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Letter to the Editor

cGAS-cGAMP Signaling and Antiviral Defense

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Cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS) is a cytosolic DNA sensor that plays an important role in innate immunity. Transfection of DNA or DNA virus infection results in the induction of type I interferon production in fibroblasts, macrophages, and dendritic cells which is dependent on cGAS. Recently, *cGas*$^{-/-}$ mice have been reported to be more vulnerable to fatal infection with herpes simplex virus 1 (HSV1) as compared to wild-type mice.

Key Words: Cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS), Type I interferons, DNA sensor, Antiviral defense

In Science on 20th September 2013, Li *et al* reported the roles of cGAS-cGAMP signaling in antiviral defense and immune adjuvant effects (1). Mammalian immune system evolved a range of innate signaling receptors to respond potential harmful stimuli (2). Cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS) is a cytosolic DNA sensor that is responsible for induction of interferons and other cytokines upon DNA stimulation and helps in protection of host cells (3). Several DNA sensors have been reported previously including DNA dependent activator of interferon regulatory factors (DAI), DEAD box polypeptide 41 (DDX41), Ku70 and interferon inducible protein 16 (IFI16). All of these cytosolic DNA sensors are dependent on protein known as stimulator of interferon genes (STING) (4, 5). STING is a transmembrane-spanning endoplasmic reticulum-resident protein which is critical for interferon-β (IFN-β) induction by *L. monocytogenes* and herpes simplex virus 1 (HSV1) infection (4). Production of effective IFNs in murine embryonic fibroblasts, macrophages and dendritic cells against HSV1 or *L. monocytogenes* infection is dependent on STING. STING is critical for host defense against DNA pathogens as STING knockout mice are highly susceptible to HSV1 infection (6). STING is also required for a normal immune response to DNA vaccines (4).

Stimulation of STING turns on IκB Kinase (IKK) and TANK binding kinase (TBK1), which in turn, activates the transcription factors nuclear factor-κB (NF-κB) and interferon regulatory factor 3 (IRF3), respectively (5). These transcriptional factors trigger the induction of type I interferons and other cytokines that play roles in protection of host (5). Researchers have shown the importance of STING; however, the molecular mechanisms that stimulate STING remain obscure. Here, we summarize the results of Li *et al* that cGAS-cGAMP signaling plays critical roles in antiviral defense and immune adjuvant effects (1).

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To study the function of cGAS *in vivo*, *cGas*-defecient (*cGas*$^{-/-}$) mice were generated (1). *cGas*$^{-/-}$ lung fibroblasts and bone marrow-derived macrophages (BMDMs) were unable to produce cGasRNA, whereas, *cGas*$^{+/-}$ cells produced intermediate levels of cGasRNA (1). Golden ticket (gt/gt) mouse has point mutation which results in deletion of STING expression. Li *et al* transfected interferon stimulatory DNA (ISD) or DNAs from herring testis (HT-DNA) and *Escherichia coli* into the lung fibroblasts from wild-type, *cGas*$^{+/-}$, *cGas*$^{-/-}$, and *Sting*$^{gt/gt}$ mice. The lung fibroblasts from wild type and *cGas*$^{+/-}$ mice showed robust production of IFN-β protein. However, the *cGas*$^{-/-}$ and *Sting*$^{gt/gt}$ cells were unable to produce IFN-β (1). Poly (I:C) induces IFN-β through the RIG-I-like receptor (RLR) pathway and poly (dA:dT) is known to induce type I interferon through the RNA polymerase III-RIG-I-MAVS pathway. Production of IFN-β both in case of poly (I:C) or poly (dA:dT) was independent of cGAS or STING (1). They infected the lung fibroblasts with the HSV1, vaccinia virus (VACV) and a mutant strain of HSV1 known as d109 and showed that IFN-β induction was almost completely abolished in *cGas*$^{-/-}$ and *Sting*$^{gt/gt}$ cells, but moderately inhibited in *cGas*$^{+/-}$ cells (1). As a control they measured IFN-β induction by Sendai virus, an RNA virus, which activate the RIG-1 pathway, and found that there is no role of *cGas* or *Sting* (1). Transfection of HT-DNA or infection by wild type HSV1 to *cGas*$^{-/-}$ cells showed that IRF3 activation is dependent on *cGas*. IRF3 activation by Sendai virus was not dependent on *cGas* (1). Thus, activation of IRF3 and cytokine induction by DNA viruses in mouse lung fibroblasts is dependent on cGAS (1).

Production of IFN-β upon HT-DNA or ISD stimulation was dependent on *cGas* and *Sting* in BMDMs (1). However, BMDMs may possess another compensatory pathway, as IFN-β induction by wild type HSV1 was severely but not entirely blocked in either *cGas*$^{-/-}$ or *Sting*$^{-/-}$ cells (1). Transfection of HT-DNA or ISD failed to induce IFN-α or IFN-β in conventional dendritic cells (DCs), from the *cGas*$^{-/-}$ and *Sting*$^{gt/gt}$ mice (1). TLR9 is expressed in plasmacytoid DCs and induces type 1 interferons by synthetic CpG DNA. Stimulation of plasmacytoid DCs by CpG DNA induced significant production of IFN-α and IFN-β even in the *cGas*$^{-/-}$ and *Sting*$^{gt/gt}$ (1). However, other forms of DNA including poly (dA:dT), ISD, and genomic DNA from *E. coli* and *Vibrio cholerae* were able to induce IFN-α in the presence of liposome and was dependent on cGAS or STING (1). Thus, by these experiments they showed that cGAS is critical for detection of natural DNA and DNA virus infections in DCs (1). By using HSV1, they infected wild type and *cGas*$^{-/-}$ mice intravenously and showed that the sera of wild type mice contained elevated levels of IFN-α and IFN-β. However, levels of IFN-α and IFN-β were significantly reduced in the *cGas*$^{-/-}$ mice infected with HSV1. *cGas*$^{-/-}$ mice were highly susceptible to HSV1 infection at the dose of 1×10^6 plaque forming unit per mouse, highlighted by lethality 4 days after infection. In contrast three out of five wild type mice showed symptoms on day 6 and died soon (1). The brain viral titers of *cGas*$^{-/-}$ mice were measured on day 3 after infection, which showed high levels of HSV1 in all *cGas*$^{-/-}$ mice. However, wild type mice showed no detectable levels of HSV1 in the brain. Thus, by *in vivo* experiment they showed that cGAS is essential for immune defense against HSV1 infection (1).

They hypothesized that the 2'3'cGAMP, a product of cGAS, may be applicable to the field of development of DNA vaccine through the immune stimulatory effect of DNA (1). Intramuscular injection of ovalbumin in the presence of 2'3'cGAMP into wild type mice enhanced production of OVA-specific antibodies as compared to *Sting*$^{gt/gt}$. They showed that 2'3'cGAMP work as an immune adjuvant that stimulates antigen-specific T cell and B cell responses.

Li *et al* showed that cGAS plays a critical role in the induction of type I interferons during DNA viral infection. Most of the DNA molecules found in nature completely use cGAS-cGAMP-STING pathway to stimulate type I interferon. However, further studies are required for detailed *in vivo* function of other putative DNA sensors. In future it could be possible to develop 2'3'cGAMP as an adjuvant for vaccines to prevent or cure human diseases.

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