamate in mixed neuronal/glia cultures caused excitotoxic cell death and subsequent release of HMGB1 protein into the extracellular medium. The authors could not detect HMGB1 synthesis or release from cultures of rat microglia or astrocytes after incubation with kainic acid, glutamate, or inflammatory mediators, such as tumor necrosis factor-α, ATP, IL-1β, or any combination of these three factors. However, ictal activity itself, hypoxia, and interactions with neurons may determine the rate of synthesis and release of HMGB1 from rat glial populations. In fact, HMGB1–TLR4 antagonists reduced the number of seizures, seizure duration, and increased latency to seizure onset in the bicuculline-induced nonlesional model of seizures. Thus, these data suggest that ictal activity in neurons is sufficient to facilitate HMGB1 synthesis and then subsequent release outside the cell.

One of the most compelling yet puzzling aspects of this study is the extent to which the HMGB1–TLR4 signaling pathway influences chronic epilepsy. Maroso et al. demonstrated that HMGB1 and TLR4 receptor antagonists were effective in blocking both acute seizures triggered by kainic acid and bicuculline, and in reducing seizure recurrence in chronic epileptic C57BL/6 mice. The downstream signaling events, denoted by presumed activation of Src protein kinases, phosphorylation of NR2B, and increased NMDA-mediated Ca^{+2} influx, can be blocked by ifenprodil, a sensitive blocker of NR2B-containing NMDA receptors. Ifenprodil blocks the proconvulsant effects of HMGB1 in the kainic acid model of acute seizures and decreases seizure recurrence in chronically epileptic C57BL/6 mice. However, ifenprodil fails to block acute seizures from kainic acid in the absence of the proconvulsant HMGB1 protein, suggesting inflammatory processes are necessary for the antiseizure effect of ifenprodil. An alternative hypothesis here might be that IL-1β signaling mediates some or all of the HMGB1 effects on ictogenesis and seizure recurrence. To evaluate the role of IL-1β in the HMGB1–TLR4 signaling, the most straightforward approach is to compare intraperitoneal injection to intrahippocampal injections of kainic acid in C57BL/6, mice, as the first model (i.e., with intraperitoneal injections) does not result in spontaneous seizures (4). The data in Maroso et al. do not provide evidence for continuous HMGB1 synthesis and activity, nor does it distinguish the role of IL-1β pathway in these processes. However, given the proposed role of proinflammatory cytokines in astroctye synapses and neurodevelopmental disorders accompanied by epilepsy, including Fragile X and autism, developmental programmed cell death or neural activity-induced cell stress from any process may stimulate the HMGB1–TLR4 axis, promoting aberrant synaptic connectivity, neuronal excitability, and epileptogenesis (5–7).

Changes in glial and neuronal networks, cell death, and hyperexcitability all contribute to epileptogenesis. The contribution of HMGB1–TLR4 signaling to epileptogenesis or ictogenesis remains to be solved. HMGB1–TLR4 signaling may use only parts of the IL-1β pathways and thus, be a completely separate pathophysiology. Alternatively, HMGB1 may completely control the IL-1β pathway through modifications of intracellular signaling components or primary regulation of IL-1β, itself. The lack of cell death as a requirement and the presence of common signaling molecules for the proepileptogenic actions of IL-1β and HMGB1 suggest that pharmacologic interventions along this pathway may prove effective for current drug-resistant epilepsies, especially intractable pediatric epilepsies.

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