REVIEW

Caspases control antiviral innate immunity

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Caspases are a family of cysteine proteases whose functions have been scrutinized intensively in recent years. Beyond their established roles in programmed cell death and inflammatory response, some caspases are also fundamental players in antiviral immunity by fine-tuning the levels of antiviral signaling adapters and cytokines, such as type I interferons, which serves as a major, sophisticated weapon against viruses. Viral infections can result in inflammasome activation and the initiation of cell death, including apoptosis and pyroptosis, and multiple caspases are significantly involved in these processes. This review will focus on the cutting-edge discoveries regarding the multifaceted roles of caspases in antiviral innate immunity.

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INTRODUCTION

Viral infections can induce systemic immune responses, which are shaped by specific signaling cascades between the host and the pathogen. Host cells are equipped with an array of multiple germline-encoded pattern recognition receptors (PRRs) to detect pathogen-associated molecular patterns (PAMPs). Viral PAMPs are often nucleic acid-based, including double-stranded (ds) RNA, single-stranded (ss) RNA and viral DNA.¹ In some cases, hosts also detect viral proteins, such as viral fusion glycoproteins, respiratory syncytial virus (RSV)-F protein and Ebola virus GP1 proteins. Current studies have shown that PRRs detect viral nucleic acids mainly consisting of several Toll-like receptor (TLR) family members and various cytosolic sensors. TLRs 3, 7, 8, 9 and 13 can recognize viral nucleic acids in the endosomal compartment. Viral RNA serves as a ligand for both cytosolic NLRP3 inflammasomes and RIG-I-like receptors (RLRs), such as RIG-I, MDA-5 and DHX58 (DEXH box polypeptide 58 or LGP2).^{2–4} Thus far, the cytoplasmic DNA sensors identified include DNA-dependent activator of IFN-regulator factors, DEAD box polypeptide 41, RNA polymerase III, STING (also known as MITA/ERIS/MPYS/TEM173), cGAS, polyglutamine-binding protein 1 (PQB1),⁵ DNA-dependent protein kinase,⁶ sex-determining region

Y-box 2,⁷ meiotic recombination 11 homolog A,⁸ AIM2 and IFI16 inflammasomes.^{9–17}

Upon detecting viral nucleic acids, PRRs activate a set of adapter proteins and relay the signals to downstream transcriptional factors to drive the production of IFNs and IFN-stimulated genes (ISGs), induce the secretion of inflammatory cytokines and further initiate pyroptosis, a lytic, programmed cell death.

Insufficient production of IFNs and inflammatory cytokines cause the deficiency of viral infection clearance and leads to chronic viral infections, while excessive amounts of IFNs and inflammatory cytokines contribute to autoimmune and auto-inflammatory diseases. Meanwhile, concomitant uncontrolled pyroptosis is also very detrimental to the host. Accordingly, mammals have developed a series of critical downstream mediators to fine-tune the antiviral innate immune system, aiming to balance immune responses against viral pathogens at an appropriate level.

Among the various mediators, caspases are a family of genes situated at the nexus of vital regulatory networks, as they balance inflammation, antiviral innate immunity and cell death. This review will focus on how caspases control antiviral innate immunity on multiple levels.

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Caspases and signaling pathways

Caspases are initially synthesized as inactive monomeric zymogens (procaspases) that only obtain catalytic activity upon an appropriate stimulus (Table 1). While 18 mammalian caspases have recently been identified, the newly discovered caspases-15 through -18, except for caspase-16, are absent in placental mammals. The other 12 members of the caspase family are traditionally classified into two groups according to their function.^{19,20} The apoptotic caspases (caspases-2, -3, -6, -7, -8, -9 and -10 in mammals) function in the initiation and execution of a morphologically different programmed cell death called apoptosis. The inflammatory caspases (caspases-1, -4, -5 and -12 in humans and caspases-1, -11 and -12 in mice) mediate innate immune response by cleaving proinflammatory cytokine precursors (for example, pro-IL-1 β and pro-IL-18) to initiate inflammation.⁸⁰ Inflammatory caspases are also involved in another programmed cell death known as pyroptosis.^{19,21–24} The functions of caspases-2, -10, -14 and -16 are currently somewhat controversial and enigmatic and are less easily categorized (Table 1).

The mitochondrial-related intrinsic apoptosis pathway is predominantly controlled by the Bcl-2 family and Bcl-2 homology domain-3 (BH3)-only proteins (the pro-death proteins BAK and BAX are essential effectors).^{21,25,26} In this pathway, apoptotic stimuli (for example, cytotoxic drugs, DNA damage) activate the BH3-only proteins, resulting in activation of the pro-apoptotic effectors BAK/BAX and the induction of MOMP (Figure 1). Subsequently, the mitochondrial proteins, such as cytochrome c, efflux into the cytoplasm, initiating the assembly of the apoptosome, including APAF-1, and further activating caspase-9. Ultimately, apoptosis ensues after the

cleavage and activation of the executioner caspases (caspases-3 and -7) by caspase-9^(refs 27,28) (Figure 1).

The extrinsic apoptosis pathway is death receptor (DR)-mediated. DR members include TNF receptor-1, first apoptosis signal (FAS, also known as APO-1 or CD95), DR3, TRAIL-R1 (TNF-related apoptosis-inducing ligand receptor-1 or DR4) and TRAIL-R2 (or DR-5 in human). The DR ligands include tumor necrosis factor (TNF), CD95-L (CD95 ligand or Fas-L), TRAIL (or Apo-L) and TNF-like ligand 1A. Upon activation, the DR death domain recruits adapter proteins to form the DISC comprising FADD, TRADD, caspase-8 (caspase-10 in human cells) and other components.²⁹ The activated initiator caspase-8 is capable of either initiating the extrinsic apoptotic pathway by cleaving and activating the apoptotic effector caspases (-3, -6, -7) or amplifying intrinsic mitochondrial apoptosis by cleaving BID to a truncated form (tBID)³⁰ (Figure 1). In certain conditions, when the levels of FLIP_L (long isoform of the FLICE-like inhibitory protein) are decreased and upon genotoxic stress, caspase-8 associates with RIPK1 and FADD to form a multiple protein complex called the 'riposome', leading to apoptosis.^{6,31–33} (Figure 1).

Inflammatory caspases function as central adapters of innate immunity, mainly by controlling the assembly of larger, multiple protein complexes called inflammasomes. Canonical inflammasomes are often characterized as scaffold proteins containing members of the NLR (nucleotide-binding-and-oligomerization domain and leucine-rich-repeat-containing) family, the AIM2-like receptor family or the tripartite motif (TRIM, of which pyrin is the only member of inflammasomes) family and caspase-1 molecules together to trigger the autocleavage and activation of caspase-1.³⁴ The inflammasomes currently known to be engaged in antiviral innate immunity include the NLRP3 inflammasome,^{2,35–38} the RIG-I inflammasome³⁹ and the AIM2 inflammasome.^{10–12} The noncanonical inflammasomes comprise caspases-4 and -5 in humans and caspase-11 in mice, which directly recognize lipopolysaccharides (LPS).^{40–43} Both canonical caspase-1 and noncanonical caspase-11 inflammasomes trigger two sets of inflammatory responses: the cleavage and release of proinflammatory cytokines, including IL-1 β , IL-18 and IL-33,⁴⁴ and the induction of pyroptosis.^{34,45}

Caspase-1-dependent antiviral inflammasomes

Caspase-1 plays a vital role in inflammatory responses by assembling a large (700 kDa) molecular structure called an inflammasome.⁴⁶ The conversion of pro-caspase-1 into catalytically active caspase-1 is tightly connected with the maturation of pro-IL-1 β , pro-IL-18 and most likely pro-IL-33 in some cases. To date, four distinct caspase-1-dependent inflammasomes have been identified as being involved in antiviral innate immunity: the RIG-I inflammasome,^{39,47} the AIM2 inflammasome,^{10–12} the IFI16 inflammasome and the NLRP3 inflammasome.^{2,36–38} The adapter ASC provides a platform between RIG-I, NLRP3, AIM2 or IFI16 and procaspase-1 through a homotypical association with N-terminal PYDs (pyrin domains) and C-terminal CARDs. Once the

Table 1 Classification of caspases

	Caspases	Genomic location		Domain Structure
		Human	Mouse	
Apoptosis Initiator	Caspase-2	7q34-7q35	6B2.1	452
	Caspase-9	1p36.21	4E1	416
	Caspase-8	2q33-2q34	1B	479
	Caspase-10	2q33-2q34	Absent	521
Apoptosis Effector	Caspase-3	4q34	8B1.1	277
	Caspase-6	4q25	3H1	293
	Caspase-7	10q25	19D2	303
Inflammation	Caspase-1	11q23	9A1	404
	Caspase-4	11q22.2-11q22.3	Absent	377
	Caspase-5	11q22.2-11q22.2	Absent	434
	Caspase-11	Absent	9A1	373
	Caspase-12	11q22.3	9A1	419
			Casp-12L	213
			Casp-12S	213
	Caspase-14	19p13.1	10C1	242
	Caspase-16	16p13.3	17A3.3	183

CARD, caspase activation and recruitment domain;
DED, death effector domain; L, long form; S, short form

The apoptotic caspases have been subdivided by their mechanism of action into initiator caspases (caspases-2, -8, -9 and -10) and effector caspases (caspases-3, -6 and -7). Caspase-10 is only present in the mouse genome. The initiator caspases are involved in various apoptotic pathways, which fall into either the intrinsic or extrinsic category.^{18,137}

filamentous inflammasome complex is assembled, pro-caspase-1 is initially autoproteolytically cut into a p35 CARD-containing fragment and a p10 fragment. Sequentially, p35 is further digested into a p20 fragment and a CARD, and then two p20 subunits heterodimerize with two p10 subunits to produce a bioactive caspase-1. Activated caspase-1 then mediates the processing of pro-IL-1 β and pro-IL-18 at cysteine residues adjacent to aspartic acid residues. The secreted and active cytokines lead to downstream inflammatory responses (Figure 2).

AIM2 and IFI16 inflammasomes. AIM2 and IFI16 belong to a family of interferon-inducible HIN-200 proteins, which contain an N-terminal pyrin domain for sensing cytoplasmic DNA and a C-terminal domain (oligonucleotide/oligosaccharide-binding domain) for interacting with ASC to activate caspase-1 and caspase-8. Caspase-1 mediates IL-1 β and IL-18 maturation and pyroptosis, while caspase-8 induces apoptosis. The family includes IFI16, MNDNA, IFIX and AIM2 in humans and IFI202, IFI203, IFI204, IFI205, Pyin1 and AIM2 in mice. Four groups have discovered that AIM2 senses cytoplasmic dsDNA, including viral DNA (for example, adenovirus and retrovirus). A report using cultured cells showed that vaccinia virus (VACV) and mouse cytomegalovirus (mCMV) triggered the autoactivation of caspase-1 through AIM2. Moreover, *Aim2*-deficient mice infected with mCMV revealed low IL-18 levels, decreased IFN γ -expressing nature killer cells and higher mCMV viral titers compared to control mice.⁵⁶ Intriguingly, current work revealed that AIM2 could also recognize RNA viruses, such as Chikungunya virus (CHIKV) and West Nile virus in a caspase-1-dependent manner in human dermal fibroblasts. Similarly, inhibition of the inflammasome via caspase-1 silencing enhanced CHIKV replication in resident skin cells.⁵⁷ Furthermore, skin fibroblasts infected with Zika virus (a ssRNA virus) displayed elevated levels of AIM2 and IL-1 β transcripts.⁵⁸ More intensive studies are needed to explore the mechanism of how AIM2 recognizes RNA viruses

Figure 2 Caspase-1-mediated inflammasomes involved in antiviral defense. In responses to viral PAMPs, RIG-I, NLRP3, AIM2 and IFI16 assemble caspase-1-activating inflammasome complexes in an ASC-dependent manner. Activated caspase-1 drives the cleavage and maturation of the proinflammatory cytokines pro-IL-1 β and pro-IL-18. Inflammatory caspases cleave gasdermin D, causing pyroptosis, which promotes the release of matured IL-1 β and IL-18. The NLRP3 inflammasome recognizes multiple PAMPs, including viral RNA, in combination with ion flux or ROS. The RIG-I and AIM2 inflammasomes recognize viral ssRNA and dsDNA, respectively. The AIM2 inflammasome could recruit caspase-8 to induce apoptosis. The IFI16 inflammasome specifically recognizes KSHV dsDNA in the nucleus. RIG-I, retinoic acid inducible gene-I; NLRP3, nucleotide-binding oligomerization-like receptor family, pyrin domain-containing protein 3; AIM2, absent in myeloma 2; IFI16, γ -interferon-inducible protein 16; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; IL-1 β , interleukin-1 β ; IL-18, interleukin-18; KSHV, Kaposi's sarcoma-associated herpes virus.

to activate inflammasomes and the subsequent procession of caspase-1 and IL-1 β /IL-18.

AIM2 is located in the cytosol to recognize viruses, and the AIM2-like receptor IFI16 is localized predominantly in the nucleus, despite small amounts of cytosolic IFI16 being observed in some cells. In addition to the engagement of I-IFN induction in response to viral dsDNA, such as in VACV and herpes simplex virus type 1 (HSV-1), IFI16 has also been proven to sense KSHV genomes and associate with ASC and pro-caspase-1 in the nucleus⁵⁹ (Figure 2). However, the mechanism of IFI16 inflammasome redistribution to the cytoplasm remains unknown. Other studies have showed that IFI16 induces the expression of both I-IFNs and caspase-1-dependent inflammatory cytokine maturation in response to human immunodeficiency virus-1 (HIV-1) replication.^{60–62}

NLRP3 inflammasomes. Until now, the NLRP3 inflammasome is the most well-studied member of the NLR family, and it can activate caspase-1 and induce the production of IL-1 β and IL-18 in response to a wide range of PAMPs and endogenous and environmental stimuli.³⁴ Until now, NLRP3 is the only member of this family shown to be engaged in viral infection, including influenza A virus, SeV, vaccinia virus and encephalomyocarditis virus.^{2,35,37,39} Although several studies have revealed that the shared mechanism of a diversity of potential activators can enable NLRP3 to oligomerize and

recruit both ASC and pro-caspase-1 through the PYD domain, there is little evidence of NLRP3 directly binding to these ligands (Figure 2). Dual distinct signaling levels account for NLRP3 activation in terms of viral infection.^{63–65} The entry of influenza A virus into the host cell leads to the release of ions and the production of ROS by NADPH (nicotinamide adenine dinucleotide phosphate) in the cytosol. In the meantime, the viral M2 channel is induced and transferred to the acidified trans-Golgi apparatus, facilitating the efflux of hydrogen ions to activate the NLRP3 inflammasome. Likewise, ROS acts as secondary messengers to augment the virus-induced NLRP3 inflammasome.^{2,65} Modified vaccinia virus Ankara-mediated NLRP3 inflammasomes have also been demonstrated to require TLR2-dependent signaling.⁶⁶

Caspase-dependent pyroptosis

Cumulative reports have discovered that inflammasomes are not only capable of maturing proinflammatory cytokines, but also promote 'pyroptosis', a distinct inflammatory caspase-dependent cell death.⁶⁷ Accordingly, the inflammatory caspases-1, -4, -5 and -11 are also called pyroptotic caspases^{40,41,68,69} (Figure 2).

Pyroptosis cell death was initially observed in *Shigella flexneri*-infected macrophages in 1992, and it was defined as apoptosis because it shared common features, such as nuclear condensation, DNA fragmentation and caspase dependence.⁷⁰

It was not until 2001 that researchers further determined that pyroptosis characteristics are distinct from those of apoptosis. First, pyroptosis is programmed by inflammatory caspases. Second, inflammatory caspase activation leads to plasma membrane pore formation and, thus, permeabilizes the cells to small molecules, such as water and ions. Third, the water/ion influx and osmotic lysis cause massive leakage of cytosolic substances.^{68,71} Fourth, pyroptotic cell death requires the cleavage of gasdermin D by caspases-1, -4, -5 and -11, generating the N-terminal fragment responsible for pyroptosis initiation. However, the C-terminal fragment remains an enigma.^{72–74} Finally, pyroptosis causes DNA damage, whereas the nucleus remains intact following the activation of ADP-ribose polymerase and consummation of NAD⁺ and ATP.⁷⁵ Summarily, innate immunity is armed with pyroptosis to dispose of pathogen-laden cells and eliminate invading microbe protective niches by enabling them to be removed by a secondary phagocyte.

Pyroptosis is triggered by either caspase-1 or caspases-4, -5 and -11, and proinflammatory cytokine maturation is dispensable for pyroptosis. However, some differences exist between pyroptosis induced by caspase-1 and pyroptosis induced by caspases-4, -5 and -11.^{76–80} Caspase-1-dependent pyroptosis is initiated by various ligands, which activate canonical inflammasomes, and occurs mainly in macrophage or dendritic cells,

while pyroptosis dependent upon caspases-4, -5 and -11 is directly triggered by bacterial stimuli, for which the production of IL-1 β /IL-18 is nearly negligible.^{81,82}

Caspase-1 is currently the only member known to be involved in virus-induced pyroptosis. During HIV infection, caspase-1-dependent pyroptosis is critical for CD4⁺ T-cell depletion, implicating HIV pathogenesis.⁶¹ It is remarkably noted that caspase-1-deficient mice develop normally, disputing that the blockade of caspase-1 activity exerts beneficial rather than detrimental effects in HIV patients. Despite these important roles, the mechanisms of how inflammatory caspases drive pyroptosis need further study.

Caspases regulate the antiviral innate signaling pathways

cGAS-STING antiviral pathway. Recent work has demolished the traditional definition of apoptotic caspases, as it has become obvious that apoptosis caspases can control antiviral innate immunity through non-death processes. Two studies have showed that caspases-9, -3 and -7 involved in the intrinsic apoptotic pathway negatively regulate the induction of I-IFNs response by controlling cGAS and STING signaling^{83,84} (Figure 3).

Succumbing to DNA viral infection, the cGAS/STING pathway is triggered by the recognition of cytosolic dsDNA, including dsDNA from HSV-1, murine gammaherpesvirus 68,

Figure 3 Caspases control the cGAS-STING signaling pathway. Cytosolic DNA from virus or self-DNA from mitochondria or the nucleus could activate cyclic GMP-AMP synthase cGAS, which catalyzes the production of cGAMP in the presence of ATP and GTP. cGAMP binds and activates the ER-localized adapter protein STING, which then recruits and activates TBK1. This is followed by the phosphorylation and activation of the transcription factors IRF3, NF- κ B and STAT6, leading to I-IFN, proinflammatory cytokines and chemokine production. The apoptotic caspases-9, -3 and -7 could inhibit cGAS/STING/TBK1/IRF3-mediated IFN α/β production, while the inflammatory caspase-1 could cleave cGAS to control innate immunity during inflammasome activation. cGAS, cGAMP/cAMP synthase; STING, stimulator of IFN genes; TBK1, Tank-binding kinase 1; IRF3, IFN regulatory factor 3; NF- κ B, nuclear factor κ B; STAT6, signal transducer and activator of transcription 6; mtDNA, mitochondrial DNA.

KSHV, VACV, adenovirus, human cytomegalovirus, human papillomavirus, hepatitis B virus, by the DNA sensor cGAS.^{85–92} In addition, retroviruses, such as HIV-1 and human T-cell leukemia virus-1, which contain ssRNA genomes, require reverse-transcribed DNA to propagate their infection. With the aid of the proximal sensor PQBP1, cGAS recognizes immunogenic HIV-1 DNA and then gains its enzymatic activity to synthesize the secondary messenger 2'3'-cGAMP from ATP and GTP, following the interaction and activation of the endoplasmic membrane protein STING through a series of structural changes. Activated STING recruits the kinase TBK1 to phosphorylate the transcriptional factors IRF3 and STAT6, which robustly stimulates I-IFNs (IFN α and IFN β) and STAT6-specific chemokines, respectively.^{93,94} Moreover, STING also activates IKK to phosphorylate I κ B α (inhibitor of κ B) following its degradation and NF- κ B activation, thus controlling DNA, cytokine production and cell survival (Figure 3).⁹⁵

In addition to viral DNA stimulation, HSV-1 has been shown to induce cell stress and stimulate the generation of mtDNA.⁹⁶ In addition, intrinsic apoptosis reportedly mediates BAK/BAX-mediated mitochondrial damage to release mtDNA into the cytosol, which, in turn, triggers the cGAS/STING-mediated antiviral immune responses and establishes a potent state of viral resistance. By contrast, mtDNA-triggered cGAS/STING-dependent I-IFN production can be inhibited by the activation of apoptotic caspases-3, -7 and -9, indicating the crucial function of caspases in restraining cell-intrinsic immune response to maintain immunological silence in apoptotic cells^{83,84} (Figure 3). A deficiency in caspase-9, -3 or -7 results in an intensive resistance to viral infection both *in vitro* and *in vivo*. The HSC (hematopoietic stem cell) niche is highly sensitive to the effects of I-IFNs, which predominantly determine the cellular vulnerability to viral infections. The loss of either caspase-9, -3 or -7 impairs HSC function, ascribed by the elevated level of mtDNA-triggered apoptotic I-IFNs. Moreover, the pharmaceutical inhibition of caspases could perturb I-IFN production and HSC dysfunction. The mechanisms of the caspase-dependent suppression of I-IFNs elucidated that either caspase-3 or -7 could prevent cGAS activation by detecting mtDNA or inhibiting some components of I-IFN signaling, whose intermediates, such as IRF3, are reportedly targeted by caspases.⁹⁷ However, the precise contribution of caspases to the regulation of I-IFNs signaling needs to be further established.

In addition to the apoptotic caspases, inflammatory pyroptotic caspases are also found to negatively regulate the cGAS/STING pathway during inflammatory activity.⁹⁸ Jiang's group was the first to identify that pyroptotic caspases directly cleave cGAS at the N terminus upon inflammasome activation, resulting in reduced cGAMP production and cytokine secretion. Caspase-1 has been shown to bind directly and cleave human cGAS at D^{140/157} during canonical inflammation activation, releasing the less-conserved N terminus of human cGAS (*hcGAS*-N160) and the highly conserved nucleotidyl-transferase/Mab 21 domains. Current studies have demonstrated that *hcGAS*-N160 promotes the activity of full-length

hcGAS upon sensing host- or pathogen-derived dsDNA.¹³⁷ Compared to WT mice, peritoneal macrophages from ASC^{-/-} and *Casp1*^{-/-} mice were shown to produce significantly higher levels of I-IFNs and IL-6, rendering host resistance to DNA viruses, such as HSV-1 or Vaccinia virus infection, but not RNA virus infection, such as SeV. Similarly, inhibition of the caspase-1 inflammasome potentiates DNA virus-induced I-IFN production. Alternatively, upon LPS-induced noncanonical inflammasome activation, caspases-4, -5 and -11 cleave cGAS in a manner distinct from caspase-1.

All these data suggest that apoptotic/pyroptotic caspases can either positively or negatively regulate virus/DAMP (damage-associated molecular pattern)-induced I-IFN signaling and canonical/noncanonical inflammatory responses.

RIG-I/Mda-5-mediated antiviral pathway. Caspase-8 and -10 are recognized as initiator caspases that induce DR-mediated extrinsic apoptosis (for example, Fas, TNFR and TRAIL). Recently, more studies have emphasized the relationship between caspases-8 and -10 and antiviral I-IFN production.^{100–104} The cytosolic RNA helicase RIG-I and Mda-5 sense RNA viruses and then recruit the mitochondrial adapter MAVS (also called IPS1/VISA/CARDIF) to activate transcription factors, including IRF3 and NF- κ B. The kinase RIPK1 is recruited by MAVS at mitochondria and undergoes K63-linked polyubiquitination following the activation of antiviral responses via IRF3-dependent I-IFN production. Remarkably, caspase-8 cleaves the polyubiquitinated RIPK1 to release a RIPK1 fragment, which inactivates IRF3 and suppresses RIG-I/Mda-5-mediated antiviral I-IFN responses¹⁰¹ (Figure 4). In response to RNA viral infection (for example, SeV and VSV) or poly (I:C) stimulation, caspase-8 deficiency in fibroblastoid cells or hepatocytes results in the increased activation of IRF3.¹⁰¹ Moreover, RIG-I-mediated signaling activates caspase-8 to initiate an important intermediate process in the ubiquitination/proteasome-dependent degradation of IRF3, modulating dsRNA-dependent gene induction.¹⁰⁵ Interestingly, another group has reported the opposite result. By interacting with FADD, caspases-8, and -10 are required for RIG-I/Mda-5-induced NF- κ B and IRF3 activation. Caspase-10 knockdown cells and cells isolated from caspase-8-deficient mice display an impaired ability to produce proinflammatory cytokines in response to dsRNA.¹⁰⁰ Analogously, compared to the full-length form, transfection with the DED domain of caspase-8 and -10 enhances the activity of NF- κ B. Furthermore, dsRNA treatment or overexpression of a constitutively active RIG-I/Mda-5 mutant significantly abolished NF- κ B activation in murine embryonic fibroblasts isolated from both caspase-8 knockout mice and HEK293T cells in which caspase-8 and -10 was knocked down. Interestingly, IFN- β stimulation remains intact in caspase-8-deficient cells. The above data suggest that caspase-8 and -10 are critical elements for RIG-I/Mda-5-mediated NF- κ B antiviral signaling.^{100,106}

In addition to its effective function in I-IFN stimulation (IFN- α/β), MAVS is suggested to mediate apoptosis from its mitochondrial site of action upon RNA viral infection, such as

Figure 4 Caspases regulate RLR and TLR antiviral signaling. RIG-I and Mda-5 recognize viral RNA and associate with MAVS localized in mitochondria and then transduce signal through multiple adapters, leading to the activation of IRF3/7 via the kinase TBK1. Simultaneously, MAVS triggers NF- κ B via TRADD, FADD and caspases-8 and -10. In addition, host cells could mount a caspase-3-mediated apoptotic response to some viruses, such as SeV, via MAVS. The RNA genomes of viruses internalized in the endosomes are detected by members of the TLR family of receptors, including TLR3, TLR7, TLR8 and TLR9, which recruit adapter proteins, such as TRIF and MyD88. Subsequently, TRIF and MyD88 transduce signals to the downstream transcription factors IRF3/7 and NF- κ B via TBK1 and the IKK complex, respectively. Notably, in some cell types, both TRIF and MyD88 can trigger RIPK3 and further form a cell death-inducing complex that includes RIPK1, FADD and caspase-8. Caspase-8 is also reportedly involved in the maturation of pro-IL-18/1 β . Caspase-12 could suppress RIG-I signaling by cleaving TRIM25. By inhibiting multiple adapters in the RLR/TLR antiviral signaling pathway, such as TBK1, IRF3, RIPK1 and RIPK3, caspase-8 has even more powerful functions in controlling antiviral innate immunity. MDA-5, melanoma differentiation-associated protein 5; MAVS, mitochondrial antiviral signaling protein; TRIF, TIR-domain-containing adapter-inducing interferon- β ; MyD88, myeloid differentiation primary response gene; RIPK1, receptor-interacting serine/threonine-protein kinase 1; IKK, inhibitor of NF- κ B kinase.

SeV, dengue virus, reovirus and semliki forest virus (SFV).^{107–111} MAVS-mediated apoptosis requires the activation of caspases-3, -8 and -9, and MAVS^{-/-} fibroblasts are resistant to SeV-induced apoptosis.^{108,110,112,113} It has been observed that MAVS-deficient cells have decreased caspase-8 and -3 activity. Moreover, SFV reportedly requires MDA-5 and MAVS but not TRAF2, IRF3/7, IFN- β , IFNAR, PKR or RNase L for apoptosis induction. This pro-apoptotic pathway involves the SFV-induced recruitment of caspase-8 to MAVS in mitochondria, and FADD is unnecessary. Sequentially, activated caspase-8 cleaves and activates caspase-3 for apoptosis using a Bax/Bak-independent mechanism.¹¹⁴ Furthermore, HRV1a (human rhinovirus 1a) infection was shown to induce a stronger increase of IFN- β than caspase-3-mediated MAVS cleavage and apoptosis.¹¹⁵ Virus-encoded viroporins, such as 6K protein from Sindbis virus, 2B/3A from poliovirus, E protein from mouse hepatitis virus, M2 from influenza A virus and p7/NS4A from HCV, can change the permeability of host cells and have a critical influence on virus budding. These viroporins are reportedly able to induce caspase-3-dependent apoptosis in a mitochondrial manner.¹¹⁶

Caspase-12 was recently suggested to control RIG-I signaling cascades.¹¹⁷ Caspase-12 has been shown to regulate the TRIM25-mediated ubiquitination of RIG-I, leading to the downregulation of I-IFN response. Caspase-12-deficient mice were shown to be highly predisposed to West Nile virus encephalitis and to have aggravated neurological symptoms^{117,118} (Figure 4).

TLRs antiviral pathway. TLR3 has been identified as a key sensor for viral dsRNA or its analog poly (I:C) and interacts with a vital TLR adapter, TRIF, culminating in the activation of NF- κ B and IRF3 and the transcriptional induction of their target genes, including TNF- α , IL-6 and IFN- α/β (Figure 4).

Picornavirus infection-induced TLR3/TRIF reportedly recruits a 2 MDa intracellular cell death complex called the 'riposome', containing RIP1, FADD, caspases-8 and -10 and isoforms of the caspase inhibitor cFLIP. cFLIP(S) enhances riposome assembly, while cFLIP(L) exerts the opposite function. The caspase-8-cFLIP(L) complex has adequate catalytic activity to cleave the RIP1 kinase. Caspase-8 reportedly negatively controls TLR3 signaling in macrophages and BM-

derived dendritic cells via caspase-8-interacting proteins, such as FADD, cFLIP, RIP and TRAIL-R¹¹⁹ (Figure 4).

NF- κ B plays a vital role in the transcriptional activation of many inflammation-related and anti-apoptotic genes in response to inflammatory cytokines, PAMPs, growth factors and cellular stress states. NF- κ B is located in the cytosol in an inactive form, which is inhibited by I κ B. After multiple signaling cascades, IKK phosphorylates I κ B, and the subsequent degradation of I κ B releases NF- κ B after it translocates to the nucleus and, finally, activates its targets. In B cells, caspase-1 was shown to activate NF- κ B to induce inflammation in a different manner.¹²⁰ This novel signaling is mediated by the CARD of procaspase-1 and results in the stimulation of NF- κ B and p38 MAPK in a RIP2-dependent manner. In addition, this pathway is not shared by caspase-11 and -12.^{120,121}

Recent data have indicated that caspase-8 is critical for the activation of NF- κ B signaling, which results in the induction of proinflammatory cytokines. However, many studies of the function of caspase-8 in modulating NF- κ B signaling have thus far been restricted in anti-bacterial/fungal innate immunity, adaptive immunity and cancer.^{80,122–125} Moreover, to date, there is no consensus on the molecular mechanism underpinning how caspase-8 mediates NF- κ B signaling or whether caspase-8 can regulate the NF- κ B activation involved in antiviral innate immunity, and these phenomena need to be studied in more detail. Caspase-8 has also been demonstrated to exert critical functions in B-cell activation and expansion in response to TLR3 but not TLR9 by associating with the IKK complex and, in turn, modulating NF- κ B translocation.¹²⁴ These findings are consistent with the increased susceptibility of human patients deficient for caspase-8 to viral infections.¹²²

In addition, the existence of the involvement of TRIF in the maturation of IL-1 β upon TLR3 activation has been demonstrated. Surprisingly, poly (I:C)-stimulated-IL-1 β production was still observed in caspase-1-deficient cells but was suppressed by caspase-8 inhibitors and caspase-8 RNAi silencing. Accordingly, researchers proposed that a comparable TRIF-RIP1-FADD-caspase-8 cascade leads to IL-1 β production.¹²⁶ Moreover, melanoma cells and HaCaT human keratinocytes stimulated by poly (I:C) were shown to be susceptible to apoptosis associated with the TLR3/TRIF/caspase-8 complex¹²⁷ (Figure 4).

By contrast, caspase-8 has also been shown to prevent inflammasome activity, IFN response and necroptosis, a programmed cell death resulting from caspase-8, RIPK1 and RIPK3.^{128,129} Compared to WT or *Ripk3*^{-/-} BMDMs, *Ripk3*^{-/-} *Casp8*^{-/-} BMDMs have been shown to display decreased levels of IL-1 β and poly (I:C) /TLR3-induced inflammasome priming.¹³⁰

TLR7 and TLR9 recognize ssRNA and unmethylated CpG DNA, respectively, in endosomes and are tightly connected to the pathogenesis of SLE (systemic lupus erythematosus). Except for TLR3, most TLRs signal through MyD88 to activate NF- κ B signaling. Caspase-8 also reportedly serves as a molecular rheostat to restrict MyD88-dependent dendritic cell activation.¹³¹ When stimulated by CpG (TLR9 agonist) or

imiquimod (TLR7 agonist), *Cre*^{CD11c}*Casp8*^{fl/fl} BMDCs exhibit increased expression of IL-12/23p40, TNF α , IL-6 and IL-1 β without inducing cell death. The loss of caspase-8 in DCs causes hyper-responsiveness to TLR activation, indicating that caspase-8 suppresses MyD88 signaling. However, it was proposed that this suppressive activity of caspase-8 is related to MyD88 recruitment but not its catalytic activity.¹³¹ These data provide a relationship between caspase-8 and augmented TLR responses to pathogenetic stimuli.

Caspase-8-deficient keratinocytes exhibit enhanced gene activation by DNA transfection. Meanwhile, TBK1 and IRF3 are constitutively phosphorylated in the epidermis of the *Casp8*^{F/-} K5-Cre mice, and the well-known ISGs are also induced. Moreover, in response to DNA transfection, caspase-8-deficient keratinocytes yield a higher level of IRF3-targeted genes than the caspase-8-overexpressed cells. In addition, since MyD88, TRIF and most TLR adapters were ruled out, caspase-8 was further proven to regulate IRF3 or TBK1 directly, but the exact molecular mechanism needs to be further clarified¹³² (Figure 4).

Caspases regulate viral ER stress-induced apoptosis

Caspase-12 is an inflammatory caspase located in the endoplasmic reticulum (ER). Early studies indicated that caspase-12 is an ER stress response caspase.¹³³ Unbalanced ER-Ca²⁺ homeostasis and accumulated misfolded, unassembled or aggregated proteins in the ER lumen reportedly stimulate ER stress signaling. Furthermore, viruses utilize the ER as an integrated unit for their life cycles, leading to some levels of ER stress. Mild ER stress affects protein synthesis initiation and can downregulate cell proliferation, whereas acute or persistent ER stress can cause cell death induced by caspase-12.¹³⁴ Studies in caspase-12-deficient mice emphasized the principle function of caspase-12 in ER stress-mediated apoptosis.¹³³ In addition, viral infection might induce caspase-12-dependent apoptosis.^{18,135} The RNA virus BVDV (bovine viral diarrhea virus) and related flavivirus-infected MDBK (Madin-Darby bovine kidney) cells have increased expression of caspase-12 and decreased levels of the anti-apoptotic protein Bcl-2. Caspase-12 cleaves pro-caspase-7 to its active caspase-7 form. The detailed mechanisms of how ER stress signaling activates caspase-12 have not yet been elucidated. However, current studies have proven that other cytosolic caspases can be recruited by activated caspase-12 in the ER membrane, and thus, the apoptotic signal is greatly boosted.¹³⁶ In A549 epithelial cells infected with RSV, caspase-9 is not activated, caspases-3 and -8 are modestly activated, and caspase-12 is strongly activated. Moreover, RSV-stimulated apoptosis seems to occur through a caspase-12-dependent ER stress response, which is uncoupled from NF- κ B activation.¹³⁵

CONCLUSIONS

Viral PAMPs are recognized differentially by innate PRRs to initiate antiviral immune responses in a systematic manner. The complicated antiviral innate immune signaling network has co-evolved with viruses to defend against and restrain

autoimmunity via a sophisticated state-of-the-art feedback scheme. The caspase family members are architects of the body and sit at the nexus of this dynamic regulatory framework. In the past few decades, significant advances in understanding the critical functions of caspases for organism homeostasis and especially novel and exciting discoveries have unveiled the multifaceted nature of individual caspases in the antiviral innate immune system. Studies on caspases have provided an interesting point of view that caspases act as molecular 'decision-makers' because of their ability to fine-tune antiviral innate immunity and because caspases have both proinflammatory and anti-inflammatory functions. Moreover, recent studies have uncovered the capacity of caspases to mediate nearly all the PRRs involved in innate immunity by acting as a molecular switch on whether to respond to an insult by initiating inflammation or potentiating I-IFN response to eliminate invading viruses, or by preventing inflammation to maintain immunological silence by dismantling insulted or damaged cells. Increasingly distinguished and sophisticated discoveries have challenged the traditional definition of caspase family members. However, contradiction and controversy regarding the regulation of innate immunity by caspases exist, and the understanding of the molecular basis of these phenomena remains in infancy. Caspases are double-edged swords. The inappropriate activation of caspases and dysregulation of the antiviral innate immune signaling controlled by caspases has dire consequences for human health and is associated with an extensive list of human diseases. Ongoing studies will provide us with a more comprehensive understanding of caspase mechanisms and may shed new light on therapeutic options for the various caspase-related human diseases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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