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SPOTLIGHT

Pink1/Parkin link inflammation, mitochondrial stress, and neurodegeneration

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What causes inflammation in age-related neurodegenerative diseases remains a mystery. Sliter et al. (2018. *Nature.* https://doi.org/10.1038/s41586-018-0448-9) show that, when damaged mitochondria cannot be removed by mitophagy, stress from exercise or mitochondrial DNA mutations activates the proinflammatory cGAS–STING pathway that may contribute to Parkinson's disease.

An exciting new realm of cell biology is the role of mitochondria in triggering and regulating the immune system. In collaboration with the endoplasmic reticulum, mitochondria are platforms for RNA and DNA sensing during antiviral innate immune signaling and, due to their bacterial origin, contain "pathogen-like" components (e.g., mitochondrial DNA [mtDNA]) that can stimulate various innate immune receptors to promote inflammation (West and Shadel, 2017). Mitochondria also become damaged and dysfunctional with age or by environmental stressors, raising the question of whether chronic inflammation associated with age-related disease, including common neurodegenerative diseases like Parkinson's and Alzheimer's, is due to mitochondria-induced inflammation (mitoflammation; Fig. 1).

Parkinson's disease (PD) is a common neurodegenerative disorder and involves progressive loss of dopaminergic neurons in the substantia nigra region of the brain (Herrero et al., 2015). Non-familial, idiopathic PD usually occurs after the age of 50, indicating that aging itself is a major risk factor, which could have both genetic and environmental components. Mutations in the *PINK1* or *PARKIN* genes cause familial, autosomal recessive inherited forms of PD. Pink1 and Parkin work together in the same cellular process, called mitophagy, whereby damaged mitochondria are targeted for degradation by lysosomes. The discovery of these genes was preceded by several lines of evidence suggesting that mitochondrial dysfunction is involved in PD. This included the realization that certain IV drug users develop PD-like symptoms because of exposure to a chemical called MPTP (Greenamyre et al., 2001). MPTP is converted to MPP^+ and taken up by dopaminergic neurons, where it inhibits mitochondrial oxidative phosphorylation complex 1. Studies in mice revealed that another complex I poison, rotenone, causes PD-like pathology and suggested that human exposure to this pesticide might be an environmental risk factor (Greenamyre et al., 2001). Thus, mitochondrial stress and the accumulation of damaged mitochondria caused by defects in Pink1/Parkin-mediated mitophagy may contribute to PD. Inflammation is thought to be a key driver of PD pathology (Herrero et al., 2015), but that mitoflammation (Fig. 1) might be involved has not been tested directly.

Making solid connections between mitophagy and PD has been stymied by lack of suitable mouse models. In *Drosophila melanogaster*, Pink1 and Parkin gene knock-outs cause defects in mitochondrial homeostasis, oxidative stress, dopaminergic neuron function, locomotion, and innate immune responses (Whitworth and Pallanck, 2017). However, no consistent phenotype has emerged from studies of *Pink1*$^{-/-}$ or *Parkin*$^{-/-}$ mice. This situation led Sliter et al. (2018) to begin layering additional mitochondrial stressors onto these genetic perturbations to seek a neurodegenerative phenotype. When they crossed *Parkin*$^{-/-}$ mice to mtDNA polymerase "Mutator" mice (that hyper-accumulate mtDNA mutations but do not display neurodegenerative phenotypes on their own), dopaminergic neurodegeneration and motor defects were observed, suggesting that the inability to remove mutated mtDNA via mitophagy might result in PD-like pathology. How this type of mitochondrial dysfunction may precipitate the neurodegenerative phenotype remained unclear. Sliter et al. (2018) have broken exciting new ground by showing that, in the absence of Pink1 or Parkin, mtDNA mutational stress or exhaustive exercise results in activation of the DNA-sensing cGAS–STING pathway and an inflammatory phenotype, connecting mitoflammation to aspects of PD pathology for the first time.

In their study, Sliter et al. (2018) subjected wild-type, *Pink1*$^{-/-}$, and *Parkin*$^{-/-}$ mice to exhaustive exercise, which increases mitophagy in the heart. Strikingly, multiple cytokines (i.e., IL-6, -12, and -13; IFNβ; CXCL1; and CCL2 and 4) were elevated in the serum of *Pink1*$^{-/-}$ or *Parkin*$^{-/-}$ mice, which persisted

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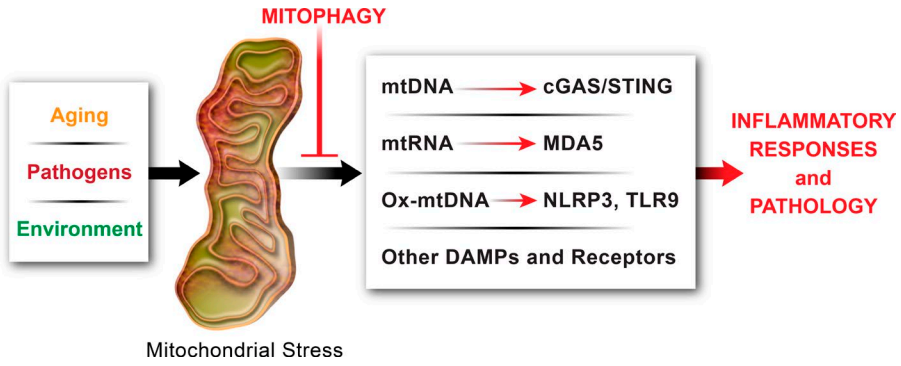

Figure 1. **Mitoflammation.** Mitochondria are targets of stress by many factors, including aging, pathogens, and the environment (e.g., toxins like MPP^+ and rotenone). Mitochondrial stress can result in the release of damage-associated molecular patterns (DAMPs; shown to the right of the mitochondrion) that activate innate immune receptors and downstream signaling. Ox-mtDNA: oxidized mtDNA. Other DAMPs include reactive oxygen species, ATP, *N*-formyl peptides, and TFAM. Chronic activation of these pathways results in inflammation and associated pathology. By removing damaged mitochondria, mitophagy is predicted to help prevent mitoflammation. Sliter et al. (2018) indicate that mitophagy prevents activation of the mtDNA–cGAS–STING pathway.

several days after exercise. A more extensive but overlapping set of cytokines was also observed in Mutator/*Parkin*$^{-/-}$ mice that show PD-like neurodegeneration. Prior studies revealed that mtDNA can gain access to the cytoplasm of cells and activate the DNA-sensing cGAS–STING innate immune pathway, leading to proinflammatory type I IFN and NF-κB signaling, and cytokine production (West et al., 2015; West and Shadel, 2017). Genetic inactivation of STING in the *Parkin*$^{-/-}$ and Mutator/*Parkin*$^{-/-}$ mice prevented exercise and age-dependent cytokine production, respectively. Remarkably, lack of STING also rescued the neurodegenerative and locomotor defects in the Mutator/*Parkin*$^{-/-}$ mice. Finally, Sliter et al. (2018) found increased mtDNA in the circulation in both models of mitoflammation, leading them to conclude that mtDNA activates cGAS–STING under these circumstances. They go on to speculate that this might also be driving the observed inflammatory pathology in PD, based on their own and other published analyses of circulating cytokine profiles and anti-DNA antibodies in PD patients. If true, then inhibiting the cGAS–STING pathway or finding ways to reduce release of mtDNA into the cytoplasm or circulation may be of therapeutic value for PD.

While connecting mitoflammation to PD is a huge first step, several key questions remain from the Sliter et al. (2018) study. First, more work is needed to implicate mtDNA by itself as the cGAS–STING agonist and demonstrate increased cytoplasmic mtDNA and its binding to cGAS, which is important because nuclear DNA can also activate this pathway. Although it is known that circulating mtDNA is inflammatory (West and Shadel, 2017), this is usually sensed by TLR9, which can trigger type I IFN and NF-kB signaling, leading to similar cytokine profiles to that observed by Sliter et al. (2018). Thus, the connection between the circulating mtDNA observed and cGAS–STING activation, which is usually a result of cytoplasmic DNA sensing, is not immediately evident, and whether TLR9 is involved needs to be tested. Second, the mechanism underlying increased cytoplasmic and circulating mtDNA when mitophagy is compromised is not clear. Third, the requirement for severe mitochondrial stress on top of loss of Pink1-Parkin–mediated mitophagy to observe the neurodegenerative phenotype in mice leaves open the question of what triggers idiopathic PD in humans. Extending from Sliter et al. (2018) and focusing on inflammation, it is noteworthy that neurotoxicity due to inflammation, and type I interferon in particular, has been documented in rotenone and MPTP mouse models of PD (Main et al., 2017). These models involve inhibition of complex I activity, which can be accompanied by increased mitochondrial reactive oxygen species production. Thus, the possibility that oxidized mtDNA might be the inflammatory agonist is worth considering. Finally, although Pink1 and Parkin mediate mitophagy, these proteins are also involved in the generation of mitochondrial-derived vesicles and mitochondrial antigen presentation (Matheoud et al., 2016) that could be involved in mtDNA release and/or the inflammatory phenotypes observed.

In summary, the study by Sliter et al. (2018) has illuminated an important connection between mitochondrial stress and inflammation in the context of PD. This should spur additional studies of how mitoflammation pathways are involved in other neurodegenerative diseases and the chronic inflammation associated with aging that drives other common diseases. In addition to activation of cGAS–STING, mtDNA activates other immune receptors (West and Shadel, 2017). Two recent studies show that in addition to TLR9, newly synthesized, oxidized mtDNA and double-stranded mitochondrial RNA can activate the Nlrp3 inflammasome and the RNA-sensing immune receptor MDA5 (Dhir et al., 2018; Zhong et al., 2018), respectively. How cellular nucleic acid sensors differentially respond to these various nucleic acid agonists, the cell-type specificity of these inflammatory responses, and how they are involved in disease and aging are important future areas of research.

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