# Inhibition of NLRP3 inflammasome as a therapeutic intervention in crystal-induced nephropathy



doi: 10.1016/j.kint.2016.05.003

# Inhibition of NLRP3 inflammasome as a therapeutic intervention in crystal-induced nephropathy

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Key wards; iGLuc, inflammasome, crystal, NLRP3

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Abstract; 75words/75words
Total; 1530words/1500words

**Abstract**

Inflammasomes are important components of innate immunity system. Dysregulation of inflammasomes activation is central to the pathogenesis of inflammatory diseases. The measurement of inflammasomes activity especially in *in vivo* settings should give us important information to regulate inflammatory events. Ludwig-Portugall and Bartok *et al*. clearly visualized the intrarenal activation of inflammasome in a murine model of crystal nephropathy using a proteolytic luciferase-based reporter, and showed that the blockade of NLRP3 inflammasome may prevent renal injuries.

Inflammasomes are important components of innate immunity system that is evolutionally conserved as a first line host defense against pathogens.[1] Inflammasomes are activated by pathogen-associated molecular patterns (PAMPs) on invading pathognes or damage-associated molecular patterns (DAMPs) released from host damaged cells to produce pro-inflammatory cytokines interleukin (IL)-1β and IL-18 through germline-encoded pattern recognition receptors (PRRs) in host cells. PRRs consist of four different families such as Toll-like receptors (TLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene (RIG)-1 receptors (RLRs), and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). This system is required to maintain host homeostasis, however, aberrant activation of inflammasomes has been known to be central to the pathogenesis of various inflammatory disorders.

Among PRRs, NLRs is known to be activated by intracellular molecular patterns to form NLRs inflammasomes by binding with apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) adaptor protein and pro-caspase-1. The activation of NLRs inflammasomes leads to autocatalytic activation of caspase-1, thereby inducing the cleavage of pro-IL-1β and pro-IL-18 into mature form of IL-1β and IL-18. Thus far, NLRs inflammasomes have been reported to be activated by crystal structures including monosodium urate crystals, calcium pyrophosphate crystals and cholesterol crystals.[2] The kidney is prone to intrarenal formation/deposition of crystals due to the concentration of the primary glomerular filtrate, and a number of acute and chronic renal injuries are thought to be induced in association with the crystal formation/deposition, suggesting the involvement of NLRs inflammasomes. Recently, calcium oxalate crystals have been shown to be capable of inducing renal inflammation by NLRP3 (NLR family, pyrin domain containing 3) inflammasome activation.[3] Therefore, the measurement of inflammasome activity especially in *in vivo* settings should give us important information on various renal diseases including crystal-induced renal injuries. Currently, the gold-standard technique to assess the inflammasome activity is the analysis of caspase-1 cleavage using western blotting, however, this is a time consuming method and is difficult to use as a high-throughput screening.

In this issue, Ludwig-Portugall and Bartok et al.[4] clearly visualized the intrarenal activation of inflammasome in a murine model of crystal nephropathy induced by adenine-enriched diet. Prior to this study, Bartok et al.[5] developed a biosensor which is a pro-IL-1β-Gaussia luciferase (iGLuc) fusion construct that reports the proteolytic activity of caspase-1 (which means the inflammasome activation) by the production of visible light. GLuc activity is almost completely suppressed under the control state by the formation of iGLuc aggregates. However, the treatment with the stimuli leading to caspase-1 activation induces the monomerization of iGLuc through the cleavage of pro-IL-1β to mature form of IL-1β, and activates iGLuc. In addition, iGLuc construct is able to be transduced into mouse primary bone marrow-derived macrophages or dendritic cells. Therefore, the authors in this issue applied this new tool to the nephrology field to monitor *in vivo* inflammasome activity. They expressed this iGLuc reporter construct in hematopoietic progenitors isolated from the bone marrow of donor mice using lentiviral transfection, and transferred these cells into irradiated recipient mice to visualize inflammasome activation using *in vivo* bioluminescent imaging system. Using this system, a luminescent signal was observed on day12 in the kidneys of adenine-rich diet fed mice and continued to increase on day 16. These results indicate that the activation of inflammasome in bone marrow-derived cells is induced in the course of adenine-induced crystal nephropathy and iGLuc system is a powerful tool to monitor the *in vivo* activation of inflammasome (Figure 1).

Next, the authors in this study investigated the role of NLRP3 inflammasome in the development of adenine-induced crystal nephropathy characterized by renal dysfunction and tubulointerstitial fibrosis. They used NLRP3-deficient mice and a potent and selective NLRP3 inhibitor CP-456,773, previously known as both CRID3 and MCC950.[6] These genetic deletion or pharmacological antagonism of NLRP3 protected mice from adenine-induced renal dysfunction and tubulointerstitial fibrosis, blunting the increases in renal IL-1β and IL-18 production and caspase-1 activation compared to control animals. These results suggest that NLRP3 inflammasome plays essential roles in the pathogenesis of adenine-induced crystal nephropathy as previously reported in calcium oxalate crystals (Figure 1).

As mentioned above, the luminescent signal of iGLuc was observed in the kidneys of mice fed with adenine-rich diet, however, the treatment with CP-456,773 reduced the signal intensity. In this study, the luminescent iGLuc signal was coming from the transferred bone marrow-derived cells transfected with iGLuc reporter construct. Of the bone marrow-derived cells, recent study has demonstrated that $CD11c^+$ dendtritic cells (DCs) have essential roles in calcium oxalate crystals-induced renal inflammation by NLRP3 inflammasome activation.[3] Therefore, to determine whether $CD11c^+$ DCs contributes to the development of adenine-induced crystal nephropathy through NLRP3 inflammasome activation, the authors in this study performed a series of experiments using mice expressing diphtheria-toxin receptor on $CD11c^+$ DCs ($CD11c^+$DTR mice) and isolated renal $CD11c^+$ DCs. First, $CD11c^+$DTR mice were exposed to DT for the depletion of $CD11c^+$ DCs, and showed the significant reduction of tubulointerstitial fibrosis as well as the levels of serum BUN and creatinine. Next, renal $CD11c^+$ DCs were isolated from the kidneys to examine the role of NLRP3 inflammasome in these cells. Renal $CD11c^+$ DCs isolated from wild type mice showed the increased amount of mature IL-1β and IL-18 by adenine-enriched food, but $CD11c^+$ DCs from NLRP3-deficient mice or CP456,773-treated mice had lower levels of those cytokines. In addition to that, renal $CD11c^+$ DCs from adenine-fed mice without CP-456,773 treatment showed strong luminescent signal, and this was greatly reduced by the treatment with CP-456,773. Taken altogether, renal $CD11c^+$ DCs may have significant roles to cause adenine-induced crystal nephropathy through their NLRP3 inflammasome activation (Figure 1).

Despite the fact that NLRP3 inflammasome especially in $CD11c^+$ DCs significantly contributes to the pathogenesis of adenine-induced crystal nephropathy as shown in this study, there seems to be several issues to be clarified. First, the role of renal resident cells in the pathogenesis of crystal nephropathy was not elucidated in detail. Not only innate immune cells, but also intrinsic renal cells such as tubular epithelial cells (TECs) are capable of secreting mature IL-1β and IL-18, and have been reported to have all the components necessary for inflammasome activation.[7] Therefore, TECs may also be important as the source of mature IL-1β dependent on NLRP3 inflammasome. Further studies using

TECs-specific deletion of NLRP3 inflammasome will be required to address the role of inflammasome activation in TECs. Technical advance of iGLuc transfection would also be required to see the cell-specific inflammasomes activation even in resident cells. Second, the mechanism(s) by which NLRP3 inflammasome is activated are still unclear. Recently, damaged TECs by calcium oxalate crystals have been known to release adenosine triphosphate (ATP), an endogenous NLRP3 agonist that stimulates $CD11c^+$ DCs to secrete mature IL-1β, thereby contributing to the pathogenesis of calcium oxalate nephropathy.[3] Besides ATP, various stimuli such as mitochondrial reactive oxygen species and potassium efflux have been recognized as endogenous activators of NLRP3 inflammasome.[8,9] Uptake of calcium oxalate crystals by $CD11c^+$ DCs is also able to induce NLRP3-mediated IL-1β secretion via potassium efflux.[3] It would be reasonable to speculate that these endogenous stimuli are involved in adenine-induced nephropathy as well. Third, PRRs may have crosstalk among them to activate each other in response to inflammatory stimuli. The expression of pro-IL-1β and IL-18 is regulated by nuclear factor-κB, which is controlled by various PRRs such as TLRs, therefore, the cooperation of NLRP3 and other PRRs could be critical for the secretion of IL-1β and IL-18. To clarify it in this model, comprehensive analyses of PRRs could be important. More detailed study to address above-mentioned questions would be required to precisely understand the mechanisms of crystal-induced nephropathy dependent NLRP3 inflammasome.

Taken altogether, this study clearly showed that a potent and selective NLRP3 inhibitor CP-456,773 significantly inhibited the activation of NLRP3 inflammasome in $CD11c^+$ DCs and the resulting IL-1β and IL-18 production, thereby contributing to the attenuation of tubulointerstitial inflammation and fibrosis in crystal-induced nephropathy. Currently, there is no effective treatment targeting inflammation for patients with primary hyperoxaluria or taking chemotherapy resulting in hyperuricosuria. In addition, NLRP3 inflammasome has been reported to be implicated in the various sterile inflammation including ischemia-reperfusion injury, atherosclerosis and diabetic nephropathy.

This study may open the door of the new possibilities of the inhibitors of NLRP3 inflammasome for various inflammatory diseases.

Since the discovery of inflammasomes, many sorts of inflammatory diseases have been known to be involved in the dysregulation of inflammasomes. To clarify the mechanisms of inflammasome activation and develop beneficial clinical drugs against inflammatory diseases, simple and precise method(s) to estimate inflammasomes activation is surely required. The iGLuc reporter system used in this study would be a suitable tool for that. Moreover, this technique can be applied to the investigation of other proteases as well by modifying its construct. Further progress on this system would provide us the great possibilities to investigate various diseases associated with proteolytic events.

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**Figure legend**

**Figure 1. *In vivo* detection of inflammasome activation using iGLuc and the role of NLRP3 inflammasome in a murine model of adenine-induced crystal nephropathy.**

iGLuc is a fusion construct of pro-IL-1β and Gaussia luciferase (GLuc) that reports the proteolytic activity of caspase-1 (which means the inflammasome activation) by the production of visible light. GLuc activity is almost completely suppressed under the control state by the formation of iGLuc aggregates. The treatment with the stimuli leading to caspase-1 activation induces the monomerization of iGLuc through the cleavage of pro-IL-1β to mature form of IL-1β, and activates iGLuc. In a murine model of adenine-induced crystal nephropathy, bone marrow-derived cells (BMDCs) transfected with iGLU were transferred into irradiated recipient mice, and high luminescent signal was detected in the kidneys, indicating that the activation of inflammasome in bone marrow-derived cells is induced in the course of adenine-induced crystal nephropathy. In addition, a potent and selective NLRP3 inhibitor CP-456,773 significantly inhibited the activation of NLRP3 inflammasome and the resulting IL-1β and IL-18 production, thereby contributing to the attenuation of tubulointerstitial inflammation and fibrosis in crystal-induced nephropathy.

ASC; apoptosis-associated speck-like protein containing a caspase recruitment domain, DCs; dendritic cells, NF-κB; nuclear factor-κB, NLRs; nucleotide-binding oligomerization domain-like receptors, TLRs; Toll-like receptors.