Regulation of cGAS-STING pathway - Implications for systemic lupus erythematosus

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Received August 31, 2021 accepted October 07, 2021

Abstract

Type I interferon (IFN-I) is implicated in the pathogenesis of systemic lupus erythematosus (SLE) and the closely associated monogenic autoinflammatory disorders termed the “interferonopathies.” Recently, the cytosolic DNA sensor cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS) and its downstream signaling adaptor stimulator of interferon genes (STING) have been identified as having important, if not central, roles in driving IFN-I expression in response to self-DNA. This review highlights the many ways in which this pathway is regulated in order to prevent self-DNA recognition and underlines the importance of maintaining tight control in order to prevent autoimmune disease. We will discuss the murine and human studies that have implicated the cGAS-STING pathway as being an important contributor to breakdown in tolerance in SLE and highlight the potential therapeutic application of this knowledge for the treatment of SLE.

Keywords

systemic lupus erythematosus • cGAS • STING • type I interferon

Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that has multi-organ involvement and presents with clinically varying phenotypic expression. It is more prevalent in females, with a female-to-male incidence ratio of approximately 9:1 during the fourth decade of life and may be seen more frequently in non-white populations.^[1] Manifestations of SLE include leukopenia, mucocutaneous lesions, vasculitis, arthritis, nephritis, and central nervous system (CNS) disease, among many others, with periods of flares and remissions. There is also a wide spectrum of disease manifestations from relatively mild symptoms to organ-threatening disease.^[2] The pathogenesis for this disease is complex and still relatively unknown, with several immunopathogenic pathways recently identified as possible contributory factors. The understanding of some of these pathways has resulted in potential new therapeutic targets, such as B cell activating factor (BAFF) or B lymphocyte stimulator (BLyS) and IFN- α (IFN α), among others. This has resulted in the approval of belimumab in 2011, which was the first agent specifically approved for SLE treatment since hydroxychloroquine in 1958.^[3, 4]

A more recent potential target has emerged in the literature, involving the cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS) and stimulator of interferon genes (STING) pathway, which induces type I interferon (IFN-I) in SLE.^[5] The involvement of the cGAS-STING pathway in SLE has largely stemmed from the discovery of monogenic autoinflammatory or autoimmune disorders that were characterized by defective regulation of the cGAS-STING pathway and overproduction of type I IFN, leading to them being described as “type I interferonopathies.”^[6, 7] The potential that cGAS-STING may also be dysregulated in SLE, another type I interferonopathy, was rapidly realized. cGAS is a cytosolic double-stranded DNA (dsDNA) sensor discovered in 2013 that is indispensable for host anti-viral immunity.^[8] Once activated, it synthesizes the second messenger cyclic GMP-AMP (cGAMP) that activates STING, an endoplasmic reticulum (ER) resident adaptor protein critical for induction of IFN- β (IFN β) downstream of immunostimulatory cytosolic DNA.^[9–11] Here, we discuss new insights into how the cGAS-STING signaling pathway is regulated, the

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studies supporting a role for cGAS-STING in the pathogenesis of SLE, and the potential therapeutic implications of targeting this pathway in developing treatments for SLE.

Type I IFNs and their role in SLE

IFN-I is a well-studied factor implicated in the pathogenesis of SLE, first reported as increased in active SLE serum as far back as the 1970s.^[12, 13] The IFN-I family is a major subgroup of cytokine proteins that are expressed in response to detection of viral, bacterial, or self-RNA and DNA by a group of pattern recognition receptors described as nucleic acid sensors. Subtypes include IFN α , of which there are 13 identified isoforms, as well as IFN β , IFN-epsilon (IFN ϵ), IFN-kappa (IFN κ), and IFN-omega (IFN ω).^[14] IFN-I bind to type 1 interferon receptors – which are a complex of two proteins, IFNAR1 and IFNAR2 – initiating a signal transduction cascade through activation of the Janus kinase/Signal transducer and activator of transcription (JAK/STAT) pathway, ultimately inducing the expression of IFN stimulated genes (ISGs).^[15–19] Studies have shown that serum IFN-I levels and ISGs are elevated in peripheral blood mononuclear cells (PBMCs) of patients with SLE and positively correlate with disease activity.^[13, 20–33] Elevated levels of IFN-I activity have also been positively correlated with anti-double-stranded DNA (anti-dsDNA) antibody positivity and hypocomplementemia in patients with SLE.^[27] Furthermore, skin or kidney samples from patients with cutaneous lupus or lupus nephritis have demonstrated elevated ISG activity.^[34–36] In addition, treatment of patients with viral infections such as hepatitis C or even malignancy with IFN α has resulted in induction of SLE symptoms in recipients.^[37, 38] These findings have suggested IFN-I as a significant cause for the pathologic manifestations seen in SLE.^[39]

RNA/DNA sensors not only recognize viral and bacterial RNA/DNA but can also recognize self or endogenous nucleic acids. Tight regulation of these sensors is therefore required to prevent their activation, type I IFN production, and development of SLE-like disease. The methods to prevent inappropriate recognition include intracellular and extracellular RNA- and DNA-degrading enzymes, in addition to sequestration or compartmentalization of nucleic acids to prevent detection (such as in the nucleus or mitochondria). However, when cells are damaged or stressed, nucleic acids are released extracellularly (either via apoptosis or necrosis or via release in exosomes) or into the cytosol from subcellular compartments such as the mitochondria or nucleus, providing the potential for activation of nucleic acid sensors.^[40, 41] Sources of cytosolic DNA in SLE include mitochondrial DNA,^[42] endogenous retroviral elements,^[43] genomic DNA, and oxidized DNA damage products.^[44] Whereas the involvement of endosomal toll-like receptor (TLR)7 and TLR9 in nucleic acid sensing in SLE has been known for nearly 20 years,^[45–48] the role that the cytosolic DNA sensor cGAS might play in SLE pathogenesis or type I IFN

production has only recently been uncovered.^[49] Importantly, while TLR7 and TLR9 expression is largely restricted to subsets of immune cells (myeloid and B cells predominantly), cGAS and STING are broadly expressed in immune and non-immune cells, thus increasing the potential that defective cGAS signaling in either may be important in IFN-driven autoimmune disease.^[50] Interestingly, both cGAS and STING are also ISGs, which is a positive feature in viral infection but less so when considering the potential amplifying effect their enhanced expression might have on IFN expression in the context of SLE.^[49]

Regulation of cGAS-STING Signaling

Much of our understanding regarding the importance of regulating cGAS-STING signaling has come from the discoveries of monogenic IFN-driven diseases such as Aicardi-Goutier syndrome (AGS), which is caused by mutations in *TREX1*, a DNase that prevents cytosolic DNA detection via DNA degradation, and STING-associated vasculopathy with onset in infancy (SAVI), which is driven by activating mutations in *STING*.^[6, 7] More recently an additional monogenic SLE-like disease was discovered where mutations in the *COPA* gene result in inappropriate trafficking and regulation of STING and enhanced IFN production.^[51, 52]

Activation of the cGAS-STING pathway (outlined in Figure 1) is initiated by binding of dsDNA to cGAS, triggering a conformational change in the active site which then catalyzes the formation of cGAMP from adenosine triphosphate (ATP) and guanosine triphosphate (GTP).^[8, 53, 54] cGAMP acts as a second messenger and binds to STING, embedded in the ER membrane, triggering a conformational change that promotes dimerization of STING.^[55, 56] The STING dimer then translocates from the ER to the Golgi, where it recruits TANK-binding kinase 1 (TBK1) to form STING-TBK1 aggregates – the STING signalsome – which binds and recruits the transcription factor IRF3.^[55, 57] Recruitment of IRF3 is facilitated by phosphorylation of serine 366 on STING by TBK1, which in turn phosphorylates IRF3 once it binds.^[58, 59] Clustering of STING at the Golgi also promotes palmitoylation and full activation of STING.^[60] STING can also cluster with I kappa B ($\text{I}\kappa\text{B}$) Kinase (IKK) and drive activation of NF κB following the phosphorylation and degradation of $\text{I}\kappa\text{B}$.^[57, 58, 61, 62] IRF3 and NF κB translocate into the nucleus and function together to induce the expression of IFN β and pro-inflammatory cytokines.^[54, 63]

Regulation of cGAS-STING signaling is achieved on a number of levels as shown in Figure 1 – access to cGAMP, localization of the various components, and posttranslational modifications that alter the stability and function of either cGAS or STING (reviewed by Wan^[64]). For example, localization of both cGAS and STING is closely coupled with function. cGAS was first thought to be solely a cytosolic protein, thus preventing inappropriate access to self-DNA.^[65] However, later studies

Figure 1: Overview of the cGAS-STING pathway. cGAS activity can be negatively regulated via phosphorylation of Serine 291 by CDK1 and AKT, triggering sequestration of cGAS to histone. cGAS binding to PIP2 also restricts its ability to interact with and sense free cytosolic DNA. Untethered cGAS may freely bind cytosolic viral, mitochondrial, bacterial, or damaged nuclear DNA and form homodimers to generate cGAMP. cGAMP generated this way or transported into the cytosol via transporter proteins SLC19A1, P2RX7, or LRRC8A:C bind to and activate STING, which then homodimerizes and translocates to the Golgi. cGAMP-bound STING homodimers recruit TBK1, which drives STING phosphorylation at Serine 366 to initiate IRF3 and IKK/NFκB phosphorylation, nuclear translocation, and transcription of the interferon-driven immune response. AMP, adenosine monophosphate; CDK1, cyclin-dependent kinase 1; cGAMP, 2'3'-cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP); cGAS, cyclic GMP-AMP synthase; IFN, interferon; IRF3, IFN response factor 3; IKK, IκB kinase; LRRC8A:C, leucine-rich repeat-containing protein 8A:C; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; P2RX7, P2X purinoceptor 7; PIP2, phosphatidylinositol; STING, stimulator of IFN genes; TBK1, tank-binding kinase 1. Figure created with Biorender.com.

found that cGAS can be found in the nucleus, with one study showing that nuclear tethering was essential for preventing autoreactivity,^[66] and additional studies showing that chromatin tethering was via interactions with the histone H2A-H2B dimer leading to cGAS inactivation in order to block recognition of DNA during mitosis.^[67, 68] cGAS also interacts with the plasma membrane inner leaflet via binding to phosphoinositol 4,5-bisphosphate (PIP2), an interaction that sequesters cGAS and prevents self-DNA detection and activation.^[65] Alterations that result in mislocalization of STING also impact its activity. COPA syndrome is a recently identified inherited disorder that results from point mutations in the *COPA* gene. COPA is an integral part of the transport machinery that facilitates retrograde transport of proteins from the Golgi back to the ER.

COPA mutations are associated with enhanced STING signaling from the Golgi as a result of failure to return STING signaling complexes to the ER.^[52, 69–72] Interaction with the calcium sensor stromal interaction molecule 1 (STIM1) also acts to inhibit STING by retaining it in the ER. Not surprisingly, deletion of *Stim1* results in increased IFN and ISG expression following viral infection.^[73, 74]

cGAS-STING activity can also be regulated through availability of the second messenger cGAMP. Recent evidence is emerging that in addition to being synthesized by cGAS, cGAMP can be transferred between cells or taken up from the extracellular milieu by cGAMP importers to directly activate STING. Regarding between cell transfer, two independent studies

showed how cGAS activation by DNA virus or retrovirus drove encapsulation of cGAMP into viral particles that could then trigger an antiviral state in a STING-dependent manner in nearby target cells.^[75, 76] cGAMP is also released by dead and dying cells into the extracellular milieu and thereby facilitates its role as a damage-associated molecular patterns (DAMP) or alarmin. Due to its negative charge, cGAMP must be actively imported into cells to have an effect on STING. While there is some evidence that it is internalized via clathrin-mediated endocytosis, recently the reduced folate carrier SLC19A1, which is highly expressed on myeloid cells, has been shown by two independent groups to act as a cGAMP importer on monocytes and macrophages.^[77, 78] Interestingly, an association between polymorphisms in SLC19A1 with increased risk of developing SLE has been demonstrated, making SLC19A1 an interesting target for further evaluation in SLE.^[79] In addition to SLC19A1, two other cGAMP importers have recently been described – P2RX7 which is found on tumor-associated macrophages and facilitates cGAMP uptake in an ATP-dependent manner,^[80] and LRRC8A:C which is found on endothelial cells and facilitates cGAMP uptake.^[81] cGAMP is also targeted by extracellular hydrolase ENPP1, suggesting an additional way in which cGAMP levels and activity may be dysregulated in SLE.^[82, 83] Thus, understanding how cGAMP and the proteins that regulate its stability and uptake into cells will be important in understanding its contribution to cGAS-STING driven expression of IFN β in SLE.

Posttranslational modifications also regulate the activity and stability of both cGAS and STING. B lymphocyte kinase (BLK) for example phosphorylates tyrosine 215 of cGAS in human skin fibroblast cells to promote cytosolic retention,^[84]

whereas AKT and CDK1 phosphorylate murine cGAS on serine 291 to inhibit its enzymatic activity; CDK1-induced phosphorylation promotes chromatin tethering during mitosis in order to prevent recognition of self-DNA.^[85–87] Whether any alterations of cGAS phosphorylation occur in SLE has yet to be examined. A number of E3 ligases have also been identified as promoting cGAS activation, whether by monoubiquitination (TRIM14, TRIM56) or K27-linked polyubiquitination (RNF185).^[88–90] Glutamylation and acetylation are other means of regulating cGAS activity in a dynamic way (reviewed by Wu^[91]), although whether these forms of post-translational modifications are altered in SLE or in specific immune cell subsets is currently unknown.

Evidence for Role for cGAS-STING Pathway in SLE from Murine Studies

Multiple studies using murine models with lupus-like features have implicated the cGAS-STING pathway in the pathogenesis of lupus as outlined in Table 1.^[92] Much of the initial work in this respect came from studies using the *Trex1*^{−/−} mice.^[93] TREX1 is an exonuclease that digests DNA in the cytoplasm.^[94–96] Discussed above, loss of function mutations in *TREX1* in humans is associated with the autoimmune and inflammatory disease AGS, an IFN-I mediated infantile disease characterized by encephalopathy and SLE.^[97–105] whereas *Trex1* deficiency in mice manifests as a noninfectious autoimmune inflammatory myocarditis, driven by self-DNA recognition via the cGAS-STING sensing pathway.^[106–109] Using *Trex1*-deficient mice, Gao *et al.* demonstrated that complete deletion of *cGas* increased their survival rates and led to the elimination of

Table 1: Mouse Disease Models implicating cGAS-STING in autoimmune disease indicating molecular cause of disease and phenotype

Model	Molecular cause of disease	Notable phenotypes	References
<i>Trex1</i> ^{−/−}	Lacking cytosolic DNA exonuclease activity	<ul style="list-style-type: none"> Multi-organ inflammation ISG induction Autoantibody production Aberrant T cell activation 	[93, 99, 107, 109, 110]
129/B6. <i>Fcgr2b</i> ^{−/−}	Lack of inhibitory signal to autoreactive B cells	<ul style="list-style-type: none"> Autoantibody production Fatal glomerulonephritis 	[113, 115]
<i>Stim1</i> ^{−/−}	Spontaneous anterograde transport and activation of STING	<ul style="list-style-type: none"> ISG induction 	[73, 74]
<i>COPA</i> ^{E241K/E241K}	Ineffective retrograde transport and deactivation of STING	<ul style="list-style-type: none"> ISG induction Perturbed thymocyte development 	[51, 69, 71]
<i>hSTING</i> -R284M <i>hSTING</i> -N154S	Constitutively active STING	<ul style="list-style-type: none"> ISG induction Increased immune infiltrate in tissues Lymphopenia 	[116]
<i>Ogg1</i> ^{−/−}	Raised 8-OHdG Activation of STING	<ul style="list-style-type: none"> Enhanced ISG induction Spontaneous skin lesions in pristane model 	[126]
UVB exposure (500 mJ/cm ²)	DNA damage and activation of neutrophils	<ul style="list-style-type: none"> ISG induction in skin Neutrophil trafficking to kidneys 	[124, 125]

8-OHdG, 8-Oxo-2'-deoxyguanosine; IFN, interferon; ISG, IFN-stimulated gene; STING, stimulator of interferon genes; STIM1, stromal interaction molecule 1; UVB, ultraviolet B light.

Table 2: cGAS-STING in human autoimmune/autoinflammatory disorders highlighting disease name, gene involved, symptoms and molecular cause of disease

Disease	Affected allele(s)	Molecular cause of disease	Notable symptoms	References
Aicardi-Goutières syndrome	<i>TREX1</i>	Lacking cytosolic DNA exonuclease activity	<ul style="list-style-type: none"> Multi-organ inflammation ISG induction Autoantibody production Aberrant T cell activation 	[98, 100, 102–104, 145]
SLE	<i>DNASE1</i> <i>FCGR2B</i> <i>TMEM173</i> <i>TREX1</i>	Multiple	<ul style="list-style-type: none"> Autoantibody production Fatal glomerulonephritis ISG induction 	[97, 113, 117]
CRAC Channelopathies	<i>STIM1</i>	Spontaneous anterograde transport and activation of STING	<ul style="list-style-type: none"> Combined immunodeficiency Autoantibody production ISG induction 	[130]
SAVI	<i>TMEM173</i>	Constitutive activation of STING	<ul style="list-style-type: none"> ILD ISG induction 	[127–129]
COPA Syndrome	<i>COPA</i>	Ineffective retrograde transport and deactivation of STING	<ul style="list-style-type: none"> Autoantibody development ILD ISG induction Arthritis 	[51, 69, 71]

COPA, coatomer subunit alpha; CRAC, calcium release activated channel; IFN, interferon; ISG, IFN-stimulated gene; ILD, interstitial lung disease; STING, stimulator of interferon genes; STIM1, stromal interaction molecule 1; SAVI, STING-associated vasculopathy with onset in infancy; SLE, systemic lupus erythematosus.

inflammatory pathology within organ tissues as well as an overall decrease in ISGs.^[108] In addition, deletion of *cGas* in the *Trex1*-deficient mice significantly reduced production of autoantibodies and the number of activated T cells.^[108] These findings were also reported in a separate study performed by Gray *et al.*, who observed an increase in survival, a decrease in inflammatory destruction of tissue, and a decrease in autoreactivity and IFN-I production in *Trex1/cGas* double knockout mice.^[110] These findings strongly suggest that cGAS activation plays an important role in the development of the pathology seen in SLE. The lethal autoimmune and myocarditis phenotype of the *Trex1*^{-/-} mice is also rescued by deletion of *STING*, confirming the role for this pathway in this model.^[106, 107, 111]

Additional evidence supporting a central role for cGAS-STING pathway in SLE has come from work using mice deficient in *Fcgr2b*, a SLE-susceptibility gene, deletion of which is known to cause lupus-like disease in mice.^[112–114] In a study conducted by Thim-Uam *et al.*, researchers utilized *Fcgr2b*-deficient mice on the 129 background backcrossed to C57BL6 mice (129/B6.*Fcgr2b*^{-/-}), which carries an autoimmune susceptibility locus containing interferon-inducible *Ifi200* family genes, including *Ifi202*, which is thought to utilize STING. These mice develop overt autoreactivity and fatal lupus-like disease. STING expression was found to be increased in 129/B6.*Fcgr2b*^{-/-} mice and disruption of STING signaling by crossing these mice to *Sting*^{gt/gt} mice rescued the autoimmune phenotype.^[115] In addition, when compared with the sera from the *Fcgr2b*^{-/-} mice, sera from the *Fcgr2b*^{-/-}.*Sting*^{gt/gt} mice contained lower antinuclear antibody (ANA) and anti-dsDNA antibody levels, indicating the significant role STING plays in developing autoimmunity. Evaluation

of kidney tissue from the *Fcgr2b*-deficient mice exhibited upregulation of ISG expression in the presence of STING and demonstrated diffuse proliferative glomerulonephritis in comparison to the *Fcgr2b*^{-/-}.*Sting*^{gt/gt} mice. Thim-Uam *et al.* also conducted an adoptive transfer of STING-activated bone marrow dendritic cells from *Fcgr2b*-deficient samples into wildtype and double-deficient mice.^[115] In the wildtype mice, this adoptive transfer resulted in the development of autoimmunity, including production of anti-dsDNA and expansion of T and B cells. In the *Fcgr2b*^{-/-}.*Sting*^{gt/gt} mice, the adoptive transfer resulted in increased levels of anti-dsDNA. In both groups, the adoptive transfer resulted in increased immune complex deposition and inflammatory cell infiltration in kidney tissues. In this murine model, STING was implicated in the immunopathogenesis and development of autoimmune manifestations observed in SLE. This is supported by analysis of mice bearing a constitutive active mutation in STING, which resulted in enhanced ISG expression, immune infiltration into tissues, and an SLE-like disease.^[116] Although the majority of murine studies substantiate the role of the cGAS-STING pathway in augmenting IFN-I responses and producing the inflammatory pathologies seen in SLE, studies have conversely described a suppressive role for STING in both the MRL^{lpr} and pristane models.^[117, 118] Crossing *STING*^{-/-} with the MRL^{lpr} mice resulted in enhanced splenomegaly, lymphadenopathy, autoantibody production, and increased proteinuria in comparison to wildtype mice. Similar findings were also observed in *STING*^{-/-} animals using the pristane model of IFN-driven SLE. To explain these findings the authors showed that macrophages from *STING*^{-/-} mice were hyperresponsive to TLR7 and TLR9 ligands, and that expression of negative regulators of these pathways (A20, SOCS1, and SOCS3) were decreased in both mice and macrophages

from the STING-deficient mice.^[118] Similar opposing roles for STING and TLR-driven pathways have also been shown in models of infection.^[119] Thus, it will be important to understand the relative contribution of STING and TLRs to IFN induction in human SLE in order to support targeting it therapeutically.

Recently, the role of cGAS-STING signaling in regulating UVB-driven IFN-I responses in the skin and subsequent systemic disease has been elucidated in both murine and human studies. A large majority of SLE patients exhibit some form of sensitivity to UVB, which consequently can manifest in localized skin disease or provoke systemic SLE flares.^[120] Critically, exposure to UVB drives oxidative stress, oxidized DNA damage, and accumulation of 8-OH-dG lesions in the skin.^[121, 122] Oxidized DNA, including 8-OH-dG, is also abundant in UV-exposed skin lesion of lupus patients. Critically, it is insensitive to TREX1 degradation and immune sensing of UV-damaged DNA is dependent on both cGAS and STING is sensed by cGAS.^[123] Subsequent studies by Skopelja-Gardner *et al.* demonstrated that exposure of healthy human skin to a single dose of UVB resulted in an increased IFN-I signature, thereby indicating UVB exposure as an important source of IFN-I activation in skin.^[124] In mice, the investigators showed that a single dose of UVB exposure resulted in increased ISG expression in the skin in keeping with analysis of human skin.^[124] Furthermore, increased ISG expression in both peripheral blood cells and kidney tissue following UVB exposure to skin indicated that UVB exposure can induce a systemic inflammatory response. When exposing cGAS-deficient mice to UVB, researchers observed a near-complete elimination of early cutaneous ISG expression. In addition, peripheral blood from cGAS-deficient mice contained lower levels of ISG in comparison to wildtype mice following UVB exposure, implicating cGAS as a consequential factor contributing to a systemic immune response. Interestingly, Skopelja-Gardner *et al.* also observed that the skin of cGAS-deficient mice, in comparison to the skin of wildtype mice, had significantly reduced neutrophil and inflammatory monocyte infiltration as well as decreased chemokine expression following UVB exposure.^[124] A follow-up study demonstrated that skin exposure to UVB triggered migration of neutrophils to the kidney from the skin, demonstrating a direct link between skin inflammation by UV light and kidney injury.^[125] The reduction of inflammatory cell infiltration in the setting of cGAS deficiency indicates the importance of the cGAS-STING pathway in augmenting the innate immune cell response. Such findings from this murine model demonstrate how UVB exposure, a common cause of flares in SLE, can incite both local and systemic IFN-I responses, predominantly induced by the cGAS-STING pathway. Interestingly, the DNA repair enzyme *Ogg1* catalyzes the repair of 8-OH-dG DNA lesions that form following UVB exposure in the skin. Deletion of *Ogg1* in mice resulted in accelerated skin inflammation in the pristane model of IFN-inducible SLE, with further analysis implicating the cGAS-STING in driving enhanced IFN β expression detected in the

mice. Furthermore, *OGG1* expression in lesional areas from SLE with cutaneous lupus erythematosus (CLE) was reduced compared with non-lesional areas, suggesting that *OGG1* protects against cutaneous involvement in SLE by reducing 8-OH-dG-driven IFN responses.^[126]

Evidence for Role for cGAS-STING Pathway in SLE Patients

The first indication that the cGAS-STING pathway may be clinically important came from the discovery of an extremely rare autoinflammatory disease driven by autosomal dominant mutations in *TMEM173*, the gene encoding STING, presenting with clinical and histological evidence of systemic inflammation, vasculopathy, and pulmonary inflammation.^[127] Termed SAVI, patients typically presented with systemic inflammation, interstitial lung disease (ILD) on computed tomography of the chest, and severe cutaneous vasculopathy.^[128] Patients also demonstrated a marked increase in ISG expression in their peripheral blood, indicating that *TMEM173* mutations led to a gain of function in STING, resulting in increased interferon production, marking SAVI as an interferonopathy.^[127, 129] Compared with healthy controls, skin biopsies of SAVI patients with active lesions revealed upregulated inflammatory markers in their vascular endothelial cells. Such results implicate the cGAS-STING pathway in augmenting the inflammatory process, thereby contributing to the clinical and histologic signs of inflammation. More recently, hereditary mutations in the calcium sensor *STIM1*, which acts to restrain STING, have been reported to result in a syndrome of immunodeficiency (recurrent bacterial and viral infections), autoimmune hemolytic anemia and hepatosplenomegaly, resulting from impaired lymphocyte activation in the patients.^[130] As discussed above, mutations in *COPA*, which gives rise to COPA syndrome, manifests as either diffuse alveolar hemorrhage or ILD. As discussed above, multiple groups have not only shown a role for type I IFN in driving the pathology of COPA but have also shown that failure to relocate STING from the Golgi post activation results in overactivation of STING-induced IFN induction.^[51, 52, 69–72, 131–134] Recent work has also shown that C9orf72, a protein associated with the development of amyotrophic lateral sclerosis (ALS), regulates STING stability and that the absence of C9orf72 results in STING-induced inflammatory disease.^[135] Thus, enhanced exposure to cytosolic DNA through incomplete DNA degradation in the case of AGS or a reduction in activity of DNA repair pathways targeting oxidized lesions, or lack of regulation of STING may contribute to enhanced signaling through the cGAS-STING pathway in SLE. Research into how precisely this pathway is dysregulated in SLE using cells and tissues from patients is ongoing and is ultimately required to address this.

In SLE patients, the role of the cGAS-STING pathway was first demonstrated by An *et al.*^[5] When cGAS expression was

measured in the PBMCs from SLE patients and healthy patients, cGAS expression in the SLE patients was significantly higher than in the healthy controls, with approximately half of the SLE patients measured demonstrating a modest increase in cGAS expression. Additionally, when comparing cGAS expression in PBMCs from SLE patients to an inflammatory disease control group consisting of rheumatoid arthritis (RA) patients, cGAS expression was found to be lower in the RA samples than in the SLE samples. After demonstrating an increase in cGAS expression in the SLE samples, an investigation into the downstream effects and levels of cGAMP was examined using mass spectrometry. Importantly, 15% of SLE patients had detectable cGAMP, while no cGAMP was detectable in the samples from the RA patients and the healthy control group, indicating more enzymatic activity of cGAS in the SLE group.^[6] These results suggest a potential role for cGAS in driving IFN β expression in SLE.

In support of a role for cGAS in SLE, Kato *et al.* measured serum IFN-I activity and ISG activity in Japanese patients with SLE, systemic sclerosis (SSc), primary Sjogren syndrome (SjS), and healthy controls.^[136] In comparison to the other samples, sera from the SLE patients were shown to have significantly higher IFN-I bioactivity and ISG activity. In addition, Kato *et al.* demonstrated that *STING*-knockout reporter cells had reduced ISG-inducing activity when stimulated with SLE sera, indicating that the cGAS-STING pathway plays a role in activation of ISG. Furthermore, a correlation was found between STING-dependent ISG-inducing activity and IFN-I independent ISG-inducing activity of the sera from the SLE patients, suggesting that the activation of the cGAS-STING pathway may contribute to IFN-I levels in SLE.

The effect of the cGAS-STING pathway on IFN-I production in SLE patients was again demonstrated by Murayama *et al.*^[137] Using PBMCs from SLE patients and healthy patients, the frequency of IFN- α producing cells was measured after the samples were stimulated with cGAMP. The frequency of IFN- α producing cells in the SLE patients was significantly higher when compared with the healthy controls, indicating that cGAMP activation of the cGAS-STING pathway increased IFN- α production. Additionally, STING expression in monocytes from SLE patients was increased in comparison to the healthy patients.^[137] Furthermore, when cGAMP stimulated monocytes from healthy controls were treated with IFN- α , there was an increase in the production of IFN- α and STING expression, as well as an increase in the presence of TBK1, indicating that elevated levels of IFN- α seen in SLE may augment activation of the cGAS-STING pathway. Murayama *et al.* also found a positive correlation between the frequency of IFN- α producing monocytes with SLE disease activity and serum IFN- α levels.

Although studies are limited thus far in patients, there is growing appreciation of the role of the cGAS-STING pathway in

the activation and production of IFN-I and its role in human disease (as outlined in Table 2), which is fundamental to the systemic inflammation observed in SLE. However, more extensive studies are needed to understand whether defects in this pathway are common to all SLE patients or whether regulation of these pathways is only altered in a specific subset or subgroup.

Therapeutic implications of cGAS-STING and Future Directions

While there is relatively limited research on the cGAS-STING pathway (and even fewer studies using human subjects), contemporary findings have demonstrated compelling evidence supporting this pathway as a key mediator in the production of IFN-I and the development of systemic inflammation in SLE. Also, while treatment for SLE has generally involved broad-based immunosuppression, there is a growing interest in more targeted therapies, resulting in the approval of belimumab, a monoclonal antibody to BAFF or BlyS, and most recently, anifrolumab in August 2021. Additionally, some commonly used therapies for SLE, primarily antimalarials such as hydroxychloroquine, have been shown to attenuate the cGAS-STING pathway to some degree.^[138, 139] More recently, therapeutic agents targeting the cGAS-STING pathway have been described. Lama *et al.* identified human-cGAS small-molecule inhibitors effective in human macrophages using high-throughput screening and targeted medicinal chemistry optimization, highlighting the potential therapeutic development of cGAS antagonists.^[140] Other small molecules targeting the active site have also been described and tested in murine studies (reviewed by McWhirter and Jefferies^[49], Decout *et al.*^[139]). Understanding the localization of cGAS activity may also provide another prospective therapeutic target in the cGAS-STING pathway. As described previously, cGAS activity has been shown to be dependent on subcellular localization, such as plasma tethering, which allows for interaction with cytosolic DNA and subsequent activation.^[65, 141, 142] cGAS is also typically thought to be compartmentalized to the cytosol but has been shown to exist within the nucleus and is closely tethered to intact nuclear chromatin.^[66, 143] Thus, targeting localization of cGAS may be another opportunity for developing potential therapeutic agents.

STING antagonists have targeted STING palmitoylation – a necessary step to fully activate STING – inhibiting type I IFN responses and signs of systemic inflammation in the *Trex1*^{-/-} mice.^[60, 144] Furthermore, Haag *et al.* identified a potent small-molecule inhibitor of STING identified as H-151, which binds to STING at the transmembrane cysteine residue at position 91. As H-151 was shown to inhibit both murine and human STING activation, its clinical considerations are significant and may potentially be used in the development of cGAS-STING targeting agents.^[144] The challenge currently is translating the utility of these targeting strategies in animal models to

human diseases, to provide more precise knowledge of the roles of the cGAS-STING pathway in IFN-driven disease and SLE. Thus, continued research investigating the relationship between SLE and the cGAS-STING pathway is essential for

the development of this new class of promising immunomodulating agents, thereby expanding the therapeutic treatments currently available for SLE patients.

Conflict of Interest

There is no conflict of interest declared.

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