Inflammatory Caspases in Innate Immunity and Inflammation

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Abstract

Caspases are best known for their role in apoptosis. More recently, they have gained prominence as critical mediators of innate immune responses. The so-called ‘inflammatory caspases’ include human caspase-1, -4, -5 and -12 and murine caspase-1, -11 and -12. Of these, caspase-1 is best characterized and serves as the prototype for our understanding of the processing, activation and function of inflammatory caspases. Like their apoptotic counterparts, inflammatory caspases are produced as inactive zymogens and require activation to become proteolytically active. Caspase-1 is activated within the inflammasome, a large cytosolic protein complex that is induced by a growing number of endogenous, microbial, chemical or environmental stimuli. The importance of caspase-1 in initiating innate immune responses is demonstrated by its role in cleaving pro-IL-1 β and pro-IL-18 to their biologically active forms. New functions have also been implicated, as these proteases and the mechanisms underlying their activation and regulation emerge as important mediators of human health and disease.

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Introduction

Caspases (cysteine *aspartic acid proteases*) are a family of highly conserved cysteine proteases that play key roles in apoptosis, development and inflammation [1]. In general, caspases exist in the cell as inactive pro-forms that require either proteolytic cleavage or oligomerization to become activated [2] (fig. 1a). Caspases share a common structure, consisting of a 3- to 24-kDa N-terminal prodomain, a 17- to 21-kDa central, large domain, and a 10- to 13-kDa small C-terminal catalytic subunit domain. All caspases contain an active cysteine residue in their large central domain and show specificity in cleavage after an aspartate residue in their respective substrates. Caspases can be classified according to their function as either apoptotic initiator, apoptotic effector or inflammatory caspases (fig. 1b).

The apoptotic initiator caspases (caspase-2, -8, -9 and -10) share homology with the *Caenorhabditis elegans* cell death gene, *ced-3* [3], and possess a long N-terminal prodomain that contains two death effector domains (DED; caspase-8 and -10) or a single caspase recruitment domain (CARD; caspase-2 and -9; fig. 1b). Pro-caspase-8 and -10 are activated to initiate apoptosis by recruitment to the death-inducing signaling complex (DISC) via homotypic DED interactions with the adaptor protein FADD (Fas-associated protein with death domain). This extrinsic pathway is induced following activation by

members of the tumor necrosis factor receptor superfamily, such as TNFR1 or CD95 [4]. Caspase-9, on the other hand, is the canonical caspase in the intrinsic mitochondrial pathway of apoptosis [5]. Following release of cytochrome c and dATP from the mitochondria, Apaf1 complexes with cytochrome c. This results in the recruitment and proteolytic activation of pro-caspase-9 which in turn activates caspase-3, -6 and -7 [6]. Caspase-3, along with caspase-6 and -7, are apoptotic effector caspases [7] and possess a short (20- to 30-amino acid) N-terminal prodomain. They can cleave a multitude of substrates including structural components of the cytoskeleton, nuclear proteins, protein kinases, cell cycle proteins, or DNA metabolism and repair proteins [reviewed in ref. 8]. Therefore, apoptotic effector caspases are responsible for the 'programmed' destruction of a cell.

The final category of caspases constitutes the inflammatory caspases, which includes human caspase-1, -4, -5 and -12 and murine caspase-1, -11 and -12 [reviewed in ref. 9]. These caspases possess a single CARD at the N-terminus and, as their name suggests, play an important function in initiating inflammatory immune responses. The inflammatory caspases of humans, chimpanzees, monkeys and rodents share significant structural homology and are organized in a single gene locus [9]. While human and mouse caspase-1 is highly conserved, human caspase-4 and caspase-5 have been suggested to originate from a duplication of caspase-11 [10]. Caspase-11 is therefore the murine orthologue of human caspase-4 and -5. Caspase-1, formerly known as interleukin (IL)-1 β -converting enzyme (ICE), was the first mammalian caspase to be described [11]. This caspase was shown to proteolytically convert the inactive precursor of the 31-kDa IL-1 β cytokine into its 17-kDa biologically active form, an important step in the initiation of inflammatory responses [11]. In this review, the latest advances in our understanding of the activation, function and regulation of the inflammatory caspases are discussed, with particular focus on caspase-1, the best characterized inflammatory caspase to date. In addition, clinical implications of deregulated inflammatory caspase responses are explored, highlighting their important role in human health and disease.

Activation of Inflammatory Caspases

As mentioned, in most cases caspases undergo proteolytic processing to become active proteases. Major advances in our understanding of inflammatory caspase

activation came with the discovery of the inflammasome, a large protein complex that activates pro-caspase-1 [1, 12]. The inflammasome consists of three major components: (1) a receptor such as a NOD-like receptor (NLR), which acts as an intracellular sensor and induces complex formation; (2) in most cases the adaptor protein ASC (apoptosis speck-like protein containing a CARD), which recruits pro-caspase-1 to the complex through homotypic CARD interactions, and (3) pro-caspase-1, which is auto-proteolytically cleaved, resulting in the generation of active caspase-1 homodimers [1, 12] (fig. 2).

To date, the NLR family consists of 22 human receptors that have been classified according to a common domain structure. This structure consists of a C-terminal leucine-rich repeat (LRR) domain, presumably involved in direct or indirect ligand sensing, a NACHT domain that allows oligomerization, and an N-terminal effector domain that transduces downstream signaling events. NLRs are further classified into subfamilies based on their downstream function and whether their N-terminal effector domain consists of a CARD, a pyrin (PYD) or a baculovirus inhibitor of apoptosis repeat (BIR) domain [1, 13]. Accordingly, the nucleotide-binding oligomerization domain (NOD) subfamily members possess an N-terminal CARD and induce a RIP2-dependent signaling cascade ultimately leading to the activation of NF κ B and potent cytokine expression [1, 13, 14]. Conversely, the nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing (NLRP) subfamily members contain an N-terminal PYD. While the function of several NLRPs is still elusive, the best-characterized NLRPs will be discussed later. Despite containing an N-terminal CARD, NLRC4 (also known as IPAF) is functionally more closely related to the NLRP subfamily than to the NOD subfamily, as it has been shown to form inflammasomes. Finally, NAIP is unique among the NLRs, as it contains three N-terminal BIR domains. Several studies showed that NAIP is able to restrict *Legionella* growth in macrophages [15–17], however, it is unclear whether this involves caspase-1 activation [18].

While the function of most NLRPs remains elusive, NLRP1, NLRP3 and the NLRP-related protein, NLRC4, have been extensively characterized and are known to form inflammasomes that activate pro-caspase-1 [14]. NLRP3 requires the adaptor protein ASC to recruit and activate pro-caspase-1, whereas NLRP1 and NLRC4 also seem to be able to recruit caspase-1 directly through homotypic CARD interactions, independently of ASC [1]. Furthermore, in addition to caspase-1, other caspases

Fig. 2. Activators and inhibitors of caspase-1. Several factors can modulate the activation of caspase-1. Activators are largely comprised of inflammasome scaffolding proteins of the NOD-like receptor (NLR) family such as NLRP3, NLRP1 and NLRC4. In response to microbial products or danger signals, these scaffolding molecules recruit caspase-1 into a macromolecular complex and result in its activation. Inhibitors of caspase-1 include CrmA, PI-9 and the CARD-only proteins, COP, INCA and ICEBERG. More recently, caspase-12 was identified as an additional potent natural inhibitor of caspase-1.

might be recruited to the inflammasome complex. In the case of the NLRP1 inflammasome, recruitment of caspase-5 was shown to take place [12]. More recently, AIM2 (absent in melanoma 2) was described as an inflammasome-forming protein [19–22]. Similar to NLRP3, AIM2 contains a PYD domain and is able to form an inflammasome consisting of ASC and pro-caspase-1 following intracellular DNA stimulation.

NLRP3 is induced to form an inflammasome and activate pro-caspase-1 by a wide variety of chemically and biologically unrelated PAMPs (pathogen-associated molecular patterns) and DAMPs (danger-associated molecular patterns) [1]. Such diversity in activating stimuli beckons just how inflammasome complexes become activated. Although this has been the subject of intense and ongoing investigation, the exact mechanism remains elusive. The presence of an LRR domain implies that NLRP3 may bind its ligands directly, in a mechanism analogous to Toll-like receptors (TLRs). Yet no evidence in support of this hypothesis has been published to date. Therefore, an alternative hypothesis has been put forward whereby

NLRP3 is proposed to detect an intermediate molecule that is generated by a common mechanism elicited by all NLRP3 activators. In line with this, it has been shown that potassium efflux is essential for NLRP3 activation [23]. In an evolutionary sense, the release of potassium may represent a danger signal, as some bacteria express pore-forming toxins, such as nigericin, leading to the release of potassium [24, 25]. However, it remains unclear whether potassium efflux itself represents an NLRP3 stimulus or whether low intracellular potassium provides a favorable environment for inflammasome assembly following the appearance of an as yet undefined primary stimulus. More recently, it was shown that NLRP3 activation by amyloid- β or crystals such as silica and aluminum salts depends on lysosomal leakage and the release of the lysosomal protease cathepsin B into the cytosol [26]. In this model, cathepsin B might induce a signaling cascade eventually leading to inflammasome formation. Reactive oxygen species might represent another common NLRP3 activator, which is induced by a range of PAMPs and DAMPs. Indeed, inhibition of reactive oxy-

gen species production with chemical inhibitors, as well as shRNA against proteins of the NADPH oxidase complex, leads to a reduction in pro-caspase-1 activation and mature IL-1 β production [27, 28]. Clearly, the mode of inflammasome activation could be stimulus as well as cell type specific. Further research is thus required to elucidate the mechanisms underlying the activation of NLRP3, and other NLR, in order to fully understand inflammasome formation and inflammatory caspase activation.

Downstream Functions of Inflammatory Caspases

Cytokine Activation

The best-defined function of active caspase-1 is proteolytic processing of inflammatory cytokines, pro-IL-1 β and pro-IL-18, to their biologically active forms [1]. By controlling the signaling of these cytokines, caspase-1 greatly influences innate immunity and inflammation. In addition to pro-IL-1 β and pro-IL-18 activation, further inflammatory functions of caspase-1 are beginning to emerge [1, 29–31]. These include the regulation of unconventional protein secretion. A study by Keller et al. [32] describes the requirement of caspase-1 activation for the secretion of several cytokines, such as IL-1 α and FGF-2. Via homotypic CARD interactions, caspase-1 was shown to interact with the serine/threonine kinase RIP2 to directly activate NF κ B [33]. Furthermore, evidence suggests that competition exists between the inflammasome adaptor molecule ASC and RIP2 for caspase-1 interaction, resulting in a shift towards either inflammasome formation or NF κ B activation, depending on the ratio of ASC to RIP2 [34]. It is important to note, however, that caspase-1-dependent NF κ B activation has only been demonstrated in vitro and awaits confirmation in vivo.

Pyroptosis

While the importance of caspases in apoptosis has been extensively highlighted, caspases are also implicated in pyroptosis, a recently described pathway of programmed cell death that is distinct from apoptosis [31]. Unlike apoptosis which is silent with respect to inflammation, pyroptosis is characterized by rapid plasma membrane rupture, release of danger signals into the extracellular milieu and subsequent induction of inflammatory immune responses [35]. Furthermore, unlike intrinsic apoptosis, pyroptosis is dependent on caspase-1 activation, occurs independently of caspase-3, -6 and -8,

and does not require the breakdown of mitochondrial integrity and subsequent release of cytochrome c [31, 36]. Upon infection with particular pathogens, cell death was shown to be redirected from apoptosis to pyroptosis, which might represent a host mechanism to combat infection, by inducing increased inflammation [37]. Although a requirement for caspase-1 activation was demonstrated, events downstream of caspase-1 are still unknown. Therefore, it is still unclear how caspase-1 induces programmed cell death distinct from apoptosis.

Endoplasmic Reticulum Stress

Endoplasmic reticulum (ER) stress can arise from protein accumulation inside the ER or excessive protein trafficking through the ER, e.g. during viral infection. Activation of inflammatory caspase-12 is a well-established hallmark of ER stress. More recently, activation of caspase-4 as a result of ER stress was also demonstrated [38]. In accordance with these results, suppression of caspase-4 or -12 activation was shown to abrogate ER-stress-induced apoptosis, possibly linking these caspases to the pathogenesis of neurodegenerative disorders [38–40]. However, the function of inflammatory caspases in ER stress remains under debate, as several recent studies have challenged the aforementioned evidence [41–43].

Regulation of Inflammatory Caspases

In order to control the potent inflammatory responses induced by inflammatory caspases, stringent regulation must be in place. Several mechanisms have been elucidated so far. Of these, three proteins were identified that directly inhibit the enzymatic activity of caspase-1 [44–48]. These proteins, known as COP (CARD only protein), INCA (inhibitory CARD) and ICEBERG (also known as CARD18), are encoded in the same gene locus as human caspase-1, -4 and -5. Their structure is highly analogous to the C-terminal CARD of inflammatory caspases, thus enabling these proteins to directly inhibit caspase-1 via competitive homotypic CARD interactions. However, little is known about the regulation and expression of these proteins in a physiological context. Regulating caspase-1 activation through a similar mechanism as COPs, pyrin and POP (pyrin only protein) 1 and 2 interact with the pyrin domains of ASC and other NLRPs, thereby inhibiting inflammasome assembly and consequently caspase-1 activation [49–51]. In addition to COPs and POPs, several other endogenous inhibitors of inflammatory caspases with distinct mode of actions have been de-

scribed. PI-9 (serpin protease inhibitor 9) was shown to negatively regulate caspase-1, -4 and -8 through binding to the activation loop of these caspases [52, 53] (fig. 2). More recently, the anti-apoptotic proteins Bcl-2 and Bcl-X_L were shown to interfere with NLRP1 oligomerization, possibly due to inhibiting ATP binding to NLRP1 [54].

Alternative splicing, tissue-specific distribution and gene induction by inflammatory stimuli add a layer of complexity to endogenous mechanisms of inflammatory caspase regulation. In most tissues and cells other than monocytes, macrophages and dendritic cells, low-level inflammatory caspase expression likely serves as a safeguard for activation under legitimate immune threat [55]. Further to this, inflammatory caspase expression can be induced upon exposure to IFN γ , TNF, LPS and other bacterial products [43, 55–61].

In addition to the endogenous regulatory mechanisms discussed above, pathogens have also evolved strategies to evade inflammatory caspase responses. The cowpox virus protein, CrmA (cytokine response modifier A), is one such example. CrmA inhibits caspase-1 and subsequent IL-1 β proteolysis, thus suppressing the inflammatory response during cowpox virus infection [62] (fig. 2). Similarly, the poxviral gene product M13L-PYD is able to inhibit caspase-1 activation through homotypic PYD-PYD interactions with ASC [63]. Finally, mycobacteria have been shown to inhibit caspase-1 activation. This represents one of the mechanisms by which mycobacteria achieve immune suppression and parasitize host macrophages [64].

A recent publication indicates the existence of cross-talk between TLR signaling and caspase-1. While TLR signaling induces pro-IL-1 β production, NLR signaling is required for caspase-1 activation and mature IL-1 β secretion. Miggin et al. [65] present data showing interaction between Mal (MyD88 adaptor-like), an adaptor molecule used by TLR2 and TLR4, and caspase-1. This physical interaction leads to the cleavage of Mal and, subsequently, the inhibition of TLR2 and TLR4 signaling. These results thus imply the existence of a negative feedback loop, whereby the activation of caspase-1 can terminate, among others, pro-IL-1 β expression.

Finally, it could be shown that abrogation of NF κ B signaling in neutrophils and macrophages results in increased susceptibility to endotoxin shock due to elevated IL-1 β levels in the serum of these mice [66]. These results suggest an inhibitory effect of NF κ B signaling on pro-IL-1 β processing possibly through the induction of inflammasome/caspase inhibitors. These data are supported by recent findings by our group showing that effector and

memory T cells can efficiently suppress macrophage caspase-1 activation in response to NLRP1 and NLRP3 stimuli in an antigen-dependent way. The same effect is indeed achieved by incubating the antigen-presenting cells with ligands of the TNF family expressed on activated T cells, such as the potent NF κ B activator CD40L. This finding infers the existence of a cross-talk mechanism from the adaptive to the innate immune cells, whereby the quality of the inflammatory mediators produced by the latter can be controlled, avoiding inadequate responses.

Clinical Implications of Deregulated Inflammatory Caspase Responses

The most important function of inflammatory caspases is to protect the organism against intrinsic and extrinsic danger and to induce inflammation when a protective inflammatory response is required. Inflammatory caspases mediate responses to danger signals such as ATP [25], monosodium urate [68] or UVB irradiation [69], and aid in the clearance of many pathogens including *Salmonella* [70], *Shigella* [71], *Legionella* (together with caspase-7) [72] and *Candida albicans* [73]. In support of this, caspase-1 has been described to cleave enzymes involved in glycolysis, thereby regulating metabolism during infections [74]. However, as discussed below, inappropriate activation of caspase-1 may be detrimental.

Overactivation of caspase-1 due to missense mutations in the NACHT domain of NLRP3, the domain responsible for oligomerization, can cause hereditary auto-inflammatory diseases, such as familial cold urticaria, Muckle-Wells syndrome and neonatal-onset multisystem inflammatory disease (NOMID; also known as chronic infantile neurologic, cutaneous, articular syndrome, CINCA) [75]. These disorders are clinically characterized by periodic fever accompanied by skin rashes, amyloid depositions and, in the case of NOMID, neurological complications and hearing impairment. As macrophages from these patients display spontaneous processing and secretion of IL-1 β [76], treatment of patients with IL-1 receptor antagonists dramatically diminishes disease severity and progression by blocking the downstream effects of IL-1 β , thus counteracting the effects of increased caspase-1 activity [77, 78].

Familial Mediterranean fever is an autosomal-recessive disease with similar clinical symptoms to Muckle-Wells syndrome, characterized by mutations in the protein known as pyrin. However, the impact of pyrin muta-

tions on inflammasomes is controversial. On the one hand, mutations in the C-terminal part of pyrin can enable the protein to interact with the PYD of ASC, blocking the recruitment of ASC to the inflammasome and thereby diminishing caspase-1 activation [79]. Recent data, however, describe a direct interaction between the SPRY domain of pyrin with NLRP3, leading to the assembly of the inflammasome complex and enhanced IL-1 β secretion [80, 81].

Non-hereditary diseases can also be exacerbated by deregulated inflammatory caspase responses. Polymorphisms in human caspase-12, resulting in the synthesis of a full-length caspase-12 zymogen (caspase-12L), are strongly associated with increased susceptibility to severe infections such as bacterial-induced sepsis. An inhibitory effect of caspase-12L on caspase-1 activation was suggested as the underlying mechanism for this observation [43]. Deregulated caspase-1 responses are also linked to cardiovascular disease [82], inflammatory bowel disease [83], psoriasis [84] and neurological diseases such as Huntington's disease [85] (table 1). However, the actual impact of caspase-1 on the pathogenesis of these diseases requires further investigation.

Conclusion

In addition to their well-characterized role in apoptosis, caspases have emerged as critical mediators of innate immunity and inflammatory responses. In accordance with their apoptotic counterparts, the so-called inflammatory caspases are produced as inactive zymogens that require cleavage to generate their active, proteolytic forms. Caspase-1 represents the best-studied inflammatory caspase to date. Activation of caspase-1, and probably of other inflammatory caspases, requires assembly of large cytosolic protein complexes termed inflammasomes that are induced by a growing number of microbial and chemical environmental stimuli. Once activated, caspase-1 cleaves pro-IL-1 β and pro-IL-18 to their biologically active forms, initiating a potent inflammatory response. In addition, inflammatory caspases have recently been implicated in pyroptosis and, albeit controversial, in ER stress responses.

As inflammatory caspases are important regulators of innate immune responses, their activity is controlled by stringent regulatory mechanisms. Such mechanisms include endogenous inhibitory proteins that compete for pro-caspase-1 binding and inhibit caspase-1 activation, as well as tissue-specific and inflammation-inducible

Table 1. Diseases with possible involvement of inflammasomes or inflammatory caspases

<i>Hereditary syndromes with known site of mutations</i>	
Muckle-Wells syndrome	NLRP3 [86]
Familial cold autoinflammatory syndrome/urticaria	NLRP3 [86]
Neonatal-onset multisystem inflammatory disease (NOMID, CINCA)	NLRP3 [75]
Familial Mediterranean fever	pyrin [79]
Pyogenic arthritis, pyoderma gangrenosum and acne syndrome	PSTPIP1 [87]
<i>Diseases with involvement of inflammatory caspases or their effectors</i>	
Gout	NLRP3 [68]
Pseudogout	NLRP3 [68]
Contact hypersensitivity	NLRP3 [88, 89]
Vitiligo	NLRP1 [90]
Malaria	NLRP3 [67]
Hydatidiform moles	NLRP7 [91]
Crohn's disease (SNP)	NLRP3 [99]
Type 2 diabetes	IL-1 β [92]
Hypertension	NLRP3 [93]
Fever syndromes	NLRP12 [94]
Brain injury	NLRP1 [95]
Psoriasis	caspase-1 [84]
Huntington's disease	caspase-1 [85]
Sjögren's syndrome	caspase-1 [96]
<i>Legionella pneumophila</i> infection	caspase-7 [72]
Sepsis	caspase-12 [97]
Epidermal water loss and UV damage	caspase-14 [98]

gene expression patterns. Failure to sufficiently regulate inflammatory caspase activation can manifest in severe autoinflammatory diseases such as hereditary fever syndromes, or dramatically increase susceptibility to bacterial sepsis.

Although the field of innate immunity and caspase-1 activation in particular has made enormous progress, the exact mechanism of inflammasome activation and possibly new functions of inflammatory caspases need to be addressed to understand their suitability as putative targets for the treatment of inflammatory diseases.

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