


## REVIEW

### Cytokines Focus

# Inflammasome signaling and regulation of interleukin-1 family cytokines

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**Specific IL-1 family cytokines are expressed by cells as cytosolic pro-forms that require cleavage for their activity and cellular release. IL-1 $\beta$ , IL-18, and IL-37 maturation and secretion is governed by inflammatory caspases within signaling platforms called inflammasomes. By inducing pyroptosis, inflammasomes can also drive the release of the alarmin IL-1 $\alpha$ . Recent advances have transformed our mechanistic understanding of inflammasome signaling, cell death decisions, and cytokine activation and secretion. Here, we provide an updated view of inflammasome signaling; mechanisms underpinning IL-1 $\alpha$ , IL-1 $\beta$ , IL-18, and IL-37 maturation and release; and the functions of these cytokines in protective and pathological inflammation.**

## Introduction

IL-1 $\alpha$  and IL-1 $\beta$  are potent proinflammatory cytokines in immunity and immune pathology. IL-1 $\alpha/\beta$  (collectively termed IL-1) is the founding member of the IL-1 family of cytokines, which also includes IL-1 receptor antagonist (IL-1Ra), IL-18, IL-33, IL-36Ra, IL-36 $\alpha$ , IL-37, IL-36 $\beta$ , IL-36 $\gamma$ , and IL-38 (Dinarello et al., 2010). The IL-1 family of cytokines possesses a multitude of biological activities in immunity and inflammation. Many members of this family are proinflammatory (e.g., IL-1 $\alpha$  and IL-1 $\beta$ ), while some are anti-inflammatory (e.g., IL-37, IL-38, IL-1Ra), and others can be pro- or anti-inflammatory, depending on the context (e.g., IL-18).

Specific members of the IL-1 family are expressed by cells as cytosolic pro-forms that require cleavage for secretion of their active forms. Maturation and secretion of IL-1 $\beta$ , IL-18, and IL-37 are mediated by inflammatory caspases within inflammasome signaling complexes (Monteleone et al., 2015). Inflammasome signaling often induces pyroptosis, a form of programmed cell lysis that allows the passive release of alarmins, including IL-1 $\alpha$ . Research over the last few years has transformed our understanding of inflammasome biology, and in particular, the molecular mechanisms underlying the activation and secretion of these IL-1 cytokines. This article will briefly overview our current understanding of the functions of IL-1 $\alpha$ , IL-1 $\beta$ , IL-18, and IL-37 in host defense and diseases (Table 1); for further mechanistic details therein, we refer the reader to recent reviews (Chen and Schroder, 2013; Mantovani et al., 2019). We then provide an updated understanding of inflammasome signaling and inflammasome-driven maturation and secretion of IL-1 family cytokines in host defense and disease.

## IL-1 family members promote or suppress inflammation

### IL-1 $\alpha/\beta$ : The archetypal proinflammatory cytokine

IL-1 $\alpha$  and IL-1 $\beta$  are produced during infection and in a wide range of inflammatory diseases (Dinarello, 2009). While generally host-protective during infection, IL-1 contributes to several inflammatory pathologies, such as rheumatoid arthritis, osteoarthritis, gout, and type 2 diabetes. Elevated IL-1 signaling also drives the pathogenesis of several hereditary auto-inflammatory diseases, including cryopyrin-associated periodic syndromes, familial Mediterranean fever, and TNF receptor-associated periodic syndrome (Gabay et al., 2010; Dinarello et al., 2012; Lopalco et al., 2015). IL-1 $\alpha$  and IL-1 $\beta$  signal via a common cell surface receptor (Table 1). These cytokines thus induce many overlapping biological effects systemically and at sites of local inflammation. IL-1 activates cells of the innate and adaptive immune system and exerts a wide range of biological activities that are protective during infection, such as promoting fever, vasodilation, hematopoiesis, angiogenesis, the acute phase response, leukocyte attraction and extravasation, lymphocyte activation, and antibody synthesis (Garlanda et al., 2013). IL-1 $\alpha$  and IL-1 $\beta$  are, however, expressed by different cell types, and their activity is controlled by distinct mechanisms, engendering some differences in their functions. IL-1 $\alpha$  is expressed in a wide range of cell types, including keratinocytes, epithelial cells, endothelial cells, fibroblasts, and hepatocytes, whereas IL-1 $\beta$  is primarily produced by myeloid cells (Hacham et al., 2002; Dinarello, 2011). Both cytokines can be released extracellularly, but IL-1 $\alpha$  can also translocate to the nucleus to restrain its proinflammatory

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Table 1. **Specific members of the IL-1 family and their receptors and function**

IL-1 family member	Receptor complex	Function
IL-1 $\alpha$	IL-1R1/IL-1R3	Proinflammatory
IL-1 $\beta$	IL-1R1/IL-1R3	Proinflammatory
IL-18	IL-1R5/IL-1R7	Proinflammatory Anti-inflammatory
IL-37	IL-1R5/IL-1R8	Anti-inflammatory

activity (Cohen et al., 2010) or allow it to function as a proinflammatory transcription factor (Werman et al., 2004). IL-1 $\alpha$  is an alarmin released by dying cells to initiate the early phase of sterile inflammation, while IL-1 $\beta$  is produced by inflammasomes at sites of tissue infection or sterile injury. Both IL-1 $\alpha$  and IL-1 $\beta$  induce local inflammation and the recruitment and activation of neutrophils, monocytes, and macrophages, with some distinctions in timing and activity (Rider et al., 2011). These cytokines thus serve overlapping functions to coordinate local inflammation.

#### IL-18: A moonlighting inflammatory modulator?

IL-18 is constitutively expressed in myeloid cells and epithelial cells, such as keratinocytes. IL-18 signals via a heteromeric receptor (Table 1), and this interaction is antagonized by the IL-18-binding protein. IL-18 is generally considered to be a proinflammatory cytokine, as it up-regulates cell adhesion molecules for leukocyte trafficking, induces nitric oxide synthesis and chemokine production, and instructs adaptive immunity (Dinarello, 2018). A key function of IL-18 is to cooperate with IL-12 in inducing IFN- $\gamma$  production from T helper cells and natural killer cells, leading to natural killer cell activation, T helper type 1 cell skewing, up-regulated antigen presentation, and antiviral and antitumor functions (Schroder et al., 2004). Several diseases are driven by elevated production of IL-18, including multiple sclerosis, inflammatory bowel disease, Crohn's disease, rheumatoid arthritis, systemic lupus erythematosus, and graft-versus-host disease (Dinarello, 2007; Dinarello et al., 2013). Despite its proinflammatory functions, a growing number of studies indicate important physiological functions for IL-18 in homeostasis. IL-18 deficiency in mice leads to hyperphagia, obesity, and insulin resistance (Netea et al., 2006; Zorrilla et al., 2007). IL-18 insufficiency is also associated with colitis and macular degeneration in mice (Chen et al., 2007; Elinav et al., 2011; Hirota et al., 2011; Doyle et al., 2012). Interestingly, IL-18 exerts a protective function in suppressing aberrant neuronal transmission in Alzheimer's disease (Tzeng et al., 2018). Thus IL-18 has both harmful and protective functions in inflammatory pathologies.

#### IL-37: An immune suppressor

IL-37 is an anti-inflammatory cytokine expressed by human blood monocytes, tissue macrophages, lymphocytes, and synovial, neoplastic, and epithelial cells (Nold et al., 2010). Human IL-37 lacks a mouse orthologue, so many studies characterizing IL-37 function employ overexpression of the human cytokine in murine cells, where it exerts several anti-inflammatory

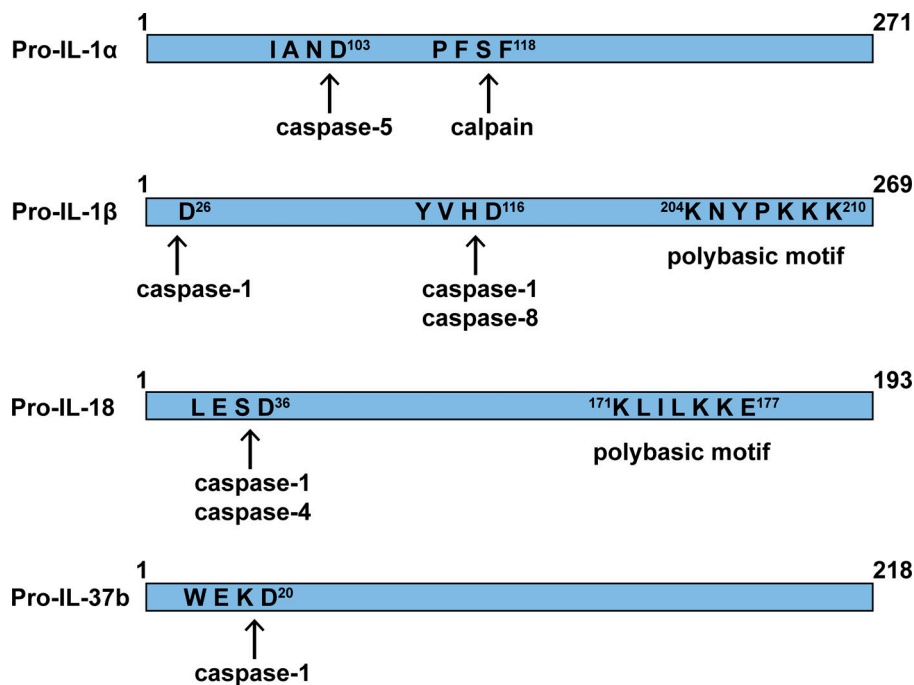
functions. IL-37 translocates to the nucleus to down-regulate expression of inflammatory mediators (Sharma et al., 2008; Nold et al., 2010). The anti-inflammatory program of IL-37 is, however, primarily mediated via signaling through its receptor (Table 1; Molgora et al., 2016). Some inflammatory and diabetic diseases are associated with lower levels of IL-37, suggestive of a dysregulated anti-inflammatory program (Ballak et al., 2014; Zeng et al., 2017). Somewhat counterintuitively, expression of this anti-inflammatory cytokine is elevated in several human disease conditions, which may represent a mechanism to attempt to dampen pathological inflammatory responses. For example, IL-37 is elevated in patients with inflammatory bowel disease (Imaeda et al., 2013; Weidlich et al., 2014) and rheumatoid arthritis (Zhao et al., 2014; Xia et al., 2015a,b; Yang et al., 2015).

#### Inflammasomes govern the maturation of IL-1 $\beta$ , IL-18, and IL-37

IL-1 $\beta$  and IL-18 are expressed as inactive precursors (pro-IL-1 $\beta$  and pro-IL-18) and require processing at specific sites (Fig. 1) for their activity and secretion (Afonina et al., 2015). IL-37 cleavage also enhances its biological activity (Fig. 1). The maturation and secretion of these cytokines are controlled by cysteine proteases called caspases within inflammasomes. Inflammasomes are activation platforms for the inflammatory caspases, caspase-1 (canonical inflammasomes), or caspase-4/5/11 (noncanonical inflammasomes), but these signaling platforms also crosstalk to apoptotic caspases (e.g., caspase-8) for the regulation of IL-1 cytokines.

#### Canonical inflammasomes cleave IL-1 $\beta$ and IL-18 to generate the mature cytokines

Canonical inflammasomes are composed of an inflammasome sensor protein bound to pro-caspase-1, usually via an adapter, ASC (apoptosis-associated speck-like protein containing the caspase activation and recruitment domain [CARD]). Canonical inflammasome sensor proteins are NLRP1, NLRP3, NLRP6, NAIP/NLRC4, AIM2, and PYRIN, which each respond to specific signals. These sensors recognize pathogen- and danger-associated molecular patterns indicative of infection, cellular damage, or cell stress (Schroder and Tschoopp, 2010). Recognition of these signals triggers sensor protein oligomerization, which in turn drives ASC recruitment and polymerization into a  $\sim 1\text{-}\mu\text{M}$  structure (the "ASC speck"). The CARD of ASC, or the CARD of the inflammasome sensor protein (NLRP1 and NLRC4), recruits caspase-1 monomers via CARD-CARD interactions. Clustering upon the inflammasome promotes caspase-1 dimerization, thereby unleashing protease function (Boucher et al., 2018; Fig. 2 A). Caspase-1 contains two linker regions that are sensitive to autoproteolysis. A CARD domain linker connects the N-terminal CARD domain to the C-terminal protease domain, while the protease domain contains large (p20) and small (p10) enzymatic subunits separated by an interdomain linker. Upon inflammasome-mediated dimerization, caspase-1 autocleaves at the interdomain linker to generate a fully active p33/p10 species, which remains bound to the inflammasome (Fig. 2 A). Cleavage at this interdomain linker is required for caspase-1 to process pro-IL-1 $\beta$  in macrophages (Broz et al., 2010), which occurs at two sites (D26 and D116 of pro-IL-1 $\beta$ ) to produce either a 26-kD



**Figure 1. Human IL-1 family cytokines regulated by inflammatory caspases.** For each cytokine, amino acid positions and specific caspase cleavage sites are indicated. IL-1 $\beta$  and IL-18 each contain a polybasic motif within the mature cytokines.

product or the mature 17-kD form (Afonina et al., 2015; Fig. 1). Caspase-1 cleaves pro-IL-18 at residue D36 to convert the inactive 23-kD precursor into the 18-kD active cytokine (van de Veerdonk et al., 2011; Fig. 1). Caspase-1 also cleaves another substrate, gasdermin D (GSDMD), to generate a pore-forming fragment that targets the plasma membrane (Aglietti et al., 2016; Ding et al., 2016; Liu et al., 2016; Sborgi et al., 2016). If the GSDMD pore burden in the plasma membrane overwhelms the natural membrane-repair mechanisms of the cell (Rühl et al., 2018), a lytic form of cell death called pyroptosis ensues (He et al., 2015; Kayagaki et al., 2015; Shi et al., 2015; Chen et al., 2018).

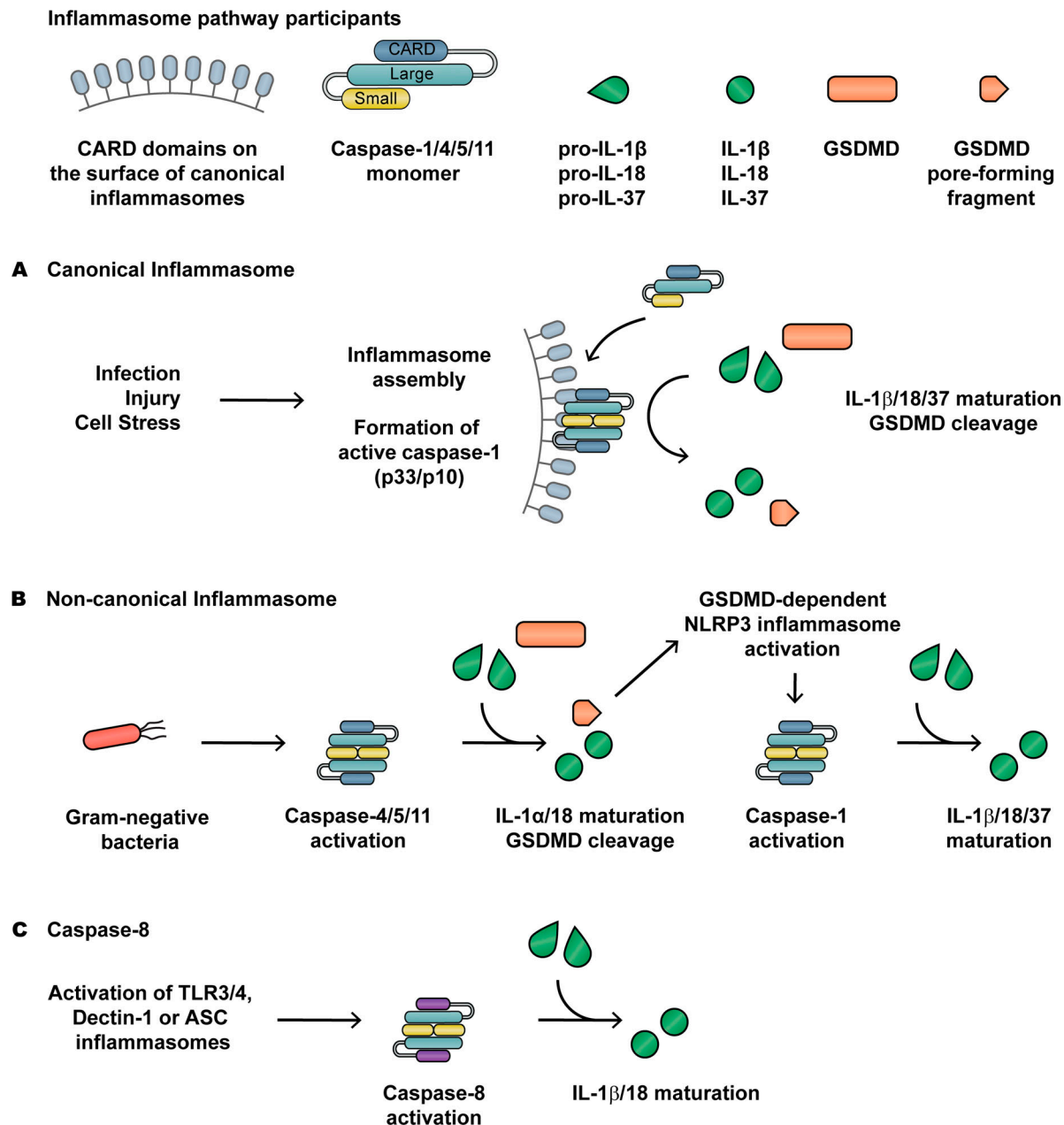
Following full caspase-1 activation by autoprocessing at the interdomain linker, caspase-1 self-cleaves again, this time at the CARD domain linker, to generate p20/p10. This releases caspase-1 from the inflammasome and terminates protease activity and cytokine processing. Since active p33/p10 is a transient species, caspase-1-dependent cytokine processing occurs within a set time frame (Boucher et al., 2018). Interestingly, the duration of caspase-1 activity is dictated by the size of the signaling platform (and the resulting number of available binding sites for caspase-1), which differs between distinct inflammasomes and cell types. Macrophages form large ASC inflammasomes that rapidly activate caspase-1 but also turn over caspase-1 protease activity quickly, so mature IL-1 $\beta$  is generated within a short time frame. Neutrophils express substantially less ASC per cell and so form smaller signaling hubs with prolonged caspase-1 activity and sustained kinetics of IL-1 $\beta$  maturation (Chen et al., 2014; Boucher et al., 2018). Likewise, small inflammasomes, such as the macrophage NLRC4-caspase-1 inflammasome that signals independently of ASC, facilitate prolonged caspase-1 activity (Boucher et al., 2018). Thus, the kinetics of IL-1 family cytokine production is dictated by the identity of both the inflammasome and the cell.

#### Canonical inflammasomes cleave IL-37 to up-regulate cytokine activity

While pro-IL-37 is active in vitro and in vivo, IL-37 maturation promotes its biological activity (Moretti et al., 2014; Li et al., 2015). IL-37 maturation appears to be primarily mediated by caspase-1 (Kumar et al., 2002; Sharma et al., 2008; Nold et al., 2010; Bulau et al., 2014). The IL-37 transcript undergoes alternative splicing to generate five isoforms (IL-37a–e), of which IL-37b–e contain a caspase-1 cleavage site (D20; Fig. 1; Kumar et al., 2002; Bulau et al., 2014). To date, only IL-37b is studied in detail. The 25-kD pro-IL-37b is cleaved to generate a 19-kD mature form in cells stimulated with bacterial LPS plus ATP to activate the NLRP3 inflammasome in a caspase-dependent manner (Bulau et al., 2014). Mature IL-37 can be secreted or can translocate to the nucleus to suppress proinflammatory cytokine expression (Sharma et al., 2008; Bulau et al., 2014). Pro-IL-37 can also be released into the extracellular space (Bulau et al., 2014), where it could be a target for processing by yet-undefined extracellular enzymes to yield functional forms with variable biological activity and anti-inflammatory properties.

#### Regulation of IL-1 cytokines by the noncanonical inflammasome

The noncanonical inflammasome is a lipid-caspase complex that enables the activation of caspase-4 and caspase-5 in humans and caspase-11 in mice. This pathway provides host defense against cytosolic Gram-negative bacteria, as it allows cytosolic detection of bacterial LPS to be coupled to cell death and inflammatory responses (Kayagaki et al., 2011, 2013; Shi et al., 2014). The noncanonical inflammasome is reported to function without a dedicated sensor protein (Shi et al., 2014). Caspase-4, caspase-5, and caspase-11 directly interact with LPS (Shi et al., 2014), but physiological bacterial detection by these caspases occurs with the assistance of guanylate-binding proteins (Liu et al., 2018), whose functions in LPS sensing await full clarification. LPS



**Figure 2. IL-1 family cytokine processing by canonical, noncanonical, and caspase-8 inflammasomes.** (A) Infection, cell damage, and cell stress signals trigger the formation of canonical inflammasomes, which recruit monomeric caspase-1 through CARD–CARD interactions. Caspase-1 dimerizes and self-cleaves to generate the fully active p33/p10 species that cleaves and activates IL-1 $\beta$ , IL-18, and IL-37. Caspase-1 also processes GSDMD, leading to formation of GSDMD pores. (B) The noncanonical inflammasome senses cytosolic bacterial LPS, activates caspase-4 and caspase-5 in humans and caspase-11 in mice, and induces the formation of GSDMD pores. Caspase-5 and caspase-11 directly cleave IL-1 $\alpha$ , while caspase-4 and caspase-11 directly cleave IL-18. Signaling through the GSDMD/NLRP3 axis induces caspase-1 activation, leading to further cytokine processing. (C) Under certain conditions, active caspase-8 can directly process IL-1 $\beta$  and IL-18, either upon the inflammasome or independently of the inflammasome pathway.

interaction with two or more caspase-11 monomers triggers the dimerization of the caspase protease domains to induce basal protease activity (Ross et al., 2018). This leads to auto-processing at the caspase-11 interdomain linker (residue D285), generating a p32/p10 species with full protease activity (Lee et al., 2018; Ross et al., 2018). Caspase-4/5/11 then cleave GSDMD to trigger GSDMD pores and initiate pyroptotic cell lysis (He et al., 2015; Kayagaki et al., 2015; Shi et al., 2015; Aglietti et al., 2016; Ding et al., 2016; Liu et al., 2016), or

pyroptosis-associated expulsion of neutrophil extracellular traps (Chen et al., 2018). These caspases are not reported to directly cleave IL-1 $\beta$  or IL-37 but can indirectly mediate cytokine maturation (Fig. 2 B). GSDMD membrane pores induce ionic flux and resultant NLRP3 inflammasome assembly and caspase-1 activation (Rühl and Broz, 2015). Thus, signaling by the noncanonical inflammasome indirectly leads to caspase-1-dependent IL-1 $\beta$  and IL-18 secretion (Kayagaki et al., 2011, 2015; Rathinam et al., 2012).



The caspase-4 inflammasome appears able to directly cleave IL-18 in response to enteric pathogens, such as *Salmonella enterica* and *Shigella flexneri* (Kobayashi et al., 2013; Knodler et al., 2014). Similarly, caspase-11, but not caspase-1, was required for *Salmonella*-induced IL-18 maturation from cecal explants (Knodler et al., 2014). Hence, caspase-4 and caspase-11 non-canonical inflammasomes may be able to directly control IL-18 maturation and release, thereby mediating host defense in the intestinal mucosa.

IL-1 $\alpha$  is expressed as a 31-kD precursor with some biological activity, but this is dramatically increased by cleavage to the 19-kD mature form (Afonina et al., 2015; Wiggins et al., 2019). IL-1 $\alpha$  is usually processed by calpain during necrosis (Burzynski et al., 2015; Wiggins et al., 2019), but can also be cleaved by caspase-5 and caspase-11 to promote IL-1 $\alpha$ -specific cytokine activity (Wiggins et al., 2019). In a fully recombinant system, pro-IL-1 $\alpha$  was cleaved to its mature form by caspase-5 and -11, but not caspase-1 or caspase-4 (Wiggins et al., 2019). Caspase-5 and caspase-11 cleaved IL-1 $\alpha$  at D103, a highly conserved site in multiple mammalian species, while calpain cleaves at a distinct site (Wiggins et al., 2019). This study also demonstrated that IL-1 $\alpha$ -dependent senescence-associated secretory phenotype required caspase-5 and caspase-11 in vitro and in vivo (Wiggins et al., 2019). Thus, IL-1 $\alpha$  is a direct substrate of specific inflammatory caspases during noncanonical inflammasome activation and senescence.

#### IL-1 $\beta$ and IL-18 maturation by caspase-8

Caspase-8 is traditionally classified as an apoptotic initiator caspase of the extrinsic cell death pathway but is more recently implicated as a participant in inflammasome pathways and an alternative protease for IL-1 $\beta$  and IL-18 maturation (Fig. 2 C). Various forms of cell stress (e.g., inhibition of protein translation, chemotherapeutics, or endoplasmic reticulum stress) enable macrophages and dendritic cells to produce mature IL-1 $\beta$  and IL-18 in response to TLR3 or TLR4 stimulation through a pathway that requires caspase-8, but not caspase-1. Here, TLR3/4 engages TRIF (TIR-domain-containing adapter-inducing IFN- $\beta$ ), and TRIF recruits the stress-induced RIP1/FADD/caspase-8 (receptor-interacting protein; FAS-associated death domain) complex, leading to caspase-8 activation (Blander, 2014) and IL-1 $\beta$ /18 maturation (Maelfait et al., 2008; Bossaller et al., 2012; Antonopoulos et al., 2013; Shenderov et al., 2014; Moriwaki et al., 2015). Fungal pathogens detected by dectin-1 can also trigger caspase-8-dependent IL-1 $\beta$  maturation (Ketelut-Carneiro et al., 2018). Here, dectin-1 signaling induces signaling by the CARD9-BCL10-MALT1 complex to activate caspase-8 (Blander, 2014). Caspase-8 directly cleaves IL-1 $\beta$  and IL-18 to their mature forms (Maelfait et al., 2008; Bossaller et al., 2012; Gringhuis et al., 2012; Antonopoulos et al., 2013; Shenderov et al., 2014) and targets the same IL-1 $\beta$  cleavage site (D116) as caspase-1 (Maelfait et al., 2008). Caspase-8 is primarily responsible for IL-1 $\beta$  maturation during infection with fungal pathogens such as *Candida albicans* and *Aspergillus fumigatus* and bacterial pathogens such as *Mycobacterium bovis* and *Mycobacterium leprae* (Gringhuis et al., 2012).

Caspase-8 can also modulate IL-1 $\beta$  processing via the inflammasome pathway. Caspase-8 positively regulates NLRP3

inflammasome activation (Allam et al., 2014; Gurung et al., 2014) and pro-IL-1 $\beta$  synthesis (Gurung et al., 2014). Caspase-8 is also recruited to canonical ASC inflammasomes (Man et al., 2013; Vajjhala et al., 2015), suggesting that caspase-8 may contribute to IL-1 $\beta$  maturation upon the inflammasome, particularly in the absence of caspase-1 (Antonopoulos et al., 2015).

#### Secretory pathways for inflammasome-dependent IL-1 family cytokines

##### IL-1 $\beta$ secretion requires maturation and trafficking to the plasma membrane

IL-1 $\beta$ , IL-18, and IL-37 are synthesized in the cytosol and released via an unconventional secretory pathway that bypasses the conventional ER/Golgi trafficking route. IL-1 $\beta$ /18 were originally believed to be passively released upon inflammasome-driven cell rupture (Brough and Rothwell, 2007; Liu et al., 2014; Shirasaki et al., 2014; Cullen et al., 2015). However, accumulating evidence suggests that IL-1 secretion precedes cell rupture in pyroptotic cells (Perregaux and Gabel, 1994; Verhoef et al., 2004; Fink and Cookson, 2006; Brough and Rothwell, 2007; Monteleone et al., 2015; Evavold et al., 2018) and indeed can also occur in nonpyroptotic cells (Kang et al., 2013; Chen et al., 2014; Conos et al., 2016; Gaidt et al., 2016; Wolf et al., 2016; Zanoni et al., 2016; Diamond et al., 2017; Monteleone et al., 2018).

Caspase-1 activation has long been associated with the secretion of mature IL-1 $\beta$ , but until recently, the specific activities of caspase-1 that supported IL-1 $\beta$  secretion were unclear. Herein, one critical function of caspase-1 is cytokine maturation itself, as only mature IL-1 $\beta$  is actively secreted by macrophages while the pro-form is passively released during cell lysis (Monteleone et al., 2018). In resting cells, pro-IL-1 $\beta$  has an overall negative charge, which would be repelled from the negatively charged plasma membrane (Monteleone et al., 2018). The isoelectric point of IL-1 $\beta$  shifts upon maturation, with mature IL-1 $\beta$  exhibiting an overall positive charge, largely because it contains a polybasic motif that is highly conserved between human and mouse IL-1 $\beta$  and -18 (Monteleone et al., 2018). Mature IL-1 $\beta$ , but not pro-IL-1 $\beta$ , colocalized with negatively charged phosphatidylinositol 4,5-bisphosphate (PIP2) in the plasma membrane, and both mature IL-1 $\beta$  and PIP2 were particularly enriched in surface projections and ruffles (Monteleone et al., 2018). Indeed, mutation of the polybasic motif prevented the relocation of mature IL-1 $\beta$  to the PIP2-enriched membrane domains and also its secretion (Monteleone et al., 2018). IL-1 $\beta$  maturation thus directs this cytokine to traffic to the plasma membrane to facilitate its secretion from the cell. A second function of caspase-1, the generation of GSDMD pores, is also important for IL-1 $\beta$  secretion. GSDMD pores insert in the plasma membrane through interaction with phospholipids such as PIP2 (Ding et al., 2016; Liu et al., 2016) and were recently shown to serve as a portal for IL-1 $\beta$  exit from the cell (Evavold et al., 2018). Thus, IL-1 $\beta$  maturation and cotrafficking to PIP2-enriched membrane microdomains alongside GSDMD likely coordinates IL-1 $\beta$  exit through GSDMD pores. As discussed below, this appears to be a major mechanism for IL-1 $\beta$  secretion from both living cells and cells undergoing pyroptosis.

### IL-1 $\beta$ secretion from hyperactivated cells

Pro-IL-1 $\beta$  is not expressed in resting myeloid cells, and its induction requires cell “activation,” typically by a TLR ligand or specific proinflammatory cytokines (e.g., IL-1). In addition to pro-IL-1 $\beta$  expression, myeloid cell activation induces antimicrobial responses and the production of proinflammatory mediators to drive inflammatory responses. Mature IL-1 $\beta$  is not present within this suite of secreted mediators unless the cell additionally receives an inflammasome-activating stimulus that induces pro-IL-1 $\beta$  processing to its mature form. As discussed above, inflammasome signaling and cell secretion of mature IL-1 $\beta$  are often accompanied by pyroptosis. In specific circumstances, however, cells release IL-1 $\beta$  while maintaining viability. As these viable cells have received both a classical phagocyte activation signal and an inflammasome activation signal (a cell state termed “hyperactivation”), they secrete IL-1 $\beta$  in addition to other inflammatory mediators (Evavold et al., 2018).

Hyperactivation can be observed in several cell types and with specific triggers. IL-1 $\beta$  secretion proceeded without concurrent pyroptotic cell death in neutrophils exposed to *Salmonella* or intracellular flagellin (Chen et al., 2014; Monteleone et al., 2018), monocytes stimulated with extracellular LPS (Gaidt et al., 2016), and dendritic cells exposed to oxidized phospholipids (Zanoni et al., 2016). Live macrophages also released inflammasome-mediated IL-1 $\beta$  and IL-18 upon exposure to bacterial N-acetyl glucosamine (Wolf et al., 2016). Cell hyperactivation appears to occur when inflammasome signaling elicits only a small number of GSDMD pores, where the pore burden is subthreshold for inducing pyroptosis but sufficient for facilitating IL-1 secretion (Chen et al., 2014; Evavold et al., 2018; Monteleone et al., 2018). In hyperactive cells, the total amount of caspase-1 activity at one time may be low but is prolonged (Boucher et al., 2018) and likely to cause sustained GSDMD pore formation and IL-1 $\beta$  transit through these pores before membranes are repaired (Fig. 3; Rühl et al., 2018). Thus, hyperactivation may be dictated by the nature of inflammasomes and the resultant kinetics of caspase-1 turnover (Boucher et al., 2018).

Mature IL-1 $\beta$  is also secreted by a slow, GSDMD-independent pathway, regardless of immediate cell fate (continued viability or pyroptosis; Fig. 3). While early secretion of IL-1 $\beta$  from NLRC4-activated neutrophils required GSDMD, GSDMD was dispensable for later release, and in vivo secretion of IL-1 $\beta$  required caspase-1, but not GSDMD, 6 h after *Salmonella* infection (Monteleone et al., 2018). The sole requirement for caspase-1 herein appears to be cytokine processing, as IL-1 $\beta$  maturation was necessary and sufficient for IL-1 $\beta$  secretion in inflammasome-unstimulated, resting macrophages (Monteleone et al., 2018). A recent report identified another, perhaps related, GSDMD-independent secretory pathway involving the endosome docking and fusion protein, early endosomal autoantigen 1 (EEA1). Caspase-1 cleaved EEA1 at D127/132, and this induced EEA1 and IL-1 $\beta$  release in a manner that required EEA1, but not GSDMD (Baroja-Mazo et al., 2019). While the mechanisms of GSDMD-independent IL-1 $\beta$  release remain to be fully defined, they are likely to contribute to IL-1 $\beta$  secretion from cells that do not express GSDMD or in situations of cell hyperactivation.

### IL-1 $\beta$ is secreted through GSDMD pores in pyroptotic cells before cell rupture

In pyroptotic cells, IL-1 $\beta$  release and cell lysis are often closely temporally associated (Brough and Rothwell, 2007; Liu et al., 2014; Shirasaki et al., 2014). However, the osmoprotectant glycine slows membrane rupture and resultant passive protein release in macrophages, without affecting the formation or function of GSDMD pores or the kinetics of mature IL-1 $\beta$  secretion (Verhoef et al., 2004, 2005; Fink and Cookson, 2006; Pelegrin et al., 2008; Evavold et al., 2018; Monteleone et al., 2018). Further, the membrane-stabilizing agent punicalagin suppressed inflammasome-induced cell death and release of IL-1 $\beta$  and IL-18 from mouse macrophages, suggesting that it inhibits GSDMD pore insertion into the plasma membrane, and IL-1 $\beta$ /18 passage through these pores (Martín-Sánchez et al., 2016; Tapia et al., 2019). These data collectively suggest that IL-1 $\beta$  is secreted via GSDMD pores before cell rupture during the process of pyroptosis (Fig. 3).

### IL-1 $\beta$ secretion is negatively regulated by membrane repair

A recent study found that calcium influx through GSDMD pores serves as a signal for cells to initiate membrane repair by recruiting the endosomal sorting complexes required for transport (ESCRT) machinery to damaged membrane areas (Rühl et al., 2018). Here, the ESCRT machinery repairs damaged membrane by removing GSDMD pores in the form of ectosomes, which tempers cell death and mature IL-1 $\beta$  release in inflammasome-stimulated macrophages (Rühl et al., 2018), and may facilitate cell hyperactivation in some settings. The delayed nature of this response appears to allow some cytokine release through GSDMD pores before membrane repair (Fig. 3) while restraining cell rupture (Evavold et al., 2018; Heilig et al., 2018).

### Cell fate decisions affect IL-1 $\beta$ secretion pathways and kinetics

Combined, these studies suggest that the cell fate decisions of inflammasome-activated cells dictate IL-1 $\beta$ , and likely IL-18, release kinetics and the extent of the ensuing inflammatory response. As a danger detection and immune alert system, inflammasomes recognize potential threats to organisms and respond by inducing immune system activation. Hyperactivation appears to occur when inflammasomes detect a moderate level of danger, where the appropriate response for the cell is to incorporate IL-1 cytokines into the secreted repertoire while remaining viable to provide immunomodulatory and immune defense functions. Indeed, in mouse dendritic cells, hyperactivation led to stronger antigen-specific T cell responses compared with traditional activation states (Zanoni et al., 2016). Alternatively, when inflammasomes detect a serious threat to the organism, it may be appropriate to induce the strongest possible inflammatory response. In this case, the cell undergoes pyroptosis in addition to IL-1 $\beta$ /18 release in order to engage the strong immunomodulatory properties of alarmins. In the case of neutrophils, this also serves to harness the antimicrobial properties of pyroptosis-associated neutrophil extracellular traps. While our understanding of the signaling mechanisms controlling these cell fate decisions remains rudimentary, they are

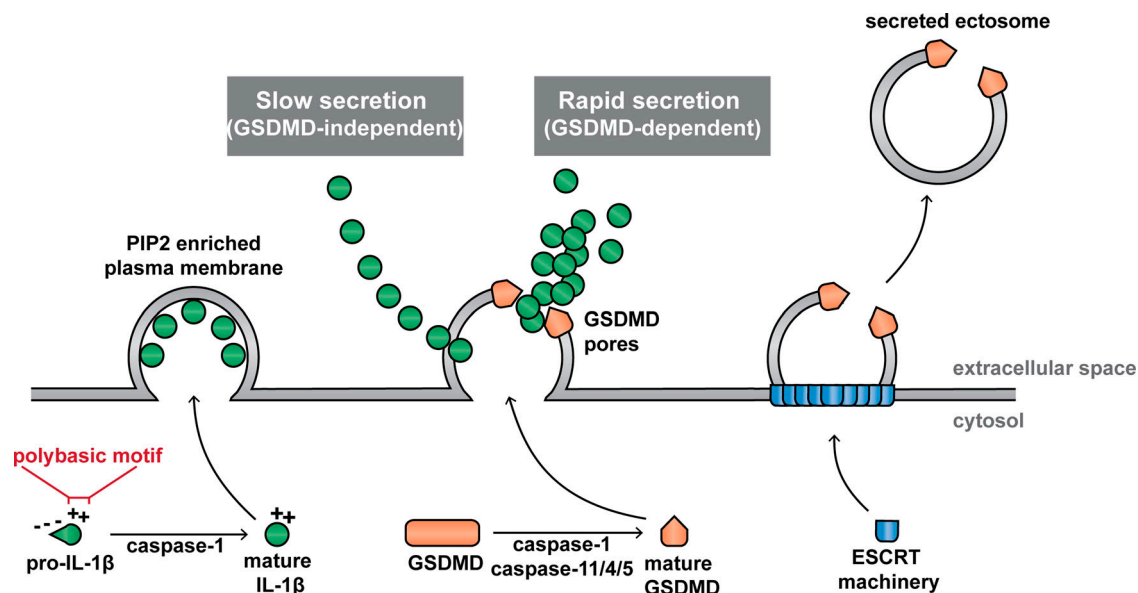


Figure 3. **Model for IL-1 $\beta$  active secretion from the plasma membrane.** IL-1 $\beta$  cleavage by caspase-1 alters the isoelectric point of IL-1 $\beta$ , leading to IL-1 $\beta$  trafficking to PIP2-enriched plasma membrane domains in a manner requiring the polybasic motif of the mature cytokine. IL-1 $\beta$  is released to the extracellular space by at least two mechanisms: (1) through a yet-uncharacterized, GSDMD-independent mechanism; and (2) through GSDMD pores that were generated by canonical and noncanonical inflammasome signaling. The ESCRT machinery is recruited to sites of GSDMD pore formation to repair the membrane. If the GSDMD pore burden in the plasma membrane is high enough to overwhelm membrane repair mechanisms, the cell will die by pyroptosis.

likely underpinned by the extent and duration of caspase activity within inflammasomes (Boucher et al., 2018).

#### IL-1 $\alpha$ is secreted via an alternate pathway to IL-1 $\beta$

IL-1 $\alpha$  is generally considered to be an immune alarmin that is passively released during cell lysis (Monteleone et al., 2015). IL-1 $\alpha$  is released by pyroptotic cells and cells undergoing specific inflammasome-independent forms of cell death, such as lysosomal cell death induced by crystals and particles (Gross et al., 2012). In keeping with a distinct route of exit to IL-1 $\beta$ , punicalagin did not inhibit IL-1 $\alpha$  release, suggesting that IL-1 $\alpha$  does not pass through GSDMD pores (Martín-Sánchez et al., 2016). Pro-IL-1 $\alpha$  is bound to an intracellular receptor, IL-1R2, to prevent its release during forms of necrosis that do not activate the inflammasome pathway (Zheng et al., 2013; Burzynski et al., 2015). Inflammasome activity during cell death liberates IL-1 $\alpha$  from dying cells, as caspase-1 and caspase-5 cleave IL-1R2 to enable subsequent IL-1 $\alpha$  processing, cellular release, and signaling (Zheng et al., 2013). Intriguingly, IL-1 $\alpha$  appears able to be secreted from living cells in some circumstances (Tapia et al., 2019), such as the senescence-associated secretory phenotype (Gardner et al., 2015; Wiggins et al., 2019). The mechanism underpinning IL-1 $\alpha$  secretion from living cells awaits clarification.

#### Concluding remarks

With the recent remarkable progress in the inflammasome field, we are beginning to understand the distinct functions of inflammasomes in IL-1 family cytokine processing versus secretion. Both processes are important, ultimately, for the biological activities of IL-1 $\alpha$ , IL-1 $\beta$ , IL-18, and IL-37. Distinctions in the cytokine repertoire for different inflammasomes are emerging,

particularly with respect to cytokine processing by the non-canonical inflammasome. Emerging physiological and anti-inflammatory functions for IL-18 and IL-37 also highlight the multifaceted nature of inflammasome action in homeostasis, immune activation and inhibition. IL-1 $\alpha$ , IL-1 $\beta$ , and IL-18 drive pathology in a range of human diseases. Biological antagonists of IL-1 are effective treatments for many of these conditions, such as gouty arthritis (So et al., 2010) and autoinflammatory disease (Dinarello, 2018), presenting these cytokines as validated drug targets. New agents that block the generation or release of mature IL-1 family cytokines (e.g., inflammasome or GSDMD inhibitors) are currently under development as potential first-in-class anti-inflammatory therapies.

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K. Schroder is a co-inventor on patent applications for NLRP3 inhibitors, which have been licensed to Inflazome, Ltd., a company headquartered in Dublin, Ireland. Inflazome is developing drugs that target the NLRP3 inflammasome to address unmet clinical needs in inflammatory disease. K. Schroder served on the Scientific Advisory Board of Inflazome in 2016-2017. The remaining authors have no competing financial interests.

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