Microglial TLR9: Guardians of Homeostatic Hippocampal Neurogenesis

TLR9 Signalling in Microglia Attenuates Seizure-Induced Aberrant Neurogenesis in the Adult Hippocampus.

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Pathological conditions such as epilepsy cause misregulation of adult neural stem/progenitor populations in the adult hippocampus in mice, and the resulting abnormal neurogenesis leads to impairment in learning and memory. However, how animals cope with abnormal neurogenesis remains unknown. Here we show that microglia in the mouse hippocampus attenuate convulsive seizure-mediated aberrant neurogenesis through the activation of Toll-like receptor 9 (TLR9), an innate immune sensor known to recognize microbial DNA and trigger inflammatory responses. We found that microglia sense self-DNA from degenerating neurons following seizure, and secrete tumour necrosis factor-α, resulting in attenuation of aberrant neurogenesis. Furthermore, TLR9 deficiency exacerbated seizure-induced cognitive decline and recurrent seizure severity. Our findings thus suggest the existence of bidirectional communication between the innate immune and nervous systems for the maintenance of adult brain integrity.

Commentary

Epileptogenesis is a multifaceted process in which various cellular components contribute to the development of epilepsy. Among them, aberrant hippocampal neurogenesis induced by acute seizures is thought to be among the crucial players in the generation of spontaneous recurrent seizures and memory impairment (1, 2). However, the cellular and molecular mechanisms that regulate seizure-induced aberrant neurogenesis in the hippocampus remain largely unknown. In this study, Matsuda et al. elegantly showed that microglial activation after kainic acid (KA)-induced seizures could affect chronic seizure susceptibility and memory function by modulating the proliferation of hippocampal neural progenitors. With genetic and pharmacological manipulation of microglial activation, in addition to a conditioned medium-based cell culture system, the authors demonstrated microglial mechanisms controlling seizure-induced aberrant hippocampal neurogenesis. In their study, self-DNAs—possibly derived from nearby dead or degenerating neurons—could stimulate microglial Toll-like receptor 9 (TLR9), which triggered TNF-α production via the NF-κB signaling pathway, resulting in the reduced proliferation of neural progenitor cells in the epileptic dentate gyrus.

Microglia (resident immune cells in the brain) have long been considered to play deleterious roles in epileptogenesis (3). Activated by various brain insults, microglia produce and secrete proinflammatory cytokines such as interleukin-1 (IL-1) and TNF-α. This cytokine surge turns on downstream signaling cascades, leading to histopathological outcomes and functional impairment. However, other studies have suggested that microglia have positive roles in epilepsy by secreting anti-inflammatory cytokines, including IL-4, IL-10, and TGF-β. The paper discussed here supports the beneficial side of microglia. For example, both inhibition of microglial activation using minocycline or suppression of TNF-α by thalidomide aggravated seizure-induced enhanced proliferation in the dentate gyrus. Interestingly, considering that minocycline is an inhibitor of proinflammatory cytokine-producing microglia, the authors suggest a novel mechanism of proinflammatory microglia affecting epileptogenesis and a new positive effect of microglia in epilepsy. Since microglia can display pro- and anti-inflammatory functional states—depending on the different combinations of inducing factors, which largely fluctuates during the course of epileptogenesis—it will be interesting to further investigate the temporal pattern of alternating microglial states and their specific roles in aberrant hippocampal neurogenesis.

TLRs are pattern-recognition receptors that respond to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). Ever since the first human TLR was identified (4), many additional TLRs have been extensively studied in the brain. In adult hippocampus, TLR4 deficiency was shown to induce the proliferation of hippocampal progenitor cells (5). Moreover, TLRs are involved in cell fate choice. For example, TLR2 deletion promotes astrocyte differentiation at the expense of neurogenesis, while TLR4 deficiency promotes neuronal differentiation without affecting astrocyte differentiation (5). As expected, TLRs can also affect the survival of newborn granule cells, given that TLR3-deficient mice showed an increased number of adult-generated mature neurons (6). As for TLR9, the authors found no difference in the

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proliferation of hippocampal progenitors by microglial TLR9 knockout under physiological conditions. However, after acute seizures, the authors' careful assessments unveiled that TLR9 specifically influenced progenitor proliferation without affecting neuronal differentiation or the survival of newborn neurons when normalized to the rate of progenitor proliferation. In regard to the roles of TLRs in epilepsy, very little information is available except for TLR4, in which inhibition led to the reduction of acute and chronic seizures (7). Moreover, in a follow-up study by the same group, doublecortin (DCX) immunoreactivity was examined by TLR4 deletion using a model of intrahippocampal KA injection, but the authors could not draw a conclusion due to lack of DCX-positive cells in wild-type animals. Thus, it is noteworthy that this is the first report showing that TLRs can modulate seizure-induced aberrant neurogenesis.

Constant surveillance of extracellular or endogenous molecular patterns by TLRs is critical for maintaining tissue homeostasis. To achieve a wide range of defense mechanisms, each TLR is assigned to recognize specific ligands. For example, TLR7 can identify single stranded RNAs, while TLR9 senses unmethylated CpG sequences in microbial and self-DNA. Interestingly, the authors observed that (unlike TLR9 deletion) TLR7 knockout did not influence seizure-induced hippocampal neurogenesis. They further confirmed that depletion of self-RNAs using RNase-treated conditioned media was not able to reduce microglial KA-induced TNF-α expression, excluding self-RNAs from the microglial mechanism of controlling aberrant neurogenesis. One possible reason is that RNAs can be used to regulate neuron-glia communication considering the fact that exosomes, a secretory vesicle, contain mRNAs and miRNAs. On the contrary, DNAs are often released into the extracellular space only when cells are damaged, meaning an emergency situation. Since enhanced proliferative activities of neural stem cells is one of the common seizure-induced environmental changes, microglial TLR9-mediated sensing of self-DNAs—but not self-RNAs via TLR7—may initiate compensatory mechanisms to recover hippocampal homeostasis. Additionally, although the authors suggested the source of self-DNAs would be degenerating hippocampal neurons after seizures, it could be other cell types including amplifying progenitors in the subgranular zone of the dentate gyrus based on the observation that the majority of newborn cells die within 1 to 4 days after their birth (8) and their close location to microglia, as demonstrated in this paper.

To investigate the functional roles of microglial TLR9 in epilepsy, the authors focused on two of the most pervasive issues in patients with epilepsy: recurrent seizures and cognitive deficits. Interestingly, in a repeated KA injection model of seizures, TLR9 knock-out mice developed more severe seizures when compared to wild-type mice. Similarly, minocycline treatment of wild-type mice increased the severity of KA-induced seizures. Learning and memory was examined next. Subjected to a novel object location test, KA-injected wild-type animals exhibited a lower preference rate that was near chance levels compared to sham mice, suggesting impaired memory functions. Moreover, additional minocycline treatment to block microglial activation further deteriorated KA-associated memory deficits, similar to the level of TLR9 knock-out mice. This finding was supported by a prior study showing that pharmacological TLR9 activation ameliorated spatial memory deficits associated with Alzheimer's disease (9), in line with the beneficial effects of TLR9 after a brain insult. However, as continuous stimulation of TLR9 by CpG DNA injection under physiological conditions resulted in memory impairment as evaluated by Morris water maze testing (10), further studies are warranted to define the context-specific roles of TLR9.

Despite intense demands of developing therapies to prevent epileptogenesis, there have been no approved disease-modifying drugs to date. As Matsuda and colleagues nicely describe non-cell autonomous mechanisms influencing aberrant hippocampal neurogenesis, future comprehensive strategies will need to target neuronal and non-neuronal cells, such as immune system components, to develop true anti-epileptogenic therapies.

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