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Microglial Cells in Epilepsy: Not That Bad After All?

Microglial Depletion Aggravates the Severity of Acute and Chronic Seizures in Mice

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Microglia are the resident immune cells of the center nervous system and participate in various neurological diseases. Here, we determined the function of microglia in epileptogenesis using microglial ablation approaches. Three different microglia-specific genetic tools were used, CX3CR1$^{CreER/+}$: R26$^{iDTA/+}$, CX3CR1$^{CreER/+}$: R26$^{iDTR/+}$, and CX3CR1$^{CreER/+}$: Csf1r$^{Flox/Flox}$ mice. We found that microglial depletion led to worse kainic acid (KA)–induced status epilepticus, higher mortality rate, and increased neuronal degeneration in the hippocampus. In KA-induced chronic spontaneous recurrent seizures, microglial depletion increased seizure frequency, interictal spiking, and seizure duration. Therefore, microglial depletion aggravates the severity of KA-induced acute and chronic seizures. Interestingly, microglial repopulation reversed the effects of depletion upon KA-induced status epilepticus. Our results demonstrate a beneficial role of microglia in suppressing both acute and chronic seizures, suggesting that microglia are a potential therapeutic target for the management of epilepsy.

Commentary

A wealth of evidence implicates neuroinflammatory processes in the pathogenesis of epilepsy.[1] In human cases, and in animal models of acquired and genetic disease, a wide variety of inflammatory mediators are elevated in epileptogenic brain tissue, and targeting these in experimental models can suppress seizures, and impede progression of epileptogenesis, implicating causal involvement in disease development.[2] The primary source of these inflammatory molecules is attributed to activation of glial cells—both microglia and astrocytes—as well as damaged neurons and endothelial cells. The specific contributions of each of these cell types to the broad inflammatory cascades have not yet been clearly articulated, although it is logical to suggest that, since these cells play complementary physiological roles in the central nervous system (CNS), they may also differentially contribute to neuroinflammation in epilepsy. The current study by Wu et al[3] seeks to explore the specific contribution of microglial cells in the pathogenesis of epilepsy.

In addition to conducting a range of homeostatic functions necessary for CNS health, including synaptic pruning during development and synaptic plasticity and neurogenesis in adulthood, microglia are recognized as resident immune cells of the brain. CNS challenges such as invading pathogens, protein aggregates or brain injury, trigger these cells to become "activated," causing a morphological and molecular shift in their phenotype designed to overcome these challenges. These responses are now recognized to be diverse and wide-ranging, and can be both beneficial and deleterious to disease development and progression. For example, microglial phagocytosis of cell debris or toxic proteins would enhance CNS function, but excessive release of pro-inflammatory cytokines could promote dysfunction. These cells can therefore play a variety of roles, through surveillance to pathogenic, and anywhere in between.[4]

With respect to epilepsy, microglia are heavily implicated in pathophysiological processes through their association with neuroinflammation, and strategies to manipulate these cells for therapeutic benefit are emerging.[5] In addition, evidence is accumulating describing neuroinflammation-independent actions of microglia which may also be relevant.[6] Microglia can be rapidly activated by seizures, such as those triggered by chemoconvulsants.[7] They are also found to be activated in chronic epilepsy, both in brain tissue of patients with temporal lobe epilepsy,[8] and in rodent models.[9] In these cases, they are generally considered to play detrimental roles, adopting hyper-ramified or amoeboid morphology and promoting pro-inflammatory cascades and neurodegeneration. However, reactive microglia can also trigger epilepsy in the absence of pronounced neuroinflammation.[10] The influence of microglia on epilepsy development, and the underlying mechanisms, is therefore complex and requires further study.

To address this, Wu et al developed 3 different transgenic mouse lines to allow them to selectively deplete microglial cells, and investigated the influence of this manipulation on a range of outcomes relating to epilepsy. These cells were targeted by utilizing CX3CR1-Cre mice, since this chemokine receptor is highly expressed on microglia cells. Temporal control of cell death was afforded by the use of tamoxifen-inducible Cre, and cell death was triggered using either diphtheria toxin or excision of the CSF-1 receptor, which is essential for microglial survival. The authors provide convincing evidence that microglia are depleted in all 3 mouse lines, evidenced by loss of IBA1 immunostaining, although they do not extend their histological evaluation to establish the specificity of cell depletion to microglia. Interestingly, despite persisting with either diphtheria or tamoxifen injections, microglia depletion only persists for a couple of weeks, before these strategies lose effectiveness. At this time, new microglia begin to repopulate the brain,[11] an event which also has the potential to impact chronic neurological disease.[12]

In the first series of studies, they examined how loss of microglia impacted seizures and neurodegeneration induced by a chemoconvulsant. Depletion of microglia prior to intracerebroventricular administration of kainic acid (KA) resulted in more severe behavioral seizures during status epilepticus (SE), and greater mortality. Exacerbated hippocampal neuronal degeneration occurring in the subsequent days after SE was also noted in microglia-depleted mice, possibly a consequence of the more severe seizures observed in these mice. This study demonstrated clear detrimental consequences of the absence of microglia on drug-induced seizures and associated neuronal cell death, suggesting that these cells normally protect against such effects.

They next examined the influence of microglial depletion in a model of chronic epilepsy, this time induced by intrahippocampal KA. Epilepsy was allowed to develop over 20 days following KA, at which point microglia depletion was triggered. Epilepsy severity was assessed 1 to 2 weeks later at a time when microglial depletion was maximal, using electrographic assessment of spontaneous seizures and spikes. In all 3 mouse lines, depletion of microglia resulted in more frequent spikes and seizures, suggesting that microglial cells play disease-limiting roles in chronic epilepsy. This seems counterintuitive, since dogma suggests that pro-inflammatory processes appear to contribute to epilepsy.[1] However, other evidence supports a protective role: for example, SE promotes a dramatic increase in microglia–neuron interactions mediated by CX3CR1, and loss of this signaling factor leads to more severe seizures.[13]

The authors noted that after a period of 1 to 2 weeks, despite continuing with tamoxifen administration to induce Cre expression, microglial cells began to repopulate the brain. This was consistent across models and is known to occur when the factor provoking microglial depletion is removed.[14] For the final experiment, the authors returned to their acute seizure model and asked whether depletion and subsequent repopulation of microglia prior to SE impacted seizure severity. In this case, mice with repopulated microglia behaved no differently from controls, suggesting that once these cells are replaced, they perform similar functions as original microglial cells. Although not characterized in this study, after SE, these cells adopt an activated phenotype.[9] One intriguing question which remains unanswered concerns depletion of these activated microglial cells after SE: do the subsequent repopulated cells adopt an activated phenotype? Or are they "resting," potentially promoting a regenerative environment and improve epilepsy pathogenesis. In addition, it would be valuable to characterize the neuroinflammatory response in chronic epilepsy when microglia are not present. Is this limited by removal of microglia, or do existing astrocytes and neurons maintain this environment? Such a question might inform about the mechanism of effect of microglial depletion.

The study from Wu et al comprehensively demonstrates that depletion of microglia intensifies the severity of drug-induced SE and enhances disease severity in chronic epilepsy, suggesting that native cells have antiepileptic properties, both at rest, and in pathological environments. This appears contrary to the majority of the literature, which suggests that microglia contribute to the chronic and pathogenic neuroinflammation observed in epilepsy, although neuroinflammation-independent roles are now emerging also. The mechanism of the observed neuroprotective and antiepileptic effects may either be direct—endogenous microglia could provide a protective role through maintenance of glutamate homeostasis and suppression of ictogenesis; or indirect—the consequences of microglia depletion on brain structure and function may cause nonspecific adverse effects, such as degradation of brain cytoarchitecture, which manifests here as enhanced seizure susceptibility. Indeed, some reports suggest that depletion of microglia, from the healthy brain, leads to astrogliosis,[11] so this may synergize with the existing neuroinflammation to exacerbate disease. However, if the former is true, this study promotes the idea that enhancing endogenous microglial functions may result in therapeutic benefits for people with epilepsy.

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