Research Highlight

STING-ing the Antiviral Pathway

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The cytosolic DNA sensing pathway has remained poorly defined thus far. A recent study by Ishikawa et al. demonstrates that STING is essential for DNA-mediated type I IFN production and host defence against DNA pathogens.

The past decade has witnessed tremendous progress in our understanding of the innate host response to infection and the pattern recognition receptors that sense and respond to infectious pathogens. A subgroup of endosome localized Toll-like receptors (TLR3, 7, 8, 9) detect nucleic acids-particular viral DNA/RNA and can be distinguished from surface expressed TLRs (TLR1, 2, 4, 5, 6, 10) that recognize bacterial and fungal cell wall components, as well as some viral proteins (Kawai and Akira, 2009). Distinct from the TLR-dependent nucleic acid-sensing system, RIG-I-like receptors (RLRs), the retinoic acid-inducible gene-1 (RIG-I) and melanoma differentiation-associated gene-5 (MDA-5), are novel cytoplasmic RNA helicases that recognize viral RNA present within the cytoplasm (Kawai and Akira, 2009). In addition, Takaoka et al. (2007) identified a cytosolic DNA sensor, DNA-dependent activation of interferon (DAI) regulatory factors or Z-DNA binding protein 1 (ZBP-1), which was involved in B-DNA-mediated IRF3 activation and interferon β (IFNβ) induction; further investigation, however, argued that other DNA sensing pathways exist, underlying the complexity of the mechanisms that sense foreign nucleic acids.

The HIN-200 family member,Absent in melanoma 2 (AIM2), was recently characterized as the elusive cytoplasmic dsDNA sensor. AIM2 mediates caspase-1 activation and pro-IL-1β maturation following direct binding to dsDNA but not ssDNA, irrespective of source and sequence of the dsDNA (Hornung et al., 2009). Interestingly, the AIM2 inflammasome was dispensable for dsDNA-mediated IFNβ induction (Hornung et al., 2009).

The group of Glen Barber at the University of Miami demonstrated that a novel protein termed STING was critical for the non-CpG intracellular DNA-mediated induction of type I IFN and was essential for host defence against DNA pathogens (Ishikawa et al., 2009). STING (also called MITA, MYP5 and ERIS), an endoplasmic reticulum (ER) resident transmembrane protein, interacted with both MAVS and RIG-I, and exerted a potent antiviral effect via the production of type I IFNs (Ishikawa and Barber, 2008; Zhong et al., 2008; Sun et al., 2009). Ishikawa and Barber (2008) concluded that STING was involved in RIG-I-mediated type I IFN induction but did not affect the TLR pathway. Although STING interacted directly with RIG-I, it did not co-immunoprecipitate with MDA-5. Loss of STING rendered cells unable to induce IFNβ production in response to transfected B-form DNA (poly dA-dT), the DNA of HSV-1 or Listeria monocytogenes (Ishikawa and Barber, 2008). The Shu group found that MITA and MAVS formed a complex localized to the outer mitochondrial membrane. Another interesting difference was that TBK1-mediated phosphorylation of MITA was critical for virus-triggered activation of IRF3 (Zhong et al., 2008). Sun et al. (2009) further reinforced the ER localization of STING by highlighting the importance of ER retention/retrieval sequences RYR and RIR for ER retention and protein integrity. Moreover, ERIS dimerization was found to be critical in initiating type I IFN production in response to virus infection.

In an article published in the October issue of Nature, Ishikawa et al. (2009) provided answers to the involvement of STING in dsDNA-mediated immune responses and further insight into the localization of STING. A systematic approach was taken to compare the induction of IFNβ in STING−/− murine embryonic fibroblasts to a variety of DNA ligands and found that IFNβ production was totally abrogated in response to synthetic dsDNA containing or lacking CpG sequences, as well as viral DNA from HSV-1 and −2 or L. monocytogenes. Further analysis in STING−/− macrophages transfected with interferon stimulatory DNA (ISD), revealed that STING functioned independently of the AIM2 inflammasome and did not lead to caspase-1 activation and subsequent IL-1β cleavage. In addition, STING served as an adaptor for intracellular-DNA-mediated production of type I IFN in various immune cells. However, STING−/− plasmacytoid dendritic cells showed a largely intact type I IFN response to exogenous CpG DNA, thus pointing to a TLR9 response that is STING-independent. In vivo analysis of STING−/− mice infected with HSV-1 or vesicular stomatitis virus revealed the importance of STING in eliciting a type I IFN response not only to DNA viruses, but also to negative strand RNA viruses. In contrast, poly I:C tranfection or encephalomyocarditis virus infection, which elicits a MDA5-dependent response, did not induce IFNβ induction. Thus, it...
seems that STING is critical for bridging RIG-I-mediated signalling and is dispensable for an immune response initiated by MDAs.

The investigators next undertook an elegant approach to determine whether any virus targets STING for suppression. Flaviviruses such as hepatitis C virus encode a NS3-4A protease complex that specifically targets MAVS for cleavage as part of its immune evasion strategy. Bioinformatic analysis revealed that both flaviviruses yellow fever and Dengue virus encode a viral product NS4B that exhibited strong homology with the amino terminus of STING; NS4B inhibited STING-mediated IFNβ activation, presumably by direct interaction (Ishikawa et al., 2009).

Previously, it was shown that TBK1 is a key signalling molecule for DNA-vaccine-induced immunogenicity, as well as immune activation by B-form DNA (Ishii et al., 2008). To evaluate whether STING was involved in this signalling pathway, STING−/− or control mice were immunized with plasmid DNA encoding the ovalbumin gene. STING−/− mice showed considerably less serum OVA-specific IgG, as well as reduced IFN-γ secretion (Ishikawa et al., 2009). Thus, the STING molecule is required for both an effective innate and adaptive immune response.

Previous studies localized STING to both the mitochondria and the ER. Ishikawa and Barber demonstrated that STING co-immunoprecipitated with the components of the TRAP complex (TRAPβ) and the translocon adaptor SEC61β whose cellular function involves protein translocation across the ER membrane following translation (Ishikawa and Barber, 2008). Interestingly, Chien et al. (2006) demonstrated that the exocyst complex recruits and activates TBK1 facilitating an immune response to virus infection. This observation led the authors to suggest that STING is a junction point linking the RIG-I and dsDNA-mediated innate immune responses to the translocon, so it may detect translocating viral RNAs, as well as B-form DNAs to induce antiviral immunity via the exocyst-recruited TBK1. Using confocal microscopy and fractionation studies, Ishikawa et al. revealed that STING associated with microsomes, a complex of continuous membranes that comprise the ER. Thus, the discrepancy observed in STING localization can be attributed to the mitochondria-associated ER-membrane, which is involved in various cellular ‘housekeeping’ functions, including the transmission of Ca2+ from the ER to the mitochondria to stimulate oxidative metabolism. Interestingly, after HSV-1 infection and ISD stimulation, both STING and TBK1 translocated to the ER via the Golgi to perinuclear vesicules. Moreover, DNA stimulation leads to the translocation of STING from the ER to Sec5, a subunit of the exocyst complex containing vesicules. In accordance with this observation, knockdown of Sec5 by siRNA led to a significant reduction of intracellular DNA-mediated type I IFN response. Thus, a STING/TBK1 complex is formed following DNA stimulation and this complex is shuttled to endosomal compartments that associate with Sec5 and lead to a robust innate immune response.

Figure 1 Intracellular RNA and DNA sensors of the innate immune response. RIG-I is a cytoplasmic RNA receptor that senses incoming viral RNA and triggers the IFN response via its downstream adaptor molecule MAVS. RNA polymerase III (Pol III) detects cytoplasmic microbial DNA that is transcribed and presented to the RIG-I sensor in the form of 5′-tripophosphates dsRNA, triggering RIG-I activation. In addition, DAI is cytoplasmic sensor for viral DNA that induces the production of type I IFN in a TBK1-IRF3-dependent pathway. The efficient induction of a type I IFN response to viral RNA and DNA requires the central adaptor molecule STING. Additional cytoplasmic DNA sensors for recognition of dsGC-rich foreign DNA might exist and remain to be discovered.
Although this report clearly establishes a role for STING as the central adaptor mediating both intracellular DNA and negative-stranded RNA virus IFN responses in all cell types (Figure 1), an important question remains as to the receptor molecule that bridges the gap between intracellular DNA detection. Recently, two independent groups have identified a previously undefined cytosolic DNA-sensing pathway that depends on the RNA polymerase III-mediated conversion of viral DNA, as well as Legionella pneumophilia DNA into 5′-triphosphate dsRNA that activates the RIG-I signalling pathway (Ablasser et al., 2009; Chiu et al., 2009). Pol III may be one of the cytosolic DNA sensors involved in the detection of dsAT-rich DNA. Finally, are there additional cytosolic DNA receptors distinguishing dsAT-rich versus dsGC-rich DNA that remain to be discovered? Taken together, the discovery of STING has shed light on the complexities of innate immune signalling in response to DNA and RNA viruses.

References


