Published in final edited form as:

Curr Opin Immunol. 2018 February ; 50: 82–87. doi:10.1016/j.coi.2017.11.004.

Cytosolic sensing of immuno-stimulatory DNA, the enemy within

Rekha Dhanwani, Mariko Takahashi, and Sonia Sharma

La Jolla Institute for Allergy & Immunology, 9420 Athena Circle, La Jolla, CA 92037, United States

Abstract

In the cytoplasm, DNA is sensed as a universal danger signal by the innate immune system. Cyclic GMP–AMP synthase (cGAS) is a cytosolic DNA sensor/enzyme that catalyzes formation of 2′–5′-cGAMP, an atypical cyclic di-nucleotide second messenger that binds and activates the Stimulator of Interferon Genes (STING), resulting in recruitment of Tank Binding Kinase 1 (TBK1), activation of the transcription factor Interferon Regulatory Factor 3 (IRF3), and *trans*-activation of innate immune response genes, including type I Interferon cytokines (IFN-I). Activation of the pro-inflammatory cGAS–STING–IRF3 response is triggered by direct recognition of the DNA genomes of bacteria and viruses, but also during RNA virus infection, neoplastic transformation, tumor immunotherapy and systemic auto-inflammatory diseases. In these circumstances, the source of immuno-stimulatory DNA has often represented a fundamental yet poorly understood aspect of the response. This review focuses on recent findings related to cGAS activation by an array of self-derived DNA substrates, including endogenous retroviral elements, mitochondrial DNA (mtDNA) and micronuclei generated as a result of genotoxic stress and DNA damage. These findings emphasize the role of the cGAS axis as a cell-intrinsic innate immune response to a wide variety of genomic insults.

Introduction

Cell-intrinsic innate immunity represents the early, essential and ubiquitous defensive response of susceptible cells to pathogenic threats. At sites of infection or exposure, innate immunity is initiated by sensing of common pathogen-associated molecular signatures by germline-encoded innate immune receptors, commonly termed pattern-recognition receptors (PRR) [1]. Immuno-stimulatory DNA encoded by invading and/or replicating intracellular pathogens is a potent innate immune ligand that elicits high levels of IFN-I and many other pro-inflammatory mediators in immune and stromal cells. Although Toll-Like Receptor 9 (TLR9) is a PRR for immuno-stimulatory DNA localized in endosomes, distinct receptors exist for sensing of cytoplasmic DNA [2^{*}]; of these, the sensor/enzyme cGAS has emerged as a widely expressed, essential molecule for cytoplasmic DNA recognition [2^{*},3^{*}]. cGAS dimers recognize B-form DNA, independently of its sequence context [4^{**}], by binding to the sugar-phosphate backbone without contacting individual bases [5–8]. DNA binding induces a conformational change in the active site of the enzyme, which relieves auto-inhibition and allows for an atypical phosphodiester bond to be formed between the 2′ hydroxyl group of GMP and the 5′ phosphate of AMP, which is followed by cyclization to

Corresponding author: Sharma, Sonia (soniasharma@lji.org).

form an additional canonical 3′–5′ linkage. Short DNA fragments of 15–20 base pairs bind efficiently to cGAS and induce its activity *in vitro* [8]; however, longer DNA elements are required to fully activate cGAS, particularly in cells [9]. A cooperative mechanism by which longer DNA elements are recognized by a network of cGAS dimers has been recently described [10]. Assembly of a single dimer appears to nucleate formation of additional structures by inducing U-turn bends in flanking DNA, which favors recognition and binding by additional cGAS dimers that assemble ladder-like along the strand of DNA. The formation of U-bend structures is also enhanced by endogenous DNA-associated proteins like HMGB1, TFAM and Hu proteins [10]. In this way, cGAS recognizes a wide variety of DNA substrates, including microbial genomes, endogenous retroviral elements, and more recently mitochondrial DNA (mtDNA) and chromatinized nuclear DNA. This review focuses on recent studies demonstrating cGAS activation by different DNA species in wide-ranging scenarios of infection, auto-inflammatory disease, cancer and DNA damage.

DNA sensing during infections

cGAS is essential for early innate sensing and control of a diverse and ever-increasing number of DNA-encoding viruses and intracellular bacterial pathogens [2*]. The human retroviruses HIV1 and HIV2 are also detected by cGAS, upon cytosolic accumulation of reverse transcribed cDNA originating from damaged capsids or generated during active replication. Viral replication is highly sensitive to the effects of IFN-I and IFN-stimulated genes [11,12], and early innate sensing through the cGAS axis is essential for controlling infections. Mice deficient in cGAS fail to mount an IFN-I response to DNA-encoding herpes simplex virus, vaccinia virus and murine γ -herpesvirus 68 [13**,14**], rendering knockout mice highly susceptible to lethal infection. For other DNA-encoding pathogens the scenario is more complex, with the cGAS–STING pathway driving initial detection as a part of a greater bi-phasic response to sub-lethal infection. Human and mouse cytomegalovirus potently activate cGAS–STING signaling, and STING and cGAS knock-out mice initially exhibit impaired levels of IFN-I and high viral titers in organs [15]; as infection progresses and cGAS–STING independent, TLR9-dependent IFN-I responses develop in myeloid cells, the second wave of IFN-I is actually higher in the knock-out mice, presumably triggered by higher viral loads. Despite a strong secondary response, it is likely that failure to control initial infection in pathogen-susceptible cells promotes increased cytomegalovirus latency, a key factor driving viral persistence and pathogenesis long-term.

RNA viruses also trigger cGAS and STING signaling. One mechanism involves cytosolic sensing of viral membrane fusion, as observed during influenza A infection, which results in activation of STING in a cGAS-independent manner [16]. However, *in vitro* studies indicate that cGAS exerts broad anti-viral effects against numerous RNA-encoding viruses, and cGAS knock-out mice display impaired *in vivo* responses to infection with west Nile virus [14**]. Although A-form RNA can occupy the active site of cGAS [9], it is not capable of relieving auto-inhibition of the enzyme, suggesting a distinct mechanism of action for RNA pathogens. RNA:DNA hybrid molecules are capable of binding cGAS and inducing its activity [17], although it is not known if these structures can accumulate in the cytoplasm during RNA virus infection. Activation of cGAS by cytosolic mtDNA has been demonstrated in several contexts [18*,19*,20*,21*]. Deficiency of pro-apoptotic caspases

results in permeabilization of the mitochondrial outer membrane and causes mtDNA leakage into the cytosol, potentially activating cGAS–STING responses [18*,19*]. Haplo insufficiency of TFAM, a DNA-binding protein of the inner mitochondrial membrane, causes aberrant mtDNA packaging and cytosolic leakage [20*]; interestingly, TFAM and other DNA binding proteins actually promote cGAS recognition of long DNA strands by inducing U-bend structures that nucleate dimer binding [10]. The vaccine adjuvant chitosan also produces cGAS and STING-dependent IFN-I responses through mitochondrial stress and cytosolic mtDNA accumulation [21*]. Notably, mtDNA leakage occurs during natural herpes simplex virus infection [20*], and recent studies also point to mtDNA as an innate sensor for the RNA-encoding virus Dengue [22,23]. As a strategy for evading innate immune detection by the host, the viral protease cofactor NS2B degrades cGAS [22], which is activated during the course of Dengue infection by mitochondrial damage and mtDNA leakage [22,23]. cGAMP molecules can traffic to adjacent cells via gap junctions [24**], and the mtDNA-dependent innate immune response to Dengue is important for limiting spread of the infection to neighboring cells [23]. It is highly likely that other DNA and RNA-encoding pathogens also induce and evade mitochondrial stress and mtDNA-related responses during the course of infection, providing an extra layer of cell-intrinsic protection against those pathogens that successfully mask or sequester their genomes.

DNA sensing during inflammation

Sensing of self-DNA by cGAS signaling is significant in that it implicates the pathway in settings of inflammation other than infectious disease. Indeed, induction of IFN-I responses in the absence of discernible microbial infection, a process called ‘sterile inflammation,’ was initially described in the context of rare human monogenic diseases of auto-inflammation [25*]. Regarding cytosolic DNA sensing, loss-of-function mutations in the cytoplasmic DNA endonuclease TREX1 [26,27**,28,29], or ribonuclease RNase H2 [30–33,34**] directly drive cGAS–STING pathway activation by self-DNA, precipitating a multi-organ inflammatory disease called Aicardi–Goutieres Syndrome (AGS). In TREX1-deficient cells, retroviral DNA elements from the cellular genome spontaneously accumulate in the cytoplasm [27**], whereas deficiency of RNase H2 precipitates genomic instability [32,33,34**]. In addition to rare monogenic syndromes, inappropriate activation of IFN-I responses through cGAS and STING is also implicated in common, chronic inflammatory diseases. Bai *et al.* have shown that aberrant activation of the cGAS–STING–IFN-I pathway exacerbates obesity-induced inflammation and insulin resistance [35]. Genetic ablation of Dsba-L, a protein chaperone localized to the mitochondrial matrix, enhanced mtDNA leakage and cGAS–STING activation in adipocytes, while over-expression protected against inflammatory responses and liver damage in obese mice [35,36]. Myocardial infarction also drives a classical IFN-I response downstream of cGAS–STING activation [37]. The mechanism involves extrinsic sensing of cellular debris and self-DNA by cardiac macrophages, and it is not yet known if cell-intrinsic DNA sensing may also play a role in non-myeloid cell types during MI.

Cancer, cancer therapy and genotoxic stress

The protective role of IFN-I as part of the host immune response to cancer is well documented. Mice deficient in IFN-I signaling display enhanced susceptibility to tumor

formation [38^{*}], and IFN-I signaling is critical for the development of anti-tumor activity of NK cells [39^{*}] as well as antigen cross-priming of tumor-specific CD8⁺ T cells by DC [40^{*}, 41^{*}]. Among an array innate immune signaling molecules examined for an essential role in tumor growth and immunogenicity, STING and cGAS appear to be uniquely indispensable for natural or spontaneous anti-tumor immunity [42^{**},43^{**}], as well as the immunogenic effects of irradiation [44^{**}], CD47 blockade [45], and immune checkpoint therapy [46]. Accordingly, cGAMP is a potent anti-tumor agent against many solid and suspension tumor types [47–50]. In addition to modulation of tumor immunogenicity, STING agonists have been demonstrated to directly affect tumor cells by promoting induction of apoptosis in breast tumor cells [51], transformed B cells [50] and transformed T cells [52]. Notably, the cell-intrinsic response of tumor cells to STING agonists is variable, depending on cancer cell type and whether the signaling pathway is intact.

Similar to scenarios of infection and inflammation, activation of cGAS–STING in tumors occurs via both extrinsic and intrinsic sensing of self-DNA. Antigen presenting DC internalize tumor-derived DNA via phagocytosis of tumor cell debris [42^{**},43^{**}], creating an IFN-I-driven autocrine loop that drives CD8⁺ T cell priming and activation [42^{**},43^{**},44^{**}, 53]. Although extrinsic sensing of tumor cell debris is confined to cells with phagocytic capability, it appears that ubiquitous cell-intrinsic mechanisms exist for sensing genotoxic stress and DNA damage, which has long been appreciated as a robust inducer of the IFN-I response [54,55]. As part of a cell-intrinsic replicative stress response, prostate cancer cells continuously shed genomic DNA into the cytosol due to cleavage of DNA at stalled replication forks by the DNA repair endonuclease MUS81, which results in activation of STING-dependent cytosolic DNA sensing and contributes to tumor immunogenicity and rejection [56]. Extra-chromosomal telomere repeat DNA (ECTR) fragments, generated by trimming of telomeres elongated through the pro-oncogenic alternative lengthening of telomeres (ALT) pathway, accumulate in ~10–15% of cancer cells and can also trigger cGAS–STING–IFN-I signaling [57]. Notably, in ALT cancer cells cytosolic DNA sensing is defective, suggesting that loss of cell-intrinsic self-DNA sensing is requirement for transformation and maintenance of ALT cells. Mammary tumor cells subjected to increasing doses of radiation therapy also accumulate DNA in the cytoplasm, which correlates with cGAS-mediated expression of IFN-I and IFN-I stimulated genes [58]. TREX1 is an important regulator of the intrinsic process, as it is up-regulated at high doses of radiation and drives attenuation of the immunogenic signal. Recently, five independent studies have shed new insight into the role of the cGAS–STING axis in maintenance of genomic integrity by intrinsic sensing of genotoxic stress [34^{**},59^{**},60^{**},61^{**},62^{**}]. In cells deficient in ribonuclease RNase H2, a causative factor in inflammatory AGS that is involved in DNA synthesis and repair [30–33,34^{**}], cGAS specifically localizes to micronuclei, which are formed from mis-segregation of chromosomal DNA, where it initiates an inflammatory response [34^{**}]. Similarly, continuous passaging of cells [59^{**},60^{**}], hyperoxic conditions [60^{**}] or ionizing radiation [61^{**},62^{**}] also activate cGAS-mediated sensing of damaged genomic DNA fragments or micronuclei, with the resulting inflammatory response driving cellular senescence. Interestingly, recognition of chromatinized DNA substrates contained within micronuclei by cGAS requires the breakdown of the micronuclear membrane, which can only occur during mitotic progression [34^{**},62^{**}]. The ability of the cGAS pathway to

impair cellular immortalization by sensing genotoxic stress in a cell-intrinsic manner underscores a key role in preventing tumorigenesis, one that is distinct from immuno-modulation.

Conclusions

IFN-I are profoundly immuno-modulatory cytokines that exert pleiotropic effects on cells and tissues. As a nearly-universal defensive response to pathogens and cell or tissue damage, IFN-I are secreted by many different stromal and immune cell types during the course of infection, tissue damage, neoplastic transformation and tumor therapy. Accordingly, there exist many different pathways to IFN-I production that engage distinct but overlapping innate immune signal transduction cascades. The localization, timing and origin of the IFN-I response is crucial to its effectiveness, and early responses are particularly important for limiting damage and developing effective immunity. The cGAS–STING–IRF3 is a ubiquitous cellular mechanism for triggering robust IFN-I responses at directly at the site of infection, exposure or damage. Initially established for its essential role as a defensive response to intracellular bacteria and viruses encoding DNA genomes, it has become clear that innate immune sensing of a wide variety of mis-localized or aberrant DNA structures and fragments is a strategy for detecting pathogenic threats. With physiological relevance established as an intrinsic and extrinsic determinant of immunity to microbial pathogens, genotoxic stress, malignant transformation, and tumor growth, boosting or restoring cytosolic DNA sensing is of obvious clinical importance in the context of infection and cancer. However, lessons from inflammatory disease indicate that non-specific or excessive activation of this nearly-ubiquitous response can have dire consequences in terms of immune-related adverse events and systemic inflammation, the so-called ‘Achilles heel’ of immune-related therapy.

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spontaneous immortalization (Refs. [59^{**},60^{**}]) or irradiation (Refs. [61^{**},62^{**}]), resulting in activation of an inflammatory program that drives cellular senescence. [PubMed: 28759889]

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