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The Inflammatory Caspases: Key Players in the Host Response to Pathogenic Invasion and Sepsis

Amal Nadiri, Melissa K. Wolinski, and Maya Saleh

Caspases are cysteinyl-aspartate-specific proteinases known for their role in apoptosis (cell death or apoptotic caspases) and proinflammatory cytokine maturation (inflammatory caspases). The inflammatory caspases were among the first to be discovered, but only recently have the mechanisms leading to their activation and inhibition begun to be elucidated. In this review, we examine the biochemistry, substrates, and function of this unique family of inflammatory proteases, highlight the most recent findings regarding their regulatory mechanisms, and discuss what remains to be understood about their roles in health and disease. The Journal of Immunology, 2006, 177: 4239–4245.

Caspases are essential proteases for the initiation and execution of apoptosis, and for the processing and maturation of the inflammatory cytokines IL-1β and IL-18 (1). In humans, the caspase family includes 13 members that are classified into three groups according to their phylogenetic relationship (Fig. 1A), which seems to correlate with functional relatedness (2). The cell death caspases are initiators (caspase-2, -8, -9, and -10) and executioners (caspase-3, -6, and -7) of apoptosis. The initiator caspases sense death signals, and activate more downstream executioner caspases, which cleave cellular substrates mediating the changes associated with apoptosis. However, this simplistic cascade is not the whole story, for “apoptotic” caspases, especially caspase-8, appear to have additional functions unrelated to cell death. Caspase-8 has been shown to be required for T cell homeostasis, proliferation, and activation (3, 4), and for cell motility under nonapoptotic conditions (5). It remains to be determined whether other apoptotic caspases, besides caspase-8, function in promoting cellular survival.

Both inflammatory and apoptotic caspases are synthesized as inactive zymogens and share a common conserved structure composed of a prodomain and a catalytic region (Fig. 1B). The prodomain contains a caspase-recruitment domain (CARD), which is also present in the cell death initiator caspase-2 and -9, suggesting that these caspases might interact with similar cellular partners and may therefore share common mechanisms of activation. The CARD domain contains a “death fold” structure also found in the death domain, death effector domain, and pyrin domain (PYD). The caspase catalytic region is composed of both large and small subunits with a conserved QACXG active site sequence (where X is R, Q, or G) found in the large subunit (Fig. 1B). Caspases recognize a tetrapeptide motif in their substrates and have an absolute specificity for an aspartic acid residue at the scissile bond. The substrate specificities for caspase-1, -4, -5, and -11 have been determined using small peptides in vitro, and were found to be similar with a preference for the sequence Trp-Glu-His-Asp (WEHD). However, caspase-12 seems to have acquired alterations in its catalytic function, as it only recognizes and cleaves itself in rodents (W. Shao and M. Saleh, manuscript in preparation) and appears inactive in humans (6). It is unclear whether the inflammatory caspases play a direct role in apoptosis, as we lack mechanistic information about both their execution of cell death and their cellular “apoptotic” substrates. In this study, we review the mechanisms regulating the inflammatory caspases, their function in inflammation and innate immunity, and what we have recently uncovered about their roles in both sepsis and the host immune response to pathogenic infection.

Gene orthology and chromosomal localization

The human inflammatory caspases include caspase-1 (IL-1β-converting enzyme (ICE)), -4, -5, and -12. Mice have one fewer inflammatory caspase; caspase-5 is absent, probably having arisen by tandem gene duplication of caspase-4 in higher species. Mouse caspase-11 appears to be the ortholog of human caspase-4. The genes for the inflammatory caspases cluster together on human chromosome 11q22.2-q22.3 and on a syntenic region on mouse chromosome 9A1. They are arranged from telomere to centromere as caspase-1, -5, -4, and -12 in humans and as caspase-1, -11, and -12 in mice (Fig. 1C). Two demonstrated inhibitors of caspase-1, Iceberg and Cop or pseudo-ICE, which are CARD-only proteins, are found in close proximity to caspase-1 in the human but not the murine locus. The arrangement, proximity, as well as the exon-intron structure of the inflammatory caspases, suggest that they originated from the same ancestral gene(s).
Expression patterns and gene regulation

The inflammatory caspases are held in check at different levels. Alternative splicing, tissue-specific distribution and gene induction by inflammatory mediators add to the complexity of their regulation. In total, there are 18 transcripts encoded by the human inflammatory caspases and 3 by their murine orthologs (Fig. 2). Alternative splicing of human caspase-1 yields five different transcripts and that of human caspase-4 and -12 results in three and nine distinct transcripts, respectively. Human caspase-5 as well as murine caspase-1, -11, and -12 each generate one transcript (7–12).

The expression patterns of the inflammatory caspases have been studied extensively. Caspase-1 and -5 share a similar pattern of distribution. They are expressed at low levels in most tissues, but are constitutively expressed at high levels in monocytes, macrophages, and to a lesser extent in neutrophils (13). Transcription from the caspase-5 gene is induced by LPS. In the nervous system, caspase-1 but not caspase-5, is found at higher levels in neurodegenerative diseases such as Huntington's and amyotrophic lateral sclerosis (14). Unlike caspase-1, caspase-5 is highly expressed in the brain and the colon.

Caspase-4 differs from caspase-1 and -5 in that it is normally present at high levels in most tissues. Its expression, however, is lower in the brain and colon than caspase-1 and -5, respectively. One important characteristic of its gene regulation is its robust induction by IFN-γ (15–17).

Like caspase-1, mouse caspase-11 is expressed in the hematopoietic and the CNS but found at low levels in most tissues. It is highly induced by LPS and IFN-γ (18–21). In addition, it is induced in the duodenum and jejunum following intestinal mucositis, and in the brain following hypoxic and ischemic brain injury (11, 22).

Caspase-12 is expressed in almost all tissues at the RNA level with the highest expression observed in the lung, stomach, and small intestine (23, 24). Its constitutive protein expression, however, is restricted to skeletal muscle, heart, brain, liver, eye, and testis. Its expression is low in the lymph nodes, thymus, and spleen (25), but is highly inducible by IFN-γ and bacterial components in various cells including splenocytes and macrophages (24).

Substrates

Very little is known about the physiologic substrates of the inflammatory caspases. Caspase-1 is known to cleave pro-IL-1β (26), pro-IL-18 (27), the related IL-1 family member IL-1F7b, also known as IL-1H4 (28), and more recently IL-33 (29). It has also been shown in vitro to process actin (30), the kinase PITSLRE (31), and parkin (32). However, the relevance of this in the inflammatory response and programmed cell death remains unclear. Caspase-4 has been shown to process pro-IL-18 and IL-1F7b inefficiently (28), and was suggested to cleave caspase-3 into its active form (33). Caspase-5 was also reported to cleave caspase-3 (34) and was shown to process the transcription factor Max in vitro (35). The only substrates known for caspase-11 are caspase-1 (36) and -3 (37). Although it is clear that the inflammatory caspases, with the exception of caspase-12, are catalytically efficient and can process multiple cellular proteins in vitro (S. Roy, J. R. Sharom, C. Houde, W. Shao, T. P. Loisel, J. P. Vallancourt, M. Saleh, and D. W. Nicholson, unpublished observations), we still do not know of their in vivo substrates. The finding that caspase-1 is secreted from macrophages upon activation presents one possible explanation for the lack of identified caspase-1 targets (38). Whether caspase-4, -5, and -11 are also secreted upon activation, and whether the secretion of activated inflammatory caspases occurs in other tissues such as neurons, which are reported to undergo apoptosis.
“downstream” of the activation of caspase-1 and -11, remains to be determined.

Biological activities of the prime caspase-1 substrates IL-1β and IL-18

The role of caspase-1 in the maturation of IL-1β and IL-18 renders it a key player in response to pathogenic infection as well as in inflammatory and autoimmune disorders. IL-1β and IL-18 are key cytokines in these conditions (39). Although they share certain proinflammatory activities, they also have very important individual functions. For example, IL-1β but not IL-18 is anorectic (40, 41), pyrogenic (42, 43), results in skin rashes and urticaria, induces hepatic-acute phase proteins (44, 45), up-regulates prostanoid synthesis, and is involved in inflammatory pain hypersensitivity (46). IL-1β is also implicated in destructive joint and bone disease (47), tumor angiogenesis and invasiveness (48), and toxicity of insulin-producing pancreatic islets β cells (49), and neurons in stroke and neurodegeneration (50). IL-18, in contrast, was originally known for its ability to stimulate IFN-γ production (51, 52) and to mediate T cell polarization. Now we know that it possesses many other functions including modulation of the heart contractile force (53), up-regulation of adhesion molecules and NO synthesis (54), and regulation of energy intake and insulin sensitivity (55). Therefore, inhibition of caspase-1 is an attractive therapeutic strategy aimed at blocking the effects of both cytokines in inflammatory and autoimmune diseases. Although we know of many biological effects of IL-1β and IL-18, we still lack information on the exact mechanisms by which caspase-1 is activated, and by which these cytokines are matured and secreted.

Activators of the inflammatory caspases

Our knowledge about the mechanisms leading to caspase-1 activation has increased tremendously in the last few years. We knew for some time that bacterial products, ATP or Nigericin which alter the intracellular ionic milieu resulting in cytosolic acidification, lead to caspase-1 activation (56, 57). However, it was not clear how these signals converged on caspase activation. The characterization of the “inflammasome” as the macromolecular complex required for caspase-1 activation solved a big piece of the puzzle (38). The inflammasome resembles in many aspects the apoptosome, which is assembled during mitochondrial apoptosis to activate caspase-9 (58). In both cases, the caspases are brought in close proximity in response to an activating signal via a scaffolding molecule. Apoptosis protease activating factor 1 is such a molecule in the apoptosome. Through its CARD domain it binds the CARD domain of caspase-9 and results in its activation in response to cytochrome c release from the mitochondria. In the case of the inflammasome, members of the NOD-leucine-rich repeat (NLR) family act as regulators of caspase-1 activity (59) (Fig. 3). Although NOD1 and NOD2 activate the NF-κB pathway upon sensing of pathogens (60, 61), NACHT, LRR, and PYD-containing protein (NALP)1, 2, 3 (38, 62), neuronal apoptosis inhibitor protein (NAIP) 5 (63), and ice protease activating protein (IPAF) (64, 65) have all been shown to activate caspase-1. It is interesting to note that these proteins share common structural determinants with apoptosis protease activating factor 1, as they are able to recruit caspases and oligomerize following the binding of nucleotides. The questions that remain center around what ligand binds the scaffolding molecule to stimulate inflammasome assembly, and what determines the specificity of that binding. Although cytochrome c is the stimulus activating the apoptosome, the ligand for the inflammasome is unknown. Recently, multiple groups investigating the NALP-3 inflammasome examined this question and showed that ATP, the toxin Nigericin (66), bacterial RNA (67), gout-associated monosodium urate, and calcium pyrophosphate dihydrate crystals (68) induce NALP-3 inflammasome activity. Similarly, Salmonella flagellin has been recently reported to activate the IPAF inflammasome (69, 70). It is unclear, despite this observed induction of the inflammasome, whether these stimuli interact directly with NALP-3 or IPAF. Moreover, one riddle
remains: what determines which inflammasome is assembled in response to a specific ligand? It is clear from both the NALP-3-deficient mice (66–68) and from patients with the autoinflammatory disorders Muckle Wells syndrome, familial cold urticaria, and chronic infantile neurologic cutaneous and articular syndrome which are associated with NALP-3 mutations, that other NALPs cannot compensate for the loss of NALP-3 (59). Similarly, NAIP-5, but not NALPs or NODs, is the specific molecule engaged in response to Legionella pneumophila (63), indicating a high degree of specificity in the inflammasome response and activation. The CARD-containing kinase receptor-interacting protein 2, known for its function as a mediator of NOD2 signaling, was also shown to function in the oligomerization and activation of caspase-1 (71, 72). We do not know, however, whether a "ligand" would directly activate RIP2 to induce its effects on caspase-1 or whether NOD2 is involved in the RIP2-caspase-1 complex. In the mouse CNS, caspase-1 and -11 have been reported to be activated by oxygen and glucose deprivation as well as following spinal cord injury (73, 74). Whether caspase activation in neurons is also mediated through an inflammasome remains to be determined. Interestingly, RIP2 appears to be the activator of caspase-1 in this context (75). NOD2, in contrast, is suggested to assemble a caspase-1 inflammasome in the gut which is hyperactivated in Crohn’s disease due to a mutation in its LRR (76). However, this awaits further characterization at the molecular level. The activation of caspase-4 and -5 has not been analyzed extensively in the studies mentioned above. In most instances, the activation of caspase-1 was assessed by determining the levels of secreted IL-1β as a "read-out" and observation of caspase-1 processing. The lack of "inflammatory" substrates for caspase-4 and -5 led researchers to examine the processing of these caspases as a read-out for activation. Caspase-5 was shown to be processed in the NALP-1 inflammasome (38), however, we do not know whether the cleaved caspase-5 was active, and whether its was processed by caspase-1 or by a caspase-5 auto-cleavage event. Consequently, we ignore whether the enzymatic activity of caspase-5 is necessary in this context. The activation of caspase-4 during inflammation has not been studied. In contrast, caspase-4 has been reported to be activated by endoplasmic reticulum (ER) stress and to induce apoptosis in response to this stress as well as the neurotropic peptide amyloid β, a fibril associated with localized amyloidosis seen in Alzheimer’s disease (17, 77). Caspase-4 was also reported to act downstream of Fas ligation and to activate caspase-3 (33).

Inhibitors of caspase-1

Our understanding of the regulation of the inflammatory caspases, especially caspase-1, has been fueled by several important studies reporting on caspase-1 inhibitors. A first look at the inhibition of caspase-1 came from studies of the viral protein cytokine response modifier (CrmA) known to dampen the cytokine response (78). Using purified protein incubation techniques and HPLC, CrmA was shown to inhibit caspase-1-mediated cleavage of pro-IL-1β. This regulation of caspase-1 by CrmA was supported by later studies (as reviewed in Ref. 79). Another proposed endogenous caspase-1 inhibitor is the proteinase inhibitor, serpin PI-9. It is constitutively expressed within human vascular smooth muscle cells, and was shown to be responsible for caspase-1 inhibition and the resulting loss of pro-IL-1β and pro-IL-18 processing (80). In addition to CrmA and PI-9, caspase-1 enzymatic activity has been shown to be inhibited by the CARD-only proteins ICEBERG and COP (71, 81, 82). These proteins physically interact with caspase-1. Therefore, it is conceivable that they inhibit caspase-1 by acting as dominant-negative regulators interfering with the recruitment of caspase-1 to an activating inflammasome. More recently, we have shown that rodent caspase-12 also blocks caspase-1 catalysis as described in more detail below (24).

Caspase-12

A single nucleotide polymorphism (SNP) (T125GA → CGA) in exon 4 of the human caspase-12 gene results in the synthesis of either a truncated CARD-only protein or a full-length protein (Fig. 1) (23). The majority of the human population has the premature stop codon at this position. However, in 20% of people of African descent an arginine is present, resulting in read-through and synthesis of a full-length caspase-12 "proenzyme" (6). We have previously shown that the presence of the caspase-12 full-length variant dampened the inflammatory response of its carriers to endotoxins and was associated with severe sepsis and sepsis-related mortality (6). A recent study examining the age, geographical origin, and spread of the human caspase-12 deleted form suggested that the stop codon arose in Africa ~100,000 years ago (Fig. 4). This mutation was initially neutral, but around 60,000 years ago, following the migration out of Africa, a positive selection force is believed to have driven it to near fixation (83). The increase in population sizes and densities in Europe and Asia and their exposure to rising infectious diseases and sepsis is believed to have constituted a selective pressure favoring the truncated caspase-12 variant (83). The positive selection advantage of the truncation of caspase-12 is therefore proposed to be sepsis resistance.

In all other species sequenced to date, including rodents, and Old and New World primates, a coding amino acid exists at the position equivalent to that of the human caspase-12 SNP, resulting in the production of a full-length caspase-12 protein (6). In mice, caspase-12 was initially proposed to mediate ER-stress induced apoptosis (84). However, we and others have shown that caspase-12 deficient cells, in both humans and mice, are as
sensitive to ER stress-induced apoptosis as caspase-12-proficient cells (6, 24, 25, 85, 86). Murine caspase-12 appears to function in inflammation similarly to the human caspase-12 full-length variant. We have recently shown that caspase-12-deficient mice are more resistant to sepsis, and are able to clear bacterial pathogens more efficiently than wild-type mice. Therefore, our results indicate that as in humans, caspase-12 is also deleterious in mice. Caspase-12 seems to exert its negative effects on inflammation through inhibition of caspase-1 (Fig. 5). Caspase-12-/- splenocytes secrete higher levels of mature IL-1β and IL-18 as compared with wild-type splenocytes, in response to various purified bacterial ligands (TLR/NOD2 ligands). Interestingly, the enzymatic function of caspase-12 is dispensable for its inhibitory effect on caspase-1, as mutation of the caspase-12 catalytic cysteine to alanine did not abrogate its inhibitory effects on both caspase-1 catalysis and IL-1β processing (24).

Therefore, caspase-12 could constitute a sepsis therapeutic target for populations of African descent. In contrast, the finding that caspase-1 stimulation is necessary for sepsis survival suggests that activators of caspase-1 would also be beneficial as sepsis therapeutics in all ethnic backgrounds. Whether small molecular compounds could be synthesized to activate caspase-1 transiently in sepsis remains to be determined. Alternatively, recombinant mature IL-18 could prove beneficial in sepsis patients. In support of this hypothesis, rIFN-γ seems to improve sepsis survival in human sepsis clinical trials (87, 88).

Caspase inhibitors in the treatment of inflammatory disorders

In contrast to sepsis, it is tempting to hypothesize that reducing the formation and release of mature IL-1β and IL-18 by caspase-1 inhibitors would offer a useful therapeutic option for inflammatory and autoimmune diseases. This hypothesis is supported by the finding that the irreversible tetrapeptide caspase-1 inhibitor, YVAD-CMK, strongly reduced IL-1β and IL-18 production in human osteoarthritis cartilage explants (89). However, this compound was ineffective when administered orally. Other more specific caspase-1 inhibitors have been more recently described: 1) pralnacasan is an orally bioavailable pro-drug which, when metabolized, appears to be a selective, nonpeptidic inhibitor of caspase-1. In vitro, pralnacasan inhibited LPS-induced IL-1β and IL-18 release by human PBMCs (90, 91). In vivo, oral administration of pralnacasan inhibited the elevation of serum IL-1β levels observed in a mouse model of osteoarthritis (90), and reduced dextran sulfate sodium-induced murine colitis and Th1 T cell activation (91). 2) A second orally active caspase-1 inhibitor that has been recently described is VX-765. It has been shown to block IL-1β secretion in LPS-stimulated human PBMCs from familial cold autoinflammatory syndrome and control subjects (92). Altogether, these studies are promising and support a potential role for caspase-1 inhibitors in the treatment of autoinflammatory disorders.

Conclusions

Our understanding of the inflammatory caspases and their regulatory mechanisms has recently improved substantially. Multiple new players have now been identified to modulate the function of these enzymes. The discovery of the caspase-12 polymorphism and the characterization of its function in sepsis and the host response to pathogenic infection has emphasized the essential role of these caspases in innate immunity. In parallel, the identification of the NLR family and its regulation of the inflammatory caspases has provided an important step forward in our knowledge of the tight and highly specific mechanisms of activation of these enzymes. Intense investigation is currently underway worldwide to answer the many remaining questions: what determines the specificity in inflammasome assembly and activation? Does inhibition of caspase-1 by caspase-12 in individuals of African descent provide an advantage in certain conditions? Are there other caspase-1 substrates essential for its effects in innate immunity? What are the roles of caspase-4 and -5 in inflammation?

References


