

Review

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Inflammatory caspases: key regulators of inflammation and cell death

Abstract: The innate immune system represents the first line of defence against infectious agents, and co-ordinates cellular and molecular mechanisms that result in effective inflammatory and anti-microbial responses against pathogens. Infection and cellular stress trigger assembly of canonical and noncanonical inflammasome complexes that activate the inflammatory caspases-1 and -11, respectively. These inflammatory caspases play key roles in innate immune responses by inducing pyroptosis to halt intracellular replication of pathogens, and by engaging the extracellular release of pro-inflammatory cytokines and danger signals. In addition, the inflammatory caspases-4, -5 and -11 were recently shown to directly bind microbial components. Although the immune roles of caspase-12 are debated, it was proposed to dampen inflammatory responses by interfering with caspase-1 activation and other innate immune pathways. Here, we recapitulate the reported roles of inflammatory caspases with an emphasis on recent insights into their biological functions.

Keywords: caspase; infection; inflammasome; inflammation; NOD-like receptor; pyroptosis.

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Introduction

Cysteine-dependent aspartate-specific proteases (caspases) comprise an evolutionary conserved family of

metazoan proteases with well-established roles in apoptosis and inflammation (Lamkanfi et al., 2002). They are broadly classified based on their demonstrated or assumed roles in apoptosis (caspases-2, -3, -6, -7, -8, -9 and -10) and inflammatory signalling (caspases-1, -4, -5, -11 and -12). Caspase-14 is generally positioned outside of these apoptotic and inflammatory clusters because it is thought to be uniquely involved in terminal differentiation of keratinocytes (Denecker et al., 2007; Hoste et al., 2011; Seidelin et al., 2013).

Apoptotic caspases are further segregated into ‘initiator’ and ‘executioner’ caspases based on their activation mechanisms. Initiator caspases (caspases-2, -8, -9 and -10) exist as latent zymogens that gain proteolytic activity upon their recruitment into multi-protein platforms that promote their proximity-induced autoactivation such as the death-inducing signalling complex (DISC) that activates caspases-8 and -10, and the apoptosome that drives caspase-9 activation, respectively (Bratton and Salvesen, 2010; Lavrik and Krammer, 2012). In addition to other protease groups such as cathepsins and granzymes, initiator caspases cleave the apoptotic executioner caspases-3, -6 and -7, the activation of which ultimately results in the emergence of the biochemical and morphological hallmarks of apoptosis (Cornelis et al., 2007; Yazdi et al., 2010).

Caspase-12 was initially thought to promote endoplasmic reticulum stress-induced apoptosis (see below), but this and other inflammatory caspases (i.e. caspases-1, -11 and -12 in rodents, and caspases-1, -4 and -5 in humans) are currently thought to be largely dispensable for apoptosis signalling (Kuida et al., 1995; Wang et al., 1998). Instead, they are believed to control inflammatory and host defence responses during infection by modulating the maturation and secretion of inflammatory mediators, by inducing a lytic cell death mode termed pyroptosis, and by engaging other mechanisms that contribute to pathogen eradication (Lamkanfi, 2011).

In the next sections, we will separately review current understanding of the molecular activation mechanisms and (patho)physiological roles of each inflammatory caspase.

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Caspase-1 activation in canonical inflammasomes triggers cytokine release and pyroptosis

Caspase-1 was originally discovered for its ability to cleave and convert the inflammatory cytokine pro-interleukin (IL)-1 β into its bioactive form (Cerretti et al., 1992). Caspase-1 was subsequently shown to be the principal protease that processes proIL-1 β and proIL-18 under most pathological conditions (Kuida et al., 1995; Li et al., 1995; Ghayur et al., 1997; Gu et al., 1997). However, additional mechanisms of proIL-1 β maturation have recently been described (Guma et al., 2009; Joosten et al., 2009; Mayer-Barber et al., 2010; Bossaller et al., 2012).

Caspase-1 itself is produced as a cytosolic precursor that undergoes proximity-induced autoactivation. Auto-cleavage of human caspase-1 after D103 and D122 releases the linker that separates the amino-terminal caspase recruitment domain (CARD) from the catalytic domain. Removal of a second linker that is located inside the protease domain by cleavage after residues D297 and D316 triggers conformational changes around the catalytic pocket that render the protease active (Elliott et al., 2009). Recent work with mouse caspase-1, however, has shown that caspase-1 autoprocessing is not a prerequisite for its activation. Recruitment of caspase-1 in large multi-protein complexes known as ‘canonical inflammasomes’ is sufficient for its activation in the absence of autoprocessing (Broz et al., 2010b; Van Opendenbosch et al., 2014).

Several canonical inflammasomes have been characterized in recent years, with those assembled by NLRP1, NLRP3, NLRC4, AIM2 and PYRIN among the best characterized (Figure 1). The NLRs NLRP2, NLRP6, NLRP7 and NLRP12 have also been proposed to form inflammasomes, but further characterization is required to clarify the functions of these complexes (Lamkanfi and Dixit, 2014).

The Nlrp1 inflammasomes

Nlrp1a is one of three murine Nlrp1 isoforms. They have in common that they are composed of an amino-terminal nucleotide-binding and oligomerization (NACHT) domain, a stretch of centrally located leucine-rich repeat (LRR) motifs followed by a function to find (FIIND) domain and a carboxy-terminal CARD motif with which they recruit caspase-1 (Masters et al., 2012). Nlrp1a is expressed in haematopoietic progenitor cells, and has been implicated in inflammasome assembly in response to infection

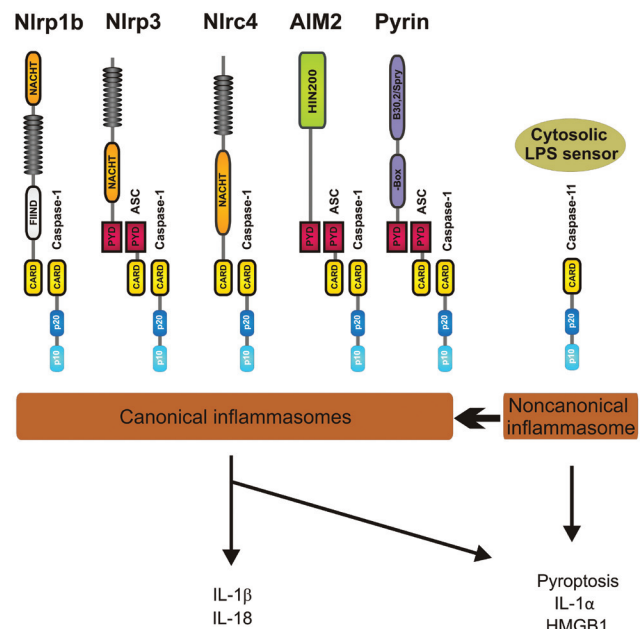


Figure 1 Composition and effector mechanisms of canonical and noncanonical inflammasomes.

Canonical and noncanonical inflammasome pathways lead to cytokine secretion and cell death. Canonical inflammasomes such as the murine Nlrp1b, Nlrp3, Nlrp4, AIM2 and Pyrin lead to activation of caspase-1, which induces the secretion of the inflammatory cytokines IL-1 β , IL-18 and pyroptosis. The murine NLRs (Nlrp1b, Nlrp3 and Nlrp4) harbour a NACHT domain followed by a variable number of LRRs, as well as an N-terminal CARD or PYD motif. Additionally, Nlrp1b contains a FIIND domain but lacks the PYD domain found in the human NLRP1 inflammasome. The murine gene *MEFV* encodes PYD at the N-terminus which facilitates interaction with other domains, followed by a B-box domain. AIM2 contains a PYD domain at the N-terminus followed by a HIN200 domain. The protein adaptor ASC is required for the assembly of Nlrp3, AIM2 and Pyrin inflammasome. Activation of the noncanonical inflammasome is mediated by caspase-11 and it will either trigger pyroptosis directly or activate the canonical Nlrp3 inflammasome leading to secretion of the inflammatory cytokines IL-1 β and IL-18.

(Masters et al., 2012). Nlrp1a activation triggers pyroptosis and release of bioactive IL-1 β , excessive secretion of which may result in neutrophilia.

The Nlrp1b inflammasome activates caspase-1 in murine macrophages that have been intoxicated with lethal toxin (LeTx) of *Bacillus anthracis*, the causative agent of anthrax (Boyden and Dietrich, 2006). LeTx is a bipartite toxin that consists of the protective antigen (PA) and lethal factor (LF) subunits. PA is a pore-forming protein that protects LF from degradation in phagolysosomes by shuttling it into the cytosol of intoxicated cells (Bann, 2012). The metalloprotease activity of LF is required to induce Nlrp1b inflammasome-mediated pyroptosis and to promote secretion of IL-1 β and IL-18 (Fink et al., 2008).

Interestingly, the central FIIND domain of Nlrp1b spontaneously undergoes autocatalytic cleavage, which was shown to be critical for subsequent LeTx-induced inflammasome activation (Frew et al., 2012). Moreover, deletion of the inflammasome adaptor apoptosis-associated speck-like protein containing CARD (ASC) blocked LeTx-induced caspase-1 autoprocessing, but not the Nlrp1b- and caspase-1-dependent induction of pyroptosis and IL-1 β secretion (Van Opdenbosch et al., 2014). This shows that caspase-1 autoprocessing is not critical for caspase-1 activation, although it may further enhance protease activity by locking caspase-1 in an active conformation.

Unlike rodents, humans express a single NLRP1 isoform. The human ortholog differs from its murine counterparts in that it contains an additional pyrin motif at the amino-terminus (D’Osualdo and Reed, 2012). Mutations in *NLRP1* are associated with vitiligo-associated autoimmune disease (Jin et al., 2007), autoimmune Addison’s disease (Zurawek et al., 2010) and type I diabetes (Magitta et al., 2009). However, more work is needed to clarify the underlying molecular mechanisms by which these mutations trigger autoimmunity.

The Nlrp3 inflammasome

The Nlrp3 inflammasome arguably is the most studied inflammasome. It is implicated in host defence against a wide range of bacterial (*Staphylococcus aureus*, *Escherichia coli*, *Citrobacter rodentium*) (He et al., 2010; Gurung et al., 2012), viral (influenza A) (Allen et al., 2009; Thomas et al., 2009), fungal (*Candida albicans*, *Aspergillus fumigatus*; Gross et al., 2009) and parasitic (*Leishmania amazonensis*) infections (Lamkanfi and Dixit, 2012; Lima et al., 2013). In addition, the Nlrp3 inflammasome is activated by damage-associated molecular patterns (DAMPs) such as adenosine triphosphate (ATP), uric acid crystals, amyloid β fibrils and hyaluronan that are thought to trigger inflammation under sterile conditions. Also exogenous crystalline particles and inhaled nanomaterials such as cigarette smoke, diesel exhaust particles and silica activate this inflammasome, suggesting possible roles in pulmonary inflammation (Brusselle et al., 2014).

Unlike blood monocytes that activate the Nlrp3 inflammasome in response to TLR ligands alone (Netea et al., 2009), it is now well-established that activation of the Nlrp3 inflammasome requires two signals in murine macrophages. Owing to its low basal expression levels in the latter cells, a priming signal is firstly required to promote the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)-dependent transcriptional

upregulation of Nlrp3 (Bauernfeind et al., 2009). Recent work also suggested transcription-independent mechanisms of Nlrp3 priming (Juliana et al., 2012; Schroder et al., 2012). However, neither of these signals is sufficient to activate the Nlrp3 inflammasome by themselves. The infectious agents, DAMPs and crystalline particles mentioned above are thought to trigger a common signal that activates the Nlrp3 inflammasome. Several molecular mechanisms have been described, including the extracellular release of K⁺ ions (Muñoz-Planillo et al., 2013), the release of oxidized DNA from damaged mitochondria (Zhou et al., 2011), cytosolic release of lysosomal cathepsins (Hornung et al., 2008), translocation of Nlrp3 to mitochondria (Misawa et al., 2013) and cardiolipin recognition in the cytosolic compartment (Iyer et al., 2013). However, the precise mechanisms of Nlrp3 activation remain a topic of intense research, and future work will undoubtedly shed more light on this aspect.

Activation of the Nlrp3 inflammasome is of clinical relevance because mutations in *NLRP3* cause three hereditary periodic fever syndromes that are collectively referred to as cryopyrin-associated periodic fever syndromes (CAPS). These autosomal dominant gain-of-function mutations in *NLRP3* may cause conformational changes in NLRP3 that result in the loss of autoinhibitory mechanisms (Lu et al., 2014). In addition to these rare autoinflammatory syndromes, defective NLRP3 inflammasome activation has been implicated in common autoimmune diseases such as inflammatory bowel disease (Villani et al., 2009; Zaki et al., 2011), Alzheimer’s disease (Heneka et al., 2013), age-related macular degeneration (Doyle et al., 2012) and several other ailments (Lamkanfi and Dixit, 2012, 2014).

The AIM2 inflammasome

Whereas NLRP1 and NLRP3 are NLRs, absent in melanoma 2 (AIM2) is a cytosolic member of the intracellular HIN200 receptor family. Binding of dsDNA to its HIN200 domain triggers assembly of an inflammasome that contains ASC and caspase-1. AIM2 inflammasome activation contributes to host defence against intracellular bacterial pathogens such as *Listeria monocytogenes* and *Francisella tularensis* as well as against a subset of DNA viruses that includes vaccinia virus and cytomegalovirus (Fernandes-Alnemri et al., 2010; Jones et al., 2010; Rathinam et al., 2010; Tsuchiya et al., 2010).

Interestingly, AIM2 was originally discovered as a protein that was markedly downregulated in a substantial set of melanoma and other cancer cells (Lee et al., 2012). Although the precise roles of AIM2 in carcinogenesis have

remained obscure, these observations suggest that it may act as a tumor suppressor. Interestingly, deregulated AIM2 expression has also been linked to systemic lupus erythematosus (SLE) and psoriasis (Dombrowski et al., 2011; Zhang et al., 2013), although studies in larger patients groups are needed to further substantiate a causal relationship and to shed light on the mechanisms by which its absence causes autoimmunity.

The Nlrc4 inflammasome

Caspase-1 is activated by the Nlrc4 inflammasome upon infection with intracellular pathogens such as *Salmonella enterica serovar Typhimurium* (*Salmonella Typhimurium*), *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Shigella flexneri* and *Burkholderia thailandensis* (Lamkanfi and Dixit, 2012). These pathogens have in common that they express flagellin – the principal component of bacterial flagella – and/or specialized type III secretion systems (Lamkanfi and Dixit, 2014). These bacterial factors are recognized by members of the NAIP proteins, which act upstream of Nlrc4 in inflammasome activation (Kofoed and Vance, 2011; Zhao et al., 2011). In addition to these NAIP proteins, *S. Typhimurium*- and flagellin-induced caspase-1 activation was shown to require phosphorylation of Nlrc4 on Ser533 (Qu et al., 2012). As in the Nlrp1b inflammasome, ASC was dispensable for Nlrc4 inflammasome-induced pyroptosis induction, but required for caspase-1 autoprocessing (Broz et al., 2010b; Van Opdenbosch et al., 2014). Interestingly, these inflammasomes differed in their requirement for ASC for cytokine secretion because ASC-deficient macrophages were severely hampered in their ability to secrete mature IL-1 β and IL-18 when infected with *S. Typhimurium* (Mariathasan et al., 2004; Broz et al., 2010b; Van Opdenbosch et al., 2014). Recent work demonstrated that the ASC CARD domain has prion-like characteristics (Cai et al., 2014; Lu et al., 2014), in line with the observation that inflammasome-activating agents trigger formation of ‘ASC specks’, large filamentous structures in the perinuclear region of cells that were treated with inflammasome-activating agents (Broz et al., 2010a,b; Jones et al., 2010; Van Opdenbosch et al., 2014). Detailed analysis of the physiological role of these specks is somewhat hampered by the fact that ASC is critical for assembly of the Nlrp3 and AIM2 inflammasomes themselves (Sagulenko et al., 2013). Although ASC is required for caspase-1 automaturation, ASC specks are unlikely to be directly required for inflammasome-driven pyroptosis and cytokine secretion because ASC was not essential for Nlrp1b-mediated cytokine secretion, nor

for Nlrp1b- and Nlrc4-mediated pyroptosis (Mariathasan et al., 2004; Broz et al., 2010b; Van Opdenbosch et al., 2014). Recent work has shed light on the possible roles of ASC specks by demonstrating that extracellular release of ASC specks from pyroptotic cells promoted inflammation by extending the maturation of caspase-1 and IL-1 β to the extracellular milieu and surrounding cells that have internalized specks, as well as by enhancing the recruitment of neutrophils that contribute to the release of additional inflammatory mediators (Baroja-Mazo et al., 2014; Franklin et al., 2014).

The Pysin inflammasome

Mutations in the Pysin-encoding gene *MEFV* cause familial Mediterranean fever (FMF), the most prevalent auto-inflammatory disease (Bernot et al., 1997; Consortium, 1997). Pysin is composed of an amino-terminal PYD domain, centrally located zinc-finger B-box and coiled-coil domains, and a carboxy-terminal B30.2 domain. The amino-terminal PYD domain was shown to interact with ASC, and the C-terminal B30.2 domain with caspase-1 in overexpression studies (Chae et al., 2006, 2008). In agreement, Pysin was shown to assemble an ASC- and caspase-1-containing inflammasome in response to infection with *Burkholderia cenocepacia*, an opportunistic pathogen that causes respiratory inflammation in cystic fibrosis patients (Gavrillin et al., 2012). More recently, Pysin was demonstrated to sense the Rho-glucosylating activity of cytotoxin (TcdB) of *Clostridium difficile*, a pathogen that causes nosocomial diarrhoea (Xu et al., 2014). Pysin also triggered inflammasome activation by Rho-inactivating toxins of *Vibrio parahaemolyticus*, *Histophilus somni*, *Clostridium botulinum* and upon *B. cenocepacia* infection. In agreement, the Pysin inflammasome proved to be critical for controlling intra-macrophage growth of *B. cenocepacia*, and Pysin-deficient mice were markedly protected from *B. cenocepacia*-induced lung inflammation (Xu et al., 2014).

Caspase-11: a cytosolic sensor of lipopolysaccharides

Caspase-11 is produced as isoforms of 42 and 38 kDa, both originating from the *caspase-11* gene that is located in the inflammatory caspase locus on chromosome 9 (Wang et al., 1996). The 38 kDa isoform likely arises from the usage of an alternative start codon because both isoforms

are produced upon transient transfection of a mouse caspase-11 cDNA-expressing plasmid in L929 cells (Kang et al., 2000). Human caspases-4 and -5 are duplicated counterparts of murine caspase-11 (Lamkanfi et al., 2002). These human homologs share a sequence homology of 60% and 55% in relation to murine caspase-11, respectively, at the protein level, the highest between inflammatory caspases. Mouse caspase-11 and caspase-4 have in common that their mRNAs have a similar tissue distribution. Caspase-11 mRNA is ubiquitously expressed in many organs and its protein expression is predominant in skeletal muscle, heart and brain (Kalai et al., 2003). Conversely, caspase-11 and human caspase-5 both have low basal expression levels in naive macrophages, and both are highly upregulated by lipopolysaccharides (LPS) and interferon-gamma (IFN- γ) (Van de Craen et al., 1997; Lin et al., 2000; Lamkanfi et al., 2002).

Although further work is needed to establish which of the two human paralogues represents the functional counterpart of mouse caspase-11, significant progress was recently made in understanding the (patho)physiological roles of caspase-11. LPS-stimulated bone marrow-derived macrophages (BMDMs) of *caspase-11*^{-/-} mice were defective in IL-1 β secretion when exposed to cholera toxin b (CtB) or when infected with Gram-negative enteropathogens such as *C. rodentium*, *Vibrio cholerae* and *E. coli* (Kayagaki et al., 2011). Subsequent studies demonstrated that caspase-11 expression and activation downstream of Toll-like receptor 4 (TLR) and TIR-domain-containing adapter-inducing IFN- β (TRIF)-dependent production of type I interferons contributed to IL-1 β production by a broad array of Gram-negative bacterial pathogens (Broz et al., 2012; Gurung et al., 2012; Rathinam et al., 2012). In contrast, caspase-11 was dispensable for IL-1 β secretion by Gram-positive bacteria. Caspase-11 is unable to mature proIL-1 β itself, but triggers caspase-1 activation by the ‘canonical’ Nlrp3 inflammasome to induce IL-1 β production (Kayagaki et al., 2011). Indeed, NLRP3, ASC and caspase-1 were dispensable for caspase-11 activation, but they were critical for *C. rodentium*- and *E. coli*-induced IL-1 β secretion (Kayagaki et al., 2011).

Unlike IL-1 β secretion, caspase-11 induced pyroptotic cell death of infected cells autonomously, without a requirement for a functional Nlrp3 inflammasome (Kayagaki et al., 2011). Pyroptosis is considered a pro-inflammatory cell death mode that is characterized by early cell lysis and release of the intracellular content into the surrounding tissue. Concomitant with the secretion of the inflammatory cytokines IL-1 β and IL-18 by canonical inflammasomes, the release of DAMPs such as S100 proteins and high mobility group box1 (HMGB1) promotes inflammation (Lamkanfi,

2011). This cell death mode has been proposed as an efficacious mechanism to clear intracellular pathogens (Miao et al., 2011; Broz et al., 2012). Pyroptosis has been observed in monocytes, macrophages and dendritic cells when infected with *S. Typhimurium*, *F. tularensis*, *L. pneumophila* and *B. anthracis*, but also occurs upon inflammasome activation by non-infectious agents (Broz et al., 2012; Gurung et al., 2012; Rathinam et al., 2012).

Interestingly, caspase-11 activation was induced specifically by the acylated lipid A component of LPS in most Gram-negative bacteria, explaining why caspase-11 specifically reacted to intracellular Gram-negative pathogens. Notably, synthetic lipid A and LPS isolated from *E. coli* and *S. Typhimurium*-activated caspase-11 independently of the established membrane-bound LPS receptor TLR4 (Hagar et al., 2013; Kayagaki et al., 2013). Instead, caspase-11 activation required LPS to be present in the cytosolic compartment (Hagar et al., 2013; Kayagaki et al., 2013). This observation suggested the existence of an intracellular LPS-sensor that triggers caspase-11 activation. In agreement, recent work showed the CARD motif of recombinant caspase-11 to bind LPS specifically and with high affinity (Figure 1), suggesting caspase-11 to act both as the LPS-sensor and -effector in infected macrophages (Shi et al., 2014). Indeed, *caspase-11*^{-/-} mice were more resistant to LPS-induced lethality (Wang et al., 1998; Kayagaki et al., 2011). Moreover, caspase-11 activation contributed importantly to LPS-induced lethality of TLR4-deficient mice that had been pre-stimulated with non-lethal doses of the TLR3 agonist polyinosinic:polycytidylic acid (polyI:C) to induce caspase-11 expression (Hagar et al., 2013; Kayagaki et al., 2013). In contrast, a high percentage of *caspase-11*^{-/-} mice survived this treatment regimen. Thus, caspase-11 contributed to LPS-induced lethality independently of TLR4 and caspase-1. Concurrently, caspase-11-induced pyroptosis was proposed to mediate host defence against intracellularly-replicating Gram-negative bacteria (Hagar et al., 2013; Kayagaki et al., 2013), and to protect against dextran-sodium sulfate (DSS)-induced colitis (Demon et al., 2014). The identification of biomarkers for pyroptosis may shed further light on how caspase-11-mediated pyroptosis modulates pathology of infectious and non-infectious diseases *in vivo*.

Caspase-12: a negative regulator of immune signalling

The geographic distribution of caspase-12 expression in humans has intrigued investigators for nearly a decade

(Saleh et al., 2004; Kachapati et al., 2006; Xue et al., 2006; Ferwerda et al., 2009; Hervella et al., 2012). Remarkably, full-length caspase-12 (Casp12L) is absent in Caucasians, but expressed in some ethnic groups living in North and Sub-Saharan Africa, together comprising 20–30% of the African population (Kachapati et al., 2006; Xue et al., 2006). In addition, the Casp12L allele is found in populations of the Middle East and South-East Asia, albeit at lower frequencies (Kachapati et al., 2006; Hervella et al., 2012). Casp12L is also found in a fraction of the African-American population that was suggested to be more susceptible to septic shock (Saleh et al., 2004), although another study failed to detect significant differences in TLR ligand-induced inflammatory cytokine expression profiles of haematopoietic cells from individuals expressing Casp12L or the short isoform of caspase-12 (Casp12S) (Ferwerda et al., 2009).

The loss of Casp12L in Europe predates a domestic lifestyle because genotyping of tooth samples of 24 pre-Neolithic individuals from six archaeological sites in Spain showed that they were all homozygous for a C>T single nucleotide polymorphism (SNP; rs497116) that gives rise to Casp12S that largely corresponds to its amino-terminal CARD domain (Fischer et al., 2002; Hervella et al., 2012). The fixation of the Casp12S allele in Caucasians, large parts of Africa, the Americas and Asia suggested that it conferred a selective advantage against bacterial infections or other environmental factors soon after its emergence in Africa 100 000–500 000 years ago (Xue et al., 2006). However, the precise environmental factors that drove the Casp12S allele to near-fixation during human evolution remain unclear (Ferwerda et al., 2009). Remarkably, this selective pressure is unlikely to involve caspase-12 proteolytic activity because both the Casp12L and Casp12S alleles contain an additional missense mutation that converted the catalytic SHG motif found in other caspases to an SHS motif in human caspase-12 (Fischer et al., 2002). When introduced in human caspase-1, this mutation prevented cleavage of proIL-1 β into its mature form, confirming the critical importance of this evolutionary conserved motif for caspase activity (Rocher et al., 1997). Interestingly, although mouse caspase-12 has an intact SHG motif, no validated substrates have been reported for the recombinant protease apart from it undergoing autocleavage after D319 into separate 20 kDa and 10 kDa catalytic subunits upon its production in *E. coli* (Roy et al., 2008). While this may suggest that a co-factor is required for its optimal activation, it has also raised the possibility that – as in humans – mouse caspase-12 may regulate cellular processes by interfering with protein-protein interactions in multi-protein complexes.

The roles and signalling mechanisms of caspase-12 are not fully understood. Caspase-12 was originally proposed to be critical for endoplasmic reticulum (ER) stress-induced apoptosis (Nakagawa et al., 2000), but this has been contested by other studies (Kalai et al., 2003; Saleh et al., 2006). More recently, caspase-12 was proposed to negatively regulate inflammasome activation by recruiting caspase-1 away from its activating complexes (Saleh et al., 2006). In agreement, caspase-12-deficient splenocytes had increased production of mature IL-1 β and IL-18 when stimulated with TLR ligands (Saleh et al., 2006). In addition, caspase-12 knockout mice were found to be hypersusceptible to LPS-induced endotoxemia. These mice were also shown to be more resistant to septic shock when subjected to the colon ascendens stent peritonitis (CASP) model because they cleared the infection more efficiently than wild-type controls (Saleh et al., 2006). De-repression of inflammasome signalling in caspase-12-deficient mice also was proposed to promote resistance to DSS-induced colitis (Dupaul-Chicoine et al., 2010).

In another study from these investigators, cecum and colon samples of caspase-12-deficient mice were found to harbour up to 10-fold higher levels of the anti-microbial peptide β -defensin 1 relative to control C57BL/6 mice (LeBlanc et al., 2008). Regulation of β -defensin 1 expression by caspase-12 was not a result of differential caspase-1 regulation and IL-1 β production, but rather involved modulation of NOD1/2 signalling. Caspase-12 was shown to recruit the NOD1/2-adaptor kinase RIP2. The absence of caspase-12 thus increased NOD1/2-mediated activation of mitogen-activated protein (MAP) kinase and NF- κ B signalling in the gastro-intestinal tract (LeBlanc et al., 2008). Caspase-12 knockout mice were demonstrated to clear the malaria parasite *Plasmodium chabaudi* better than control mice because of increased cytokine production in the absence of caspase-12 (Labbe et al., 2010). This was not because of enhanced inflammasome and NOD1/2 signalling, but rather because NF- κ B activation was increased in the absence of caspase-12-mediated sequestration of the central NF- κ B modulator I κ B kinase (IKK) γ /NEMO (Labbe et al., 2010). Caspase-12 also was shown to contribute to effective host defence responses against West Nile Virus (WNV) infection by enhancing type I interferon production by the intracellular RNA receptor RIG-I (Wang et al., 2010). Consequently, caspase-12-deficient mouse embryonic fibroblasts had reduced IFN- β levels and increased viral titers when challenged with WNV (Wang et al., 2010).

In conclusion, caspase-12 appears to employ a diverse set of molecular mechanisms to modulate innate immune signalling: it inhibits inflammasomes by sequestering caspase-1, it dampens NOD1/2 signalling by recruiting

RIP2, and it prevents activation of the inflammatory transcription factor NF- κ B at the level of the IKK complex (Figure 2). In contrast, caspase-12 contributed positively to WNV-induced type I IFNs production upstream of RIG-I. Further work is needed to fully understand the spatio-temporal factors that define the molecular mechanisms by which caspase-12 modulates inflammation and host defence responses *in vivo*.

Concluding remarks

Over the past years, research into the mechanisms and immune roles of inflammatory caspases uncovered new functions of these proteases and revealed the signalling pathways in which they operate. Recruitment of caspase-1

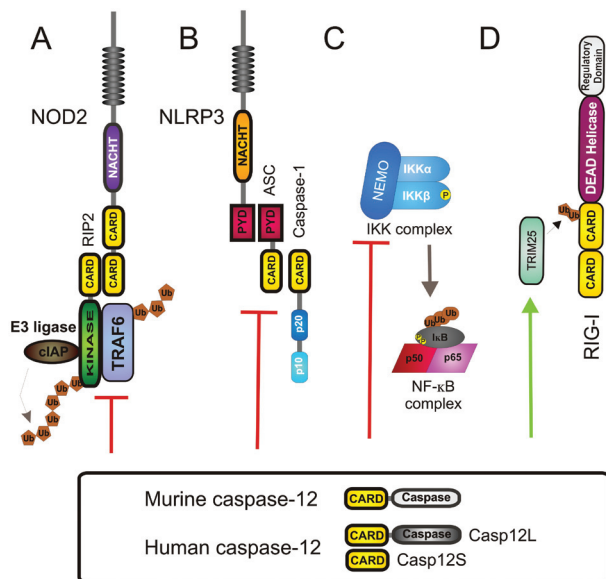


Figure 2 Schematic depiction of proposed caspase-12 effector mechanisms.

(A) Upon bacterial infection, the CARD domain of the pattern recognition receptor NOD2 establishes a homotypic CARD-CARD interaction with the CARD domain of RIP2 kinase. Next, the ubiquitin E3-ligases cIAP1 and -2 interact with RIP2 and conjugate K63-polyubiquitin chains to the RIP2 kinase domain, leading to NF- κ B activation. Caspase-12 dampens the NOD2-RIP2 interaction by displacing TRAF6 from RIP2 and consequently reducing the inflammatory response by dampening NF- κ B activation. (B) The NLRP3 inflammasome and its regulation by caspase-12 as a dominant negative regulator are depicted. (C) Caspase-12 inhibits NF- κ B signalling by targeting the IKK complex. Caspase-12 displaces NEMO from the complex, leading to an inadequate assembly of the IKK complex. (D) Upon WNV infection, caspase-12 modulates TRIM25-mediated ubiquitination of RIG-I, thus leading to enhanced type I interferon production.

in multi-protein complexes termed inflammasomes seems sufficient for it to become activated without the necessity of autoprocessing. The Pyrin inflammasome now also is better understood and was demonstrated to trigger inflammasome activation by *B. cenocepacia* or Rho-inactivating toxins of *V. parahaemolyticus*. Caspase-11 was shown to sense intracellular LPS directly and to induce pyroptosis, thus preventing replication of Gram-negative pathogens. In addition, caspase-11 was shown to contribute to LPS-induced lethality in the absence of TLR4 and to contribute to host defence against *E. coli*, *C. rodentium*, *S. Typhimurium* and *S. flexneri*. Finally, caspase-12 was proposed to modulate immune signalling through diverse molecular mechanisms, but it is generally believed to play an inhibitory role in the innate immune system.

Despite such rapid progress in our understanding of these key inflammatory proteases, many questions remain unanswered. For instance, the molecular mechanisms leading to activation of the Nlrp3 inflammasome remain unclear. The mechanism by which caspase-11 drives pyroptosis, and how it modulates pathology of infectious and non-infection diseases *in vivo* require further work. Finally, the roles of caspase-12 remain incompletely understood, and further analysis of the signalling mechanisms and physiological roles of this caspase will undoubtedly break new ground.

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