Review

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Response of host inflammasomes to viral infection

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Inflammasomes are multiprotein complexes that induce downstream immune responses to specific pathogens, environmental stimuli, and host cell damage. Components of specific viruses activate different inflammasomes; for example, the influenza A virus M2 protein and encephalomyocarditis virus (EMCV) 2B protein activate the nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain (PYD)-containing 3 (NLRP3) inflammasome, whereas viral double-stranded RNA (dsRNA) activates the retinoic acid inducible gene-I (RIG-I) inflammasome. Once activated in response to viral infection, inflammasomes induce the activation of caspases and the release of mature forms of interleukin-1β (IL-1β) and IL-18. Here we review the association between viral infection and inflammasome activation. Identifying the mechanisms underlying virus-induced inflammasome activation is important if we are to develop novel therapeutic strategies to target viruses.

Overview of inflammasomes

Many of the cells that constitute the front line of host defenses express germline-encoded receptors called pattern recognition receptors (PRRs) [1,2]. PRRs detect microbial and viral components known as pathogen-associated molecular patterns (PAMPs) [3] and rapidly initiate innate immune responses, including the secretion of cytokines and chemokines (see Glossary) and the maturation and differentiation of immune cells, which then mount an adaptive immune response. PRRs are specific for certain PAMPs and are expressed by different cell types. For example, Toll-like receptors (TLRs) [4,5] and C-type lectin receptors (CLRs) [6] bind extracellular and endosomal PAMPs, whereas NLRs [7], IFI200 family member absent in melanoma 2 (AIM2) [8], and RIG-I-like helicases (RLHs) [9] are specific for intracellular PAMPs.

Proinflammatory stimuli, including some PAMPs, induce expression of the inactive pro-forms of IL-1β and IL-18, which are important proinflammatory cytokines that stimulate the production of adhesion molecules and chemokines, which in turn trigger immune and inflammatory responses [10]. Maturation of these pro-cytokines requires the proteolytic cleavage of active proinflammatory caspases, particularly caspase-1, to release the active cytokine [11]. Caspase-1 is a cysteine protease present in the cytoplasm as an inactive zymogen. The autocleavage process yields an active caspase-1 p10/p20 tetramer, which is regulated by multiprotein complexes called inflammasomes [12].

Several distinct inflammasomes have been identified, including the NLRP3 inflammasome, the NLRP1 inflammasome, the AIM2 inflammasome, and the RIG-I inflammasome. After PRRs detect the presence of specific PAMPs, they act as scaffold proteins for specific inflammasome complexes, leading to the activation of caspases and cytokines. Recently, researchers have begun to appreciate that viruses activate inflammasomes, which in turn mediate antiviral responses. Here we review the role and activation mechanisms of inflammasomes during viral infection (Table 1).

The NLRP3 inflammasome

NLRP3 belongs to the NLR family, which comprises 22 genes in humans and at least 34 in mice. The NLRs are multidomain proteins comprising an N-terminal caspase recruitment domain (CARD), a PYD, or a baculovirus inhibitor of apoptosis repeat domain (BIR), a central nucleotide-binding and oligomerization domain (NACHT) (also termed NOD), and C-terminal leucine-rich repeats (LRRs) [13,14]. The N-terminal domain mediates signal transduction via direct interaction with other CARD- or

Glossary

Adaptive immune response: includes humoral responses, mediated by B cells to recognize antigens and produce antibody-secreting plasma cells and memory cells, and cell-mediated responses, mediated by T cells to destroy antigen-presenting cells and to release more cytokines.
Caspases: cysteine proteases that cleave, for example, cytokines, the cytoskeleton, nuclear lamina proteins, caspases, and inhibitor of caspase-activated DNase. Caspases are important in regulating apoptosis.
Cytokines and chemokines: cytokines are small proteins produced by immune cells that are involved in regulating immune responses to extrinsic threats and diseases; chemokines are a type of cytokine that induce chemotaxis to trigger the movement of target cells toward sites of inflammation.
Interleukins: a group of cytokines that bind to specific receptors on the plasma membrane to induce downstream signaling pathways stimulating immune cell proliferation and differentiation.
Mitophagy: a mitochondrion degradation pathway by lysosomes to eliminate damaged mitochondria and maintain normal biological activities of cells such as the production of energy, ion storage, and cell proliferation.
Phagocytosis: a process by which cells devour extracellular material by the formation of phagosomes.
Viroporins: viral proteins with ion channel activities. Viroporin oligomers can form pores on membrane structures to facilitate virus release and disturb the intracellular ionic balance.

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Table 1. Virus-induced inflammasome activation

PRR	Virus	Activator	Possible mechanisms involved in activation	Refs
NLRP3	Influenza virus	M2 viroporin, PB1-F2, and vRNA[a]	Mitochondrial biological function [$\Delta\Psi$(m)] and Mfn2, H^+, cathepsin B, and ROS	[34,39–41]
	EMCV	2B viroporin	Mitochondrial biological function [$\Delta\Psi$(m)] and Mfn2, Ca^{2+} flux	[34,42,49]
	HRV	2B viroporin	NLRC5 and Ca^{2+} flux	[43]
	RSV	SH viroporin	Monovalent cation	[44]
	HCV	vRNA	ROS and K^+ efflux	[46,47]
	JEV		ROS and K^+ efflux	[54]
	Sendai virus		MAVS	[36]
	RVFV		MAVS	[55]
	Dengue virus		CLEC5A	[56]
	HSV-1, VSV, WNV, rabies virus, and VACV Ankara			[48–53]
NLRP1	LCMV			[60]
AIM2	VACV and mCMV	dsDNA		[62,64]
RIG-I	Influenza virus	dsRNA replication intermediates	MAVS, type I IFN, and IFNAR1	[75,76]
	VSV	dsRNA replication intermediates	K^+ efflux	[77]

[a]Viral RNA.

PYD-containing proteins. The NLR family contains several subfamilies, which are categorized according to the N-terminal domain. The central NACHT domain mediates oligomerization and serves as a scaffold protein for inflammasomes, which is believed to be the crucial step of inflammasome activation. Previous studies indicate that NLRP3 mutations within NACHT induce higher inflammasome activities and are correlated with several autoinflammatory diseases [15,16]. The LRRs are thought to act as a ligand sensor. The domain structure of NLRP3 (from N terminus to C terminus) is PYD–NACHT–LRRs.

Several PAMPs from pathogens including *Candida albicans* [17], *Listeria monocytogenes*, *Neisseria gonorrhoeae* [18,19], and Sendai and influenza viruses [20] activate NLRP3. In addition, NLRP3 is also activated by host damage-associated molecular patterns (DAMPs), including extracellular ATP, hyaluronan, and monosodium urate (MSU) crystals produced from injured or necrotic cells [18,21,22], and by environmental stimuli such as asbestos and silica aggregates, aluminum (alum), and UVB irradiation [23–25].

The NLRP3 inflammasome is the most studied inflammasome and comprises NLRP3, the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), and procaspase-1. On activation, NLRP3 forms a homo-oligomer via its NACHT domain and directly interacts with ASC through its PYD. ASC directly interacts with procaspase-1 through the CARD. Formation of the NLRP3 inflammasome induces the activation of caspase-1 and the production of mature IL-1β and IL-18 [11].

Three major mechanisms of NLRP3 inflammasome activation have been proposed (Figure 1). The first is the ion channel model. When the concentration of extracellular ATP increases, it stimulates both potassium ion efflux through the P2X7 ATP-gated ion channel and membrane pannexin-1 pore formation, which facilitates the influx of PAMPs and DAMPs [26]. The second is the lysosomal rupture model. Several NLRP3 activators, such as silica and asbestos aggregates, enter cells via phagocytosis, which induces lysosome collapse and the release of lysosomal contents, particularly cathepsin B. Cathepsin B plays an important role in activating NLRP3 [25]. The third is the reactive oxygen species (ROS) model. NLRP3 activators induce the production of ROS by NADPH oxidase, which then activates NLRP3. ROS-sensitive thioredoxin-interacting protein (TXNIP) is thought to be involved in this NLRP3 activation mechanism. In addition, the increased ROS concentration induces the efflux of potassium ions to further activate NLRP3 [27,28].

Besides potassium ions, the mobilization of calcium ions induces NLRP3 inflammasome activation. Elevated concentrations of extracellular calcium ions activate the NLRP3 inflammasome through G protein-coupled calcium-sensing receptors (CaSRs) or G protein-coupled receptor family C group 6 member A (GPRC6A) and phospholipase C (PLC) to cleavage phosphatidylinositol 4,5-bisphosphate (PIP2) to diacyl glycerol (DAG) and inositol trisphosphate ($InsP_3$, IP_3). $InsP_3$ induces the release of calcium ions from the endoplasmic reticulum (ER) via the $InsP_3$ receptor, causing higher concentrations of intracellular calcium ions to trigger inflammasome assembly and IL-1β production [29,30]. The calcium efflux from ER induces store-operated Ca^{2+} entry (SOCE)-dependent calcium influx from the extracellular space, and this process is important in NLRP3 inflammasome activation [31]. The activated CaSR also induces a decrease of intracellular cAMP to trigger the activation of the NLRP3 inflammasome in a dependent manner [30]. In addition, treatment with charged liposomes induces production of ROS, and the release of IL-1β and the calcium influx induced by charged liposomes is ROS dependent. Deficiency in or inhibition of transient receptor potential melastatin 2 (TRPM2), a cationic nonselective channel, blocks the calcium influx and liposome-induced NLRP3 inflammasome activation [32]. These observations suggest that calcium ions serve as a regulator of inflammasome activation (Figure 1).

Recent studies also demonstrate that mitochondria are involved in activating the NLRP3 inflammasome. NLRP3 activators such as MSU, alum, and nigericin induce