

Blowing Off Steam: Virus Inhibition of cGAS DNA Sensing

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Detection of viral DNA is essential for eliciting mammalian innate immunity. However, viruses have acquired effective mechanisms for blocking host defense. Indeed, in this issue of *Cell Host & Microbe*, Wu et al. (2015) discover a herpesviral strategy for inhibiting the prominent host sensor of viral DNA, cGAS.

For all metazoa, continual surveillance of the intracellular milieu is essential for both detecting and responding to viral infections. To this end, cells utilize specialized receptor proteins termed “sensors” that recognize pathogen-derived moieties, such as viral DNA, to subsequently initiate antiviral responses. In mammals, these events are required for mobilizing the innate and adaptive arms of the immune system and, ultimately, for pathogen clearance. Consequently, these cellular mechanisms exert extreme selective pressures on viruses, driving the evolution of unique viral gene products that target and disable important host defenses. Understanding virus immune evasion strategies used to inhibit host sensors of viral DNA can accelerate the design of effective therapeutics that directly hinder pathogen fitness.

In this issue, Wu et al. (2015) make a substantial leap forward in this research area by discovering a viral strategy for inhibiting one of the most prominent sensors of viral DNA, cGAS (cyclic GMP-AMP synthase). To place this study in its biological context, recent work has demonstrated that cGAS directly binds to foreign DNA in the cytoplasm, triggering a cascade of events that culminates in the expression of antiviral cytokines (Figure 1, steps 1–6) (Sun et al., 2013; Wu et al., 2013). Specifically, cGAS catalyzes the production of cGAMP (cyclic guanosine monophosphate–adenosine monophosphate) from cellular ATP and GTP pools. In turn, the cGAMP second messenger binds to the ER transmembrane adaptor protein STING (stimulator of interferon genes), triggering activation of the protein kinase TBK-1 and IRF3 (interferon regulatory factor 3) (Ablasser et al., 2013). Subsequently, IRF3 translocates into the nucleus where it orchestrates the

expression of immune and inflammatory genes, such as interferons (*ifn*). Under-scoring the significance of this sensor in recognizing multiple pathogens, cGAS was shown to be required for triggering immune responses during infection with several DNA viruses and bacterial pathogens. Interestingly, however, cGAS (also known as C6ORF150 and Mab-21 domain containing 1, MB21D1) was initially found as a potent inhibitor of several RNA viruses in a screen of over 380 interferon-stimulated genes (Schoggins et al., 2011). This suggests that cGAS may possess additional broad-acting antiviral activities. Along these lines, cGAS was also recently demonstrated to interact with and stabilize another DNA sensor, the interferon inducible protein IFI16 (Orzalli et al., 2015). Initially identified as a cytoplasmic sensor, several groups have later demonstrated that IFI16 also acts as a nuclear DNA sensor, being required for STING-dependent IFN expression in response to infections with the nuclear-replicating viruses herpes simplex virus 1 (HSV-1) and human cytomegalovirus (HCMV).

Although the discovery of DNA sensors is a major step forward in understanding the barriers to pathogen replication, it represents only one side of the host-pathogen interaction. On the opposing side are the diverse viral immune evasion strategies, which have remained less characterized. Progress has been made in recent years, in which a few virus factors that inhibit DNA sensors during herpesvirus infections have been identified. During HSV-1 infection, the viral E3 ubiquitin ligase ICP0 was shown to promote the proteasome-dependent degradation of IFI16 (Orzalli et al., 2012) (Figure 1, step 8). In contrast, during HCMV infection, the viral tegument protein pUL83 was

shown to bind IFI16, preventing its DNA-dependent oligomerization (Li et al., 2013) (Figure 1, step 9). Both of these viral strategies effectively abate IFI16- and STING-dependent IFN expression. Surprisingly, given the enormously expanded interest in DNA sensing, no immunoevasion mechanism targeting cGAS has yet been described.

Here, Wu et al. (2015) address this important gap in knowledge by identifying a viral strategy for inhibiting cGAS. The study is a true tour de force with respect to the diversity of cellular, biochemical, and molecular techniques employed to reveal a virus immunoevasion mechanism during infection with Kaposi sarcoma-associated herpesvirus (KSHV). Specifically, the authors define the poorly characterized tegument protein ORF52 as a potent inhibitor of the central cGAS-STING signaling axis (Figure 1, step 7). For this, each KSHV open-reading frame (>80) was individually assayed for its ability to attenuate an IFN reporter driven by cGAS activity. Of the KSHV ORFs that reduced IFN reporter stimulation, only ORF52 displayed both DNA-binding activity and cytoplasmic localization. ORF52 also inhibited the stimulation of IRF3 in human monocytes upon challenge with either DNA substrates or Vaccinia virus, a DNA virus that replicates in the cytoplasm. Interestingly, cells stimulated with cGAMP still initiated immune signaling independent of ORF52 expression, suggesting that ORF52 may directly inhibit cGAS function, rather than affecting a downstream pathway component. To substantiate this model, the authors demonstrated in vitro that purified ORF52 drastically reduces cGAS production of cGAMP in the presence of DNA. Thus, ORF52 may function directly

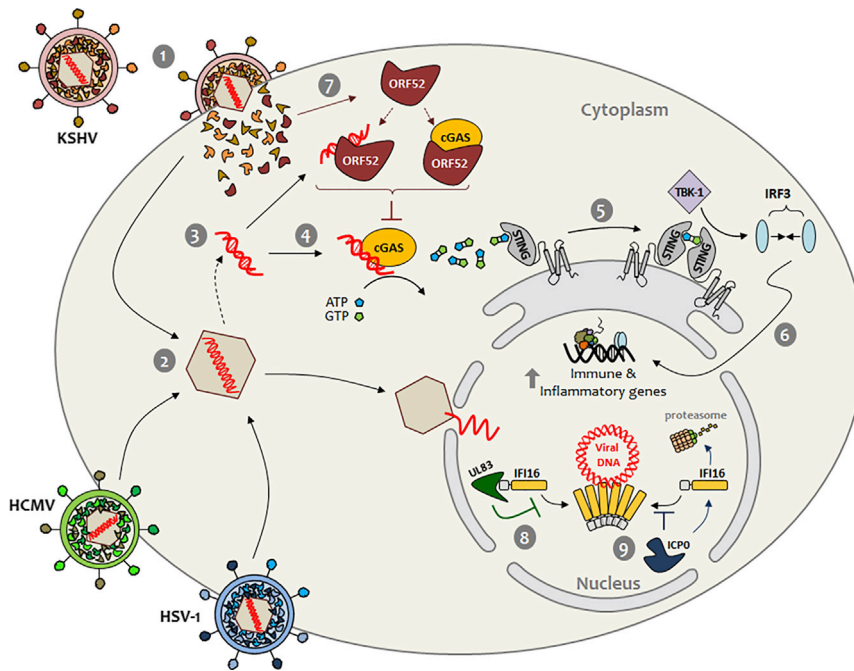


Figure 1. Herpesvirus Strategies for Abating Host DNA Sensing

Fusion of the viral lipid envelope with the plasma membrane of host cells releases viral tegument proteins and the nucleocapsid containing the virus double-stranded DNA genome (1-2). During its transit to the nucleus, the nucleocapsid may be disrupted, releasing viral DNA into the cytosol (3). Here cGAS binds to the viral DNA, stimulating cGAMP production from ATP and GTP (4). Subsequently, cGAMP triggers STING to activate protein kinase TBK-1 (5), in turn activating transcription factor IRF3. Upon dimerization, IRF3 enters the nucleus and stimulates antiviral gene expression (6). As shown by Wu et al. (2015), during KSHV infection, the tegument protein ORF52 obstructs cGAS function through the sequestration of viral DNA substrate and/or an interaction, which directly alters cGAS enzymatic activity (7). In contrast, the HSV-1 E3 ubiquitin ligase ICP0 promotes degradation of the nuclear DNA sensor IFI16 (8), whereas the HCMV tegument protein UL83 inhibits IFI16 by blocking its oligomerization (9).

by modulating cGAS (i.e., its DNA binding or catalysis of cGAMP production) or indirectly by sequestering its DNA substrate. The authors provide substantial evidence for both ORF52 functions. Using DNA competitive binding assays, ORF52 was shown to affect the ability of cGAS to bind its DNA substrate. Furthermore, using an impressive mutagenesis screen, the authors demonstrate that upon loss of binding to DNA, ORF52 can no longer inhibit cGAMP production *in vitro*. The authors go on to establish by immunoprecipitation that ORF52 and cGAS can also interact in a DNA-independent manner, mapping the domains that mediate this interaction. Thus, although ORF52 may to some extent compete with cGAS for its DNA substrate, it seemingly has a second mechanism for directly targeting cGAS. Furthermore, this inhibitory mechanism seems to be specific for cGAS, as ORF52 expression did not affect the functions of AIM2,

another cytoplasmic DNA sensor. This virus immune evasion mechanism was validated in both human monocytes and epithelial cells, as the authors show that KSHV-induced immune responses through the cGAS-STING signaling axis are significantly elevated in the absence of ORF52. Mechanism aside, the positive impact of ORF52 on KSHV fitness is underscored by its conserved function across the gammaherpesvirus family. Similar to their KSHV counterpart, ORF52 homologs from human Epstein-Barr virus, Rhesus monkey rhadinovirus, and Murid gammaherpesvirus 68 all bind to both cGAS and DNA, inhibit cGAMP production *in vitro*, and attenuate IRF3 activation upon DNA challenge. Altogether, Wu et al. (2015) provide considerable evidence that ORF52 is a critical KSHV factor targeting a central component of the mammalian immune system—the cGAS-STING signaling axis.

Considering all the recent findings in DNA sensing, this is clearly an exciting and evolving research area. So, it is not surprising that many questions still remain. For instance, like all herpesviruses, KSHV deposits its DNA genome into the nucleus, where it is replicated by the viral DNA polymerase. It is therefore challenging to reconcile how cGAS, thought to be predominantly cytoplasmic, has the opportunity to sense the viral genome. The disruption of the viral capsid within the cytosol may explain this phenomenon, but this remains to be further explored. Also, herpesviruses can establish a latent infection characterized by a stably integrated, but transcriptionally silent, viral genome within certain cell types. This raises further questions about how cGAS sensing would be affected during productive versus latent viral infection. Additionally, cGAS has been observed to interact with the DNA sensor IFI16, promoting IFI16-dependent responses to other herpesviruses. Therefore, there are likely additional components and crosstalk pathways that require further characterization. Reinforcing the immunomodulatory role of the cGAS-STING axis, recent reports showed that cGAMP produced in response to HIV-1 and mouse CMV infections is packaged into progeny virions and transferred to naive cells, stimulating their immune responses (Bridgeman et al., 2015; Gentili et al., 2015). This may serve to rapidly disseminate IFN signaling locally or to slow the progress of subsequent *de novo* infections. Thus, further understanding the interaction between pathogens and the cGAS-STING axis, as well as continued research for new mechanisms of viral immune evasion, is a high priority in the race toward rational drug design. For example, additional clarification of the mechanism by which ORF52 specifically inhibits cGAS has the promise to be an important contributor to drug design efforts and to expand the current understanding of the antagonistic interplay between pathogens and host immunity.

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S. aureus Toxins Join the DARC Side

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Staphylococcus aureus, like other bacterial pathogens, scavenges host iron for growth through incompletely understood mechanisms. In this issue of *Cell Host & Microbe*, Spaan et al. (2015) demonstrate that two *Staphylococcus aureus* leukotoxins, HlgAB and LukED, target the Duffy antigen receptor for chemokines on erythrocytes, resulting in lysis and iron release.

A bacterial pathogen entering a mammalian host is placing a substantial bet on its ability to withstand iron starvation. Microbial access to iron, which is essential for growth, is limited by sequestration within host cells, limited solubility of free iron at neutral pH, and binding by host molecules. Effective bacterial pathogens have developed multipronged approaches to liberate iron from the grip of the host (Skaar, 2010). Such strategies include production of small molecules that bind free iron with exceptional affinity (siderophores); binding and uptake of iron-associated host factors such as lactoferrin, transferrin, and hemoglobin; and liberation of iron from intracellular pools using cytotoxins. The outcome of infection, both systemic and localized, may hinge on the host's ability to withstand or to directly counteract (such as through the production of siderophore-binding lipocalins) bacterial attempts to gain access to iron (Ganz and Nemeth, 2015).

Staphylococcus aureus is an efficient colonizer and a particularly vexing human pathogen that is responsible for an astonishing worldwide burden of disease. Within the host, *S. aureus* preferentially uses hemoglobin as a source of iron (Pishchany et al., 2014), but its specific means of gaining access to that target have re-

mained unclear. Given the diverse array of pore-forming toxins (PFTs) produced by *S. aureus*—including α -, β -, and γ -hemolysins; other bicomponent cytolytins such as the Pantone-Valentine leukocidin; and the phenol-soluble modulins—as well as the large reservoir of hemoglobin-bound iron contained in circulating erythrocytes, it has been hypothesized that PFT-mediated hemolysis likely provides a source of iron for *S. aureus* in vivo. However, to date, the question of which of these PFTs target human erythrocytes in vivo has not been resolved. A clear understanding has been confounded by the fact that α -toxin efficiently lyses rabbit but not human erythrocytes, and several other *S. aureus* toxins are either inactivated by serum factors or selectively target leukocytes rather than erythrocytes.

In this issue of *Cell Host & Microbe*, Spaan et al. (2015) take a major step forward in understanding the interplay among *S. aureus* toxins, erythrocytes, and pathogenesis by demonstrating that two *S. aureus* bicomponent cytolytins, HlgAB and LukED, exploit the Duffy antigen receptor for chemokines ([DARC]; also called atypical chemokine receptor 1) as a cellular receptor to mediate erythrocyte lysis, thus releasing iron. DARC, a seven-transmembrane protein expressed primar-

ily on erythrocytes and endothelial cells, has a history as an important factor in human infectious diseases (Horuk, 2015). As an essential receptor for invasion of erythrocytes by *Plasmodium vivax* and *P. knowlesi*, DARC has served as a model to understand malaria pathogenesis and parasite-host interactions. At the level of human populations, there is strong evidence for selection on specific DARC allelic variants. A promoter polymorphism that alters GATA-1 transcription factor binding and selectively abolishes DARC expression on erythrocytes has reached fixation in human populations in sub-Saharan Africa and is thought to be a major factor in the absence of *P. vivax* disease from that region. Other variants, including the FY*B allele used by Spaan et al. (2015), decrease but do not eliminate cell surface DARC.

DARC also acts as a promiscuous decoy (non-signaling) chemokine receptor on erythrocytes and endothelial cells. It directly binds several chemokine ligands and can buffer serum concentrations of such proteins by sequestering them from traditional, signal-transducing receptors (Dawson et al., 2000). This “chemokine sink” effect prevents rapid, high-amplitude changes in chemokine concentrations, alters local and systemic immunity, and