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SPOTLIGHT

Necroptosis is SARMful to your health

Brian A. Pierchala

Necroptosis is a cell death pathway involved in inflammation and disease. In this issue, Ko et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.201912047) link SARM1, the executioner of Wallerian degeneration of axons, to necroptosis, revealing a unique form of axonal disassembly likely involved in neurodegenerative disorders.

Injuries to the nervous system can result in severing of axons from the cell bodies of neurons. Outside of the central nervous system in the periphery, neurons have an impressive amount of regenerative capacity and can regrow axons great distances to reconnect to their targets. For this to happen, the severed distal axon stump must be cleared so the new axon can regenerate, akin to getting a train wreck off the tracks so the new train can pass by. This occurs by a process known as Wallerian degeneration, and over the past 30 years, many of the molecular events that constitute this orderly axon destruction have been identified. The executioner in this process is Sterile-α and Toll/interleukin 1 Receptor Motif Containing Protein 1 (SARM1). SARM1 has nicotinamide adenine dinucleotide (NAD) degrading activity, and upon SARM1 activation, NAD levels in the axon plummet. This causes an energy collapse and the influx of calcium , leading to activation of calcium-dependent proteases and axon granulation (1). A key enzyme involved in the synthesis of NAD in axons, nicotinamide mononucleotide adenyltransferase 2 (NMNAT2), is transported into axons and plays the central role in the production and maintenance of axonal NAD levels. Importantly, NMNAT2 has a short half-life that is highly regulated by phosphorylation, ubiquitylation, and palmitoylation. The degradation and depletion of NMNAT2 from axons triggers the activation of SARM1 by a mechanism that is unclear, leading to axonal destruction (2).

There is a growing appreciation that Wallerian degeneration is not only involved in the regeneration process after nerve injury but is involved in neurodegenerative diseases. In rodent modes of glaucoma, retinal ganglion cells (RGCs) are exposed to increased intraocular pressure and/or inflammation and degenerate and die. Increasing NMNAT activity in glaucoma models, either with or without providing nicotinamide, a precursor of NAD, reduces the axonal degeneration of RGCs (2). To determine whether SARM1 is involved in this process, Ko et al. examined whether RGCs of *Sarm1*$^{-/-}$ mice were protected from degeneration in an inflammation-induced model of glaucoma involving injection of TNFα into the vitreous fluid (3). Indeed, SARM1 deletion completely prevented both axon degeneration and the subsequent death of RGC cell bodies.

In regards to the mechanism by which RGCs die in glaucoma, evidence exists for the involvement of both apoptosis and necroptosis. Apoptosis, sometimes called cellular suicide, culminates with the activation of cysteine proteases known as caspases, resulting in nuclear condensation, the formation of apoptotic bodies and their removal by local phagocytic cells. Necroptosis, in contrast, is triggered by activation of the serine/threonine kinases RIPK1 and RIPK3, resulting in the phosphorylation and activation of mixed lineage kinase-like (MLKL). MLKL is the final executioner of necroptosis that translocates to the plasma membrane and causes its rupture (4). Apoptosis generally does not cause inflammation, whereas necroptosis is well known to induce inflammation (5). In their examination of the TNFα model of glaucoma, the authors detected phosphorylation of both RIPK3 and MLKL in optic nerves, but not caspase activation, suggesting that necroptosis was the predominant cell death pathway involved.

To better understand the mechanisms behind TNFα-induced necroptosis, the authors established a primary sensory neuron culture system in which TNFα application, in the presence of caspase inhibition, induces MLKL phosphorylation and necroptotic cell death. Remarkably, initiation of necroptosis selectively in axons, either with the application of TNFα or the direct activation of MLKL, was completely dependent upon SARM1. Even more surprising were their observations that MLKL activation in axons was incapable of directly inducing calcium influx, and instead required SARM1. This was in stark contrast to TNFα-mediated necroptosis in the cell bodies that induced canonical MLKL-dependent influx of calcium that was independent of SARM1 (Fig. 1). This novel form of axonal necroptosis appeared to require the entire Wallerian degeneration pathway because MLKL activation induced the depletion of NMNAT2 from axons, along with the depletion of Stathmin2/SCG10, an axonal survival protein that is coregulated with NMNAT2 (6, 7; Fig. 1). Consistent with this, expression of a stabilized form of NMNAT1

Department of Anatomy, Cell Biology & Physiology, Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, IN.

Correspondence to Brian A. Pierchala: brpierch@iu.edu.

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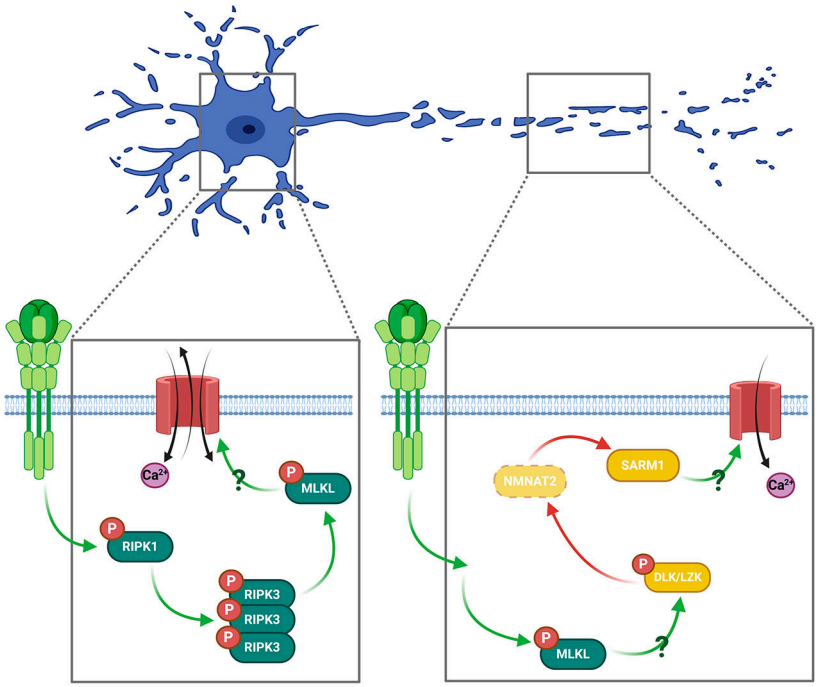

Figure 1. **SARM1 is critical for necroptosis in axons.** TNF-α treatment of neuronal cell bodies (left panel) or axons (right panel) in the presence of caspase inhibitors triggers necroptosis. In cell bodies, necroptosis proceeds via the canonical necrosome pathway in which RIPK1 phosphorylates RIPK3 leading to its aggregation. RIPK3 subsequently phosphorylates and activates MLKL, which translocates to the plasma membrane and induces rupture and calcium entry. Necroptosis in axons proceeds initially via the necrosome pathway to the activation of MLKL. MLKL in axons triggers the degradation of NMNAT2, most likely via activation of a DLK/LZK MAP kinase cascade. This results in SARM1 activation and the destruction of axons through a mechanism involving metabolic collapse and calcium influx. Question marks indicate that the mechanism underlying the event is unknown. Green arrows and red arrows are activation and inhibition, respectively.

potently inhibited axonal necroptosis. Furthermore, augmentation of NAD or ATP production in axons, thereby acting downstream of SARM1, inhibited necroptosis.

This study raises several important questions. Perhaps the most obvious is why MLKL cannot induce membrane rupture and calcium influx in axons. Although MLKL can form membrane channels, as well as interact with TRP channels, the mechanism of MLKL-mediated membrane permeability is still unclear (5, 8, 9). This underscores the potential utility of the sensory neuron system described by Ko et al. for addressing this question, given canonical necroptosis and axonal necroptosis can be interrogated within the same cell. Importantly, axonal injury and disease initiate retrograde stress signals that elicit powerful regenerative and degenerative cell body responses. The authors made an intriguing observation that necroptosis in axons occurred without any apparent morphological changes in cell body. It would be interesting to determine whether axonal necroptosis initiates retrograde signals to "notify" the cell body, so to speak, for better or worse. The "Wallerian necroptosis" described by Ko et al. will surely have relevance in neurodegenerative diseases such as ALS, for example, in which both necroptosis and Wallerian degeneration have been implicated (2, 10).

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References

1. Figley, M.D., and A. DiAntonio. 2020. *Curr. Opin. Neurobiol.* https://doi.org/10.1016/j.conb.2020.02.012
2. Coleman, M.P., and A. Höke. 2020. *Nat. Rev. Neurosci.* https://doi.org/10.1038/s41583-020-0269-3
3. Ko, K.W., et al. 2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.201912047
4. Heckmann, B.L., et al. 2019. *Cell Death Differ.* https://doi.org/10.1038/s41418-018-0195-3
5. Zhou, W., and J. Yuan. 2014. *Semin. Cell Dev. Biol.* https://doi.org/10.1016/j.semcdb.2014.07.013
6. Summers, D.W., et al. 2020. *Mol. Neurobiol.* https://doi.org/10.1007/s12035-019-01796-2
7. Walker, L.J., et al. 2017. *eLife.* https://doi.org/10.7554/eLife.22540
8. Cai, Z., et al. 2014. *Nat. Cell Biol.* https://doi.org/10.1038/ncb2883
9. Xia, B., et al. 2016. *Cell Res.* https://doi.org/10.1038/cr.2016.26
10. Ito, Y., et al. 2016. *Science.* https://doi.org/10.1126/science.aaf6803