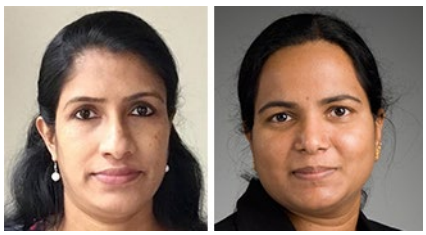
cGAMP: A tale of two signals

In this issue of JEM, Swanson et al. (https://doi.org/10.1084/jem.20171749) report an unanticipated role for cGAMP in priming and activation of inflammasomes in addition to its well-characterized function as an endogenous second messenger inducing type I interferons in the cytosolic DNA-sensing pathway.

DNA, when inadvertently present in the cytosol, acts as a danger signal alarming the host of infection or cellular damage and triggers a potent innate immune response characterized by the induction of type I IFNs and proinflammatory cytokines. The two major DNA sensors mediating these responses are the cyclic GMP-AMP synthase (cGAS) and the inflammasome forming receptor absent in melanoma 2 (AIM2; Wu and Chen, 2014). cGAS upon DNA binding catalyzes the production of cyclic GMP-AMP (cGAMP), which acts as an endogenous second messenger that triggers type I IFN production through the stimulator of IFN genes (STING) pathway (Sun et al., 2013; Wu et al., 2013). AIM2 also directly binds to cytoplasmic dsDNA and assembles an inflammasome complex with the adaptor molecule apoptosis speck-containing protein (ASC) and procaspase-1, leading to activation of caspase-1 and subsequent processing and secretion of proinflammatory cytokines IL-1β and IL-18 (Sharma and Kanneganti, 2016). Although both of these DNA-sensing pathways are important in infections, autoimmune diseases, and cancer, their functional intersection and cross-regulation are less studied (Cai et al., 2014; Man et al., 2016). In this issue, Swanson et al. report the positive cross-talk between the IFN and inflammasome pathways in response to transfected DNA and DNA virus infection where cGAMP mediates both the induction of IFNs as well as the priming and activation of an AIM2–NLRP3–ASC inflammasome complex (see figure).

Cytosolic delivery of 2′3′-cGAMP is known to induce IFNβ secretion. In addition to IFNβ secretion, Swanson et al. (2017) found activation of caspase-1 and secretion of inflammasome-dependent cytokines IL-1β and IL-18 from LPS-primed murine bone marrow–derived macrophages (BMDMs) transfected with 2′3′-cGAMP or bacterial cyclic dinucleotides (CDNs). This response is not restricted to murine cells, and transfection of 2′3′-cGAMP or CDNs also induces secretion of IL-1β from primary human macrophages and dendritic cells. Using BMDMs from mice lacking different inflammasome components, the authors identified the role for both AIM2 and the canonical NLRP3 inflammasome in 2′3′-cGAMP–induced IL-1β secretion. In transfected cells, cGAMP localizes with AIM2, NLRP3, ASC, and caspase-1, suggesting the formation of inflammasome complexes containing both the AIM2 and NLRP3 sensors. Association of cGAMP with components of the inflammasome complex supports a direct role for cGAMP in facilitating inflammasome assembly and activation. Interestingly, unlike other inflammasome-activating stimuli, transfection of cGAMP did not induce pyroptosis in BMDMs (Sharma and Kanneganti, 2016). Previous studies have reported concurrent activation of AIM2 and NLRP3 inflammasomes in response to diverse stimuli (Kim et al., 2010; Kalantari et al., 2014; Karki et al., 2015). Whether cGAMP is also involved in assembling the "dual inflammasome complexes" in response to these stimuli and how the assembly and activation of these effector complexes is mediated warrants future studies.

To delineate the upstream and downstream components involved in cGAMP-mediated inflammasome activation, Swanson et al. (2017) probed the role of DNA sensor cGAS and the adaptor STING. Transfection of dsDNA analogue dA:dT revealed the role of cGAS in enhancing DNA-mediated AIM2 inflammasome activation. Whereas cGAS is dispensable for cGAMP-mediated responses as expected because of its upstream function, STING is required for both IFNβ production and optimal inflammasome activation in response to cGAMP. In addition to providing the second signal to induce inflammasome activation, cGAMP also promotes up-regulation of inflammasome components through STING-dependent IFNβ production and subsequent IFN feedback loop. Collectively, these observations demonstrated the functional role of cGAMP in providing both the priming and activation signals for inflammasomes. Importantly, 2′3′-cGAMP similarly triggers AIM2- and NLRP3-dependent inflammasome activation and IL-1β secretion in the lungs after intranasal administration (Swanson et al., 2017). To further underscore the relevance of their findings, the authors explored cGAS-dependent inflammasome activation in mice infected with murine cytomegalovirus (MCMV). In this model, ablation of cGAS impaired inflammasome activation and control of virus replication independently of type I IFNs. Whereas IFN signaling itself is important in limiting MCMV replication, lack of inflammasome activation



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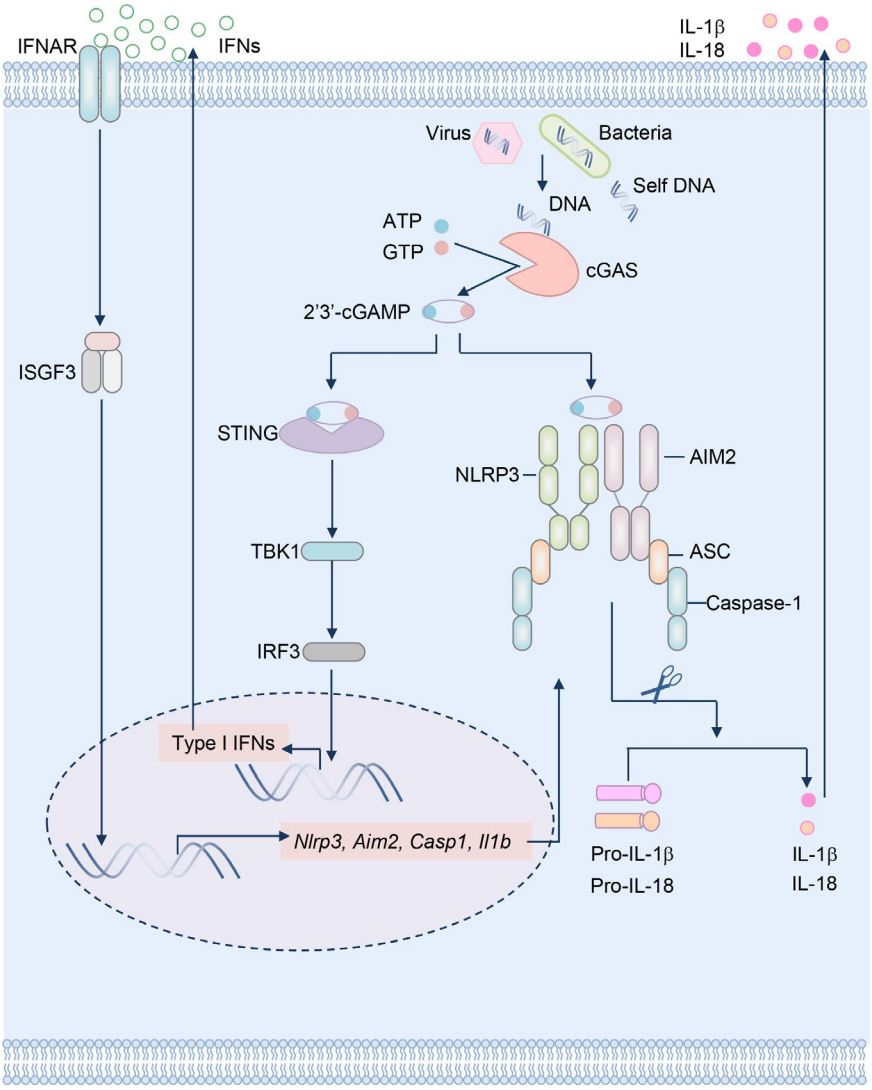

cGAMP activates divergent signaling cascades to induce IFN production and inflammasome activation. cGAS forms a complex with cytosolic DNA and synthesizes 2′3′-cGAMP from ATP and GTP. cGAMP subsequently binds and activates adaptor STING to transduce signals that induce type I IFN production. The IFN feedback loop promotes up-regulation of inflammasome components and thus provides the priming signal. cGAMP also associate with AIM2 and NLRP3 sensors and facilitate the assembly and activation of the inflammasome complex independently of the adaptor STING.

further enhanced virus replication in *Ifnar1*$^{-/-}$*cGAS*$^{-/-}$ DKO mice. Based on these data, Swanson et al. (2017) suggested a "double role" for cGAS in control of MCMV infection—one through the production of IFNs and the second through the activation of inflammasomes. Whether administration of cGAMP can rescue the phenotype observed in *cGAS*$^{-/-}$ mice infected with MCMV is an interesting question. Future studies are also needed to explore whether the cGAS–cGAMP–STING pathway is directly involved in facilitating inflammasome assembly and activation during other bacterial and viral infections.

The study by Swanson et al. (2017) reports a previously uncharacterized role for cGAMP in priming and activation of inflammasomes. Although colocalization of cGAMP with inflammasome components was demonstrated, it is not known whether cGAMP directly binds to AIM2 and NLRP3 to trigger inflammasome assembly. Another interesting aspect that warrants future investigation is the lack of pyroptosis upon cGAMP-mediated inflammasome activation. The current study did not report whether gasdermin D, the caspase-1 substrate and executioner of pyroptosis, is cleaved and activated in response to cGAMP (Shi et al., 2017). It is also intriguing that IL-1β secretion, which is impaired in gasdermin D–deficient cells, occurs even in the absence of pyroptosis. Whether cells transfected with cGAMP maintain plasma membrane integrity and how cGAMP differentially regulates IL-1β and IL-18 secretion compared with other inflammasome-activating stimuli are not known.

In accordance with the findings reported by Swanson et al. (2017), a recent study by Gaidt et al. (2017) also demonstrated the role for cGAS-STING signaling in facilitating inflammasome activation in human myeloid cells. Both these studies found the involvement of the cGAS–cGAMP–STING axis in promoting inflammasome assembly and activation in the DNA-sensing pathway; however, the species-specific differences between human and murine cells led to distinct observations in some aspects. Unlike murine cells where both AIM2 and NLRP3 are involved in inflammasome activation in response to DNA or cGAMP, AIM2 is dispensable for DNA-dependent activation of the cGAS–STING–NLRP3 inflammasome in human myeloid cells. Despite the species- and cell type–specific differences, both the studies found lack of pyroptosis when inflammasome activation is triggered by the cGAS–STING axis. In human cells, IL-1β secretion upon DNA stimulation occurs independently of gasdermin D, and STING activation triggers a unique lysosomal cell death program upstream of NLRP3 (Gaidt et al., 2017). Whether STING trafficking to the lysosome and subsequent lysosomal cell death also occurs in murine cells warrants future studies. Regardless of the species-specific differences, both of these studies collectively reveal a novel role for cGAS–cGAMP–STING

signaling in promoting inflammasome activation in response to cytosolic DNA and further confirm the importance of this pathway in host-protective responses during bacterial and viral infections.

The cGAS–cGAMP–STING DNA-sensing pathway has critical roles in infectious and inflammatory diseases and cancer and is identified as a potential target for therapeutic interventions (Tao et al., 2016). Most of the observations so far are based on the IFN-dependent functions driven by this signaling axis. Identification of additional immune pathways regulated by the cGAS–STING axis helps to further extend the range of disease conditions that can be benefited by cautious targeting of this pathway. The newly described role of cGAS–cGAMP–STING signaling in facilitating inflammasome-dependent responses adds mechanistic insights and also provides a greater understanding regarding the possible benefits in modulating this signaling axis for therapeutic purposes. Inflammasomes and inflammasome-dependent cytokines IL-1β and IL-18 have diverse functions in various aspects of innate and adaptive immunity, and their importance in antimicrobial immunity, cancer, and autoimmune and autoinflammatory diseases is well established. It is conceivable that both the arms of cGAMP–STING signaling and their cross-talk are critical in mediating the downstream effects. Modulating these responses in a controlled manner using either agonists or antagonists offers a plausible approach to harness the beneficial effects. With the current knowledge, activators of this pathway appear to be useful in promoting host defense during infections where IFNs and inflammasome responses are protective. These agonists might also be useful in enhancing antitumor immunity. cGAMP itself was identified as an effective vaccine adjuvant and immunotherapeutic agent for cancer treatment. Administration of 2′3′-cGAMP as an adjuvant was shown to boost antigen-specific T cell activation and antibody production (Sun et al., 2013). Moreover, cGAMP also enhances the antitumor effects of immune checkpoint therapy using PD-L1 blockade by increasing DC activation, antigen presentation, and activation of cytotoxic T cells (Wang et al., 2017). Although these studies show how agonists of the cGAS–STING pathway can be used to enhance antimicrobial and antitumor immunity, inhibitors might be useful in the treatment of autoimmune and sterile inflammatory conditions associated with aberrant or chronic activation of cGAS–STING signaling and subsequent IFN and inflammasome responses (Tao et al., 2016). Because both IFNs and inflammasomes, the downstream effectors of cGAS–cGAMP–STING signaling, are important in health and disease, understanding the balance between beneficial and detrimental effects is of paramount importance in developing therapeutics that target this pathway.

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