Enhanced p62 expression through impaired proteasomal degradation is involved in caspase-1 activation in monosodium urate crystal-induced interleukin-1β expression

Jung-Yoon Choe¹,2, Hyun-Young Jung¹, Ki-Yeun Park² and Seong-Kyu Kim¹,2

Abstract

Objective. Evidence for the role of autophagy in the regulation of inflammation, especially IL-1β expression in response to monosodium urate (MSU) crystals, is presented. This study investigated the role of p62, a selective autophagy receptor in autophagy, in IL-1β production in MSU crystal-induced inflammation.

Methods. IL-1β, TNF-α and IL-6 mRNA expression was measured by quantitative real-time PCR (qRT-PCR). Autophagy-related molecules such as p62, Cullin-3, microtubule-associated protein 1 light-chain 3 (LC3) I/I, ubiquitin, caspase-1 and mitogen-activated protein kinase (MAPK)-related proteins were measured by immunoblotting. Small interfering RNAs (siRNAs) for Atg16L1, IL-1β and p62 were used to silence each target gene.

Results. MSU crystals accelerate the process of autophagosome formation and also induce impairment of proteasomal degradation, resulting in p62 accumulation in autophagy. Enhanced p62 accumulation by MSU crystals leads to IL-1β expression through activation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK), but not p38, of the MAPK pathway and is also involved in activation of caspase-1 in inflammasomes. Impaired autophagosome formation by Atg16L1 siRNA significantly amplified p62 levels, thereby producing enhanced inflammatory responses, including overexpression of IL-1β under stimulation of MSU crystals. IL-1β also induces p62 protein, and blocking IL-1β under stimulation of MSU crystals greatly reduced p62 levels.

Conclusion. This study demonstrates that enhanced p62 expression through impaired proteasomal degradation by MSU crystals plays a crucial role in caspase-1 activation in MSU crystal-induced IL-1β production. p62 is required for activation of inflammasomes during acute inflammation in gout.

Key words: monosodium urate, autophagy, autophagy-related genes, interleukin-1β, p62.

Introduction

The precise pathogenic mechanisms for gout have not been clarified, although the roles of inflammatory cells, including neutrophils, monocytes and macrophages, and of pro-inflammatory cytokines, including IL-1β, CXCL-8 (IL-8) and TNF-α, have been elucidated in monosodium urate (MSU)-mediated inflammation [1, 2]. Of these inflammatory molecules, IL-1β is a crucial inflammatory molecule produced by immune cells, including macrophages, monocytes and dendritic cells in the pathogenesis of gout.

A pro-molecule of IL-1β, pro-IL-1β, is cleaved into active IL-1β by the enzyme caspase-1 and then active IL-1β is secreted from the cell [2]. The inflammasome has been established as a molecular platform for caspase-1 activation [3]. Danger signals that trigger NALP3 inflammasomes include extracellular adenosine triphosphate (ATP), infected cells or injured tissues, and particulate elements such as...
silica [4]. Recently, important investigations have determined that the pivotal role IL-1β plays in gouty inflammation is derived from NALP3 inflammasome activation through Toll-like receptors by MSU crystal and is a danger signal in the innate immune system [5–8].

Autophagy is an essential homeostatic process involving lysosomal degradation of damaged or aged cytoplasmic organelles and soluble macromolecules [9, 10]. In recent years, evidence has accumulated suggesting that autophagy is critical for regulating autoimmunity and inflammation. Saitoh et al. [11] demonstrated that lipopolysaccharide (LPS) induced IL-1β secretion in embryonic liver macrophages from Atg16L−/− mice, suggesting the role of autophagy in inflammatory responses. Inhibition of autophagy stimulates the production of IL-1β by bone marrow-derived macrophages in an NLRP3-dependent manner [12]. Autophagy is also found to be required for the activation of caspase-1 and the secretion of IL-1β mediated by NALP3 inflammasomes [13]. In crystal-related inflammation, loss of functional Atg16L1 and Atg5 genes impairs autophagosome formation and significantly enhances MSU crystal-induced IL-1β production [11, 14].

p62/SQSTM1 is a multifunctional adaptor molecule in autophagosome formation, promoting degradation of poly-ubiquitinated proteins through a proteasomal pathway [15, 16]. In recent research, p62 accumulation by impaired lysosomal and proteasomal pathways was found to be associated with enhanced inflammatory response by IL-1β overexpression through the mitogen-activated protein kinase (MAPK) pathway [17]. Although there is growing evidence of a close interaction between autophagy and MSU crystal-induced inflammation in gout [11, 14, 18], little information on the delicate linkage between p62, a selective autophagy receptor for the ubiquitinated protein aggregates in autophagy, and MSU crystal-induced inflammation, especially IL-1β production, is available. Therefore this study investigated the role of p62 in the regulation of MSU crystal-induced inflammation.

Materials and methods

Cell culture

Murine macrophage RAW 264.7 cells and human myelomonocytic cell line THP-1 were purchased from the Korean Cell Line Bank (Seoul, Korea) and maintained in DMEM or Roswell Park Memorial Institute (RPMI) 1640 (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) (HyClone, Logan, UT, USA), 100 U/ml penicillin and 100 μg/ml streptomycin. THP-1 cells (1 × 10⁶/well) were differentiated in six-well plates by treatment with 50 nM phorbol 12-myristate 13-acetate (PMA) (Sigma, St Louis, MO, USA). After 2 days, non-adherent cells were removed and adherent cells were treated with MSU crystals.

Preparation of MSU crystals

MSU crystals were prepared as described by Scanu et al. [19]. MSU crystal formation was observed by light microscopy. Endotoxin assessment was performed using the ToxinSensor Chromogenic LAL Endotoxin Assay kit (GenScript, Piscataway, NJ, USA) according to the manufacturer’s instructions. The experimental concentration of MSU crystals (0.5 mg/ml) was determined to be endotoxin free (<0.025 EU/ml).

Quantitative real-time PCR

Cells were incubated in 24-well plates containing 1 × 10⁶ cells/well and treated with 0.5 mg MSU crystals/ml for 2 h at 37°C. Total RNA was extracted with Trizol reagent (Gibco). For reverse transcription, complementary DNAs were made with the ReverTra Ace-α-kit (Toyobo, Osaka, Japan). Nuclease-free water was added to bring the final volume to 20 μl and the reaction was incubated at 42°C for 20 min, followed by heating at 99°C for 5 min, and stored at 4°C or −70°C.

Reverse transcriptase polymerase chain reaction

Reverse transcriptase polymerase chain reaction (RT-PCR) was performed in a 20 μl final volume containing cDNA, forward and reverse primers and New, Economic & Wide applicable Taq (Bionics, Seoul, Korea) master mixtures. The PCR products were detected on 1.2% agarose gel containing ethidium bromide.

Immunoblotting assay

Raw 264.7 cells were treated with MSU crystals (0.5 mg/ml) for 1 h and differentiated THP-1 cells (adherent cells) were treated with MSU crystals (0.2 mg/ml) for 1 h. After cells were harvested, pellets were resuspended in lysis buffer [1 M Tris-HCl, pH 8.0, 5 M NaCl, 10% Nonidet P40, protease inhibitor cocktail 1 tablet (Roche Diagnostics, Mannheim, Germany)], incubated on ice for 10 min and centrifuged at 12 000 rpm for 10 min at 4°C. The protein concentration was determined by a Bio-Rad protein assay kit. Proteins (50–70 μg) were separated by 13% SDS-PAGE and transferred to nitrocellulose membrane (Bio-Rad). The membrane was blocked in 5% skim milk (BD Bioscience, San Francisco, CA, USA) for 1 h at room temperature and incubated with primary antibodies at 4°C overnight. The secondary antibodies were conjugated to horseradish peroxidase and incubated for 1 h at room temperature. Reactive proteins were detected using the SuperSignal West Pico chemiluminescent kit (Thermo Scientific, Rockford, IL, USA). Densitometry was analysed
and quantified with Quantity One software (Bio-Rad). Primary antibodies including phosphorylated extracellular signal-regulated kinase (p-ERK), total ERK, phosphorylated c-Jun NH2-terminal kinase (p-JNK), total JNK (Santa Cruz Biotechnology, Dallas, TX, USA), phosphorylated p38 (p-p38), total p38 (Cell Signalling Technologies, Beverly, MA, USA), caspase-1 (Santa Cruz Biotechnology), Cul-3 (Cell Signalling Technologies), microtubule-associated protein light chain 3 (LC3) (Abcam, Cambridge, MA, USA), IL-1β (Abcam), p62 (Sigma-Aldrich, St Louis, MO, USA), total nuclear factor κB (NF-κB; Santa Cruz Biotechnology) and phosphorylated NF-κB (Abcam) were used for immunoblotting.

Immunofluorescence staining

Cells were seeded on coverslips and then fixed in 3.5% paraformaldehyde solution for 10 min at room temperature. Cells were then washed with PBS and permeabilized with Triton-X 100 (0.1% Triton-X 100 in PBS). Cells were washed and incubated with blocking buffer for 30 min at room temperature. Cells were incubated with primary antibody diluted in blocking buffer for 1 h. After washing with 0.1% Tween 20 in PBS, the slides were incubated with goat anti-rabbit IgG-FITC (Santa Cruz Biotechnology) for 30 min, and nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI) in the dark. Cover slips were mounted with fluorescent mounting medium (Dako, Glostrup, Denmark) and detected by fluorescence microscopy (TE2000-U, Nikon Instruments, Melville, NY, USA).

Transfection of small interfering RNA (siRNA)

Cells (1 × 10^5 cells/well) were seeded in 24-well plates and transfected with siRNA using Opti-MEM media (Invitrogen, Carlsbad, CA, USA). In brief, 50 ng of siRNA (non-targeting negative control siRNA; Med GC, mouse Atg16L1; MSS246476, mouse p62; MSS207330, human Atg16L1; HSS182825, and human p62; HSS113118) was mixed with Opti-MEM media. Separately, lipofectamine RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA) was mixed with Opti-MEM and then the mixtures were combined for 15 min at room temperature. The mixture was then added to DMEM supplemented with 10% FBS and incubated for 72 h.

Statistical analysis

Data are presented as mean (±SEM). Statistical analysis was performed using a nonparametric Mann-Whitney U test or paired Wilcoxon’s signed rank test. P-values < 0.05 were considered statistically significant. Data were analysed using SPSS version 13.0 for Windows (SPSS, Chicago, IL, USA).

Results

MSU crystals stimulate production of IL-1β through the MAPK pathway

IL-1β expression was highly detectable by RT-PCR when induced by MSU crystals at a concentration of 0.5 mg/ml in mice macrophage cell line RAW 264.7 cells (Fig. 1A). The maximum IL-1β protein expression was detected after 2 h of incubation (Fig. 1B). Consistently, qRT-PCR revealed that mRNA IL-1β expression under stimulation of MSU crystals (0.5 mg/ml) was 10 times greater at 2 h than non-treated macrophages (P < 0.01) (Fig. 1C). In addition, MSU crystals activated phosphorylation of all MAPKs, including ERK, JNK and p38, at 0.5 and 1 h (Fig. 1D).

MSU crystals induce autophagosome formation

MSU crystals significantly increased LC3-II expression in the protein level, a marker for autophagosome formation, compared with untreated murine cells (P < 0.05 for the ratio of LC3-II to LC3-I) (Fig. 2A), implicating MSU crystals in autophagy formation. After treatment with 3-methyladenine (3-MA), an autophagy inhibitor that inhibits phosphoinositide 3-kinase (PI3K) [21], LC3-II expression in both MSU-treated and untreated murine macrophages was markedly less (Fig. 2A). Experimental macrophages transfected with Atg16L1 siRNA (siAtg16L1) significantly suppress LC3-II expression, indicating blocked autophagosome formation (Fig. 2B). In the experiment in control cells, we consistently identified enhancement of LC3-II expression in MSU crystal-treated macrophages compared with non-treated cells (P < 0.05). For the IF assay, cells were transfected with Atg16L1 siRNA for 72 h and treated with MSU crystals (0.5 mg/ml) for 24 h or treated with only MSU crystals for 24 h. The results of IF staining showed that murine macrophages treated with MSU crystals had enhanced LC3-positive autophagosomes (Fig. 2C). Macrophages with autophagosomes, as stained by LC3, were significantly reduced in siAtg16L1-transfected cells.

MSU crystals enhance p62 accumulation by impaired proteasomal degradation

We investigated the effect of MSU crystals on p62 expression in autophagy. Western blot analysis indicated that p62 protein levels after treating cells with MSU crystals at 0.5 and 1.0 mg/ml in RAW 264.7 cells increased significantly compared with untreated cells (Fig. 3A). Fig. 3B illustrates the increased p62 protein level after MSU crystal stimulation at 0.1 and 0.2 mg/ml in THP-1 cells. In addition, MSU crystals (0.5 mg/ml) increased p62 protein levels at 1, 24 and 48 h compared with non-treated murine macrophages (Fig. 3C).

In addition, MG132, a proteasome inhibitor, induced a significant accumulation of p62, Cullin-3 and ubiquitin in a time-dependent manner (Fig. 3D). Cullin-3, a ubiquitin E3 ligase, interacts with its adaptor protein, Keap 1, and p62 in the autophagy process [22]. Simultaneously, murine macrophages treated with proteasome inhibitor (10 μM) for 4 and 12 h significantly increased IL-1β mRNA expression compared with non-treated cells (P < 0.05 and P < 0.01, respectively). This implies that p62 protein levels by impaired proteasomal degradation in autophagy are positively associated with IL-1β expression. MSU crystals alone increased p62, Cullin-3 and ubiquitin protein levels, similar with experimental macrophages treated...
with MG132 (Fig. 3E). Enhanced expression of these proteins was synergistically amplified by adding MG132. qRT-PCR analysis indicated consistently increased IL-1β mRNA expressions in macrophages treated with MSU crystals and/or MG132 (P < 0.05, P < 0.01 and P < 0.01 compared with non-treated cells). The result in a similar experiment using THP-1 cells was consistent with that in RAW 264.7 cells (Fig. 3F). We noted that an increase in ubiquitinated protein by MSU crystals might be related to suppressed proteasomal degradation in autophagy.

**p62 is involved in the activation of MARK signalling and caspase-1 in inflammasomes**

Western blot analysis indicated that p62 expression and phosphorylation of ERK, JNK and p-38 were significantly increased in MSU crystal-stimulated macrophages compared with non-treated cells with MSU crystals (Fig. 4A). In macrophages transfected with p62 siRNA under stimulation of MSU crystals, p62 expression and two MAPKs, including ERK and JNK but not p-38, was significantly attenuated compared with those in negative control siRNA macrophages. In addition, MSU crystal-stimulated RAW 264.7 cells significantly promoted activation of procaspase-1 to cleaved caspase-1, proportionally with increasing p62 levels, whereas activation of this enzyme and p62 levels was markedly reduced in macrophages transfected with p62 siRNA (Fig. 4B). Whereas negative control siRNA cells enhanced expression of cleaved caspase-1 in the response to MSU crystals, the cleaved caspase-1 level was significantly attenuated in THP-1 cells transfected with p62 siRNA (Fig. 4C).

We measured p62 levels in siAtg16L1-transfected and control macrophages treated with or without MSU crystals. MSU crystal-induced p62 accumulation was markedly amplified by Atg16L1 deficiency compared with negative control siRNA macrophages (Fig. 4D). When stimulated with MSU crystals, illustrated in Fig. 4A, phosphorylation of all MAPKs was noted in controls cells. However, phosphorylation of ERK and JNK in the MAPK pathway was more prominent in siAtg16L1 murine macrophages compared with negative control siRNA murine macrophages. This finding is compatible with that of a
previous study [17]. siAtg16L1-transfected murine macrophages after stimulation of MSU crystals produce more cleaved caspase-1 protein than negative control siRNA cells (Fig. 4E). Similarly, more enhanced cleaved caspase-1 protein expression was noted in siAtg16L1-transfected THP-1 cells (Fig. 4F).

Next, we identified whether suppressing autophagosome formation using Atg16L1 siRNA enhanced the production of pro-inflammatory cytokines such as IL-1β, TNF-α and IL-6. Using qRT-PCR, IL-1β and IL-6 expression was significantly higher in macrophages transfected with siAtg16L1 than untransfected cells under stimulation...
of MSU crystals [4-fold for IL-1β (P < 0.01) and 2-fold for IL-6 (P < 0.05)] (Fig. 4G). TNF-α, however, did not change significantly (P > 0.05), though its expression tended to increase. These results suggest that p62 is involved in activation of the MAPK signal pathway and caspase-1 in inflammasomes.

p62 level is reduced by IL-1β blockade

We investigated the effects of IL-1β on p62 and autophagosome formation. IL-1β-stimulated macrophages also enhanced production of p62 protein and promoted autophagosome formation, indicating LC3-II activation (Fig. 5A).

IL-1β stimulation in siAtg16L1-transfected cells promoted more enhanced IL-1β and p62 expression compared with negative control siRNA macrophages in each experiment using RAW 264.7 cells and THP-1 cells (Fig. 5B and C, respectively). Additionally, IL-1β deficiency by IL-1β siRNA markedly reduced p62 expression compared with negative control siRNA cells (Fig. 5D).

Discussion

Autophagy is an important determinant of cellular homeostasis that controls the intracellular organelles and...
pathogen-related molecules [9, 10]. With regard to MSU crystal-induced inflammation, the loss of function in autophagy leads to enhanced inflammatory responses such as increased production of IL-1β in response to MSU crystals [11, 14]. The aim of this study was to determine the role of p62, an adaptor molecule in autophagy, in the regulation of MSU crystal-induced IL-1β production.

We found that MSU crystals activate the MAPK signalling pathways and also stimulate accumulation of p62 through inhibiting proteasomal degradation. Enhanced p62 induced phosphorylation of ERK and JNK for pro-IL-1β production and activation of enzyme pro-caspase-1 into cleaved caspase-1, as summarized in Fig. 6. Overall, our data suggest that p62 expression plays a crucial role in
the pathogenesis of MSU crystal-induced inflammation. Furthermore, p62 is required for activation of inflammasomes during acute inflammation and could be considered as a therapeutic target in gout inflammation.

Autophagy is a cellular pathway involved in clearing damaged or aged organelles and proteins in the cytoplasm through fusion of an autophagosome and a lysosome and lysosomal degradation [9, 10]. Among these complicated mechanisms in autophagy, formation of the Atg5–Atg12–Atg16L complex plays an essential role in expanding a phagophore from an isolation membrane and also in cargo recognition. In particular, Atg16L1 has been shown to be associated with inflammatory and immune responses, including antibacterial response, antigen presentation and suppression of proinflammatory cytokines [9]. Loss of Atg16L1 function resulted in intestinal injury through disrupted secretion of granules containing antimicrobial peptides from intestinal Paneth cells of mice and in patients with Crohn’s disease due to an Atg16L1 mutation [23]. In addition, Atg16L1-deficient macrophages showed increased LPS-induced IL-1β production via inflammasome activation as well as suppressed autophagy formation [11]. IL-1β production was also significantly increased in MSU crystal-stimulated Atg16L1-deficient macrophages. These results suggest that a definite role of autophagy in MSU crystal-induced inflammation. Recently another study demonstrated that enhanced IL-1β production suppressed autophagy by down-regulating Atg6 or Beclin, which is known as Atg6 and is required to initiate autophagy, in THP1 macrophages [14]. Danger signals from the innate immune system, such as LPS, ATP and MSU crystals, may enhance the production of aberrant proinflammatory cytokines, including IL-1β, when autophagy is disrupted. Consistently our study also observed that MSU crystal-induced IL-1β release in RAW 264.7 cells occurred more prominently when autophagy was impaired by the loss of functional Atg16L1.

p62 has been identified as an intracellular protein with diverse roles as a scaffold protein in cell signalling, including the NF-κB pathway, and in turning over poly-ubiquitinated protein aggregates [24]. p62 has an
LC3-interacting region (LIR) and a ubiquitin-associated (UBA) domain. p62 is critical for shuttling ubiquitinated substrates for proteasomal degradation [15]. Some evidence suggests that p62, in a complex with ubiquitinated substrates through LIR and UBA domains, is targeted for proteasomal degradation during autophagy [17, 22, 25]. The interaction of Keap1, a stress sensor of Cullin-3 ubiquitin E3 ligase, and p62 led to Keap1 degradation by autophagy [22]. However, aberrant Atg7 expression results in these molecules accumulating in the liver. These results suggest that disturbed autophagosome formation is responsible for p62 accumulation. Recently p62 accumulation has been shown to enhance the inflammatory response by activating NF-κB and MAPK pathways by IL-1β stimulation in Atg16L1-deficient cells [17]. In this study, MSU crystals induced p62 production. When cells were stimulated with MSU crystals, ubiquitinated protein expression was more prominent compared with non-treated cells. MSU crystals might disrupt proteasomal degradation. Xu et al. [26] reported that angiotensin II increased 26S proteasome activity in endothelial cells in vitro, whereas uric acid reversed the proteasome activity. Therefore p62 accumulation in this study might be explained by MSU crystals suppressing proteasomal degradation of poly-ubiquitinated protein aggregates. In addition, we found that p62 accumulation due to MG132, a proteasome inhibitor, resulted in significantly greater IL-1β production. These results suggest that aberrant p62 accumulation through suppressed proteasomal degradation in autophagy leads to amplified inflammatory responses through signal cascades related to inflammation.

p62 regulates various signal cascades, especially as a scaffold protein for NF-κB activation in the TNF-α and IL-1β signalling pathways [24]. Moreover, siRNA-induced p62 knockdown in Atg16L1-deficient mouse embryonic fibroblasts suppressed NF-κB and ERK activation through the IL-1β signalling pathway [17]. In contrast, insulin resistance and obesity in p62 knockout mice were due to a hyperactive ERK pathway in adipogenesis [27]. In this study, increased p62 in Atg16L1-deficient cells was related to ERK and JNK activation. Diverse cellular signal transduction pathways, including NF-κB, MAPK, Syk tyrosine kinase and Src family tyrosine kinases, were involved in acute inflammatory responses in gout [28]. From this study, p62 protein seems to play a crucial role in MSU crystal-induced inflammation through the MAPK pathway. We also identified enhanced phosphorylation of NF-κB in Atg16L1-deficient macrophages and attenuation of NF-κB activation in p62-deficient macrophages (data not shown), consistent with previous findings [17, 24]. In addition, we found that IL-1β induced p62 protein expression in control macrophages and p62 was suppressed in macrophages transfected with IL-1β siRNA. This finding provides a rationale for IL-1 target production.

**Fig. 6** Proposed model for the role of p62 in MSU crystal-induced IL-1β expression.

MSU crystals activate cleavage of pro-IL-1β into active IL-1β through the MAPK signalling pathway. p62, through impaired proteasomal degradation mediated by MSU crystals, is accumulated in the cytoplasm in the process of autophagy. p62 plays a role in the activation of ERK and JNK, but not p38, in the MAPK signalling pathway and in the transition to the active form of caspase-1, thereby leading to production of active IL-1β. In addition, excess accumulation of p62 could have occurred in impaired autophagy, such as the loss of Atg16L1, thus amplifying inflammatory responses.
therapy since IL-1β blockade could reduce the burden of pathogenic activity by p62 in MSU crystal-induced inflammation.

The present study demonstrated that p62 is involved in the activation of caspase-1 as an important component within the inflammasome complex. Although a clear association between autophagy and inflammasome activation in MSU crystal-mediated inflammation has been established in earlier investigations [11–14], the role of signalling adaptor p62 in the regulation of inflammasomes, especially caspase-1, has remained unclear. In this study, p62-deficient macrophages using p62 siRNA suppressed activation of caspase-1, together with down-regulation of p62. In contrast, we observed up-regulation of caspase-1 in Atg16L1-deficient macrophages presenting with overexpression of p62. Here, the question of how p62 participates in the activation of caspase-1 has been raised. p62-dependent caspase-1 activation in MSU crystal-induced inflammation is consistent with other reports demonstrating that p62 mediated caspase-1 activation triggered by Mycobacteria abscessus [29]. However, the mechanism of how p62 is involved in the inflammasome complex, consisting of NALP, apoptosis-associated speck-like protein (ASC) adaptor and caspase-1, is unclear. Recently p62 was found to be located in the same membrane compartment as inflammasome and caspase-1 in Shigella flexneri-infected non-myeloid cells [30]. Based on this observation in an infectious model, it possibly suggests that p62, presenting a function of promoting protein aggregation as ubiquitination, might be involved in oligomerization of inflammasome components. However, the function of p62 in activating caspase-1 should be further investigated.

In conclusion, this study demonstrated that MSU crystals, a danger signal in the innate immune system, stimulate IL-1β release by inducing p62 accumulation in autophagy. Increased IL-1β production occurred when autophagosome formation was impaired due to p62 overexpression by inhibited autophagic or lysosomal degradation. p62 could be associated with acute inflammatory responses to MSU crystals and also could be a possible therapeutic target in gout inflammation.

**Rheumatology key messages**

- Monosodium urate (MSU) crystals induce p62 accumulation through impaired proteasomal degradation in autophagy process.
- Enhanced p62 accumulation promotes IL-1β expression through activation of mitogen-activated protein kinase signalling and caspase-1 in MSU crystal-induced inflammation.
- p62 could be a therapeutic target in the control of gout inflammation and potentially regulated by IL-1β blocking therapy.

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