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STING-in Vaccine Adjuvants



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Cytosolic DNA Sensors (CDSs): a STING in the tail

The innate immune system provides the first line of defense against infectious pathogens and serves to limit their early proliferation. It is also vital in priming and activating the adaptive immune system. Innate immune detection of intracellular DNA derived from viruses and invasive bacteria is important to initiate an effective protective response. This crucial step depends on cytosolic DNA sensors (CDSs), which upon activation trigger the production of type I interferons (IFNs) and the induction of IFN-responsive genes and proinflammatory chemokines. Although the identity of these CDSs is not fully uncovered, much progress has been made in understanding the signaling pathways triggered by these sensors.

Cytosolic DNA-mediated production of type I IFNs (mainly IFN- β) requires the transcription factor IRF3 regulatory factor 3 (IRF3), which is activated upon phosphorylation by TANK-binding-kinase-1 (TBK1)¹. Recently, a new molecule, STING (stimulator of IFN genes), has been shown to be essential for the TBK1-IRF3-dependent induction of IFN- β by transfected DNA ligands and intracellular DNA produced by pathogens after infection^{2,3}. STING (also known as MITA, MPYS and ERIS) is a transmembrane protein that resides in the endoplasmic reticulum (ER)²⁻⁶. In response to cytosolic DNA, STING forms dimers and translocates from the ER to the Golgi then to punctate cytosolic structures where it colocalizes with TBK1, leading to the phosphorylation of IRF3. How STING stimulates TBK1-dependent IRF3 activation was recently elucidated by Tanaka and Chen. They found that, upon cytosolic DNA sensing, the C-terminal tail of STING acts as a scaffold protein to promote the phosphorylation of IRF3 by TBK1⁷.

Until very recently, STING in addition to its role as an adaptor protein was also thought to function as a sensor of cyclic dinucleotides. Burdette *et al.* first demonstrated that STING binds directly to the bacterial molecule cyclic diguanylate monophosphate (c-di-GMP)⁸. This finding was confirmed by several teams who examined the structure of STING bound to c-di-GMP⁹⁻¹¹, including Cheng and colleagues, however their data suggest that STING is not the primary sensor of c-di-GMP¹². Rather, they indicate that DDX41, an identified CDS (see next page), functions as a direct receptor for cyclic dinucleotides upstream of STING. The authors hypothesized that DDX41 binds to c-di-GMP then forms a complex with STING to activate the IFN response.

Exciting new developments reveal that STING participates in another aspect of innate immunity, autophagy. Autophagy plays a critical role in host defense responses to pathogens by promoting the elimination of microbes that enter into the cytosol by their sequestration into autophagosomes and their delivery to the lysosome. Recent studies have reported that DNA viruses and intracellular bacteria induce autophagy and that this process is dependent on cytosolic genomic DNA and STING¹³⁻¹⁵. Robust induction of autophagy was also observed after transfection of various double stranded (ds) DNA species, such as poly(dA:dT), poly(dG:dC) or plasmid DNA, but not single stranded (ss) DNA, dsRNA or ssRNA¹⁶. Interestingly, activated STING was shown to relocate to unidentified membrane-bound compartments where it colocalizes with LC3, a hallmark of autophagy, and ATg9a. The latter protein was reported to regulate the interaction between STING and TBK1 after dsDNA stimulation¹⁶. The E3 ubiquitin ligases TRIM 56 and TRIM32 were also found to regulate STING by mediating its dimerization through K63-linked ubiquitination^{17,18}.

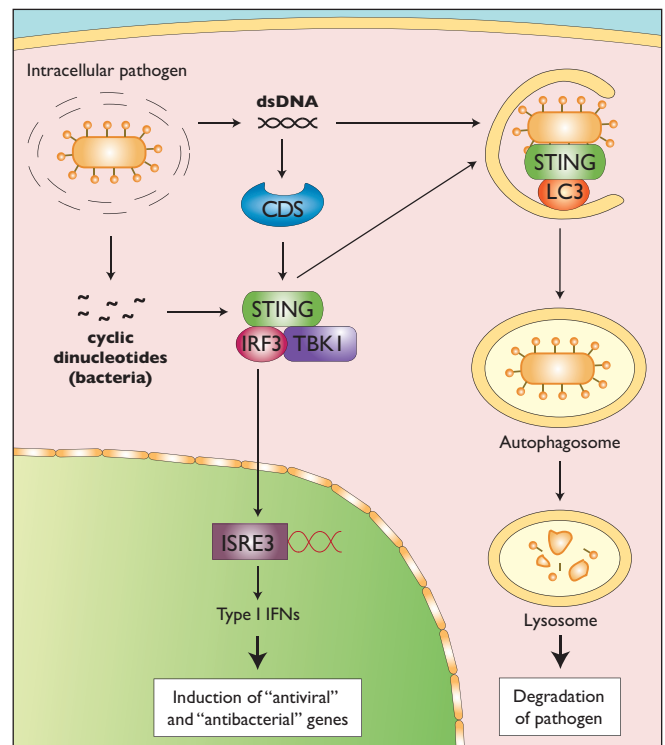
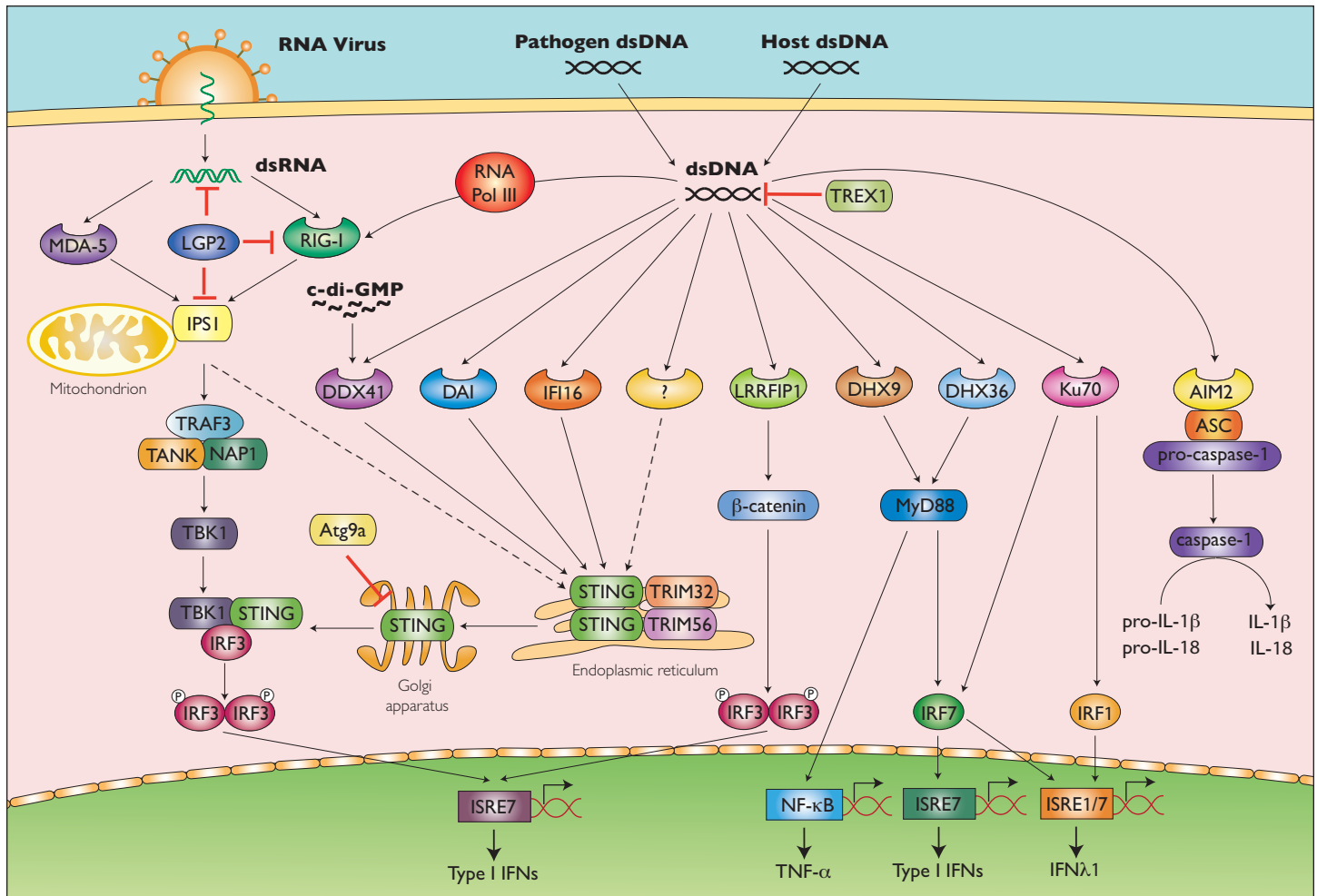


Figure 1: STING in the host response to intracellular pathogens. Linking type I IFN response and autophagy for better defense

Several cytosolic DNA sensors upstream of STING have been proposed. DNA-dependent activator of IRFs (DAI) was the first CDS discovered based on the ability of transfected poly(dA:dT) to induce IFN- β ¹⁹. However, the role of DAI has been shown to be very cell-type specific and cells derived from DAI-deficient mice responded normally to dsDNA ligands²⁰. While analyzing immune responses to dsDNA regions derived from vaccinia virus (VACV-70) or Herpes simplex virus 1 (HSV-60) genomes, Unterholzner *et al.* identified IFI16 as a DNA binding protein mediating IFN- β induction²¹. Interestingly, IFI16 belongs to a new family of pattern recognition receptors that contain the pyrin and HIN domain (PYHIN), termed AIM2-like receptors (ALRs). AIM2 is a STING-independent cytosolic DNA sensor that forms an inflammasome with ASC to trigger caspase-1 activation and the secretion of the pro-inflammatory cytokines IL-1 β and IL-18²⁰. Members of the DExD/H-box

helicase superfamily have also been reported to function as cytosolic DNA sensors. While DHX36 and DHX9 were identified as STING-independent but MyD88-dependent sensors of CpG-containing DNA in plasmacytoid dendritic cells, DDX41 was found to bind various dsDNA ligands and localize with STING to promote IFN- β expression²². Other CDSs have been reported to function independently of STING: RNA Pol III, LRRFIP1 and Ku70²⁰. Unlike cytosolic RNA sensors (RIG-I, MDA-5), which detect structural moieties specific to pathogen RNA, such as 5'-triphosphates, it is not clear whether cytosolic DNA sensors can recognize any particular structural motif of DNA that would discriminate between self and non-self. This suggests that CDSs may have a role not only in anti-microbial innate immune responses but also in autoimmunity. A multitude of CDSs have been described but whether they are all true receptors remains an open question.



1. Stetson DB & Medzhitov R. 2006. Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. *Immunity*. 24(1):93-103. 2. Ishikawa H. & Barber GN., 2008. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature*. 455(7213):674-8. 3. Ishikawa H. *et al.*, 2009. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature*. 461(7265):788-92. 4. Zhong B. *et al.*, 2008. The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. *Immunity*. 29(4):538-50. 5. Jin L. *et al.*, 2008. MPYS, a novel membrane tetraspanner, is associated with major histocompatibility complex class II and mediates transduction of apoptotic signals. *Mol Cell Biol*. 28(16):5014-26. 6. Sun W. *et al.*, 2009. ERIS, an endoplasmic reticulum IFN stimulator; activates innate immune signaling through dimerization. *PNAS* 106(21):8653-8. 7. Tanaka Y. & Chen ZJ., 2012. STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway. *Sci Signal*. 5(214):ra20. 8. Burdette DL. *et al.*, 2010. STING is a direct innate immune sensor of cyclic di-GMP. *Nature*. 478(7370):515-8. 9. Yin Q. *et al.*, 2012. Cyclic di-GMP sensing via the innate immune signaling protein STING. *Mol Cell*. 46(6):735-45. 10. Ouyang S. *et al.*, 2012. Structural analysis of the STING adaptor protein reveals a hydrophobic dimer interface and mode of cyclic di-GMP binding. *Immunity*. 36(6):1073-86. 11. Shu C. *et al.*, 2012. Structure of STING bound to cyclic di-GMP reveals the mechanism of cyclic dinucleotide recognition by the immune system. *Nat Struct Mol Biol*. 19(7):722-4. 12. Parvatyar K. *et al.*, 2012. The helicase DDX41 recognizes the bacterial secondary messengers cyclic di-GMP and

cyclic di-AMP to activate a type I interferon immune response. *Nat Immunol*. [Ahead of print]. 13. McFarlane S. *et al.*, 2011. Early induction of autophagy in human fibroblasts after infection with human cytomegalovirus or herpes simplex virus 1. *J Virol*. 85(9):4212-21. 14. Rasmussen SB. *et al.*, 2011. Activation of autophagy by α -herpesviruses in myeloid cells is mediated by cytoplasmic viral DNA through a mechanism dependent on stimulator of IFN genes. *J Immunol*. 187(10):5268-76. 15. Watson RO. *et al.*, 2012. Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. *Cell*. 150(4):803-15. 16. Saitoh T. & Akira S., 2010. Regulation of innate immune responses by autophagy-related proteins. *J Cell Biol*. 189(6):925-35. 17. Tsuchida T. *et al.*, 2010. The ubiquitin ligase TRIM56 regulates innate immune responses to intracellular double-stranded DNA. *Immunity*. 33(5):765-76. 18. Zhang J. *et al.*, 2012. TRIM32 protein modulates type I interferon induction and cellular antiviral response by targeting MITA/STING protein for K63-linked ubiquitination. *J Biol Chem*. 2012. Aug 17;287(34):28646-55. 19. Takaoka A. *et al.*, 2007. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature*. 448(7152):501-5. 20. Keating SE. *et al.*, 2011. Cytosolic DNA sensors regulating type I interferon induction. *Trends Immunol*. 32(12):574-81 (review). 21. Unterholzner L. *et al.*, 2010. IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol*. 11(11):997-1004. 22. Zhang Z. *et al.*, 2011. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat Immunol*. 12(10):959-

Nucleic Acid Sensors: Expression and Silencing

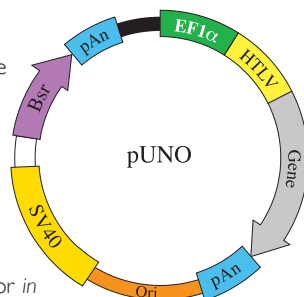
In vivoGen provides a comprehensive collection of genes, as well as shRNAs, that target genes involved in the signaling mediated by nucleic acid sensors (see illustration on opposite page). The genes are available in an expression vector with a strong and ubiquitous mammalian promoter, ready-to-use in protein expression studies. Similarly, short hairpin RNAs (shRNAs) are available as ready-made psiRNA plasmids validated for gene silencing.

Genes in pUNO Plasmids

Description

Genes involved in nucleic acid sensing are available in the pUNO plasmid. The genes are cloned from the ATG to the Stop codon, excluding introns and untranslated regions, and are expressed constitutively at high levels by the EF1 α /HTLV composite promoter. All genes are fully sequenced, and their sequences are available on our website.

pUNO plasmids can be used directly for *in vitro* or *in vivo* transfection experiments. They are selectable with blasticidin in both *E. coli* and mammalian cells. To facilitate the excision and subcloning of the gene of interest into another vector, each gene is flanked by unique restriction sites that are compatible with many others.



Contents and Storage

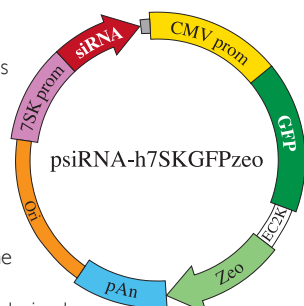
Each pUNO plasmid is provided as a lyophilized transformed *E. coli* strain on a paper disk. Transformed strains are shipped at room temperature and should be stored at -20°C. Lyophilized *E. coli* cells are stable for at least 1 year when properly stored. Each plasmid is provided with 4 pouches of *E. coli* Fast-Media® Blas (2 TB and 2 Agar).

shRNAs in psiRNA Plasmids

Description

Short hairpin RNAs (shRNAs) targeting the genes involved in nucleic acid sensing are expressed by a family of ready-made psiRNA plasmids. The functionality of these shRNAs has been described in the literature or validated in house. Ready-made psiRNA plasmids eliminate the need to design and clone several siRNA sequences before identifying an effective one. They express shRNAs that silence the expression of a target gene by >70%.

Ready-made psiRNAs are psiRNA-h7SKGFPzeo-derived plasmids that express high amounts of shRNAs through the human 7SK RNA Pol III promoter. They feature a GFP::Zeo fusion gene which confers both reporter and antibiotic resistance activities allowing simple monitoring of transfection efficiency and selection with Zeocin™ in both *E. coli* and mammalian cells.



Contents and Storage

Ready-made psiRNA plasmids are available alone or in a kit. Ready-made psiRNA plasmids are provided as 20 µg of lyophilized DNA. Ready-made psiRNA kits contain 20 µg of a Ready-made psiRNA plasmid, 20 µg of a control psiRNA plasmid (psiRNA-Luc), 1 vial of LyoComp GT116, 4 pouches of Fast-Media® Zeo. Products are shipped at room temperature. Store at -20°C.

GENE NAME	SPECIES	pUNO	psiRNA*
AIM2	Human	puno l-haim2	psirna42-haim2
	Mouse	puno l-maim2	psirna42-maim2
ASC	Human	puno-hasca	psirna42-hasc
	Mouse	puno l-masc	psirna42-masc
Caspase 1	Human	porf-hcasp1	psirna42-hcasp1
	Mouse	porf-mcasp1	psirna42-mcasp1
DAI / ZBP1	Human	puno l-hzbp1	psirna42-hdai
	Mouse	puno l-mzbp1a	psirna42-mdai
DDX41	Human	puno l-hddx41	psirna42-hddx41
	Mouse	puno l-mddx41	psirna42-mddx41
DHX9	Human	puno l-hdhx9	psirna42-hdhx9
DHX36	Mouse	puno l-mdhx36	-
IFI16	Human	puno l-hifi16	psirna42-hifi16
	Mouse	puno l-mifi16	psirna42-mifi16
IPS-1	Human	puno-hips1	psirna42-hips1
	Mouse	puno-mips1	psirna42-mips1
IRF1	Human	puno-hirf1	psirna42-hirf1
	Mouse	puno-mirf1	psirna42-mirf1
IRF3	Human	puno-hirf3	psirna42-hirf3
	Mouse	puno-mirf3	psirna42-mirf3
IRF7	Human	puno l-hirf7	psirna42-hirf7
	Mouse	puno-mirf7	psirna42-mirf7
Ku70/XRCC6	Human	puno l-hxrcc6	psirna42-hku70
	Mouse	puno l-mxrcc6	psirna72-mku70
LGP2	Human	puno l-hlgp2	psirna42-hlgp2
	Mouse	puno l-mlgp2	psirna42-mlgp2
LRRFIP1	Human	puno l-hLRRFIP1b	psirna42-hlrrfip1
	Mouse	-	psirna42-mlrrfip1
MDA-5	Human	puno l-hmda5	psirna42-hmda5
	Mouse	puno l-mmda5	psirna42-mmda5
NAPI	Human	puno-hnap1	psirna42-hnap1
	Mouse	puno-mnap1	psirna42-mnap1
RIG-I	Human	puno l-hrigi	psirna42-hrigi
	Mouse	puno-mrigi	psirna42-mrigi
STING	Human	puno l-hsting	psirna42-hsting
	Mouse	puno l-msting	psirna42-msting
TANK	Human	puno l-htank	psirna42-htank
	Mouse	puno l-mtankb	psirna42-mtank
TBK1	Human	puno-htbk1	psirna42-htbk1
	Mouse	puno-mtbk1	psirna42-mtbk1
TRAF3	Human	puno-htraf3	psirna42-htraf3
	Mouse	puno-mtraf3	psirna42-mtraf3
TREX1	Human	puno-htrex1	psirna42-htrex1
	Mouse	puno-mtrex1	psirna42-mtrex1
TRIM32	Human	puno l-htrim32	psirna42-htrim32
TRIM56	Human	puno l-htrim56	psirna42-htrim56
	Mouse	puno l-mtrim56	psirna42-mtrim56

* For the catalog code of the kit, replace psirna42 by ksirna42

CDS and STING Ligands

Intracellular DNA from pathogens is recognized by multiple cytosolic DNA sensors (CDSs), which display contextual preferences for the recognition of DNA. InvivoGen provides a comprehensive collection of double-stranded DNA ligands known to stimulate CDSs and single-stranded DNA that serve as negative controls. These ligands are provided either naked or already complexed to a transfection reagent (LyoVec™).

➤ CDS Ligands

HSV-60 and VACV-70

NEW

HSV-60 and VACV-70 are 60 bp and 70 bp oligonucleotides, respectively, containing viral DNA motifs¹. HSV-60 derives from the herpes simplex virus 1 genome, while VACV-70 derives from the vaccinia virus DNA. Transfected HSV-60 and VACV-70 were shown to potently induce IFN- β in a TLR-, DAI- and RNA Pol III-independent, but STING-, TBK1- and IRF3-dependent manner. Both oligonucleotides are recognized by IFI16¹.

HSV-60c and VACV-70c are single-stranded oligonucleotides which, unlike their double-stranded counterparts, HSV-60 and VACV-70, respectively, are not IFN-inducers¹.

ISD

NEW

ISD (interferon stimulatory DNA) is a 45-bp non-CpG oligomer from the *Listeria monocytogenes* genome. When transfected into various cell types, including plasmacytoid and conventional DCs, macrophages and murine embryonic fibroblasts, ISD strongly enhances the expression of IFN- β ². This ISD-induced response is mediated by the STING-TBK1-IRF3 signaling axis^{2,3}.

ISD Control is a non-immunostimulatory single-stranded oligonucleotide with the same sequence as its double-stranded counterpart.

pCpGfree-giant

pCpGfree-giant is a CpG-free non-coding high molecular weight (~15 kb) plasmid containing AT-rich regions. When transfected in ISG-reporter cells (see next page), pCpGfree-giant induces strong reporter activity. This activity requires STING as cells deficient for STING fail to respond to transfected pCpGfree-giant (see data next page). The CDSs of pCpGfree-giant have not been identified yet, but they probably include IFI16 and DDX41.

Poly(dA:dT) & poly(dG:dC)

Poly(dA:dT) and poly(dG:dC) are repetitive synthetic double-stranded DNA sequences of poly(dA:dT)•poly(dT:dA) and poly(dG:dC)•poly(dC:dG), respectively. Poly(dA:dT) is a synthetic analog of the B-DNA form while poly(dG:dC) is a synthetic analog of the Z-DNA form. Poly(dA:dT) is recognized by several sensors, including DAI⁴, IFI16¹, LRRFIP1⁵ and DDX41⁶. So far, only LRRFIP1 has been clearly identified as a sensor for poly(dG:dC)⁵.

Poly(dA) is a repetitive synthetic single-stranded DNA sequence of polydeoxyadenylic acid with no IFN stimulatory property.

➤ STING Ligands

c-di-AMP and c-di-GMP

NEW

Cyclic diadenylate monophosphate (c-di-AMP) and cyclic diguanylate monophosphate (c-di-GMP) are second messenger molecules produced in bacteria but not in mammals. Both c-di-AMP and c-di-GMP can induce a strong immune response *in vitro* and *in vivo*. It was recently found that these cyclic dinucleotides induce the production of type I interferons in a STING dependent manner⁷. Additionally, STING was shown to directly sense these cyclic dinucleotides⁸, although a very recent study suggests that DDX41 may function upstream of STING⁹.

PRODUCT	QTY	CAT. CODE
CDS Ligands		
HSV-60 Naked	200 μ g	tlrl-hsv60n
HSV-60 / LyoVec	100 μ g	tlrl-hsv60c
HSV-60c Naked (control)	200 μ g	tlrl-hsv60cn
HSV-60c / LyoVec (control)	100 μ g	tlrl-hsv60cc
ISD Naked	200 μ g	tlrl-isdn
ISD / LyoVec	100 μ g	tlrl-isdc
ISD Control Naked	200 μ g	tlrl-isdcn
ISD Control / LyoVec	100 μ g	tlrl-isdcc
pCpGfree-giant Naked	200 μ g	tlrl-cpgfn
pCpGfree-giant / LyoVec	100 μ g	tlrl-cpgfc
Poly(dA) Naked	200 μ g	tlrl-pan
Poly(dA) / LyoVec	100 μ g	tlrl-pac
Poly(dA:dT) Naked	200 μ g	tlrl-patn
Poly(dA:dT) / LyoVec	100 μ g	tlrl-patc
Poly(dG:dC) Naked	200 μ g	tlrl-pgcn
Poly(dG:dC) / LyoVec	100 μ g	tlrl-pgcc
VACV-70 Naked	200 μ g	tlrl-vav70n
VACV-70 / LyoVec	100 μ g	tlrl-vav70c
VACV-70c Naked (control)	200 μ g	tlrl-vav70cn
VACV-70c / LyoVec (control)	100 μ g	tlrl-vav70cc
STING Ligands		
c-di-AMP	1 mg	tlrl-cda
c-di-GMP	1 mg	tlrl-cdg

Contents and Storage

All CDS ligands are available **naked or complexed** with the cationic lipid LyoVec™ to facilitate their uptake. STING ligands are provided naked. CDS and STING ligands are provided lyophilized. Products are shipped at room temperature and should be stored at -20°C.

Related Products

- 5' Triphosphate blunt-end double-stranded (ds) RNA is a ligand for RIG-I, unlike blunt-end dsRNA, which thus serves as a negative control. InvivoGen provides both products naked or complexed with LyoVec™.
 - 5'ppp-dsRNA Naked, 100 μ g (tlrl-3prna-100)
 - 5'ppp-dsRNA / LyoVec, 100 μ g (tlrl-3prnalv-100)
 - 5'ppp-dsRNA Control Naked, 100 μ g (tlrl-3prnac-100)
 - 5'ppp-dsRNA Control / LyoVec, 100 μ g (tlrl-3prnacvl-100)
- Products are also available as 25 μ g units.

CDS Reporter Cell Lines

CDS and STING ligands trigger the production of type I IFNs and the induction of interferon stimulated genes (ISG) through interferon regulatory factors (IRFs). In order to facilitate their study, InvivoGen has developed stable reporter cells in two well established immune cell models, the human monocytic THP-1 cell line and the murine RAW 264.7 macrophages. They feature a novel reporter gene encoding a secreted luciferase, Lucia®.

➤ ISG-Luciferase Reporter Cells

THP1-Lucia™ ISG Cells

NEW

THP1-Lucia™ ISG cells were derived from the human monocytic cell line THP-1, which represents a model of choice to study the activation and signaling of CDSs. Indeed, THP-1 cells have been shown to express all the CDSs identified so far^{6,10,11}, with the exception of DAI¹².

THP1-Lucia™ ISG cells express the secreted luciferase Lucia® reporter gene under the control of an IRF-inducible promoter. This composite promoter is comprised of five IFN-stimulated response elements (ISRE) fused to an ISG54 minimal promoter, which is unresponsive to activators of the NF-κB or AP-1 pathways. As a result, THP1-Lucia™ ISG cells allow the monitoring of the IRF pathway by determining the activity of Lucia®. The levels of IRF-induced Lucia® in the cell culture supernatant are readily assessed with QUANTI-Luc™, a Lucia® detection reagent. THP1-Lucia™ ISG cells are resistant to Zeocin™.

THP1-Lucia™ ISG cells are highly responsive to transfected double-stranded nucleic acids, such as CDS and STING ligands (see figure).

RAW-Lucia™ ISG Cells

NEW

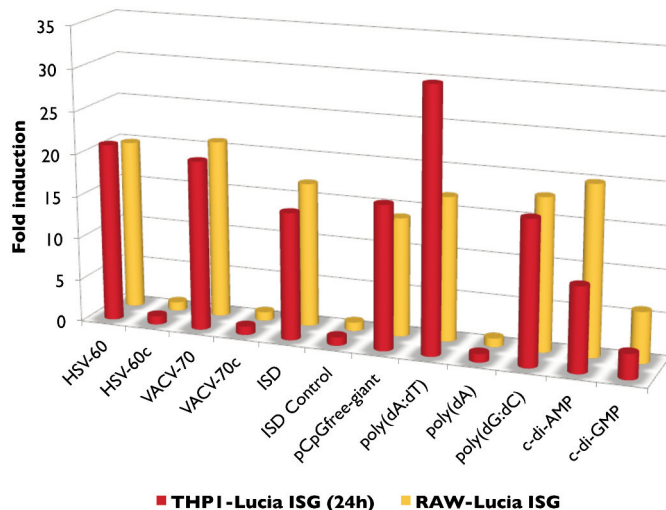
RAW-Lucia™ ISG cells were generated from the murine RAW 264.7 macrophage cell line by stable integration of an interferon regulatory factor (IRF)-inducible Lucia® reporter construct. RAW 264.7 have been reported to express IFI16¹³, DDX41 and AIM2¹⁴.

RAW-Lucia™ ISG cells express the Lucia™ gene under the control of an ISG54 minimal promoter in conjunction with five IFN-stimulated response elements. Thus, RAW-Lucia™ ISG cells allow the monitoring of IRF activation by determining the activity of Lucia®. The levels of IRF-induced Lucia® in the cell culture supernatant can be easily monitored using QUANTI-Luc™, a Lucia® detection reagent. RAW-Lucia™ ISG cells are resistant to Zeocin™.

RAW-Lucia™ ISG cells are responsive to murine IFN-α and IFN-β but do not respond to their human counterparts. They are also responsive to PRR ligands that trigger the IFN signaling pathway, such as transfected double-stranded nucleic acids (see figure).

References:

1. Unterholzner L. et al., 2010. IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol.* 11(11):997-1004. 2. Stetson DB & Medzhitov R. 2006. Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. *Immunity.* 24(1):93-103. 3. Ishikawa H. et al., 2009. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature.* 461(7265):788-92. 4. Takaoka A. et al., 2007. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature.* 448(7152):501-5. 5. Yang P. et al., 2010. The cytosolic nucleic acid sensor LRRFIP1 mediates the production of type I interferon via a beta-catenin-dependent pathway. *Nat Immunol.* 11(6):487-94. 6. Zhang Z. et al., 2011. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat Immunol.* 12(10):959-65. 7. Jin L. et al., 2011. MPYS is required for IFN response factor 3 activation and type I IFN production in the response of cultured phagocytes to bacterial second messengers cyclic-di-AMP and cyclic-di-GMP. *J Immunol.* 187(5):2595-601. 8. Burdette DL. et al., 2011. STING is a direct innate immune sensor of cyclic di-GMP. *Nature.* 478(7370):515-8. 9. Parvatiyar K. et al., 2012. The helicase DDX41 recognizes the bacterial secondary messengers cyclic di-GMP and cyclic di-AMP to activate a type I interferon immune response. *Nat Immunol.* [Ahead of print]. 10. Veeranki S. et al., 2011. IFI16 protein mediates the anti-inflammatory actions of the type-I interferons through suppression of activation of caspase-1 by inflammasomes. *PLoS One.* 6(10):e27040. 11. Arakawa R. et al., 2010. Characterization of LRRFIP1. *Biochem Cell Biol.* 88(6):899-906. 12. Lippmann J. et al., 2010. IFNβ responses induced by intracellular bacteria or cytosolic DNA in different human cells do not require ZBP1 (DLM-1/DAI). *Cell Microbiol.* 10(12):2579-88. 13. Kawasaki T. et al., 2011. Recognition of nucleic acids by pattern-recognition receptors and its relevance in autoimmunity. *Immunol Rev.* 243(1):61-73. 14. Stein SC & Falck-Pedersen E., 2012. Sensing adenovirus infection: activation of interferon regulatory factor 3 in RAW 264.7 cells. *J Virol.* 86(8):4527-37.



IRF response of THP1-Lucia™ ISG and RAW-Lucia™ ISG cells. Cells were transfected with CDS ligands (1 µg/ml of HSV-60, HSV-60c, VACV-70, VACV-70c, ISD, ISD Control or 0.3 µg/ml pCpGfree-giant, poly(dA:dT), poly(dA), poly(dG:dC) using LyoVec™ or directly stimulated with 10 µg/ml c-di-AMP or c-di-GMP. After 24h incubation, the levels of IRF-induced Lucia® were determined using QUANTI-Luc™.

PRODUCT	QUANTITY	CAT. CODE
THP1-Lucia™ ISG Cells	5-7 × 10 ⁶ cells	thpl-isg
RAW-Lucia™ ISG Cells	5-7 × 10 ⁶ cells	rawl-isg

Contents

THP1-Lucia™ ISG cells are grown in RPMI medium, 2mM L-glutamine, 10% FBS supplemented with 100 µg/ml Zeocin™. RAW-Lucia™ ISG cells are grown in DMEM medium, 2 mM L-glutamine, 10% FBS supplemented with 100 µg/ml Normocin™ and 200 µg/ml Zeocin™. THP1-Lucia™ ISG and RAW-Lucia™ ISG cells are provided frozen in a cryotube containing 5-7 × 10⁶ cells and supplied with 50 mg of Normocin™, 10 mg Zeocin™ and 1 pouch of QUANTI-Luc™.

Also Available

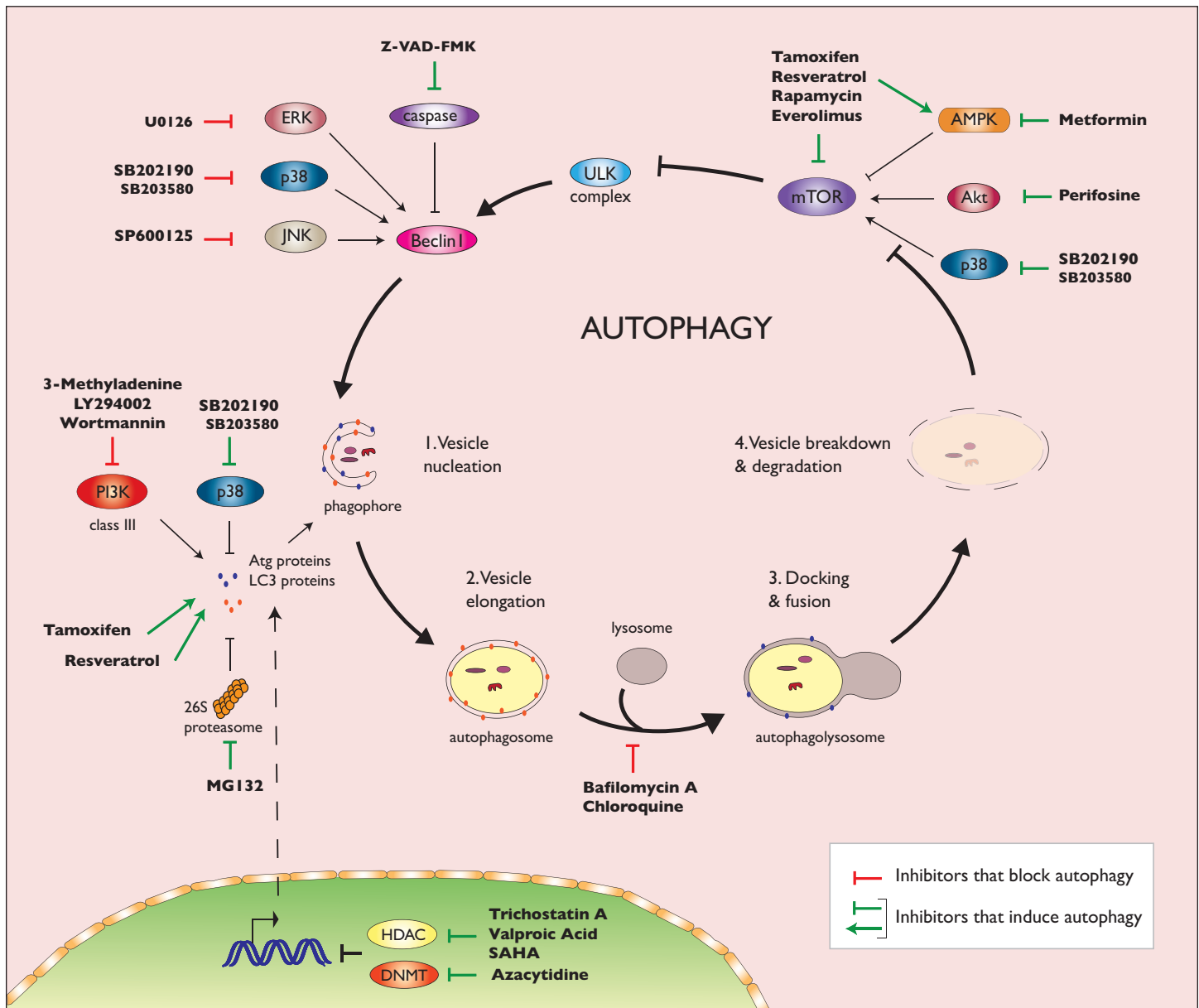
THP1-Blue™ ISG and RAW-Blue™ ISG cells feature an IRF-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene which expression is driven by the same promoter construct than their Lucia® counterparts. Levels of SEAP in the cell supernatant can be determined using QUANTI-Blue™.

Coming Soon

THP1-Blue™ defSTING and THP1-Blue™ defTBK1 are ISG-SEAP reporter cells knock-down for STING or TBK1 expression, respectively. SEAP production is highly reduced in response to cytosolic nucleic acids compared to the parental cell line, THP1-Blue™ ISG.

Autophagy

Autophagy is an orchestrated homeostatic process to eliminate unwanted proteins and damaged organelles. The autophagic process is also used to remove intracellular microbial pathogens. Several signaling pathways sense different types of cell stress, ranging from nutrient deprivation to microbial invasion, and converge to regulate autophagy at multiple stages of the process. A number of inhibitors that target molecules involved in these pathways can impact autophagy at different levels of the process, as depicted in the illustration below, and can be used to study autophagy in cells.



Inhibitors that block autophagy

The primary step in inducing autophagy involves membrane nucleation, controlled by ULK complex and Beclin I. Inhibitors of positive regulators of the ULK complex and Beclin I have been demonstrated to block autophagy. These include inhibitors to the MAP kinases, JNK1, ERK and p38. The induction of Atg protein and LC3 proteins is required for vesicle expansion and formation. Inhibitors of the class III PI3 kinases can block autophagy. In a later step of the autophagic process, inhibitors that inhibit lysosome acidification essentially block the formation of autophagosome and autophagolysosome.

Inhibitors that induce autophagy

mTOR is a major negative regulatory axis of autophagy and is influenced by several nutrient signaling pathways. Direct inhibitors of mTOR and those of pathways activating mTOR, subsequently induce autophagy by inhibiting mTOR. In addition, Beclin I is negatively regulated by caspases, the inhibitors of which act to promote Beclin I action to induce the initial stages of autophagy. Furthermore, inhibitors of the 26S proteasome and the epigenetic regulators, HDACs and DNMTs, result in the increase of Atg and LC3 proteins levels essential to the process of autophagy.

➤ Inhibitors of Autophagy

Inhibitors of Beclin I

SP600125 - JNK Inhibitor

SP600125 is a potent, and selective inhibitor of c-Jun N-terminal kinase (JNK). Inhibition of JNK activity by SP600125 is usually associated with downregulation of Beclin-I and reduced autophagy.

U0126 - MEK1/MEK2 Inhibitor

U0126 is a selective inhibitor of the MAP kinase kinases, MEK1 and MEK2, and thus of ERK activation. U0126 can be used to study the role of ERK, a MAPK involved in the induction of autophagy.

Inhibitors of autophagosome / autophagolysosome formation

3-Methyladenine, LY294002 & Wortmannin - PI3K Inhibitors

The PI3K inhibitors, 3-methyladenine (3MA), LY294002 and wortmannin have a net inhibitory effect on autophagy through blocking the class III PI3Ks that are critical during the late stage of vesicle expansion.

SB203580 & SB202190 - p38 MAP Kinase Inhibitors

SB203580 and its close relative, SB202190, are widely used to assess the physiological roles of p38 α and p38 β MAPKs. Several studies suggest that p38 MAPKs regulate distinct phases of autophagy. p38 can elicit autophagy via Beclin I. Contrarily, p38 α has also been reported to inhibit autophagy by interfering with the trafficking of Atg9.

Bafilomycin A1 & Chloroquine - Endosomal Acidification Inhibitor

Bafilomycin A1 and chloroquine are known inhibitors of the late phase of autophagy. They both cause a disruption in the pH of acidic vesicles preventing the fusion of autophagosomes with lysosomes

PRODUCT	QUANTITY	CAT. CODE
3-Methyladenine	50 mg	tlrl-3ma
5-Aza-cytidine	100 mg	inh-aza
Bafilomycin A1	10 μ g	tlrl-baf
Chloroquine	250 mg	tlrl-chq
Everolimus	5 mg	tlrl-eve
LY294002	5 mg	tlrl-ly29
Metformin	1 g	tlrl-metf
MG-132	5 mg	tlrl-mg132
Perifosine	5 mg	tlrl-peri
Rapamycin	5 mg	tlrl-rap
Resveratrol	100 mg	tlrl-resv
SAHA	25 mg	inh-saha
SB202190	5 mg	tlrl-sb90
SB203580	5 mg	tlrl-sb20
SP600125	10 mg	tlrl-sp60
Tamoxifen	200 mg	tlrl-txf
Trichostatin A	1 mg	met-tsa-1
U0126	5 mg	tlrl-u0126
Valproic acid	5 g	inh-vpa
Wortmannin	5 mg	tlrl-wtm
Z-VAD-FMK	1 mg	tlrl-vad

➤ Activators of Autophagy

Inhibitors of mTOR activation

Metformin - AMPK Activator

Metformin activates adenosine monophosphate-activated protein kinase (AMPK), an enzyme that coordinates control of cell growth and autophagy. Metformin can be used to induce autophagy in an AMPK-dependent manner.

Perifosine - Akt Inhibitor

Perifosine is the best characterized inhibitor of Akt. mTOR is a target for Akt, the activation of which suppresses autophagy. Therefore, treatment with perifosine, alone or in combination with rapamycin, induces autophagy.

Rapamycin & Everolimus - mTOR Inhibitors

Rapamycin (sirolimus) and its analog, everolimus (RAD001), are potent inhibitors of mTOR, the master negative regulator of autophagy. Both inhibitors are powerful inducers of autophagy.

Resveratrol - mTOR Inhibitor

Resveratrol (3,4',5-trihydroxy-trans-stilbene) is a polyphenol found in plants displaying autophagy-promoting activities. Resveratrol induces autophagy through its capacity to both inhibit mTOR and activate AMPK.

Tamoxifen - Estrogen Receptor Antagonist

Tamoxifen is an antagonist of the estrogen receptor known to induce autophagy. Tamoxifen stimulates autophagy by increasing the intracellular level of ceramide, which inhibits mTOR activation and/or stimulates expression of Atg genes.

Activators of autophagosome formation

MG-132 - 26S Proteasome Inhibitor

MG-132 selectively blocks the proteolytic activity of the 26S proteasome and is used to disrupt the proteasome-regulated degradation of proteins. Recent studies suggest that inhibition of the proteasome by MG-132 can induce autophagy by stabilizing Atg protein levels.

SAHA, Trichostatin A & Valproic acid - HDAC Inhibitors

SAHA (Vorinostat), Trichostatin A and Valproic Acid are histone deacetylase (HDAC) inhibitors. HDACs epigenetically silence the transcription of Atg and LC3 genes. Thus, HDAC inhibitors can lead to an increase of Atg and LC3 proteins levels and promote autophagy.

5-Aza-cytidine - DNA Methyltransferase Inhibitor

5-aza-cytidine (AZA) is a potent inhibitor of DNA methyltransferase I (DNMT1). Similar to HDAC inhibitors, AZA can enhance the expression of Atg and LC3 and hence induce autophagy.

Z-VAD-FMK - Caspase Inhibitor

Z-VAD-FMK is a cell-permeable pan-caspase inhibitor that irreversibly binds to the catalytic site of caspase proteases. Through the inhibition of caspases 3, 7 and 8, Beclin I is stabilized and autophagy is promoted.

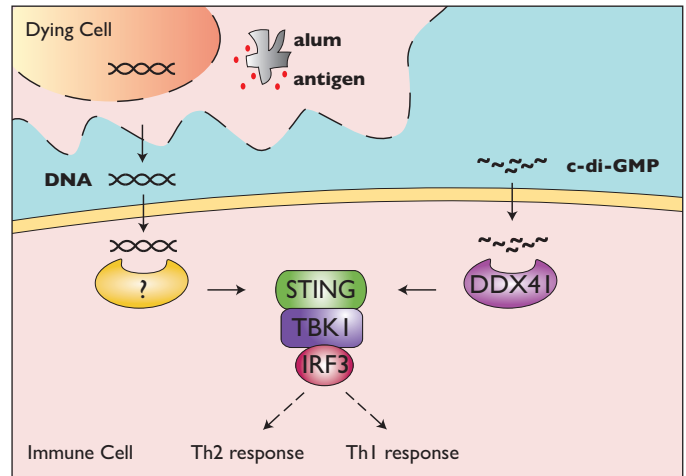
Warning: The net effect of many inhibitors on autophagy is dependent on the induction cue, cell-type and the stage of the autophagic process. Differential or off-target effects by any one particular inhibitor may be seen. It is advisable to confirm effects through use of multiple inhibitors.

Contents and Storage

Products are provided as solids and shipped at room temperature. Store at room temperature, 4°C or -20°C according to the product label. Products are stable at least 6 months.

STING-in Vaccine Adjuvants

Adjuvants enhance and direct the adaptive immune response to vaccine antigens through various mechanisms, some of which are still poorly understood. Recently, STING and more generally the host nucleic acid sensing machinery were shown to play an essential role in vaccination. Aluminum salt formulations (referred to as alum) have been used for decades as effective and safe vaccine adjuvants. Alum mostly potentiates IgG1 and IgE production through the promotion of Th2 cell responses. It has long been known that alum causes cell death and the release of numerous molecules that act as damage-associated molecular patterns (DAMPs). Two of these DAMPs, which are both connected to nucleic acid biology, have been identified. Uric acid¹, which is the end product of the degradation of purines, and host cell DNA² have been shown to accumulate at sites of alum injection and to induce T cell responses and the production of IgG1 and IgE. The signaling pathways activated by uric acid in this context have not been identified, nor the cytosolic DNA sensor(s) triggered by host DNA in alum immunization^{1,2}. However, the (STING)-TBK1-IRF3 axis has been shown to control the IgE response². The STING-TBK1-IRF3 axis has also been involved in the adjuvant activity of c-di-GMP. Cyclic-di-GMP is a bacterial signaling molecule with strong immunostimulatory activity, which is currently being investigated as a mucosal adjuvant^{3,4}. Several studies have shown that intranasal immunization with c-di-GMP promotes predominantly Th1 responses, essential for the elimination of intracellular pathogens. Although its adjuvancy mechanism is not yet understood, c-di-GMP is known to activate the innate immune system⁵ and induce the production of type I IFNs through STING-TBK1-IRF3⁶, the same signaling pathway used by intracellular DNA. According to a very recent study, c-di-GMP is recognized by the cytosolic DNA sensor DDX41⁷ and not by STING directly, as previously thought⁸. The data suggest that DDX41 binds to c-di-GMP then forms a complex with STING to facilitate downstream signaling and the activation of type I IFNs. It is intriguing that the STING-TBK1-IRF3 signaling pathway can promote two distinct Th responses.



1. Kool M. et al., 2008. Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. *J Exp Med.* 205(4):869-82.
2. Marichal T. et al., 2011. DNA released from dying host cells mediates aluminum adjuvant activity. *Nat Med.* 17(8):996-1002.
3. Chen W. et al., 2010. The potential of 3',5'-cyclic diguanylic acid (c-di-GMP) as an effective vaccine adjuvant. *Vaccine.* 28(18):3080-5 (review).
4. Pedersen GK. et al., 2011. Evaluation of the sublingual route for administration of influenza H5N1 virosomes in combination with the bacterial second messenger c-di-GMP. *PLoS One.* 6(11):e26973.
5. Karaolis DK. et al., 2007. Bacterial c-di-GMP is an immunostimulatory molecule. *J Immunol.* 178(4):2171-81.
6. Jin L. et al., 2011. MPYS is required for IFN response factor 3 activation and type I IFN production in the response of cultured phagocytes to bacterial second messengers cyclic-di-AMP and cyclic-di-GMP. *J Immunol.* 187(5):2595-601.
7. Parvatiyar K. et al., 2012. The helicase DDX41 recognizes the bacterial secondary messengers cyclic di-GMP and cyclic di-AMP to activate a type I interferon immune response. *Nat. Immunol.* [Ahead of print].
8. Burdette DL. et al., 2011. STING is a direct innate immune sensor of cyclic di-GMP. *Nature.* 478(7370):515-8.

InvivoGen provides different classes of vaccine adjuvants that are either already approved for use in human vaccination or under investigation. InvivoGen's adjuvants are VacciGrade™, a specific grade for *in vivo* studies. They are prepared under strict aseptic conditions. They are guaranteed sterile and thoroughly tested for the presence of endotoxins.

Alhydrogel® 2%

Alhydrogel® 2% is an aluminium hydroxide (alum) wet gel suspension and the most widely used adjuvant in licensed human vaccines. Alum causes the adsorption of protein antigens, slowing their release from the injection site (the "depot effect") and facilitating their uptake by antigen-presenting cells. Alum increases Th2 antibodies but does not promote significant Th1 cellular response. Alum has long been known to trigger the production of the proinflammatory cytokine IL-1β which was recently found to be mediated by the NLRP3 inflammasome¹. However, the contribution of the NLRP3 inflammasome in the adjuvant effect of alum remains unclear.

c-di-GMP & c-di-AMP VacciGrade™

Cyclic diguanylate monophosphate (c-di-GMP) and cyclic diadenylate monophosphate (c-di-AMP) are intracellular signaling molecules produced by bacteria. Both molecules have been shown to activate the host innate immune system, which has prompted their evaluation as vaccine adjuvants. Preclinical studies have proven that co-administered c-di-GMP elicits a Th1-biased response after systemic or mucosal vaccination², while recent data suggest that c-di-AMP promotes a balanced Th1/Th2/Th17 response pattern³.

Contents and Storage

Alhydrogel 2% is provided as a ready-to-use, sterile wet gel suspension. c-di-GMP and c-di-AMP VacciGrade™ are provided lyophilized. Products are shipped at room temperature. Store at 4°C or -20°C according to the product label.

PRODUCT	QUANTITY	CAT. CODE
Alhydrogel® 2% (Alum)	250 ml	vac-alu-250
c-di-AMP VacciGrade™	1 mg	vac-cda
c-di-GMP VacciGrade™	1 mg	vac-cdg

1. Eisenbarth SC. et al., 2008. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature.* 453(7198):1122-6.
2. Chen W. et al., 2010. The potential of 3',5'-cyclic diguanylic acid (c-di-GMP) as an effective vaccine adjuvant. *Vaccine.* 28(18):3080-5.
3. Ebensen T. et al., 2011. Bis-(3',5')-cyclic dimeric adenosine monophosphate: strong Th1/Th2/Th17 promoting mucosal adjuvant. *Vaccine.* 29(32):5210-20.

Check our website for the complete list of InvivoGen's vaccine adjuvants

<http://www.invivogen.com/vaccine-adjuvants>