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REVIEW

Strategies that modulate inflammasomes—insights from host–pathogen interactions

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Abstract The innate immune system is a dynamic and complex network for recognizing and responding to cellular insult or tissue damage after infection or injury. The primary effector mechanism of innate immunity is the generation of acute and chronic inflammatory responses through regulation of the processing and activation of proinflammatory caspases, particularly caspase 1, and cytokines, most notably IL-1β and IL-18. Inflammasomes, cytosolic multi-protein complexes that function as molecular scaffolds for caspase activation, have recently emerged as the pivotal mechanism by which host innate immune and inflammatory responses are regulated. In this review, we investigate the mechanisms by which inflammasomes are modulated, both by endogenous host systems and by microbial pathogens.

Keywords Host–pathogen interaction · Innate immunity · Pyrin domain · Immune modulation · Inflammasome inhibitors

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Abbreviations

ASC	apoptosis-associated speck-like protein containing CARD
ATP	adenosine triphosphate
CAPS	cryopyrin-associated periodic syndromes
CARD	caspase activation and recruitment domain
caspase	cysteine-aspartate proteases
COP	CARD-only protein
DDF	death domain fold
FCAS	familial cold autoinflammatory syndrome
IL	interleukin
IL-1R	IL-1 receptor
IFN	interferon
IPAF	ICE-protease activating factor
IRF	interferon response factor
LPS	lipopolysaccharide
LRR	leucine-rich repeat region
MDP	muramyl dipeptides
MWS	Muckle–Wells syndrome
NACHT	NAIP, C2TA, HET-E, and TP1 domain
NALP	NACHT-, LRR- and PYD-containing protein
NF-κB	nuclear transcription factor κB
NLR	NOD-like receptor
NOD	nucleotide binding and oligomerization domain
NOMID	neonatal-onset multisystem inflammatory disease
p38MAPK	p38 mitogen-activated protein kinase
PAMP	pathogen-associated molecular pattern
POP	PYD-only protein
PRR	pathogen recognition receptor
PYD	PYRIN domain
TIR	Toll/interleukin-1 receptor
tlpA	TIR-like protein A
TLR	Toll-like receptor

Introduction

Innate immunity is a crucial survival mechanism common to all metazoans that provides a rapid means of responding to and controlling microbial infections. In mammals, this first line of host defense against invading pathogens is provided by diverse effector elements, most notably complement, monocytes, macrophages, neutrophils, and dendritic and natural killer cells. The breadth of potential pathogens against which these effectors must respond necessitates that they possess multiple activities, including the ability to detect pathogens, mount an effective defense against microbes, and provide instruction to the adaptive immune system. Consequently, there is growing recognition that the innate immune system is a dynamic and complex system for recognizing and responding to infection or injury.

Clearly, the host must meet the challenge of defending against the presence of pathogen-derived molecules, membrane disruptions, and toxins. As a system, innate immunity operates by detecting a broad range of molecular patterns foreign to mammalian tissues, termed pathogen-associated molecular patterns (PAMPs). Among the more common PAMPs are highly conserved microbial structures such as bacterial cell-wall components (e.g., lipopolysaccharide, LPS), flagellin, and bacterial and viral nucleic-acid motifs [3, 111, 115]. It should be noted that microbial PAMPs are not the only signals capable of activating an innate immune response. Endogenous cellular products associated with tissue injury or self 'danger' signals, such as toxic compounds, defective nucleic acids, or the presence of normal cell components in atypical extracellular or intracellular locations, can also stimulate innate mechanisms [81, 106].

Recognition of these signals is achieved through constitutive expression in innate immune cells of a limited number of germ cell-encoded proteins known collectively as pathogen recognition receptors (PRRs) [33]. While adaptive immunity employs somatic recombination of the genes encoding for the T-cell receptor and the immunoglobulin heavy and light chains to generate diversity in receptors for microbial antigens, PRRs recognize microbial pathogens or ligands from damaged tissues on the basis of shared molecular structures [49]. This decreased requirement for specificity and clonal expansion of cell populations allows a limited number of highly active effector genes to induce rapid and efficient defensive responses to varied pathogens. Moreover, the limited number of PRRs encoded by a given organism suggests that these receptors target highly critical microbial patterns common to a wide range of microorganisms.

PRRs in the innate immune system function at both extracellular and intracellular levels and include soluble, membrane-bound and cytosolic molecules. The prototypical sensors, the Toll-like receptors (TLRs), recognize diverse ligands at the cell surface or within phagosomes [57]. In comparison, the nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs) regulate innate immunity in response to recognition of microbial products in the cytosol [47]. Members of the NLR family are typified by the presence of a leucine-rich repeat region (LRR) required for ligand sensing, a NOD/NACHT (NAIP, C2TA, HET-E, and TP1) domain and signaling modules such as the caspase activation and recruitment domain (CARD), PYRIN domain (PYD), or baculoviral inhibitor of apoptosis repeat domain [4]. Other cytoplasmic PRRs that appear to specifically mediate anti-viral innate immune response have also been reported. These include the retinoic acid inducible (type I) and melanona differentiation associated (Type V) genes [56, 84]. Thus, the cytosol is also an active environment for immune surveillance during host–pathogen interactions.

Activation of PRRs results in diverse physiological responses, including mobilization of soluble defense molecules, elimination of infected cells or tissues, and acquisition of specialized functions by antigen presenting and other immune cells. The most common mode of action of PRRs is to trigger intracellular signaling cascades that culminate in transcriptional activation of inflammatory mediators, such as the nuclear transcription factor (NF)-κB pathway [3, 4, 60, 111, 116]. Inflammation, a complex network of molecular and cellular interactions with the common goals of facilitating tissue repair and a return to physiological homeostasis, is the most powerful tool in the innate immunity arsenal. This response is composed of both local and systemic events that result in increased blood flow, elevated cellular metabolism, vasodilatation, leakage of fluids, cellular influx, and the release of soluble mediators, such as cytokines [87]. Cells of the innate immune system play pivotal roles in initiating, propagating, and regulating early inflammatory responses, as well as directing the infiltration of T lymphocytes and plasma cells that characterize more prolonged states of inflammation. The inflammatory response constitutes a classic two-edged sword. Normally self-regulating inflammation is essential for containing infectious microorganisms and foreign antigens and resolving damage. However, an overzealous response can become a chronic condition that is detrimental to the host and leads to severe collateral tissue damage [87].

Several members of the PYD superfamily of proteins have merged as important mediators of host innate immune and inflammatory responses against intracellular pathogens. These include NLRs that function as PYD-containing PRRs, known as PYD-NLRs, in particular the NACHT-, LRR- and PYD-containing proteins (NALPs). Activation of PYD-NLRs after recognition of microbial ligands by their LRRs results in the recruitment and oligomerization of the PYD-containing adapter protein ASC (apoptosis-associated

speck-like protein containing CARD) to form the cytosolic, multi-protein inflammasomes [18, 22, 74, 80]. Several inflammasome configurations with different constituent components have been identified and defined by the NLR protein that they contain, including the NALP-1, NALP-3 and ICE-protease activating factor (IPAF) inflammasomes [76, 77]. In each case, the inflammasome serves as a molecular scaffold that facilitates the autolytic cleavage and activation of proenzyme cysteine-aspartate proteases (caspases), in particular caspase-1, and stimulation of NF-κB signaling pathways [74]. Caspase-1 represents the central effector protein of the inflammasome, mediating such activities as the processing and release of proinflammatory cytokines, including interleukin (IL)-1β and IL-18 [39, 54, 72, 78]. These cytokines, in turn, are secreted to trigger an inflammatory cascade in surrounding innate immune cells and tissues to limit spread of the pathogen. In addition, caspase-1 activation is associated with death in certain cell types, possibly acting to restrict intracellular replication of pathogens, and the induction of membrane biogenesis pathways that promote cell survival when confronted with microbial toxins [39]. Thus, the inflammasome functions as an early sensor to detect pathogen and non-pathogen threats to the host and initiate multiple defense mechanisms.

IL-1β is one the most important soluble mediators of inflammation. Produced mainly by blood monocytes, IL-1β mediates a wide range of reactions, including fever, hypotension, the release of adrenocorticotrophic hormone, and the production of other cytokines, such as IL-6, which induce the leukocytosis, thrombocytosis, and synthesis of acute-phase proteins required to sustain an inflammatory response [20]. However, IL-1β is also a potent endogenous pyrogen whose activity can be detrimental when control of its processing and release is compromised. As with other pro-inflammatory innate immune responses, excessive or uncontrolled IL-1β production has the potential to cause damage to host tissues and acerbate pathologies with an inflammatory component. The production and secretion of IL-1β typically requires two separate events [65, 84]. The first involves priming of macrophages after recognition of PAMPs, such as LPS, which stimulates transcription of the IL-1β and accumulation of its precursor form. The second signal requires proteolytic processing by caspases to generate the mature, active form of the cytokine for secretion. Because inflammasomes have the capacity to mediate signal recognition and caspase activation, they represent attractive targets for both pathogens seeking to circumvent the immune system and therapeutic interventions in inflammatory diseases.

In this review, we focus on the strategies involved in inhibiting inflammasome activity, with special emphasis on the mechanisms used by pathogens to evade or usurp innate host immune responses. Significant strides have been made in recent studies to elucidate the role of the inflammasome in immune responses to bacteria, but there is also growing evidence to implicate these complexes in antiviral defenses. More comprehensive reviews of inflammasome structure and regulation are contained within this volume. This review highlights cellular negative regulators of inflammasome activity and the seminal immunomodulatory strategies employed by pathogens to either mimic or disrupt endogenous regulation.

Regulation of the inflammasome—insights into activation and inhibition

Specific mechanisms underlying the regulation and activation of inflammasomes are still in the nascent stages of characterization. Recent advances indicate that the inflammasome is a sophisticated and dynamic collection of entities in which constituent components are selectively employed according to the nature of the primary trigger. However, little is known about the functions, ligands, and activation mechanisms for the majority of the NLRs that initiate inflammasome assembly. What is apparent is that the many NLRs and adaptor molecules that have been implicated in inflammasome function, together with the variety of pathogens and toxins that induce innate immune and inflammatory responses, imply that diversity is a hallmark of this system.

As described above, activation of the inflammasome is thought to follow the PAMP-PRR paradigm proposed for TLR signaling pathways in which signal recognition is determined by the LRRs of specific NLRs [25, 73, 90, 94]. The natural stimuli to which these NLRs respond are unclear, but several candidates have been proposed. Given the hypothesis that inflammasomes constitute a highly conserved and specific response system that detects the presence of microorganisms, it is not surprising that diverse microbial products have been implicated as triggers. In phagocytes, LPS [17, 103, 117] and microbial peptidoglycans such as muramyl dipeptides (MDP) [29] have been shown to activate NALP1 inflammasomes. In addition, bacterial RNA [54] and the toxin Nigericin [72] have been implicated in NALP3 inflammasome formation, while flagellin from several bacterial strains has been reported to induce the IPAF inflammasome [32, 85, 88, 99]. More recently, cellular stress signals have also been shown to promote the assembly and activation of the NALP3 inflammasome. These signals include monosodium urate and calcium pyrophosphate dihydrate crystals that are released by damaged cells in conditions such as gout and pseudo-gout [78]. NALP3 has also been found to be essential for contact hypersensitivity to hapten trinitrophenyl at the sensitization phase [112]. Similarly, the exposure of cells to extracellular adenosine triphosphate (ATP), an

indicator of cytolysis under hypotonic conditions [19], generates an inflammatory response mediated by ionotropic receptor (P2X7) stimulation of the inflammasome pathway [61, 66, 108]. Thus, both microbial products and changes in the cellular environment regulate assembly of inflammasome complexes.

These findings provide insight into inflammasome function but fail to address the question of whether inflammasomes are specialized structures that are activated in a non-redundant fashion by specific stimuli or whether they are generic sensors for innate signals. For example, gain-of-function mutations in the gene coding for NALP3 are associated with three distinct autoinflammatory diseases: Muckle–Wells syndrome (MWS), familial cold autoinflammatory syndrome (FCAS), and neonatal-onset multisystem inflammatory disease (NOMID) [1, 43, 55]. These conditions, collectively termed cryopyrin-associated periodic syndromes (CAPS), are characterized by recurrent periodic fever and serosal inflammation due to increased NF-κB signaling, caspase-1 activation, and production of IL-1β [2]. The same phenotype presents when the NALP3 inflammasome is activated by pathogen-associated signals. Of interest, disorders associated with NALP3 dysfunction can be successfully treated by inhibiting the effectors of inflammasome signaling, namely, activated caspases and proinflammatory cytokines [36, 40]. Thus, it can be argued that any strategy that targets these downstream effector molecules is also a viable mechanism for inhibiting inflammasome function.

This potential is aptly demonstrated by IL-1β regulation. As described above, the production of IL-1β is critical for the control of pathogens, but excessive cytokine production is also harmful to the host. Consequently, several endogenous safeguards are in place to control the activity of IL-1β, including the regulation of gene expression, synthesis, secretion, and receptor association [20–22]. For example, the naturally occurring IL-1 receptor (IL-1R) antagonist is structurally similar to IL-1β but binds the IL-1R without eliciting a biological signal [20–22]. Any list of regulatory mechanisms controlling IL-1β activity must also include proteins that interfere with caspase-activity and therefore IL-1β processing and release. For example, the serpin proteinase inhibitor 9 (PI-9) is constitutively expressed within vascular smooth muscle cells and prevents pro-IL-1β and pro-IL-18 processing by blocking the active site of caspase-1 [6, 120]. Similarly, caspase-12 has a direct dominant-negative suppressive effect on caspase-1 catalysis [100]. The prototypical exogenous caspase-1 inhibitor is the poxvirus-encoded cytokine response modifier, CrmA, which has been shown to block caspase-1 mediated cleavage of pro-IL-1β [97].

As the above example illustrates, an in-depth analysis of the endogenous and exogenous mechanisms known to influence inflammatory cytokine processing is beyond the scope of this review. Inflammatory cascades represent the convergence of diverse pathways, and too little is known about the specific roles that inflammasomes play in these process. Thus, our discussion on modulators of inflammasomes will focus on endogenous and exogenous mediators that have been shown to specifically target components of the inflammasome complex itself.

Endogenous inhibitors of the inflammasome

Cellular or host-encoded molecules that directly target proteins in the inflammasome to regulate its function tend to be members of the death domain fold (DDF) domain family that encode a single DDF motif. Typically, these proteins function as dominant-negative inhibitors of specific signaling pathways that compete for essential binding partners and interrupt signal transmission to downstream effector proteins. Two classes of single DDF proteins have been shown to directly inhibit inflammasome function: the PYD-only proteins (POPs) and the CARD-only proteins (COPs) (Table 1).

The PYD is believed to mediate homotypic protein–protein interactions between components of signaling pathways involved in the regulation of apoptosis, NF-κB activation, and proinflammatory cytokine production [28]. Although the functions of many PYD proteins are still being determined, approximately 20 members have been identified in humans [98]. Three of these, the eponymous pyrin and the cellular POP-1 and POP-2, possess solitary PYDs and are hypothesized to regulate inflammasome formation by disrupting PYD-mediated interactions between ASC and PYD-NLR proteins [24, 109]. A role for pyrin as a regulator of inflammasome function is implied by the fact that mutations in the gene encoding the protein are associated with inheritable autoinflammatory syndromes [43]. Similarly, targeted truncation of pyrin in mice produces a phenotype in which animals are hypersensitive to LPS shock and express higher levels of IL-1β [15]. Two mechanisms have been proposed to explain how pyrin regulates caspase-1 activation and consequently IL-1β release. As noted above, the first is believed to involve an interaction between the PYDs of pyrin and ASC to prevent recruitment and activation of caspase-1 at the inflammasome by titrating the adapter molecule out of the pathway [15, 121]. More recently, it has also been proposed that an interaction occurs between the carboxy-terminal B30.2 domain of pyrin and the catalytic domains of caspase-1 to prevent autoactivation of the enzyme within the inflammasome, although the specific mechanism for this inhibition remains unclear [16].

Table 1 Cellular and microbial inhibitors of inflammasome activity

Source	Protein[a]	Target(s)[b]	Proposed effect	References
Host cell	Pyrin	ASC	Blocks inflammasome assembly	[15, 121]
		caspase-1	Directly blocks caspase activation	[16]
	cPOP-1	ASC	Inhibits inflammasome assembly and NF-κB activation	[109]
	cPOP-2	PYD-NLRs	Inhibits inflammasome assembly and NF-κB activation	[9]
	COP	caspase-1	Blocks caspase recruitment	[26]
	ICEBERG	caspase-1	Blocks caspase recruitment	[26, 46]
Virus	M13L[c]	ASC	Blocks inflammasome assembly	[51]
Bacteria	YopE	caspase-1	Blocks caspase autocleavage	[102]
	YopT	caspase-1	Blocks caspase autocleavage	[102]
	YopP	NF-κB	Blocks NF-κB signaling	[102]

[a] cPOP, cellular pyrin domain-only protein; COP, caspase recruitment domain (CARD)-only protein
[b] ASC, apoptosis apoptosis-associated speck-like protein containing CARD; PYD-NLR, pyrin domain (PYD)-containing Nod-like receptors (NLRs); NF-κB, nuclear factor κB
[c] M13L is the prototypical poxvirus-encoded POP from the leporipoxvirus, myxoma virus. Other viral POPs have been identified in representative yatapoxvirus species (Tanapox and Yaba-like disease viruses), the suipoxvirus swinepoxvirus and the leporipoxvirus Shope fibroma virus.

Thus far, two POPs have been identified in the human genome and shown to possess different substrate specificities and mechanisms of action. The existence of two distinct cellular POPs implies a versatile system for regulating inflammasome assembly that may allow for targeted suppression of specific PYD-NLR family members. cPOP1 (DASC) is predominantly expressed in macrophages and granulocytes within immune tissues and appears to inhibit ASC-dependent activation of NF-κB and inflammasome formation [109]. Structurally, cPOP1 exhibits high homology to the prototypical PYDs of pyrin and ASC, consistent with its ability to interact directly with ASC to prevent subsequent interactions between the adapter protein and both pyrin and NALP3 [109]. The structurally similar cPOP2 is expressed primarily in peripheral blood leukocytes. Unlike cPOP1, cPOP2 more closely aligns with the PYD-NLRs than ASC [9]. As a result, cPOP-2 has been shown to bind to the PYD of both ASC and several PYD-NLR proteins, including NALP3 and PAN1, to impair inflammasome-mediated activation of pro-caspase-1 and IL-1β processing [9]. Like cPOP1, cPOP2 has been shown to inhibit activation of NF-κB in response to cytokine stimulation [9], indicating that cPOP2 functions as a negative regulator of diverse innate immune and inflammatory responses. Thus, the presence of cPOPs and their ability to regulate ASC interactions have broad implications for cellular development, survival, and homeostasis.

The second class of single DFF proteins implicated in inflammasome function is the COP proteins, such as COP (pseudo-ICE) and ICEBERG [26, 46, 64]. The finding that activation of inflammatory caspases involves a cytosolic protein complex analogous to the apoptosome for apoptotic caspases has greatly advanced our understanding of the complexity of their regulation. Consequently, the mechanisms of action of COP and ICEBERG were subject to re-evaluation in light of the possibility that they may act as dominant negative regulators interfering with the recruitment of caspase-1 to the inflammasome. Both COP and ICEBERG contain CARD domains closely resembling the prodomain of caspase-1. Thus it is predicted that via CARD–CARD interactions, COP and ICEBERG inhibit pro-IL-1β processing by preventing direct interactions between the caspase-1 and adapter proteins that support oligomerization, such as ASC [26, 46, 64].

Bacterial strategies for modulating the inflammasome

As a survival and defense strategy, bacterial pathogens have acquired sophisticated mechanisms to interfere with the host responses to infection. To establish a successful replication cycle, the encoded virulence factors from both Gram-positive and Gram-negative bacteria interact with host molecules and modulate cellular processes important for host defense. Included in these mechanisms is the capacity to directly and indirectly interact with inflammasomes and modulate caspase-1 function (Fig. 1) [25, 79, 90]. Activation of caspase-1 and production of IL-1β typically lead to apoptosis in infected macrophages, a phenomenon that obviously brings into question the survival benefit derived by bacteria that activate this pathway upon infection. In the case of *Salmonella typhimurium*, it has been suggested that IL-1β may promote recruitment of host inflammatory cells to the site of infection, thereby increasing the abundance of host reservoirs for bacterial replication or altering vascular blood flow to facilitate deep tissue invasion by the pathogen [50]. Alternatively, IL-1β may simply be a by-product of the

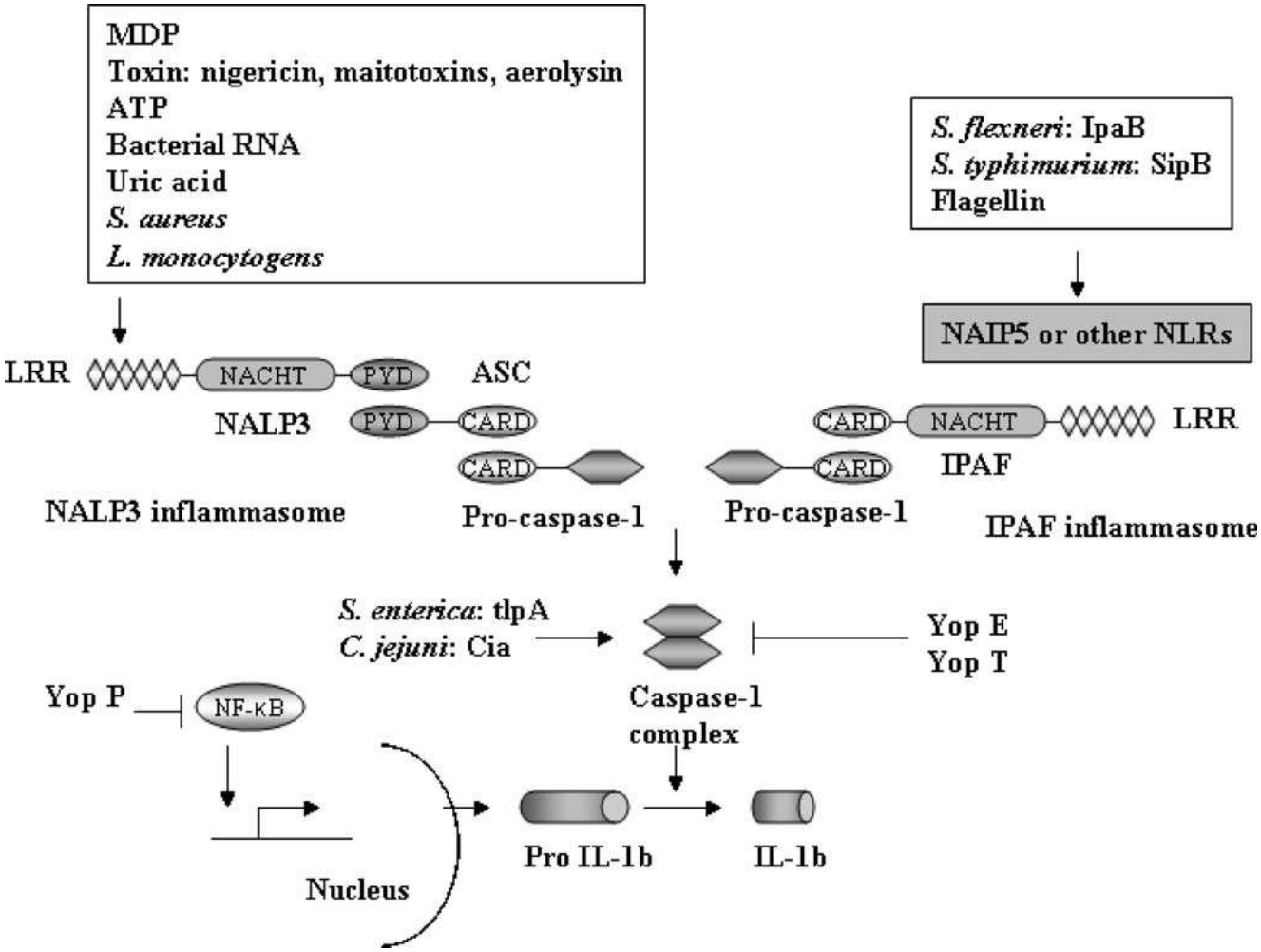

Fig. 1 Modulation of inflammasomes by bacteria. The NALP3 inflammasome is activated by bacterial products present in the cytoplasm (muramyl dipeptides, lipopolysaccharides, bacterial RNA, toxins) and cellular signals (ATP, uric acid) that stimulate TLRs. The active oligomerized NALP3 interacts with the adapter protein, ASC, through the PYD domain. The CARD domain of ASC simultaneously interacts with the CARD domain of procaspase-1, to induce conformational changes that yield active caspase-1, which then processes pro-IL-1β to active IL-1β. The bacterial type III and IV secretion system proteins, such as IpaB, SipB and flagellin, can activate caspase-1 through the IPAF inflammasome and NALP5 or other NLRs. Bacterial proteins such as tlpA and Cia can activate caspase-1, while YopE and YopT can inhibit caspase-1 function. The bacterial protein YopP can reduce IL-1β levels by inhibiting NF-κB signaling

caspase activation initiated by invading bacteria to kill the host defense cells. However, it is more likely that caspase-1 activation is the result of a defensive innate sensor and signaling mechanism in the host immune system. For example, bacterial LPS has long been recognized as a potent inducer of IL-1β. Acting alone or in concert with a secondary trigger such as ATP activation of the $P2X_7$ receptor, LPS induces a rapid efflux of potassium ions from the cytosol that activates caspase-1 and leads to the processing of pro-inflammatory cytokines [19]. However, deletion of caspase-1 or its downstream effectors does not provide resistance to *S. typhimurium* infection [62], suggesting that this pathway does not contribute to an essential pathogenic mechanism.

Studies using mice deficient for either ASC or NALP3 have shown that these components of the inflammasome are essential for the activation of caspase-1 and the subsequent release of mature IL-1β and IL-18 induced by LPS treatment [70, 72, 119]. The NALP3 inflammasome is also activated by RNA derived from several bacterial strains, including *Escheria coli*, *Listeria monocytogens*, and *Legionella pneumophila* [54], as well as bacterial peptidoglycans such as MDP [75]. This latter observation may be species-specific in that it has been demonstrated in the human monocytoid cell line, THP-1, but MDP alone does not exhibit similar activity in murine macrophages [54, 72, 112]. The mechanisms by which caspase-1 and IL-1β are activated also vary greatly according to the strain of bacterial pathogen investigated. For example, NALP3 has been found to be essential for activation of this pathway by some Gram-positive, but not some Gram-negative, bacteria

[54, 72, 112]. However, the clear involvement of inflammasomes in these processes is demonstrated by the obligate requirement for ASC in caspase-1 activation regardless of the stimuli [54, 72, 112]. Of note, earlier findings that the caspase-1 activation induced by MDP involved a mechanism that was TLR-independent, but NALP3-dependent [75], have subsequently been supported by more recent studies indicating that TLRs and NALP3 control the secretion of IL-1β and IL-18 through different intracellular pathways [54, 72, 112].

Among the other bacterial components that activate inflammasomes are flagellin and microbial toxins, the roles of which have recently unfolded. For bacterial toxins that cause changes in intracellular ion composition, such as potassium efflux associated with the aerolysin toxin from *Aeromonas hydrophila*, inflammasome assembly and caspase-1 activation occurs in a NALP3-dependent manner [39]. In comparison, the caspase-1 activation induced by the lethal anthrax toxin encoded by *Bacillus anthracis* is mediated by the NALP1 inflammasome [12]. Therefore, the presence of specific PYD-NLDs within the inflammasome appears to confer selectivity to the ability of the host to detect and respond to microbial pathogens. Similar selectively is evident with the flagellum protein, flagellin. The presence of flagellin in the cytosol of infected cells is associated with activation of the IPAF inflammasome leading to caspase activation, as has been demonstrated for flagellated bacteria such as *S. typhimurium* and *L. pneumophila* [32, 85, 99]. In fact, *S. typhimurium* mutants deficient in flagellin are unable to activate caspase-1 in infected macrophages [32, 85]. It should be noted that many bacterial pathogens are known to activate caspase-1 through the inflammasome signaling pathway, but that the effector molecules involved have yet to be identified. For example, caspase-1 activation after infection by the intracellular pathogen *Francisella tularensis* requires ASC, implicating the inflammasome in this process, but the NLR involved is unknown [34, 71].

Bacterial pathogens belonging to *Yersinia*, *Salmonella*, *Shigella*, and *Pseudomonas* species employ a "molecular syringe," termed the type III secretion system, to inject their effectors into the host cell and interfere with signaling pathways involved in host defense, including inflammation [31]. The formation of pores in the membranes of target host cells by these secretion systems triggers an ion efflux analogous to that induced by the ATP-P2X$_7$ interaction. As with that interaction, one consequence of this change in intracellular ion balance is caspase-1 activation, although the exact mechanism is still poorly understood. Given the selectivity with which these pathogens induce inflammasome assembly, it seems likely that similar selectivity would be present in the inflammasome activators and inhibitors that they have evolved. Among the *Yersinia*-encoded YOP effector proteins, both YopE and YopT have been shown to interfere with the caspase-1 mediated maturation of proIL-1β in macrophages (Table 1). This outcome is achieved by impeding the autoproteolytic activation of caspase-1 through a mechanism both related to and dependent on the ability of these proteins to inhibit the key Rho GTPases involved in cytoskeletal reorganization, namely, Rac-1 and LIM kinase-1 [102]. Another Yop protein, YopP, a bacterial modulator of NF-κB activity, can also regulate IL-1β production by inhibiting NF-κB mediated transcriptional events [102]. Conversely, the type III secretion systems of *Salmonella* and *Shigella* result in activation of caspase-1 in infected macrophages, as demonstrated for the SipB protein of *S. typhimurium* and the IpaB protein of *Shigella flexneri* [41, 42]. These proteins function either directly, by binding to caspase-1 itself, or indirectly, by intercalating within membrane lipid rafts of infected host cells and inducing actin rearrangements that activate Rac1 [37, 38]. Recent studies have shown that this activation of caspase-1 is mediated by ASC, as macrophages deficient in ASC release lower amounts of mature IL-1β [70].

The *Salmonella enterica* protein, tlpA (TIR-like protein A), closely resembles the mammalian Toll/interleukin-1 receptor (TIR) domain. Like the *Salmonella* virulence factor, SipB, tlpA induces caspase-1 activation that results in secretion of IL-1β and host cell apoptosis [89]. It has been proposed that tlpA modulates TIR-dependent signaling events linked to caspase-1 activation. Consistent with this hypothesis, loss of tlpA results in a virulent bacterial strain that loses the capacity to either promote IL-1β secretion or to survive intracellularly in human THP-1 monocytoid cells. On the other hand, over-expression of tlpA in mammalian cells suppresses the ability of mammalian TIR-containing proteins TLR-4, IL-1R, and MyD88, to induce the transactivation and DNA-binding activities of NF-κB. These findings suggest that tlpA is a virulence factor that modulates both NF-κB and IL-1β activity [89]. In comparison, the intracellular pathogen *Campylobacter jejuni* encodes a protein, the campylobacter invasion antigen, which has also been shown to induce apoptosis in THP-1 cells concurrent with caspase-1 activation and increased synthesis, processing and secretion of IL-1β. However, inhibition of IL-1β processing by blocking caspase-1 activity fails to prevent apoptosis, suggesting that for this bacterium, the process by which IL-1β induces apoptosis follows a regulatory pathway and mechanism of action distinct from that of *Salmonella* and *Shigella* [107]. Gram-positive bacteria such as *Salmonella aureus* and *Listeria monocytogenes* have been shown to directly activate inflammasomes through a process that requires NALP3 and the intracellular presence of the pathogen. To that end, macrophages deficient in NALP3 and ASC fail to secrete IL-1β and IL-18 following infection [72, 91].

Viral strategies for inhibiting the inflammasome

Innate immunity also represents the primary host defense against viral infection, culminating in the induction of antiviral responses such as the secretion of type I interferons (IFNs) and pro-inflammatory cytokines [92, 113, 114]. As with bacteria, recognition of virus infections involves the detection of conserved PAMPs, typically genomic DNA and single- and double-stranded RNA produced during viral replication [8, 11, 30, 58, 113]. Conventional dogma suggests that viral PAMPs are recognized by TLRs, in particular TLR-3, TLR-7, TLR-8 and TLR-9, after the endocytosis of viral particles [8, 11, 30, 58, 113]. After recognition, these receptors initiate signaling pathways involving NF-κB and IFN response factor (IRF) 3 and 7 that promote expression of host proteins to inhibit viral replication and promote secondary immune responses [83, 101, 105].

However, cytosolic sensor pathways involving members of the NLR family of proteins have also been shown to participate in viral recognition and to mediate anti-viral responses. Secretion of IL-1β and IL-18 that requires caspase-1 activation is commonly observed after infection of monocytes/macrophages by a variety of viruses [52, 95], Although there is little evidence to support a specific and direct antiviral function for IL-1β beyond its contribution to general inflammatory responses to infection, IL-18 is known to play an important role in viral clearance by acting synergistic with IL-12 to stimulate natural killer and cytotoxic T cells [35, 67]. Recently, Kanneganti et al. [53] have demonstrated that this response involves activation of the NALP3 inflammasome in response to either double-stranded RNA or active virus infection (Sendai and influenza viruses). Moreover, this response was abrogated in macrophages lacking either NALP3 or ASC [53]. These findings suggest that the NALP3 inflammasome functions as an essential sensor for infection by directly or indirectly detecting viral RNA produced during replication.

The demonstration that inflammasomes participate in the induction of antiviral responses in innate immune cells establishes these complexes as viable targets for inhibition by viruses in an attempt to circumvent host immunity. This hypothesis is further supported by recent work characterizing the function of poxvirus-encoded PYD-containing proteins [51]. Poxviruses employ a diverse array of proteins targeting such immune process as apoptosis; the production of IFNs, chemokines, and inflammatory cytokines; and the activity of cytotoxic T cells, natural killer cells, complement, and antibody [104]. The obvious sequence similarity between some poxvirus genes and the complementary DNA (cDNA) versions of cellular counterparts suggests that they are the product of ancestral capture, recombination, and reassortment events that occurred during coevolution of the virus and its vertebrate hosts [82]. Recently, we reported that the presence of genes in the sequenced genomes of multiple orthopoxvirus genera that share close evolutionary ancestry and are predicted to encode PYD proteins [51]. Characterization of M13L-PYD, the PYD protein encoded by the leporipoxvirus myxoma virus, revealed that it was a host range factor and virulence determinant for myxomatosis [51]. Loss of a M13L-PYD function attenuated myxoma virus virulence in rabbits, producing a phenotype in which rapid resolution of the infection was associated with an elevated host inflammatory response and an inability to replicate in blood leukocytes. Moreover, M13L-PYD colocalized and interacted with the cellular adapter protein ASC to inhibit caspase-1 activation and the subsequent processing of IL-1β and IL-18 by monocytes (Table 1 and Fig. 2) [51]. Thus, poxvirus-encoded PYD proteins may constitute a novel viral anti-inflammatory strategy.

To date, poxviruses remain the only viral family shown to possess members that encode PYD proteins. Poxvirus immunomodulatory proteins can be divided by function into three strategic classes: virostealth proteins, viromimetics (virokines and viroreceptors), and virotransducers [104]. M13L-PYD appears to be a member of this latter group, which act intracellularly to disconnect innate antiviral pathways from their biological effect. Although the exact mechanism of action for M13L-PYD remains uncertain, its structural resemblance to cPOPs and ability to interact directly with ASC [51] suggests that the protein may block inflammasome formation by preventing ASC aggregation through a homotypic interaction mediated by its PYD. Of interest, orthopoxviruses that do not have comparable PYD proteins, such as vaccinia and molluscum contagiosum viruses, encode other secreted proteins known to function as molecular scavengers to bind and sequester IL-1β [13, 118]. Comparable IL-1β scavengers are absent in poxviruses that possess PYD proteins [7], suggesting that poxvirus-encoded PYD proteins evolved as an alternate strategy for disrupting the intracellular signaling pathways leading to IL-1β processing and secretion.

Although poxviral PYD proteins may be novel in their ability to target cytosolic NLRs that serve as sensors for innate immune defenses, it should be noted that several viruses employ strategies to impede signaling by other PRRs, such as TLRs. For example, vaccinia virus encodes three products that interfere with TLR pathways, including A46R, A52R, and N1L [10, 23]. Despite limited sequence similarity with the TIR domains of cellular TLRs and IL-1R, A46R and A52R have been shown to block NF-κB activation mediated by TLR4 and the proinflammatory cytokines, IL-1β and IL-18 [10]. Similar observations were made for N1L, with the added caveats that the protein appeared to also interact with the inhibitory I-κB kinase

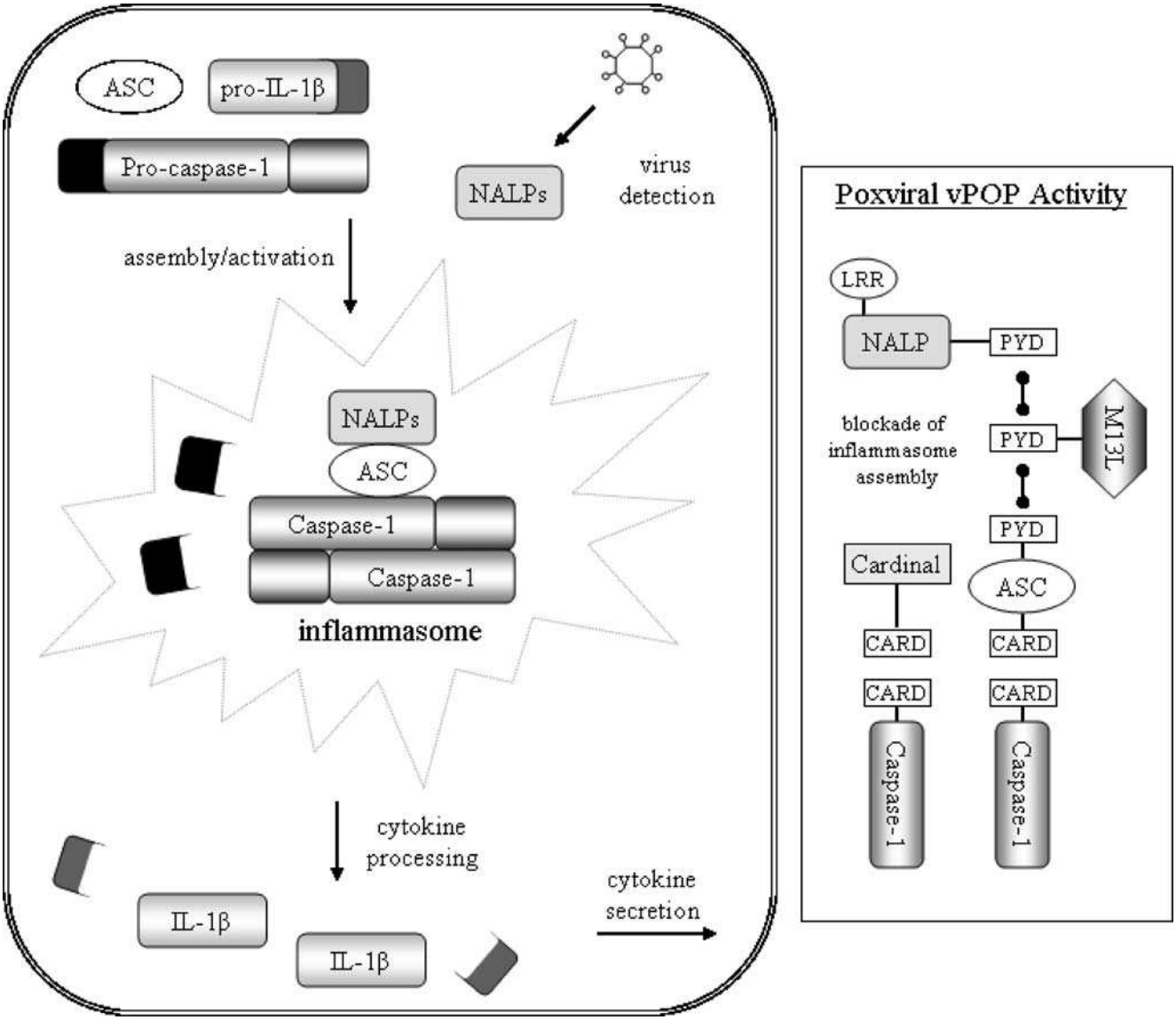

Fig. 2 Inhibition of inflammasomes by poxviruses. Activation of the inflammasome after virus infection results in procaspase-1 recruitment and autocleavage and the subsequent processing and release of proinflammatory cytokines, such as IL-1β. A NALP3-containing inflammasome is depicted with specific like protein domains (*PYD* pyrin domain; *CARD* caspase recruitment domain; *LRR* leucine-rich repeat) mediating the interaction of inflammasome components during assembly. The vPOP (M13L) encoded by the leporipoxvirus myoxma virus is believed to interact with ASC in a PYD-dependent manner to disconnect the activation signals induced by the cytosolic PYD-NLR sensors, NALPs. In doing so, M13L inhibits caspase-1 activation and the innate host inflammatory responses to infection

complex and interrupt NF-κB events induced by the tumour necrosis factor superfamily of receptors [23]. Thus, viruses must overcome or evade multiple PRR signaling actions to successfully replicate and spread, necessitating the evolution of viral modulators that target key control points, including the inflammasome.

Therapeutic applications of inflammasome inhibitors

Chronic inflammation evokes a cascade of events that can have devastating consequences, the most serious of which is major organ failure due to the accrual of abnormalities in tissue architecture. Once an individual has progressed to this extreme, therapeutic options are limited and death is the likely outcome. Given its proposed role as a regulatory platform for one of the most potent proinflammatory cytokines, IL-1β, it is not surprising that defective control of inflammasome function can lead to serious autoinflammatory disorders. These conditions are characterized by recurrent episodes of systemic inflammation in the absence of infection and are distinguished from traditional autoimmune diseases by the lack of high-titer autoantibodies or antigen-specific T cells [55, 110, 115]. Many of the

hereditary fever syndromes, such as the CAPS triad (MWS, FCAS, and NOMID) described above and familial Mediterranean fever (FMF), fall into this category. Moreover, the root cause of these syndromes is commonly a somatic mutation in a single gene encoding a key component of inflammasomes that is transmitted through Mendelian inheritance [55, 110]. However, inflammation is a complex process and it is reasonable to assume that inflammatory pathologies are underpinned by a far more complicated framework intertwining multiple known and unknown factors.

For example, mutations in the NALP3 gene, *CIAS1*, have been identified in CAPS patients around the world [5, 43]. The three conditions comprising CAPS not only share some clinical characteristics but also have distinguishing features such as increasing severity from FCAS to MWS to NOMID [115]. MWS is characterized by sensorineural deafness, rashes, systemic amyloidosis, arthritis, and frequent nonspecific limb pain [115]. In FCAS, skin lesions, swollen and painful joints, conjunctivitis, and fever after exposure to cold are observed [115]. NOMID is associated with chronic meningitis, rashes, deforming arthropathy, and neurosensory hearing loss [115]. More than 40 disease-associated mutations have been identified in *CIAS1*, many of which are associated with a single disease phenotype. However, the same genetic defect within the NALP3 can also result in a wide variety of phenotypes, indicating that additional factors are involved. The majority of these mutations are gain-of-function missense changes that fall within the NACHT/NOD domain of NALP3, resulting in chronic systemic inflammation due to increased caspase-1 activation and IL-1β release [2]. The importance of this domain has also been shown in the NLR family member, NOD2, where mutations in the NACHT region can result in other inflammatory diseases such as Crohn's disease and Blau syndrome [48].

Similarly, the autoinflamatory disease FMF is caused by mutations in the gene encoding pyrin, *MEFV*. This condition, which is most common in the Mediterranean basin and the Middle East, is characterized by recurrent fever, monoarticular arthritis, erythematous rash, abdominal pain, and systemic amyloidosis [110]. In addition, patients with FMF and *MEFV* mutations exhibit increased risk for developing other autoinflammatory diseases, including inflammatory bowel disease, vasculitis, and Beccet disease [44]. More than 50 FMF-associated mutations have been identified in the *MEFV* gene, a large percentage of which reside in the 200 residue C-terminal B30.2 domain. A recent study has shown that mutations in B30.2 prevent direct interaction of this domain with caspase-1, resulting in increased caspase-1 activation and IL-1β production [16]. This interaction is independent of its PYD-mediated interaction with ASC in the inflammasome [16].

Inflammasome-associated diseases reflect dysregulation of the processing and release of proinflammatory cytokines like IL-1β. This phenotype is a hallmark of virtually all inflammatory disease; therefore, therapeutic agents targeting these cytokines in the treatment of other autoinflammatory diseases, such as rheumatoid arthritis, also have benefit in conditions associated with inflammasome function. Among the therapeutic goals for treating these conditions are suppression of acute attacks, suppression of long-term sequela such as amyloidosis and the prevention of long-term neurological impairment. However, known therapies are far from perfect, reflecting the potential for a single factor to exponentially amplify the number of variables and interactions that ultimately determine how a disease manifests. Thus, a very small perturbation at some point within an inflammatory cascade can profoundly impact the disease profile. To that end, all elements of proinflammatory cytokine regulation are subject to intervention, be it at the level of the cytokine itself, caspase-activation or signal transduction.

The most promising therapeutic candidate is Anakinra, a recombinant human IL-1R antagonist. Using Anakinra, a complete cessation of clinical symptoms and biochemical changes were reported in MWS patients [40], while significant therapeutic benefit has also been observed in patients with NOMID [68]. Orally active inhibitors of caspase-1, such as the peptidic aldehyde Ac-YVAD-CHO and the aldehyde inhibitor Ac-WEHD-CHO, are also candidate treatments despite the potential for significant side effects if used long-term. Small molecule caspase-1 inhibitors have been proposed as alternate therapies for several autoinflammatory diseases. The majority of these inhibitors are substrate-based, having been derived from the P1–P4 tetrapeptide sequence (YVHD) that is the target site for caspase-1 in pro-IL-1 [96]. Moreover, they can function in a reversible or irreversible manner to competitively inhibit caspase-1 depending on the nature of the covalent bond they form with the active site of caspase-1. Non-selective caspase inhibitors that preferentially target caspase-1, including chloromethylketone and fluoromethylketone compounds such as Ac-YVAD-cmk, are also candidate therapies [63]. Initially developed for the treatment of inflammatory bowel disease, it is conceivable that these compounds can also be used to treat diseases associated with inflammasomes. Finally, p38 mitogen-activated protein kinase (p38MAPK) plays a central role in the regulation of proinflammatory cytokines like IL-1β at both the transcriptional and translational level. Several inhibitors of p38MAPK the prototypical pyridinyl imidazole have been characterized in vitro and some are advanced into clinical trials. However, they might block the biosynthesis of various other proinflammatory cytokines [93].

One cannot ignore the attractive therapeutic potential incorporated in the endogenous negative regulators of inflamamsome function, such as the cellular POPs, or their viral cousins identified in the genomes of several poxviruses. Although inclusion in this family of regulatory PYD proteins appears to be fairly exclusive, it is highly likely that additional members will emerge as hitherto unknown cellular and pathogen gene products are characterized. Proteins, such as POPs, which can disrupt or impede inflammatory and apoptotic networks involving inflammasomes have the potential to broadly impact a host of physiological and pathological processes at the level of both the cell and the organism. These range from cellular development, survival, and homeostasis to systemic inflammation and innate immune responses. As such, POPs may have applications in dampening adverse inflammatory processes by blocking pro-inflammatory signal upstream and downstream of the inflammasome complex, impeding inflammasome assembly or limiting the duration of inflammasomes. For example, cPOP2 has been shown to disrupt the NALP3 inflammasome [9]. Given the number of pathologies attributed to excessive and uncontrolled NALP3 activity, therapies built on the premise of increasing cPOP2 expression and/or activity may be useful in the treatment of multiple conditions. Moreover, the central role that NALP3 and other PYD-NLRs are postulated to play in inflammation greatly expands the spectrum of pathologies where POP intervention could be beneficial. Another intriguing therapeutic application of POPs may lie in their ability to inhibit NF-κB signaling pathways. Aberrant NF-κB signaling is linked to the pathologies of multiple inflammatory diseases, making it an attractive yet elusive therapeutic target in efforts to block the expression of proinflammatory genes [14, 27, 45]. Current NF-κB inhibitors have limited therapeutic benefit because of the toxicity inherent in blocking such a critical pathway, providing potential opportunity for more selective inhibitors of NF-κB activity.

Conclusion

Deregulated IL-1β production and chronic inflammation are hallmarks of many autoimmune diseases that present both systemically and within the central nervous system, including rheumatoid arthritis and multiple sclerosis [59, 86]. There is no question that many microbial pathogens understand the workings of host inflammasomes better than we do. Generally, the consequences of bacterial or viral modulation of inflammasome activities provide a more supportive environment for either the pathogen replication or subsequent dissemination. It is conceivable that future generations of inflammasome inhibitors could be patterned on the microbial modulators themselves, similar to how secreted anti-inflammatory regulators from viruses [69] have been co-opted to treat clinical diseases that are mediated by chronically dysregulated pro-inflammatory cascades. Whether the discovery of the inflammasome truly constitutes the "Rosetta stone" of innate immunity and inflammatory responses [25] will be determined by history. What is abundantly clear, however, is that an improved understanding of the components of inflammasomes and the interactions that govern their function will elucidate fundamental mechanisms of inflammatory cytokine production applicable to diverse pathologies. This understanding may give rise to new insights into biologically relevant targets to control inflammatory diseases or infections that will complement or replace existing therapies that are hindered by limited clinical efficacy or excessive adverse complications.

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