LETTERS

Gout-associated uric acid crystals activate the NALP3 inflammasome

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Development of the acute and chronic inflammatory responses known as gout and pseudogout are associated with the deposition of monosodium urate (MSU) or calcium pyrophosphate dihydrate (CPPD) crystals, respectively, in joints and periarticular tissues. Although MSU crystals were first identified as the aetiological agent of gout in the eighteenth century[1] and more recently as a 'danger signal' released from dying cells[2], little is known about the molecular mechanisms underlying MSU- or CPPD-induced inflammation. Here we show that MSU and CPPD engage the caspase-1-activating NALP3 (also called cryopyrin) inflammasome, resulting in the production of active interleukin (IL)-1β and IL-18. Macrophages from mice deficient in various components of the inflammasome such as caspase-1, ASC and NALP3 are defective in crystal-induced IL-1β activation. Moreover, an impaired neutrophil influx is found in an *in vivo* model of crystal-induced peritonitis in inflammasome-deficient mice or mice deficient in the IL-1β receptor (IL-1R). These findings provide insight into the molecular processes underlying the inflammatory conditions of gout and pseudogout, and further support a pivotal role of the inflammasome in several autoinflammatory diseases.

The notion of autoinflammatory diseases delineates a heterogeneous group of pathologies characterized by spontaneous periodic inflammation and fever in the absence of infectious or autoimmune causes[3]. Hereditary periodic fevers, systemic onset juvenile idiopathic arthritis, Still's disease, Behçet's disease and the metabolic disorders gout and pseudogout are examples of such inflammatory maladies. Increased production of the inflammatory cytokine IL-1β was recently identified as the cause of several autoinflammatory diseases, providing clear evidence for a pivotal role of this cytokine in triggering autoinflammation[4–8]. IL-1β, also known as the endogenous pyrogen, is a highly inflammatory cytokine whose production is tightly controlled by at least three distinct steps[9]. The first step involves the production of the pro-IL-1β protein (p35); this is followed by cleavage of the precursor pro-IL-1β to produce the active IL-1β protein (p17), and finally IL-1β is released into the extracellular environment. The middle step, processing of pro-IL-1β, involves the activation of a caspase-1-activating complex, the best characterized being the inflammasome[10,11].

Upon activation, the inflammasome is formed by a member of the NALP protein family, such as NALP1, NALP2 or NALP3, and the adaptor protein ASC that connects the NALPs with caspase-1 (ref. 12). Signals and mechanisms leading to inflammasome activation are still poorly understood. Muramyl dipeptide (MDP), a degradation product of the bacterial cell wall component peptidoglycan and contaminant of crude lipopolysaccharide (LPS), was recently shown to activate a NALP3 inflammasome[13] through the leucine-rich repeat domain of NALP3, suggesting that NALPs, like Toll-like receptors (TLRs), are fundamental for microbial detection[14]. However, the inflammasome is also proficient in sensing stress or endogenous danger signals, such as extracellular ATP or hypotonic stress[10,11,15]. Recently, MSU crystals were identified as a danger signal formed after release of uric acid from dying cells[2]. This observation, and the well-known role of uric acid crystals in gouty arthritis[16], prompted us to investigate whether MSU crystals could activate the inflammasome.

Cells from the differentiated monocytic cell line THP1 were incubated with MSU crystals. Maturation of IL-1β was indeed detected after stimulation with as little as $10\,\mu g\,ml^{-1}$ of the crystals (Fig. 1a). The caspase-1 dependency of the pro-IL-1β cleavage was confirmed by addition of the caspase-1 inhibitor zYVAD-fmk, which completely blocked MSU-induced IL-1β activation (Fig. 1a). CPPD, another type of pathogenic crystal involved in calcium pyrophosphate deposition disease, also known as pseudogout, was as active as MSU (Fig. 1b). Crystal-induced IL-1β processing was specific for pathogenic agents, as the non-inflammatory allopurinol or diamond crystals and particulate elements such as zymosan and aluminium powder failed to induce pro-IL-1β processing (Fig. 1c), despite their similar size and/or chemical composition. Compared to the known activators of the inflammasome (that is, crude LPS, ATP), MSU and CPPD were more active[11,13] (Fig. 1c). This superior potency was particularly evident when analysing processing of pro-IL-18, the second known substrate of caspase-1 (Fig. 1c). Previously, we demonstrated that the inflammatory caspases are cleaved and released along with active IL-1β after activation of the inflammasome[13]. This was also observed when cells were treated with MSU and CPPD (Fig. 1c, d). In order to exclude the possibility that crystal-mediated activation of caspase-1 is a unique property of the THP1 cell line only, MSU and CPPD were added to purified human monocytes. As shown in Fig. 1d, a strong response to both pathogenic crystals was also elicited in primary cells.

In order to provide direct evidence for the involvement of the inflammasome in crystal-induced inflammation, we analysed peritoneal macrophages (PMΦs) derived from mice deficient in various key proteins of the inflammasome complex or other proinflammatory pathways. Given the absence and/or rapid degradation of pro-IL-1β in PMΦs *ex vivo*, and because we failed to see any direct induction of the transcription or translation of pro-IL-1β by MSU or CPPD, we stimulated TLR4 in PMΦs with highly purified LPS to induce the synthesis of the cytokine[11,13]. Consistent with our previous findings in human monocytes, murine PMΦs stimulated with MSU or CPPD activated caspase-1 and secreted mature IL-1β (Fig. 2a). Maturation was abolished in PMΦs from caspase-1-deficient mice, confirming the specificity of the activation. As expected, MyD88-deficient PMΦs did not produce mature IL-1β due to their defective TLR signalling, resulting in a failure to produce pro-IL-1β after LPS pre-stimulation (Fig. 2a). Nevertheless, $MyD88^{-/-}$ PMΦs still activated caspase-1 (Fig. 2a), further suggesting that this activation is

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TLR independent and is consistent with a possible involvement of the inflammasome[13,15]. ASC is a crucial adaptor protein required for the recruitment of caspase-1 to the NALP platform of inflammasomes[12]. ASC-deficient PMΦs did not produce any mature IL-1β after stimulation by MSU and CPPD crystals (Fig. 2b).

The human genome harbours a repertoire of 14 NALPs. It is currently not clear how many of them form inflammasomes. NALP3 is expressed in both monocytes and macrophages and is well conserved in human and mouse. Its ability to form an inflammasome and to drive inflammation in humans is well supported by its implication in many hereditary autoinflammatory syndromes[4]. We considered that the NALP3 inflammasome was possibly implicated in crystal-induced caspase-1 activation and we therefore generated NALP3-deficient mice (Supplementary Fig. 1 and V.P., F.M. and J.T., manuscript in preparation). Similar to PMΦs from *Asc*$^{-/-}$ mice, IL-1β release was impaired in NALP3-deficient PMΦs upon MSU and CPPD exposure (Fig. 2c). IL-1β induction by ATP, the other known non-microbial stimulus of inflammasomes, was also dependent on NALP3 (Fig. 2c). Whereas blocking of the ATP receptor $P2X_7$ inhibited ATP-driven inflammasome activation, it had no effect on MSU-induced activation, indicating that the two inflammasome-activating pathways act independently (Supplementary Fig. 2).

In addition to cytokines whose activity is dependent on caspase-1 activation, MSU and CPPD are known to induce release of other cytokines such as TNF[17,18], suggesting additional, inflammasome-independent activities of the crystals. When assaying the release of TNF, we realized that the production of TNF was relatively slow and was preceded by the release of IL-1β[19] (Fig. 3a). It was therefore possible that TNF secretion was initiated, at least in part, by the released mature IL-1β. Indeed, blocking the maturation of IL-1β with zYVAD-fmk considerably reduced the production of TNF induced by MSU and CPPD, without affecting TNF production by the TLR2 agonist zymosan (Fig. 3a). Similarly, IL-1ra, a natural inhibitor of IL-1 signalling, significantly affected the production of TNF and IL-6 by human monocytes (Fig. 3b). These results suggest that the processing of IL-1β is a proximal event in the inflammatory cascade initiated by pathogenic crystals, possibly explaining the extraordinary success of IL-1ra in the treatment of some auto-inflammatory diseases[20,21].

Colchicine is another drug that is frequently used for the treatment of autoinflammatory diseases, including familial Mediterranean fever, acute gout and pseudogout episodes[22]. Pre-treatment with intravenous colchicine before intra-articular MSU injections greatly reduces inflammation[23], suggesting that colchicine targets the initial phase of inflammation. We therefore investigated the role of colchicine in crystal-induced maturation of IL-1β. As shown in Fig. 3c, pre-treatment with colchicine, but not its solvent ethanol, completely blocked the processing of IL-1β. In contrast, colchicine did not affect IL-1β activation by extracellular ATP, indicating that the drug acts upstream of inflammasome activation. Taken together, the above

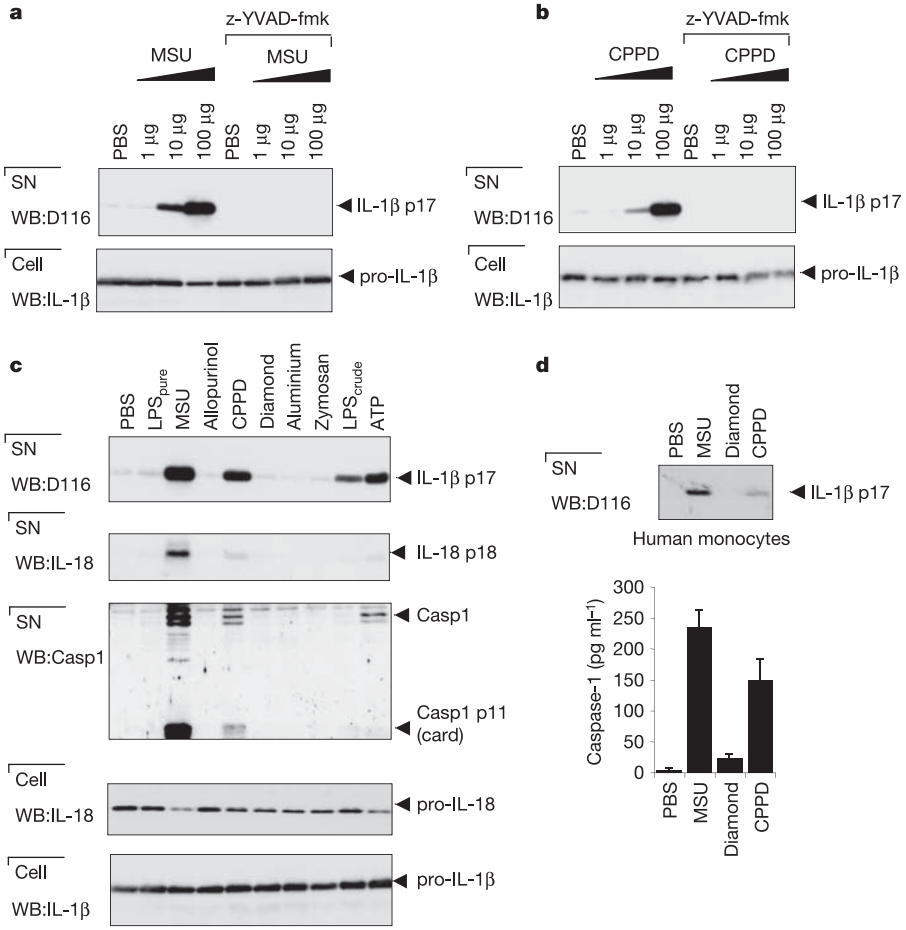

Figure 1 | Monosodium urate (MSU) and calcium pyrophosphate dihydrate (CPPD) crystals activate IL-1β cleavage and release. a–c, THP1 cells were stimulated for 6 h with the indicated amounts (per ml) of MSU crystals (**a**), CPPD crystals (**b**) or with 50 μg ml^{-1} of pure LPS, MSU crystals, allopurinol crystals, CPPD crystals, diamond crystals, aluminium particles, zymosan, crude preparations of LPS, or 5 mM of extracellular ATP as indicated (**c**). Supernatants (SN) were analysed for the presence of mature IL-1β, IL-18 or caspase-1, and cell extracts (Cell) for the presence of pro-IL-1β and pro-IL-18. WB, western blot. **d**, Human monocytes were stimulated with 50 μg ml^{-1} of the indicated crystals for 6 h and analysed by western blot for IL-1β activation or by ELISA for released caspase-1 and IL-1β. Values are ±s.e.m.

results indicate that crystals are proinflammatory by virtue of their capacity to activate the NALP3 inflammasome.

Clinically, gout and pseudogout are associated with oedema and erythema of the joints, with consequent severe pain, conditions that are associated with strong infiltration of neutrophils in the intra-articular and periarticular spaces. This marked neutrophil influx can be reproduced experimentally in mice by intraperitoneal injection of crystals[24]. We used this well-established model to investigate the *in vivo* role of the inflammasome in crystal-induced inflammation. MSU, CPPD or allopurinol crystals were injected and the peritoneal recruitment of neutrophils was analysed 6 h later. Both MSU and CPPD crystals elicited a considerable increase in the recruitment of neutrophils compared with PBS or allopurinol when injected in wild-type C57BL/6 mice (Fig. 4a). Although neutrophil influx was slightly increased in BALB/c mice, both BALB/c and C57BL/6 strains adequately reproduced crystal-induced inflammation (Fig. 4 and data not shown). Importantly, when pathogenic crystals were injected in mice deficient in caspase-1 or ASC, neutrophil influx was markedly impaired (Fig. 4b, c), indicating a pivotal role of the inflammasome and IL-1β in this process. In agreement with this notion was the observation that IL-1R-deficient mice exhibited a similarly reduced recruitment of neutrophils after MSU and CPPD injection (Fig. 4d). In contrast, zymosan-induced neutrophil influx was not affected by ASC or IL-1R deficiency.

Gout and pseudogout are two common causes of inflammatory joint diseases. Despite differences underlying their pathogenesis, their clinical presentation and treatment share many common features. On the basis of our findings that pathogenic crystal-mediated IL-1β maturation requires the inflammasome components NALP3, ASC and caspase-1, we propose that both aetiological agents of gout and pseudogout (that is, MSU and CPPD) mediate inflammation in an inflammasome-dependent manner. This notion is further supported by clinical data demonstrating that colchicine, a drug able to resolve the initial inflammatory phase of both gout and pseudogout, blocks IL-1β maturation by MSU and CPPD. Combining colchicine's known mode of action as an inhibitor of microtubule assembly with the observation that colchicine blocks crystal-induced IL-1β generation upstream of inflammasome activation (Fig. 3c), it is likely that the drug acts at the level of crystal endocytosis and/or presentation to the inflammasome[25]. Notably, MSU uptake was also proposed to be partly dependent on the presence of TLR2 and TLR4 (ref. 26). The mechanism whereby endocytosed MSU and CPPD are sensed by the NALP3 inflammasome is currently not known, nor is it clear whether the crystals directly interact with NALP3 or whether sensing occurs via intermediary protein(s). Because inflammasome-activating MSU crystals and inflammasome neutral allopurinol are chemically and structurally similar and, additionally, are both internalized, inflammasomes must have the capacity to distinguish between subtle differences in crystal surface charge or form.

Microbial components (pathogen-associated molecular patterns

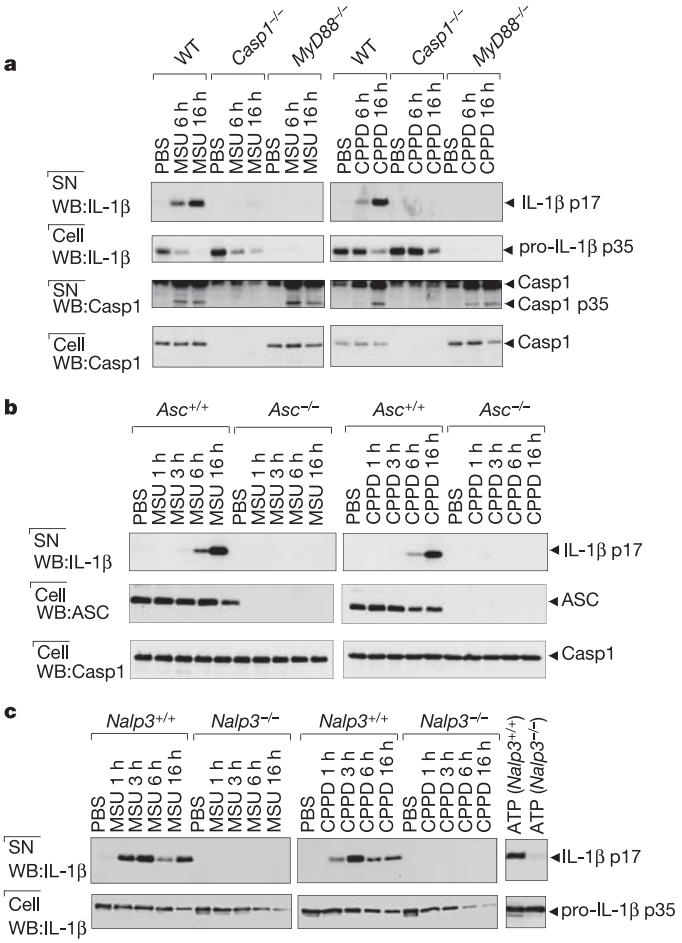

Figure 2 | The NALP3 inflammasome is required for the maturation of IL-1β. a–c, Mouse macrophages from wild-type (WT), caspase-1 (Casp1)- or MyD88-deficient mice (**a**), ASC-deficient mice or littermate controls (**b**), and NALP3-deficient mice or littermate controls (**c**) were stimulated as indicated in the presence of ultra-pure LPS (1 μg ml^{-1}, Alexis or Invivogen) in order to induce the synthesis of precursor pro-IL-1β. In **c**, ultra-pure LPS was added 1 h before stimulation. Supernatant (SN) or cell extracts (Cell) were analysed by western blot as indicated.

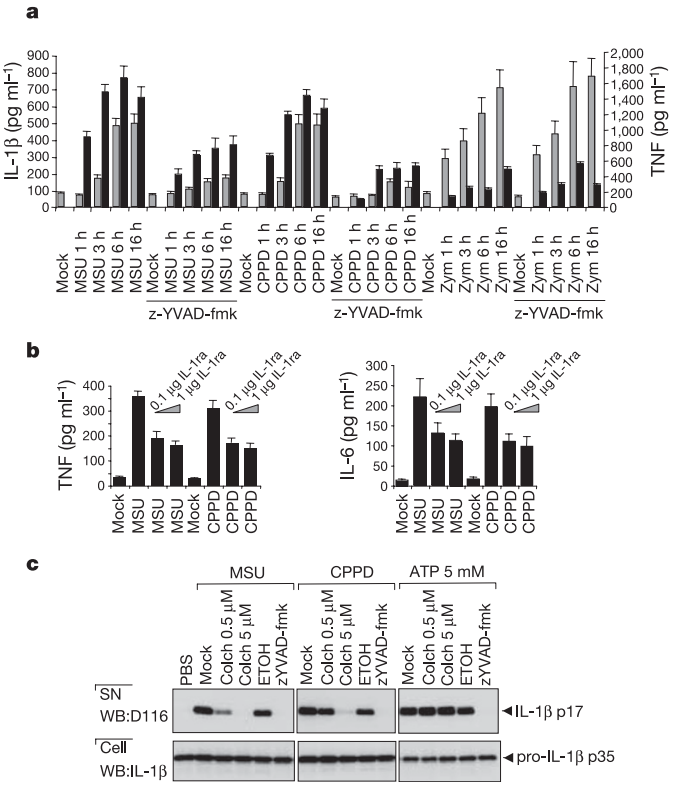

Figure 3 | IL-1β maturation is an early event after MSU and CPPD stimulation, and is blocked by colchicine. a, THP1 cells were stimulated with MSU, CPPD or zymosan (Zym) for the indicated times in the presence or absence of the caspase-1 inhibitor zYVAD-fmk. Supernatants were analysed for TNF (grey bars) and IL-1β (black bars) production by ELISA. Values are ±s.e.m. **b**, Human monocytes were incubated with MSU or CPPD in the presence of two concentrations of IL-1ra. TNF and IL-6 production was monitored by ELISA. Values are ±s.e.m. **c**, THP1 cells were stimulated with MSU, CPPD or ATP in the presence or absence of colchicine (Colch). Maturation of IL-1β was analysed by western blot.

(PAMPs)) provide signals that alert our immune system to danger and promote the innate generation of immunity[27]. However, PAMPs (non-self) are not the only triggers of innate immunity. Innate immunity is able to recognize abnormal self or danger signals, such as uric acid released by injured cells[2,28]. How these danger signals are recognized by cells is mostly unknown, but based on our results inflammasomes probably constitute some of the long-sought proximal sensors for stress or danger signals designed to initiate inflammation.

In addition to gouty inflammation, the NALP3 inflammasome is also implicated in other autoinflammatory diseases. Specific gain-of-function mutations in the NALP3 protein lead to three related familial autoinflammatory diseases: Muckle–Wells syndrome, familial cold autoinflammatory syndrome and chronic infantile neurologic cutaneous and articular syndrome[4,29]. In patients with these diseases, mutations in NALP3 lead to a constitutive processing of IL-1β[30]. In the case of gout and pseudogout, aberrant NALP3 inflammasome activation is not genetic, but mediated by local deposition of crystals. Importantly, inflammation in hereditary periodic fevers patients with mutations in NALP3 can be markedly improved by treatments designated to block IL-1β[20,21]. Owing to the similarity between NALP3-mediated hereditary periodic fevers and gout and pseudogout, we can anticipate that similar treatments could benefit gout and pseudogout patients. It is also reasonable to foresee that further identification of additional inflammasome-activating endogenous danger signals will probably shed some light on the molecular aetiology of other autoinflammatory diseases such as systemic onset juvenile idiopathic arthritis and Behçet's disease that share similarity with hereditary periodic fevers, gout or pseudogout.

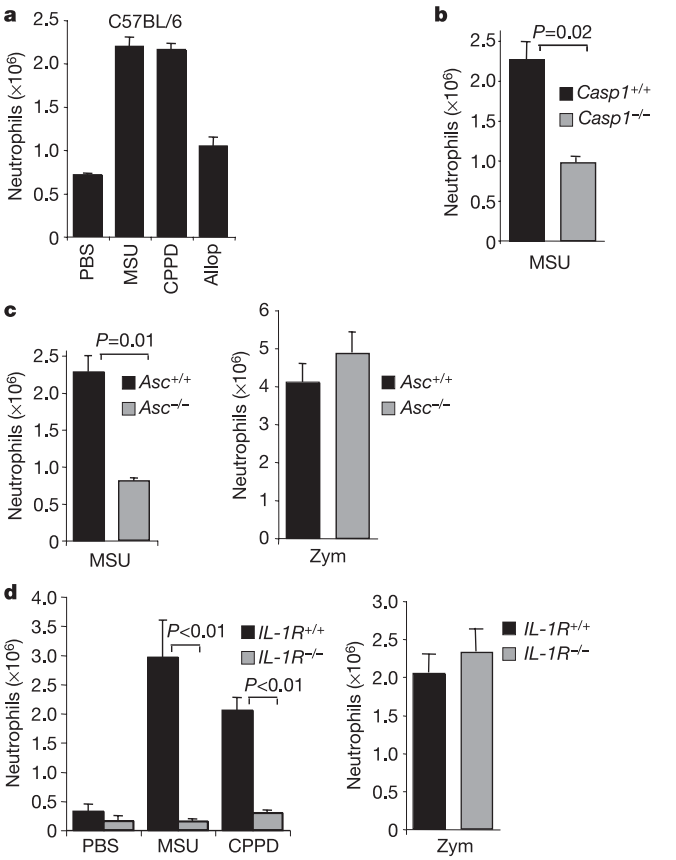

Figure 4 | Role of the inflammasome in a mouse model of crystal-mediated peritonitis. a–d, The indicated wild-type or mutant mice received 0.5 ml (intraperitoneally) of sterile PBS alone or supplemented with 1 mg of the indicated crystals or 0.2 mg of zymosan. Neutrophil influx was quantified 6 h later (values are ±s.e.m. of $n = 4$–6 mice per group). Unpaired Student's *t*-test was used to calculate *P* values. Allop, allopurinol.

METHODS

Primary human monocyte and THP1 preparation and stimulation. THP1 cells were stimulated for 3 h with 0.5 μM of PMA the day before stimulation, as described[10]. This treatment increases the phagocytic properties of the cells and induces a constitutive production of pro-IL-1β. Human monocytes were purified as described previously[30]. All cells were stimulated in OptiMEM medium as indicated. Human mature IL-1β was detected with a specific antibody directed against the cleaved epitope (D116) from Cell Signaling.

Mouse macrophage preparation. Eight-to-twelve-week-old mice of indicated genotypes were injected intraperitoneally with 4% thioglycollate solution, and macrophages were collected by peritoneal lavage 3 days later. Cells were plated at the density of 7×10^5 cells in 12-well dishes and non-adherent cells were removed after 3 h. Cells were cultured in RPMI complemented with 10% FCS, sodium pyruvate, penicillin/streptomycin and L-glutamine. All cells were stimulated in OptiMEM medium.

***In vivo* mouse peritonitis model.** Peritonitis was induced by injection of 1 mg of crystals or 0.2 mg of zymosan in 0.5-ml sterile PBS. After 6 h, mice were killed by CO_2 exposure and peritoneal cavities were washed with 10 ml of PBS. The lavage fluids were analysed for PMN recruitment by FACS using the neutrophil marker Ly-6G (1A8, BD Biosciences).

Mice and reagents. NALP3 targeting vector (Supplementary Fig. 1) was electroporated into C57BL/6 embryonic stem (ES) cells (Ozgene). Homologous recombinant ES cells were identified by Southern blot analysis and microinjected into C57BL/6 blastocysts. Offspring were backcrossed to C57BL/6 mice and germline transmission was confirmed by PCR of tail genomic DNA. Additional details on mice, preparation of crystals and reagents are given in the Supplementary Information.

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1. Wollaston, H. W. On gouty and urinary concretions. *Phil. Trans.* **87,** 386–400 (1797).
2. Shi, Y., Evans, J. E. & Rock, K. L. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* **425,** 516–521 (2003).
3. Galon, J., Aksentijevich, I., McDermott, M. F., O'Shea, J. J. & Kastner, D. L. TNFRSF1A mutations and autoinflammatory syndromes. *Curr. Opin. Immunol.* **12,** 479–486 (2000).
4. Martinon, F. & Tschopp, J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* **117,** 561–574 (2004).
5. Stojanov, S. & Kastner, D. L. Familial autoinflammatory diseases: genetics, pathogenesis and treatment. *Curr. Opin. Rheumatol.* **17,** 586–599 (2005).
6. Dinarello, C. A. Blocking IL-1 in systemic inflammation. *J. Exp. Med.* **201,** 1355–1359 (2005).
7. Shoham, N. G. *et al.* Pyrin binds the PSTPIP1/CD2BP1 protein, defining familial Mediterranean fever and PAPA syndrome as disorders in the same pathway. *Proc. Natl Acad. Sci. USA* **100,** 13501–13506 (2003).
8. Chae, J. J. *et al.* Targeted disruption of pyrin, the FMF protein, causes heightened sensitivity to endotoxin and a defect in macrophage apoptosis. *Mol. Cell* **11,** 591–604 (2003).
9. Burns, K., Martinon, F. & Tschopp, J. New insights into the mechanism of IL-1β maturation. *Curr. Opin. Immunol.* **15,** 26–30 (2003).
10. Martinon, F., Burns, K. & Tschopp, J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-β. *Mol. Cell* **10,** 417–426 (2002).
11. Mariathasan, S. *et al.* Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* **430,** 213–218 (2004).
12. Tschopp, J., Martinon, F. & Burns, K. NALPs: a novel protein family involved in inflammation. *Nature Rev. Mol. Cell Biol.* **4,** 95–104 (2003).
13. Martinon, F., Agostini, L., Meylan, E. & Tschopp, J. Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. *Curr. Biol.* **14,** 1929–1934 (2004).
14. Martinon, F. & Tschopp, J. NLRs join TLRs as innate sensors of pathogens. *Trends Immunol.* **26,** 447–454 (2005).
15. Yamamoto, M. *et al.* ASC is essential for LPS-induced activation of procaspase-1 independently of TLR-associated signal adaptor molecules. *Genes Cells* **9,** 1055–1067 (2004).
16. Faires, J. S. & McCarty, D. J. Acute arthritis in man and dog after intrasynovial infection of sodium urate crystals. *Lancet* **280,** 682–685 (1962).
17. Dalbeth, N. & Haskard, D. O. Mechanisms of inflammation in gout. *Rheumatology (Oxford)* **44,** 1090–1096 (2005).
18. Meng, Z. H., Hudson, A. P., Schumacher, H. R. Jr, Baker, J. F. & Baker, D. G. Monosodium urate, hydroxyapatite, and calcium pyrophosphate crystals induce tumour necrosis factor-alpha expression in a mononuclear cell line. *J. Rheumatol.* **24,** 2385–2388 (1997).

19. Chapman, P. T. *et al.* Endothelial activation in monosodium urate monohydrate crystal-induced inflammation: *in vitro* and *in vivo* studies on the roles of tumor necrosis factor alpha and interleukin-1. *Arthritis Rheum.* **40,** 955–965 (1997).
20. Hoffman, H. M. *et al.* Prevention of cold-associated acute inflammation in familial cold autoinflammatory syndrome by interleukin-1 receptor antagonist. *Lancet* **364,** 1779–1785 (2004).
21. Hawkins, P. N., Lachmann, H. J. & McDermott, M. F. Interleukin-1-receptor antagonist in the Muckle–Wells syndrome. *N. Engl. J. Med.* **348,** 2583–2584 (2003).
22. Molad, Y. Update on colchicine and its mechanism of action. *Curr. Rheumatol. Rep.* **4,** 252–256 (2002).
23. Malawista, S. E. & Seegmiller, J. E. The effect of pretreatment with colchicine on the inflammatory response to microcrystalline urate: A model for gouty inflammation. *Ann. Intern. Med.* **62,** 648–657 (1965).
24. Getting, S. J. *et al.* Molecular determinants of monosodium urate crystal-induced murine peritonitis: a role for endogenous mast cells and a distinct requirement for endothelial-derived selectins. *J. Pharmacol. Exp. Ther.* **283,** 123–130 (1997).
25. Goldfinger, S. E., Howell, R. R. & Seegmiller, J. E. Suppression of metabolic accompaniments of phagocytosis by colchicine. *Arthritis Rheum.* **8,** 1112–1122 (1965).
26. Liu-Bryan, R., Scott, P., Sydlaske, A., Rose, D. M. & Terkeltaub, R. Innate immunity conferred by Toll-like receptors 2 and 4 and myeloid differentiation factor 88 expression is pivotal to monosodium urate monohydrate crystal-induced inflammation. *Arthritis Rheum.* **52,** 2936–2946 (2005).
27. Janeway, C. A. Jr & Medzhitov, R. Innate immune recognition. *Annu. Rev. Immunol.* **20,** 197–216 (2002).
28. Matzinger, P. The danger model: a renewed sense of self. *Science* **296,** 301–305 (2002).
29. Hoffman, H. M., Mueller, J. L., Broide, D. H., Wanderer, A. A. & Kolodner, R. D. Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle–Wells syndrome. *Nature Genet.* **29,** 301–305 (2001).
30. Agostini, L. *et al.* NALP3 forms an IL-1β-processing inflammasome with increased activity in Muckle–Wells autoinflammatory disorder. *Immunity* **20,** 319–325 (2004).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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