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Self-DNA, STING-dependent signaling and the origins of autoinflammatory disease

Jeonghyun Ahn and Glen N Barber

Self-DNA has long been considered a key cause of inflammatory and autoimmune disease, although the exact origin and general mechanisms of action have remained to be elucidated. Recently, new insight has been gained into our understanding of those innate immune pathways and sensors that are responsible for instigating self-DNA triggered autoinflammatory events in the cell. One such sensor referred to as STING (for stimulator of interferon genes) has been found to be seminal for controlling cytosolic-DNA induced cytokine production, and may be responsible for a wide variety of inflammatory diseases including systemic lupus erythematosus (SLE), Aicardi-Goutieres syndrome (AGS) and STING-associated vasculopathy with onset of infancy (SAVI). STING may also be involved with augmenting certain types of carcinogen induced cancer. Aside from generating valuable information into mechanisms underlining innate immune gene regulation, these findings offer new opportunities to generate innovative therapeutics which may help treat such diseases.

Addresses
Department of Cell Biology and Sylvester Comprehensive Cancer Center, University of Miami School of Medicine, Miami, FL, USA

Corresponding author: Barber, Glen N (gbarber@med.miami.edu)

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Importance of STING in antimicrobial host defense

STING (stimulator of interferon genes) is a 348 amino acid transmembrane containing protein that resides as a dimer in the endoplasmic reticulum (ER) of epithelial, endothelial cells as well as a variety of hematopoietic cells such as macrophages and dendritic cells (DCs) [1••,2]. STING (TMEM173/MPYS/MITA) has been found to be essential for triggering the production of various cytokines including type I IFNs in response to the detection of pathogen related dsDNA in the cytosol of the cell, or cyclic di-nucleotide (CDNs) such as cyclic di-AMP produced from intracellular bacteria [1••,2,3••,4]. However, it has recently been found that STING may also be responsible for causing a variety of autoinflammatory diseases, such as forms of systemic lupus erythematosus (SLE) as a result of becoming activated by self-DNA [5••,6••,7••,8••,9•]. These important findings have shed significant insight not only into mechanisms of host defense and plausible reasons underlining pathogenesis but also into the causes of inflammatory disease and even cancer. Such information enables the design of new drugs that target the STING signaling pathway, with the objective of generating treatments that may alleviate these types of inflammatory disease.

The cell appears to have evolved a number of sensors able to recognize anomalous DNA species present in the cytosol and to trigger innate immune responses. These include Toll receptor 9 (TLR9) mainly expressed in plasmacytoid dendritic cells (pDCs), and B cells and which recognizes approximately 21 nucleotides of unmethylated CG rich DNA [10,11]. Another sensor is AIM2 (absent in melanoma 2), which in response to associating with dsDNA facilitates the activation of pro-inflammatory IL1β and IL18 [12]. Mammalian STING is known to bind to dsDNA species weakly though is strongly activated by CDNs generated directly from bacteria such as *Listeria monocytogenes* [3••,4,13,14]. A key dsDNA interacting protein that expedites STING activity is the cyclic GMP–AMP synthase (cGAS/Mab-21 Domain Containing Protein/C6orf150) which in the presence of ATP and GTP generates the production of non-canonical CDNs (cyclic di-GMP–AMP [cGAMP-c[G(2′,5′)pA(3′,5′)p]) [4,15••]. These CDNs bind to STING dimers and trigger STING/Tank-binding kinase 1 (TBK1) autophagy-related trafficking from the ER to endosomal/lysosomal perinuclear regions that harbor the transcription factors IRF3 and NF-κB [16]. TBK1 activates IRF3 and perhaps NF-κB phosphorylation and translocation which induces innate immune gene transcription [11,16].

STING-deficient mice are viable, but extremely sensitive to DNA pathogens such as herpes simplex I (HSV1) since they fail to generate protective type I IFN as well as other cytokines [2]. In addition, STING is important for protection against RNA viruses through mechanisms that are unclear but that do not appear to involve STING trafficking or innate immune gene induction [2]. STING is further required for eradicating bacteria, such as *Mycobacterim tuberculosis* (*M. tuberculosis*) and may achieve this by delivering such microbes to autophagosomes for degradation [17]. Indeed, it is becoming rapidly evident that microbes, such as Dengue virus have devised a number of

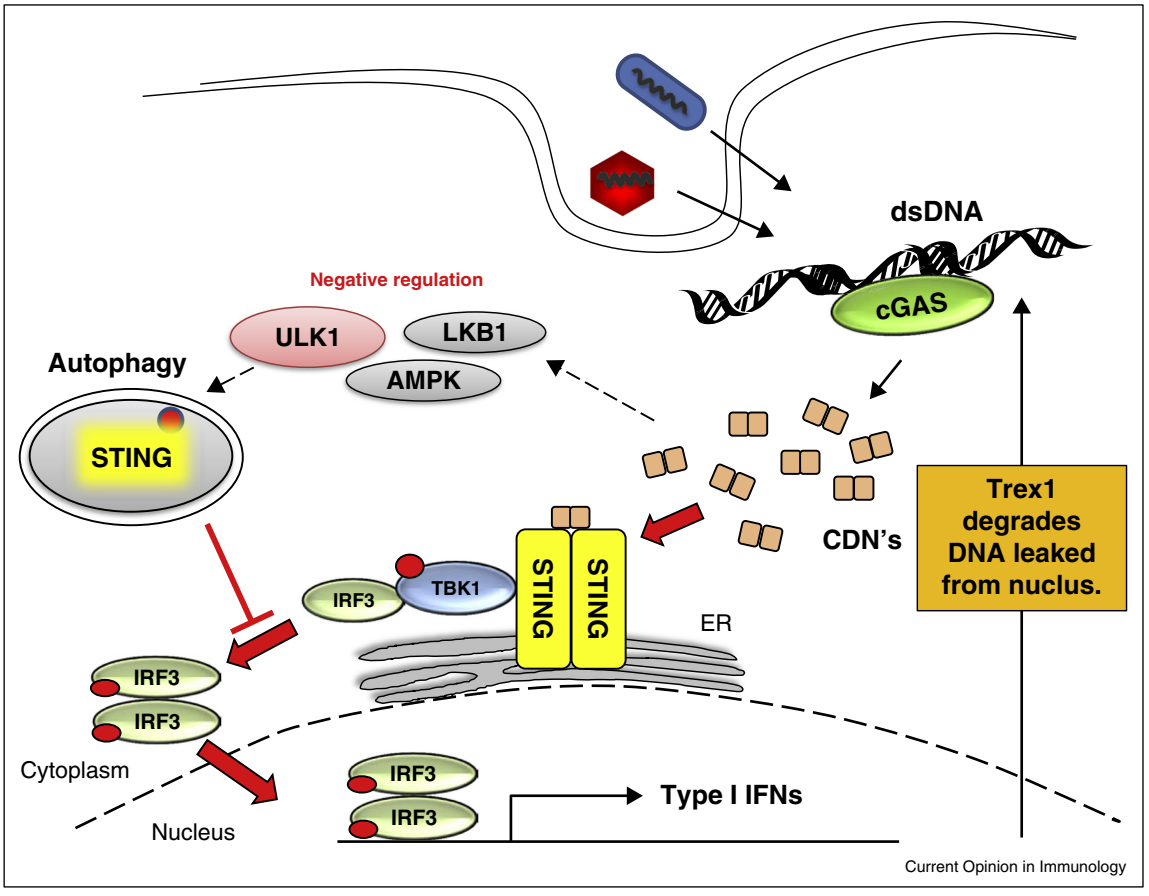
ways of inhibiting STING function, presumably to avoid host defense countermeasures instigated by this cellular sensor [18] (Figure 1).

STING activation by self DNA and autoinflammatory diseases

Although type I IFNs are well known for host defense against microbial infections, aberrant innate immune signaling though failure to distinguish between self and foreign nucleotides can result in inflammatory diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis [19–22]. Several animal models have been used for the study of inflammatory and autoimmune disease with a number involving loss of deoxyribonuclease (DNase) function. For example, DNase I knockout mice exhibit immune complex nephritis, while DNase II-deficient mice die before birth due to self-DNA-induced cytokine toxicity. DNase III (Trex 1) deficient mice, in contrast, suffer from inflammatory myocarditis and have a median lifespan of approximately 10 weeks [23••,24,25]. Thus, the inability to appropriately degrade self-DNA can lead to inflammation and defects in some of these exonucleases have now been shown to occur in humans exhibiting similar disease [26,27].

For example, the Nagata laboratory first showed that phagocytes in DNase II deficient mice lacked the ability to effectively digest DNA left over from engulfed apoptotic cells [28,29]. Accumulated self DNA leaks from lysosomal compartments and stimulates the production of cytokines including type I IFN, leading to lethal anemia [28,29]. Embryonic lethality could not be rescued by crossing *DNase II*$^{-/-}$ mice onto a TLR-negative background, indicating that the responsible cellular sensor did not involve TLR9 [28]. Lethality could be rescued, however, by crossing *DNase II*$^{-/-}$ mice with *IFNr*$^{-/-}$ mice, suggesting that type I IFN was responsible, in large part, for the death of the embryos [30•]. Nevertheless, such mice were found to suffer from severe arthritis due to the copious prevalence of other cytokines such as TNFα [30•]. An important role for STING in manifesting this phenotype was demonstrated by crossing *DNase II*$^{-/-}$ mice with

Figure 1



Recognition of accumulated dsDNA and CDNs. STING localizes in the endoplasmic reticulum (ER) and recognizes cyclic di-nucleotides (CDNs). CDNs are produced from intracellular microbes or by cyclic GMP–AMP synthase (cGAS) binding to DNA. These CDNs bind to STING dimers and trigger tank-binding kinase 1 (TBK1) activation to phosphorylate IRF3 which induces type I IFNs transcription. After STING activation, STING is phosphorylated by serine/threonine UNC-51-like kinase (ULK1/ATG1) and IRF3 function is suppressed through a negative feedback loop.

STING$^{-/-}$ mice and observing that the resultant progeny were born and were relatively healthy without any evidence of arthritis even after 1 year of age [5••]. Indeed, *DNase II*$^{-/-}$ and *STING*$^{-/-}$ mice exhibited dramatically reduced type I IFN/cytokine production clearly demonstrating that STING dependent signaling was responsible for this severe inflammatory disease manifested from inappropriately digested apoptotic DNA. In addition, engulfed necrotic cells similarly trigger cytokine production through STING signaling [5••].

The severe consequences that defects in DNaseII activity cause the host may explain why little information exists into the prevalence of DNaseII mutations in humans. However, defects in DNaseI have been reported in certain patients suffering from inflammatory bowled disease and perhaps lupus [25,31]. As mentioned mice lacking DNaseI exhibit lupus like nephritis although the causes, include putative role for STING signaling are not yet clear. What is more evident is that STING instigates inflammation responsible for the early death of TREX1-deficient mice. Trex1 is a major nuclear 3′ → 5′ DNA exonuclease 1 that targets both ssDNA and dsDNA as substrates [24]. Patients suffering from Aicardi-Goutieres Syndrome (AGS) and severe forms of SLE have been found to exhibit mutations in Trex1, characterized by high cytokine levels and inflammation of the central nervous system including encephalopathy. Most individuals with AGS do not survive childhood [32••,33–35]. However, while *Trex1*$^{-/-}$ mice exhibit high levels of cytokines such as TNF-α and IL1-β and generally die within 10 weeks of birth, *Trex1*$^{-/-}$ *STING*$^{-/-}$ mice are completely viable, exhibit dramatically reduced cytokine activity, negligible anti-nuclear antibody (ANA) and do not suffer any significant inflammation of key organs [8••,9•].

Of note was that immunohistochemical analysis demonstrated that cardiomyocytes do not express STING. However, high levels of infiltrating inflammatory CD68 or CD11C positive cells which do express STING were found in the hearts of *Trex1*$^{-/-}$ mice [9•]. In fact, Bone marrow derived macrophages (BMDM) or dendritic cells (BMDC) from *Trex1*$^{-/-}$ mice were found to *constitutively* produce high levels of innate immune proteins such as Ifit3, Cxcl10 in a STING-dependent manner [9•]. The importance of STING-signaling in generating 'smouldering' hematopoietic cells was confirmed by bone marrow transplantation studies. For example, the introduction of wild type bone marrow containing Trex1, into *Trex1*$^{-/-}$ mice prevented inflammatory disease [9•]. An additional study also noted that the inflammatory activity of macrophages was dampened in the presence of Trex1 [36]. Therefore, loss of Trex1 facilitates STING activity and cytokine production, predominantly in cells of the hematopoietic lineage, to cause inflammatory disease.

While these studies seemed to point to STING-dependent signaling as the culprit behind Trex1-related disease, the source of the STING 'aggravator' was unclear. It had been proposed that Trex1 may play a role in eliminating endogenous retroviruses [37]. However, retroviral genome activity was not detected in hematopoietic cells lacking Trex1 using a non-PCR based assay based on nanostring technology [9•]. Furthermore, certain bacteria were not thought to be provoking STING in the absence of Trex1 since antibiotic treatment of *Trex1*$^{-/-}$ mice did not reduce the myocarditis or inflammatory disease [9•]. The possibility that extracellular DNA from dying neutrophils (NETosis) could be activating STING in *Trex1*$^{-/-}$ mice was also largely eliminated [9•]. It has been reported that Trex1$^{-/-}$ cells show a defect in cell division (G1/S transition) and exhibit sustained ATM-dependent checkpoint activation which leads to an accumulation of ssDNA in the cytoplasm [38•]. Thus, Trex1 could plausibly play a role in eliminating aberrant DNA species, which can reside in the cytosol following the cell division process. Indeed, further studies indicated that synchronized bone marrow derived dendritic cells (BMDC) from *Trex1*$^{-/-}$ mice exhibit notably higher levels of cytokine production after being released from G2 arrest compared to similarly treated BMDC from WT mice [9•]. Thus, Trex1 may be required to eliminate leftover DNA resulting from the cell division process that may otherwise antagonize innate immune pathways by triggering cGAS-generated CDNs which augment STING function [39•].

Finally, damaged-associated DNA modification caused by the oxidation of DNA (8-hydroxyguanosine; 8-OHG) induced by UV-irradiation or pathogen elicited ROS has also been found to strongly trigger STING-dependent signaling [40•]. Such modified DNA avoided efficient Trex1 degradation, presumably to ensure that STING signaling occurred to trigger host immune responses to eliminate such cells. However, such DNA may also be responsible for instigating certain types of lupus, by avoiding nuclease degradation and irritating cytosolic DNA sensors.

The above cases have involved loss of function of a cellular protein that that may help to restrain STING activity by eliminating nucleic acid activators. However, a recent report indicates that mutations in STING itself can also exist to cause autoinflammatory disease. For example, patients suffering from vascular and pulmonary syndrome (VAPS), a systemic inflammatory disease causing lesions of the ears, nose and cheeks were found to exhibit point mutations in exon 5 of STING (N154S, V155M, and V147L) [7••]. *In vitro* studies indicated that such STING variants inherited a gain of function phenotype and were able to robustly stimulate the production of an IFNβ1 reporter construct [7••]. It is not yet clear why such mutations exhibit this behavior, but it is plausible that they are unable to be effectively repressed by as yet uncharacterized negative regulators of STING. These amino acid changes may cause STING to become

active without robust ligand activation. This new role for STING in VAPS is now referred to as STING-associated vasculopathy with onset in infancy (SAVI) [7••]. Of note was that JAK inhibitors could inhibit STING-generated cytokine upregulation in SAVI patient derived cells, thus opening the door for possible treatment for these, as well as other STING governed inflammatory disease.

The role of STING in influencing inflammation driven cancer

Chronic inflammation is also known to enhance both the initiation and promotion of tumor development through generating reactive oxygen species (ROS) which can damage DNA, and cytokines and growth factors which can stimulate cell growth and differentiation [41,42]. In mice, carcinogens such as 7,12-dimethylbenz(a)anthracene (DMBA) are known to be potent inflammatory agents which promote cutaneous skin cancer although the initiating mechanisms remained to be fully resolved [43,44]. A recent study indicated that DMBA, a polyaromatic hydrocarbon (PAH) which causes genomic damage by forming DNA adducts, additionally induces chromatin leakage into the cytosol of the cell. This event may be responsible for stimulating proinflammatory cytokine production with concomitant phagocytic infiltration and the development of skin polyps [6••]. However, mice lacking STING exhibited dramatically reduced cytokine production and subsequent skin cancer when treated with DMBA compared to control mice [6••]. Possibly, DNA damaged keratinocytes and residual dendritic cells intrinsically produce STING-dependent proinflammatory cytokines which trigger tumor initiation as well as attract inflammatory cells. Infiltrating phagocytes engulf damaged keratinocytes with the DNA from the dying cells further activating extrinsic STING signaling and the production of additional cytokine/growth factors that boost tumorigenesis. The importance of STING in hematopoietic cells was demonstrated by transferring bone marrow from wild type mice into irradiated *Sting*$^{-/-}$ mice and demonstrating that the recipients became susceptible to DMBA-induced carcinogenesis [6••]. Thus, STING may play a key role in inflammation associated cancer caused by chronic exposure to carcinogens. These findings are reminiscent to that of DMBA treated MyD88-deficient mice, which were also found to be resistant to skin cancer [45]. The reported study indicated that MyD88 was downstream of STING and likely enforced signaling events through IL1β, IL18 or other receptors by cytokines originally produced via STING [6••]. It is also noteworthy that the anti-cancer drugs cisplatin and etoposide, also DNA-adduct forming agents, similarly induced nucleosome release into the cytoplasm and activated STING controlled cytokine production [6••]. Thus, it is possible that some of the immunostimulatory anti-cancer events instigated by chemotherapeutic reagents may, in part, involve the STING pathway [6••]. Finally, a number of pathogens, such as hepatitis B and V viruses are now known to be associated with cancer and may achieve this at least in part through triggering inflammatory responses. It remains to be seen however whether STING plays a role in these processes, or in other types of cancer.

Concluding remarks

Significant progress has been recently achieved in elucidating the role of innate immune signaling in the cause of inflammatory disease. The influence of STING-controlled signaling in facilitating these malaises is emerging as a key factor in these processes. New insight has been gained into the nature of the self-DNA responsible for pro-inflammatory cytokine production including those 'activators' triggering STING. The importance of preventing self-DNA from accumulating in the cytosol is becoming readily evident, as well as our understanding of how the cell strides to achieve this [16,46]. In addition, it is clear that the cell has gone to significant lengths to ensure that STING is not 'switched on' for long periods. These studies provide new opportunities to design drugs that may prevent these recently discovered signaling pathways from being overstimulated. Such therapeutics may be useful not in the treatment of a variety of diseases such as arthritis, SLE, SAVI and AGS, but also in certain types of cancer, where inflammation may play a key role.

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