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United Again: STING and the Police

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Neuroinflammation is a common feature of aging and neurodegeneration. In this issue of *Neuron*, Mathur et al. (2017) report that the antiviral drug Ganciclovir induces an interferon type I response in microglia through activation of the STING pathway, inhibiting inflammation and leading to protection in a model of multiple sclerosis.

For several decades, the brain has been viewed as an immune-privileged organ and the inflammation supposed to occur there mostly as a consequence of blood-brain barrier disruption and infiltration of peripheral immune cells. Increasing evidence, however, suggests that several challenges arising in the periphery, such as systemic infection, but also aging or neurodegeneration, are sufficient to cause innate immune cerebral activation, referred to as neuroinflammation. Microglia, the resident innate immune cells of the brain, and eventually infiltrating peripheral immune cells, jointly contribute to the neuroinflammation by secreting a wide array of pro- and anti-inflammatory mediators. Microglia have been viewed as the "police" of the brain, supporting where necessary and defending the brain's homeostasis when needed. Of note, several of these mediators can adopt both qualities depending on their concentration, tissue context, and duration of release. The interferons well reflect such duality with ambiguous results of interferon type I (IFNβ) and II (IFNγ) in neuroinflammation (Deczkowska et al., 2016). Thus, type I IFNs play pivotal roles for antiviral responses by inducing apoptosis of virally infected cells. Cytosolic double-stranded DNA (dsDNA) sensing induces type I interferon production through activation of the cGAS-STING pathway. Binding of dsDNA by the nucleotidyltransferase cGAS initiates the synthesis of the cyclic dinucleotide second messenger molecule cGAMP, which in turn activates the endoplasmic reticulum (ER)-resident transmembrane receptor STING (encoded by TMEM173). STING subsequently exits the ER toward the Golgi to initiate further signaling cascades involving the recruitment and activation of TBK1 kinase, IRF3, and NFκB transcription factors, ultimately leading to the production of IFNβ (Gaidt et al., 2017; Takeuchi and Akira, 2010). Despite having emerged as a potential drug target for immune regulation, the role of STING for neuroinflammation has been elusive so far.

In this issue of *Neuron*, Mathur and colleagues (Mathur et al., 2017) describe that the FDA-approved drug Ganciclovir (GCV) induces a type I interferon response dependent on a functional STING and not by acting on its canonical target the thymidine kinase. Mathur et al. (2017) found that GCV reduced inflammation in cultured microglia and related cell lines and, notably, in a mouse model of multiple sclerosis (experimental autoimmune encephalomyelitis, EAE), bringing attention to STING as a novel pathway regulating microgliosis and neuroinflammation. Previously, it has been demonstrated that GCV, administered at therapeutic doses, reduced neuroinflammation and infiltration of peripheral immune cells in an EAE model of multiple sclerosis (Ding et al., 2014). In the present work, Mathur et al. (2017) found that, by using BV2 cells, primary microglia cultures, and human iPSC-derived microglia, GCV led to an upregulation of several antiviral proteins, including CXCL10 and IFNβ, at the mRNA and protein levels. Most importantly, this action of GCV occurred independently of its well-characterized action on the viral thymidine kinases (tks). Microglia isolated from tk1 knockout mice showed increased CXCL10 and IFNβ levels when treated with GCV, suggesting that tk is dispensable for this new mechanism of action of GCV. Further experiments showed that GCV signals through the Jak/Stat signaling transduction pathway to finally induce the upregulation of CXCL10.

Pharmacological inhibition and genetic silencing of either Jak or Stat1 confirmed these results. Since recent evidence suggested that type I interferon responses involve the synthesis of the cyclic dinucleotide second messenger cGMP, which activates STING, subsequently leading to the induction to CXCL10 and IFNβ (Gaidt et al., 2017), Mathur et al. (2017) hypothesized that GCV may mimic cyclic dinucleotide and activate the STING pathway. Consistent with this, GCV required a functional STING or its downstream signaling partners. Based on these initial *in vitro* data, the authors then sought to determine the role of STING for microglial cell activation and neuroinflammation. To take these findings further, Mathur et al. (2017) employed an EAE mouse model of multiple sclerosis using wild-type (WT) and $STING^{tg/tg}$ (lacking functional STING) mice. As a major novel element to our understanding of cerebral innate immune activation, they found that STING was exclusively expressed by microglia in the brain. Furthermore, they discovered that EAE induction increased STING expression in both myeloid ($Iba1^{+}$) and microglia ($Tmem119^{+}$) cells. GCV treatment reverted this increase in STING expression almost completely. In keeping with previous results (Ding et al., 2014), GCV treatment reduced the incidence and lethality of EAE. Moreover, treatment with GCV reduced the number of proliferative myeloid cells, the expression of the microglial activation marker CD68, and the number of infiltrating peripheral immune cells, suggesting that reduced neuroinflammation accounts for the beneficial effect of GCV in EAE. Importantly, these effects were shown to be STING dependent. $STING^{tg/tg}$ mice were refractory to GCV

treatment, since GCV failed to reduce disease severity and neuroinflammation. In order to get a deeper insight on the anti-inflammatory action of GCV in the context of EAE, microglia from GCV-treated WT and STING$^{tg/tg}$ mice were isolated and RNA sequencing was performed. Treatment with GCV mostly modulated inflammation-associated genes in WT mice with EAE, but not in STING$^{tg/tg}$ mice, supporting previous observations that GCV requires a functional STING for mitigating microgliosis. A unique type of damage-associated microglia (DAM) has been recently described for neurodegenerative disease (Keren-Shaul et al., 2017). Interestingly, DAM genes were found to increase in EAE and to be downregulated by GCV in a STING-dependent manner.

Together, this study by Mathur et al. (2017) sheds light on the role of STING as a potent immune regulator of neuroinflammation. Mathur et al. (2017) found that STING is exclusively expressed by microglia upregulated during EAE. Sting activation by GCV reduced EAE severity by reducing microgliosis and neuroinflammation. GCV is a widely used antiviral drug, whose canonical pathway involves the viral tk in order to inhibit viral replication. Notably, the type I interferon response induced by GCV was not mediated by its canonical pathway, but through STING. Aiming to translate these findings to the clinic, the search for an optimal GCV dose seems to be important as high interferon responses may lead to interferonopathies (Rodero and Crow, 2016), and low doses of GCV may not affect neuroinflammation (Skripuletz et al., 2015). In the end, STING and the police are united and back on stage, "the message in the bottle" is out, and STING is positioned as a novel and important player, not so much as musician, but as a signaling element in the field of neuroinflammation.

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The Locomotion Tug-of-War: Cholinergic and Dopaminergic Interactions Outside the Striatum

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In this issue of *Neuron*, Moehle et al. (2017) demonstrate that presynaptic muscarinic receptors counteract the effects of dopamine in an output nucleus of the basal ganglia. They provide intracellular, anatomical, and network-level mechanisms for this cholinergic-dopaminergic interplay.

The basal ganglia play a critical role in generation of locomotion and selection of motor plans to perform behaviors necessary for survival, such as running from a predator or chasing prey. Decades of anatomical and functional work have delineated the nuclei that make up this circuit, and it is well known that the balance between two neuromodulators—dopamine and acetylcholine—plays a critical role in basal ganglia function (Dudman and Gerfen, 2015). Traditionally, the field focused on this interaction between dopamine (from the midbrain) and acetylcholine (from local cholinergic interneurons) within the confines of the striatum. However, the striatum is not the only site of cholinergic influence on the basal ganglia (Picciotto et al., 2012). In this issue of *Neuron*, Moehle et al. (2017) use sophisticated pharmacological, transgenic, and viral manipulations to probe extra-striatal regulation of the basal ganglia through cholinergic signaling.