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Revving up cGAS

By Chris Cain, Senior Writer

Cytosolic DNA is a well-known trigger of innate immunity, but it was only last year that its primary sensor, cyclic GMP-AMP synthase, was identified.[1] Now, the first *in vivo* functional study of the protein has confirmed its essential role in antiviral immunity and strengthened the case for developing modulators of cyclic GMP-AMP signaling, including agonists that serve as adjuvants or inhibitors for autoimmune conditions.[2]

A core component of the innate immune response is its ability to detect and react to foreign molecules, including DNA or RNA, during viral or bacterial infections. The toll-like receptors (TLRs), which are localized to the cell surface and in endosomes, are the best-understood system for this recognition. For example, foreign DNA is detected by TLR9, which recognizes unmethylated CpG sites commonly found in bacterial and viral DNA.

"STING has been implicated in certain autoimmune diseases, and I'm pretty sure they would also be cGAS dependent, so it would be quite interesting to design analogs of cGAMP that inhibit STING or to inhibit cGAS itself."

—Veit Hornung, University of Bonn

At least 25 companies are developing agonists or antagonists of TLRs for a variety of indications, and 9 companies are developing products that act upon TLR9.

Recently it has become clear that TLR9-independent pathways of DNA sensing also exist. For example, transmembrane protein 173 (STING; TMEM173), an endoplasmic reticulum protein, was shown to be required for intracellular DNA–mediated, TLR9-independent immunity. Mice lacking Sting are particularly susceptible to viral and bacterial infection and have an impaired type I interferon (IFN) response.

Although there was widespread agreement that STING is a required component of the immune response to intracellular DNA, it remained hotly debated how intracellular DNA was being recognized by cells.

One hypothesis is that STING is a direct DNA sensor,[3] along with a handful of other proteins. Separately, studies have shown that STING recognizes another substrate, cyclic-di-GMP, a secondary signaling molecule widely produced and used by bacteria.[4]

Last December, a team from **The University of Texas Southwestern Medical Center** filled in the missing piece of the puzzle with the discovery of cyclic GMP-AMP synthase (cGAS). The group, led by **Howard Hughes Medical Institute** investigator and UT Southwestern Medical Center professor of molecular biology Zhijian Chen, biochemically mapped out a pathway in which cGAS directly binds and is activated by cytosolic DNA, which leads to the production of a previously unknown signaling molecule, cyclic GMP-AMP (cGAMP).

cGAMP then binds and activates STING, triggering a signaling cascade that leads to the production of IFN (*see* **Figure 1, "Cyclic GMP-AMP signaling in the immune response"**).[1,5]

Veit Hornung, professor of clinical biochemistry at the **University of Bonn**, told *SciBX* that the study was a breakthrough. "How DNA is sensed in the cytosol was one of the last big questions left in the field of pattern recognition. The field had been very complicated prior to the discovery of cGAS," he said. "Now it has become clear that this is the primary route for cytosolic DNA sensing."

Over the last 10 months, a host of labs including Hornung's have characterized the pathway in additional detail, solving crystal structures of both cGAS in complex with DNA and STING in complex with cGAMP.[6–10]

Now, Chen's group has provided the best evidence to date to support the functional importance of this signaling pathway in antiviral immunity.

In one series of experiments, his group sought to determine whether cGAS mediates an immune response to retroviral infection.[11] In cells lacking *cGas*, infection with HIV, simian immunodeficiency virus (SIV) or murine leukemia virus (MLV) triggered no IFN response, whereas infection with a control RNA virus led to a strong IFN response.

In cultured human cells that express cGAS, infection with HIV induced the production of cGAMP as measured by mass spectrometry.

In a second series of experiments, Chen's team generated *cGas* knockout mice and characterized their response to DNA transfection and DNA-virus infection *in vitro* and *in vivo*.[2] In fibroblasts, macrophages and dendritic cells, *cGas* knockout eliminated the production of IFN in response to purified DNA or infection with herpes simplex virus (HSV), whereas wild-type cells generated expected levels of IFN.

In *cGas* knockout mice, HSV infection generated significantly less IFN response and decreased survival compared with infected mice that had intact *cGas*.

Finally, the group showed that cGAMP can act as a vaccine adjuvant. In mice, the model antigen ovalbumin plus cGAMP induced an IFN response and increased antibody production and antigen-specific $CD8^+$ T cell responses compared with ovalbumin alone.

Results were published in two separate *Science* articles.

cGAMP ramp

Chen said that the results provide the first evidence that cGAMP could act as an adjuvant but noted that it is still far from clear how the molecule would stack up against other approaches.

"This approach mimics the stimulatory effect of DNA, which is mediated by cGAS, so by using this molecule you can more directly trigger an immune response," he said. "It's a bit premature to say how this will compare with other adjuvants; studies would have to be done side by side, and in the context of viral infections."

Chen and Christophe Desmet, a research fellow in cellular and molecular immunology at the **University of Liege**, said that one potential advantage could be cGAMP's ability to induce a robust $CD8^+$ T cell response, which is characteristic of DNA-based vaccines.

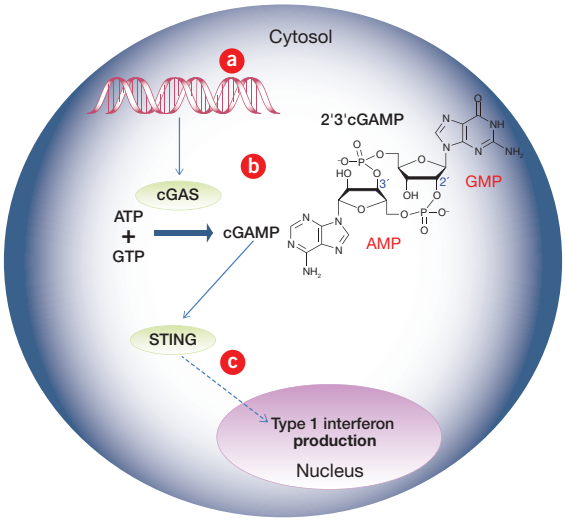

Figure 1. Cyclic GMP-AMP signaling in the immune response. Although cytosolic DNA (**a**) has long been known to trigger an immune response, it was only this year that its primary molecular sensor was identified. The sensor, cyclic GMP-AMP synthase (cGAS), is activated by binding directly to DNA, and it subsequently synthesizes cyclic GMP-AMP (cGAMP) from ATP and GTP (**b**). cGAMP is a soluble molecule that can be transmitted from cell to cell between tight junctions. It directly binds to transmembrane protein 173 (STING; TMEM173), triggering a signaling cascade that leads to the production of type I interferon (**c**).

There are two therapeutic options for manipulating the pathway. To stimulate an immune response against a foreign infection, cGAMP could be given as an adjuvant that induces a type I interferon response. To prevent inflammation, which can be caused by host DNA entering the cytoplasm, inhibitors of cGAMP could be developed.

Multiple DNA-based influenza and HIV vaccines are in development with the goal of increasing efficacy by generating a robust $CD8^+$ T cell response.

However, Desmet cautioned, "DNA vaccines generally don't work well in humans. They work fine in mice but then they are not strongly immunogenic in humans, and we still don't know why that is."

Last year, Desmet led a team that showed that the commonly used adjuvant alum induces an immune response in part by triggering DNA release from host cells.[12] He thus wants to see studies exploring whether cGAS could be contributing to alum's function.

Hornung said that it makes sense to test how cGAMP behaves as an adjuvant given the evidence that it is a key DNA-sensing pathway. He did say that there is little industry appetite for developing new adjuvants for prophylactic vaccines given the extremely high safety bar.

In February, the FDA issued a complete response letter for **Dynavax Technologies Corp.**'s Heplisav HBV vaccine, which is formulated with an immunostimulatory DNA adjuvant that agonizes TLR9.[13]

"cGAMP is a very potent interferon producer, and some companies may not like that idea, though this may be more emotional than rational," Hornung said.

Doing the opposite

Hornung argued that a more promising therapeutic tack may be to develop and test inhibitors of cGAS in autoimmune indications.

"STING has been implicated in certain autoimmune diseases, and I'm pretty sure they would also be cGAS dependent, so it would be quite interesting to design analogs of cGAMP that inhibit STING or to inhibit cGAS itself," he said. He added that multiple labs, including his own, are pursuing the strategy.

Chen said that his lab also is pursuing the target. "cGAS is quite amenable to small molecule inhibition and could be a very attractive target for treating autoimmune diseases," he said.

Last year, researchers at the **University of Miami Miller School of Medicine** showed that knocking out *STING* could prevent DNA-induced inflammatory disease in a mouse model.[14]

Ken Ishii, project leader at the Laboratory of Adjuvant Innovation at the **National Institute of Biomedical Innovation** and adjunct professor of vaccine science at **Osaka University**, agreed that blocking the pathway may be a more attractive avenue than adjuvant design.

"cGAS, as an enzyme critical in the DNA-STING-interferon-autoimmunity pathway, should be one of the best candidates to target for developing therapeutic approaches for many autoimmune diseases, including SLE [systemic lupus erythematosus]," he said.

He added that the possible role of cGAS in autoimmune diseases raises safety concerns about using cGAMP at high concentrations as an adjuvant.

Chen said that thus far, "*cGas* knockout phenotypes are virtually identical to the *Sting* knockout." He did say there could be differences uncovered as the model is characterized in more detail.

Hornung and Desmet agreed that the knockout mouse will likely be rapidly tested in models of bacterial and viral infection and autoimmune disease.

Hornung is continuing to study the effect of cGAMP *in vivo*. Last month in work published in *Nature*, his lab showed that cGAMP can spread from cell to cell to propagate an immune response.[15]

The pace of research on cGAMP continues to heat up. Last week, University of Miami Miller School of Medicine researchers published in *Cell* that even as cGAMP activates STING directly, it can also act on a separate AMP-activated protein kinase (AMPK) pathway to suppress sustained STING activation.[16]

This suggests that cGAMP can both trigger and put the brakes on an immune response and reinforces the fact that cGAMP's functions are not fully fleshed out.

Chen's group has filed patent applications covering the use of cGAMP as an adjuvant and targeting cGAS to treat autoimmune diseases. Their licensing status was not disclosed.

Cain, C. *SciBX* 6(40); doi:10.1038/scibx.2013.1117
Published online Oct. 17, 2013

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