This Week in The Journal

Mitochondrial Protein in Neurons Limits CNS Inflammation

Micah Harland, Sandy Torres, Jingyi Liu, and Xinglong Wang

(see pages 1756–1765)

When pathogens invade peripheral tissues, they elicit an immune response that includes the production of cytokines and other inflammatory mediators. These mediators can enter the brain, where they alter neural activity to produce sickness behaviors, such as lethargy, loss of appetite, and social avoidance. Prolonged inflammation can cause cognitive deficits and depression of mood that persist after the peripheral infection is resolved. In severe cases, widespread hyperactivation of the immune system produces sepsis, which may lead to degeneration in the brain and peripheral organs and can ultimately result in death.

Persistent CNS inflammation, whether from sepsis or neurodegenerative diseases, is typically accompanied by mitochondrial dysfunction, which may exacerbate neuronal damage. To investigate the consequences of neuronal mitochondrial dysfunction during sepsis in mice, Harland et al. overexpressed mitofusion 2 (Mfn2), a major regulator of mitochondrial function, selectively in neurons, then intraperitoneally injected the bacterial membrane component lipopolysaccharide (LPS) to induce sepsis.

Wild-type and mutant mice showed similar spleen enlargement and elevation of proinflammatory cytokines IL-1 β and TNF α in the blood, suggesting similar levels of peripheral immune-system activation. Peripheral tissue damage was also similar between genotypes. Despite this, neuronal Mfn2 overexpression increased survival: whereas most wild-type mice died within 1 week of infection, those overexpressing Mfn2 survived at least 2 weeks. Mfn2-overexpressing mice also exhibited less sickness behavior than wild-type mice.

As expected, overexpressing Mfn2 reduced fragmentation of neuronal mitochondria after LPS injection. Although brain levels of several inflammatory molecules were comparable in wild-type and

Mfn2-overexpressing mice, levels of IL-1 β (the main inflammatory agent in the brain) were lower in mutants, suggesting reduced neuroinflammation. Correspondingly, microglia were less activated in Mfn2-overexpressing brains than in controls. This was attributable to elevated levels of the chemokine CX3CL1, which is produced by neurons and inhibits microglial activation. Indeed, knocking down CX3CL1 restored LPS-induced microglial activation in Mfn2-overexpressing mice.

These results are remarkable for several reasons. First, they suggest that the mitochondrial protein Mfn2 can regulate neuronal gene expression; how this occurs is unknown. Furthermore, they show that improving mitochondrial health might reduce neuroinflammation. Finally, they suggest that improving mitochondrial health and/or reducing inflammation in the brain is sufficient to prevent the multiorgan failure that typically causes death in sepsis.

Treating endothelial cell cultures with NMDA plus tPA (right) causes cells to shrink, as indicated by reduced area of f-actin staining (green), compared with controls (left). See Mehra, Guérit, et al. for details.

Endothelial NMDA Receptors Increase BBB Permeability

Anupriya Mehra, Sylvaine Guérit, Richard Macrez, Fabien Gosselet, Emmanuel Sevin, et al.

(see pages 1778–1787)

In many inflammatory conditions, the blood–brain and blood–spinal-cord barriers (BBB and BSCB) break down, allowing influx of peripheral immune cells. One contributor to barrier breakdown is tissue plasminogen activator (tPA), a protease released by neurons and many other cell types. tPA can promote BBB breakdown by activating other proteases that in

turn break down the extracellular matrix surrounding blood vessels; it also increases BBB permeability by enhancing activation of NMDA receptors (NMDARs) in vascular endothelial cells. Indeed, tPA has been shown to increase NMDAR activation in neurons, and an antibody that blocks tPA–NMDAR interactions reduces permeability of the BSCB. Mehra, Guérit, et al. have confirmed that tPA-dependent activation of NMDARs increases permeability of endothelial barriers *in vitro* and have uncovered some of the downstream effectors.

NMDAR subunits GluN1, GluN2B, and GluN3A were colocalized near tight junction proteins in spinal cord endothelial cells. GluN3-containing NMDARs are less permeable to calcium than other NMDARs, and consistent with this, application of glutamate did not increase calcium levels in cultured epithelial cells, even when tPA was present. Nevertheless, application of NMDA in the presence of tPA caused epithelial cells to shrink, creating gaps between cells and thus increasing the permeability of cell monolayers. These effects were accompanied by an increase in RhoA activity and phosphorylation of myosin light chain (MLC) in epithelial cells, and they were prevented by a Rho inhibitor, an inhibitor of the Rho-associated kinase (ROCK), and the antibody targeting NMDAR–tPA interactions. Treatment of cells with glycine and tPA, but not with either alone, also induced cell shrinkage and increased monolayer permeability while activating RhoA and increasing MLC phosphorylation, and these effects were prevented by blocking the glycine binding site of NMDARs, as well as by ROCK inhibition and blocking NMDAR–tPA interactions.

These data suggest that glutamate and glycine can increase BBB permeability in the presence of tPA by binding to NMDARs, thus activating a Rho-dependent signaling cascade that leads to phosphorylation of myosin light chain. This likely leads to rearrangement of the actin cytoskeleton, resulting in cell shrinkage and the opening of gaps between cells. Future work should determine whether and when this happens *in vivo*.

This Week in The Journal was written by Teresa Esch, Ph.D.
<https://doi.org/10.1523/JNEUROSCI.twij.40.8.2020>