STING mediates neurodegeneration and neuroinflammation in nigrostriatal α-synucleinopathy

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In idiopathic Parkinson’s disease (PD), pathologic αSyn aggregates drive oxidative and nitrative stress that may cause genomic and mitochondrial DNA damage. These events are associated with activation of the cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING) immune pathway, but it is not known whether STING is activated in or contributes to α-synucleinopathies. Herein, we used primary cell cultures and the in vitro model of αSyn preformed fibril (αSyn-PFF) mouse model of PD to demonstrate that αSyn pathology causes STING-dependent neuroinflammation and dopaminergic neurodegeneration. In microglia-astrocyte cultures, αSyn-PFFs induced DNA double-strand break (DSB) damage response signaling (γH2A.X), as well as TBK1 activation that was blocked by STING inhibition. In the αSyn-PFF mouse model, we similarly observed TBK1 activation and increased γH2A.X within striatal microglia prior to the onset of dopaminergic neurodegeneration. Using STING-deficient (Stinggt) mice, we demonstrated that striatal interferon activation in the α-Syn PFF model is STING-dependent. Furthermore, Stinggt mice were protected from α-Syn PFF-induced motor deficits, pathologic αSyn accumulation, and dopaminergic neuron loss. We also observed upregulation of STING protein in the substantia nigra pars compacta (SNpc) of human PD patients that correlated significantly with pathologic αSyn accumulation. STING was similarly upregulated in microglia cultures treated with αSyn-PFFs, which primed the pathway to mount stronger interferon responses when exposed to a STING agonist. Our results suggest that microglial STING activation contributes to both the neuroinflammation and neurodegeneration arising from α-synucleinopathies, including PD.

STING | Parkinson’s disease | neurodegeneration | inflammation | alpha-synuclein

In idiopathic Parkinson’s disease (PD), dopaminergic neurons of the substantia nigra pars compacta (SNpc) progressively degenerate within a milieu of α-synuclein (αSyn) proteopathy, neuroinflammation, and reactive gliosis (1–3). These phenomena are increasingly appreciated to be interdependent and not wholly dissociable from each other in their relationships to neurodegeneration in PD. Ordered, insoluble, and fibrillar species of αSyn protein template further aggregation of endogenous αSyn, catalyzing the spread of α-synucleinopathy throughout neural circuits (4, 5). In the process, αSyn aggregates disperse toxic oligomeric forms throughout the synaptic, axonal, and somatic cellular compartments (6, 7). These oligomers are understood to interfere with a host of normal cellular processes critical for neuronal viability, including proteostasis, mitochondrial activity, and DNA integrity and repair (8). Furthermore, αSyn aggregates engage both innate and adaptive immune processes, similar to classic pathogen-associated molecular patterns (PAMPs) (9–13). Therefore, neuroinflammation in PD and other α-synucleinopathies is not merely an epiphenomenon of neuronal damage but rather intrinsically linked to αSyn pathology.

Current research at the intersection of immunity and neurodegeneration in PD is focused on identifying the upstream signaling mechanisms that drive damaging chronic neuroinflammation. Recent studies implicate classic pattern recognition receptor (PRR)-based mechanisms, such as toll-like receptor (TLR) and nucleotide-binding oligomerization domain-like receptor (NLR) signaling, as mediators of inflammation in α-synucleinopathies (9–11, 14, 15). A distinct element of innate immunity not previously investigated in relationship to αSyn pathology is the cyclic GMP-AMP synthase (cGAS) sensor of cytosolic double-stranded DNA (dsDNA) and its mediator, stimulator of interferon genes (STING) (16, 17). The cGAS/STING system initiates an inflammatory antiviral program through both type-I interferon (IFN-I) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling (18, 19). cGAS/STING has been extensively studied in the context of cytosolic viral or mitochondrial DNA (mtDNA); however, genomic self-DNA also activates cGAS/STING aberrantly in the context of nuclear dysfunction, particularly when micronucleation

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and DNA breaks are present (20–24). In neurons, αSyn aggregates cause DSBs in genomic DNA due to increased nitrative stress (25), suggesting a connection between DNA damage and α-synucleinopathies. However, it is not currently known whether αSyn aggregation is capable of driving cGAS/STING-dependent neuroinflammation that contributes to neurodegeneration in PD.

In the present work, we used primary mouse cell cultures and the intrastriatal αSyn preformed fibril (αSyn-PFF) model of PD (5) to examine whether cGAS/STING activation is a consequence of pathologic αSyn aggregation. We report that αSyn aggregates cause DSBs in microglial DNA and STING-dependent microglial inflammation that occurs prior to the onset of neurodegeneration. Furthermore, STING knockout was neuroprotective in the αSyn-PFF model, reducing inflammation, motor deficits, αSyn pathology, and SNpc neuron degeneration. STING was up-regulated in the SNpc of human PD patients and primed for maximal activation in primary microglia treated with αSyn-PFFs. These results suggest that cGAS/STING activation may exacerbate pathologic neuroinflammation in α-synucleinopathies such as idiopathic PD.

Results

αSyn-PFFs Cause Glial Genotoxicity and cGAS/STING Activation In Vitro. To determine potential cellular sources of cGAS/STING activation, we examined cytoplasmic expression of pathway components in primary mouse cell cultures treated with αSyn-PFF (Fig. 1A). In primary neuronal cultures, we detected no STING expression and little or no expression of cGAS or their effector TBK1. By contrast, STING was strongly expressed in mixed glial cultures enriched for microglia and astrocytes (MA cultures). Therefore, we investigated whether αSyn-PFFs induce genotoxic stress or cGAS/STING activation in MA cultures. We found increased Ser139-phosphorylated H2AX (γH2AX), a marker of DSBs (26), in MA cultures after 24 h of αSyn-PFF treatment (Fig. 1B and D and SI Appendix, Fig. S1A). This time course aligned with accumulation of Ser172-phosphorylated TBK1 (pTBK1) in noncytoplasmic cell fractions (SI Appendix, Fig. S1A), reflecting relocalization to lipid rafts at the trans-Golgi network (27, 28). H151, a selective inhibitor of STING activation, blocked the appearance of pTBK1 in the MA cultures (Fig. 1C and E), suggesting that TBK1 auto-phosphorylates in a STING-dependent manner. Notably, αSyn-PFFs caused a significant increase in the cGAS product and STING activator 2′3′-cGAMP (Fig. 1B) of MA cultures, further supporting canonical cGAS/STING activation. In separate experiments with microglia-depleted cultures, this pattern was not observed, suggesting that microglia are important for STING activation in vitro (SI Appendix, Fig. S1B).

Striatal Interferon Induction in the αSyn-PFF Mouse Model of PD Is STING-Dependent. Given our observation that αSyn-PFFs caused nuclear DSBs and cGAS/STING activation in vitro, we next investigated whether this occurs in vivo. In the αSyn-PFF mouse model of PD (5), microglial activation and reactive astrogliosis precede and accompany the dopaminergic SNpc neurodegeneration that is reliably observed after 6 mo postinjection (14, 29, 30). Therefore, we investigated whether DNA damage and cGAS/STING activation may contribute to neuroinflammation related to α-synucleinopathy in the PFF model. We focused on microglia, which are the principal immune cells of the CNS and were previously shown to be the main cell type expressing STING in the CNS (31, 32). At 3 mo after bilateral striatal αSyn-PFF injection, prior to the onset of SNpc degeneration, we observed a substantial and significant increase in the number of pTBK1+ cells in the striatum (Fig. 2 A and B). Notably, pTBK1 intensity was increased in both Iba1+ and Iba1− cells, suggesting that α-synucleinopathy activates pTBK1 via multiple cellular mechanisms. The relative area of Iba1+ signal that colocalized with pTBK1 was also significantly increased in the αSyn-PFF group (Fig. 2B), indicating a larger proportion of microglia with activated TBK1. We also found that the PFF-injected mice had significantly increased nuclear or peri-nuclear γH2AX foci in the striatum (Fig. 2 C and D and SI Appendix, Movies S1 and S2) at 3 mo after bilateral striatal injection. Furthermore, this evidence of genomic DNA damage was highly concentrated in microglia as indicated by the increased amount of Iba1+ cells with γH2AX expression (Fig. 2D and SI Appendix, Movies S3 and S4). The nuclear and perinuclear localization of γH2AX may both reflect formation of micronuclei, which have previously been shown to associate with both cGAS and γH2AX (20, 33).

Because our results suggested that α-synucleinopathy induces DNA DSBs in microglia in vitro and in vivo, we next tested the mechanistic hypothesis that this causes cGAS/STING-
Fig. 2. Striatal α-synucleinopathy causes microglial DNA damage and STING-dependent interferon induction. (A) Representative immunofluorescence staining for striatal pTBK1 in tissues prepared from WT mice 3 mo after bilateral striatal injection with αSyn-PFF. (B) Quantification of the signal intensity for pTBK1 in microglia (Iba1−) or nonmicroglial (Iba1+) cells of the striatum and the total microglial pTBK1 as measured by the proportion of Iba1 signal with colocalized pTBK1. All data are mean ± SEM. P values from unpaired t tests (pTBK Iba1−: t = 12.65; pTBK Iba1+: t = 14.8; Iba1+ proportion pTBK+: t = 3.18, all df = 7). (C) Representative immunofluorescence staining with orthogonal Z-stack views for striatal γH2AX in tissues prepared from WT mice 3 mo after bilateral striatal injection with αSyn-PFF. (D) Quantification of the total nuclear (Hoechst+) and microglial (Iba1+) γH2AX measured by the proportion with colocalized γH2AX. All data are mean ± SEM P values from unpaired t tests (γH2AX: t = 3.56; Iba1+: γH2AX: t = 4.59, all df = 8). (E) qPCR was used to measure transcription of Sting and inflammatory markers in striatal tissue of WT or Sting−/− mice 3 mo after unilateral striatal injection with αSyn-PFF or PBS vehicle. All data are mean ± SEM P values from unpaired t test for Sting upregulation in WT mice injected with PFF (t = 5.27, df = 9) and two-way ANOVA with Tukey post hoc multiple comparisons tests for all other targets (test statistics in SI Appendix, Dataset S1).

The intrastriatal αSyn-PFF experiment was repeated with a comparison group of Golden-ticket mice (Sting−/−) mice, which lack STING protein due to a mutant missense allele in Sting (34). qPCR was then used to characterize the induction of inflammatory genes. In wild-type (WT) mice, unilateral αSyn-PFF injection increased transcription of Sting in the ipsilateral striatum after 3 mo, suggesting the pathway might be primed for activation prior to SNpc degeneration (Fig. 2E). Consistent with this idea, WT mice injected with αSyn-PFF exhibited upregulation of several interferon signaling genes associated with cGAS/STING activation, such as Cxcl10, Ifit1, Ifit3, and Ifi27 (Fig. 2E). We observed significant reduction of this interferon response in Sting−/− mice, suggesting that STING mediates striatal interferon activation in the αSyn-PFF model. Interestingly, rescue of complement gene transcription (C1qa, C3, and C4) and common inflammatory markers (e.g., Tnf, Il1α) were relatively attenuated and not statistically significant, suggesting that striatal STING signaling is predominantly associated with interferon activation at 3 mo postinjection.

STING Knockout Is Neuroprotective in the αSyn-PFF Model. To determine whether reducing cGAS/STING activation may ameliorate nigrostriatal dysfunction and dopaminergic neurodegeneration secondary to αSyn proteopathy, we examined behavioral and biochemical markers of PD-like deficits in WT
and Sting<sup>−/−</sup> mice injected in bilateral striatum with αSyn-PFFs. At 9 mo postinjection, WT mice exhibited motor deficits on the accelerating rotarod, grip strength test, and pole test (Fig. 3 A–C). These deficits were not observed in the Sting<sup>−/−</sup> mice (Fig. 3 A–C), suggesting that these mice are protected from motor deficits in the αSyn-PFF model. Immunohistochemical analysis of SNpc brain sections from these mice indicated that αSyn-PFFs induced dopaminergic SNpc neurodegeneration in the WT mice that was attenuated in the Sting<sup>−/−</sup> group (Fig. 3 D–F). Relative to WT mice, there was also a significant reduction in pathologic phosphorylated αSyn (pS129-αSyn) in SNpc dopaminergic neurons of the Sting<sup>−/−</sup> mice (Fig. 3 G and H). Finally, in the striatum, WT αSyn-PFF mice exhibited a significant reduction of dopamine (Fig. 3 I) and its metabolites homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), and 3-methoxytyramine (3-MT), which was not observed in the Sting<sup>−/−</sup> mice (SI Appendix, Fig. S2). Collectively, these results indicate that Sting knockout mice are generally protected from the clinico-pathologic PD-like signs that are usually observed in the αSyn-PFF model. In vitro, the STING inhibitor H-151 did not protect primary neuronal cultures from αSyn-PFF cytotoxicity (SI Appendix, Fig. S3), which corroborates the notion that STING-dependent neurotoxicity in the SNpc largely arises from non–cell-autonomous signaling or inflammation.

**STING Is Upregulated in Human PD.** To determine whether idiopathic PD exhibits increased or altered markers of the cGAS/STING pathway, we analyzed SNpc tissue from autopsied idiopathic PD patients and age-matched controls (metadata in SI Appendix, Table S1). Compared to controls, PD patients had significantly increased STING protein expression (Fig. 4 A and B). Interestingly, we did not observe a commensurate increase in pTBK1 in the PD tissue (Fig. 4 C). Given that TBK1 functions in different pathways and cell types (35), the relative amount phosphorylated as part of STING activation may be relatively small in whole-tissue extracts or reduced in late disease. In support of our hypothesized connection between STING and pathologic α-synuclein aggregation, STING...
protein level correlated significantly and linearly with the amount of pS129-αSyn among the PD tissue samples (Fig. 4D).

αSyn-PFFs Prime Microglial cGAS/STING for Maximal Signaling. In vitro, MA cultures stimulated with αSyn-PFFs exhibit substantial upregulation of STING protein, but much less pronounced increases in cGAS or TBK1 (Fig. 5A and B). Modulation of STING protein expression could function as a priming mechanism for increasing the cGAS/STING pathway’s sensitivity to activating stimuli. Recently, this functional outcome was observed in a mouse model of Niemann-Pick disease type C due to altered cellular trafficking that increased the amount of functional microglial STING protein (36). To determine whether αSyn-PFFs cause a similar priming effect of cGAS/STING, we treated microglia with αSyn-PFFs for 24 h as a priming signal, then used the synthetic STING agonist 5,6-dimethylxanthenone-4-acetic acid (DMXAA, also known as vadimezan) to trigger immediate STING activation (Fig. 5C). We found that there was a multiplicative or synergistic effect between αSyn-PFF priming and DMXAA on transcription of several key interferon-induced genes (Fig. 5D). This was reflected in statistically significant interaction terms in two-way ANOVA analyses for Cxcl10, Ifit3, Ccl5, and Isg15 (SI Appendix, Table S2). We also examined whether αSyn-PFF priming led to increased cytokine secretion (in cell culture media) using enzyme-linked immunosorbent assay (ELISA). Similar to the gene transcription results, there was a synergistic relationship between αSyn-PFF priming and DMXAA in promoting the secretion of Cxcl10 protein, as well as IFNβ (Fig. 5E and SI Appendix, Table S3). Interestingly, DMXAA did not stimulate TNF secretion despite Tnf induction, suggesting a complex relationship between STING activation and TNF release (Fig. 5E). We did not observe IFNγ secretion in any condition, suggesting that these responses were not indirectly mediated by IFN-II signaling.

Discussion

The cellular and molecular biology of α-synucleinopathy strongly implicates multifactorial organelle damage as a consequence of αSyn aggregation (8). In turn, the nature of these deficits—which include nuclear dysfunction and DNA damage—strongly suggest the capacity to trigger canonical cGAS/STING activation. The studies presented here show that the cGAS/STING pathway is indeed activated and contributes to neurodegeneration in a mouse model of idiopathic PD. We also show that αSyn-PFFs cause both genomic DNA damage and cGAS/STING signaling both in vitro and in vivo, suggesting that genotoxicity may be upstream of cGAS/STING activation.

αSyn-PFF injections into the mouse striatum reliably engender αSyn pathology by initiating the templated misfolding of endogenous αSyn monomer, likely in the presynaptic compartment of nigrostriatal terminals (5). Here, we found striatal interferon activation within 3 mo of injection, which corresponds to an early stage of disease progression with minimal dopaminergic neurodegeneration and no significant motor impairment (25). Neuroinflammation has long been recognized as being a characteristic of PD and other neurodegenerative diseases, but only in the past decade or so has it become widely considered as something more than an epiphenomenon. This recognition, combined with slow progress in developing disease-modifying therapies, has prompted us to explore how the disruption of inflammatory cascades or loops might constitute a viable therapeutic strategy for blocking neurodegeneration. For example, we have previously shown that reducing microglia-derived neuroinflammation can reduce neurodegeneration and the accumulation of pathologic αSyn in neurons in the αSyn-PFF and A53T mouse models of PD (30). Other recent work has shown that activated microglia directly promote neuronal αSyn aggregation in PFF-based models (37). Reduced interferon signaling in microglia is also associated with lower Aβ aggregate propagation, suggesting that modulation of microglial phenotype is a viable therapeutic approach in neurodegeneration (38). The ideal disease-ameliorating modification of microglial function may be selective reduction in inflammatory activities that preserves adaptive phagocytic functions. Our studies suggest that reducing cGAS/STING activation may be useful in this regard.

Our findings also describe the nature of STING-dependent immune responses in PD. For example, we observed induction of chemokines such as Cxcl10 and Ccl5, which are important chemokines that increase infiltration of immune cells, including lymphocytes (39). Cxcl10 was also recently found to be upregulated in striatal tissue of Huntington’s diseasesepatients, along with apparent activation of cGAS/STING (23). Lymphocytic infiltration and CCL5 increases were similarly observed in the nonhuman primate MPTP parkinsonism model (40), although it is not clear how directly comparable these studies are to our αSyn-PFF model study. However, T-cell infiltration into the SNpc of human PD patients has been documented and Th17 lymphocytes increase iPSC-derived midbrain neuron...
death, suggesting a possible role for lymphocytic infiltration as a pathologic event in idiopathic PD (12). cGAS/STING also has conserved roles in autophagy induction (41); future work may clarify whether this is mechanistically important in conditions such as idiopathic PD that are marked by proteostatic dysfunction and protein aggregation.

In seeking to relate our mechanistic mouse studies to human PD, we also found that STING protein levels were upregulated in lysates of SNpc tissue isolated from human PD patients relative to age-matched controls. Interestingly, STING protein levels correlated positively with the amount of pSer129-αSyn, supporting a connection between pathologic αSyn accumulation and STING. We did not see a clear increase in the ratio of pTBK1 to TBK1 in the PD patients, though this may be related to the activity of TBK1 and its phosphorylation as part of signaling pathways other than cGAS/STING (35). For example, TBK1 is a key regulator of autophagy in both neurons and glia (42). One group reported that loss of function mutations in TBK1 can accelerate disease onset, impair autophagy, and increase SOD1 aggregation in the SOD1<sup>fl</sup>/fl ALS mouse model (43). However, point mutations that only partially reduce TBK1 kinase activity accelerated disease onset while reducing interferon activation and extending lifespan relative to full TBK1 loss. Thus, TBK1 activation is a relatively complex and dose-sensitive process that may not lead to clear changes in pTBK1 level in bulk tissue. Finally, we also note that the high degree of variability in pTBK1/TBK1 in our samples (compared to STING) may reflect biases arising from comorbid conditions and the nonspecificity of neuroinflammatory signaling to PD.

Our in vitro data suggest that the STING upregulation detected in PD tissue may be important for basal or chronic cGAS/STING-dependent inflammation. Priming microglia with αSyn-PFFs increased STING expression and amplified interferon responses elicited by the STING agonist DMXAA. An analogous mechanism that increases the amount of functional STING in microglia contributes to neurologic disease in Niemann-Pick disease (36). Increased STING expression could also theoretically reduce its activation threshold with respect to intracellular cGAMP concentrations. Finally, basal cGAS activity increases as a function of cellular senescence (21, 22, 33, 44), suggesting that increased STING expression in persons with PD could amplify the effects of inflammation on the CNS and further accelerate senescence. Normalizing or disrupting this cGAS/STING signaling may therefore be therapeutically useful for reducing damaging chronic neuroinflammation in PD.

In summary, our work demonstrates that STING is activated by the effects of αSyn aggregation in a model of idiopathic PD and that its knockout ameliorates neuroinflammation and neurodegeneration. Other recent publications have revealed pathologic STING activation in neurodegenerative disease models (23, 24, 36, 45–48), suggesting a wider significance of antiviral cGAS/STING signaling to neurologic disease. Our laboratory has also demonstrated that α-synucleinopathy causes neurodegeneration through genotoxic stress in neurons that leads to cell death via parthanatos (25, 49). The present work complements this line of research by demonstrating pathologic consequences of DNA damage in glial cells. Interventions that preserve the integrity or repair of genomic DNA may therefore be an effective upstream approach to halt pathologic processes across diverse cell types in the CNS during neurodegeneration.

**Materials and Methods**

**Mice.** C57BL/6 (WT) and “Goldenticket” (34) Sting<sup>fl</sup> (Jackson stock #017537) adult mice were obtained from Jackson Laboratory. CD1 mice were from Charles River. Animal usage in this project followed guidelines put forth by the Johns Hopkins University Animal Care and Use Committee (ACUC). The procedures are part of our ACUC-approved protocol, MO20M262.

**Postmortem SNpc Tissue.** Frozen SNpc tissue extracts from autopsied PD patients (n = 5) and age-matched controls (n = 5) were obtained from the Division of Neuropathology, Department of Pathology at Johns Hopkins School of Medicine.

**Primary Cell Culture.** Primary glial cerebrocortical cultures were established by dissociating tissue from PD-P2 mouse pups and plating in a growth medium (DMEM/F12 with 10% FBS, 50 μg/mL penicillin, 50 μg/mL streptomycin, 2 mM L-glutamine, 100 μM nonessential amino acids, and 1 mM sodium pyruvate).
Microglia were isolated via immunomagnetic separation with the EasySep Mouse CD11b Positive Selection Kit (Stemcell Tech). Astrocytes were isolated by serial passaging as in previous work (30). Alternatively, no selection method was used to maintain mixed microglia/astrocyte cultures. Primary neuron cultures were prepared similar to previous work (30).

αSyn-PFF Preparation and Use. Recombinant mouse αSyn-PFFs were made as described previously (4) with minor modifications. PFFs were sonicated at 20% amplitude for 30 seconds (0.5s on/off) with a probe-tip sonicator (Branson Digital Sonifier, Danbury, CT). For neurotoxicity experiments, αSyn-PFFs were added to cell culture media to a final concentration of 5 μg/mL per convention. For experiments in astrocytes, microglia, or microglia-astrocyte cultures, a concentration of 2 μg/mL was used unless otherwise specified. Strial coordinates for stereotactic injection were +0.2 mm (AP), +2 mm (ML), and +2.8 mm (DV).

Behavioral Assays. All behavioral assays were performed as previously described (25).

HPLC. Striatal biogenic amines were measured using high-performance liquid chromatography (HPLC) with electrochemical detection (ECD) as previously described (25).

Quantitative PCR. RNA was extracted from homogenized striatal tissues or cells using TRIzol reagent (Invitrogen, Waltham, MA). Total RNA was reverse transcribed into cDNA via a ViiA7 Real-Time PCR System (Applied Biosystems). Primer sequences used are listed in the supplement (SI Appendix, Table 54).

Immunohistochemistry and Stereology. PFA-fixed cryopreserved brains were sectioned at 30 μm thickness using a sliding microtome (Microm HM 450, ThermoFisher, Waltham, MA) for SNpc cell counts, immunohistochemistry for tyrosine hydroxylase (TH) and astrocytic activation in microglia. For TH immunohistochemistry, TH neurolite-like aggregates. Nat. Protoc. 9, 2135–2146 (2014).

K. C. Luk et al. Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science 338, 949–953 (2012).

R. Cassella et al., The release of toxic oligomers from α-synuclein fibrils induces dysfunction in neuronal cells. Nat. Commun. 12, 1814 (2021).

I. A. Volpirelli-Daley et al., Exogenous α-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. Neurol 72, 57–71 (2011).

Y. C. Wong, D. Kriau, α-synuclein toxicity in neurodegeneration: Mechanism and therapeutic strategies. Nat. Med. 23, 1–3 (2017).

C. Kim et al., Neurodegeneration: Autophagic α-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. Nat. Commun. 4, 1562 (2013).

S. G. Danieli et al., Activation of MYD88-dependent TLR2 signaling by mistfolded α-synuclein, a protein linked to neurodegenerative disorders. Sci. Signal. 8, ra5 (2015).

N. Panicker et al., Fyn kinase regulates mistfolded α-synuclein uptake and NLRP3 inflammasome activation in microglia. J. Exp. Med. 216, 1411–1430 (2019).

A. Sommer et al., TH17 lymphocytes induce neuronal cell death in a human iPSC-based model of Parkinson’s disease. Cell Stem Cell 23, 123–131.e1 (2020).

C. S. Lindemuth Aitchell et al., α-Synuclein-specific Cell reactive is associated with preclinical and early Parkinson’s disease. Nat. Commun. 11, 1920 (2020).

R. Gordon et al., Inflammasome inhibition prevents α-synuclein pathology and dopamine neurodegeneration in mice. Sci. Transl. Med. 10, eaax4066 (2018).

L. Feliner et al., Full-like receptor 4 is required for α-synuclein dependent activation of microglia and astrogliosis. Glia 64, 349–360 (2016).

A. Decot et al., J. D. Katz, S. Venkatraman, A. Ablasar, The γH2AX–STING pathway as a therapeutic target in inflammatory diseases. Nat. Rev. Immunol. 21, 548–569 (2021).

A. Ablasar et al., γH2AX triggers a 2’–5’ linked cyclic dinucleotide second messenger that activates STING. Nature 508, 380–384 (2013).

A. Ablasar et al., The γH2AX–STING pathway as a therapeutic target in inflammatory diseases. Nat. Rev. Immunol. 21, 548–569 (2021).

N. Panicker et al., Fyn kinase regulates mistfolded α-synuclein uptake and NLRP3 inflammasome activation in microglia. J. Exp. Med. 216, 1411–1430 (2019).

L. Fellner et al., Full-like receptor 4 is required for α-synuclein dependent activation of microglia and astrogliosis. Glia 64, 349–360 (2016).

A. Decot et al., J. D. Katz, S. Venkatraman, A. Ablasar, The γH2AX–STING pathway as a therapeutic target in inflammatory diseases. Nat. Rev. Immunol. 21, 548–569 (2021).

A. Ablasar et al., γH2AX triggers a 2’–5’ linked cyclic dinucleotide second messenger that activates STING. Nature 508, 380–384 (2013).

K. C. Luk et al., γH2AX and IκBα reductase to mediate STING-induced NF-κB responses in myeloid cells. Cell Rep. 31, 107492 (2020).

sections were imaged at equal exposure settings and total TH+ neurons were counted for each image. pS129α-Syn+ and TH+ (double-positive) cells were then counted and divided by the total number of TH+ cells. For pTBK1 and γH2AX analyses, images were acquired using a Zeiss LSM 880 confocal scanning microscope and quantification of colocalization or intensity was performed using the Zen software (Carl Zeiss).

ELISA. Cytokines were measured in cell culture media and detergent-soluble brain tissue fractions with DuoSet ELISA development systems (R&D Systems): mouse CXCL10/IP-10/CRG-2 (DY466); mouse IFNβ (DYB234); mouse IFNγ (DY485); and mouse TNF (DY410). 2’3’-cGAMP was measured using the 2’3’-cGAMP ELISA kit from Cayman Chemical (S01700).

Data Availability. Correspondence and requests for materials and access to datasets should be addressed to T.M.D. and V.L.D. All study data are included in the article and/or SI Appendix.

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36. T. T. Chu et al., Tonic prime-boost of STING signalling mediates Niemann-Pick disease type C. Nature 596, 570-575 (2021).
37. M. Guo et al., Microglial exosomes facilitate α-synuclein transmission in Parkinson’s disease. Brain 143, 1476-1497 (2020).
38. P. d’Errico et al., Microglia contribute to the propagation of Aβ into unaffected brain tissue. Nat. Neurosci. 25, 20-25 (2021).
39. R. E. Marques, R. Guabiraba, R. C. Russo, M. M. Teixeira, Targeting CCL5 in inflammation. Expert Opin. Ther. Targets 17, 1439-1460 (2013).
40. J. Seo et al., Chronic infiltration of T lymphocytes into the brain in a non-human primate model of Parkinson’s disease. Neuroscience 431, 73-85 (2020).
41. X. Gui et al., Autophagy induction via STING trafficking is a primordial function of the cGAS pathway. Nature 567, 262-266 (2019).
42. J. A. Oakes, M. C. Davies, M. G. Collins, TBK1: A new player in ALS linking autophagy and neuroinflammation. Mol. Brain 10, 5 (2017).
43. V. Gerbino et al., The loss of TBK1 kinase activity in motor neurons or in all cell types differentially impacts ALS disease progression in SOD1 mice. Neuron 106, 789-805 e5 (2020).
44. H. Yang, H. Wang, J. Ren, Q. Chen, Z. J. Chen, cGAS is essential for cellular senescence. Proc. Natl. Acad. Sci. U.S.A. 114, E4612-E4620 (2017).
45. C. H. Yu et al., TDP-43 triggers mitochondrial DNA release via mPTP to activate cGAS/STING in ALS. Cell 183, 636-649 e18 (2020).
46. D. A. Sliter et al., Parkin and PINK1 mitigate STING-induced inflammation. Nature 561, 238-262 (2018).
47. Y. Hou et al., NAD+ supplementation reduces neuroinflammation and cell senescence in a transgenic mouse model of Alzheimer’s disease via cGAS-STING. Proc. Natl. Acad. Sci. U.S.A. 118, e2011226118 (2021).
48. A. Nazmi et al., Chronic neurodegeneration induces type I interferon synthesis via STING, shaping microglial phenotype and accelerating disease progression. Glia 67, 1254-1276 (2019).
49. A. A. Fatokun, V. L. Dawson, T. M. Dawson, Parthanatos: Mitochondrial-linked mechanisms and therapeutic opportunities. Br. J. Pharmacol. 171, 2000-2016 (2014).