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Review

The Emerging Roles of STING in Bacterial Infections

Fabio V. Marinho,[1,2,†] Sulayman Benmerzoug,[1,2,†] Sergio C. Oliveira,[2] Bernhard Ryffel,[1,3] and V.F.J. Quesniaux[1,3,*]

The STING (Stimulator of Interferon Genes) protein connects microorganism cytosolic sensing with effector functions of the host cell by sensing directly cyclic dinucleotides (CDNs), originating from pathogens or from the host upon DNA recognition. Although STING activation favors effective immune responses against viral infections, its role during bacterial diseases is controversial, ranging from protective to detrimental effects for the host. In this review, we summarize important features of the STING activation pathway and recent highlights about the role of STING in bacterial infections by *Chlamydia, Listeria, Francisella*, *Brucella, Shigella, Salmonella*, *Streptococcus*, and *Neisseria* genera, with a special focus on mycobacteria.

Trends

The mammalian adaptor protein STING (Stimulator of Interferon Genes) senses cyclic dinucleotides and nucleic acids originating from viruses, bacteria, and from host cells.

STING is found as a dimer in the endoplasmic reticulum, but it can also be associated with mitochondrial membranes.

STING activation induces type I interferon responses and thus favors effective immune response against viral infections.

Recently, STING activation has also been associated with proinflammatory cytokines and chemokine expression, and with autophagy.

The role of STING during bacterial diseases is controversial, ranging from protective to detrimental effects for the host. Thus, rational manipulation of the STING pathway will require careful investigations of host STING–pathogen interactions.

STING: Linking Sensing to Effectiveness

Innate immunity came in light in the late 1990s after the discovery of Toll-like receptors (TLRs), leading to intense investigation of their activation, signaling, and role in diverse pathologies [1]. This research field recently expanded to include a new dimension with the novel cytosolic surveillance systems. The identification of **STING** (Stimulator of Interferon Genes, see Glossary) adaptor protein [2] represented an important milestone for nucleotide sensing research. Nucleotide recognition provides a general mechanism for detecting microorganisms and is involved in diverse pathological scenarios. STING connects microbial cytosolic sensing with host cell effector functions. Its role as a sensor of cyclic dinucleotides (**CDNs**), and as a link between DNA sensing and cell activation, confers on STING a key role in host immune response. As DNA is present in most microorganisms (excepted RNA viruses), and CDNs are crucial for bacterial metabolism, these oligonucleotides are considered pathogen-associated molecular patterns (**PAMPs**). Depending on the type of microorganisms, PAMPs from extracellular pathogens are mainly recognized by TLRs, while those from intracellular pathogens are predominantly sensed by NLRs (NOD-like receptors) and RLRs (RIG-I-like receptors) [1]. STING recognizes CDNs or, in association with intracellular sensors, DNA from viruses [2–5], bacteria [6–9], and from host cells, thus recognizing self-DNA [10], to control host immune responses [2,11].

STING's function has been extensively studied during viral infections owing to its major role in the induction of type I interferons (**IFNs**), as reviewed recently [12]. Furthermore, STING plays a role in the development of autoinflammatory diseases, cancer, and other sterile inflammations [13]. However, less is known about the extent of involvement of STING and its related sensors in the immune responses to protozoa, fungi, or helminths. Concerning bacteria, the outcome of STING activation, leading to IFNβ production, may be beneficial or detrimental for the host, making STING a friend or foe to the bacterial pathogen, depending on the infection. Given the crucial role of CDNs in bacterial metabolism, such as biofilm formation and protein function

[1]CNRS, UMR7355, Orleans, France
[2]Department of Biochemistry and Immunology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[3]Experimental and Molecular Immunology and Neurogenetics, University of Orleans, France
[†]These authors contributed equally to this work.

*Correspondence: quesniaux@cnrs-orleans.fr (V.F.J. Quesniaux).

[14,15], STING activation is not limited to bacteria containing secretion systems. In this review, we present the recent findings on the role of STING during bacterial infections.

Dissecting STING Signaling

STING Activation

Microbial components, such as lipopolysaccharide, CpG DNA, and CDNs, are recognized by membrane and intracellular receptors to induce the expression of multiple host defense genes [1,8,16]. Several studies concerning *Chlamydia muridarum*, *Streptococcus pyogenes, Mycobacterium tuberculosis*, and other bacteria reveal STING-dependent activation of type I IFN [6–9]. CDNs are important for bacterial physiology [14,15]. These PAMPs consisting of dinucleotide monophosphate circularized by 5′3′ phosphodiester bonds are recognized by STING (Figure 1). This process leads to TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3) activation, culminating in a type I IFN response [8,17]. Mammalian 2′3′ cGAMP (cGAMP) similarly triggers type I IFN production after DNA sensing [18]. Apart from microbial sensing, STING can also be activated by self-DNA [11]. Mitochondria are an important source of intracellular DNA (mtDNA) [19]. After cellular stress, mtDNA is released into the cytosol and induces the expression of type I IFN genes in a STING-dependent manner [20]. Furthermore, mtDNA participates in the adjuvant mechanism of chitosan during vaccine therapy, since chitosan promotes mitochondria damage, leading to type I IFN-dependent dendritic cell (DC) activation and triggering adaptive immunity [21].

CDN binding to STING induces its migration from the endoplasmic reticulum (**ER**) to form perinuclear punctate structures. This intracellular trafficking is mediated by iRhom2/TRAPβ [22]. Moreover, iRhom2 protein recruits the deubiquitinase EIF3S5 which maintains the stability of STING during its trafficking [22]. STING migration from ER to the perinuclear region is essential to recruit TBK1, leading to phosphorylation and activation of IRF3 [23]. Indeed, TBK1 phosphorylates the carboxy-terminal tail domain of STING that results in IRF3 recruitment and phosphorylation [24]. Following IRF3 activation, the STING–TBK1–IRF3 complex is dissociated, and STING is readily degraded after K48-linked polyubiquitination via RNF5/TRIM30α. Phosphorylated IRF3 homodimers translocate to the nucleus to activate the expression of innate immune response genes, such as type I IFN-regulated genes [25–27]. However, it is not fully understood whether the STING–TBK1 complex migrates to the perinuclear region or if STING moves alone from the ER to recruit TBK1 directly in the Golgi [28,29]. Interestingly, TBK1 also regulates IRF7 expression [30].

The STING–TBK1 axis phosphorylates IκB, yielding NF-κB release [31], translocation to the nucleus, and NF-κB-dependent gene expression involved in cellular stress, tumor progression, inflammation, and immunity [32]. Moreover, STING–TBK1 engagement can lead to activation Signal Transducer and Activator of Transcription 6 (STAT6) through phosphorylation of serine 407 by TBK1 and of tyrosine 641 by an unidentified kinase [33]. Phosphorylated STAT6 translocates to the nucleus, inducing upregulation of chemokines such as CCL2 and CCL20. Interestingly, STING-induced activation of STAT6 is distinct from that induced by cytokines like IL-4 or IL-13 [33].

Besides type I IFN production, STING activation can lead to **autophagy**, a cellular degradation process where cytoplasmic constituents are degraded, that may play a prominent role in antibacterial defense. It involves engulfment of cargo by double-membrane autophagosomes which then fuse with the lysosome. Autophagy core components belong to the Autophagy Related Gene (Atg) proteins family, but the process also involves Bcl-2 family members and various transcription factors [34]. Autophagic elimination of bacteria is a key defense mechanism to control infection. Autophagy can target pathogens within phagosomes, in damaged vacuoles, or in the cytosol [34]. Some Atg proteins regulate the STING pathway, as will be

Glossary

AIM2 (Absent in Melanoma 2): this sensor protein recognizes cytosolic DNA and triggers inflammasome activation, pyroptosis, and release of IL-1β and IL-18.

Autophagy: a conserved process in eukaryotes whereby cytoplasmic components are enveloped and sequestered by membranous structures that subsequently fuse to lysosomes for degradation. This process can be triggered in response to nutrient-limiting conditions to generate substrates for energy metabolism and protein synthesis. It also plays an important role in innate defense against invading intracellular pathogens, by sequestering them into autophagosomes and delivering them to the lysosome.

CDNs (cyclic dinucleotides): made up of nucleotides circularized by phosphodiester bonds. The generally found CDNs are c-di-AMP, c-di-GMP, 3′3′cGAMP (all from bacterial source) and 2′3′cGAMP (mammalian).

cGAS (cyclic GMP-AMP synthase): also known as Mb21d1. This protein belongs to the nucleotidyltransferase protein family and catalyzes the formation of the 2′3′cGAMP from ATP and GTP upon DNA recognition. The response is irrespective of nucleic acid sequence.

ER (endoplasmic reticulum): consists of a continuous membrane system that forms several flattened sacks within the cytoplasm of eukaryotic cells. It is important for synthesis, folding, modification, and transport of proteins.

IFNs (interferons): these cytokines were named due to their capacity to elicit cellular antiviral responses, 'interfering' with viral replication. They are classified as type I (the most studied are IFNβ and IFNα), type II (IFNγ), and type III (IFNλ).

IRF (interferon regulatory factor): consists in a transcription factor family of nine members in mammals, important in immunity and other biological processes. IRF3 and IRF7 are particularly important in evoking type I IFN responses.

PAMPs (pathogen-associated molecular patterns): conserved motives or molecules found in pathogens and important for eliciting innate immune responses.

SS (secretion system): the bacterial secretion system function to