



Alpha-synuclein–mediated DNA damage, STING activation, and neuroinflammation in Parkinson’s disease

David G. Standaert^{a,1} and Gabrielle M. Childers^a

Parkinson’s disease (PD) is a progressive and debilitating neurodegenerative movement disorder that currently affects more than 7 million people worldwide, and the prevalence is growing rapidly with the aging of populations (1). Despite over 200 y of observation and study of PD, there are still no treatments which have been proven to alter the progression of the disease (2). A characteristic pathological feature found in most cases of PD is the aggregation and misfolding of alpha-synuclein (α -syn) protein. A strong causal link has been formed between α -syn aggregation and activation of both innate and adaptive inflammatory responses in the central nervous system, including gliosis, increased microglial antigen presentation, T cell infiltration, and increased inflammatory cytokines and chemokines (3). These observations have led to a search for targetable immune signaling pathways that might be exploited to slow or halt PD disease progression. In PNAS, Hinkle et al. reveal a mechanism linking misfolded α -syn to type-1 interferon responses in microglia and downstream neurodegeneration, opening doors to new therapeutic approaches targeting the innate immune response in PD (4).

In their report, Hinkle et al. (4) describe the role of the cGAS/STING pathway in the brain’s response to pathological fibrils of α -syn. This innate immune pathway serves to protect against viral and bacterial infection by recognition of fragments of double-stranded DNA (dsDNA). dsDNA fragments can bind to cGAMP synthase (cGAS), which in turn activates the protein stimulator of interferon genes (STING) and the effector protein TBK1 to promote the expression of type-1 interferons (5). While the primary target of cGAS is dsDNA derived from invading pathogens, cGAS can also recognize fragmented self-DNA, arising either from released mitochondrial DNA or damaged genomic DNA (6). Using primary mixed glial cultures, Hinkle et al. show that treatment of the cells with α -syn preformed fibrils (PFFs) initiates dsDNA breaks in microglia, activating the cGAS/STING pathway (Fig. 1). This genotoxic damage and cGAS/STING activation in microglia are recapitulated *in vivo* after the direct injection of PFFs into the striatum of mice, a model of Parkinson’s disease. Furthermore, they found that mice without functional STING (STING^{fl/fl} mice) have reduced inflammatory responses after injection of PFFs and are protected from PFF-induced neurodegeneration. The authors also provide supporting biochemical evidence for activation of this pathway in human PD postmortem tissue and show that this activation is correlated to α -syn burden. From these data, the authors suggest that the ability of misfolded α -syn to cause genomic DNA damage to microglia and trigger cGAS/STING-driven neuroinflammation is a key mechanism of neurodegeneration in PD.

This is not the first time that the cGAS/STING pathway has been implicated in PD. Biallelic mutations in the ubiquitin ligase Parkin or in the kinase PINK1, proteins involved in the homeostasis of mitochondria, cause autosomal recessive forms of PD. Parkin- and PINK1-related PD generally lack the α -syn aggregates seen in more common sporadic forms of PD but nevertheless appear to engage cGAS/STING. In mouse models of Parkin or PINK1 deficiency, exhaustive exercise triggers STING through release of fragmented DNA from mitochondria, and blockade of the cGAS/STING pathway can protect the animals from dopaminergic neurodegeneration (7). Consistent with these observations, in patients biallelic for either Parkin or PINK1 gene mutations there are increased levels of mitochondrial DNA in the serum as well as evidence of inflammation (8).

Activation of STING is also associated with the rare but devastating alpha-synucleinopathy multiple system atrophy (MSA). MSA is defined by α -syn aggregates in oligodendrocytes, progressive autonomic failure, and rapid neurodegeneration involving either the basal ganglia (MSA-parkinsonian subtype, MSA-P) or cerebellum (MSA-cerebellar subtype, MSA-C) (9). As in PD, neuroinflammation has been observed in MSA postmortem brains with gliosis, increased myeloid antigen presentation, and T cell infiltration (10). Interestingly, recent studies in human postmortem MSA tissue found evidence of STING activation in astrocytes in the basal ganglia of MSA-P patients and the striatum of MSA-C patients (11). While DNA damage has not been directly examined in MSA, the activation of cGAS/STING implies that it is present.

Both α -syn pathology and neuroinflammation are present in PD, but the linkage between the two has not been clearly explained; Hinkle et al. (4) put forth a mechanism which gives innate immune cells a central role. Activated by α -syn genotoxicity, microglia could be the site at which neuroinflammation is triggered in sporadic PD. Activation and dysregulation of STING has been a pathway of interest for inflammatory diseases spanning many conditions from autoimmunity to cancer, and many small molecules have

Author affiliations: ^aDepartment of Neurology, University of Alabama at Birmingham, Birmingham, AL 35252

Author contributions: D.G.S. and G.M.C. wrote the paper.

The authors declare no competing interest.

Copyright © 2022 the Author(s). Published by PNAS. This article is distributed under [Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 \(CC BY-NC-ND\)](https://creativecommons.org/licenses/by-nc-nd/4.0/).

See companion article, “STING mediates neurodegeneration and neuroinflammation in nigrostriatal α -synucleinopathy,” [10.1073/pnas.2118819119](https://doi.org/10.1073/pnas.2118819119).

¹To whom correspondence may be addressed. Email: dstandaert@uabmc.edu.

Published April 21, 2022.

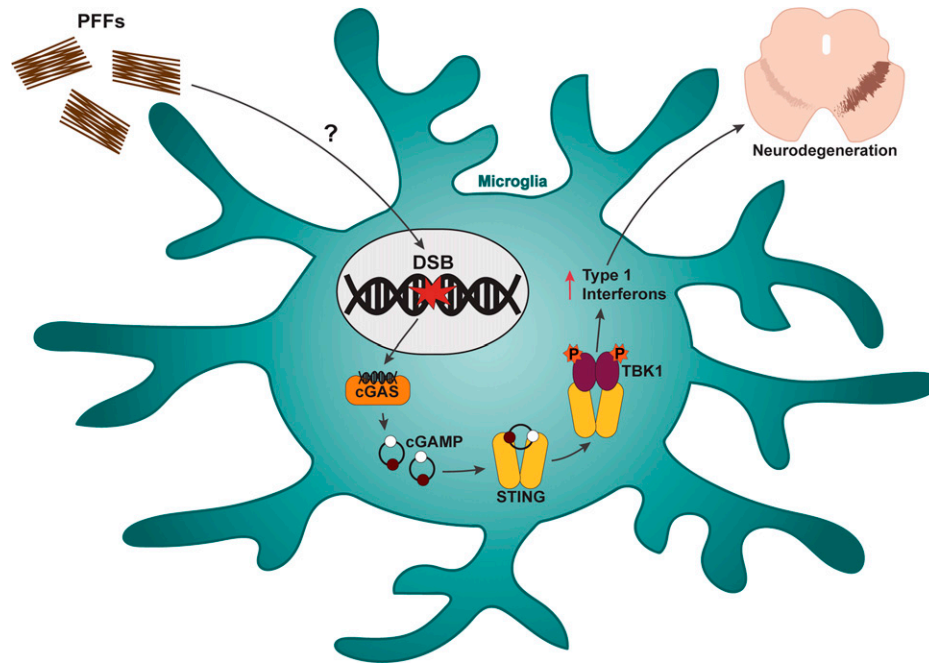


Fig. 1. Proposed mechanism by which α -syn aggregates activate the cGAS/STING pathway. α -syn fibril accumulation in microglia results in double-stranded breaks (DSB) in genomic DNA. Misplaced self-DNA is sensed by cyclic GMP-AMP synthase (cGAS), producing 2'3' cyclic GMP-AMP (cGAMP). cGAMP acts on STING dimers which recruit TANK-binding kinase 1 (TBK1) and promotes auto-phosphorylation. pTBK1 is then able to act on transcription factors that up-regulate type-1 interferon expression. Activation of this innate immune pathway in microglia leads to neurodegeneration in the substantia nigra.

been developed to target the DNA binding site and active site of cGAS as well as to limit STING activation and oligomerization (12). These observations suggest that cGAS or STING inhibition could be a viable therapeutic approach for the slowing of the progression of PD.

There are, however, several critical questions which remain to be answered. PD has a long clinical course, with a prodromal state that may last a decade or more. Is the cGAS/STING pathway activated chronically from preclinical to late-stage disease, or is there a more limited period of peak activation that results in the subsequent activation of other inflammatory cascades? For example, Hinkle et al. (4) show that activation of cGAS/STING results in microglial up-regulation of proinflammatory chemokines including CXCL10, which is known to promote the trafficking of T cells to the area of inflammation. Given that adaptive immune populations such as CD4 T cells are also important for α -syn-driven neuroinflammation and neurodegeneration (13), will inhibiting glial activation and cGAS/STING after the onset of PD be enough to also resolve activation of the adaptive immune system, which maintains immunological memory?

Additionally, it remains unclear how α -syn causes the dsDNA breaks in microglia. In the experiments reported, both microglia and astrocytes were present. Is the phagocytosis of these fibrils causing cellular dysfunction and organelle damage to the microglia? Previous studies have shown that α -syn fibrils cause decreases in microglia phagocytic capacity (14), but there is currently no other evidence that α -syn causes direct damage to microglia. Additionally, astrocyte activation states have been shown to be affected by α -syn in vitro, including cytokine expression (15), but these studies have not been extended to glial mixed cultures. Do astrocytes create a toxic microenvironment in response to α -syn fibrils that results in microglial stress? If microglia are damaged directly by misfolded α -syn, will limiting activation of damage-associated immune pathways alter disease progression? Or will abnormal α -syn still trigger immune activation through other pathways and lead to tissue damage regardless? Understanding these interactions and continuing to explore the role inflammatory processes play in driving PD are essential for the development of much-needed disease-modifying treatments for PD and related disorders.

1. GBD 2016 Neurology Collaborators, Global, regional, and national burden of neurological disorders, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* **18**, 459-480 (2019).
2. J. A. Obeso et al., Past, present, and future of Parkinson's disease: A special essay on the 200th anniversary of the Shaking Palsy. *Mov. Disord.* **32**, 1264-1310 (2017).
3. A. M. Schonhoff, G. P. Williams, Z. D. Wallen, D. G. Standaert, A. S. Harms, Innate and adaptive immune responses in Parkinson's disease. *Prog. Brain Res.* **252**, 169-216 (2020).
4. J. T. Hinkle et al., STING mediates neurodegeneration and neuroinflammation in nigrostriatal α -synucleinopathy. *Proc. Natl. Acad. Sci. U.S.A.* **119**, 10.1073/pnas.2118819119 (2022).
5. N. Liu, X. Pang, H. Zhang, P. Ji, The cGAS-STING pathway in bacterial infection and bacterial immunity. *Front. Immunol.* **12**, 814709 (2022).
6. K. P. Hopfner, V. Hornung, Molecular mechanisms and cellular functions of cGAS-STING signalling. *Nat. Rev. Mol. Cell Biol.* **21**, 501-521 (2020).
7. D. A. Sliter et al., Parkin and PINK1 mitigate STING-induced inflammation. *Nature* **561**, 258-262 (2018).
8. M. Borsche et al., Mitochondrial damage-associated inflammation highlights biomarkers in PRKN/PINK1 parkinsonism. *Brain* **143**, 3041-3051 (2020).
9. A. Fanciulli, G. K. Wenning, Multiple-system atrophy. *N. Engl. J. Med.* **372**, 249-263 (2015).
10. G. P. Williams et al., T cell infiltration in both human multiple system atrophy and a novel mouse model of the disease. *Acta Neuropathol.* **139**, 855-874 (2020).
11. Y. Inoue et al., The stimulator of interferon genes (STING) pathway is upregulated in striatal astrocytes of patients with multiple system atrophy. *Neurosci. Lett.* **757**, 135972 (2021).
12. A. Decout, J. D. Katz, S. Venkatraman, A. Ablasser, The cGAS-STING pathway as a therapeutic target in inflammatory diseases. *Nat. Rev. Immunol.* **21**, 548-569 (2021).
13. G. P. Williams et al., CD4 T cells mediate brain inflammation and neurodegeneration in a mouse model of Parkinson's disease. *Brain* **144**, 2047-2059. (2021).
14. S. Bido et al., Microglia-specific overexpression of α -synuclein leads to severe dopaminergic neurodegeneration by phagocytic exhaustion and oxidative toxicity. *Nat. Commun.* **12**, 6237 (2021).
15. C. Chavarría, S. Rodríguez-Bottero, C. Quijano, P. Cassina, J. M. Souza, Impact of monomeric, oligomeric and fibrillar alpha-synuclein on astrocyte reactivity and toxicity to neurons. *Biochem. J.* **475**, 3153-3169 (2018).