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Review

NLRP3 and pyroptosis blockers for treating inflammatory diseases

Rebecca C. Coll,[1,*] Kate Schroder,[2,*] and Pablo Pelegrín[3,*]

The nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing protein 3 (NLRP3) inflammasome has emerged as a key mediator of pathological inflammation in many diseases and is an exciting drug target. Here, we review the molecular basis of NLRP3 inhibition by drug-like small molecules under development as novel therapeutics. We also summarize recent strategies to block pyroptosis as a novel approach to suppress chronic inflammation. Major recent developments in this area include the elucidation of mechanisms of action (MoAs) by which small molecules block NLRP3 inflammasome assembly and gasdermin D (GSDMD)-induced pyroptosis. We also discuss the status of clinical trials using agents that block specific components of the NLRP3 pathway, including their potential clinical applications for the treatment of many diseases.

Highlights

The nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing protein 3 (NLRP3) inflammasome is a key mediator of pathological inflammation in many diseases.

There are several drug-like small molecules under clinical development as novel therapeutics targeting the NLRP3 inflammasome.

Pyroptosis is a unique approach to suppress chronic inflammation.

Understanding the mechanism of action by which small molecules block the NLRP3 inflammasome allows better molecular optimization.

Different clinical trials are running using agents that inhibit the NLRP3 inflammasome.

Inflammasomes are novel targets for anti-inflammatory therapies

NLRP3 (see Glossary) **inflammasome** is an intracellular protein complex that has emerged as a key mediator of inflammation in many pathologies. NLRP3 activation by microbes and danger signals (Figure 1) leads to the release of the proinflammatory cytokines **IL-1**β and IL-18, as well as to **GSDMD**-mediated pyroptosis, a proinflammatory type of cell death (Box 1).

The past 5 years have seen rapid progress in the development of small-molecule inhibitors targeting the NLRP3 inflammasome pathway, with several NLRP3 and GSDMD inhibitors alleviating pathology in more than 100 preclinical disease models [1,2] (Tables 1 and 2 and Figure 2). These promising findings have fueled the rapid progression of NLRP3 inhibitors through human trials toward the clinic (Box 2), with GSDMD inhibitors closely following. Thus, it is both important and timely to review the landscape of emerging inflammasome drug candidates and their potential clinical applications. It is becoming particularly pressing to understand which inflammasome inhibitors to prioritize for trials in specific disease indications. Here, we discuss priority disease areas for NLRP3 inflammasome blockers, as well as the advantages and disadvantages of directly blocking the inflammasome (via NLRP3 inhibition) versus suppressing GSDMD-induced pyroptosis.

The inflammasomes

The inflammasome pathway is an important innate immune effector mechanism triggered in response to infection and tissue injury. Inflammasomes elicit an inflammatory response that aims to eliminate invading pathogens and repair damaged tissues, restoring homeostasis. Inflammasome assembly involves a series of protein oligomerization events induced after the recognition of specific molecular patterns from pathogens, cell damage, or altered homeostatic conditions, resulting in the formation of large multiprotein signaling platforms that activate the protease caspase-1 (Figure 1). The best-described inflammasomes are formed by the following sensor proteins: (i) nucleotide-binding oligomerization domain with leucine-rich repeat (NLR) and pyrin domain-containing 1 (NLRP1); (ii) NLRP3 (Figure 1); (iii) NLRP6; (iv) NLR family apoptosis inhibitory protein (NAIP), which

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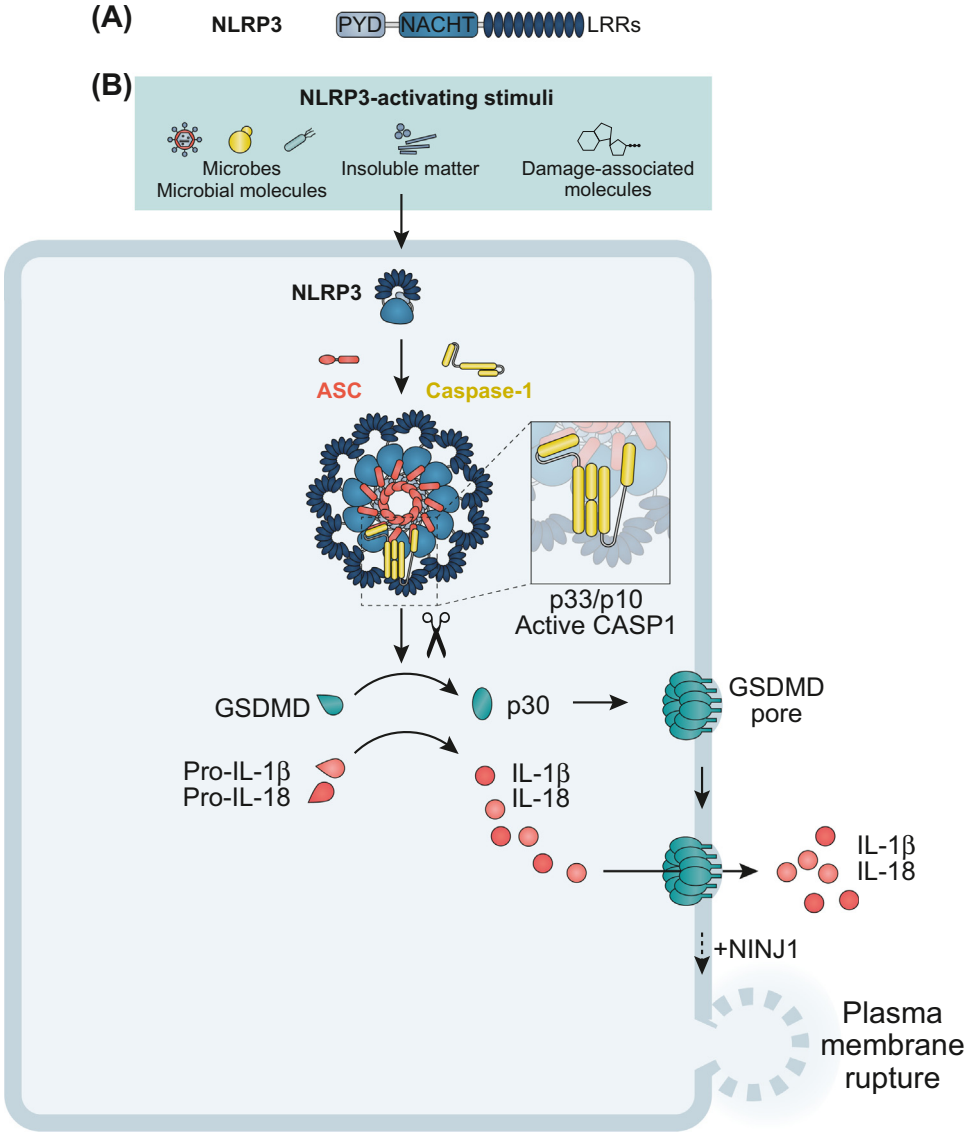


Figure 1. The nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing protein 3 (NLRP3) inflammasome signaling pathway, which can result in cell pyroptosis. (A) Domain structure of NLRP3, with an N-terminal Pyrin domain (PYD), a central NACHT domain, and a C-terminal leucine-rich repeat (LRR) domain. (B) A range of stimuli, including microbes, particulate matter (misfolded proteins, crystals, and nanoparticles), and damage-associated molecules (e.g., extracellular ATP), induce the formation of NLRP3 oligomers and allow recruitment of the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) and the zymogen caspase-1, leading to activation of caspase-1 and the cleavage of gasdermin D (GSDMD). Caspase-1 also processes pro-IL-1β and pro-IL-18 to their mature forms. The N-terminal domain of GSDMD (p30) forms pores in the plasma membrane and facilitates the release of IL-1β and IL-18. The plasma membrane protein ninjurin-1 (NINJ1) mediates the extent of plasma membrane rupture, inducing pyroptotic cell death. Abbreviation: CARD, caspase activation and recruitment domain.

functions in concert with the NLR family, caspase activation and recruitment domain (CARD)-containing 4 (NLRC4); (v) absent in melanoma 2 (AIM2); and (vi) Pyrin (PYD) [3]. These inflammasome sensor proteins recognize distinct perturbations of homeostasis and form oligomers able to recruit the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) into the

Glossary

Cryopyrin-associated periodic syndromes (CAPS): rare autoinflammatory disorders resulting from gain-of-function mutations in *NLRP3* and characterized by periodic fevers, sterile urticaria, and joint inflammation. CAPS incorporate three overlapping disease entities: familial cold autoinflammatory syndrome (FCAS), Muckle–Wells syndrome (MWS), and neonatal-onset multisystem autoinflammatory syndrome (NOMID).

Gasdermin D (GSDMD): pore-forming protein that induces pyroptosis downstream of inflammasome activation.

Inflammasome: signaling complex that activates inflammatory caspases. Most inflammasomes are multiprotein assemblies that activate the caspase-1 protease. The so-called 'non-canonical inflammasome' is a protein–lipid assembly that activates the protease function of caspase-11 in mice and caspase-4 and caspase-5 in humans.

Interleukin-1 (IL-1): a family of 11 cytokines that includes IL-1β and IL-18. IL-1β and IL-18 are produced as inactive precursor proteins that require caspase-1 cleavage to generate their secreted, bioactive form.

NACHT: NAIP (NLR family apoptosis inhibitory protein), CIITA (i.e., C2TA or MHC class II transcription activator), HET-E (incompatibility locus protein from *Podospora anserina*), and TEP1 (i.e., TP1 or telomerase-associated protein) is the central nucleotide-binding and oligomerization domain of NLR receptors, including NLRP3. This acronym reflects some of the proteins that contain this particular domain.

Nucleotide-binding oligomerization domain, leucine-rich repeat receptor and pyrin-domain containing-protein 3 (NLRP3): sensor protein of the NLRP3 inflammasome.

Purinergic receptor type 2, family X, subunit 7 (P2X7): ATP-gated cationic receptor that effluxes potassium from the cells, inducing NLRP3 inflammasome activation.

Box 1. Pyroptosis: a gasdermin-meditated type of inflammatory cell death

Pyroptosis is a type of programmed necrotic cell death characterized by the permeabilization of the plasma membrane by gasdermin protein family members, leading to the subsequent release of intracellular contents (for recent review, see [4]). The presence of cytosolic constituents in the extracellular milieu is a signal that the innate immune system recognizes through specific receptors that elicit an inflammatory response aimed to repair tissue damage. For example, proinflammatory cytokines of the IL-1 family, such as the alarmin IL-1β, lack a signal peptide and, thus, their cellular release requires permeabilization of the plasma membrane [112]. Gasdermins are an evolutionary conserved family of proteins that share two well-defined structural domains: an N-terminal domain with intrinsic pore-forming properties and a C-terminal domain that represses the pore-forming activities of the N-terminal domain, connected by a linker sequence [4]. Linker cleavage of gasdermin proteins liberates the N-terminal fragment, which oligomerizes and forms pores in cellular membranes, including the plasma membrane. Several distinct proteases cleave the linker of specific gasdermin family members. GSDMD is processed by caspase-1/-4/-5/-8/-11 and neutrophil elastase, whereas GSDMB is processed by granzyme A, and GSDME is cleaved by caspase-3 [4,113,114]. GSDMD plasma membrane pores are positively regulated by ROS [40] and negatively regulated by the scission of damaged membranes by the endosomal sorting complexes required for transport (ESCRT) machinery [4]. GSDMD pores are a negatively charged conduit for the direct passage of mature IL-1 cytokines [115], which have a neutral or positively charged surface [37]. In many settings, the extent of plasma membrane rupture induced by GSDMD pores in the dying cells is regulated by the protein ninjurin-1 [116], leading to the release of intracellular contents, including HMGB1, inflammasome oligomers, and mitochondrial DNA, or even inducing the expulsion of neutrophil nuclear DNA leading to NETosis [4,39,115,117].

inflammasome through PYD–PYD or CARD–CARD homotypic domain interactions (Figure 1). ASC then oligomerizes to form helical filaments in which the zymogen pro-caspase-1 is recruited via CARD–CARD domain interactions to favor its activation [3]. Caspase-1 subsequently induces the proteolytic maturation of proinflammatory cytokines of the IL-1 family, such as IL-1β and IL-18 (Figure 1) [3]. Caspase-1 also processes the protein GSDMD, which releases its lytic N-terminal domain (N-GSDMD), which binds to the plasma membrane and oligomerizes to form pores, allowing the release of mature IL-1β and IL-18 (Figure 1). If the burden of plasma membrane pores is sufficiently high, this results in a proinflammatory necrotic type of cell death called pyroptosis (Box 1) [4]. During pyroptosis, the release of intracellular contents, including inflammasome oligomers, amplifies the inflammatory response and contributes to inflammatory pathology [5].

Among inflammasomes, the NLRP3 inflammasome is the most promiscuous, because it can be activated in response to diverse triggers, including pathogen- and danger-associated molecular patterns. Activators of the NLRP3 inflammasome include an array of viral and bacterial proteins (e.g., bacterial toxins, such as nigericin or viroporins), intracellular molecules released into the extracellular fluid upon cellular damage (such as extracellular ATP), which engages the **purinergic receptor type 2, family X, subunit 7 (P2X7)**, and insoluble particles [such as uric acid crystals, amyloid-β (Aβ) depositions, or silica particles] [2,3]. Most of these triggers activate NLRP3 by inducing a decrease in the cytosolic concentration of potassium ions (K^+), which promotes a conformational change in the inactive NLRP3 oligomer, allowing it to interact with negatively charged lipids in the dispersed *trans*-Golgi network, thereby promoting the formation of active NLRP3 oligomers that initiate inflammasome signaling [6,7]. NLRP3 can be also activated through the non-canonical inflammasome pathway, in which intracellular bacterial lipopolysaccharide (LPS) and arachidonic acid-derived oxidized phospholipids activate caspase-4/5 (caspase-11 in mice). These caspases cleave GSDMD, promoting pore formation by inducing the insertion of the N-GSDMD into the plasma membrane. GSDMD pores allows K^+ efflux and the subsequent activation of the NLRP3 inflammasome, activating caspase-1. In the non-canonical inflammasome pathway, the activation of IL-1β and IL-18 depends on caspase-1, whereas the activation of GSDMD and pyroptosis development depends on caspase-4/5/11 [2].

Pathological functions for NLRP3 signaling in humans were first discovered in studies that characterized a group of rare autoinflammatory diseases called **cryopyrin-associated periodic syndromes (CAPS)**. These patients harbor gain-of-function mutations in *NLRP3* that promote

Table 1. NLRP3 inhibitors[a]

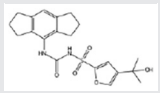
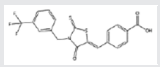
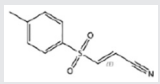
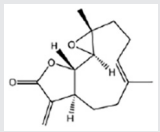
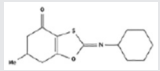
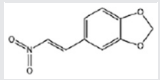
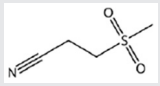
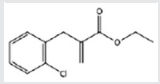
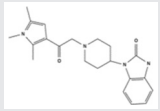
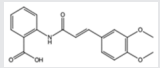
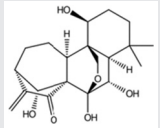
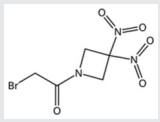
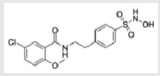
Inhibitor	MoA	IC_{50}[b]	Off-target effects (IC_{50})[c]	NLRP3-associated disease model[d]
MCC950 	Binds to NACHT domain, locking NLRP3 in an inactive conformation Inhibits ATPase activity	7.5 nM	Carbonic anhydrase 2 (11 μM)	AD, PD, EAE, ischemic stroke, TBI, T2DM, age-related metabolic syndrome, diabetes-related co-morbidities: nephropathy, endothelial dysfunction, atherosclerosis, retinopathy, encephalopathy, cognitive impairment, ischemia–reperfusion injury, liver injury; NAFLD, NASH, liver fibrosis, IBD, colitis, and models reviewed in [1]
CY-09 	Binds to Walker A site in NACHT domain Inhibits ATPase activity	6 μM	Cytochrome P450 enzymes 1A2 (19 μM), 2C9 (8 μM), 2C19 (>50 μM), 2D6 (>50 μM), 3A4 (26 μM)	Stroke, T2DM, diabetic retinopathy, diabetic liver injury, NAFLD, peritonitis, Muckle–Wells syndrome, pain, osteoarthritis, myocardial infarction, epilepsy, osteosarcoma, acute kidney injury, hemolytic uremic syndrome
Bay-11-7082 	Inhibits ATPase activity Binding site unknown	~3 μM	IKK (10 μM), USP7 (0.19 μM), USP21 (0.96 μM)	TBI, spinal cord injury, T2DM, IBD, psoriasis, lupus nephritis, acute kidney injury, acute lung injury, wound healing
Parthenolide 	Inhibits ATPase activity Binding site unknown	1.4 μM	IKK-β (7.5 μM), caspase-1 (10 μM), Hsp72 (>10 μM), FAK1 (>10 μM), RPL10 (>10 μM), JAK2 (4 μM)	N/A
BOT-4-one 	Inhibits ATPase activity Binding site unknown	1.28 μM	IKK-β (~10 μM)	Peritonitis, dermatitis, psoriasis, arthritis
MNS 	Inhibits ATPase activity Binding site unknown	~3 μM	Syk (2.5 μM), Src kinase (29.3 μM), p97 (1.7 μM)	Burn wound healing, airway inflammation
OLT1177 	Inhibits ATPase activity Binding site unknown	ND	*In vivo* NF-κB, IκB, IKK Increases oxidative metabolism *in vivo*	AD, EAE, colitis, arthritis, allergic asthma, acute kidney injury
INF39 	Inhibits ATPase activity Binding site unknown	~10 μM	Unknown	Spinal cord injury, IBD, colitis, drug-induced liver injury, pancreatitis, myocardial infarction
HS-203873 	Inhibits ATPase activity by competing with ATP binding	~50 μM	Unknown	N/A
Tranilast 	Binds to NACHT disrupting NLRP3–NLRP3 interactions and blocking oligomerization	~25 μM	Numerous, e.g., TRPV2 (<75 μM), inhibition of histamine (100 μM), PGE2 (30 μM), and cytokine release (TGF-β, IL-6, IL-8, IL-5), collagen synthesis	T2DM, NASH, colitis, peritonitis, Muckle–Wells syndrome, atherosclerosis, nanoparticle cerebral toxicity, myocardial infarction
Oridonin 	Covalently modifies Cys279 in NACHT and attenuates NLRP3–NEK7 interaction	0.75 μM	Numerous, e.g., AKT1 (8.4 μM), AKT2 (8.9 μM), HSF1, cMyc, NF-κB, JAK/STAT, p38 MAPK	TBI, T2DM, alcohol-induced hepatotoxicity, liver fibrosis, IBD, ischemia–reperfusion injury, peritonitis, gouty arthritis, myocardial infarction, hemolytic uremic syndrome, acute lung injury, acute liver injury, ventilator-induced lung injury, hearing loss, pleurisy

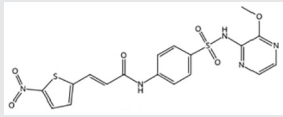
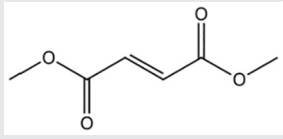
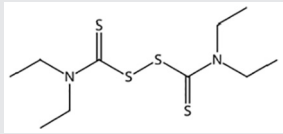
Table 1. (continued)

Inhibitor	MoA	IC_{50}[b]	Off-target effects (IC_{50})[c]	NLRP3-associated disease model[d]
RRx-001 	Binds to Cys409 in NACHT and attenuates NLRP3–NEK7 interaction	117 nM	Numerous, e.g., inhibition of glucose 6-phosphate dehydrogenase and NF-κB, hemoglobin binding, apoptosis	EAE, DSS colitis, allergic asthma
JC-171 	Attenuates NLRP3–ASC interaction. Binding site unknown	8.45 μM	Unknown	EAE

[a]Abbreviations: AKT kinase, protein kinase B; DSS, dextran sulfate sodium; EAE, experimental autoimmune encephalomyelitis; FAK1, focal adhesion kinase 1; HSF1, heat shock factor 1; Hsp72, heat shock protein 72; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; N/A, not applicable; NAFLD, nonalcoholic fatty liver disease; ND, Not determined ; PGE2, prostaglandin E2; RPL10, ribosomal protein L10; STAT, signal transducer and activator of transcription; SYK, spleen tyrosine kinase; TBI, traumatic brain injury; TGF-β, transforming growth factor-β; TRPV2, transient receptor potential cation channel subfamily V member 2; USP, ubiquitin-specific protease.
[b]IC_{50} values are included for IL-1β release from mouse macrophages stimulated with LPS and ATP or nigericin.
[c]Known off-target inhibitory effects are summarized with IC_{50} values presented where possible.
[d]Disease models in which inhibitors have been effective are summarized.

inflammasome activation and result in an inflammation-related phenotype characterized by periodic fevers, sterile urticaria, and joint inflammation [2,3]. The wild-type NLRP3 inflammasome is activated and contributes to the inflammatory response during infection with viruses [including influenza, severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), or chikungunya), fungi (e.g., *Candida albicans*), and bacteria [2,8]; here, the NLRP3 inflammasome not only mediates an important innate immune defense mechanism, but can also be responsible for

Table 2. GSDMD inhibitors[a]

Inhibitor	MoA	IC_{50}[b]	Off-target effects (IC_{50})[c]	GSDMD-associated disease model[d]
Necrosulfonamide 	Binds directly to C191 blocking GSDMD oligomerization	~10 μM	MLKL (0.2 μM)	LPS-induced sepsis, LPS-induced pulmonary fibrosis
Dimethyl fumarate 	Succinates C191, blocking GSDMD oligomerization	<10 μM	Numerous, e.g., aldehyde dehydrogenase (0.15–1.45 μM), dopamine beta-hydroxylase (<10 μM), caspase-1 (~0.25 μM), caspase-3 (~2 μM), GSDME (<10 μM)	LPS-induced sepsis, familial Mediterranean fever, autoimmune encephalitis
Disulfiram 	Modifies C191, blocking GSDMD oligomerization	~10 μM	Numerous, e.g., activates Nrf2, inhibits NFkB signaling, GAPDH, cyclooxygenase 2, NLRP3 signaling	LPS-induced sepsis, pneumonia, renal fibrosis, gouty arthritis
Antioxidants and mTORC1 inhibitors	Block ROS-induced GSDMD oligomerization	N/A	N/A	N/A

[a]Abbreviations: GAPDH, glyceraldehyde 3-phosphate dehydrogenase; N/A, not applicable; Nrf2, nuclear factor erythroid 2-related factor 2.
[b]IC_{50} values are included for inflammasome-stimulated cell death.
[c]Known off-target inhibitory effects are summarized, with IC_{50} values presented where possible.
[d]Disease models in which inhibitors have been effective are summarized.

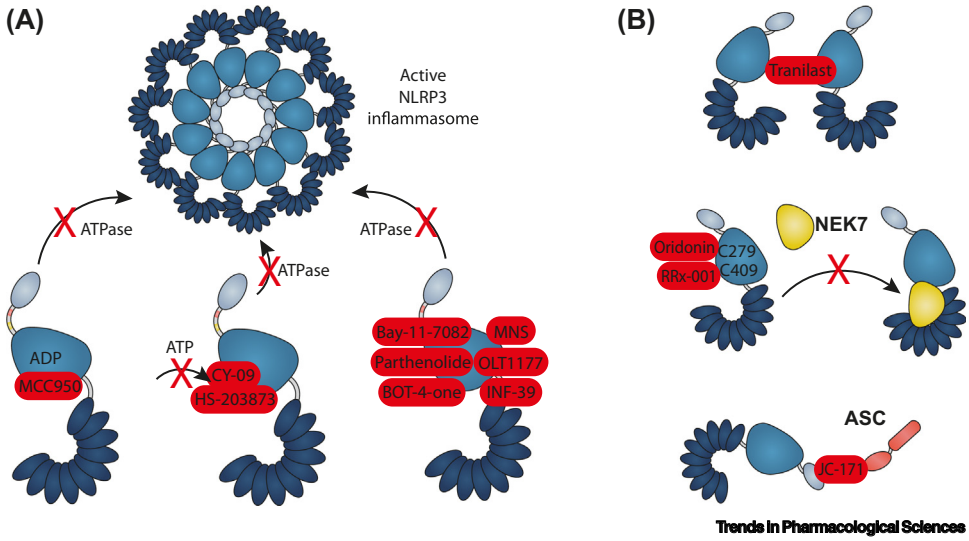


Figure 2. Mechanism of action of nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing protein 3 (NLRP3) inhibitors. (A) Inhibitors that block the ATPase activity of NLRP3. MCC950 binds to the NACHT domain, preventing NLRP3 ATPase activity and keeping NLRP3 inactive [14–18]. CY-09 and HS-203873 compete with ATP binding, preventing ATPase activity [22,30,31]. BAY-11-7082, parthenolide, 3,4-methylenedioxy-β-nitrostyrene (MNS), OLT1177, BOT-4-one, and INF-39 all inhibit the ATPase activity of NLRP3, but their binding sites are not yet described [23,24,26–28]. (B) Inhibitors that block NLRP3 protein–protein interactions. Tranilast binds to NACHT and inhibits NLRP3–NLRP3 interactions blocking NLRP3 oligomerization [33]. Oridonin binds to C279 and RRx-001 binds to C409, both of which attenuate Never in mitosis gen A-related kinase 7 (NEK7)–NLRP3 interactions [32,34]. JC-171 attenuates NLRP3–adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) interactions [35]. Abbreviation: CARD, caspase activation and recruitment domain.

immunopathology in some infection settings. NLRP3 targeting would be expected to have fewer immunosuppressive effects compared with existing anti-IL-1 therapies, because different pathogen-recognizing inflammasomes, such as NLRP1 and NLRC4, could still produce IL-1β [2]. One peculiarity of the NLRP3 inflammasome is its propensity for dysregulation during the initiation and course of many chronic inflammatory diseases, including gout, rheumatoid arthritis, and inflammatory bowel disease (IBD) [2]. The pathophysiological functions of the NLRP3 inflammasome are also emerging in several metabolic and neuroinflammatory diseases, in which NLRP3 appears to drive a subclinical inflammatory state several years before clinical symptoms

Box 2. Early clinical trials of NLRP3 blockers

Five companies have initiated clinical trials with specific NLRP3 inhibitors, although the results from most of these are not yet published. IFM Tre was acquired by Novartis in 2019, and their molecule IFM 2427 (later named DFV890) has reportedly completed Phase I safety trials. DFV890 will be tested in Phase II trials for the CAPS subtype familial cold autoinflammatory syndrome (NCT04868968) and knee osteoarthritis (NCT04886258). DFV890 has completed a Phase II trial for coronavirus disease 2019 (COVID-19) pneumonia (NCT04382053). DFV890 administrated to patients with impaired respiratory function because of COVID-19 pneumonia had no effect on disease severity as measured by the Acute Physiology and Chronic Health Evaluation II score, serum C-reactive protein levels, clinical status, or requirement for mechanical ventilation. Inflazome tested two molecules in Phase I trials. Inzomelid (IZD174; NCT04086602) is a brain-penetrant molecule, whereas Somalix (IZD334) is peripherally restricted and was tested in a Phase I and a Phase IB trial in CAPS (NCT04015076). No results from these trials have been released, but Roche acquired Inflazome in 2020[i] and Phase II trials might be initiated in the near future. In June 2020, NodThera[ii] announced a Phase I trial with their NLRP3 inhibitor NT-0167, but no further information on this trial is available. Recently, NodThera announced that they initiated a Phase I safety trial with a lead NLRP3 inhibitor NT-0796 (ACTRN12621001082897)[iii,iv]. Zydus Cadila developed MCC950-derivative molecules [20,36] and has initiated a Phase I safety trial for their molecule ZYIL1 (NCT04972188)[v,vi]. In addition, OLT1177 (aka dapansutrile) is being developed by Olatec Therapeutics and has concluded a Phase IB trial (NCT03534297) with no serious reported adverse events, in which it improved left ventricular ejection fraction in patients with heart failure and reduced ejection fraction. Dapansutrile was also tested in a Phase IIA trial in which it reduced joint pain during gout flares (EudraCT 2016-000943-14) [96,118].

appear. Therefore, NLRP3 is involved in the pathogenesis of atherosclerosis, diabetes, metabolic syndrome, and nonalcoholic steatohepatitis (NASH), as well as in pathologies of the central nervous system, including Alzheimer's (AD) and Parkinson's disease (PD), multiple sclerosis (MS), and stroke [2].

Drug-like small molecules blocking NLRP3

Numerous small molecules that directly or indirectly inhibit NLRP3 signaling have been reported. Here, we focus on direct inhibitors of NLRP3 the MoAs of which are identified (Table 1 and Figure 2). The **NACHT** domain mediates the oligomerization of active NLRP3 and contains a nucleotide-binding domain (NBD) that hydrolyzes ATP. ATP binding and hydrolysis are essential for NLRP3 function, because mutating the Walker A ATP-binding site blocks NLRP3 signaling [9]. Disrupting the ability of NLRP3 to hydrolyze ATP is a common mode of action for several small molecules (Table 1 and Figure 2).

Before the description of the inflammasome in 2002, research at Pfizer identified a group of sulfonylurea-containing compounds (e.g., glyburide) that blocked IL-1β processing in response to prototypical NLRP3 stimuli, such as LPS and ATP [10]. Based on these studies, several cytokine release inhibitory drugs (CRIDs) were developed that blocked IL-1β release from human monocytes with reported IC_{50}s in the nanomolar range [11]. In 2009, Lamkanfi and colleagues linked sulfonylureas to NLRP3 when they demonstrated that glyburide specifically inhibited NLRP3 signaling [12]. Building on these observations, Coll *et al.* [13] investigated one of the compounds developed at Pfizer called CRID3 (aka CP-456,773), renaming this compound MCC950. The authors demonstrated that MCC950 (CRID3) was a nanomolar potent and specific inhibitor of NLRP3 signaling that was active in several models of inflammatory disease (Table 1 and Figure 2) [13]. MCC950 (CRID3) has since become the most widely used tool compound for NLRP3 inhibition in research studies, with MCC950 being the more commonly used name for this compound. MoA studies demonstrated that MCC950 directly and noncovalently interacts with the NACHT domain (Table 1 and Figure 2) [14–16]. MCC950 blocks the ATPase activity of NLRP3 [14], and intramolecular bioluminescence resonance energy transfer (BRET) assays show that MCC950 can shift NLRP3 from an active to an inactive conformation [15].

Recently, the biophysical basis of the MoA of MCC950 has become clearer, with reports of the cryo-electron (cryo-EM) microscopy structure of full-length human NLRP3 in complex with ADP and MCC950 [17] and the crystal structure of the human NLRP3 NACHT domain in complex with the MCC950 derivative NP3-146 [18]. Both of these structures of inactive NLRP3 observe that, remarkably, MCC950/NP3-146 forms interactions with multiple subdomains in NACHT, including the NBD, helical domain 1 (HD1), winged-helix domain (WHD), and helical domain 2 (HD2). Within this binding pocket, the central sulfonylurea of MCC950/NP3-146 forms a hydrogen bond with the Walker A site and also interacts with two key arginine residues (R351 and R578). These structures reveal how MCC950/NP3-146 specifically locks NLRP3 in an inactive state, preventing ATP hydrolysis and the structural rearrangements necessary to form an active oligomer that nucleates inflammasome assembly. Importantly, MCC950 also interacts with active NLRP3 and inhibits hyperactive CAPS-mutant NLRP3 [14,15]. The structural basis for the ability of MCC950 to inhibit the active NLRP3 conformer remains to be determined. However, MCC950 does not inhibit the mouse NLRP3 L351P hyperactive mutation (corresponding to human L353P) because this mutation affects MCC950 binding to NLRP3 [16].

Several MCC950 derivative molecules that potently inhibit NLRP3 are reported, including *N*-cyanosulfoximineurea derivatives [19] and alkenyl sulfonylurea derivatives [20]. These derivatives may have a similar MoA to MCC950. As mentioned above, the type 2 diabetes mellitus (T2DM) drug,

glyburide, is also a sulfonylurea that inhibits NLRP3 [12] and several other sulfonylureas, such as sulofenur and glimepiride, can also inhibit NLRP3 signaling [21]. Interestingly, when the cyclohexylurea group of glyburide is removed, it can still inhibit NLRP3 signaling [12]; thus, the MoA of these sulfonylureas might be distinct from that of MCC950, and further investigations are required to determine whether they directly interact with NLRP3.

Several covalent modifiers of the NLRP3 NACHT are now described. A compound screen for NLRP3 inhibitors identified the cystic fibrosis transmembrane conductance regulator (CFTR) channel inhibitor C172, which prompted the authors to test the capacity of related compounds to inhibit NLRP3 activity [22]. They identified CY-09, an analog of C172 that inhibits NLRP3 in the low micromolar range without affecting CFTR function. CY-09 directly interacts with NLRP3, as shown by microscale thermophoresis assays and interaction studies with biotinylated-CY-09 (Table 1 and Figure 2). CY-09 appears to compete with ATP for binding at the Walker A site to inhibit the ATPase activity of recombinant NLRP3 [22].

Several other compounds block the ATPase activity of NLRP3, but their binding sites are not yet identified. The vinyl sulfone Bay 11-7082, the sesquiterpene lactone parthenolide, and the benzoxathiole derivative BOT-4-one all block the ATPase activity of NLRP3 (Table 1 and Figure 2) [23,24], but also have other effects. For example, all three compounds inhibit IκB kinases (IKKs), which are part of the signaling pathway that activates the transcription factor nuclear factor (NF)-κB. NF-κB induces the transcription of pro-IL-1β and NLRP3, a process known as inflammasome priming [25]. Therefore, compounds that inhibit NF-κB block priming and suppress IL-1β and NLRP3 activity independently of their direct effects on NLRP3. Parthenolide also inhibits caspase-1 and, thus, inhibits signaling by all canonical inflammasomes. In a small screen for kinase inhibitors, 3,4-methylenedioxy-β-nitrostyrene (MNS) was identified as a low micromolar inhibitor of NLRP3 signaling (Table 1 and Figure 2). This compound is a Syk kinase inhibitor, but suppresses NLRP3 activity independently of Syk by directly interacting with NLRP3 to block ATPase activity [26]. OLT1177 (aka dapansutrile) is a β-sulfonyl nitrile compound that partially suppresses NLRP3 signaling (Table 1 and Figure 2). OLT1177 was shown to not only attenuate NLRP3 ATPase activity, but also significantly inhibit the NF-κB pathway *in vivo,* suggesting that it also affects NLRP3 priming [27].

Based on previous work on electrophilic acrylic acids, Cocco and colleagues developed INF39 [28]. This compound is active in high micromolar concentrations (10–100 μM) and does not inhibit AIM2 or NLRC4 [29]. INF39 is a covalent inhibitor that attenuates NLRP3 structural changes as detected by BRET and suppresses NLRP3 ATPase activity (Table 1 and Figure 2). The capacity of NLRP3 to bind ATP can be exploited by using immobilized ATP matrices to purify NLRP3, which was the approach allowing Liao and colleagues [30] to identify a small molecule, HS-203873, that disrupted NLRP3–ATP interactions. HS-203873 was then confirmed to attenuate NLRP3 ATPase activity and signaling (Table 1 and Figure 2). Building on this work, a pharmacophore hybridization strategy using INF39 and HS-203873 identified several new compounds that could reduce NLRP3 ATPase activity. Molecular modeling of compounds such as INF120 with inactive ADP-bound NLRP3 suggests that they sit in a binding pocket that closely interacts with the ADP pocket [31].

Molecules that directly bind to NLRP3 and inhibit signaling by affecting protein–protein interactions (PPIs) are also identified. Oridonin is derived from traditional Chinese medicine and has broad anti-inflammatory activities, including inhibiting NLRP3 signaling. Microscale thermophoresis and pull-down studies with biotinylated oridonin confirmed that this compound directly interacts with NLRP3 to covalently modify the NLRP3 residue C279 in the NACHT domain (Table 1 and

Figure 2); this attenuates NLRP3 interactions with Never in mitosis gen A-related kinase 7 (NEK7), suppressing inflammasome formation [32]. The same authors also identified two other existing compounds, tranilast and RRx-001, as NLRP3 inhibitors. Tranilast is an antiallergy drug that inhibits NLRP3 signaling at micromolar concentrations. Based on pull-down studies with biotinylated tranilast, this drug appears to interact with the NLRP3 NACHT domain to disrupt NLRP3 intermolecular interactions, blocking NLRP3 oligomerization (Table 1 and Figure 2) [33]. RRx-001 was developed as an anticancer molecule, but also potently inhibits NLRP3. RRx-001 covalently interacts with NLRP3 and blocks NLRP3–NEK7 interactions (Table 1 and Figure 2). Mutation of NLRP3 NACHT residue C409 abrogates the RRx-001 interaction, suggesting this as its binding site [34]. The hydroxyl sulfonamide JC-171 blocks NLRP3-dependent IL-1β release from mouse macrophages at micromolar concentrations. In co-immunoprecipitation assays, JC-171 decreased the levels of ASC pulled down by NLRP3, suggesting that it disrupts NLRP3–ASC PPIs (Table 1 and Figure 2) [35].

Blocking NLRP3 ATPase activity and PPIs has emerged as effective modes of NLRP3 inhibition. For potency and specificity, MCC950 remains the tool compound of choice for targeting NLRP3. Although reported off-target effects of MCC950 are minimal, a recent report observed that, at high micromolar concentrations, MCC950 could inhibit carbonic anhydrase 2 (CA2) (Table 1) [36]. This contrasts with a previous study that identified CA2 as a potential binding partner of CRIDs/MCC950, but found that these compounds did not affect CA2 enzymatic activity [11]. It has also been reported that MCC950 is able to block Cl^- efflux from nigericin-activated macrophages [22]. Based on the recent cryo-EM and crystal structures of NLRP3 [7,17,18], we anticipate that new structure-guided drug design, coupled with improved NLRP3 protein expression techniques, will facilitate the development of next-generation, highly specific NLRP3 inhibitors with distinct MoAs.

Blocking GSDMD: novel pyroptotic inhibitors

GSDMD is a key effector of inflammasome signaling, because it controls pyroptosis and the resultant release of proinflammatory cellular contents (Box 1). Deficiency in GSDMD dramatically decreases acute IL-1β release upon inflammasome activation [4]. Given that GSDMD controls the release of IL-1β downstream of multiple inflammasomes, GSDMD is an attractive target to pharmacologically modulate signaling by many inflammasomes in distinct diseases. Although pyroptosis inhibitors will block cell lysis and passive release of proinflammatory cell contents, they will not block the inflammasome-driven maturation of IL-1β or IL-18 or other backup forms of cell death; thus, cytokine-mediated inflammatory pathology remains possible despite GSDMD blockade. Indeed, although GSDMD deficiency slows the kinetics of IL-1β release [37], substantial GSDMD-independent IL-1β release is observed at late time points in specific settings [37].

The first pyroptosis blocker described was the complex natural polyphenol, punicalagin, which specifically impairs inflammasome-driven membrane permeabilization without affecting caspase-1 activation or processing of IL-1β or GSDMD [38,39]. Although its MoA is incompletely understood, punicalagin affects plasma membrane fluidity and, thus, may interfere with insertion or oligomerization of N-GSDMD in the plasma membrane [38]. Punicalagin is also a potent antioxidant, and reactive oxygen species (ROS) facilitate pore assembly by N-GSDMD [40]; therefore, punicalagin could also affect pyroptosis by scavenging ROS. Given that GSDMD oligomerization is blocked by reducing agents and the replacement of cysteines at amino acid positions 39 and 192 (homologs of human C38 and C191) to Ala impairs N-GSDMD oligomerization [41], the formation of disulfide bonds between the Cys residues could promote oligomerization and pore formation. In fact, pharmacological agents that directly bind or modify GSDMD C191 impair pore formation and pyroptosis (Table 2), and this is the mechanism by which necrosulfonamide, dimethyl fumarate, and disulfiram

block GSDMD activity [42–44]. Given that these compounds also react with exposed Cys residues of other proteins, they have several off-target effects (Table 2). For example, necrosulfonamide blocks mixed lineage kinase domain-like pseudokinase (MLKL) oligomerization and necroptosis [45], whereas disulfiram also suppresses caspase-1 activity [43] (Table 2).

Targeting NLRP3 in preclinical disease models

Given that the NLRP3 inflammasome is implicated in driving the pathophysiology of many diseases, numerous preclinical studies using small-molecule blockers targeting NLRP3 in distinct animal models of disease have been conducted. Here, we discuss the impact of NLRP3 inhibitors in animal models of metabolic, inflammatory, and neurological disease. We focus on these pathologies because they are common human diseases that lack effective therapies and, thus, present opportunities for widespread and significant impacts on patient care, being potential priority diseases for the clinical application of inflammasome inhibitors.

Neurological disorders

Neuroinflammation driven by the NLRP3 inflammasome has been found to be important in the aging brain; in neurodegenerative diseases, such as MS, AD, and PD; and in the detrimental response to stroke [2]. Here, we summarize preclinical studies performed with NLRP3 inhibitors in rodent models of neurological diseases.

Alzheimer's disease

Neuroinflammation is considered a risk factor for the development of AD, and several innate immune and inflammatory pathways are associated with this condition [46]. Among them, the NLRP3 inflammasome is activated in microglia by Aβ aggregates, inducing the release of IL-1β driving AD symptomatology [2,46]. The nonsteroidal anti-inflammatory drug fenamate is an indirect blocker of NLRP3 activation via inhibition of the volume-regulated anion channel. Fenamate has therapeutic effects in a model of memory loss induced by Aβ administration and in the triple-transgenic mouse model of AD expressing transgenes for PS1 (methionine 146 to valine mutation), APP_{Swe}, and Tau (proline 301 to leucine mutation), a mouse model that progressively develops both Aβ deposition and Tau pathology [2]. This shows that AD symptoms can be ameliorated by blocking the inflammatory response. Indeed, other NLRP3 blockers, such as MCC950 and OLT1177 (Table 1 and Figure 2), restored cognitive function, including learning and memory ability, in the amyloid precursor protein (APP)/presenilin 1 mouse model of AD [47,48]. MCC950 also suppressed disease in other animal models of AD, including genetic models (e.g., senescence accelerated mouse-prone 8) and models of induced disease (in which disease is induced by the intracerebroventricular infusion of Aβ oligomers, or by streptozotocin), in which MCC950 improves spatial memory ability and abolishes memory impairments [49–51]. In addition, MCC950 impaired exogenously seeded Tau pathology in Tau transgenic mice and blocked Tau-induced IL-1β release in microglia [52,53]. Thus, NLRP3 is a promising therapeutic target for preventing the neuroinflammation associated with AD, to improve cognitive function.

Parkinson's disease

The development of PD is accompanied by the loss of dopaminergic neurons, the appearance of α-synuclein aggregates in the form of Lewy bodies, the activation of microglia, and the increase of proinflammatory cytokines, such as IL-1β. Aggregates of α-synuclein activate NLRP3 in microglia and induce the release of IL-1β [46]. In several mouse models of PD, MCC950 (Table 1 and Figure 2) administration improved behavioral dysfunctions by mitigating motor deficits, reducing nigrostriatal dopaminergic degeneration, and decreasing neuroinflammation, alongside inhibiting proinflammatory microglia activation [54–56]. Therefore, NLRP3 blockers are potential disease-modifying treatments for PD.

Multiple sclerosis

Chronic inflammatory demyelination of central nervous system neurons characterizes MS, which gives rise to diffuse neurodegeneration in the entire brain [57]. Experimental autoimmune encephalomyelitis (EAE) is the most commonly used animal model of MS. Although MS is a complex condition, EAE reproduces the key pathological features of MS, such as axonal demyelination and inflammation. In EAE, several NLRP3 blockers, namely, MCC950, OLT1177, and JC-171 (Table 1 and Figure 2), can attenuate disease severity and prevent cognitive and functional deficits and can also improve hippocampal pathology and reduce neuronal demyelination [13,35,58–60]. In particular, JC-171 was effective in both prophylactic and therapeutic settings, suggesting that NLRP3 blockers have potential as treatment for established disease in MS [35]. Indeed, patients with MS with increased inflammasome function and IL-1β production have significantly faster disease progression [60].

Stroke

The NLRP3 inflammasome drives inflammation in ischemia–reperfusion injury after transient middle cerebral artery occlusion. Treatment with CY-09 or MCC950 (Table 1 and Figure 2) in this model attenuates neurological deficits and long-term cognitive dysfunction [61] as well as reduces infarct volume and protects blood–brain barrier integrity [62–66]. MCC950 also reduces brain injury and inflammation in a model of intracerebral hemorrhage and in the control cortical impact model of experimental traumatic brain injury [67,68]. However, MCC950 treatment did not affect injury caused by ferric chloride ($FeCl_3$) in a model of thrombotic stroke [69]. The disease-driving role of the NLRP3 inflammasome following stroke suggests that NLRP3 blockers have therapeutic potential in stroke.

Metabolic disorders

Type 2 diabetes mellitus

A low-grade chronic inflammatory response is one mechanism leading to the development of insulin resistance, T2DM, and associated comorbidities, including liver and kidney damage. In particular, the NLRP3 inflammasome is activated in response to several altered metabolic signals in T2DM, leading not only to the activation of caspase-1 and production of IL-1β, but also to pancreatic β cell failure [32,33]. The NLRP3 blockers CY-09, tranilast, and oridonin (Table 1 and Figure 2) have proven successful for reducing body weight and blood glucose, as well as promoting insulin sensitivity in a high-fat diet-induced animal model of T2DM [22,32,33]. In keeping with this, the NLRP3 inhibitor MCC950 (Table 1 and Figure 2) was able to reduce body weight and blood glucose and increase insulin sensitivity in aged mice, suggesting that NLRP3 inhibitors provide therapy in age-related metabolic syndrome [70]. However, in the diabetic *db/db* mice, a genetic murine model of diabetes, MCC950 did not affect blood glucose or insulin resistance [71,72]. However, MCC950 did improve several diabetes-associated comorbidities, including renal failure in diabetic *db/db* mice, which develop kidney injury [72,73], and vascular endothelial dysfunction and atherosclerosis in adiponectin- or apolipoprotein-knockout mice, respectively [74,75]. However, MCC950 increased renal inflammation in streptozotocin-induced diabetic mice [76]. MCC950 ameliorated the neurological deficit and encephalopathy observed in the diabetic *db/db* mice and in the high-fat diet-induced diabetes model, improving anxiety- and depression-like behaviors [77–79]. Glyburide treatment in patients with diabetes reduced mortality in sepsis compared with patients not taking glyburide or in patients without diabetes, suggesting that glyburide exerts anti-inflammatory effects on the immune system by blocking the NLRP3 inflammasome [80]. The NLRP3 inhibitor CY-09 (Table 1 and Figure 2) also protected against diabetic retinopathy and acute liver injury in a streptozotocin-induced hyperglycemic mouse model [81,82].

NASH, liver damage, and fibrosis

Liver steatosis or nonalcoholic fatty liver disease (NAFLD) is the result of the accumulation of fat in the liver and is a common complication in diabetes. Liver steatosis could evolve to NASH when

inflammatory cytokines are produced in the liver, promoting hepatic inflammation, fibrosis, and loss of function. Liver steatosis, NASH, and fibrosis are all associated with NLRP3 inflammasome signaling [83]. A high-fat diet in mice is commonly used to model NAFLD, and the use of the NLRP3 blockers CY-09, tranilast, or oridonin (Table 1 and Figure 2) decreased the accumulation of intracellular lipid droplets in the liver and improved liver steatosis [22,32,84,85]. In particular, CY-09 also improved hepatic steatosis after bariatric surgery [85] and was able to suppress inflammatory cytokine expression in the liver [22]. Liver steatosis is also an age-related symptom and, in old mice, treatment with MCC950 reduced liver fat accumulation and improved hepatic function [70]. A diet deficient in methionine and choline results in liver inflammation, NASH, and fibrosis, and co-administration of NLRP3 inhibitors (MCC950, tranilast, or IFM-514; Table 1 and Figure 2) with this diet suppressed the severity of hepatic inflammation and fibrosis [83,86,87]. NLRP3 blockade also decreased macrophage and neutrophil infiltration to the liver [83] and caspase-1 activation [87] and downregulated expression of TGF-β and its target genes, which drive collagen production [86]. Liver fibrosis also occurs in the helminthic infectious disease schistosomiasis, in which MCC950 treatment reduced infection-induced hepatic fibrosis markers [88]. Liver injury and fibrosis also develop during bile duct ligation, in which MCC950 treatment to inhibit NLRP3 activation and IL-1β and IL-18 production alleviated neutrophil infiltration and hepatic cell death [89]. Alcohol addiction and severe alcohol consumption is a frequent cause of liver damage and fibrosis. The NLRP3 blocker oridonin (Table 1 and Figure 2) attenuated liver inflammation and injury in a model of chronic alcohol consumption [90]. Intriguingly, MCC950 also reduced alcohol consumption and preference [91], and the alcoholism drug disulfiram (Table 2) suppressed NLRP3 signaling by inhibiting GSDMD [43]. NLRP3 blockers were also successfully used in other types of hepatic damage, in particular in models of acute liver injury induced by thioacetamide treatment during severe hyperglycemia. In this model, CY-09 (Table 1 and Figure 2) alleviated acute liver injury by reducing levels of transaminases in blood, improving histological liver damage, and decreasing hepatic cell death [82]. Similarly, oridonin treatment attenuated carbon tetrachloride-induced chronic liver injury and fibrosis [92].

Intestinal inflammation

Although the NLRP3 inflammasome is implicated in the development of IBD, including Crohn's disease and ulcerative colitis [2], the etiology of these diseases in humans is complex and associated with chronic dysfunction of the immune response in the intestine. Animal models of intestinal inflammation often include toxic compounds in the drinking water to induce intestinal epithelial damage and subsequent inflammation; here, the most widely used chemical is synthetic sulfated polysaccharide dextran sulfate sodium (DSS) [93]. DSS activates pathological NLRP3 inflammasome signaling, because DSS-induced IBD is a model in which inflammatory symptoms strongly depend on this inflammasome [94]. The NLRP3 blockers MCC950, OLT1177, INF39, tranilast, or the benzo[d]imidazole derivate Fc11a-2 (Table 1 and Figure 2) successfully treated DSS-induced colitis [95–101]. In particular, these compounds improved body-weight gain, decreased disease activity index, attenuated colon shortening, and reduced histopathological scores of intestinal damage and intestinal macroscopic damage index [95–101]. When combined with other drugs, NLRP3 inhibitors potentiated their protective effect in DSS-induced colitis, suggesting their use in preventing the onset of colitis. MCC950 synergized with the NF-κB inhibitor celastrol [102] and metformin [103]. OLT1177 (Table 1 and Figure 2) similarly boosted the effect of the purinergic inhibitor brilliant blue G [104], and INF39 potentiated the effects of arctigenin, a phenylpropanoid dibenzylbutyrolactone ligand [100]. However, therapeutic treatment with OLT1177 after murine DSS treatment and the establishment of disease did not affect colitis disease indices [99]. In other models of colitis, NLRP3 blockers improved intestinal disease. In the Winnie mouse model of spontaneous chronic colitis, MCC950 treatment improved body-weight gain, colon length, disease activity index, and histopathological scores and reduced proinflammatory

cytokine production [105]. Colitis caused by di- or trinitrobenzene sulfonic acid or by oxazolone was ameliorated by treatment with the NLRP3 blockers INF39, oridonin, and tranilast (Table 1 and Figure 2) [28,106–108]. In these models, NLRP3 inhibitors suppressed Th1/Th17 cells and reduced mast cell, neutrophil, and macrophage infiltration in the intestine [28,106–108]. Therefore, NLRP3 blockade is an interesting and promising strategy for the management of bowel inflammation.

Concluding remarks and future perspectives

Although some NLRP3 inhibitors are entering early clinical trials (Box 2), the best clinical indications for NLRP3 inflammasome blockade remain to be determined. Choosing an indication for NLRP3 inhibitors is challenging given the breadth of conditions that are associated with NLRP3 (see Outstanding questions). Early proof-of-concept trials will likely focus on CAPS, a set of rare NLRP3 hereditary diseases, but given limited patient populations, other conditions must be chosen to robustly test NLRP3 inhibitor efficacy. Despite the success of NLRP3 blockers in preventing or treating over a hundred preclinical animal models of disease, we need to determine when, where, and how NLRP3 is activated in human disease to be able to translate preclinical studies into effective clinical therapies. In any case, preclinical models suggest that chronic inflammation related to NLRP3-activating mutations, digestive, metabolic, or neurodegenerative diseases would be promising indications for Phase II clinical trials. However, such trials require patient stratification to enable selection of individuals with strong NLRP3 activation. There is currently a lack of biomarkers to indicate NLRP3 inflammasome activity and for assessing target engagement. Approaches such as measuring extracellular vesicle RNA signatures specifically associated with NLRP3 or determining NLRP3 activity in blood cells could be fruitful [109,110]. The involvement of other inflammasomes in diseases is still emerging, with several nonmicrobial activators of NLRC4, AIM2, PYD, and NLRP1 inflammasomes now identified. Therapies that block the action of multiple inflammasomes, such as molecules that block ASC oligomerization [111], could be promising approaches as pan-inflammasome therapeutics. Similarly, compounds that block GSDMD and pyroptosis to suppress the execution phase of inflammasome signaling are emerging as novel approaches to ameliorate inflammation, but could be bypassed by the execution of alternative forms of cell death. Thus, inflammasome blockers show enormous promise as a new generation of anti-inflammatory drugs for a wide range of conditions currently lacking curative treatments.

Outstanding questions

What clinical applications will show the best efficacy for NLRP3-targeting molecules?

When, where, and how is NLRP3 activated in human disease?

Would pan-inflammasome inhibitors offer additional therapeutic benefit to NLRP3-specific blockers?

Will GSDMD and pyroptosis inhibitors be effective anti-inflammatory therapies?

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Declaration of interests

R.C.C. and K.S. are co-inventors on patent applications for NLRP3 inhibitors, which have been licensed to Inflazome Ltd. K.S. served on the Scientific Advisory Board of Inflazome in 2016–2017 and serves as a consultant to Quench Bio and Novartis. R.C.C. is a consultant for BioAge Labs. P.P. is co-inventor on a patent application to use the NLRP3 inflammasome as biomarker of disease, which has been licensed to Viva In Vitro Diagnostics SL, a company co-funded by P.P. P.P. is also scientific consultant of Glenmark Ltd.

Resources

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