A new lead to NLRP3 inhibition

The discovery of a small molecule inhibitor that targets the inflammasome sensor NLRP3 offers a new path for the development of selective inflammasome blockers with potential therapeutic benefit in a wide range of human diseases (in this issue, see Jiang et al., https://doi.org/10.1084/jem.20171419).

Insight from Mohamed Lamkanfi and Vishva M. Dixit

Inflammasomes are cytosolic multi-protein complexes that form in response to diverse cellular insults to promote proximity-induced autoprocessing of caspase-1 (Lamkanfi and Dixit, 2014). Each complex is assembled by sensor proteins that respond to particular pathogenic conditions (see figure). Pyrin/TRIM20 is a member of the tripartite motif (TRIM) family that assembles an inflammasome in myeloid cells that have been infected with *Burkholderia cenocepacia*, *Clostridium difficile*, and various other pathogens that produce RhoA GTPase–inactivating toxins. AIM2 (absent in melanoma 2) of the HIN200/AIM2-like receptor (ALR) family builds an inflammasome in myeloid and intestinal epithelial cells when it detects viral, bacterial, and host-derived double-stranded DNA (dsDNA) in the cytosol. Distinct inflammasomes are nucleated by members of the intracellular nucleotide-binding domain and leucine-rich repeat containing (NLR) receptor family as well. The NLRP1b inflammasome is activated by *Bacillus anthracis* lethal toxin, whereas flagellin and type III secretion systems (T3SS) of virulent bacterial pathogens such as *Salmonella enterica* and *Pseudomonas aeruginosa* trigger the NLRC4 inflammasome. Each of these inflammasomes has been implicated in human diseases, but the NLRP3 inflammasome responds to the broadest array of medically relevant pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), and insults. Indeed, DAMPs like extracellular ATP and hyaluronic acid, medically relevant crystals such as alum, CCPD, MSU, silica, and asbestos, ionophores such as nigericin, and β-fibrils such as β-amyloid can all engage the NLRP3 inflammasome (see figure). Moreover, the major component of the outer membrane of Gram-negative bacteria, LPS, activates NLRP3 through a noncanonical pathway involving caspase-11. The inflammasome adaptor apoptosis-associated speck-like protein containing a CARD (ASC) connects sensor components within the inflammasome to caspase-1 and is observed in a micrometer-sized supramolecular fibril structure in the stimulated cell named the "ASC speck." Caspase-1 proteolytically matures IL-1β and IL-18, two highly potent inflammatory cytokines that also act as co-stimulators of T cell functions (Dinarello, 2009). Caspase-1 and the related inflammatory caspases 4, 5, and 11 also cleave gasdermin D to induce pyroptosis, which is a proinflammatory form of regulated necrosis that is intrinsically associated with the passive release of IL-1β and IL-18 into the extracellular milieu along with DAMPs such as IL-1α, HMGB1, and ATP (Kayagaki et al., 2011; Shi et al., 2014).

Insight into the signaling mechanisms by which inflammasomes respond to particular DAMPs and PAMPs offers new entry points to block caspase-1 activation with more precision. Given its role in driving caspase-1 activation in the context of diverse pathological conditions, the NLRP3 inflammasome is increasingly regarded as an attractive target for reducing destructive inflammation in autoinflammatory, rheumatic, metabolic, cardiovascular, and neurodegenerative diseases. In this issue, Jiang et al. identify CY-09 as a novel inhibitor of the NLRP3 inflammasome that acts as a competitive inhibitor of nucleotide binding to the central NLRP3 NACHT domain. Binding of ATP/dATP to NLRP3 was shown previously to be required for NLRP3-dependent IL-1β secretion (Duncan et al., 2007). Several compounds that interfere with the intrinsic ATPase activity of NLRP3 have been reported (Juliana et al., 2010; He et al., 2014), but they target additional proteins with roles in innate immunity, suggesting that their selectivity profile would need significant optimization before widespread adoption as inflammasome-targeting agents. In contrast, Jiang et al. (2017) show that CY-09, unlike the closely related compound cystic fibrosis transmembrane receptor $(CFTR)_{inh}$-172, inhibits neither CFTR gating nor TLR signaling. Moreover, micromolar concentrations of CY-09 selectively inhibited the NLRP3 inflammasome without impeding caspase-1 activation by the NLRC4 and AIM2 inflammasomes (Jiang et al., 2017). It would be interesting to analyze CY-09 against the NLRP1b and Pyrin inflammasomes to further narrow down its inflammasome selectivity profile.

The NLRP3 inflammasome uniquely requires a two-step mechanism for activation: transcriptional up-regulation of NLRP3 and pro-IL-1β, for example by TLR signaling, serves as a priming step for subsequent activation by NLRP3 agonists (Bauernfeind et al., 2009). Importantly, CY-09 did not interfere with TLR4 priming, but instead

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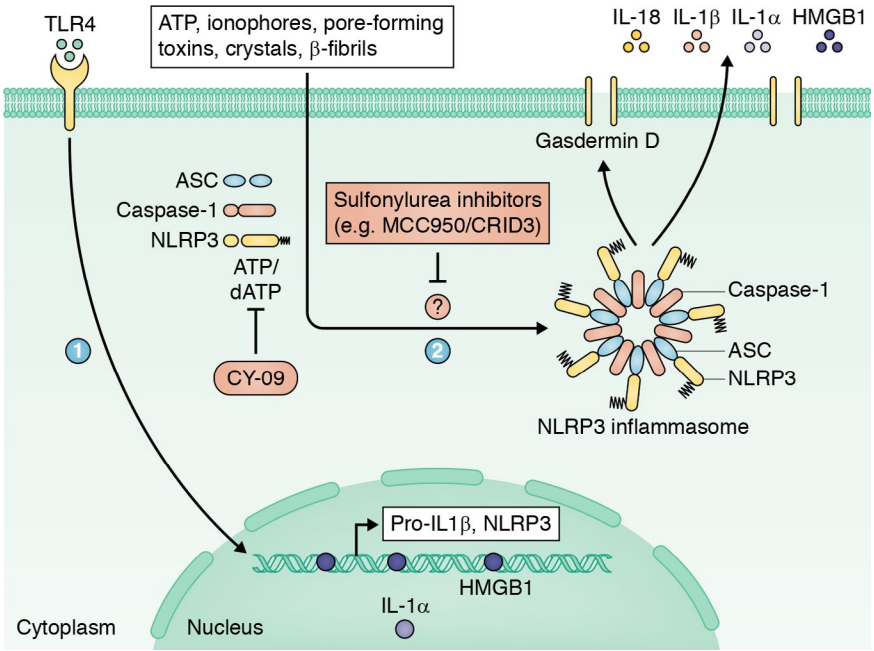
blocked NLRP3 oligomerization and ASC recruitment in the activation step (Jiang et al., 2017). Glyburide was the first compound shown to selectively inhibit PAMP-, DAMP-, and crystal-induced activation of the NLRP3 inflammasome without interfering with other inflammasome pathways (Lamkanfi et al., 2009). Currently, MCC950/CRID3 and related diarylsulfonylurea compounds that are structurally related to glyburide are the most potent reported inhibitors of the NLRP3 inflammasome with IC_{50} values around 20 nM (Perregaux et al., 2001; Coll et al., 2015; Primiano et al., 2016). MCC950/CRID3 was shown to block activation of the NLRP3 inflammasome in ex vivo–stimulated murine and human macrophages and monocytes. However, unlike for CY-09, the molecular target and mechanism by which MCC950/CRID3 and related sulfonylurea molecules inhibit the NLRP3 inflammasome remains enigmatic.

Jiang et al. (2017) also showed that CY-09 can be used for limiting NLRP3 inflammasome activation in vivo. CY-09 showed favorable in vivo and ex vivo pharmacokinetic properties for stability, safety, and oral bioavailability, resembling those of MCC950/CRID3 (Coll et al., 2015; Primiano et al., 2016; Jiang et al., 2017). MCC950/CRID3 was shown to inhibit NLRP3-dependent pathology in preclinical disease models, including the *Nlrp3*$^{Ala350Val\text{-}neoR}$ knock-in mouse model of CAPS (cryopyrin-associated autoinflammatory syndromes), the experimental autoimmune encephalomyelitis mouse model of multiple sclerosis, the imiquimod cream–induced mouse model of skin inflammation, and house dust mite–induced acute airway inflammation in mice (Coll et al., 2015; Primiano et al., 2016). CY-09 also prevented neonatal lethality in the *Nlrp3*$^{Ala350Val\text{-}neoR}$ knock-in mouse model of CAPS, it improved diabetic symptoms in mice given a high-fat diet and blocked NLRP3-dependent caspase-1 activation and IL-1β secretion from human peripheral blood mononuclear cells (PBMCs) of healthy donors and synovial fluid cells of gouty arthritis patients (Jiang et al., 2017).



Activation of the NLRP3 inflammasome and its inhibition by CY-09 and sulfonylurea compounds. Activation of the NLRP3 inflammasome involves two steps. First, TLR4 stimulation induces transcriptional up-regulation of NLRP3 and the inflammasome substrate proIL-1β. In the second activation step, NLRP3 agonists such as ATP, the ionophore nigericin, pore-forming toxins and internalized crystals, and β-fibrils trigger NLRP3 oligomerization, ASC speck formation, and inflammasome-mediated caspase-1 autoactivation. Caspase-1 cleaves its cytokine substrates IL-1β and IL-18, and it induces pyroptosis through cleavage of gasdermin D, which promotes the passive release of IL-1β and IL-18 along with DAMPs such as IL-1α and HMGB1. CY-09 inhibits NLRP3 inflammasome assembly by blocking ATP/dATP binding in the central NACHT domain, whereas the target and mechanism of action of sulfonylurea compound MCC950/CRID3 are unknown.

The findings presented by Jiang et al. (2017) suggest that further development of CY-09 might provide a novel avenue toward therapeutic inhibition of the NLRP3 inflammasome. IL-1 blocking biologics have demonstrated clinical efficacy in rheumatic diseases (gout, pseudogout, systemic juvenile idiopathic arthritis, and Still's disease) and rare hereditary monogenetic autoinflammatory diseases such as CAPS and familial Mediterranean fever. In addition, a recent study found that IL-1β inhibition significantly reduced the risk for stroke and cardiovascular events in coronary artery disease patients with inflammatory atherosclerosis (Ridker et al., 2017a). The IL-1β–targeting therapy also appeared to protect these patients from lung cancer and lung cancer–associated mortality (Ridker et al., 2017b). However, as with other cytokine-blocking therapies, IL-1β neutralization comes with an increased risk for serious life-threatening infections and sepsis. Therapies acting upstream of IL-1β secretion and targeting select inflammasome complexes have the potential for improved safety because the nontargeted inflammasomes should still mediate immune defense when the patient faces an acute infection. Selective inflammasome targeting could also have increased therapeutic potency through the simultaneous blockade of IL-1β, IL-18, and pyroptosis. A major task ahead is to develop more potent CY-09 analogues that target the NLRP3 inflammasome in the low nanomolar range IC_{50} values noted for MCC950/CRID3. Historically, generating selective inhibitors of ATP-binding pockets has been

challenging. Therefore, it would also be critical to profile any CY-09 analogues for cross-reactivity against the enzymatic activity of a broad panel of protein kinases and ATPases. Nevertheless, it is a goal worth pursuing given the tremendous benefits selective inflammasome inhibition might offer to patients.

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