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Intracellular innate immunity in gouty arthritis: role of NALP3 inflammasome

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Abstract

Gout is an inflammatory disease caused by the deposition of monosodium urate (MSU) crystals in the joint. Recent studies have significantly advanced our knowledge on the understanding of mechanisms underlying MSU crystal-induced inflammation. MSU crystals act as a ‘danger signal’ that can be recognized by pattern recognition receptors both at cell surface and cytoplasm, indicating the importance of innate immunity in gout. This review focuses on the critical role of intracellular NALP3 inflammasome in MSU crystal-induced inflammation.

Keywords

gout; innate immunity; NALP3 inflammasome

In gout, the deposition of monosodium urate (MSU) crystals in articular and periarticular tissues can be asymptomatic or can be associated with the pathogenesis of acute severe joint inflammation. Over time, acute intermittent attacks can progress to chronic gouty arthropathy that may lead to bone and cartilage destruction.¹ Innate immunity is the evolutionary conserved component of the immune system. It is the first line of defense that protects the host from invading pathogens with the molecular signature known as pathogen-associated molecular patterns (PAMPs). In addition, innate immunity can protect the host from danger-associated molecular patterns (DAMPs), which are endogenous signals released by cells that are in a dangerous situation, such as stress, damage, injury, necrotic death or environmental insult.² Unlike adaptive immune response, stereotypic innate immune ‘early induced’ response does not directly induce immunologic memory or lasting protective immunity, consistent with the nature of the recurrent acute gouty attack. Thus, innate rather than adaptive immunity is critical in acute gouty inflammation. This review outlines recent advances in the understanding of innate immunity, with a focus on intracellular innate immunity, in MSU crystal-induced inflammation.

INNATE IMMUNE RECOGNITION OF MSU CRYSTALS IN INITIATION OF ACUTE GOUTY INFLAMMATION

The pathological hallmark of acute gout is neutrophil influx into the synovium and joint fluid.^{1,3} As neutrophils are absent in the normal joint, the interaction of MSU crystals with resident cells in the joint (principally synovial lining cells) is believed to be the primary factor for triggering acute neutrophil ingress and paroxysms of gouty inflammation episodes.^{1,3} One of the important outcomes of the MSU crystal stimulation of cells is the induction of expression of nuclear factor- κ B and AP-1 transcription factor-dependent genes, including a broad array of inflammatory mediators, such as interleukin (IL)-1, tumor necrosis factor- α , IL-6, CXCL8 and cyclo-oxygenase 2.^{1,3,4}

How do MSU crystals initially transduce signaling to produce a cascade of cell activation that leads to inflammation? The innate immune system comprises a range of receptors and soluble proteins that detect pathogens or cellular products released by damaged or dying cells through innate immune pattern recognition receptors, leading to cell activation.² Toll-like receptors (TLRs), the type I transmembrane receptors, are one family of these innate immune pattern recognition receptors.^{2,5} The leucine-rich repeats (LRRs) in the ectodomain of TLRs are considered to mediate recognition of pathogens or danger signals.^{2,5} Recently, TLR2, TLR4 and their cell surface adaptor CD14, which is another pattern recognition receptor, were shown to be essential in MSU crystal-induced acute inflammation using the mouse air pouch model.^{6,7} In this context, the innate immune system facilitates cellular responses through recognition of MSU crystals by CD14, TLR2 and TLR4, and the downstream adaptor MyD88-dependent signaling directly promotes MSU crystal-induced acute inflammatory responses, including leukocyte infiltration and release of cytokines such as IL-1 β .^{6,7} MyD88-dependent IL-1 receptor (IL-1R), TLR2 and TLR4 pathways were also shown to be required for MSU crystal-induced inflammation in the mouse lung injury model.⁸ However, only MyD88-dependent IL-1R pathway, but not MyD88-dependent TLR pathway, was reported to be necessary for MSU crystal-induced inflammation using the mouse peritonitis model.⁹ The possible explanation for the differential results is that the local resident cells may be different in the mouse models applied in these studies, and these cells may respond to MSU crystal-stimulation through different mechanisms. Alternatively, the methods used for preparing MSU crystals in these studies may allow MSU crystals to be recognized by TLRs in one way, but not in other ways.

INTRACELLULAR INNATE IMMUNE RESPONSE TO MSU CRYSTALS

Although it has been known for a long time that IL-1 β is produced in monocytes and macrophages by MSU crystals *in vitro*,^{10,11} IL-1 β was only recently identified as the pivotal cytokine in gouty inflammation. This was based on the findings of significantly reduced inflammatory responses in mice treated with IL-1-neutralizing antibodies or in mice deficient in IL-1 receptor (IL-1R), and in mice deficient in MyD88, which is also a critical downstream adaptor in the IL-1R signaling pathway.⁹

IL-1 β and its maturation

Interleukin-1 β , originally identified as endogenous pyrogen, is released by many cell types. It is a potent proinflammatory cytokine,^{12,13} belongs to a cytokine family that also includes IL-1 α , IL-18 and IL-33, and IL-1 receptor antagonist (IL-1Ra).^{12,13} IL-1 β has multiple functions. It is an important mediator of inflammatory responses, and is involved in a variety of cellular activities, including cell proliferation, differentiation and apoptosis.^{12,13} The synthesis, processing and release of IL-1 β are tightly controlled.^{12,13} First, IL-1 β is produced as an inactive pro-molecule (pro-IL-1 β) by a number of signaling pathways including the MyD88-dependent TLR and MyD88-dependent IL-1R signaling pathways. Next, accumulated pro-IL-1 β in the cytoplasm is cleaved by active caspase-1 (also known as IL-1-converting enzyme), resulting in the generation of mature IL-1 β that is released to the outside of the cell.¹⁴ The biological activity of IL-1 β is directly dependent on the activity of caspase-1.

Caspase-1 and inflammasome

Caspase-1 is one of the inflammatory caspases. Mice that lack caspase-1 are unable to produce mature IL-1 β .¹⁵ The activity of caspase-1 is tightly regulated and requires recruitment and dimerization of enzyme within a molecular platform named as the inflammasome.^{14,16} Briefly, the inflammasome is a complex composed of procaspase-1, the cytoplasmic protein ASC (apoptosis-associated speck-like protein containing a caspase-associated recruitment domain), and the intra-cellular receptor containing a pyrin domain at N terminus, a central nucleotide-binding domain and a C-terminal LRR domain.^{14,16} There are three prototypes of inflammasomes—the NALP1 inflammasome, the NALP3 (also known as cryopyrin, NLRP3) inflammasome and the IPAF inflammasome. Activation of NALP3 inflammasome leads to activate caspase-1, resulting in pro-IL-1 β processing and mature IL-1 β secretion.^{14,16} Mutations (gain-of-function) in NALP3 protein have been linked to a number of hereditary autoimmune syndromes, such as familial cold urticaria or familial cold autoinflammatory syndrome, Muckle-Wells syndrome and chronic infantile neurological cutaneous and articular syndrome.^{17,18} These are due to spontaneous IL-1 β production, which probably resulted from the abnormal protein conformation responsible for an increase in NALP3 inflammasome activity.^{19–21}

NALP3 inflammasome in MSU crystal-induced inflammation

Monosodium urate crystals were recently shown to induce IL-1 β release by activation of NALP3 inflammasome²² (Figure 1). Peritoneal macrophages from mice deficient in each component of NALP3 inflammasome (for example, caspase-1, ASC and NALP3) were unable to process pro-IL-1 β and release active IL-1 β in response to MSU crystals.²² In addition, *in vivo* peritonitis studies demonstrated that mice deficient in caspase-1 and ASC have significant reduced inflammatory responses (neutrophil influx and IL-1 β release) to MSU crystals compared with wild-type mice.²² These observations indicate that NALP3 inflammasome has a critical role in MSU crystal-induced inflammation.

Several studies have shown that NALP3 inflammasome activation can be triggered by various PAMPs including bacterial muramyl dipeptide, a degradation product of the bacterial cell wall component peptidoglycan, the microbial toxins, RNA of bacterial and

viral origin, imidazoquinoline and cytosolic microbial and host DNA.^{23–26} In addition to MSU crystals, several other particulates such as CPPD crystals, alum, asbestos and silica, as well as extracellular ATP and amyloid- β , serve as DAMPs to activate NALP3 inflammasome.^{22,27–32} Interestingly, many of these diverse PAMPs and DAMPs engage TLRs first to induce synthesis of pro-IL-1 β through the MyD88-dependent signaling pathways. In the case of particulates, for example alum, in contrast to MSU crystals, does not need TLRs for its action.³³ This suggests that alum induces IL-1 β synthesis through other non-TLR signaling pathways. The exact mechanisms by which these diverse PAMPs and DAMPs trigger NALP3 inflammasome complex formation, which leads to caspase-1 activation and IL-1 β release, are still unknown. However, potassium efflux, reactive oxygen species production or the release of cathepsin B from the lysosome are common intermediates for NALP3 inflammasome activation triggered by these activators.^{28–32,34,35}

Similar to LRR domains in TLRs, NALP3 LRR domain is also proposed to be a ligand-sensing motif.³⁶ In this concept, NALP3 is considered to be present in the cytoplasm in an inactive form, but becomes active when the LRR domain is engaged by an agonist. This may be due to the conformational rearrangement of this molecule, which exposes the central nucleotide binding/oligomerization domain and subsequently the N-terminal effector domain (pyrin-binding domain).³⁶ Therefore, each of the activators mentioned above could engage the LRR domain of NALP3 either directly or indirectly, leading to a conformational change in NALP3 and subsequently to inflammasome assembly. Muramyl dipeptide was recently demonstrated to directly bind to recombinant NALP1, and muramyl dipeptide interaction with the LRR region of NALP1 is essential for caspase-1 activation mediated by the reconstituted NALP1 inflammasome.³⁷ These data indicate that NALPs may directly interact with their activators through their LRR domains.

Binding of the chaperone heat-shock protein 90 (Hsp90) and the co-chaperone-like, ubiquitin ligase-associated protein SGT1 to the LRR domain of NALP3 was shown to be essential for the function of NALP3 inflammasome.³⁸ Hsp90 and SGT1 maintain NALP3 in an inactive but signaling-competent state, and disassociate from NALP3 once activating signals are detected, therefore allowing conformational change of NALP3 that enables the interaction of NALP3 with other components such as ASC and procaspase-1. In the absence of Hsp90, NALP3 becomes unstable, and is degraded by the proteasome.³⁸ Thus, if NALP3 is missing the LRR domain, Hsp90 and SGT1 are no longer able to interact with NALP3. In this context, NALP3 without LRR domain would be unstable and unable to form an inflammasome complex in response to activating signals. To determine if the LRR domain of NALP3 is required for MSU crystal-induced inflammation, we recently performed studies using a novel recombinant mouse with the NALP3 LRR domain deleted (submitted to *Arthritis and Rheumatism*). We found that bone marrow-derived macrophages from these mice stimulated with MSU crystals failed to induce caspase-1 activation and IL-1 β release *in vitro*. In addition, IL-1 β release in macrophages from these mice was also impaired *in vitro* in response to several other known NALP3 activators (for example, peptidoglycan, bacterial RNA, R837, crude LPS). Furthermore, significant reduced inflammatory responses (leukocyte infiltration and IL-1 β release) to MSU crystals were observed in these mice *in vivo* using the subcutaneous air pouch model. These results suggest that the LRR domain of

NALP3 is necessary for MSU crystal-induced inflammatory responses. Whether MSU crystals directly interact with the LRR domain of NALP3 is still unknown.

Implications for therapy

Recent animal model studies using IL-1R antagonist (anakinra) and IL-1 inhibitor (mIL-1 trap) have undoubtedly demonstrated IL-1 β signaling to be a promising therapeutic target to manage MSU crystal-induced inflammatory responses.^{39,40} As further evidence, two small pilot clinical studies using either anakinra or rilonacept (IL-1trap) have demonstrated efficacy in treatment of patients with acute and chronic gout.^{39,41}

Colchicine, a drug long used to treat gout, suppresses MSU crystal-induced NALP3-driven caspase-1 activation, IL-1 β processing and release *in vitro* at micromolar concentration.²² Although this concentration is greater than that is normally prophylactic,⁴² this suggests that the therapeutic effect of colchicine may be partly due to the inhibition of NALP3 inflammasome activation. Developing new drugs that target at NALP3 inflammasome may provide more direct and effective therapies for the treatment of gouty arthritis, especially in those patients who no longer respond to or cannot tolerate side effects of the conventional treatments with colchicine and NSAIDs. The LRR domain of NALP3 has a potential to directly or indirectly engage with a vast array of structurally unrelated PAMPs or DAMPs to activate the NALP3 inflammasome, leading to innate immune inflammatory responses. Targeting at the LRR domain of NALP3 may have a huge drug potential for host defense.

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Figure 1.

Monosodium urate (MSU) crystal-induced activation of interleukin (IL)-1 β by NALP3 inflammasome. MSU crystals are initially recognized at the cell surface by CD14, TLR2 and TLR4 that leads to synthesis of pro-IL-1 β . Once MSU crystals are taken up into the cytoplasm, they can engage NALP3 probably through the LRR domain. This results in assembly of NALP3 inflammasome that leads to trigger caspase-1 activation, promote processing of pro-IL-1 β and release of mature IL-1 β .