The cGAS-STING pathway: The role of self-DNA sensing in inflammatory lung disease

Ruihua Ma¹, Tatiana P. Ortiz Serrano¹, Jennifer Davis¹, Andrew D. Prigge²,³, Karen M. Ridge¹,4,*

¹Division of Pulmonary and Critical Care Medicine, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA
²Division of Critical Care Medicine, Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL, USA
³Ann & Robert H. Lurie Children’s Hospital of Chicago, Chicago, IL, USA
⁴Department of Cell and Developmental Biology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Abstract

The presence of DNA in the cytosol is usually a sign of microbial infections, which alerts the host innate immune system to mount a defense response. Cyclic GMP-AMP synthase (cGAS) is a critical cytosolic DNA sensor that elicits robust innate immune responses through the production of the second messenger, cyclic GMP-AMP (cGAMP), which binds and activates stimulator of interferon genes (STING). However, cGAS binds to DNA irrespective of DNA sequence, therefore, self-DNA leaked from the nucleus or mitochondria can also serve as a cGAS ligand to activate this pathway and trigger extensive inflammatory responses. Dysregulation of the cGAS-STING pathway is responsible for a broad array of inflammatory and autoimmune diseases. Recently, evidence has shown that self-DNA release and cGAS-STING pathway over-activation can drive lung disease, making this pathway a promising therapeutic target for inflammatory lung disease. Here, we review recent advances on the cGAS-STING pathway governing self-DNA sensing, highlighting its role in pulmonary disease.

Keywords

AIM2; autoimmune disease; cGAMP; DAMP; pulmonary disease

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Correspondence Ruihua Ma, Division of Pulmonary and Critical Care Medicine, Department of Medicine, Northwestern University Feinberg School of Medicine, 303 E. Superior, 5-305, Chicago, IL 60611, USA. ruihua.ma@northwestern.edu.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.
INTRODUCTION

An organism has to efficiently recognize and eliminate continuous microbial insults to maintain host survival and homeostasis. The innate immune system protects the host from microbial infection by utilizing pattern recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs) and orchestrate proper host defense.\(^1\)\(^-\)\(^5\) In addition, a plethora of damage-associated molecular patterns (DAMPs), such as nucleic acids from uncontrolled death of host cells, are also recognized by PRRs, which elicit innate immune responses, further activate the adaptive immune system, and contribute to inflammatory diseases, such as autoimmune disease, ischemic injuries, trauma, and cancer. Thus, recognition of aberrant nucleic acids has emerged as a critical mechanism of host defense, which is mediated by the endosomal or cytosolic nucleic acid sensors. Endosomal nucleic acids are detected by Toll-like receptor (TLR) 3, TLR7, TLR8, TLR9, and TLR13. TLR3 acts as a sensor for double-stranded (ds) RNA, TLR7 and TLR8 sense single-stranded (ss) RNA, and TLR9 detects bacterial and viral DNA, specifically CpG hypomethylated DNA. In the cytosol, viral RNA and aberrant small endogenous RNAs are recognized by retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), followed by aggregation of mitochondrial antiviral-signaling protein (MAVS) and the subsequent activation of the transcription factors nuclear factor-κB (NF-κB) and interferon regulatory factor 3 (IRF3).\(^6\)\(^-\)\(^9\)

DNA usually resides in the nucleus and mitochondria in eukaryotic cells. The aberrant presence of DNA in cytoplasm either from infection or cellular damage elicits robust immune responses leading to inflammasome activation and transcription of genes encoding type I interferon (IFN) and inflammation, which are both beneficial and detrimental to the host. Absent in melanoma 2 (AIM2) was identified as a DNA sensor responsible for inflammasome activation in response to cytosolic double-stranded DNA (dsDNA).\(^10\)\(^,\)\(^11\) Upon binding to dsDNA, AIM2 recruits the adaptor protein ASC to forms a caspase-1-activating inflammasome, a cysteine protease that triggers gasdermin D mediated-pyroptosis, as well as the release of the inflammatory cytokines IL-1β and IL-18 (Figure 1).\(^12\)\(^-\)\(^14\) AIM2 plays an essential role in the host protection against DNA viruses and some bacterial pathogens. However, the type I IFN induction is the most robust response upon DNA stimulation, which is initiated by cyclic GMP-AMP synthase (cGAS). Discovered in 2013 as the universal cytosolic DNA sensor, cGAS is activated upon binding to dsDNA.\(^15\) Activated cGAS converts ATP and GTP into cyclic dinucleotide cyclic GMP-AMP (cGAMP). Then cGAMP binds and activates stimulator of interferon genes (STING, also known as TMEM173, MITA, ERIS, and MPYS) to induce transcription of genes encoding type I IFNs and pro-inflammatory cytokines via the transcription factors IRF3 and NF-κB, respectively (Figure 1). The cGAS-STING pathway is pivotal to the detection of intracellular DNA, and thus protection from infection, such as bacteria, DNA viruses, or reverse-transcribed retroviruses.\(^16\)\(^-\)\(^20\)

The molecular details of the cGAS-STING pathway and its essential role in eliciting protective immunity against noxious invading pathogens have been comprehensively reviewed elsewhere.\(^21\)\(^-\)\(^26\) In this review, we primarily focus on the role of the cGAS-STING pathway on self-DNA recognition, which leads to sterile inflammation and autoimmune disease, with an emphasis on the role of this pathway in pulmonary inflammatory disease.
2 cGAS-cGAMP-STING PATHWAY

The nucleotidyl transferase enzyme cGAS is the essential cytosolic DNA sensor, mediating the generation of type I interferons and other inflammatory cytokines. cGAS was discovered by the Chen group in 2013 using biochemical purification coupled with quantitative mass spectrometry, shortly after their identification of the second messenger molecule cGAMP. The interaction between cGAS and dsDNA is sequence-independent but length-dependent. Although cGAS can also bind ssDNA, it is with a relatively lower affinity (Kd ~ 1.5 μM) than for dsDNA (Kd ~ 87.6 nM). Notably, cGAS recognizes dsDNA with a preference for long dsDNA. Andreeva et al showed that long dsDNA provides more binding sites along two parallel-aligned long DNA duplexes for cGAS dimers, resulting in the formation of a ladder-like complex, which increases the stability via avidity. In addition, it has been shown that long dsDNA more efficiently promotes cGAS liquid-liquid phase separation and cGAS enzyme activity than short dsDNA. Recently, Hooy & Sohn showed that dsDNA length regulates the extent of cGAS activation, and cGAS discriminates against short dsDNA at both the initial recognition step and the signal transduction step.

Invading microbes, such as bacteria, DNA viruses, or retroviruses introduce foreign DNA to the cytosol, and leakage from nuclear or mitochondrial compartments induce self-DNA release to the cytosol. These cytosolic DNAs serve as cGAS agonists. Upon binding dsDNA, cGAS undergoes conformational changes, which induces its enzymatic activity. Active cGAS catalyzes ATP and GTP into 2′,3′-cGAMP (Figure 2). This unique endogenous second messenger comprises mixed 2′-5′ and 3′-5′ phosphodiester linkage. The 2′,3′-cGAMP then binds to and activates the adaptor protein STING. STING locates at the endoplasmic reticulum (ER) membrane and contains four transmembrane helices followed by a cytoplasmic ligand-binding and signaling domain. STING exists as a dimer with two cytoplasmic domains forming a V-shaped binding pocket facing the cytosol. Upon binding to cGAMP, the STING ligand-binding domain closes, leading to a 180° rotation of the ligand-binding domain relative to the transmembrane domain. This conformational change leads to the formation of the STING tetramer and higher-order oligomers through side-by-side packing. The conformational changes in STING induce its translocation from the ER to the Golgi through the ER-Golgi intermediate compartment (ERGIC). This translocation process is dependent on the COP-II complex and ARF GTPases, but is suppressed by the Shigella effector protein IpaJ and the Ca2+ sensor STIM1. Ubiquitination modification of STING (lysine 224) is also required for STING trafficking. After trafficking to the Golgi, STING is palmitoylated at Cys88/91, which is essential for its activation. Palmitoylation of STING facilitates its oligomerization, which may serve as a signaling platform to recruit and activate TANK-binding kinase 1 (TBK1) dimers. Structural analysis shows a conserved PLPLRT/SD motif within the C-terminal tail of STING mediates the recruitment and activation of TBK1. Then, recruited TBK1 directly phosphorylates the C-terminal tail of STING. Phosphorylated STING further binds to a positively charged surface of IRF3 and recruits IRF3 for its phosphorylation and activation by TBK1. Activated IRF3 forms a dimer and translocates to the nucleus to regulate the transcription of the type 1 interferon gene, IFNB1, which encodes interferon-β (Figure 2). By binding to its receptor, secreted interferon-β
activates the janus kinases (JAKs)-signal transducer and activator of transcription (STAT) signaling pathways to up-regulate the transcription of interferon-stimulated genes (ISGs) in a positive feedback loop (Figure 2).50,51

In parallel, the cGAS-STING pathway can also activate NF-κB-dependent signaling transduction, thus regulating the transcription of genes encoding inflammatory cytokines (Figure 2). It has been shown that activated STING induces canonical and noncanonical NF-κB activation via the TNF receptor-associated factor 6 (TRAF6)-TBK1 axis and TRAF3, respectively.52 In addition, chromosomal instability leads to the activation of the cGAS-STING pathway and downstream noncanonical NF-κB signaling, thus promoting tumor metastasis.53 Following initiation of downstream signaling, STING is degraded in the lysosome.54

Notably, STING can also be activated in a cGAS-independent manner. The detection of nuclear DNA damage by ataxia telangiectasia mutated (ATM) and interferon-ν-inducible factor 16 (IFI16) activates STING, inducing NF-kB activation in a cGAS-independent manner.55 Moreover, STING can also be directly activated by bacterial cyclic dinucleotides, such as cyclic di-GMP, cyclic di-AMP, and 3′-3′-cGAMP to regulate bacterial cellular processes.33,34,56,57 Thus, STING acts as a PRRs independent of cGAS. However, these bacterial cyclic dinucleotides show lower affinity for STING compared with 2′3′-cGAMP,32 and the clear mechanism is unknown.

3 | SELF-DNA SENSING BY THE cGAS-STING PATHWAY

The presence of DNA in the cytoplasm is usually a sign of pathogen invasion and is quickly detected by the cGAS-STING pathway to efficiently trigger anti-infection immune responses. However, self-DNA accumulation in the cytoplasm due to cellular damage could also activate a cGAS-mediated immune response. Excessive activation of cGAS by self-DNA leads to severe autoimmune disease. Herein, we review how the organism minimizes the self-DNA exposure to the cytoplasm in health, and report the cases on self-DNA induced the cGAS-STING pathway activation under sustained stress.

3.1 | Restriction of self-DNA by DNases

cGAS senses dsDNA irrespective of its sequence, therefore, it cannot discriminate self-DNA from foreign DNA. However, deoxyribonucleases (DNases) degrade self-DNA under normal conditions in different compartments to prevent aberrant activation of cGAS-mediated immune responses. To date, four different DNases have been attributed this function: DNase I, DNase IL3, DNase II, and TREX1 (or DNase III). Herein, the role of these DNases in DNA surveillance is reviewed in detail (Figure 3).

Cytosolic DNA exonuclease, TREX1, clears cytosolic DNA to prevent endogenous DNA accumulation (Figure 3).58,59 Mutations in the human TREX1 gene cause a spectrum of autoimmune disorders, including Aicardi-Goutiéres syndrome (AGS), familial chilblain lupus (FCL), retinal vasculopathy with cerebral leukodystrophy (RVCL) and systemic lupus erythematosus (SLE) (Figure 3).60-63 AGS is a leukodystrophy resulting from immune-mediated destruction of myelin that presents in infancy as progressive neurologic decline,
and is a genetic mimic of the sequelae of transplacentally acquired viral infection, with
~75% of patients being profoundly disabled in the first few years of life.\textsuperscript{64,65} Patients with
AGS that have a \textit{TREX1 mutation} show constitutive production of cGAS-mediated type I
interferon due to the accumulation of cytosolic DNA.\textsuperscript{66} \textit{Trex1}-deficient mice develop a high
level of interferons, leading to inflammatory myocarditis, lymphoid hyperplasia, vasculitis,
and kidney disease.\textsuperscript{67-71} These abnormal phenotypes can be fully rescued by \textit{Cgas} deletion.
\textsuperscript{72,73}

Lysosomal DNase II plays a central role in the clearance of dsDNA generated through
apoptosis and phagocytosis (Figure 3).\textsuperscript{74,75} Biallelic loss-of-function mutations in human
\textit{DNASE2}, associated with a loss of DNase II endonuclease activity, lead to an
autoinflammatory state with markedly enhanced type I interferon signaling (Figure 3).\textsuperscript{76}
\textit{Dnase} 2-deficient mice die of severe anemia in late embryogenesis,\textsuperscript{77} which are rescued by
\textit{Sting} deletion but not \textit{Ggas} deletion,\textsuperscript{78} indicating the involvement of a cGAS-independent
DNA sensor.

DNase I and DNase IL3 are grouped together, as they are both secreted extracellular
DNases, and deficiency in either is associated with SLE (Figure 3). SLE is a common
human autoimmune disease that can affect many different organ systems, including the
lungs, resulting in a broad spectrum of clinical disease. A heterozygous nonsense mutation
in exon 2 of human \textit{DNASE1} with decreased DNASE1 activity causes SLE.\textsuperscript{79,80} Loss-of-
function variant in \textit{DNASE1L3} causes a familial form of SLE.\textsuperscript{81} In addition, \textit{Dnase1}-
deficient mice spontaneously develop an SLE-like phenotype,\textsuperscript{82,83} and \textit{Dnase1L3} deficiency
increases the susceptibility of the mice to polygenic SLE.\textsuperscript{84} However, there is no direct
evidence showing the participation of the cGAS-STING pathway, and the underlying
mechanism of how DNase deficiency contributes to clinical disease needs to be fully
elucidated.

### 3.2 Compartmentalization

Sequestration of cGAS in the cytosol prevents it from accessing nuclear or mitochondria
dna in physiological conditions. This compartmentalization is an essential prerequisite for
the appropriate function of cytosol cGAS. However, nuclear integrity is compromised during
normal biological processes, such as mitosis. In these conditions, cGAS is accessible to
nuclear self-DNA. This conundrum is explained by a recent finding that tight nuclear
tethering maintains the resting state of cGAS and prevents autoreactivity.\textsuperscript{85}

Under certain pathological conditions such as chromosomal instability, genomic DNA is
released into the cytoplasm, where it activates cGAS-mediated immune responses.
Chromosomal instability in cancer cells has been shown to activate the cGAS-STING
pathway via micronuclei formation, thus promoting tumor progress.\textsuperscript{53,86-88} In addition, self-
dNA from dying acinar cells could activate STING signaling to promote inflammation in a
mouse acute pancreatitis model.\textsuperscript{89}

Despite nuclear DNA being the main source of cytosol dsDNA for cGAS activation,
mitochondrial DNA (mtDNA) also serves as a cell-intrinsic cGAS ligand in certain contexts.\textsuperscript{90-93} Mitochondrial apoptosis is mediated by \textit{BAK} and \textit{BAX}, two pro-death proteins that
induce mitochondrial outer membrane permeabilization. BAK and BAX promote the formation of large macro-pores in the mitochondrial outer membrane, which further induces the inner mitochondrial membrane to balloon out into the cytoplasm, resulting in mitochondrial herniation. The loss of membrane integrity allows the mtDNA to escape into the cytoplasm during apoptosis.\textsuperscript{94} These mtDNAs bind to and activate cGAS-mediated type I interferon production, thus causing inflammatory disease.\textsuperscript{91,95} The release of mtDNA to the cytosol has been described as a major driver of obesity-associated chronic inflammation through the activation of the cGAS-STING pathway.\textsuperscript{96} In addition, mtDNA released from hepatocytes due to high-fat-diet could be engulfed by liver resident macrophages to induce inflammatory cytokine secretion in a STING-dependent manner.\textsuperscript{97} Mitochondrial dysfunction and subsequent activation of the cGAS-STING signaling is a critical regulator of kidney injury and fibrosis.\textsuperscript{98,99} Recently, Huang et al reported mtDNA activates cGAS-mediated cGAMP generation, which suppresses endothelial cell proliferation, thus promoting lung inflammatory injury.\textsuperscript{100} Interestingly, in the context of infection, there is an escape of mtDNA into the cytoplasm, where it activates cGAS-mediated type I interferon generation, conferring broad pathogen resistance.\textsuperscript{101–103} However, these mtDNA-induced cGAS-mediated antiviral responses are suppressed by apoptotic caspases.\textsuperscript{104,105}

Apart from aforementioned mechanisms, cGAS is also regulated by the ionic environment. The binding between positively charged surfaces of cGAS and negatively charged DNA requires extensive ionic interactions. However, these interactions are vulnerable to cytosol salt concentrations, and thus spurious activation of cGAS by self-DNA is prevented below a certain threshold.\textsuperscript{28}

Collectively, self-DNA accumulation due to defective clearance by DNases or leakage from the nucleus or mitochondria leads to the activation of cGAS-mediated immune responses and inflammatory disease.

4. SELF-DNA ACCUMULATION IN LUNG DISEASE

Endogenous DNA accumulation is predicted to be a common disease-causing event. Self-DNA accumulation results in autoinflammatory and autoimmune disease, such as aforementioned Aicardi-Goutiéres syndrome (AGS) and systemic lupus erythematosus (SLE).\textsuperscript{72,106} Currently, many studies directly demonstrate that self-DNA is associated with lung diseases, such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), and asthma.

4.1. Cystic fibrosis

Cystic fibrosis is a multisystem disease characterized by persistent bronchopulmonary infections, pancreatic insufficiency, and increased sweat chloride concentrations. Due to abnormal transport of chloride across respiratory epithelial cells, patients with CF have thick, viscous secretions. Another contributor to the viscosity of airway secretions in patients with CF is extracellular DNA, a byproduct of degraded neutrophils.\textsuperscript{107} Recombinant human DNase I (rhDNase I), or dornase alfa, selectively cleaves extracellular DNA and reduces the viscosity of purulent sputum, and thus improving pulmonary function in patients with CF.\textsuperscript{108,109} Treatment with rhDNase I is also effective in re-establishing airway patency for the
treatment of persistent lobar atelectasis in new-born and pediatric populations.\textsuperscript{110} The use of rhDNase I for lobar atelectasis treatment was also reported in a lung cancer patient in 2019.\textsuperscript{111} In a mouse silica-induced lung inflammation model, DNase I treatment reduces the amount of dsDNA in the bronchoalveolar space, preventing STING pathway activation.\textsuperscript{112} In patients with CF, whether the cGAS-STING pathway is activated by the DNA accumulation as well as whether rhDNase also prevents STING activation is unclear and warrants further investigation.

4.2 Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease is a severe chronic inflammatory disease, characterized by increased inflammatory response in the airways which leads to critical lung damage in some cases.\textsuperscript{113} Maluf et al showed an increase in the level of DNA damage in peripheral blood of COPD patients.\textsuperscript{114} Moreover, Avriel et al showed that cell-free DNA levels can be used to identify COPD patients at an increased risk of poor outcomes. COPD patients with high serum levels of cell-free DNA had an increased 5-year mortality risk.\textsuperscript{115} However, the source of this DNA in COPD patients is unclear. COPD patients showed cellular abnormalities that corresponded to cell death associated with apoptosis and necrosis; this observation provides one possible explanation for the peripheral DNA. Intriguingly, there is also a substantial increase in mtDNA strand breaks and/or abasic sites in lung tissues of COPD patients.\textsuperscript{117} Tobacco smoking is one of the major causes of COPD, which induces oxidant-antioxidant imbalance characterized by excessive production of reactive oxygen species leading to DNA damage, cell death, and subsequent pulmonary inflammation.\textsuperscript{117} COPD patients who had once smoked or been exposed to biomass have increased DNA damage;\textsuperscript{118} this DNA damage and senescence induced the dysfunction of endothelial progenitor cells in smokers and COPD patients.\textsuperscript{119} In a mouse model of cigarette smoke, Nascimento et al found that cigarette smoke causes respiratory barrier damage, inducing self-DNA release. This self-DNA activates the cGAS-STING pathway, triggering type I IFN-dependent lung inflammation, which is attenuated in cGAS, STING or type I IFN receptor-deficient mice.\textsuperscript{120} Further investigation of whether the cGAS-STING pathway is also involved in self-DNA sensing and pathogenesis in COPD patients may lead to therapeutic targets.

4.3 Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis is a fatal interstitial lung disease characterized by irreversible destruction of lung architecture and abnormal wound healing, leading to disruption of gas exchange and death from respiratory failure.\textsuperscript{121} Ryu et al showed that mtDNA concentrations are elevated in the bronchoalveolar lavage (BAL) fluid and plasma of patients with IPF, and display robust association with disease progression and reduced survival.\textsuperscript{122} Thus, circulating mtDNA serves as a prognostic biomarker for IPF. Further studies showed that ER stress and PINK1 deficiency in lung type II alveolar epithelial cells could result in mitochondrial stress with significant oxidation and damage of mtDNA and subsequent extracellular release.\textsuperscript{123} Senescence and mitochondrial stress are mutually reinforcing age-related processes that contribute to IPF. Schuliga et al delineate mitochondrial dysfunction induced superoxide contributes to the senescent phenotype of lung fibroblasts from IPF patients.\textsuperscript{124} Additionally, the mtDNA levels in the cytosol and medium of lung fibroblasts

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from IPF patients are higher than in age-matched controls, and cGAS immunoreactivity was observed in regions of fibrosis associated with fibroblasts in lung tissue of IPF patients, indicating that cGAS-mediated self-DNA sensing is involved in lung fibroblasts from IPF patients.125

Silicosis is an interstitial lung disease caused by inhalation of silica or quartz. It is characterized by chronic inflammation leading to severe pulmonary fibrosis and is also associated with increased risk of cancer, tuberculosis, and COPD. Patients with silicosis exhibited increased plasma levels of dsDNA and higher CXCL10 concentration in sputum than healthy controls.112 In mice, silica triggers lung cell death and self-dsDNA release in the bronchoalveolar space which further activates the STING pathway.112 Of note, silica exposure-induced self-dsDNA release and STING pathway activation could potentiate the host response to M tuberculosis DNA via initiation of type 2 immunity in mice.126

4.4 Asthma

Asthma is a chronic inflammatory lung disease characterized by bronchial hyperresponsiveness, episodic exacerbations, and reversible airflow obstruction. Sputum neutrophils are associated with severe asthma,127 and contribute to airway inflammation in severe asthma via neutrophil-derived extracellular DNA,128 which is released in chromatin filaments forming weblike structures with granular proteins called neutrophil extracellular traps (NETs).129,130 High concentration of extracellular host-derived DNA in sputum positively correlates with more severe asthma, along with increases in NETs and inflammasome activation in the airways.131 Respiratory viral infections are the most common cause of allergic asthma exacerbations, and it has been shown that host self-DNA released by NETs promotes rhinovirus-induced type-2 allergic asthma exacerbation in a mouse model of allergic airway hypersensitivity.132 Moreover, it has been shown that the STING-TBK1-IRF3 axis is required for cGAMP-induced allergic inflammation.133 A recent study has directly demonstrated that there is an increased accumulation of cytosolic dsDNA in the airway epithelial cells in mice with OVA and house dust mite (HDM)-induced asthma.134 Notably, cGAS deletion in airway epithelial cells significantly attenuates the allergic airway inflammation induced by OVA or HDM.134 Collectively, these observations indicate that extracellular self-DNA contributes to the pathogenesis of asthma, and cGAS-STING signaling is involved.

5 cGAS-STING SIGNALING IN LUNG DISEASE

Transient activation of cGAS-STING signaling is essential for initiating innate immunity to microbial invasions and involves induction of type I interferons and inflammatory cytokines. However, sustained activation of this pathway is responsible for the development of inflammatory disorders and autoimmune disease. As mentioned above, chronic inflammatory lung disease exhibits self-DNA accumulation, which could possibly act as the cGAS ligands to activate the cGAS-STING pathway, and thus exacerbating the inflammatory condition (Figure 4). Targeting the cGAS-STING pathway provides potential avenues in anti-inflammatory therapy.
5.1 | A unique type I interferonopathy with lung manifestation in STING mutants

Gain-of-function mutations in the TMEM173 gene (encoding STING) lead to a newly classified autoinflammatory disease called STING-associated vasculopathy with onset in infancy (SAV).50,135,136 SAVI is an autosomal-dominant disease characterized by systemic inflammation, severe skin vasculopathy, interstitial lung disease, and recurrent bacterial infection.50,135,136 De novo and inherited TMEM173 mutations were found in SAVI patients. De novo TMEM173 mutations manifest as an early-onset (within the first 8 weeks of life) and severe phenotype, whereas inherited TMEM173 mutations manifested as a late-onset and mild phenotype.50,135,136 In 2014, Liu et al first reported SAVI caused by de novo N154S, V155M or V147L heterozygous missense mutation in TMEM173 in six unrelated children.50 During the same year, Jeremiah et al reported SAVI with inherited V155M heterozygous mutation in TMEM173 in four individuals of a family.136 Subsequently, more cases of SAVI caused by gain-of-function mutations in TMEM173 have been reported.137-142 These gain-of-function mutations in the TMEM173 gene induce spontaneous dimerization and activation of STING in the absence of cGAMP, leading to elevated transcription of IFNB1 and other gene targets of STING.50 Additionally, in patients’ fibroblasts, the gain-of-function of STING mutants mainly localized in the Golgi and the perinuclear vesicle at the steady state, a hallmark of the STING activation, indicating the constitutive activation of STING.136 Mice models of SAVI comprising N153S and V154M develop a hierarchy of immune abnormalities, lung inflammation, and fibrosis similar to that of patients with SAVI. However, these phenotypes do not depend on either IFN-α/β receptor signaling or mixed lineage kinase domain-like pseudokinase (MLKL)-dependent necroptotic cell death pathways as reported in humans.143-145 Unexpectedly, V154M mutant mice have more robust STING activation and develop lung fibrosis, while N153S mutant mice have a weaker STING activation and only develop lung inflammation, indicating murine models of SAVI mutations reflect different aspects of the human disease.144 Because lung fibrosis is a common complication of SAVI patients, the V154M mouse is a useful tool for dissecting the role of the STING pathway in pulmonary disease and provides an excellent model for assessing possible STING antagonists for the treatment of SAVI patients.

5.2 | Lung inflammation and fibrosis

Activation of cGAS-STING has been implicated in inflammation of various tissues. Interferon signaling driven by cGAS has been shown to promote noncanonical inflammasome activation in age-related macular degeneration.146 The STING signaling was reported to promote sterile inflammation in experimental acute pancreatitis,89 and cGAS-STING activation in mouse adipose tissue promotes obesity-associated chronic inflammation.96 It has also been shown that cGAS-STING activation induces tubular inflammation and progression of acute kidney injury.98 Consistent with these studies, the cGAS-STING pathway has also been linked to lung injury and inflammation. Deficiency in cGAS or STING ameliorates silica-induced lung inflammation,112 and endothelial cGAS signaling activation promotes inflammatory lung injury.100 In addition, cGAS-STING activation by self-DNA release upon cigarette smoke exposure leads to type I interferon-dependent lung inflammation.120 Recently, Han et al reported that airway epithelial cGAS is critical for the induction of allergic airway inflammation in mice.134 Altogether, these findings indicate cGAS-STING activation is a critical driver of lung inflammation.
Lung inflammation can progress to lung fibrosis, which causes scarring and damage to lung tissue. A recent study reported that cGAS augments lung fibroblast senescence involving damaged self-DNA, and targeting cGAS suppresses the senescent-like response. Moreover, the STING signaling pathway is activated in the lungs of patients with fibrotic interstitial lung disease, characterized by STING overexpression, phosphorylation and dimer formation, TBK1 and IRF3 phosphorylation, and CXCL10 production.

Collectively, these studies indicate that activation of the cGAS-STING pathway contributes to lung inflammation and fibrosis, revealing targeting of this pathway is beneficial in lung inflammatory disease.

5.3 Coronavirus disease 2019 (COVID-19)

cGAS, a universal DNA sensor, can directly bind to pathogen DNAs derived from bacteria, DNA viruses, or RNA retrovirus such as HIV-1 and elicit robust immune responses, which are essential for the protection of the organism against pathogen invasion. However, besides the direct recognition of PAMPs from the pathogen, in some cases, cGAS is also involved in DAMP-based pathogen detection in a much broader range, including RNA viruses. This is because infection with some pathogens induces tissue damage and self-DNA release, thus activating a cGAS-mediated immune response. Herpesvirus infection induces mtDNA stress and promotes the release of mtDNA into the cytosol, where it engages the cGAS-STING-IRF3-dependent signaling to enhance type I interferon antiviral responses. An in vitro study showed that pneumolysin could initiate oxidative damage to mitochondria, resulting in the subsequent release of mtDNA, which mediates IFN-β expression in macrophages.

COVID-19 caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread to produce a global pandemic. In patients with COVID-19, levels of 14 cytokines are increased; among them, CXCL10, CCL7, and IL-1 receptor antagonists are significantly higher in severe cases and are associated with increased viral load, loss of lung function, lung injury and a fatal outcome. CXCL10 is a prototypical ISG, indicating the activation of type 1 interferon response. Zuo et al reported that sera from patients with COVID-19 have elevated levels of cell-free DNA, which strongly correlates with acute-phase reactants. In addition, the severity of COVID-19 correlates directly with plasma myeloperoxidase (MPO)-DNA complexes (NETs). Self-DNA release due to cellular stress or tissue damage after SARS-CoV-2 infection could potentially activate the cGAS-STING pathway, leading to excessive production of the type 1 interferon and inflammatory cytokines, and contributing to the severity of COVID-19. Investigating the role of the cGAS-STING pathway in the pathogenesis of COVID-19 may provide a potential therapeutic strategy.

6 CONCLUSION AND FUTURE DIRECTIONS

The cGAS-STING pathway has emerged as the major pathway for DNA sensing and plays a fundamental role in microbial surveillance. The role of this pathway in antiviral immunity makes it highly attractive for antivirus therapy or vaccine adjuvants. However, cGAS is a universal DNA sensor and cannot discriminate self from non-self DNA, thus both are capable of stimulating the cGAS-STING pathway. While self-DNA sensing through cGAS...
is valuable for recognition of cellular or tissue damage, excessive activation of the cGAS-STING pathway by self-DNA becomes the underlying mechanism for inflammatory and autoimmune disease. Further studies are needed to elucidate how cGAS remains inactive during the normal biological process, such as mitosis during which the nuclear membrane is transiently breakdown.

Self-DNA release and cGAS-STING pathway activation are reported in diverse pulmonary diseases, providing promising therapeutic targets. In COPD patients, cell-free DNA levels inversely correlate with the outcome. The cGAS-STING pathway is associated with the progression of COPD in the mice, but its role in COPD patients is elusive so far. Circulating mtDNA serves as a prognostic biomarker for IPF, displaying a positive correlation with the disease progression and negative correlation with survival rate. In IPF patients, cGAS-mediated self-DNA sensing is engaged during the disease progression. The STING signaling pathway is over-activated in the lungs of patients with fibrotic interstitial lung disease, indirectly verifying the engagement of the cGAS-STING pathway in disease progression. Recently, cGAS-mediated mtDNA sensing was reported to activate a self-injurious and anti-regenerative response in the endothelium during murine acute lung injury, inspiring us to consider the potential role of this pathway in other cell types, such as epithelium and immune cells. However, the cellular source of these self-DNA fragments remains elusive, and it also remains unclear whether cell-free self-DNA can directly activate the cGAS-STING pathway in the patients with chronic lung disease. Neutrophil extracellular traps (NETs), also known as NETosis, are extracellular DNA complexed with antimicrobial proteins and are essential to fight against infectious pathogens. However, an overabundance of NETs has been implicated in a number of lung diseases, such as CF, COPD, asthma, and acute respiratory distress syndrome. Whether NETs could serve as a dsDNA source for cGAS-STING signaling activation needs further investigation.

Oxidative stress and mitochondrial dysfunction also serve as a potential dsDNA origin in pulmonary disease. Increasing evidence indicates that mitochondrial integrity and function are impaired or altered in various chronic lung diseases, such as COPD, asthma, pulmonary fibrosis, and lung cancer. In addition, reactive oxygen species (ROS) generated during inflammatory processes can oxidize DNA, leading to epithelial cell injury and death. Persistent bacterial lung infections despite the use of antibiotics lead to chronic pulmonary diseases, and the majority of the extracellular DNA derives from the host. Whether infection-induced tissue damage provides the origin of extracellular DNAs and whether these DNAs aggravate inflammation by activating cGAS-STING signaling warrant further study.

Loss-of-function mutation in DNASE induces autoimmune diseases, such as AGS and SLE. Gain-of-function mutation in STING results in SAVI. Targeting the cGAS-STING pathway with small-molecule inhibitors provides new opportunities for treating these diseases. Several antimalarial drugs (AMDs) and new molecules were identified as effective inhibitors of cGAS by blocking cGAS-dsDNA interaction and thus inhibiting IFN-β production. Because AMDs have been widely used in human diseases and have an excellent safety profile, thus provide warranty for treatment of cGAS-dependent inflammatory diseases. In addition, highly potent and selective small-molecule antagonists of STING protein have
been discovered and could attenuate pathological features of autoinflammatory disease in mice, which is promising for SAVI patients.

The COVID-19 outbreak, caused by the newly described viral pathogen SARS-CoV-2, is having a devastating effect worldwide. CXCL10 levels are significantly higher in patients with severe COVID-19. The hallmark signaling output of the cGAS-STING pathway is the transcriptional up-regulation of type I IFNs and other IRF3 target genes, such as ISGs. Although CXCL10 has diverse sources apart from the cGAS-STING pathway, a plethora of cell death in the lung might induce self-DNA accumulation. Thus, it will be essential to test the DNA concentration in the bronchoalveolar lavage. Further elucidating the role of cGAS-STING-mediated cytokine secretion in the patients with COVID-19 is critical and might provide new opportunities for the treatment.

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Abbreviations:

AGS Aicardi-Goutiéres syndrome
AIM2 absent in melanoma 2
ALI acute lung injury
AMDs antimalarial drugs
ARDS acute respiratory distress syndrome
ARF ADP-ribosylation factor
ASC apoptosis-associated speck-like protein containing a CARD
ATM ataxia telangiectasia mutated
BAK bcl-2 antagonist/killer
BAL bronchoalveolar lavage
BAX bcl-2-associated X protein
CF cystic fibrosis
cGAMP cyclic dinucleotide cyclic GMP-AMP
cGAS cyclic GMP-AMP synthase
COPD chronic obstructive pulmonary disease
| Abbreviation | Full Form |
|--------------|-----------|
| COP-II       | coat protein complex II |
| COVID-19     | coronavirus disease 2019 |
| CXCL10       | C-X-C motif chemokine ligand 10 |
| DAMPs        | damage-associated molecular patterns |
| ERGIC        | ER-Golgi intermediate compartment |
| FCL          | familial chilblain lupus |
| HMD          | house dust mite |
| IFI16        | interferon-v-inducible factor 16 |
| IFN          | interferon |
| IPF          | idiopathic pulmonary fibrosis |
| IRF3         | interferon regulatory factor 3 |
| ISGs         | interferon-stimulated genes |
| JAKs         | Janus kinases |
| IpaJ         | invasion plasmid antigen J |
| MAVs         | mitochondrial antiviral-signaling protein |
| MLKL         | mixed lineage kinase domain like pseudokinase |
| NETs         | neutrophil extracellular traps |
| NF-kB        | nuclear factor-κB |
| OVA          | ovalbumin |
| PAMPs        | pathogen-associated molecular patterns |
| PINK1        | PTEN-induced kinase 1 |
| PRRs         | pattern recognition receptors |
| rhDNase I    | recombinant human DNase I |
| RLRs         | retinoic acid-inducible gene-I (RIG-I)-like receptors |
| ROS          | reactive oxygen species |
| RVCL         | retinal vasculopathy with cerebral leukodystrophy |
| SARS-CoV-2   | severe acute respiratory syndrome coronavirus 2 |
| SAVI         | STING-associated vasculopathy with onset in infancy |
| SLE          | systemic lupus erythematosus |
STAT  signal transducer and activator of transcription
STIM1  stromal interaction molecule 1
STING  stimulator of interferon genes
TBK1  TANK-binding kinase 1
TLR  toll-like receptor
TRAF6  TNF receptor associated factor 6
TREX1  three prime repair exonuclease 1
YAP1  yes-associated protein 1

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FIGURE 1.
The DNA sensors and their signaling pathways. The mammalian cell has evolved a number of sensors able to recognize anomalous DNA present in the cytosol and to trigger innate immune responses, including TLR9, AIM2, and cGAS. TLR9 localizes in the endosomal compartment and is involved in the recognition of hypomethylated CpG-rich DNA. Upon recognition of DNA, TLR9 recruits the common TLR adaptor MyD88 and activates IRF7 and NF-κB, leading to type I IFN and proinflammatory cytokine production. AIM2 and cGAS are located in the cytoplasm. AIM2 is composed of a C-terminal HIN-200 domain and an N-terminal pyrin domain. Cytosolic dsDNA binds to the HIN-200 domain of AIM2, leading to the activation of AIM2. The pyrin domain of activated AIM2 recruits and interacts with the pyrin domain of ASC, and the CARD of ASC binds to the CARD of pro-caspase-1, forming the AIM2 inflammasome. Active caspase-1 cleaves pro-IL-1β and pro-IL-18, resulting in the release of mature IL-1β and IL-18. Meanwhile, active caspase-1 induces pyroptosis via the proteolytic cleavages of the N-terminal fragment of gasdermin D. Cytosolic DNA binds to and activates cGAS, inducing the production of cGAMP. Then cGAMP binds to its ER adaptor STING, leading to the activation of TBK1, IRF3, and NF-κB, inducing the transcription of genes encoding type I IFNs and proinflammatory cytokines. AIM2, absent in melanoma 2; cGAMP, cyclic GMP-AMP; cGAS, cyclic GMP-AMP.
AMP synthase; dsDNA, double-stranded DNA; ER, endoplasmic reticulum; IFN, interferon; STING, stimulator of interferon genes
FIGURE 2.
The cGAS-cGAMP-STING mediated DNA sensing and signaling. cGAS is an innate immune sensor recognizing a diverse array of aberrant cytosolic dsDNA, which include pathogen-derived DNA from bacteria, DNA viruses, or reverse-transcription of retroviruses, and self-DNA from nuclei or mitochondria of dead or damaged cells. Cytosolic DNA binds to and activates cGAS, which catalyzes cGAMP synthesis from GTP and ATP. cGAMP then binds to and activates STING at the ER membrane. Activated STING translocates to the Golgi compartments, where it interacts with TBK1 or IκB kinase (IKK), which is facilitated by the palmitoylation of STING. TBK1 phosphorylates STING, which in turn recruits IRF3 for phosphorylation by TBK1. Phosphorylated IRF3 dimerizes and enters the nucleus, where it stimulates the transcriptional expression of type I IFNs. In parallel, IκBα phosphorylation by IKK results in the translocation of NF-κB to the nucleus and the corresponding transcriptional expression of inflammatory cytokines. Notably, in some contexts, the synthesis and release of type I IFNs could induce the interferon-stimulated genes (ISGs) expression via the JAK/STAT signaling pathways in a positive feedback loop.
FIGURE 3.
DNases protect against cGAS-mediated immune activation. DNA is normally compartmentalized to the nucleus and mitochondria, yet high levels of DNA in the cytoplasm stimulate DNA sensors, such as cGAS. A, Different forms of cell death are associated with the release of DNA into the tissue and bloodstream. Extracellular nucleases such as DNase I and DNase IL3 provide the first line of protection by degrading the DNA. Cell surface-associated chromatin on microparticles from dying cells is degraded by DNase IL3, which contains a positively charged C-terminal peptide facilitating DNA digestion. DNase II is compartmentalized in endosomes and degrades the DNA from engulfed apoptotic debris. TREX1, localized in cytosol, is an abundant 3-5′-exonuclease, preventing endogenous DNA accumulation. DNases maintain cytosolic DNA levels under the threshold of cGAS activation, thus retaining immune silence (left); B, Dysfunction of DNases induced by mutations can lead to the accumulation of DNA in the cytoplasm, and therefore activation of cGAS-mediated immune responses. In humans, decreased DNase I activity or loss-of-function in DNASEIL3 are associated with SLE; biallelic loss-of-function mutations in human DNASE2 leads to an autoinflammatory state, called type I interferonopathy. Mutations in the human TREX1 gene cause a spectrum of autoimmune disorders, including AGS, FCL, RVCL, and SLE.
FIGURE 4.
The cGAS-STING pathway in lung disease. Lung exposure to infections or various sources of Inhaled environmental toxicants induces lung inflammation. Both infection and particulate toxicants induce host cell stress and cell death, leading to self-DNA release in the intracellular or extracellular milieu. The self-nuclear DNA or mtDNA could activate cGAS-STING pathways in various cell types, such as macrophages, dendritic cells, endothelium, and epithelium, resulting in type I IFNs and inflammatory cytokine release and lung inflammatory disease.