The NLRP3 inflammasome: molecular activation and regulation to therapeutics

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Abstract | NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) is an intracellular sensor that detects a broad range of microbial motifs, endogenous danger signals and environmental irritants, resulting in the formation and activation of the NLRP3 inflammasome. Assembly of the NLRP3 inflammasome leads to caspase 1-dependent release of the pro-inflammatory cytokines IL-1β and IL-18, as well as to gasdermin D-mediated pyroptotic cell death. Recent studies have revealed new regulators of the NLRP3 inflammasome, including new interacting or regulatory proteins, metabolic pathways and a regulatory mitochondrial hub. In this Review, we present the molecular, cell biological and biochemical bases of NLRP3 activation and regulation and describe how this mechanistic understanding is leading to potential therapeutics that target the NLRP3 inflammasome.

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The discovery that *NLRP3* (which encodes NOD-, LRR- and pyrin domain-containing protein 3) gain-of-function mutations cause the dominantly inherited autoinflammatory disease known as cryopyrin-associated periodic syndrome (CAPS) represented a major advance in inflammation research[1–3]. CAPS belongs to a group of diseases with varied severity that includes familial cold autoinflammatory syndrome, Muckle–Wells syndrome and chronic infantile neurological cutaneous and articular syndrome (also known as neonatal-onset multisystem inflammatory disorder). Early studies linked *NLRP3* to inflammation mediated by the cytokine IL-1 and revealed the involvement of NLRP3 in autoinflammatory diseases, which has been reviewed in depth elsewhere[4,5]. In parallel, the discovery that NLRP1 can form a complex with activated caspase 1 termed the inflammasome[6], followed by the revelation that NLRP3 can perform a similar function, provided a molecular basis to explain the CAPS phenotype[7]. Beyond the monogenic autoinflammatory diseases in humans, NLRP3 was shown to affect a wide range of disease models in mice, highlighting the potential application of NLRP3-targeted therapies for these diseases.

The past decade has witnessed a burgeoning appreciation of inflammasomes as critical innate immune components that orchestrate host immune homeostasis. This Review focuses on the recent advances in our understanding of the activation and intrinsic regulation of the NLRP3 inflammasome machinery, as well as the emerging pharmacological approaches that target the NLRP3 inflammasome and show potential for clinical translation.

The NLRP3 inflammasome: an overview

The NLRP3 inflammasome consists of a sensor (NLRP3), an adaptor (ASC; also known as PYCARD) and an effector (caspase 1). NLRP3 is a tripartite protein that contains an amino-terminal pyrin domain (PYD), a central NACHT domain (domain present in NAIP, CIITA, HET-E and TP1) and a carboxy-terminal leucine-rich repeat domain (LRR domain). The NACHT domain has ATPase activity that is vital for NLRP3 self-association and function[8], whereas the LRR domain is thought to induce autoinhibition by folding back onto the NACHT domain. ASC has two protein interaction domains, an amino-terminal PYD and a carboxy-terminal caspase recruitment domain (CARD). Full-length caspase 1 has an amino-terminal CARD, a central large catalytic domain (p20) and a carboxy-terminal small catalytic subunit domain (p10). Upon stimulation, NLRP3 oligomerizes through homotypic interactions between NACHT domains (FIG. 1). Oligomerized NLRP3 recruits ASC through homotypic PYD–PYD interactions and nucleates helical ASC filament formation, which also occurs through PYD–PYD interactions. Multiple ASC filaments coalesce into a single macromolecular focus, known as an ASC speck[9–11]. Assembled ASC recruits caspase 1 through CARD–CARD interactions and enables proximity-induced caspase 1 self-cleavage and activation. Caspase 1 clustered on ASC self-cleaves at the linker between p20 and p10 to generate a complex of p33 (comprising the CARD and p20) and p10, which remains bound to ASC and is proteolytically active[12]. Further processing between the CARD and

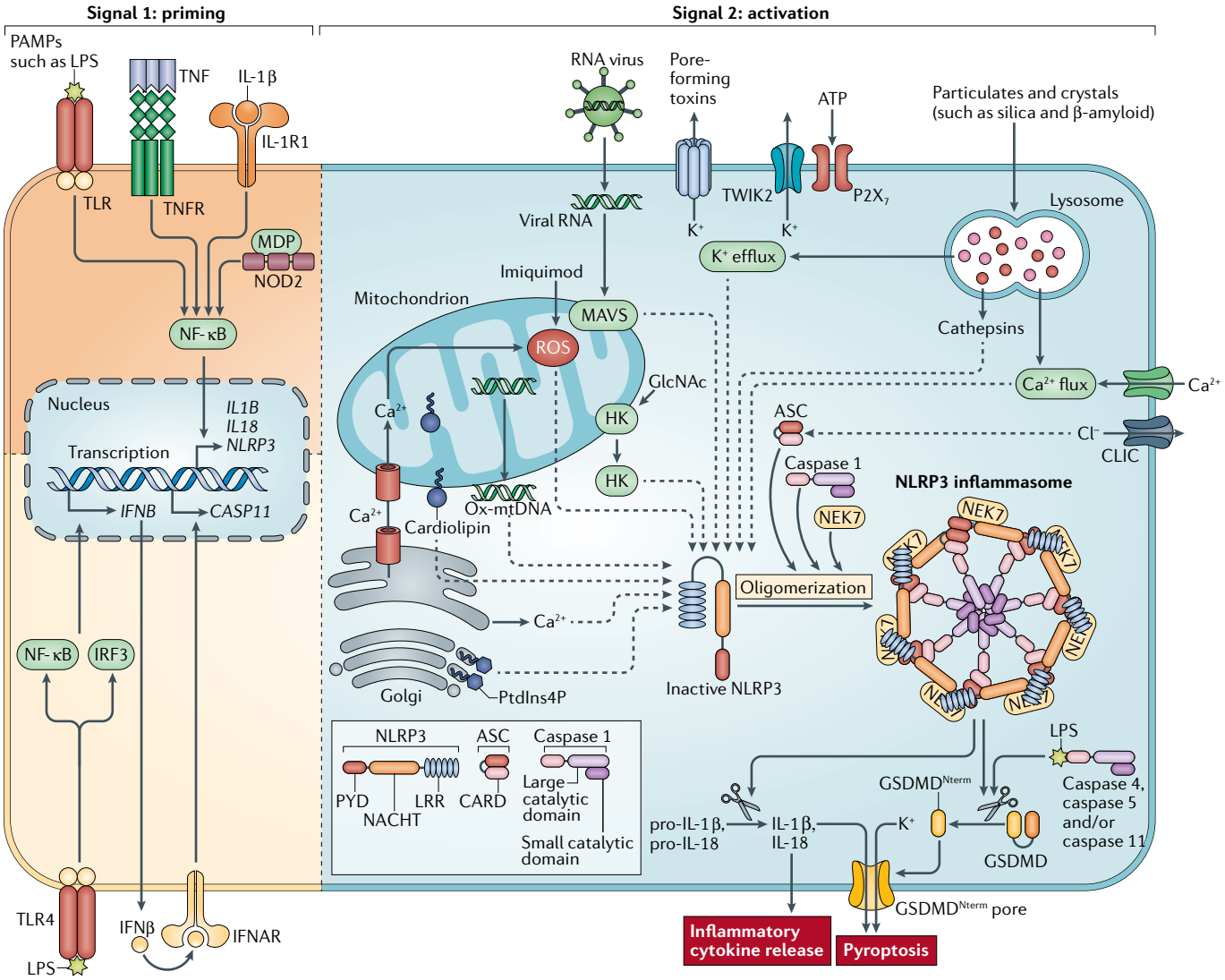

Fig. 1 | **NLRP3 inflammasome priming and activation.** The signal 1 (priming; left) is provided by the activation of cytokines or pathogen-associated molecular patterns (PAMPs), leading to the transcriptional upregulation of canonical and non-canonical NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome components. Signal 2 (activation; right) is provided by any of numerous PAMPs or damage-associated molecular patterns (DAMPs), such as particulates, crystals and ATP, that activate multiple upstream signalling events. These include K^+ efflux, Ca^+ flux, lysosomal disruption, mitochondrial reactive oxygen species (mtROS) production, the relocalization of cardiolipin to the outer mitochondrial membrane and the release of oxidized mitochondrial DNA (Ox-mtDNA), followed by Cl^- efflux. RNA viruses activate NLRP3 through mitochondrial antiviral signalling protein (MAVS) on the mitochondrial outer membrane. Formation of the inflammasome activates caspase 1, which in turn cleaves pro-IL-1β and pro-IL-18. Gasdermin D (GSDMD) is also cleaved and inserts into the membrane, forming pores and inducing pyroptosis. Upon detection of cytosolic lipopolysaccharide (LPS), caspases 4, 5 and 11 are activated and cleave GSDMD, triggering pyroptosis. CARD, caspase recruitment domain; CLIC, chloride intracellular channel protein; GlcNAc, *N*-acetylglucosamine; $GSDMD^{Nterm}$, GSDMD amino-terminal cell death domain; HK, hexokinase; IFNAR, IFNα/β receptor; IL-1R1, IL-1 receptor type 1; IRF3, interferon regulatory factor 3; LRR, leucine-rich repeat; MDP, muramyl dipeptide; NEK7, NIMA-related kinase 7; NF-κB, nuclear factor-κB; $P2X_7$, P2X purinoceptor 7; PtdIns4P, phosphatidylinositol-4-phosphate; PYD, pyrin domain; ROS, reactive oxygen species; TLR, Toll-like receptor; TNF, tumour necrosis factor; TNFR, tumour necrosis factor receptor; TWIK2, two-pore domain weak inwardly rectifying K^+ channel 2.

p20 releases p20–p10 from ASC. The released p20–p10 heterotetramer is unstable in cells, hence terminating its protease activity[12]. Recently, NIMA-related kinase 7 (NEK7), a serine-threonine kinase that is known to be involved in mitosis, was found to be essential for NLRP3 inflammasome activation[13–15]. NEK7 specifically interacts with NLRP3, but not the other inflammasome sensors NLRC4 (NOD-, LRR- and CARD-containing 4) or interferon-inducible protein AIM2. Upon inflammasome activation, the NEK7–NLRP3 interaction increases, and NEK7 oligomerizes with NLRP3 into a complex that is essential for ASC speck formation and caspase 1 activation[14,15]. Thus, NEK7 appears to be a core component specific to the NLRP3 inflammasome.

Leucine-rich repeat domain
(LRR domain). In Toll-like receptors (TLRs), the LRR domain mediates the detection of microbial components; it may serve a similar role in certain NLRs (NACHT–LRR proteins). The LRR domain of NLRs and TLRs is structurally similar. It consists of leucine-rich amino acid strands forming a peptide loop. The loops occur as tandem repeats that together form a coil or solenoid and contain constant sequences, as well as unique insertions or variable residues for each ligand.

AIM2
A sensor that combines with the adaptor protein ASC and the protease caspase 1 to form the AIM2 inflammasome. It senses cytosolic double-stranded DNA from bacteria or viruses or from mislocalized self-DNA and contributes to infection defence.

Priming the NLRP3 inflammasome

Because inflammasome activation is an inflammatory process, it must be tightly regulated. With few exceptions, inflammasome activation is considered to be a two-step process — first, it must be primed, and then it can be activated (FIG. 1). Priming serves at least two functions. The first function is to upregulate the expression of the inflammasome components NLRP3, caspase 1 and pro-IL-1β. This transcriptional upregulation can be induced through the recognition of various pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) that engage pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) or nucleotide-binding oligomerization domain-containing protein 2 (NOD2) or through cytokines such as tumour necrosis factor (TNF) and IL-1β that lead to nuclear factor-κB (NF-κB) activation and gene transcription[16–18]. In addition, priming with the TLR4 ligand lipopolysaccharide (LPS) shifts macrophage metabolism from oxidative phosphorylation to glycolysis, indirectly causing the stabilization of hypoxia-inducible factor 1α (HIF1α) and an increase in *IL1B* gene transcription[19]. The second function of priming is the induction of post-translational modifications (PTMs) of NLRP3 (FIG. 2), which stabilize NLRP3 in an auto-suppressed inactive, but signal-competent, state. Multiple PTMs have been described for NLRP3, including ubiquitylation, phosphorylation and sumoylation. PTM of NLRP3 can occur at the unstimulated, priming, activation and resolution phases. PTM of NLRP3 is further discussed in a later section.

NLRP3 activation

The priming step of inflammasome activation licenses the cell, whereas a second step occurs following the recognition of an NLRP3 activator and induces full activation and inflammasome formation. Although most PRRs have limited specificity for one or a few related PAMPs or DAMPs, NLRP3 is unique in that it is activated by a wide variety of unrelated stimuli (FIG. 1; TABLE 1). NLRP3 is activated in bacterial, viral and fungal infections, as well as in sterile inflammation mediated by endogenous DAMPs and on exposure to environmental irritants. The unifying factor of these activators is that they all induce cellular stress; cellular stress is then sensed by NLRP3. Exactly how NLRP3 senses cellular stress and which pathways are induced to culminate in NLRP3 activation and inflammasome formation remain to be fully elucidated.

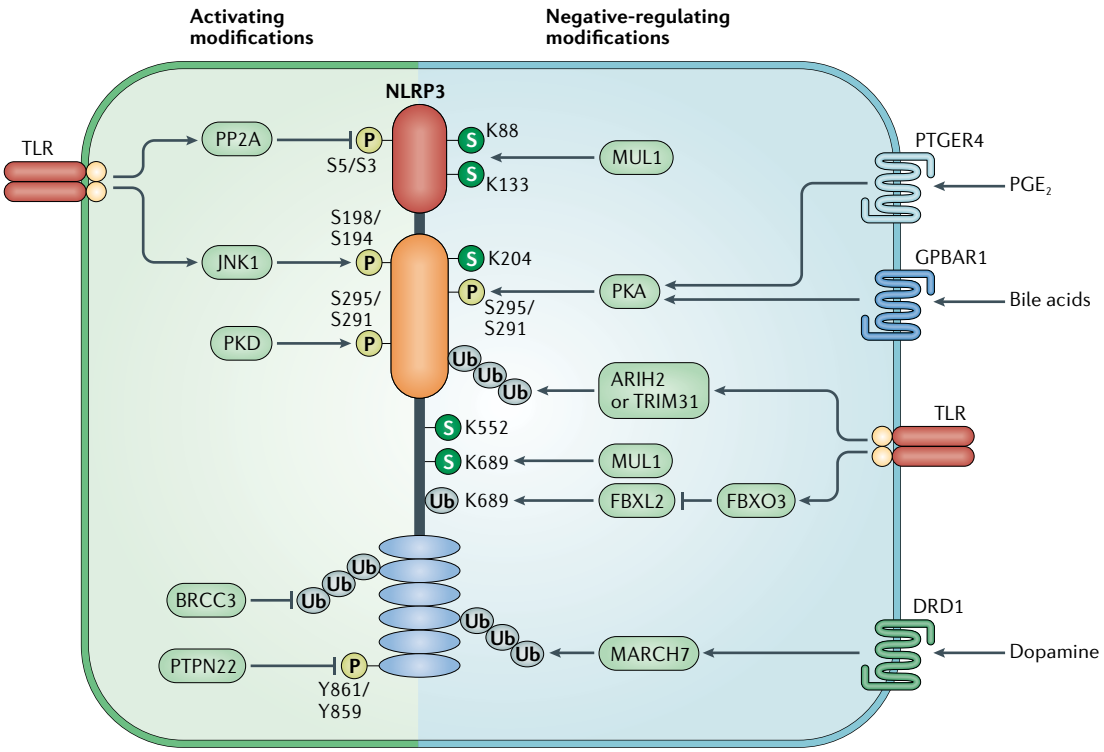

Fig. 2 | **Post-transcriptional modifications of NLRP3.** NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) is regulated by phosphorylation (P), ubiquitylation (Ub) and sumoylation (S). Activating regulatory modifications are listed on the left, and those that inhibit NLRP3 activation are on the right. Amino acid residues in both human and mouse NLRP3 are listed for phosphorylation, whereas ubiquitylation and sumoylation refer only to the mouse protein residues. ARIH2, E3 ubiquitin-protein ligase ARIH2; BRCC3, BRCA1/BRCA2-containing complex subunit 3; DRD1, D1A dopamine receptor; FBXL2, F-box/LRR-repeat protein 2; FBXO3, F-box only protein 3; GPBAR1, G protein-coupled bile acid receptor 1; JNK1, JUN N-terminal kinase 1; MARCH7, membrane-associated RING finger protein 7; MUL1, E3 SUMO protein ligase MUL1; PGE_2, prostaglandin E_2; PKA, protein kinase A; PKD, protein kinase D; PP2A, protein phosphatase 2A; PTGER4, prostaglandin E_2 receptor EP4 subtype; PTPN22, protein tyrosine phosphatase non-receptor type 22; TLR, Toll-like receptor; TRIM31, tripartite motif-containing protein 31.

Table 1 | **NLRP3 inflammasome activators**

Activator	Source	Examples	Refs
DAMP	Self-derived	ATP, cholesterol crystals, monosodium urate crystals, calcium pyrophosphate dihydrate crystals, calcium oxalate crystals, soluble uric acid, neutrophil extracellular traps, cathelicidin, α-synuclein, amyloid-β, serum amyloid A, prion protein, biglycan, hyaluronan, islet amyloid polypeptide, hydroxyapatite, haeme, oxidized mitochondrial DNA, membrane attack complex, cyclic GMP–AMP, lysophosphatidylcholine, ceramides, oxidized phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine and sphingosine	20,25,26,57,99,111,156–163
	Foreign-derived	Alum, silica, aluminium hydroxide, nanoparticles, carbon nanotubes, chitosan, palmitate (also self-derived), UVB, imiquimod (R837)/CL097 and resiquimod (R848)	28,40,164
PAMP	Bacterial	Lipopolysaccharide, peptidoglycan, muramyl dipeptide, trehalose-6,6'-dibehenate, c-di-GMP–c-di-AMP, bacterial RNA and RNA–DNA hybrid	31,165–169
		Toxins: nigericin (*Streptomyces hygroscopicus*), gramicidin (*Brevibacillus brevis*), valinomycin (*Streptomyces fulvissimus* and *Streptomyces tsusimaensis*), β-haemolysin (*Streptococcus* sp. 'group B'), α-haemolysin (*Staphylococcus aureus*), M protein (*Streptococcus* sp. 'group A'), leucocidin (*Staphylococcus aureus*), tetanolysin O (*Clostridium tetani*), pneumolysin (*Streptococcus pneumoniae*), listeriolysin O (*Listeria monocytogenes*), aerolysin (*Aeromonas hydrophila*), streptolysin O (*Streptococcus pyogenes*), enterohaemolysin (*Escherichia coli* O157:H7), haemolysin BL (*Bacillus cereus*), adenylate cyclase toxin (*Bordetella pertussis*), M protein (*Streptococcus* sp. 'group A') and maitotoxin (*Marina* spp. dinoflagellates)	170–173
	Viral	Double-stranded RNA and single-stranded RNA	62,66,67
	Fungal	β-Glucans, hyphae, mannan and zymosan	174,175

DAMP, damage-associated molecular pattern; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; PAMP, pathogen-associated molecular pattern; UVB, ultraviolet B.

NLRP3 activation is thought to include multiple upstream signals, most of which are not mutually exclusive, including efflux of potassium ions (K^+) or chloride ions (Cl^-), flux of calcium ions (Ca^{2+}), lysosomal disruption, mitochondrial dysfunction, metabolic changes and *trans*-Golgi disassembly. Although there are many data describing the upstream signalling events, many pathways are interrelated and overlapping, and the data are sometimes conflicting. Therefore, as yet, there is no consensus model of NLRP3 activation. Hence, we discuss the various upstream signals — which may act in tandem or independently — that have been mooted to have a role in NLRP3 inflammasome activation.

K^+ efflux. With few exceptions, K^+ efflux is a necessary upstream event in NLRP3 activation. It has been known for decades that nigericin, a K^+/H^+ ionophore, and ATP-mediated activation of P2X purinoceptor 7 ($P2X_7$), a ligand-gated ion channel of the purinergic receptor family, promote IL-1β maturation via K^+ efflux[20–22]. Although the P2X family receptors are membrane non-selective cation channels for sodium ions (Na^+), K^+ and Ca^{2+}, it was only recently appreciated that $P2X_7$ does not act as the ion channel for K^+ efflux[23]. After ATP stimulation, $P2X_7$ promotes Ca^{2+} and Na^+ influx and coordinates with the K^+ channel two-pore domain weak inwardly rectifying K^+ channel 2 (TWIK2), which mediates K^+ efflux[24]. In addition, NLRP3 inflammasome activation induced either by LPS treatment or by caecal ligation and puncture depends on TWIK2 (REF.[24]). LPS activation of NLRP3 in vivo is attributed to its activation of the complement cascade. The complement cascade component membrane attack complex activates NLRP3 (REFS[25,26]), while complement C3a engagement of its receptor on monocytes enhances inflammasome activation via the release of ATP[27]. In addition to ATP and pore-forming toxins, the particulate stimuli alum, silica and calcium pyrophosphate crystals all induce K^+ efflux that is critical to NLRP3 inflammasome activation[28]. Importantly, low extracellular concentrations of K^+ are sufficient to activate the NLRP3 inflammasome in THP1 cell-free lysates, as well as in cultured bone marrow-derived macrophages, whereas high extracellular concentrations of K^+ prevent its activation[28,29]. Although it has been proposed that K^+ efflux is the common denominator upstream of all NLRP3 activators, there are several reports of NLRP3 inflammasome activation independent of K^+ efflux[30–32].

Ca^{2+} flux. Ca^{2+} mobilization is also reported to be a critical upstream event in NLRP3 activation[33,34]. Mobilization of Ca^{2+} occurs by either the opening of plasma membrane channels or the release of endoplasmic reticulum (ER)-linked intracellular Ca^{2+} stores to allow the flux of Ca^{2+} to the cytosol. Both pathways are linked in that when either plasma Ca^{2+} channels open or ER-linked stores are released, the other pathway typically follows. Furthermore, K^+ efflux can regulate Ca^{2+} flux by acting as a counter ion at the plasma membrane for Ca^{2+} influx. Thus, it is often found that Ca^{2+} flux and K^+ efflux are coordinated in NLRP3 activation. For example, ATP mobilizes Ca^{2+} by inducing a weak Ca^{2+} influx via its receptor $P2X_7$ and coordinating K^+ efflux[24]. K^+ efflux promotes the release of ER-linked Ca^{2+} stores followed by the opening of plasma Ca^{2+} channels[33,35]. Additionally, NLRP3 activation induced by nigericin, alum, monosodium urate crystals and the membrane attack complex has been shown to depend on Ca^{2+} flux in addition to K^+ efflux, as noted above[25,33]. Importantly, there is contradictory evidence showing that, for some stimuli, the Ca^{2+} flux occurs downstream of both NLRP3 and caspase 1 activation and, therefore, is not critical for NLRP3 activation[36]. Thus, the role of Ca^{2+} flux in NLRP3 activation remains debated.

P2X purinoceptor 7
($P2X_7$). An ATP-gated cation channel that is expressed by haematopoietic cells and participates in cell proliferation and apoptosis. It belongs to the family of purinoceptors for ATP and is responsible for the ATP-dependent activation of NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3).

Caecal ligation and puncture
An experimental model of peritonitis in rodents, in which the caecum is ligated and then punctured, thereby forming a small hole. This leads to leakage of intestinal bacteria into the peritoneal cavity and subsequent peritoneal infection.

Cl^- efflux. Low extracellular Cl^- levels are known to enhance ATP-induced IL-1β secretion. Accordingly, Cl^- channel blockers and high extracellular Cl^- levels inhibit NLRP3 activation[37,38], suggesting a role for Cl^- efflux in NLRP3 activation. Although there is a report that nigericin-induced activation of NLRP3 does not result in changes in intracellular Cl^- levels[28], two groups have recently shown that chloride intracellular channel proteins (CLICs) are necessary for NLRP3 activation by several stimuli, including nigericin, but dispensable for AIM2 and NLRC4 inflammasome activation[37,38]. While deficiency of CLIC4 impedes nigericin-induced IL-1β release, knockdown of *Clic1* and *Clic5* in *Clic4*$^{-/-}$ bone marrow-derived macrophages further impedes IL-1β release, suggesting that CLICs might have redundant roles in NLRP3 activation[38]. CLICs are present in both the cytosol and plasma membrane, where they form anion channels. Translocation of CLIC1, CLIC4 and CLIC5 to the plasma membrane depends on the release of mitochondrial reactive oxygen species (mtROS), whereas Cl^- efflux occurs downstream of K^+ efflux[38]. Another recent study separated the contribution of K^+ efflux from Cl^- efflux by inhibiting K^+ or Cl^- efflux independently during NLRP3 activation. They showed that the role of K^+ efflux is to drive NLRP3 oligomerization, whereas the role of Cl^- efflux is to promote ASC polymerization during NLRP3 inflammasome formation[39].

Lysosomal disruption. Phagocytosis of particulates, either self-derived particulates such as uric acid and cholesterol crystals or foreign-derived particulates such as alum, silica and asbestos, causes lysosomal rupture and release of the particulates into the cytoplasm[40]. Lysosomal acidification precedes lysosomal swelling and damage and is crucial for NLRP3 activation. Additionally, lysosomal rupture induced by the lysosomotropic dipeptide Leu-Leu-OMe is sufficient to activate the NLRP3 inflammasome[40]. The use of broad spectrum cathepsin inhibitors suggested that cathepsins, which reside in the lysosomes, are important for NLRP3 activation in response to particulate stimuli. However, genetic deletion of cathepsin B, X, L or S individually or knockdown of cathepsin X individually had little effect on NLRP3 activation[41], suggesting that cathepsins might have redundant roles in NLRP3 activation. Importantly, lysosomal damage by Leu-Leu-OMe and NLRP3 particulate stimuli activates K^+ efflux and Ca^{2+} influx, suggesting that many NLRP3 activation pathways converge on either K^+ and/or Ca^{2+} flux[28,33,42].

Mitochondria. Mitochondrial dysfunction and the release of mtROS and mitochondrial DNA (mtDNA) into the cytosol are additional key upstream events implicated in NLRP3 activation. Mitochondria continually produce ROS as a by-product of oxidative phosphorylation, although during cellular stress mtROS levels are greatly increased. Mitophagy is therefore an important regulator of NLRP3 activation, as it removes damaged and dysfunctional mitochondria and reduces mtROS. Increases in damaged and dysfunctional mitochondria following treatment with inhibitors of mitophagy enhance NLRP3 inflammasome activation[43]. Early studies show that ATP and particulate stimulation induce ROS production that is necessary for NLRP3 inflammasome activation[44,45]. Imiquimod, a small-molecule adenine derivative, and the related compound CL097 were recently shown to activate the NLRP3 inflammasome via inhibition of the quinone oxidoreductases and mitochondrial complex I[31,46]. Consistent with previous reports showing that disruptors of complexes I and III induce mtROS and NLRP3 activation[43,47], imiquimod-induced NLRP3 activation was independent of K^+ flux and lysosomal disruption but dependent on mtROS production[31]. Importantly, this finding suggests that K^+ efflux is not necessary as long as mitochondrial disruption and mtROS production occur. However, because most inhibitors are known to have off-target effects, the studies that use mtROS inhibitors to show a role for ROS should be verified with other approaches. Indeed, at least one study showed that ROS inhibitors block the priming step but not activation of the NLRP3 inflammasome[48].

In addition to ROS, a role for nuclear factor erythroid 2-related factor 2 (NRF2) has also been reported in inflammasome regulation. NRF2 regulates both basal and induced levels of antioxidant genes to support cell survival during oxidative stress. By limiting ROS levels, NRF2 inhibits NLRP3 activation[49]. In addition, NRF2-driven gene transcription attenuates NF-κB activation and downregulates expression of the inflammasome components *NLRP3*, *CASP1*, *IL1B* and *IL18*, thus limiting NLRP3 inflammasome activity[50]. However, others showed that NRF2 is also necessary for NLRP3 activation[51–54] and is enriched in ASC specks[53].

In addition to mitochondrial dysfunction and mtROS release, circulating mtDNA was first found to act as a DAMP for NLRP3 activation[55] and is also a critical component of the NLRP3 activation pathway[47,56,57]. During oxidative stress, mtROS and Ca^{2+} work together to open mitochondrial permeability transition (MPT) pores[58]. The release of mtDNA into the cytoplasm was shown to depend on MPT pores and mtROS[47]. Shimada et al.[57] found that mtDNA was rapidly released into the cytoplasm after stimulation with various NLRP3 activators and importantly was oxidized. Oxidized mtDNA specifically activated the NLRP3 inflammasome, whereas non-oxidized mtDNA preferentially stimulated the AIM2 inflammasome[57]. Multiple studies show that oxidized mtDNA localizes with NLRP3 and co-immunoprecipitates with NLRP3 after activator stimulation, although these studies did not differentiate between direct or indirect interaction[56,57].

Recently, it was found that priming with the TLR2, TLR3 or TLR4 agonists results in interferon regulatory factor 1 (IRF1) activation, which in turn upregulates cytidine/uridine monophosphate kinase 2 (CMPK2); CMPK2 controls the pool of mitochondrial deoxynucleoside 5′-triphosphates (dNTPs), thus increasing mtDNA synthesis[56]. Surprisingly, this stands in contrast to earlier reports showing that IRF1 is dispensable for NLRP3 activation[59,60]. A report using a genetic deletion approach found no role for MPT pores or mitophagy in NLRP3 activation[61], whereas another study showed that mtDNA was not released into the cytosol in the absence

Mitophagy
The selective removal of mitochondria by macroautophagy under conditions of nutrient starvation or mitochondrial stress.

Oxidative stress
Cells continuously produce reactive oxygen species (ROS) such as hydrogen peroxide or superoxide anions. Under physiological conditions, mitochondria are the main source, and cellular antioxidants ensure that the redox equilibrium is maintained. During inflammatory responses (and in cancer), excessive production of ROS leads to a metabolic condition known as oxidative stress, which can lead to apoptosis and necrosis.

of NLRP3 (REF.[47]). Thus, further studies are needed to resolve the conflicting data and better understand the role of mitochondria in NLRP3 activation.

In addition to the potential role of mitochondria in NLRP3 activation, there is increasing evidence that mitochondria act as docking sites for inflammasome assembly. In unstimulated cells, NLRP3 is a cytoplasmic protein that associates with the ER, but upon activation it becomes associated with both mitochondria and the mitochondria-associated membrane (MAM)[43,62]. At least three proteins have been implicated as the connection point between NLRP3 and mitochondria. One such protein is cardiolipin. Cardiolipin is a mitochondrial phospholipid of the inner membrane that, upon mitochondrial stress, is exposed on the outer membrane, where it serves as a binding site for molecules associated with autophagy and apoptosis[63]. Cardiolipin also binds NLRP3 and full-length caspase 1 independently, and these interactions were shown to be necessary for inflammasome activation[64,65]. A second mitochondrial protein, mitochondrial antiviral signalling protein (MAVS), which is an adaptor protein in RNA sensing pathways, has been shown to be important for NLRP3 inflammasome activation during RNA viral infections and after stimulation with the synthetic RNA polyinosinic–polycytidylic acid[62,66–68]. MAVS recruits NLRP3, directing its location to the mitochondria for inflammasome activation[62,67]. Although MAVS was required for NLRP3 inflammasome activation after RNA viral infections, it appears to be non-essential for NLRP3 inflammation induced by other NLRP3 stimuli[67]. Lastly, mitofusin 2, which is found on the outer mitochondrial membrane, the ER and contact sites at the MAM, was found to be important for NLRP3 activation during RNA viral infections[68]. During viral infection, mitofusin 2 formed a complex with MAVS to support localization of NLRP3 to the mitochondria[68].

Metabolic changes. A major role of mitochondria is energy production via ATP and regulation of metabolism. As such, mitochondria are poised to detect cellular cues and metabolites to tune metabolic flux. Priming of dendritic cells (DCs) with LPS results in their switch from oxidative phosphorylation to aerobic glycolysis[69]. Phosphorylation of glucose is the first step in glycolysis and is mediated by hexokinase. During bacterial infection, degradation of the bacterial cell wall component peptidoglycan in lysosomes releases *N*-acetylglucosamine (GlcNAc). Hexokinase, which localizes at the mitochondrial surface, then binds to GlcNAc, promoting its relocalization into the cytosol[32]. GlcNAc-induced hexokinase relocalization promoted NLRP3 inflammasome activation independent of K^+ efflux. A peptide inhibitor of hexokinase was sufficient to induce hexokinase localization to the cytosol and drive inflammasome activation[32]. Importantly, during this process, although no mitochondrial membrane disruption was observed, mtDNA was detected in the cytosol. Similarly, another study reported that chemical disruption of glycolysis after the priming step activates the NLRP3 inflammasome[70]. However, the interpretation of this observation is complicated by the fact that inhibition of glycolysis during priming inhibits LPS-induced *IL1B* gene transcription[19].

Free fatty acids (FAs) that are liberated through diet or by upregulation of FA synthesis activate the NLRP3 inflammasome[71–73]. The anti-inflammatory AMP-activated protein kinase (AMPK) is an essential mediator of FA metabolism while suppressing FA-induced inflammation. One of the ways in which AMPK suppresses inflammation is by limiting ROS production and activating autophagy, thereby inhibiting NLRP3 inflammasome activation[74]. The saturated FA palmitate, however, was found to suppress AMPK activation and in turn increase ROS production and NLRP3 activation[71].

In addition to promoting NLRP3 activation, metabolic changes also negatively regulate NLRP3. Fasting and caloric restriction are known to reduce inflammation. During periods of low blood glucose, such as during caloric restriction, fasting and uncontrolled type I diabetes, metabolism switches to fatty acid oxidation, leading to the production of ketone bodies. One of these ketone bodies, β-hydroxybutyrate (BHB), inhibits NLRP3 activation induced by a wide variety of stimuli[75]. BHB also suppresses caspase 1 activation and IL-1β release in murine models of CAPS and in the urate crystal model of inflammation. Interestingly, BHB regulation of NLRP3 was not due to the starvation mechanisms of AMPK activation and autophagy, ROS decreases or glycolysis inhibition but rather was due to inhibition of K^+ efflux by an unknown mechanism.

Short-chain fatty acids (SCFAs) are fermentation products of the gut microbiota that are incorporated during FA synthesis and are reported to have anti-inflammatory effects. Butyrate, which is an SCFA that is structurally related to BHB, had no effect on ATP-induced or particulate-induced NLRP3 activation[75]. Conversely, a recent report shows that butyrate and propionate inhibit NLRP3 priming and activation by palmitate, with a major effect on reducing pro-IL-1β levels and a modest effect on inflammasome activation[76]. More investigation into whether SCFAs have a role in NLRP3 activation is warranted because metabolic regulation of NLRP3 is becoming an important subject for many diseases[77].

Trans-Golgi disassembly. Until recently, the role of the Golgi apparatus in NLRP3 activation had been underappreciated. Using a cellular reconstitution system, NLRP3 stimuli were found to promote *trans*-Golgi network disassembly into vesicles called dispersed *trans*-Golgi network (dTGN)[78]. The phospholipid phosphatidylinositol-4-phosphate on dTGN recruits NLRP3 and promotes NLRP3 aggregation that is essential for downstream ASC oligomerization and caspase 1 activation[78]. Although both K^+ efflux-dependent stimuli (nigericin) and K^+ efflux-independent stimuli (imiquimod) induce dTGN formation and NLRP3 recruitment, K^+ efflux is necessary for only NLRP3 recruitment and not for dTGN formation[78]. This suggests that K^+ efflux-dependent and mitochondria-dependent NLRP3 activation may be

Autophagy
A cytoplasmic bulk degradation system in which cytoplasmic cargo is targeted and is typically sequestered in double-membrane vesicles, leading to subsequent fusion with the lysosome. This process is essential for the response to starvation because it facilitates the recycling of cellular components. In addition, autophagy can be targeted to intracellular bacteria to restrict their growth.

Urate crystal model
A mouse model of crystal-induced peritonitis that activates the NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome.

separate pathways that converge on Golgi disassembly. Another study found that, upon activation, NLRP3 translocates to the Golgi apparatus in a complex with sterol regulatory element-binding protein 2 (SREBP2) and SREBP cleavage-activating protein (SCAP) and couples activation with SREBP2 maturation and cholesterol biosynthesis[79]. The SCAP and SREBP2 complex is required for NLRP3 activation.

Although there has been a lot of effort in understanding the upstream events during NLRP3 activation, there is still no single unifying model. Much of the evidence reported so far relies on pharmacological inhibition rather than genetic approaches. This makes it difficult to parse out indirect and off-target effects. Indeed, because most stimuli that activate NLRP3 also induce ion fluxes and organelle damage, it is hard to know whether mitochondrial dysfunction and mtROS are crucially involved or just associated with NLRP3 activation. The recent suggestion that K^+ efflux and mitochondrial dysfunction are separable events and that they both independently converge on *trans*-Golgi disassembly is intriguing[78] but requires further investigation.

NLRP3 and pyroptosis

In addition to inflammatory cytokine production, NLRP3 inflammasome activation leads to pyroptosis, which is a rapid, inflammatory form of lytic programmed cell death (FIG. 1). Recent key studies have identified that gasdermin D (GSDMD) is the mediator of pyroptosis. GSDMD has an amino-terminal cell death domain ($GSDMD^{Nterm}$), a central short linker region and a carboxy-terminal autoinhibition domain. Caspase 1 cleaves GSDMD, removing its carboxyl terminus and releasing it from intramolecular inhibition[80,81]. $GSDMD^{Nterm}$ then binds to phosphatidylinositol phosphates and phosphatidylserine in the cell membrane inner leaflet, oligomerizes and inserts into the plasma membrane, forming a 10–14 nm pore containing 16 symmetrical protomers[82], thus killing cells from within. In addition, $GSDMD^{Nterm}$ demonstrates bactericidal activity in vitro by binding to cardiolipin present in both outer and inner bacterial membranes[83]. How this direct bactericidal activity functions during infections remains elusive. Cardiolipin is also present in the inner and outer mitochondrial membranes after NLRP3 activation, but it is not clear whether $GSDMD^{Nterm}$ can permeabilize mitochondria by binding to mitochondrial cardiolipin. Another feature of GSDMD-dependent pyroptosis is that it facilitates the release of IL-1β and IL-18 via non-conventional secretion[84,85]. Pyroptosis induces the secretion of IL-1α in both full-length and calpain-processed forms, although processing does not determine its bioactivity[86]. Together, these findings reveal that GSDMD biochemically determines pyroptosis downstream of inflammasome activation. In addition to the important role played by caspase 1 in pro-IL-1β processing and pyroptosis, the apical activator caspase, caspase 8, also has a role in inducing a non-canonical pathway of IL-1β and IL-18 maturation and cell death[87–89]. Although several groups have shown caspase 8-mediated maturation of pro-IL-1β and pro-IL-18, this can occur through both NLRP3-dependent and NLRP3-independent pathways.

Pyroptosis
A lytic, inflammatory form of programmed cell death that is triggered by cleavage of gasdermin D by the inflammatory caspase 1, 4, 5 or 11. It is characterized by cytoplasmic swelling, early plasma membrane rupture and nuclear condensation. The cytoplasmic content is released into the extracellular space, and this is thought to augment inflammatory and repair responses.

Neutrophil extracellular traps
(NETs). Fibrous networks that are released into the extracellular environment by neutrophils. They are composed mainly of DNA but also contain proteins from neutrophil granules. NETs act as a mesh that traps microorganisms and exposes them to neutrophil-derived effector molecules.

Non-canonical NLRP3 activation

Cytosolic LPS during Gram-negative bacterial infection is sensed by human caspase 4 and caspase 5, and mouse caspase 11, to induce non-canonical NLRP3 inflammasome activation[90–93]. In this pathway, extracellular LPS activates TLR4, and the induced type I interferon response together with the complement C3–C3aR axis upregulates caspase 11 expression[94]. Type I interferons also induce the expression of a cluster of small GTPases, guanylate-binding proteins (GBPs) and their phylogenetic relative IRGB10[95,96]. GBPs and IRGB10 are recruited to intracellular bacteria, where they lyse bacteria and liberate LPS into the cytosol[95,96]. Direct recognition of cytosolic LPS by caspase 11 triggers its oligomerization and activation by auto-proteolytic cleavage[93,97]. Subsequently, GSDMD is cleaved by activated caspase 4, 5 or 11, and this induces pyroptosis[80,98]. The K^+ efflux caused by pyroptosis activates NLRP3–caspase 1-dependent IL-1β secretion. The oxidized phospholipid 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (oxPAPC) also binds to caspase 11 and activates this non-canonical pathway[99]. Recently, a report showed that, in mouse models of age-related macular degeneration, the non-canonical caspase 11–NLRP3 inflammasome pathway drives disease pathology and suggested that oxPAPC is important for inflammasome activation during disease[100]. Interestingly, although mtDNA was important in these models, it did not directly activate the canonical NLRP3 pathway but rather induced type I interferon production via the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) DNA sensing pathway. Conversely, a recent study suggests that oxPAPC suppresses rather than activates the caspase 11-dependent inflammasome[101]. The discrepancy between these studies requires further investigation.

In neutrophils, the detection of cytosolic LPS triggers non-canonical inflammasome activation and GSDMD cleavage by caspase 11 and activates the release of neutrophil extracellular traps (NETs; also known as NETosis)[102]. Of interest, NETosis can engage a feedforward loop that augments inflammation. NETs and the NET-associated antimicrobial peptide cathelicidin can induce NLRP3 inflammasome activation, and reciprocally, IL-18 released after inflammasome assembly can induce NETosis[103]. Together, the data reveal the critical role of the caspase 11–NLRP3 axis in intracellular bacteria surveillance.

One-step NLRP3 inflammasome activation

NLRP3 inflammasome activation does not always follow the two-step activation model. One exception is the species-specific observation that TLR4 stimulation alone is sufficient to induce the secretion of IL-1β from human and porcine monocytes but not from murine monocytes[30,104]. Given the observation that LPS induces endogenous ATP release from human monocytes[104,105], it was initially thought that LPS-induced inflammasome activation was mediated by the binding of extracellular ATP

to $P2X_7$ and the induction of K^+ efflux[105]. However, a second study revealed that in fact this pathway signals via a TRIF–RIPK1–FADD–caspase 8 pathway upstream of NLRP3 and is not associated with the usual hallmarks of inflammasome activation, including ASC speck formation, pyroptosis and K^+ efflux; it is therefore referred to as alternative NLRP3 inflammasome activation[30]. A second exception to the two-step activation model is observed in mouse bone marrow-derived DCs and mouse splenic DCs, in which LPS alone is also sufficient to activate the NLRP3 inflammasome by an unknown mechanism[106]. This one-step rapid response might contribute to maintaining sterility of blood and have implications during bacterial sepsis.

Another one-step activation pathway has been observed in mouse bone marrow-derived macrophages in which simultaneous TLR and NLRP3 stimulation leads to rapid inflammasome activation. Stimulation of TLR2, TLR4, TLR7 or TLR9, together with extracellular ATP, rapidly activates NLRP3. Although this type of NLRP3 activation does not promote IL-1β secretion and pyroptosis, it does lead to IL-18 secretion, which occurs independent of de novo transcription[107]. The transcription-independent inflammasome activation provides a fast response mechanism in tissue inflammation, as microbial infection might simultaneously engage both TLRs and NLRP3. Early pyroptosis and release of IL-18 may be important in decreasing intracellular pathogen burden.

Cooperation with other sensors

There are numerous reports of inflammasome activation involving more than one sensor[108–112]. Most of these reports include NLRP3 as one of the sensors. *Salmonella* species activate both NLRP3 and NLRC4[108], and the colocalization of NLRP3 and NLRC4 during infection has been shown by microscopic analysis[113]. The inflammasome response to lysophosphatidylcholine (also known as lysolecithin) in macrophages, microglia and astrocytes is also dependent on both of these sensors[111]. Malaria-causing species and *Aspergillus* species can activate both the NLRP3 and AIM2 inflammasomes[109,110]. During malaria infection, inflammasome activation depends on *Plasmodium* spp. genomic DNA (sensed by AIM2) and haemozoin (sensed by NLRP3)[109]. In mouse macrophages and DCs, cytosolic DNA and the DNA virus murine cytomegalovirus (MCMV) are sensed by AIM2 to form an inflammasome[114]. Cytosolic DNA is also sensed by the cGAS–STING pathway, which produces the secondary messenger cGAMP, which further promotes activation of the NLRP3 and AIM2 inflammasomes and is important for control of MCMV[112]. In contrast to murine macrophages and DCs and the human monocyte-like cell line THP1, AIM2 is dispensable for DNA-induced inflammasome activation in the human monocyte-like cell line BlaER1; in BlaER1 cells, DNA activates inflammasomes solely through a cGAS–STING–NLRP3 pathway[115]. It is currently unclear whether dual-sensing reflects two sensors activated in different cells, two sensors within the same cell each forming independent inflammasomes or two sensors cooperating to form a single inflammasome in the same cell.

PTM regulation of NLRP3

PTM by covalent addition of functional groups is important for the regulation of protein folding, localization and functional activities (FIG. 2). Various PTMs have been shown to regulate innate immunity through their effects on the activation, survival, proliferation, differentiation and migration of immune cells[116]. There is an emerging appreciation that priming not only upregulates NLRP3 expression, albeit in an inactive configuration, but also licenses NLRP3 to rapidly respond to activating stimuli. This non-transcriptional role of priming, as well as the subsequent activation phase, is at least partly regulated by PTMs.

Ubiquitylation. NLRP3 is ubiquitylated in resting macrophages and is deubiquitylated upon priming and activation[117]. F-box/LRR-repeat protein 2 (FBXL2), an SCF (SKP1-cullin-F-box) E3 ligase complex subunit, recognizes Trp73 of NLRP3 and targets it for ubiquitylation and proteasomal degradation[118]. LPS priming extends the half-life of NLRP3 by inducing another SCF subunit, FBXO3, which degrades FBXL2 (REF.[118]). In resting cells, the E3 ubiquitin ligase tripartite motif-containing protein 31 (TRIM31) interacts with the PYD of NLRP3 and induces Lys48-linked ubiquitylation and proteasomal degradation of NLRP3 (REF.[119]). LPS or IL-1β treatment further increases TRIM31 expression, suggesting that TRIM31 is a feedback suppressor of the NLRP3 inflammasome[119]. Dopamine signalling via dopamine D1 receptor increases intracellular levels of cAMP, which then binds to NLRP3 (REF.[120]). This promotes membrane-associated RING finger protein 7 (MARCH7)-dependent Lys48-linked ubiquitylation of the LRR domain of NLRP3, promoting its autophagic degradation[120]. Finally, BRCA1/BRCA2-containing complex subunit 3 (BRCC3) deubiquitylates the LRR domain of NLRP3 and is required for NLRP3 oligomerization and activation[121]. NLRP3 is not the only protein in the inflammasome that undergoes ubiquitylation. Linear ubiquitylation of ASC by the linear ubiquitin assembly complex is specifically required for NLRP3 inflammasome activation[122].

Phosphorylation. TLR priming induces the phosphorylation of NLRP3 on Ser198 (in human NLRP3; Ser194 in mouse NLRP3) by JUN N-terminal kinase 1 (JNK1; also known as MAPK8) and promotes NLRP3 self-association and activation[123]. Mechanistically, Ser198 (or Ser194) phosphorylation promotes BRCC3 binding and subsequent deubiquitylation[123]. After NLRP3 activator stimulation, protein kinase D (PKD) at the Golgi phosphorylates NLRP3 on Ser295 (in human NLRP3; Ser291 in mouse NLRP3) and promotes NLRP3 activation[124]. However, phosphorylation also can suppress NLRP3 activation. Phosphorylation at NLRP3 Ser5 (in human NLRP3; Ser3 in mouse NLRP3) within the PYD prevents inflammasome activation owing to electrostatic repulsion of the ASC PYD[125], whereas protein phosphatase 2A dephosphorylates Ser5 (or Ser3) to enhance NLRP3 nucleation with ASC[125]. In resting cells, NLRP3 is phosphorylated at Tyr861 (in human NLRP3; Tyr859 in mouse NLRP3)[126]. Upon activation, protein tyrosine

phosphatase, non-receptor type 22 (PTPN22) interacts with NLRP3 in an ASC-dependent manner and dephosphorylates NLRP3 at Tyr861 (in human NLRP3; Tyr859 in mouse NLRP3) to allow efficient NLRP3 activation[126]. Bile acids and prostaglandin E_2 can suppress the NLRP3 inflammasome by inducing protein kinase A (PKA)-mediated phosphorylation on NLRP3 Ser295 (in human NLRP3; Ser291 in mouse NLRP3)[127,128], which can occur both before and after NLRP3 activation[127]. Ser295 (or Ser291) phosphorylation attenuates NLRP3 ATPase activity and blocks NLRP3 activation[127]. It is currently unclear why PKA and PKD phosphorylate NLRP3 at the same site but have opposing effects, although Ser295 (or Ser291) phosphorylation in combination with other NLRP3 PTMs may determine the final effect, and further studies are warranted to clarify the effect.

Sumoylation. In resting cells, NLRP3 is sumoylated at multiple sites by the protein E3 SUMO protein ligase MUL1 (also known as MAPL), which restrains NLRP3 activation. Upon activation, NLRP3 becomes desumoylated by sentrin-specific protease 6 (SENP6) and SENP7, promoting inflammasome activation[129]. Notably, a defect in sumoylation of NLRP3 owing to a Lys689Arg mutation causes hyperactivation of NLRP3, which phenocopies the reported mutations found in patients with CAPS[129]. In summary, these studies highlight the delicate control of NLRP3 inflammasome activity by this dynamic landscape of PTMs.

NLRP3-interacting regulators

In addition to the proteins that form the inflammasome complex, such as ASC or pro-IL-1β, there are other proteins that interact with NLRP3. Pyrin-only proteins (POPs; also known as PYDC proteins) and CARD-only proteins (COPs) are small cytoplasmic decoy proteins that contain single homotypic protein-binding domains[130]. They function in the downregulation of inflammation. Both POPs and COPs are found only in primates. Of the four POPs (POP1–POP4), POP1 and POP2 regulate NLRP3 activation and are upregulated in response to NF-κB during priming. The PYDs of POP1 and POP2 have 64% and 37% identity, respectively, with the PYD of ASC, and in in vitro overexpression systems both POP1 and POP2 bind to ASC and inhibit the NLRP3–ASC interaction[130,131]. Similar to NF-κB, IL-1β also upregulates the expression of POP1 and POP2, thus forming a feedback loop to prevent excessive NLRP3 activation. Furthermore, POP2 negatively regulates NF-κB activation by regulating the non-canonical pathway during LPS priming[132]. Transgenic expression of POP1 and POP2 in mice reveals that they have functions beyond dampening of excessive inflammatory responses, as POP2-expressing mice are more resistant to bacterial infection than wild-type mice[133]. Mice in which monocytes, macrophages and DCs express transgenic POP1 are protected from death in an otherwise lethal LPS sepsis model and in a model of the autoinflammatory disease Muckle–Wells syndrome[134].

There are five human COPs that function to regulate caspase 1 activation. Three of them, COP1 (also known as CARD16), ICEBERG (also known as CARD18) and INCA (also known as CARD17), have 97%, 81% and 52% identity, respectively, to the CARD of caspase 1 and can bind to full-length caspase 1, thereby preventing its autoactivation and limiting inflammasome activation[130]. Because POPs and COPs are not expressed in mice, we are only beginning to understand their role during infections and diseases that activate NLRP3. With the recent development of transgenic POP mice, further studies towards the understanding of their role and regulation in diseases are expected.

Therapeutic targeting of NLRP3

An important focus of future research is to use our understanding of the molecular mechanisms of NLRP3 inflammasome activation to identify effective NLRP3 inhibitors (TABLE 2) or inhibitory pathways (FIG. 2) and assess their therapeutic potential. Excitingly, a number of NLRP3 inhibitors have been reported to date, including those that either directly inhibit NLRP3 or indirectly inhibit inflammasome components or related signalling events. A major caveat for using these inhibitors is that the inhibitory mechanism or precise target is not fully elucidated, thus representing a potential risk owing to off-target effects.

IL-1 signalling blockade is an example of successful clinical translation of basic immunology research and is currently being used in the treatment of NLRP3-driven immunopathologies. Three biologics are approved by the US Food and Drug Administration for multiple inflammatory diseases: canakinumab, an IL-1β-neutralizing antibody; anakinra, a recombinant IL-1 receptor antagonist; and rilonacept, a decoy receptor that binds IL-1β and IL-1α[135]. Two similar agents, including GSK1070806, an IL-18 blocking antibody, and MABp1, an IL-1α-neutralizing antibody, are under early development[136]. Given the initial remarkable therapeutic benefits of these approved biologics in patients with CAPS, the clinical indications of IL-1 blockade are expanding continuously. However, it is important to note that IL-1β blockade or IL-1 receptor type 1 (IL-1R1) deficiency failed to rescue the lethality in a mouse model of CAPS, suggesting that other inflammatory mediators released during NLRP3 activation may be important for disease progression[137].

Directly targeting NLRP3 by small molecules is specific, cost-effective and less invasive than cytokine blockade. Several such inhibitors have been discovered to date (TABLE 2). The diarylsulfonylurea compound MCC950 (originally reported as CRID3/CP-456773)[138] is the most potent and specific NLRP3 inhibitor. MCC950 specifically inhibits canonical and non-canonical NLRP3 inflammasome activation in both mouse and human macrophages in vitro without impairing the NLRP1, NLRC4 and AIM2 inflammasomes or TLR-mediated priming signals[139]. Of note, MCC950 is not able to inhibit the release of IL-1α and prevent cell death in response to particulate stimuli and transfected LPS because these events are NLRP3-independent. Strikingly, MCC950 demonstrated therapeutic efficacy against a variety of preclinical immunopathological models, including CAPS, experimental autoimmune encephalomyelitis[139], Alzheimer disease[140], traumatic brain injury[141], atherosclerosis[142], cardiac arrhythmias[143],

Table 2 | **NLRP3 inhibitors**

Inhibitor	~IC_{50}[a]	Inhibition mechanism	Specificity	Inhibition of priming	Clinical status	Refs
MCC950 (CP-456773)	8 nM	Unknown	NLRP3	No	Phase II	139
CY-09	5 μM	Binds Walker A motif; NACHT ATPase inhibitor	NLRP3	No	–	148
Oridonin	0.5 μM	Irreversibly binds NLRP3 Cys279 and inhibits the NLRP3–NEK7 interaction	NLRP3	No	–	153
Tranilast	25–50 μM	Binds NACHT and inhibits the NLRP3–NLRP3 interaction	NLRP3	Yes	Approved	151
MNS	2 μM	NACHT ATPase inhibitor	NLRP3	No	–	176
OLT1177 dapansutrile	• 1–100 nM (mouse) • 1 μM (human)	NACHT ATPase inhibitor	NLRP3	No	Phase II	177
Bay 11-7082	5 μM	NACHT ATPase inhibitor	• NLRP3 • NLRC4	Yes	–	148,178
BOT-4-one	0.59–1.28 μM	Alkylation; NACHT ATPase inhibitor	• NLRP3 • NLRC4	Yes	–	179
Parthenolide	5 μM	NACHT ATPase inhibitor and caspase 1 inhibitor	• NLRP3 • AIM2 • NLRC4 • NLRP1	Yes	–	148,178
INF39	10 μM	NACHT ATPase inhibitor	NLRP3	Yes	–	149

IC_{50}, median inhibitory concentration; NEK7, NIMA-related kinase 7; NLRC4, NOD-, LRR- and CARD-containing 4; NLRP, NOD-, LRR- and pyrin domain-containing protein. [a]Approximate IC_{50} of inhibitor in vitro.

myocardial infarction[144], diabetes[145], steatohepatitis[146] and colitis[147]. Although these studies provide compelling rationale for targeting NLRP3, a phase II clinical trial of MCC950 for rheumatoid arthritis was suspended owing to hepatic toxicity[4].

C172, an inhibitor for cystic fibrosis transmembrane conductance regulator channel (CFTR), and its analogue CY-09 were recently recognized as NLRP3 inhibitors in screening libraries of bioactive compounds[148]. Because CY-09 does not have CFTR-inhibitory activity, thus reducing its risks of off-target effects, it can move forward to preclinical trials[148]. CY-09 specifically inhibits the NLRP3 inflammasome but not the NLRC4 and AIM2 inflammasomes nor TLR-mediated priming signals[148]. Mechanistically, CY-09 binds to the ATP-binding Walker A motif of NLRP3 and inhibits its ATPase activity and oligomerization[149].

Tranilast, an analogue of a tryptophan metabolite, has been approved for use in treating allergy, asthma and hypertrophic scars in Korea and Japan since 1982 (REF.[150]). Only recently was its mechanism uncovered, showing that tranilast binds to the NACHT domain of NLRP3, abolishing the NLRP3–NLRP3 interaction and oligomerization without affecting its ATPase activity. Thus, it specifically inhibits NLRP3 inflammasomes but not AIM2 or NLRC4 inflammasomes[151]. Tranilast also inhibits LPS-induced pro-IL-1β and IL-6 release[147], suggesting that tranilast suppresses inflammation via a multi-target mechanism.

Isodon rubescens is an over-the-counter herbal medicine that is used for the treatment of inflammatory diseases in China[152]. Oridonin, an ent-kaurane diterpenoid, is the main ingredient in *Isodon rubescens*, and it has been shown to specifically inhibit the NLRP3 inflammasome but not AIM2 and NLRC4 inflammasomes[153]. Oridonin irreversibly binds NLRP3 Cys279 via a covalent carbon–carbon bond and blocks the NLRP3–NEK7 interaction[140]. Although oridonin has NF-κB and MAPK inhibitory effects, the dose required for this was ten times higher than that needed for NLRP3 inhibition[154]. CY-09, tranilast and oridonin also showed remarkable therapeutic effects in mouse models including CAPS, peritonitis and type 2 diabetes. Many other direct inhibitors including MNS, OLT1177, Bay 11-7082, BOT-4-one, parthenolide and INF39 target NLRP3 by inhibiting its ATPase activity; however, they have not been tested in vivo in NLRP3-dependent models[148]. Additionally, Bay 11-7082, BOT-4-one, parthenolide and INF39 have multiple biological activities and are thus unlikely to serve as specific NLRP3 inhibitors.

Several inhibitors have been shown to be ineffective in the treatment of NLRP3-associated diseases. The $P2X_7$ inhibitors AZD9056, CE-224535 and GSK1482169 were unsuccessful in the treatment of rheumatoid arthritis[135]. Glyburide is commonly used in the treatment of type 2 diabetes and acts by inhibiting ATP-sensitive K^+ channels in pancreatic β-cells and stimulating insulin release. Although glyburide potently inhibits NLRP3 activation in vitro, it requires high doses in vivo, and the mechanism of action is unknown. Additionally, the associated hypoglycaemia and perturbation of glucose metabolism limit the use of this inhibitor beyond diabetes[136]. Two caspase 1-inhibiting peptidomimetic pro-drugs, VX-740 and VX-765, reached phase II clinical trials for arthritis, epilepsy and psoriasis but were discontinued owing to hepatic toxicity[155].

Conclusions and future directions

The study of NLRP3 inflammasome activation is a rich field in immunology, with rapidly emerging insight into its mechanism of action and regulation. Discoveries of the NEK7–NLRP3 interaction, GSDMD as the pyroptosis executor and the roles for ionic flux, mitochondrial dysfunction, metabolic alteration and Golgi disassembly in NLRP3 activation represent major advances in this field.

While refinement of our understanding of NLRP3 activation continues, targeting of NLRP3 as a therapeutic for multiple diseases is rapidly progressing. Current treatment for NLRP3 pathologies focuses on inhibition of the inflammasome-derived cytokine IL-1β. Although this has been highly effective, it is not without problems. Inflammasome activation is critical for immune control of numerous pathogens, and therefore loss of IL-1β can have detrimental effects on immune defence. Many of the new therapeutics advancing to clinical trials are specific for NLRP3 activation and do not affect the function of other inflammasome sensors. With increases in the number of individuals affected by inflammatory conditions because of the Western lifestyle and ageing population, it is likely that there will be a greater need for NLRP3-specific therapeutics. Biochemical studies on structure and phase separation will unravel the molecular principles underlying inflammasome assembly. Continued profiling, refinement and repurposing of direct and specific NLRP3 inhibitors will boost future clinical translation, epitomizing the use of precision medicine in inflammasome-related disorders.

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