

Review

The inflammatory caspases: guardians against infections and sepsis

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Innate immunity is the primary host defense against invading microorganisms. Pathogen recognition, mediated through an elaborate 'microbial sensing' system comprising the Toll-like and Nod-like receptor families results in the activation of caspase-1, which is a prerequisite for pathogen clearance. Tight regulation of caspase-1 is necessary to control the magnitude of the innate immune response and protect the organism from possible damaging effects such as sepsis. Recent findings from population studies and animal models of infectious diseases and sepsis have uncovered a role for full-length caspase-12 in blocking the inflammatory response initiated by caspase-1, thus predisposing the organism to severe sepsis and sepsis-related lethality. In this review, we re-examine the relationship among the Group I caspases, their known substrates and their proposed role in apoptosis. We further discuss their function in inflammation and bacterial clearance, with an emphasis on their regulatory mechanisms during the innate immune response.

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The Innate Immune System in the Sepsis Response

The enigma of sepsis. In 1972, Lewis Thomas noted that it is the response of the individual, rather than the invading microorganism, that constitutes the disease.¹ Sepsis has been broadly defined as the host's systemic response to an infection, but generally required the confirmation of a bacterial infection and the diagnosis of any two clinical signs of the *Systemic Inflammatory Response Syndrome* (SIRS): changes in number and immaturity of white blood cells, in body temperature, heart or respiratory rates. This definition of sepsis, however, was problematic since staging of the clinical severity of the disease was difficult to perform. In 1992, the terms 'severe sepsis' and 'septic shock' were coined.² While septic shock is a rapid, highly lethal syndrome occurring 24–48 h after onset, severe sepsis is a slower syndrome that occurs 7–14 days after onset, and is accompanied by multiple organ dysfunction and a mortality rate of 30–70% (Box 1). A more recently defined staging system, *PIRO*,³ takes into account the *predisposition*, the *insult*, the *response* of the host system and the *organ dysfunction*, and is expected to improve the design and outcome of clinical trials.

The Cytokine Code in Sepsis

Inflammatory cytokines are essential mediators of the innate immune response. While it is believed that excessive cytokine

levels set the stage for capillary leakage, tissue injury and organ failure, it is also well documented that the innate immune system in septic patients is suppressed and unable to properly clear pathogens.⁴ To date, clinical trials based on anti-inflammatory strategies such as corticosteroids, anti-endotoxin antibodies and TNF α and IL-1 β -receptor antagonists have nearly all failed.⁵

Experts agree that grouping patients with heterogeneous conditions under the same diagnosis of 'severe sepsis' has led to a lack of significance and failure to reproduce results in clinical trials. Additional problems included incorrect assumptions and incorrect sepsis animal models. It was assumed, for instance, that during sepsis elevated levels of LPS, the main component of Gram-negative bacterial cell walls, were shed in the circulation. This led to injection of lethal doses of LPS in rodents as a model of sepsis. We now know that this is an endotoxic shock model and does not represent what happens in sepsis, where LPS levels are almost 200-fold lower than those found in response to LPS overdose.⁶ As a result, human trials aimed at eliminating LPS, using either hemofiltration or anti-endotoxin antibodies were carried out, but failed to treat sepsis (Box 1).⁷ In response to LPS overdose, rodents produce exponentially higher levels of TNF α and IL-1 β than those found in human sepsis, and this 'cytokine storm' causes endotoxic shock. Blocking the effects of TNF α and IL-1 β production using receptor antagonists has also proved unsuccessful in treating sepsis.

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Abbreviations: SIRS, Systemic Inflammatory Response syndrome; PIRO, *predisposition*, *insult*, *response* of the host system and *organ dysfunction*; TNF, Tumor necrosis factor; IL-1, Interleukin-1; IL-1ra, Interleukin-1 receptor antagonist; IL-18, Interleukin-18; IGIF, Interferon gamma inducing factor; IFN, Interferon; LPS, Lipopolysaccharide; TLR, Toll-like receptor; CARD, Caspase recruitment domain; ICE, Interleukin-1 beta converting enzyme; MSU, Monosodium urate; CPPD, Calcium pyrophosphate dihydrate; CINCA, Chronic Infantile Neurologic Cutaneous and Articular syndrome; ER, Endoplasmic reticulum; CrmA, cytokine response modifier A; SNP, Single nucleotide polymorphism

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Sepsis**Box 1****A Health and Financial Burden**

- Sepsis is the second most common cause of death in intensive care units
- 750,000 cases of severe sepsis are reported per year in the US, with mortality rates higher than 50%
- Treating sepsis costs US approximately \$17 billion annually

Clinical Manifestations

- Infection** - Invasion of a host sterile tissue by a microorganism
- Sepsis** - Systemic inflammatory response to an infection with 2 or more criteria of SIRS (e.g. changes in body temperature, tachycardia, tachypnea, changes in number or immaturity of WBCs)
- Severe Sepsis** - Sepsis complicated by organ dysfunction
- Septic shock** - Hypotension (despite adequate fluid resuscitation), tissue oxygen debt and multiple organ dysfunction syndrome (MODS)

Clinical Trials and Outcomes

- LPS removal using hemofiltration or anti-endotoxin antibodies has failed
- Anti-inflammatory strategies using corticosteroids, TNF α and IL-1 β -receptor antagonists have also failed to treat sepsis
- Anti-immunosuppressive approaches using INF γ have shown therapeutic promise

In contrast to immunosuppressive or anti-inflammatory approaches, stimulation of the immune system using anti-immunosuppressive strategies, such as recombinant IFN γ or caspase inhibitors, has proven beneficial in experimental models of sepsis.⁸⁻¹⁰ Our recently published results describe an association between severe sepsis and the full-length variant of Caspase-12 (Csp-12L).^{11,12} Csp-12L appears to dampen the inflammatory response, suggesting that a suppressed immune system would render an individual more prone to infections and more susceptible to sepsis. This data is, therefore, in disagreement with the notion that individuals with lower cytokines are expected to suffer a less severe form of sepsis. Although inflammatory cytokines are harmful when produced in excess, they are necessary for an initial attempt at bacterial clearance by the innate immune system. C3H/HeJ mice deficient in TLR4, which detects LPS and initiates a signaling pathway to activate cytokine production, are highly resistant to this bacterial product but are susceptible to bacterial infection.^{13,14} Similarly, humans administered with anti-TNF α therapy, or deficient in TLR4¹⁵ are also more prone to infections.

Part I – The Inflammatory Caspases: Relationship, Regulation and Substrates

Before discussing the role of the inflammatory caspases in sepsis, it is useful to briefly describe their relationship, substrates and regulatory mechanisms. Caspases are cysteinyl aspartate-specific proteinases known for their roles in apoptosis and cytokine maturation. They are classified into two subfamilies according to their structure, function and

substrate specificity as either the inflammatory (Group I) or apoptotic (Groups II and III) caspases.¹⁶

Group-I caspases include caspase-1 (ICE; Interleukin-1 β Converting Enzyme), -4, -5 and -12. Caspase-5 is absent in mice, and likely arose in higher species from tandem gene duplication of caspase-4. Mouse caspase-11 appears to be the ortholog of human caspase-4. The inflammatory caspases are found clustered together on human chromosome 11q22.2-q22.3 and on a syntenic region of mouse chromosome 9A1. They are arranged from telomere to centromere as *caspases-1, -5, -4 and -12* in humans and as *caspases-1, -11 and -12* in mice (Figure 1a). *Caspase-1* is also found in close proximity to two genes encoding CARD-only proteins: ICEBERG and COP (or pseudo-ICE). The close proximity of inflammatory caspase genes, along with their exon-intron structure supports the hypothesis that they originated from the same ancestral gene(s).

Expression patterns and gene regulation. Various cellular mechanisms ensure that the inflammatory caspases are kept under control. Alternative splicing, tissue-specific distribution and gene induction by inflammatory stimuli add to the complexity of their regulation. With the exception of caspase-4, the inflammatory caspases 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.¹⁷ Conversely, caspase-4 is normally present at high levels in most tissues. An important characteristic of its gene regulation is its robust induction by IFN γ .^{18,19}

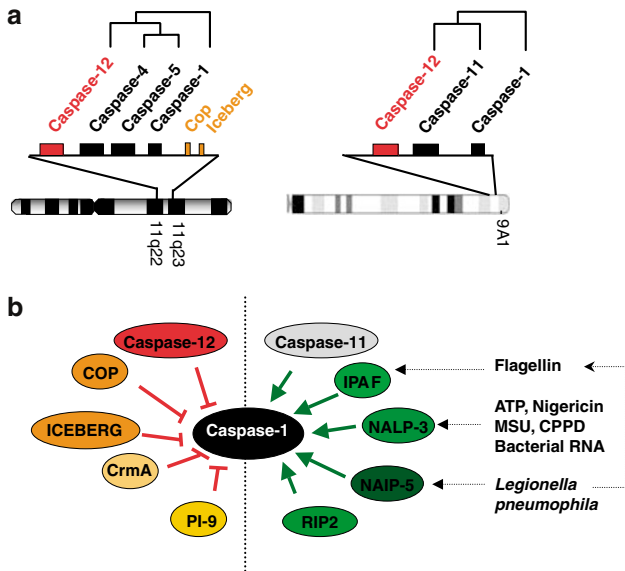


Figure 1 Phylogenetic relationship among the inflammatory caspases and modulators of caspase-1 activity. (a) Phylogenetic relationship and chromosomal localization. In humans, the group I caspases are found arranged on chromosome 11q22–23 from telomere to centromere as *caspases-1*, -4, -5, and -12. In mice, the inflammatory caspase locus is on chromosome 9A1 and the order is *caspases-1*, -11, and -12. The absence of caspases-4 and -5 in mice, and their high degree of homology to caspase-11 suggests that they may have arisen in higher primates due to gene duplication of caspase-11. *COP* and *Iceberg*, encoding two CARD-only proteins are found in the human but not the murine inflammatory caspase locus. (b) Activators and inhibitors of caspase-1. Several factors have been found which modulate the activity of caspase-1. Activators are largely comprised of inflammasome scaffolding proteins of the NOD-like receptor (NLR) family such as NALP-3, NAIP-5 and IPAF. In response to bacterial products or changes in intracellular pH, these scaffolding molecules recruit caspase-1 into a macromolecular complex and result in its activation. In addition to NLRs, the CARD-containing proteins caspase-11 and RIP2 have also been shown to be required for caspase-1 activation. Inhibitors of caspase-1 include CrmA, PI-9 and the two CARD-only proteins, COP and ICEBERG. More recently, caspase-12 was identified as an additional potent natural inhibitor of caspase-1

Caspase-12 message can be found in almost all tissues, with its highest expression observed in the lung, stomach and small intestine.²⁰ However, constitutive protein expression is restricted to skeletal muscle, heart, brain, liver, eye, testis and to a much lesser extent in the lymph nodes, thymus and spleen.²¹ Caspase-12 is also highly induced by IFN γ and bacterial products in various cell types including splenocytes and macrophages.¹² LPS has also been demonstrated to be an inducer of most inflammatory caspases.^{22–24}

Activators and inhibitors of the inflammatory caspases. Our knowledge concerning the mechanisms of caspase-1 activation has increased tremendously in the last few years. However, we still lack information on the regulatory events leading to the activation of caspases-4, -5, -11 and -12. For some time now, we have known that bacterial products, in conjunction with ATP or Nigericin (which disrupt the intracellular ionic milieu and result in cytosol acidification), lead to caspase-1 activation.^{25,26} However, it was not clear how these signals converged to activate caspase-1. Greater insight into the mechanisms of caspase-1 activation came with the characterization of the

inflammasome, a macromolecular complex required for caspase-1 activation (reviewed by Fabio Martinon and Jurg Tschopp). Although ligands for the inflammasome remain elusive, recent studies investigating the NALP-3 inflammasome showed that ATP, the toxin Nigericin,²⁷ bacterial RNA,²⁸ gout-associated monosodium urate (MSU) and calcium pyrophosphate dihydrate (CPPD) crystals²⁹ induce NALP-3 inflammasome activity (Figure 1b). Similarly, flagellin from both *Salmonella* and *Legionella* has been reported to activate the IPAF inflammasome.^{30–33} Although the characteristics of an inflammasome ligand are not known, which inflammasome is assembled in response to such a specific ligand is even more elusive. It is clear from both the NALP-3-deficient mice^{27–29} and from patients with the auto-inflammatory disorders Muckle Wells syndrome, Familial Cold Urticaria and Chronic Infantile Neurologic Cutaneous and Articular syndrome (CINCA) that other NALPs cannot compensate for the loss of NALP-3.³⁴ Similarly, NAIP-5 is the specific molecule engaged in response to *Legionella pneumophila*,³⁵ further suggesting that inflammasomes are highly specialized structures activated in a non-redundant fashion by highly specific stimuli.

In most instances, the activation of caspase-1 was assessed by monitoring IL-1 β secretion and caspase-1 processing. The lack of substrates for caspases-4 and -5 led researchers to examine the processing of these caspases as a read-out of their activation. Caspase-5 has been shown to be processed in the NALP-1 inflammasome,³⁶ however, we do not know whether the cleaved caspase-5 is active, nor whether it was processed by caspase-1 or by a caspase-5 autocleavage event. Caspase-4, on the other hand, has been suggested to be activated by endoplasmic reticulum (ER) stress and to induce apoptosis in response to this stress, as well as in response to the neurotoxic peptide amyloid β – a fibril associated with localized amyloidosis in Alzheimer’s disease.³⁷ Although these findings are interesting, they are currently controversial, as reported in reference.³⁸

In addition to the activators of caspase-1, we know of different caspase-1 inhibitors (Figure 1b). A first look at caspase-1 inhibition came from studies using the viral protein Cytokine response modifier (CrmA), which was known to dampen the cytokine response.³⁹ CrmA was shown to inhibit caspase-1 mediated cleavage of pro-IL-1 β . Another proposed caspase-1 inhibitor, serpin PI-9, 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-1 β and pro-IL-18 processing.⁴⁰ 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,^{41–43} which physically interact with caspase-1. It is, therefore, 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 caspase-12 blocks caspase-1 catalysis as we describe in more detail below.¹²

Substrates. With the exception of caspase-12, caspases-1, -4, -5 and -11 share a preference for the substrate sequence Trp-Glu-His-Asp (WEHD). In rodents, caspase-12 recognizes and cleaves itself at the ATAD³¹⁹ site

(manuscript in preparation) but does not cleave any of the caspase tetrapeptide fluorescent peptides or any cellular protein examined to date. Very little is known about the physiological substrates of the inflammatory caspases. Caspase-1 is known to cleave pro-IL-1 β ,⁴⁴ pro-IL-18,⁴⁵ the related IL-1 family member IL-1F7b (also known as IL-1H4⁴⁶), and IL-33.⁴⁷ *In vitro*, caspase-1 has also been shown to process actin,⁴⁸ the kinase PITSLRE⁴⁹ and parkin.⁵⁰ 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,⁴⁶ but has been suggested to cleave caspase-3 into its active form.⁵¹ Similarly, caspase-5 is also reported to cleave caspase-3⁵² and process the transcription factor Max *in vitro*.⁵³ The only substrates reported for caspase-11 are caspases-1⁵⁴ and -3.⁵⁵

Part II – The Inflammatory Caspases in Innate Immunity and Inflammatory Disorders

Although we know of few substrates cleaved by the inflammatory caspases, their role in modulating the IL-1 β and IL-18 pathways render them important players in the host response to pathogenic invasion. Caspase-1 deficient mice are more susceptible to bacterial infections such as *Escherichia coli*, *Listeria*, *Francisella* and *Salmonella* than their wild-type counterparts (Table 1).^{56–59} Similarly, A/J mice with polymorphisms in the caspase-1 activating protein NAIP5 are more susceptible to infection by *Legionella pneumophila* as compared to C57BL/6 mice in which caspase-1 activation is intact.³⁵ Caspase-11 has been suggested to act *in vivo* as an essential activator of caspase-1 and as its obligate partner. In support of this, caspase-11 deficient mice were shown to be incapable of producing IL-1 β and IL-18 in response to LPS stimulation.⁶⁰ Nonetheless, it seems that the requirement of caspase-11 for caspase-1 activation is not absolute and is restricted to certain stimuli, as caspase-1 could be normally activated in the absence of caspase-11 in response to *Listeria* infection (Table 1).⁶¹ This is consistent with observations in human macrophages, where caspase-5, a caspase-11-related protein, is only recruited to the NALP1 inflammasome and not other caspase-1 activating complexes. In contrast to caspase-11, caspase-12 inhibits the activity of caspase-1. Consistently, we have found that caspase-12 deficient mice are more resistant to bacterial infections and sepsis than wild-type mice (Table 1).¹² Therefore, it appears that caspase-1 is the executioner caspase in inflammation and that caspases-

11 and -12 are regulators of its activity. While caspase-1 is essential in the host defense against microorganisms, its activity needs to remain under stringent control. Excessive caspase-1 activation results in endotoxic shock. This has been demonstrated in LPS overdose animal models where both mice deficient in caspases-1 and -11 were resistant to endotoxic shock (Table 1).^{25,26,60} Another instance where excessive caspase-1 activity is deleterious is evident in human patients with the auto-inflammatory disorders Muckle Wells syndrome, Familial Cold Urticaria and CINCA. These patients suffer from uncontrolled caspase-1 activation due to mutations in the gene for the caspase-1 activating protein cryopyrin/NALP-3.³⁴ Natural caspase-1 inhibitors are therefore important in modulating its response. The inhibitory function of caspase-12 on caspase-1 appears to be a dominant effect and detrimental for the individual as it leads to increased susceptibility to infections and severe sepsis.^{11,12}

Caspase-12: a deleterious protein. Polymorphisms in human caspase-12 have been found which drastically alter its function. Most notable is the substitution of a thymidine at position 125 for a cytosine. This single nucleotide polymorphism (SNP), which occurs in exon 4, changes the codon from a stop to an arginine residue, thus resulting in read-through and synthesis of a full length protein (Figure 2a).²⁰ Most individuals express the truncated form of caspase-12. Only in about 20% of individuals of African descent is the full length variant expressed, and the consequences can be severe.¹¹ Individuals expressing the full-length variant have a dampened inflammatory response to endotoxins, and have an increased risk of developing severe sepsis.¹¹ Analysis of population genetics suggests that the truncated form of caspase-12 arose in Africa approximately 100 000 years ago. Following its migration out of Africa, a positive selection pressure is believed to have driven it to near fixation.⁶² This selection pressure is believed to be largely due to rising infectious diseases and sepsis in Europe and Asia, thus favoring the truncated caspase-12 variant and sepsis resistance (Figure 2b).⁶²

Like the human full-length variant of caspase-12, murine caspase-12 also appears to abrogate the inflammatory response. Recently, we reported that caspase-12 deficient mice are more resistant to sepsis, and are able to clear bacterial pathogens more efficiently than wild-type mice. This may largely be due to the inhibitory effect caspase-12 has on

Table 1 The role of the inflammatory caspases in the regulation of IL-1 β and IL-18 production impacts on bacterial clearance and sepsis resistance

Mouse strain	LPS overdose model	Peritonitis-induced sepsis models	<i>Listeria</i> infection model – bacterial clearance
Caspase-1 ^{-/-}	Resistant	ND	Reduced
Caspase-11 ^{-/-}	Resistant	ND	Similar to wild-type
Caspase-12 ^{-/-}	Susceptible	Resistant	Enhanced

The activity of caspase-1 is necessary for bacterial clearance and sepsis resistance: Caspase-1 deficient mice are susceptible to bacterial infections, while caspase-12 deficient mice, in which caspase-1 is de-repressed, are resistant to both infections and sepsis. Excessive caspase-1 activity however leads to endotoxic shock. Mice deficient in either caspases-1 or -11 are resistant to endotoxic shock induced by LPS overdose, while caspase-12 deficient mice are as susceptible as wild-type mice to LPS overdose. Caspase-11 seems to be required for the activation of caspase-1 in response to LPS but is dispensable during *Listeria* infection. ND: not determined

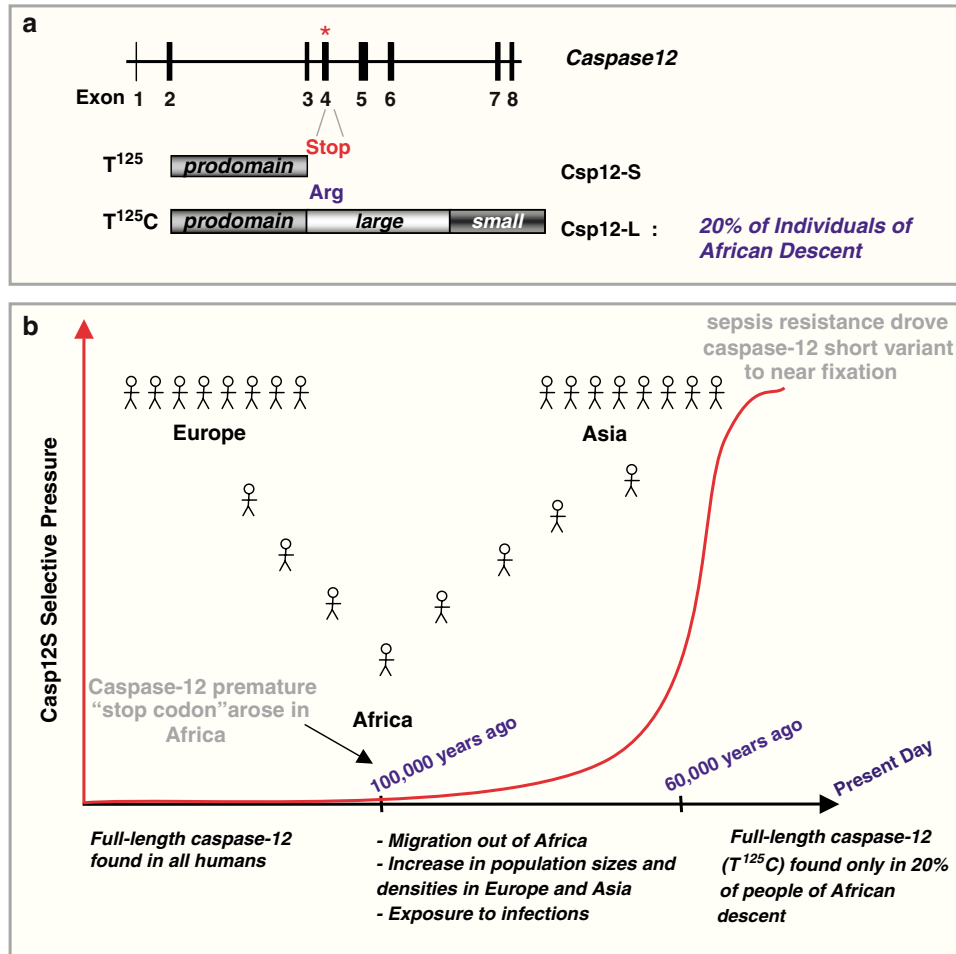


Figure 2 The human caspase-12 polymorphism and sepsis resistance. (a) The T¹²⁵C SNP, which resides in exon 4 of human *caspase-12*, encodes a premature 'stop' codon when T is present at nucleotide position 125 and an arginine when C is present at this position. The presence of the premature stop codon results in the synthesis of a truncated prodomain-only protein, Csp-12S, while the arginine at this position allows for a readthrough and the production of a full-length protein, Csp12L. (b) The importance of this SNP in sepsis resistance is paramount. Individuals expressing the full-length caspase-12 variant are more susceptible to bacterial infections and sepsis than those expressing the truncated form. It is postulated that the truncated variant arose in Africa nearly 100 000 years ago. It was not until the migration out of Africa to Europe and Asia that a selective pressure favoring this variant grew. An increased incidence of infection, along with higher population densities and sizes in Europe and Asia are believed to have constituted a selective pressure which favored the truncated variant, and hence, sepsis resistance

caspase-1 (Figure 3). This hypothesis is supported by the observation that caspase-12-deficient splenocytes secrete higher levels of mature IL-1 β and IL-18, compared to wild-type splenocytes, in response to various stimuli. Interestingly, the enzymatic function of caspase-12 is not required for its inhibitory effect on caspase-1. This was determined in studies employing caspase-12 with a catalytic cysteine-to-alanine mutation, which still blocked caspase-1 catalysis and IL-1 β processing.¹²

In light of these findings, caspase-12 could serve as a therapeutic target for the treatment of sepsis. By the same token, activators of caspase-1 should result in the same outcome, and may also hold therapeutic value. Alternatively, therapy involving recombinant mature IL-18 (a caspase-1 substrate also known as IGIF or IFN γ -Inducing Factor) could also prove effective in treating sepsis, as treatment with recombinant IFN γ seems to ameliorate the sepsis phenotype in humans.^{8,10}

Part III – The Inflammatory Caspases in Apoptosis

It is unclear whether or not the inflammatory caspases play a direct role in apoptosis, as we lack mechanistic information about their cellular 'apoptotic' substrates and the mechanisms by which they execute cell death. Here, we review reports describing a role for caspase-1 in macrophage apoptosis in response to infection by the bacteria *Shigella* and *Salmonella*, and a role for caspases-1 and -11 in neuronal apoptosis and neurodegeneration. We finally discuss the controversial role of caspase-12 in ER stress apoptosis (Figure 4).

Caspase-1 in macrophage apoptosis by *Shigella* and *Salmonella*. *Shigella* and *Salmonella*-infected macrophages undergo apoptosis. It has been postulated that the direct binding between caspase-1 and the bacterially secreted invasive proteins IpaB and SipB, respectively, results in the activation of caspase-1 and leads to cell

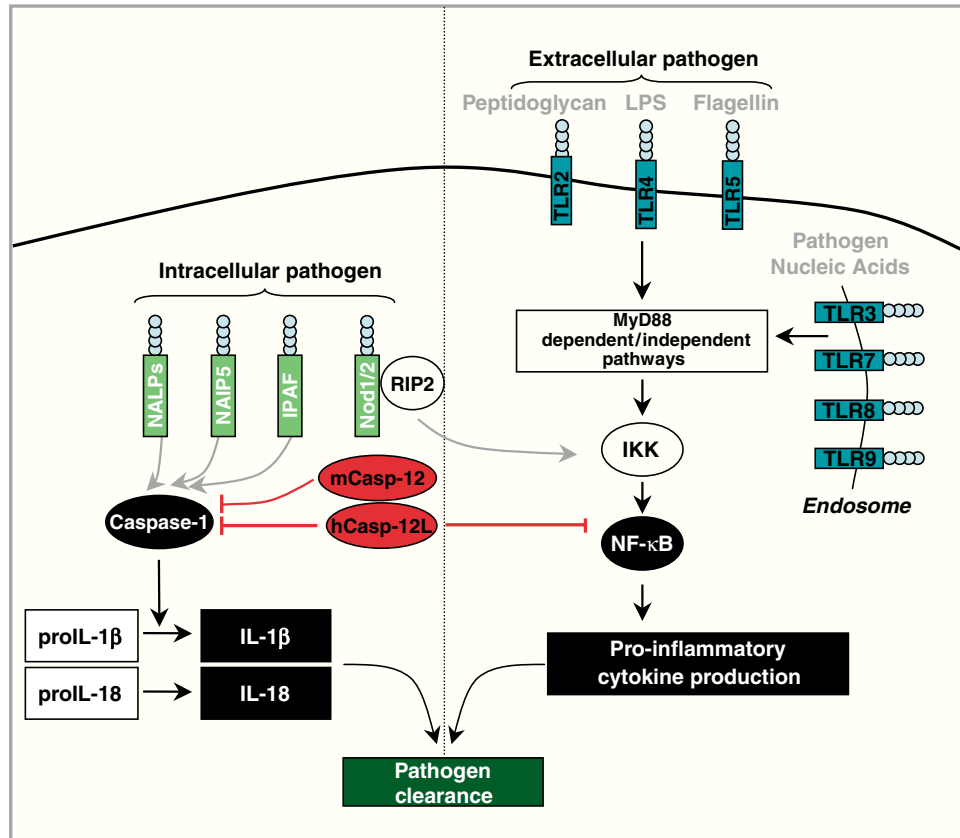


Figure 3 A model depicting the inflammatory pathways activated by extracellular and intracellular pathogens and the role of caspase-12 in modulating these pathways. The innate immune system possesses specific molecules which alert the host to pathogen infections. Extracellular pathogens commonly signal through Toll-like receptors (TLRs), leading to the eventual activation of NF- κ B and induction of its target genes. In contrast, intracellular bacteria are commonly sensed through NOD-like receptors (NLRs), which lead to the activation of caspase-1 and subsequently the processing of pro-IL-1 β and pro-IL-18. The activity of both caspase-1 and NF- κ B is necessary for pathogen clearance. Caspase-12 is shown to down regulate the inflammatory response by directly inhibiting both caspase-1 and NF- κ B activation

death.^{63,64} This hypothesis has recently been re-examined and SipB binding *per se* was shown insufficient to activate the catalytic activity of caspase-1 in the absence of flagellin-expressing bacteria. This led to the finding that caspase-1 activation involves IPAF-mediated sensing of the *Salmonella* flagellin, a process requiring SipB indirectly (Figure 4).³¹ In any event, it appears that caspase-1 is indispensable in macrophage apoptosis induced by *Shigella* or *Salmonella*. Studies using caspase-1 selective inhibitors^{63–65} as well as caspase-1 deficient cells demonstrated the requirement for caspase-1 in macrophage apoptosis.^{64,66,67} However, the molecular mechanism linking caspase-1 activation to macrophage apoptosis remains unknown. It is noteworthy that caspases-2, -3 and -11 are not engaged following caspase-1 activation and that Bcl-2 overexpression does not block *Shigella*-induced apoptosis in macrophages.⁶⁶ Although caspase-1-mediated apoptosis has been widely considered as the main pathway leading to cell death by both *Shigella* and *Salmonella*, necrosis was also described. SipB overexpression in caspase-1-deficient macrophages was also shown to induce autophagic cell death.⁶⁸

Caspases-1 and -11 in neuronal apoptosis and neurodegeneration. Early evidence that caspase-1 plays a role in neuronal cell death came from the findings that

caspase-1-deficient neurons showed prolonged survival following trophic factor withdrawal-induced apoptosis.^{69,70} Similarly, neuronal overexpression of a dominant negative and catalytically inactive (C²⁸⁵G) caspase-1 in mice resulted in an increase in resistance to neurotoxicity.⁷¹ So far, there does not appear to be a common event responsible for inducing caspase-1 activation in neurodegenerative diseases. Hypomorphic mutations in the gene encoding superoxide dismutase (SOD1) have been associated with ALS.⁷² The resulting increase in oxidative stress in SOD1-impaired mice is believed to stimulate caspase-1 activation but through a presently unknown mechanism. Neuronal cell death is also common in experimental autoimmune encephalomyelitis (EAE).⁷³ Caspase-1 mRNA levels positively correlate with the severity of EAE, and mice deficient in caspase-1, or treated with the broad spectrum caspase inhibitor zVAD-FMK, are less susceptible to EAE.⁷³ It is conceivable that caspase-1 could initiate apoptosis directly by cleaving cellular proteins to dismantle the cell, or indirectly through maturation of IL-1 β and the activation of inflammatory pathways that feed back on neurons and induce their cell death. In agreement with this hypothesis, elevated levels of IL-1 β have been found in brain biopsies or cerebrospinal fluid taken from patients suffering from brain injury, Parkinson's disease, Alzheimer's disease, epilepsy,

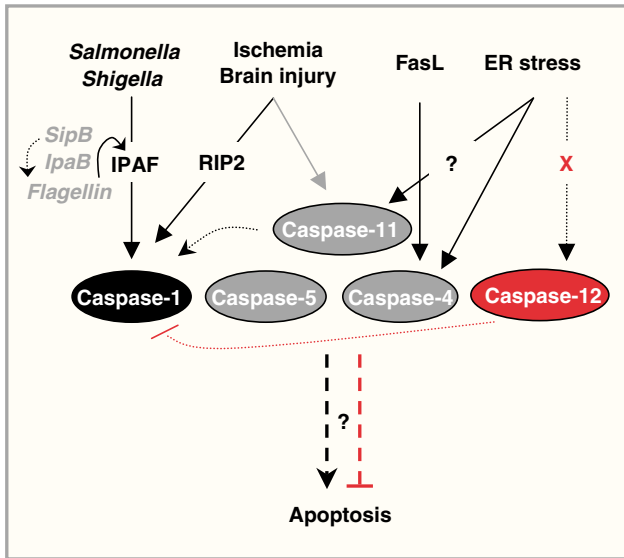


Figure 4 The role of the inflammatory caspases in apoptosis. Caspase-1 is required for macrophage apoptosis induced by the bacteria *Shigella* and *Salmonella*. This seems to be mediated through activation of caspase-1 by a flagellin-induced IPAF inflammasome. Whether the direct binding between caspase-1 and the bacterially derived proteins SipB and IpaB is required for caspase-1 activation is not clear. Caspases-1 and -11 appear to be essential in neurodegeneration. The CARD-containing kinase RIP2 is proposed to activate caspase-1 in neurons. Endoplasmic Reticulum (ER) stress induces apoptosis, however, the initiator caspase in this pathway remains elusive. Caspase-4 seems to be required for cell death in response to this stress, while caspase-12 has been recently shown to be dispensable. We still do not know how the inflammatory caspases execute apoptosis: Whether this involves direct processing of cell 'survival' substrates by the inflammatory caspases, or through activation of the executioner caspases-3, -6 or -7 remains to be elucidated

MS and other neurological disorders.⁷⁴ Additionally, IL-1Ra and IL-1 β neutralizing antibodies were shown to block apoptosis induced by trophic factor withdrawal in animal models of ischemia.⁷⁵ Similarly, caspase-11 deficient mice display a marked decrease in striatum and hippocampus apoptosis following ischemic brain trauma.⁵⁴ However, it remains unclear whether caspase-11 initiates apoptosis independently of caspase-1 or whether it acts by modulating caspase-1 activity (Figure 4). While caspase-11 was shown to be required for caspase-1 activation,⁶⁰ it was also reported to activate caspase-3 independently of caspase-1.⁵⁵ Whether caspases-1 and -11 are activated by an 'inflammasome' in the central nervous system remains to be investigated. Interestingly, the CARD-containing kinase RIP2 and the CARD-only protein COP were shown in neuronal cells to regulate caspase-1 activation and inhibition, respectively, suggesting that a macromolecular complex similar to the inflammasome might form in neurons.⁷⁶

Caspase-12 and ER stress apoptosis: a controversial issue. In rodents, caspase-12 was initially proposed to play a role in mediating ER stress apoptosis as well as cell death in response to amyloid β toxicity.⁷⁷ More recently, multiple groups failed to reproduce these results showing that caspase-12 is dispensable for apoptosis initiated by ER

stressors or other apoptotic stimuli including activators of the extrinsic or intrinsic cell death pathways.^{21,37,38,78} This controversy may exist for two main reasons. First, the levels of caspase-11 were not examined in the initial caspase-12-deficient mice.⁷⁷ The difference in results could be explained if the mice described in⁷⁷ had altered expression levels of a second caspase required for ER-stress apoptosis. Second, many of the studies reporting caspase-12 activation in response to ER stress relied on the observation of caspase-12 cleavage products on western blots as evidence for its activation. Most importantly, the catalytic activity of caspase-12 was never evaluated, and it remains unknown whether these cleavage products represent autocleavage or calpain cleavage events. Indeed, calpains have been reported to process caspase-12.^{79,80} The relevance of this processing remains to be understood. We have recently observed that rodent caspase-12 is catalytically inefficient (manuscript in preparation) and have shown that its catalytic cysteine is dispensable for its effects in inflammation¹² (see below). These results therefore suggest that caspase-12 may be incapable of efficiently processing cellular substrates and initiating apoptosis (Figure 4).

Conclusions. This past decade has seen significant advances in our understanding of the regulatory mechanisms of the inflammatory caspases. The finding that the 'inflammasome' is a highly specific mediator of caspase activation has begun to uncover the complexity of their regulation. In addition, the discovery of caspase-12 polymorphisms and the characterization of their role in sepsis and the host response to pathogenic infection has emphasized the essential role of the inflammatory caspases in innate immunity. Despite these recent advances, many questions remain to be answered. What determines the specificity of inflammasome assembly and activation? What are the advantages of caspase-1 inhibition by caspase-12 in individuals of African descent? Are there other caspase-1 substrates essential for its effects in innate immunity? Answering these questions will greatly improve our understanding of the inflammatory caspases, and may shed light on novel therapeutic targets for the treatment of sepsis and other infectious diseases.

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