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# Caspase-1: Past and Future of this Major Player in Cell Death and Inflammation

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**ABSTRACT**

Inflammation is a major physiological process required for the detection of pathogens and their elimination from an organism. It is triggered by the innate immune system that gets activated through the recognition of danger- or pathogen-associated molecular patterns by protein complexes called inflammasomes. The activation of inflammasomes does not only eliminate the replicative niche of pathogens by inducing infected cells' death (called pyroptosis) but also leads to the secretion of pro-inflammatory cytokines such as Interleukin-1β (IL-1β) and IL-18, which in turn triggers the recruitment of other immune cells to the infection site and mediates communication with neighboring resident cells. The cysteine aspartate protease Caspase-1 is the common effector enzyme of different inflammasomes and is responsible for the maturation of Gasdermin D and IL-1β required for the induction of pyroptosis and the secretion of IL-1β through the Gasdermin D pores. Several gain of function mutations in inflammasome forming receptor proteins including Caspase-1 were associated with severe auto-immune and auto-inflammatory diseases pointing out the necessity of the tight regulation of these complexes. In this review, we focused on Caspase-1 that is at the crossroad of inflammatory cell death and IL-1β secretion. We describe its discovery, Caspase-1 activator signals, its substrates and the inhibitors that have been designed. We also discuss ongoing research that reveals novel unexpected roles for this protease. This review is a good reference not only for the beginners in innate immunity and inflammation but also provides an update on Caspase-1's biology for more advanced researchers.

**Keywords:** Caspase-1, inflammasomes, pyroptosis, interleukin-1, Gasdermin D

## INTRODUCTION

Inflammation is an important physiological process triggered by tissue injury of endogenous or exogenous origin and is characterized by redness, heat, swelling and pain (1). It involves the cooperation of endothelial cells forming the blood vessels and different immune cell types that secrete several critical cytokines [Interleukin-1 (IL-1) family members IL-1β, IL-18; Tumor necrosis factor alpha (TNFα); IL-6], chemokines (IL-8) and lipids (prostaglandin) (2). Among these different signaling molecules released in the extracellular milieu, IL-1β is involved in the induction of fever and its over-secretion is associated with many pro-inflammatory disorders (3). Interestingly, unlike other mediators, IL-1 family members IL-1β and IL-18 are synthesized as precursors that lack a signal sequence triggering their secretion through the conventional endoplasmic reticulum/Golgi pathway (4). Instead, the maturation of pro-IL-1β and pro-IL-18 in the cytosol through their proteolytic cleavage by Caspase-1 is required for their secretion (4). Although the secretion pathways are not fully understood, the mechanisms that activate Caspase-1 have been characterized in the last fifteen years (5).

Synthesized as an inactive precursor itself, Caspase-1 is recruited to multiprotein complexes called inflammasomes formed by a receptor protein and the adaptor

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ASC (6). Different types of inflammasomes able to sense diverse danger and pathogen-associated molecules assemble and trigger pro-Caspase-1 cleavage into mature and active Caspase-1. Activation of Caspase-1 results in two major outcomes: the induction of an inflammatory cell death called pyroptosis by the cleavage of the Gasdermin D protein and the processing of pro-IL-1β into mature IL-1β that will be secreted from the cell through Gasdermin D pores and will mediate inflammation (7-9). Because over-activation of these complexes results in IL-1-dependent auto-inflammatory diseases, the development of inhibitors for inflammasome components and their usage in the cure of these diseases are hot topics in the field (10). This review focuses on Caspase-1 as a central player in the initiation of the immune response and mounting of the first line of immune defenses.

Caspases are proteases synthesized as zymogens with an N-terminal pro-domain that is removed by proteolytic cleavage when they get activated (11). The human Caspase family contains 12 fully characterized members (Caspase-1 to 10, Caspase-12 and Caspase-14) that can be classified into three groups according to their principal functions: inflammation, apoptosis and differentiation (Figure 1). Inflammatory Caspases family consists of Caspase-1, -4, -5 (Caspase-11 in mouse) and -12 (11-13). Apoptotic Caspases are involved in either intrinsic (mitochondria- dependent) or extrinsic (through the induction of death receptors such as Fas or Trail) pathways of apoptosis (14). While some of them initiate the apoptotic signaling cascade (Caspase-2, -9, 8, -10), others are responsible for the cleavage of substrates that mediate apoptotic cell death (Caspase-3, -6 and -7) (Figure 1). Caspase-14, on the other hand, gets activated during the terminal differentiation of keratinocytes and protects the skin against UVB radiations (15).

Caspase-1, the founder protein of the Caspase family involved in the maturation of the main inflammation mediator IL-1β, is conserved between various species from human to Drosophila (Figure 2) and is ubiquitously expressed in various cell types and tissues including immune cells such as macrophages, neutrophils and dendritic cells; cells of the nervous system, epithelial cells and intestinal cells (16). Among the other pro-inflammatory Caspases, Caspase-4, Caspase-5 and their murine homolog Caspase-11 are both receptors and effectors of the non-canonical inflammatory pathway (7). Caspase-4 and Caspase-11 directly bind a bacterial wall component - the lipopolysaccharide (LPS) - and induce cell death that in turn triggers the induction of the canonical NLRP3 pathway and leads to Caspase-1 and IL-1β activation (7). Although the non-canonical pathway is well characterized in mice, the mechanism is less understood in humans. Caspase-5 that is a gene duplication of Caspase-4, is only expressed in humans and is not implicated in the maturation of Caspase-1 and IL-1β (17). The exact role of Caspase-5 is still under investigation.

Caspase-12, the last member of the inflammatory Caspases (Figure 2), has acquired a polymorphism resulting in the expression of a short protein only containing the pro-domain. A catalytically inactive long form resulting from T125C polymorphism is found in African populations giving them susceptibility to

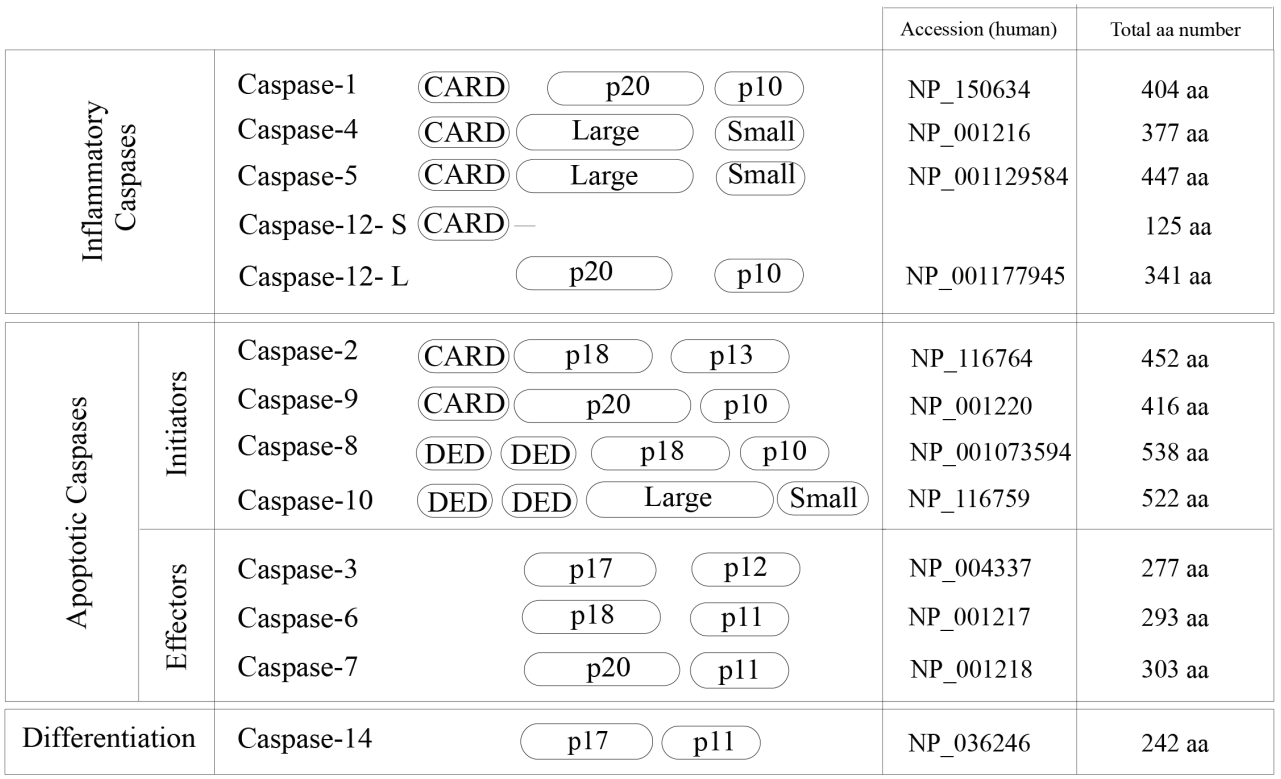


Figure 1. Functional classification and structure of Caspases.

certain infection (Figure 1) (13,18). Whereas murine Caspase-12 induces ER-dependent apoptosis in response to amyloid β stimulation, human Caspase-12 displays an anti-inflammatory role through the inhibition of NFκB pathway (13,19).

Taking into consideration the importance of inflammation in the immune response and the fact that all substrates and activator pathways are not yet fully understood, we will, in this review, focus on the founder of the Caspase family, the Caspase-1 protein by surveying its discovery, the available mouse models, the established and emerging cellular functions, the molecular mechanisms of its activation, the associated diseases and designed inhibitors.

## DISCOVERY of CASPASE-1 and GENERATION of CASPASE-1 KNOCKOUT MICE

Caspase-1 was discovered following extensive research conducted to identify the enzyme responsible for the maturation of IL-1β, an important cytokine mediator of inflammation and implicated in many pathologies. The 31 kDa precursor pro-IL-1β protein was found to be cleaved into its 17 kDa active form after the Asp116 when incubated with the cytosolic extract of human monocytes. This enzyme was called ICE for interleukin converting enzyme (20,21). ICE was synthesized as a 45 kDa inactive protein and two subunits: p20 (19,866 kDa) and p10 (10,248 kDa) were mediating its catalytic activity (22,23). Because of the presence of a catalytically essential cysteine residue in the p20 subunit of ICE and the requirement of an Aspartate in the substrate for the cleavage, ICE was nominated as "Caspase-1" for Cysteine Aspartate Protease (20,22,24).

Caspase-1 knockout mice were generated through the insertion of a neomycin selection cassette into the sixth exon of *Caspase-1* gene encoding for the active site of the enzyme resulting in the synthesis of truncated and non-functional Caspase-1 protein lacking residues important for substrate recognition and catalysis. Caspase-1 knockout (KO) mice developed normally, were fertile and contrary to expectations at that time, Caspase-1 absence had no effect on apoptosis but presented reduced IL-1β and IL-1α secretions in response to ATP or Nigericin stimulations (25,26). While two independently generated Caspase-1 KO mice were used to elucidate Caspase-1's functions for years, an important publication showed that the 129S mouse strain used to generate Caspase-1 KO mice also contains a splicing mutation in the *Caspase-11* gene that leads to the synthesis of a truncated and inactive Caspase-11 protein (Caspase-11 Δ110; 12). Thus, the previously generated Caspase-1 KO mice by using 129S mouse strain were Caspase-1/11 double KO (dKO) mice (12). These findings led to a series of experiments that elucidated the exact role of Caspase-1 that will be presented below.

## CASPASE-1 ACTIVATORY PATHWAYS

Caspase-1 is synthesized as an inactive pro-Caspase-1 enzyme and is cleaved into biologically active Caspase-1 in response to different inflammatory stimuli. Caspase-1 can be activated directly by canonical inflammasomes or indirectly following the induction of the non-canonical Caspase-11 inflammasome.

Among the canonical inflammasomes, the NLRP1 inflammasome assembles in response to anthrax lethal toxin that directly activates NLRP1b through cleavage and triggers Caspase-1

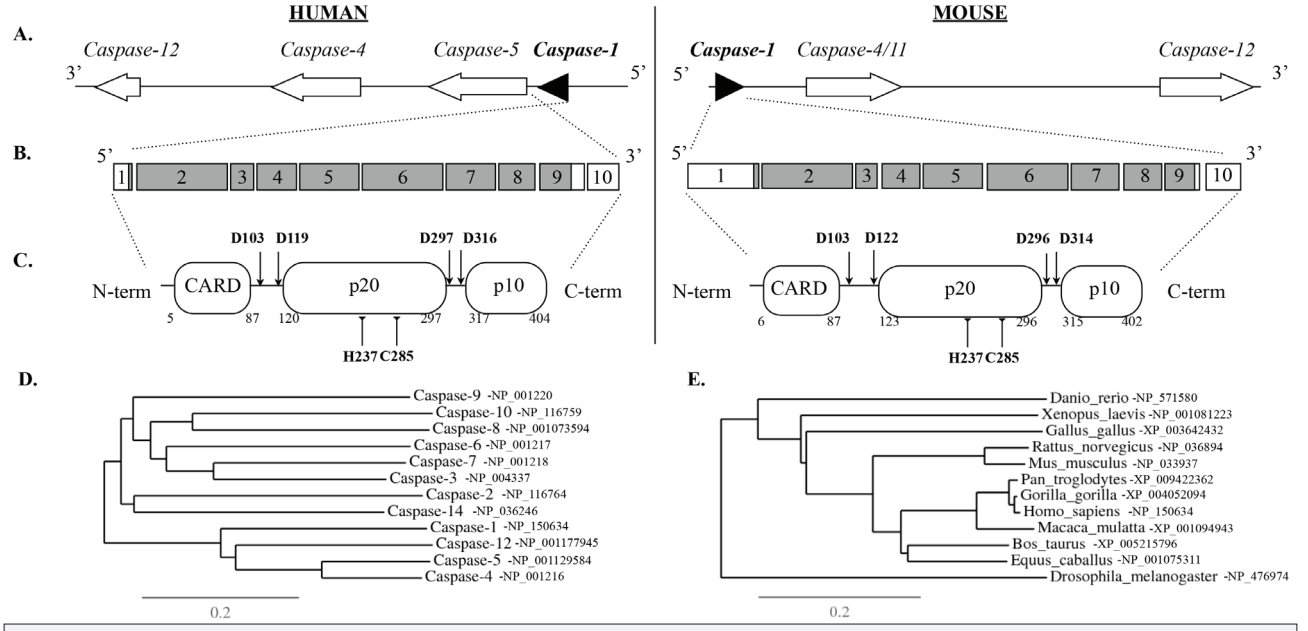


Figure 2. Genomic structure and phylogenetic anlaysis of Caspase-1.
A. Organization of the locus encoding different inflammatory Caspases. B. Exons of Caspase-1. C. Structure and critical amino acids of Caspase-1. D,E. Phylogenetic analysis of different caspases in human (D) and mouse (E).

maturation (27). The widely studied NLRP3 inflammasome is activated in response to MSU crystals, synthetic bacterial RNA and small components, bacterial toxins and ATP and *Listeria monocytogenes* infections (28-31). Caspase-1 is also directly interacting with NLRC4/IPAF protein. NLRC4/IPAF has CARD, NACHT and LRR domains and interacts with pro-Caspase-1 though its CARD domain (32). *Pseudomonas aeruginosa* stimulation assembles the NLRC4/IPAF inflammasome and activates pro-Caspase-1 (33). Finally the pyrin and HIN domain-containing protein AIM2 also formed an inflammasome and activated Caspase-1 after cytoplasmic DNA sensing (34,35). Moreover, Caspase-1 is also the effector Caspase of the NLRP7 inflammasome activated in response to microbial lipopeptides stimulations and the NLRP2 in the central nervous system through ATP induction (36,37).

The non-canonical Caspase-11 inflammasome also activates Caspase-1 but through an indirect mechanism. Cytosolic Caspase-11 recognizes lipopolysaccharide (or LPS) that is a structural component of Gram-negative bacteria such as *Salmonella typhimirium* or *Pseudomonas aeruginosa* (38,39). Active Caspase-11 cleaves Gasdermin D that forms pores into the plasma membrane and induces pyroptosis (7). Changes in ionic fluxes in the pyroptosis-undergoing cells are sensed by the NLRP3 inflammasome that gets activated and induce the processing of Caspase-1 (40). Direct processing of Caspase-1 by Caspase-11 was also proposed but needs further confirmation (41).

Caspase-1 was also described to be activated in response to apoptosis inducing stimuli. Apoptosis was first described in *C. elegans* and is mediated by the Ced-3 protein (42). Because Caspase-1's sequence was highly similar to Ced-3 and overexpression of Caspase-1 in cells induced death, Caspase-1 was considered as an inducer of apoptosis (43). The role of Caspase-1 in apoptosis was further confirmed by the finding that the stimulation of thymocytes isolated from Caspase-1 KO mice with Fas ligand did not undergo apoptosis (26). Similarly, overexpression of Caspase-1 induced apoptosis in response to Fas stimulation in colon cancer cell lines (44). Indeed, overexpression of Caspase-1 triggers apoptosis in prostate cancer cell lines in response to ionizing radiation (45). Caspase-1 also induces cell death in human neurons by proteolytic cleavage of its target Caspase-6, an effector apoptotic Caspase (46). Moreover, Caspase-1 is implicated in *Yersinia pseudotuberculosis* induced apoptosis and is directly processed by Caspase-8 after the infection (47). Besides its intracellular apoptotic function, Caspase-1 is also present in microvesicles secreted outside the cell and the treatment of lymphocytes with these Caspase-1 charged vesicles induces apoptosis (48). However, since pyroptosis was only defined in late 2000s and the only programmed cell death was considered to be apoptosis, this data has to be taken with caution and need verifications. Nonetheless, recent evidences suggest that in the absence of Gasdermin D, Caspase-1-dependent cell death followed by Caspase-3 and Caspase-9 activation can be induced in response to classical inflammasome-activator stimuli (49). These findings need to be further characterized.

**MOLECULAR MECHANISM of CASPASE-1 ACTIVATION**

Caspase-1 is synthesized as a 45 kDa precursor pro-Caspase-1 formed by a CARD domain, p20 and p10 subunits that is activated by auto-processing in response to stimulations. Pro-Caspase-1 is cleaved at Asp103, Asp119, Asp297 and Asp316 sites and the N-terminal CARD domain is released to generate p20 and p10 subunits (22) (Figure 2). *In vitro* studies demonstrate that oligomerization of pro-Caspase-1 is required prior to the self-cleavage (50). Overexpression of the p45 precursor or CARD domain lacking p30 peptide formed by p20 and p10 subunits is sufficient to induce the processing of Caspase-1 into p20 and p10 active subunits suggesting that Caspase-1 is activated by auto-processing (50) and that bringing pro-Caspases in close proximity is enough to activate the enzyme (induced proximity model). Solving the Caspase-1 crystal structure revealed that active Caspase-1 forms a tetramer constituted by a central dimer of p10 subunits and two surrounding p20 subunits (51).

The ASC protein that is formed by PYRIN and CARD domains acts as an adaptor between Caspase-1 and receptors lacking a CARD domain (52). Upon inflammasome activation, ASC and pro-Caspase-1 directly interact with each other through homotypic CARD/CARD interactions forming multimeric scaffolds, called foci or specks (52). ASC speck brings pro-Caspases-1 into close proximity and promotes their cleavage by induced proximity (52). While transfection of ASC CARD domains alone or PYRIN domains alone cannot form specks, foci formation is triggered by full length ASC or by co-transfection of ASC CARD and Caspase-1 CARD (53).

The Death Domain family is known to form filamentous structures. While overexpression of full-length ASC forms specks, overexpression of PYRIN or CARD domains alone results in filamentous structures. In our laboratory, we proposed by mutational analyses that ASC protein aggregation occurs at two levels: first of all, filament formation is induced, and these filaments compact further to form specks (54).

In conclusion, ASC and Caspase-1 oligomerize with themselves through respectively PYRIN/PYRIN and CARD/CARD domains and are maintained in an inactive state (55,56). Upon stimulation, ASC gets activated by transient PYRIN/PYRIN interactions with NLRP proteins and forms specks by recruiting Caspase-1 proteins through the interaction of its CARD domain with Caspase-1's CARD and activates Caspase-1 cleavage by induced proximity (55). We also showed in a recently published paper that ASC speck formation could be disrupted by CARD containing NLR proteins such as NLRC3 (57).

Caspase-1 activates its substrates by cleavage at a specific aspartate residue. Alignment of known Caspase-1's target proteins and *in vitro* studies with inhibitory peptides revealed that the preferential cleavage site of Caspase-1 is the "WEHD" amino acid sequence (58). Arg179 and Gln283 residues of p20 subunits and Arg341 of p10 subunit of Caspase-1 recognize WEHD sequence and cleaves after the aspartate on the target sequence

(59). Crystallography of Caspase-1 with Ac-YVAD-CHO inhibitor and mutant analyses showed that Caspase-1's catalytic site is formed by both p20 and p10 subunits (51,59). The Cysteine in the active site is the Cys285 and has an adjacent His237 and these two residues are responsible for catalysis (59).

**SUBSTRATES of CASPASE-1**

Once it gets activated, Caspase-1 is responsible for the cleavage of pro-inflammatory cytokines IL-1β, IL-18 and IL-33; the pyroptosis mediating pore forming protein Gasdermin D; MyD88 adaptor-like protein Mal and the inflammasome forming Pyrin protein.

Pro-IL-1β is the first substrate that led to the discovery of Caspase-1 (21,60). TLR receptor stimulation activates the NFκB pathway and induces the synthesis of pro-IL-1β. Pro-IL-1β is a 31 kDa precursor and is processed at Asp116 into its mature 17 kDa form by Caspase-1 and gets secreted from the cell to initiate inflammation. The second pro-inflammatory cytokine pro-IL-18 is identified as an 18 kDa IFN-γ inducing factor (or IGIF) and is cleaved by Caspase-1 at Asp35 residue in response to inflammasome activation (61). Mature IL-18 is secreted from activated cells and together with IL-12 induces the production and secretion of IFN-γ from neighboring cells (62).

Identification of Gasdermin D as a substrate of Caspase-1 was an important milestone in the inflammasome research field. Although it was clear that a programmed cell death distinct from apoptosis and depending on Caspase-1 was triggered, it is only in 2014 that pro-Gasdermin D protein was characterized as the substrate of Caspase-1. N-terminal domain of Gasdermin D was released from the inhibitory C-terminal domain and formed pores at the plasma membrane of the cells. These Gasdermin D pores not only formed conduits for IL-1β secretion but also induced pyroptosis (9,63).

Another Caspase-1 target is IL-33. While full-length IL-33 is biologically active and promotes pro-inflammatory cytokines secretion to alert the immune system, processing of IL-33 at Asp178 by Caspase-1 produces an inactive product and inhibits inflammation (64). Besides the regulation of cytokines, Caspase-1 also modulates the NFκB pathway. Caspase-1 processes Pyrin protein between residues Asp330 and Ser331 and the cleaved 30 kDa Pyrin translocates into the nucleus and activates the NFκB pathway. Mutations of the *MEFV* gene encoding Pyrin is associated with Familial Mediterranean Fever (FMF). Mutant Pyrin proteins are more susceptible to Caspase-1 cleavage and cause the aberrant inflammatory cytokine secretion observed in patients (65). MyD88-adaptor like Mal protein is another target of Caspase-1. Mal is involved in the activation of NFκB upon TLR4 and TLR2 stimulations. Whereas full-length Mal is inactive, Caspase-1 cleaved Mal induces the NFκB pathway. Caspase-1 cleaves Mal at Asp198 and generates an active 21 kDa peptide (66).

Proteomic screen of Caspase-1's substrate revealed that the apoptotic effector Caspase-7 is a Caspase-1 target. Upon *Salmonella* infection or LPS and ATP treatment of macrophages, Caspase-1 cleaves Caspase-7 at two sites: Asp23 and Asp198. Caspase-1 is required for Caspase-7 activation in response to Salmonella infection since Caspase-7 is not activated in Caspase-1 KO macrophages (67). Caspase-6 is another protein regulated by Caspase-1. Caspase-6 is expressed in neuronal cells and induces apoptosis in response to serum starvation. Caspase-1 was shown to be the upstream regulator of Caspase-6. Caspase-1 activated Caspase-6 by proteolytic cleavage and triggers cell death. Caspase-1 inhibition or depletion prevents Caspase-6 activation (46).

**CASPASE-1 IN HUMAN DISEASES**

A number of physiologically occurring *Caspase-1* variations were identified in patients with auto-inflammatory diseases and suffering from different types of cancer, but no association was established between these variations and the disease phenotype (Table 1). Indeed, the screen of tumors for *Caspase-1* mutations did not reveal any variations (Table 1).

Only neurological and cardiovascular diseases were associated with some *Caspase-1* polymorphisms. In a screen of elderly persons, rs554344 (10643C allele) and rs580253 (5352A allele) were shown to correlate with low IL-1β levels in the LPS-stimulated blood of carriers and with improvement of their memory performance (68). Thus, polymorphisms decreasing Caspase-1's activity and resulting in lower IL-1β levels had a protective effect on neurological functions.

The G+7/in6A polymorphism (also called $A^{in6}$) was significantly more represented in controls compared to patients with myocardial infarctus or with a history of cardiovascular disease. Carriers of $A^{in6}$ variation had a lower level of IL-18 in the circulating blood compared to the non-carriers. Moreover, $A^{in6}$ polymorphism resulted in a decrease of Caspase-1's mRNA levels *in vitro* (69). Thus, $A^{in6}$ has a protective effect on cardiovascular diseases by decreasing Caspase-1 levels and lowering IL-18 secretions. Taken together, these data suggest a deleterious role of Caspase-1 induced excessive IL-1β and IL-18 secretion in neurological and cardiovascular diseases.

Caspase-1 p.N263S (rs 139695105), p.K319R (rs1751523) and p.R240Q (rs45617533) polymorphisms were identified in patients with auto-inflammatory diseases and decreased both the enzyme's activity and IL-1β secretion *in vitro*. Crystal structure analysis showed that these variants affect the formation of H-bounds between the subunits of Caspase-1 dimers and destabilized the stability of the enzyme in high temperatures. Moreover, patients with homozygote R240Q polymorphism had a decrease in IL-1β secretions compared to wild-type controls (70).

Deregulation of Caspase-1's functions had also an impact on different human pathologies (Table 2). Caspase-1's inflammatory activity negatively correlates with neurological disorders such as Huntington's disease, Amyotrophic Lateral Sclerosis and with cerebral ischemic injury. Knock-in of the dominant

**Table 1.** Caspase-1's variations and association with diseases.

| Disease | Identified variations | Phenotype | References |
|---|---|---|---|
| **Auto-inflammatory** | p.N263S, p.K319R and p.R240Q | Decrease in Caspase-1 activation and IL-1β cleavage *in vitro*; R240Q has an effect on IL-1β secretion *in vivo*. | *70* |
| **Gastric cancer** | p.M345K and IV2-3C>A | Unknown. | *71* |
| | No mutation identified | Screen for *Caspase-1* mutations. | *72* |
| **Prostate cancer** | No mutation identified | Decrease in Caspase-1 levels in tissues. Screen for *Caspase-1* mutations. | *73,74* |
| **All types of cancer** | rs501192 | No difference between patient and healthy groups. | *75* |
| **Age-related cognitive functions** | rs554344 and rs580253 (A allele) | Decrease in IL-1β secretion, better cognitive function. | *68* |
| | rs488992 and rs1977989 | No effect on IL-1β secretion, no correlation with cognitive function. | |
| **Alzeihmer's disease** | rs501192, rs556205 and rs530537 | No difference between patient and healthy groups. | *76* |
| **Cardiovascular disease** | $A^{in6}$ | Decrease in Caspase-1 mRNA *in vitro*. Less IL-18 secretion in the patient sera. | *69* |

negative C285G Caspase-1 protein caused the regression of Huntington's disease whereas inhibition of Caspase-1 by zVAD-fmk had a protective effect on the SOD mutant mice model of ALS (77,78). Indeed, expression of dominant negative Caspase-1 or injection of Caspase-1 inhibitors resulted in a decrease in IL-1β levels in the injured brain, inhibited cell death and rescued the normal phenotype in a mouse model of transient ischemia (79-81).

Caspase-1 is also implicated in different types of cancer and acts as a pro- or anti- tumorigenic protein depending on which pathway it activates (inflammation or cell death respectively). Caspase-1 exerted an anti-tumorigenic effect through the induction of apoptosis in LNCaP prostate cancer lines upon TGF-β stimulation or in DU-145 prostate cancer cell lines upon irradiation (82). Caspase-1 had a tumor suppressor effect in a model of azoxymethane dextran sodium sulfate colitis-associated colorectal cancer in cooperation with NLRC4, which is known to induce a p53-dependent apoptosis (83). Finally, Caspase-1's expression is regulated by the tumor suppressor p63 and low Caspase-1 levels correlate with a mild cancer phenotype (84).

In contrast, Caspase-1 has a pro-tumorigenic impact by inducing inflammation. Caspase-1 was activated in hepatocellular carcinoma cell lines under hypoxic conditions through the HMGB1/TLR4/RAGE pathways and promoted metastasis and invasion of these cells (85). Caspase-1 was also implicated in leukemia by promoting acute myeloid leukemia cell lines' proliferation through IL-1β secretion (86). The proteins forming different inflammasomes NLRP3, AIM2 and NLRP1 were associated with colorectal, and skin cancers respectively (87-89).

As an essential protein of inflammasomes and a regulator of the inflammation mediator IL-1β, Caspase-1 deregulation was associated with different inflammatory diseases. Cryopyrin-associated periodic syndromes or CAPS are characterized by the presence of mutations in the *NLRP3* gene leading to the overactivation of NLRP3 inflammasome, thus Caspase-1 is activated and induces IL-1β secretion constitutively (90). *NLRP3* mutations were also found to cause increased IL-1β secretion in FMF and Behcet's disease (91,92). Similarly, Caspase-1 also has an impact on arthritis since its depletion declined IL-1β levels in joint and ameliorate the disease phenotype in a mouse model of chronic arthritis (93). Caspase-1 also plays a role in endometriosis. Examination of the peritoneal fluid of infertile women showed that IL-1β and Caspase-1 levels are higher compared to unaffected controls and correlate with the severity of the diseases (94).

Caspase-1 is also implicated in metabolic pathologies such as diabetes or obesity. In retinal diabetes, in the presence of high glucose concentrations, Caspase-1 gets activated and an increase in IL-1β levels was observed together with degeneration of retinal capillaries (95). An absence of Caspase-1 also caused diabetes and obesity in male mice with a high fat diet because IL-18 could not be activated and active IL-18 deficiency led to insulin resistance (96).

**Table 2.** Diseases influenced by deregulation of Caspase-1's functions.

| Disease | Induced Genetic Alterations | Phenotype | References |
|---|---|---|---|
| **Huntington's disease** | Dominant negative Caspase-1 (C285G) Knock-In. | Delay of disease progression and mortality in mice. | *77* |
| **Amyotrophic Lateral Sclerosis** | Caspase-1 inhibition by zVAD-fmk. | Protective effect. | *78* |
| **Cerebral ischemic injury** | Dominant negative Caspase-1 (C285G) Knock-In. | Decline of IL-1β levels. Resistance to trophic factors. | *79,80* |
| | Caspase-1 inhibition by zVAD-fmk and others. | Increase in tumor size through induced cell proliferation. | *81* |
| **Colon cancer** | Caspase-1 KO mice | Increase in tumor size through induced cell proliferation. | *83* |
| **Leukemia** | Caspase-1 inhibition. | Suppression of leukemia cell lines' growth. | *71* |
| **Hepatocellular carcinoma** | Caspase-1 activation during hypoxia through HMGB1-TLR4 signaling. | Promotes metastasis and invasion of hypoxic HCC or Hepa cell lines. | *70* |
| **Prostate cancer** | Overexpression of TGF-β in prostate cancer lines. | Caspase-1 activation and apoptosis induction. | *82* |
| **CAPS, FMF and Behcet's disease** | NLRP3 mutations. | Overactive Caspase-1 and enhanced IL-1β secretion. | *72-74* |
| **Arthritis** | Caspase-1 KO mice | Caspase-1 KO inhibits chronic arthritis. | *75* |
| **Diabetes** | Caspase-1 inhibition by minocycline. | Prevents capillarity degeneration induced by diabetes. | *77* |
| **Obesity** | Caspase-1 KO. | Lack of IL-18 cause obesity in male. | *78* |

**CASPASE-1 INHIBITORS**

As Caspase-1 is an important player in the crossroad of inflammation and cell death and is implicated in various diseases, Caspase-1 inhibitors were identified and designed early after its discovery. The cowpox virus expresses Cytokine Response Modifier A (CrmA) protein that inhibits Caspase-1-induced inflammation to escape the clearance of the infected cell by the host immune system (97). CrmA directly binds Caspase-1's active site through its "LVAD" sequence, forms a stable complex with the p20 subunit and prevents the IL-1β maturation. Caspases -8 and -6 are also inhibited by CrmA (98). Similarly, p35 forms an irreversible inhibitory complex with Caspase-1 and prevents IL-1β maturation *in vitro* (99). p35 also inhibits Caspases -1, -3, -6, -8 and -10 but has a higher affinity for Caspase-3 (100).

Synthetic inhibitory peptides compete with the substrate for binding to the catalytic site of the Caspases' catalytic site. The first minimal substrate found to bind Caspase-1 was Ac-Tyr-Val-Ala-Asp-CHO (Ac-YVAD-CHO) and it acted as a reversible competitive inhibitor.[14] Analyses of the target substrates sequences reveled that the 'W-E-H-D' consensus motif is recognized by Caspases -1, -4 and -5. These tetrameric peptides were engineered in order to increase cell permeability and minimize cell toxicity. They contain a benzyloxycarboxyl group (-Z) or butyloxycarboxyl group (-BOC) in N-terminal and a fluoro-methyl ketone (-FMK) or chloro-methyl ketones (-CMK) or an aldehyde (-CHO) in C-terminal (101).

Ac-WEHD-CHO was used to characterize the biochemical propriety of the Caspase-1 enzyme and is a reversible competitive inhibitor (22). Ac-WEHD-CHO has the highest affinity for Caspase-1 (Ki=0,056) but also inhibits Caspase-8 (Ki=21,1), -4 (Ki=97) and -5 (Ki=43) (90). Ac-YVAD-CHO is also a reversible inhibitor highly specific to Caspase-1 (Ki=0,76 compared to Ki= 362, 163 and 352 for Caspases -4, -5, and -8 respectively (101). Z-YVAD-FMK is a competitive and irreversible pan-Caspase inhibitor and was used in many diseases both *in vitro* and *in vivo*.

Antisense Caspase-1 oligonucleotide (5'-CCT-TGT-CGG-CCA-TGG-C-3') inhibited Caspase-1 in cells from Acute Myeloid Leukemia patients and impaired the cell proliferation and reduced the levels of secreted IL-1β (102).

VX-765 (or Belnacasan) binds Caspase-1 reversibly and inhibits LPS-induced IL-1β and IL-18 secretions in FCAS patients' cells (103). Similarly, VX-765 inhibits IL-1β secretion in mice after intravenous injection of LPS (104). VX-765 was also used in the treatment of depression in a mouse model and caused the regression of epilepsies (105-107). Pralnacasan (or VX-740) is also a reversible inhibitor specific to Caspase-1 (108). VX-740 was used in osteoarthritis, DSS-induced colitis and its active metabolite in cerebral brain ischemia and showed improvement of the symptoms of these pathologies (109-112).

## CONCLUSIONS

Caspase-1 is an important player in immunity and constitutes an essential component of the inflammasome complexes that detect and eliminate pathogens. It was first identified by its homology with the ced-3 protein that is implicated in apoptosis (20,21). Further characterization revealed that Caspase-1 cleaves to maturation an important cytokine, IL-1β, and thus was named IL-1β converting. However, further characterization of the cell death induced by Caspase-1 revealed that this cell death was physiologically and morphologically different from apoptosis and was called pyroptosis (7,8).

Besides its role in the cleavage of IL-1β and IL-18, which are two important cytokines playing an essential role in immunity and associated pathologies, Caspase-1 also induces the death of cell by the proteolysis of the Gasdermin D proteins that form pores at the plasma membrane disrupting cellular integrity and inducing pyroptosis. Gasdermin pores also constitute conduits for IL-1β release. The absence of IL-1β, IL-18 maturation and secretion and Gasdermin D cleavage and pyroptosis in Caspase-1 knockout macrophages shows that Caspase-1 is required for these events to occur.

In the induction of pyroptosis, Caspase-1 shares the common substrate Gasdermin D with Caspase-11. For long years, the use of Caspase-1 KO mice generated from cells containing a *Caspase-11* gene encoding a naturally mutated non-functional Caspase-11 protein, masked the crucial role of Caspase-11 in host defense against microorganisms. Caspase-11 and its human homologs Caspase-4 and Caspase-5 were found to directly sense lipopolysaccharide - a structural component of Gram-negative bacteria - through their CARD domain. This recognition triggered a cascade of signaling resulting in the cleavage of Gasdermin D by Caspase-11 and in the induction of pyroptosis.

Inflammasome overactivation by gain of function mutations or constitutive stimulation cause inflammatory diseases. Different types of Caspase-1 inhibitors have been designed: synthetic peptides binding to Caspase-1's substrate binding site, antisense oligonucleotides or non-peptidic molecules. Both peptidomimetics Pralnacasan and Belnacasan entered clinical trials but were withdrawn due to cellular toxicity. Targeting strategies turned to the inhibition of the final product IL-1β instead of Caspase-1. For instance, the IL-1 receptor antagonist Anakinra is used to treat inflammatory diseases. Recently, a potent NLRP3 inhibitor MCC950 was identified and entered clinical trials (113). Later studies suggested that inflammasomes are not only implicated in auto-inflammatory diseases but may also have a role in neurological disorders and different types of cancer. For instance, knockouts of inflammasome forming NLRP1 and AIM2 proteins were shown to enhance tumor growth (87-89). It is not clear yet if the phenotypes are dependent of their inflammasome forming properties (thus involving Caspase-1) or whether are they are the result of other unknown cellular functions of these proteins.

In summary, Caspase-1 is located at the crossroad of cell death and inflammation and may be the factor deciding whether the cell death should be immunologically silent (apoptosis) or active (pyroptosis). If the cell is able to clear the bacterial infection via Caspase-1-dependent inflammasome activation, then the immunological response will be silent. However, if the cell could not stop and clear the infection or the danger, the cell may activate Caspase-1 dependent pyroptosis and recruit other immune cells to the immune site. The presence of Caspase-1 into phagosomes may have a role in antigen processing and presentation to immune cells. Caspase-1 is not a simple protease but has many substrates with important cellular functions such as inflammation or cell death.

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