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Uric acid as a danger signal in gout and its comorbidities

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Abstract

Uric acid is a waste product of purine catabolism. This molecule comes to clinical attention when it nucleates to form crystals of monosodium urate (MSU) in joints or other tissues and thereby causes the inflammatory disease of gout. Patients with gout also frequently suffer from a number of co-morbid conditions including hypertension, diabetes mellitus and cardiovascular disease. Why MSU crystals trigger inflammation and are associated with comorbidities of gout has been unclear, but recent studies provide new insights these issues. Rather than simply being a waste product, uric acid could serve a pathophysiological role as a local alarm signal that alerts the immune system to cell injury and helps to trigger both innate and adaptive immune responses. The inflammatory component of these immune responses is caused when urate crystals trigger both inflammasome-dependent and independent pathways to generate the proinflammatory cytokine IL-1. The resulting bioactive IL-1 stimulates the inflammation of gout and might contribute to the development of other comorbidities. Surprisingly, the same mechanisms underlie the inflammatory response to a number of irritant particles, many of which also cause disease. These new insights help to explain the pathogenesis of gout and point to potential new therapeutic targets for this and other sterile inflammatory diseases.

Introduction

Gout is a prevalent disease, affecting 8.3 million adults in the USA in 2007–2008.[1] The disease manifests most commonly as episodes of acute and extremely painful arthritis, classically affecting the big toe but in some cases involving other joints as well.[2] In addition, hard nodules called tophi can develop in a range of locations (such as the helix of the ear, the olecranon process of the elbow and the Achilles tendons of the foot) and, in some patients, deposits in the kidney cause renal damage, which is the most serious complication of the disease. Gout is triggered when crystals of monosodium urate (MSU)—a crystallized form of uric acid—nucleate in joints, kidneys and other tissues, where they incite inflammation. Patients with gout frequently have other comorbidities, including renal disease, metabolic syndrome, diabetes mellitus and hypertension.[3]

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Review criteria
The Pubmed database was searched for relevant literature using the following keywords, alone or in combination: “gout”, “uric acid”, “MSU”, “monosodium urate”, “comorbidity”, “inflammasome”, “DAMP”, “danger signal”, “irritant particles”, “IL-1”, “NLRP3”, “ROS” and “macrophages”. We focused primarily, but not exclusively, on publications since 2000 and English-language papers The reference lists of the identified papers were used to screen for additional publications.

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The authors declare no competing interests.

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Although it has long been known that uric acid causes gout, many unresolved questions persisted. Why is the crystallized form of uric acid so inflammatory. How do these crystals actually trigger inflammation; that is, which cells, molecular sensors and mediators drive this response? What is the relationship, if any, of inflammation to uric acid, and of inflammation to other proinflammatory particles and sterile inflammatory diseases? How are comorbidities of gout related to uric acid? This article reviews recent advances in the field, with an emphasis on research since 2000 that are beginning to shed light on many of these issues. We discuss how uric acid is produced and what is known about its functions, with a focus on its role as an immunological danger signal. We explore new insights into how uric acid crystals stimulate inflammation and how these mechanisms are unexpectedly shared in the inflammation and diseases caused by many other. unrelated, irritant particles. Finally, we discuss the therapeutic implications of these insights into the underlying mechanisms of gout.

Physiology of uric acid

Uric acid is generated as part of the normal turnover of nucleic acids (Figure 1). This molecule is produced when purines, eg. from DNA or RNA, are oxidized by xanthine oxidase, an enzyme present in the peroxisomes of most cells. This process occurs continuously and, consequently, uric acid is present as a normal constituent intracellularly and in biological fluids. In healthy individuals, uric acid is present in the blood at concentrations of ~6.0 mg/dl, which is a remarkably high level given that the threshold for saturation of this molecule in biological fluids is ~7.0 mg/dl.[4] When individuals develop hyperuricaemia as a consequence of excessive intake of purines and/or genetic predisposition, uric acid saturates body fluids, and these individuals are then at risk of MSU crystals nucleating in their joints and elsewhere. As further discussed below, it is these crystals that trigger symptoms and cause disease.

Most mammals express in their livers uricase, a peroxisomal enzyme that oxidizes uric acid to allantoin, which is highly soluble, and water.[5] This endogenous uricase lowers uric acid levels somewhat and consequently these species do not develop gout under natural conditions. Humans lack uricase and therefore uric acid is an end product of purine metabolism. That the body seems to maintain this metabolite at a high level is therefore somewhat surprising. Uric acid is absorbed from the diet through the gut and, although most of it is ultimately excreted by the kidney, a substantial amount is reabsorbed by this organ (Figure 1).[5] This retention of uric acid might suggest that this molecule has some important physiological role.

A physiological role for uric acid might be expected to arise from its anti-oxidant properties. Chemically, uric acid is a reducing agent and accounts for almost half of the anti-oxidant activity in the blood.[6] As such, uric acid could alter redox potential or protect against oxidative damage, or both. Whether this anti-oxidant function is biologically important,; and whether it influences in any way the biological effects of urate discussed below, is unclear. A physiological role for the anti-oxidant effects of uric acid might be expected to be evident in individuals lacking uric acid; however, patients with the genetic condition xanthinuria—a deficiency of xanthine oxidase —completely lack uric acid yet are not known to suffer any untoward consequences other than the development of xanthine stones.[7] Therefore, high levels of uric acid do not seem to be essential in normal physiological conditions. With that being said, this finding does not rule out the possibility that uric acid has a physiological role in more- selective situations and there are hints that this might be the case. High levels of uric acid are associated with protection against a number of neurological diseases including multiple sclerosis, Parkinson disease, Alzheimer disease and amyotrophic lateral sclerosis;[8–11] however, whether this protection is attributable to uric acid's antioxidant

activity or to other of its properties is not known. As discussed next, recent studies point to a different potential pathophysiological role for uric acid, in alerting the immune system to danger.

Uric acid as a danger signal

Cell damage and necrosis is potentially dangerous to an organism; it is the manifestation of some underlying pathological process or agent that is injuring cells. As such, it is in the interest of the host to try to limit and repair such damage. If the injurious agent is a microbe, the immune system has a number of molecular sensors, such as Toll-like receptors (TLRs), NOD-like receptors (NLRs) and RIG-like receptors (RLRs), to detect and respond to pathogens.[12] The 'danger hypothesis' (Box 1) additionally proposes that the immune system has also evolved mechanisms to monitor the health of cells,[13] a concept that is now accepted as correct. The innate immune system performs this monitoring function by surveying the extracellular environment for the presence of a subset of molecules that are normally sequestered in the interior of cells but that are exposed upon loss of integrity of the plasma membrane.[14] Rupture of the cell membrane is an invariable consequence of necrosis. Therefore, by monitoring the environment for the release of intracellular molecules the immune system can detect and respond to injurious processes that pose a danger to the host, and thereby attempt to limit and repair any damage. The intracellular molecules whose release signals danger are referred to as danger signals or damage-associated molecular patterns (DAMPs).[14] Uric acid, it turns out, is one of these DAMPs.[4]

Box 1

Immunological danger

The immune system was classically thought to have evolved to protect the host against foreign invaders, and does so by first discriminating between autologous (self) molecules and foreign ones, and then responding against the latter.[117] However, the presence of a foreign substance does not always trigger an immune response and, conversely, the immune system sometimes reacts to autologous constituents. In considering these issues, Matzinger proposed the idea that the immune system actually evolved to protect the host against antigens that are dangerous or associated with a dangerous situation, regardless of whether or not they are foreign.[13] 'Danger' in this context is the potential to cause injury to the host. Thus, it was proposed that the immune system does not discriminate between antigens that were foreign versus self but rather between ones that are or are not dangerous.

Danger, adaptive immunity and uric acid

To protect us against disease, the immune system continuously monitors our tissues for the presence of antigens. To accomplish this important task, dendritic cells (DCs) are deployed in the tissues, where they collect antigens and display them as peptides bound to MHC class I and class II molecules (Box 2).[15] These cells subsequently migrate to draining lymph nodes where they report their findings to T cells.[15] T cells with the appropriate specificity will recognize the presented antigen. However, whilst recognition of antigens bound to MHC molecules is necessary, it is not sufficient to initiate a T-cell immune response. This is because T cells must also receive other activation signals from DCs,[16] and these costimulatory signals are not present under basal conditions. Instead, DCs must be stimulated in ways that cause them to express costimulatory signals (Figure 2). In order for this expression to occur, the DCs must be exposed to microbial signals or DAMPs, which are present in precisely those situations (infections and cell injury) that the immune system

needs to investigate. In other words, the requirement for DC activation enables the immune system to focus its attention on antigens that are associated with pathogenic processes. The need for DC activation explains why the simple injection of antigens does not usually induce immune responses, and why admixing such antigens with adjuvants (most often containing bacterial products) promotes such responses.[17–19]

Box 2

MHC molecules and antigen presentation

The immune system evolved MHC class I and II molecules to monitor various cellular compartments for the presence of antigens (in the form of peptide fragments). MHC class I molecules bind peptides derived from proteins degraded in the cytosol, nucleus and endoplasmic reticulum and then transport them to the cell surface for display.[118] This display enables $CD8^+$ T cells, which express receptors for peptide–MHC class I complexes, to monitor cells and detect those synthesizing foreign antigens, for example from a viral infection. By contrast, MHC class II molecules bind peptides from proteins degraded in the endocytic compartments of cells.[119] In this way $CD4^+$ T cells, which have receptors that recognize peptide–MHC class II complexes, can detect foreign antigens (for example, microbes and toxins) from the extracellular milieu that have been internalized into cells that express MHC class II (dendritic cells, macrophages and B cells).

Through these mechanisms, dying cells stimulate DC activation and, when injected into animals admixed with antigen, provide an adjuvant function that promotes T-cell and B-cell responses.[20,21] One of the immune-promoting factors isolated from dead cells was identified as uric acid.[4] Cells normally contain very high levels of this molecule intracellularly and produce even more upon death as their purines are released and metabolized.[4,22] To become immunologically active, it is thought that uric acid has to undergo a phase change to MSU crystals.[4] Pure MSU crystals have been shown to both activate DCs and augment immune responses.[4] These crystals are thought to form around dying cells when intracellular stores of uric acid are released and create a supersaturated solution in the high-sodium extracellular environment, although this mechanism has not been proven.[23] Uric acid is not the only DAMP released from dying cells but it seems to be an important one, as depleting uric acid from dead cells reduces their adjuvant activity.[4] Whether different DAMPs—HMGB, S100 proteins, ATP and DNA, to name a few—have different mechanisms of action or biological effects is unclear.

As described above, the release of uric acid and other DAMPs is thought to contribute to host defense. In some circumstances, however, this release might also contribute to disease, including autoimmune ones of rheumatological interest. By way of background, autoimmune diseases are thought to require components of both ‘nature’ and ‘nurture’. The genetic component, where allelic variants or mutations predispose an individual to autoimmune disease, is strong. In studies of identical twins, however, the concordance rate is only ~25% in many of these diseases;[24] therefore, it is thought that in addition to genetic susceptibility an environmental trigger is also required. Infection and tissue injury have been implicated as triggers of autoimmunity and these events might act by stimulating DCs to present self-antigens in ways that initiate an autoimmune response in susceptible individuals.[25–30]

Innate responses to uric acid and particles

It has long been recognized that sites of tissue necrosis become inflamed:[31] vessels vasodilate, fluid leaks cause oedema, and leukocytes infiltrate the site of injury. The net

effect of this process is that the soluble and cellular innate immune defenses are delivered to the tissue where cells are dying. This response can help to limit the injurious process and ultimately to promote repair. On the other hand, inflammation causes symptoms (pain, heat and swelling) and can itself cause damage to the tissue. Indeed, inflammation is thought to underlie or contribute to the pathogenesis of a number of medically important diseases, including ones associated with cell death (stroke and other infarcts, tissue damage from toxic agents, and so on).[14]

The release of DAMPs by dying cells stimulates tissue-resident macrophages to produce proinflammatory mediators that drive the inflammatory response.[14,32] The molecular species of DAMPs that trigger these acute responses are not fully known, but include uric acid.[22] As mentioned above, the biologically active form of uric acid is thought to be crystallized MSU that forms when intracellular stores of uric acid are released into the extracellular environment. As noted earlier, nucleation of MSU crystals in other situations, such as in gout, stimulates robust inflammation. Gout could, therefore, be viewed as a disease that is triggered when a normal bioactive mediator is overproduced and generated inappropriately (for example, in a joint).

The phenomenon that irritant particles can be highly proinflammatory is not limited to MSU crystals; indeed, many crystals and particles can stimulate inflammation and cause disease, including several diseases of rheumatological interest (Table 1). Although these various particles are structurally quite distinct, they stimulate inflammation through similar pathways.

IL-1 in sterile inflammation

Dying cells, MSU crystals and other irritant particles all cause inflammation by stimulating the production of inflammatory cytokines. For all of these stimuli, a key mediator of the inflammatory response is IL-1 (Table 1).[33] This IL-1-driven inflammation which occurs in the absence of infection differs from that in some other inflammatory diseases, such as rheumatoid arthritis, where TNF has a more dominant role than IL-1.[34] However, mice genetically deficient in the IL-1 pathway (lacking either the cytokine or its receptor) produce little inflammation in response to injection of dead cells, MSU crystals, silicates, asbestos, alum, cholesterol or other irritant particles. By contrast, cell death-induced inflammatory responses are only modestly reduced in mice lacking the TNF pathway in comparison with wild-type mice.[35] *In vitro*, dying cells and irritant particles stimulate both mouse and human macrophages to produce IL-1.[36] The IL-1 released is a potent stimulator of neutrophilic inflammation and can stimulate other tissue responses, such as fibrosis.[37]

Active IL-1 exists in two forms, IL-1α and IL-1β, which are the products of distinct but homologous genes.[38] Both of these forms of IL-1 bind to the same receptor, IL- 1R1.[38] Most work on sterile inflammation has demonstrated a role for IL-1β. However, where examined (including in response to MSU crystals), IL-1α also contributes to inflammation and does so to a substantial extent.[32,39,40]

Production of IL-1 in sterile inflammation

The production of bioactive IL-1β is a multistep process. Cells must first be stimulated in ways that induce transcription of the IL-1β gene.[41] TLR agonists (microbial molecules) and cytokines such as TNF stimulate the transcription of this gene in part by activating the transcription factor NFκB.[41,42] What stimulates this step for irritant particles *in vivo* is a bit of a mystery, because these particles are sufficient to stimulate IL-1 responses *in vivo*, yet *in vitro* require that the IL-1-producing cells first be stimulated with microbial molecules or cytokines. In any case, once produced, the IL-1β transcript encodes an inactive precursor of

the cytokine that, when translated, contains an N-terminal pro-sequence.[41] To become biologically active, another step is required in which the pro-sequence is removed.[41] Caspase 1 is the protease that cleaves pro-IL-1β into mature IL-1 β, at least in leukocytes *in vitro*;[41] as discussed below, the situation is more complex *in vivo*.

The activity of caspase-1 is tightly regulated. This protease is initially made as a zymogen (an inactive precursor) whose activity is controlled by a macromolecular complex known as the inflammasome (Figure 3). The inflammasome is made up of three components.[43,44] The first element is a NLR protein, which is thought to impart specificity and control the activity of the complex. The NLR proteins have leucine-rich repeat domains, similar to TLRs, and these are thought to be involved in substrate recognition. The NLR also contains protein-interaction domains that enable it to associate with the second component, the scaffolding protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain). ASC associates with the third element of the inflammasome, pro-caspase-1. Assembled, these three components form the inflammasomes, which can in turn form large macroscopic structures termed ‘specks’. Stimulation of the inflammasome complex leads to the cleavage and activation of caspase-1, which in turn cleaves IL-1 (Figure 3).

The NLR protein involved in sterile inflammatory responses to MSU crystals and other irritant particles is NLRP3 (Table 1). Mutant macrophages that lack this protein are unable to make active IL-1 when stimulated with irritant particles including MSU, calcium pyrophosphate, silicates, asbestos, cholesterol crystals and alum. NLRP3 is also of rheumatological interest because rare mutations in this protein lead to over-production of IL-1, which causes the autoinflammatory diseases familial cold autoinflammatory syndrome, Muckle–Wells syndrome and neonatal-onset multisystem inflammatory disease.[45–48] These diseases are characterized by fever, rash and arthritis, among other symptoms.

IL-1α is also made with a pro-sequence; however, in contrast to IL-1β, it is biologically active with or without cleavage of its *N*-terminal pro-sequence.[41] The cleavage of pro-IL-1α into mature IL-1 α is not mediated by caspase-1 but by distinct proteases including calpain (a calcium-activated cysteine protease),[49] granzyme B and possibly elastase and chymase.[50] Although the inflammasome is not involved in the processing of pro-IL-1α, surprisingly it somehow controls the release of soluble IL-1α from cells.[51–53] Perhaps this is because the inflammasome is needed to produce mature IL-1β and IL-1β has been suggested to bind to IL-1α and in so doing facilitate its release from cells.[51] IL-1α can also be expressed on the plasma membrane and the surface expression of this form is not dependent on IL-1β.

Stimulation of the NLRP3 inflammasome

The identification of NLRP3 as a sensor for MSU crystals and other irritant particles initially presented a bit of a puzzle, because this NLR protein seemed to be in the wrong cellular compartment to be able to sense the particles. The NLRP3 inflammasome is an intracellular complex that resides in the cytosol of cells. By contrast, the stimuli discussed above that trigger this complex are large macroscopic particles that, when present in the body, are initially located outside of cells. This raises the interesting question: how do these extracellular particles trigger the intracellular NLRP3 inflammasome? The first step in the process occurs when phagocytes ingest these particles (Figure 4).[54,55] Suppression of phagocytosis by treating macrophages *in vitro* with cytochalasins or colchicine (inhibitors of actin and tubulin polymerization,[56,57] respectively) blocked urate-induced NLRP3 activation. [54,58,59] as an aside, this mechanism might explain in part why colchicine is an effective therapeutic agent for acute attacks of gout, although this drug also affects other components of the inflammatory response.[60] Once the particles are in phagosomes, they can stimulate the NLRP3 inflammasome by way of at least two different mechanisms, one of

which involves reactive oxygen species (ROS) and another involves phagosomal proteases (Figure 4).

In support of a mechanism involving ROS, small-molecule scavengers of these reactive species were found to inhibit the ability of irritant particles to stimulate NLRP3 inflammasomes.[61] This finding led to a model proposing that internalized particles stimulate phagocytes to produce ROS, which in turn activate the NLRP3 inflammasomes.[62] This model was appealing because ingestion of particles such as bacteria can stimulate NADPH oxidase in phagosomes to produce ROS (known as the oxidative burst); however, the ability of irritant particles to stimulate NLRP3 inflammasomes was not diminished in mouse or human cells genetically lacking NADPH oxidase.[54,63] Therefore, if this model is correct, then there must be an alternative source of ROS. A study published in 2011 suggested that internalization of irritant particles somehow stimulates mitochondria to produce ROS, although exactly how this occurs is not yet clear.[64] It is also not entirely clear how ROS lead to inflammasome activation. Limited data shows that ROS alter the redox potential in cells in ways that lead to the release from thioredoxin of thioredoxin-interacting protein (TXNIP), a putative endogenous ligand for NLRP3 (Figure 4).[62] However, cells from TXNIP-deficient mice did not show reduced production of IL-1-β in response to MSU crystals or other particles;[65] therefore, the importance of this mechanism is unclear.

A second mechanism by which particles might stimulate NLRP3 inflammasomes in cells involves lysosomal proteases (Figure 4).[54,55] When macrophages ingest particles their phagosomes are subsequently acidified, which in turn activates acid-optimal proteases to digest the phagosomal contents. This process is part of the normal physiology by which phagocytes digest what they have ingested. Blocking vesicular acidification has been shown to inhibit the ability of irritant particles to stimulate NLRP3 inflammasomes, as was inactivation of certain cathepsins (B and L) with inhibitors or genetic mutations, in many[54,66–68] but not all studies.[69,70] Furthermore, particle-containing phagosomes have been found to leak their contents into the cytosol,[54,71] and such vesicular leakage, even without particles (e.g. caused by osmotic rupture), was shown to be sufficient to stimulate NLRP3 inflammasomes.[54] On the basis of these findings, it has been hypothesized that NLRP3 does not directly sense particles but rather senses the internal cell damage that they cause—the rupture or leakage of lysosomes or phagosomes. How does NLRP3 sense this event? Although the answer is not yet clear, it has been hypothesized that activated cathepsins stimulate NLRP3 by cleaving it into an active state or by generating a stimulatory ligand for this NLR.

Yet other mechanisms might contribute to the activation of NLRP3. For example, some evidence exists that potassium efflux can be triggered by known NLRP3 activators and that this efflux somehow promotes NLRP3-inflammasome activation.[72–74] In addition, oxidized DNA released from damaged mitochondria can directly bind to and activate the NLRP3 inflammasome.[75] A number of different mechanisms for activating NLRP3 could be interconnected. For example, it turns out that a number of NLRP3 activators (eg. alum, ATP and nigericine), potassium efflux and lysosomal damage can all lead to mitochondrial dysfunction and release of oxidized DNA.[75] Moreover, inhibition of potassium efflux rescued mitochondrial dysfunction following treatment of macrophages with NLRP3 activators (including ATP, nigericin, staurosporine, and infection of *Chlamydia pneumoniae*) and thereby reduced IL-1β secretion by these cells.[75]

Inflammasome-independent pathways

The production of bioactive IL-1β by cells in culture is absolutely dependent on the inflammasome. Consequently, macrophages genetically lacking any of the inflammasome

components fail to produce any mature IL-1β after stimulation with MSU crystals or other irritant particles *in vitro*. By contrast, IL-1β-dependent inflammation *in vivo* is often only modestly reduced in animals genetically lacking the same inflammasome components (K. L. Rock, unpublished data).[76,77] These results indicate that additional mechanisms must be operative *in vivo* that can cleave pro-IL-1β into mature IL-1β.

Indeed, in addition to caspase-1, a number of other proteases, including neutrophil serine proteases (elastase, cathepsin G and protease 3), mast cell chymase, and matrix metalloproteinases, can cleave purified pro-IL-1β into bioactive IL-1β, at least in the test tube.[41,78–81] Many of these proteases are serine proteases that are present in vacuoles of leukocytes, including neutrophils, macrophages and mast cells. Most of these IL-1-cleaving proteases are themselves produced as zymogens and require removal of a pro-sequence to become active. This activating cleavage is performed by a single vacuolar protease, cathepsin C.[82,83] Mice that lack cathepsin C (and hence the IL-1-cleaving serine proteases) produce less bioactive IL-1β and generate attenuated inflammation in response to injection with anti-collagen antibodies, zymosan, immune complexes,[84] and irritant particles (K. L. Rock, unpublished data), although responses to MSU crystals were not examined. On the other hand, these mutant animals generate normal inflammatory responses when injected with recombinant IL-1β (K. L. Rock, unpublished data). These results indicate that cathepsin C-deficient mice are fully capable of responding to IL-1β, but when injected with particles do not seem to produce enough of this cytokine to drive inflammation. Mice lacking both cathepsin C and an inflammasome component (caspase-1) show an additive defect in bioactive IL-1β production and inflammation to irritant particles (K. L. Rock, unpublished data). These results in mice implicate cathepsin C in the inflammasome-independent pathway of IL-1β processing *in vivo*, presumably through the role of cathepsin C in activating serine proteases capable of cleaving IL-1β. Consistent with these findings, chemical inhibitors of some of these serine proteases similarly inhibit IL-1β production in models of inflammatory arthritis and MSU crystal-induced inflammation *in vivo*.[76,77]

At exactly what stage the inflammasome-independent proteases cleave IL-1 is not clear. It is unclear whether they process pro-IL-1 β to mature IL-1β in cells prior to the release of the cytokine (and if so where this occurs in cells) and/or they cleave pro-IL-1β in the extracellular space after it is released from living or dying cells.

The secretion of IL-1α by cultured macrophages stimulated with MSU crystals seems to be only partially dependent on inflammasomes.[40] Possibly, this IL-1α secretion also contributes to IL-1-dependent, inflammasome-independent responses *in vivo*.

Other effects of uric acid and particles

Other than the inflammasome-dependent and inflammasome-independent mechanisms discussed above, MSU stimulates cells by way of other pathways. TLR2 signaling has been shown to be essential for chondrocyte production of nitric oxide induced by MSU or calcium pyrophosphate dihydrate,[85] and to augment inflammasome-dependent responses.[86] However, TLR2 is not required for responses to such stimuli in all situations. For example, in other settings, MSU still activated antigen-presenting cells with double-deficiency of MyD88 and Trif (the adaptor proteins required for signaling through all TLRs),[87] and in mice deficient for TLR2 the inflammation induced by MSU was not reduced in comparison to wild-type mice.[36] MSU can also bind CD14 on macrophages to enhance expression and processing of IL-1β and CXCL1 expression through p38 activation.[88] Direct binding of MSU to the plasma membrane can even lead to aggregation of the lipid rafts to induce Syk–phosphatidylinositol 3-kinase activation in DCs without the requirement of specific receptors on the plasma membrane.[87] Protein kinase C has been implicated in MSU-

stimulated neutrophil degranulation and migration.[89] MSU might also activate the complement pathway to induce inflammatory response.[56,90] Phagocytosis of MSU activates COX-2 expression and prostaglandin E_2 (PGE_2) production in human peripheral blood mononuclear cells, and treatment with colchicine has been shown to block these effects.[91] Particulates, including alum, silica and nickel oxide, have also been shown to stimulate PGE_2 production through an inflammasome-independent pathway.[92] Upon phagocytosis, these particulates cause lysosomal disruption that leads to Syk–p38 MAPK (mitogen-activated protein kinase)-$cPLA_2$ (cytosolic phospholipase A2) activation and subsequent production of $PGE_{2.92}$ Interestingly, the induced PGE_2 mediates the adjuvanticity of alum and nickel oxide in antigen-specific IgE response.[92]

Uric acid and comorbidities of gout

Patients with gout frequently have a number of comorbid conditions, which can include renal disease, hypertension, diabetes mellitus, metabolic syndrome, cardiovascular disease, lipid disorders and respiratory symptoms.[3,93,94] Some of these comorbidities, such as renal disease, can contribute to the pathogenesis of gout by elevating serum levels of uric acid; in other words, some comorbidities may be a cause rather than an effect of elevated levels of uric acid. In other cases, however, the possibility has been raised that high uric acid levels or gout, or both, might contribute to the development of comorbidities, possibly including hypertension, metabolic syndrome and atherosclerosis. Determining cause and effect of gout and its comorbidities, and elucidating the mechanisms underlying these epidemiological associations, is not trivial and at this point is unresolved. Some of these associations are briefly reviewed below, and interested readers are referred to more in-depth reviews published elsewhere.[3,93,94]

Epidemiologic studies have found an association between high levels of uric acid and hypertension, and multivariate analyses have suggested that hyperuricaemia is an independent risk factor for this comorbidity.[3,93,94] Interestingly, experimentally elevating levels of uric acid in animals raises blood pressure and this effect can be reversed by inhibiting uric acid synthesis.[3,93,94] Consistent with these findings, pharmacological lowering of uric acid levels reduced blood pressure in a small study of normal human subjects.[3,93,94] These limited studies suggest that high serum level of uric acid might somehow cause hypertension.

Similarly, human epidemiological data show associations between atherosclerosis and cardiovascular disease and gout and/or hyperuricaemia.[3,93,94] In some but not all studies, gout or hyperuricaemia, or both, have been independent risk factors for such disease.[3,93,94] Limited data show that hyperuricaemia is associated with vascular endothelial dysfunction in animals,[95–97] and that lowering uric acid levels in humans may improve vascular flow.[98] *In vitro* studies suggest that hyperuricaemia causes calcium ion overload in mitochondria to produce excess ROS, which cause endothelial dysfunction.[99] The increased ROS, at least in part, reduce endothelial production of nitric oxide and the expression of endothelial nitric oxide synthase.[100,101] Some human epidemiological data also shows a higher incidence of metabolic syndrome and insulin resistance in patients with gout as compared with the general population.[3,93,94] Interestingly, in a model of metabolic syndrome in fructose-fed animals, pharmacological lowering of uric acid levels resulted in increased insulin sensitivity.[102] However, cause and effect are not firmly established in any of these comorbidities.

If uric acid contributes to the development of comorbidities, how it would do so is not clear. One speculative possibility is that uric acid contributes to gout comorbidities through its effects as a DAMP. Both atherosclerosis and metabolic syndrome are now recognized to be

sterile inflammatory diseases, and IL-1 has been implicated in the pathogenesis of these conditions.[103,104] Data also exist, albeit much more limited, implicating inflammation and IL-1 in hypertension.[105–107] Thus, these three comorbidities probably have an IL-1 and inflammatory component. Moreover, the NLRP3 inflammasome—the same pathway of IL-1 production that is triggered by MSU[59]—has been implicated in the pathogenesis of some comorbidities of gout (atherosclerosis[104] and metabolic syndrome[103]) although it should be noted that the inflammasome is also triggered by other stimuli that can be associated with these comorbidities, such as cholesterol crystals in atherosclerosis[104]). Moreover, in asymptomatic humans an association has been observed between serum levels of uric acid and inflammatory markers.[108] Therefore, uric acid possibly contributes to the triggering of IL-1 and inflammation locally or systemically, which then contributes to the development of comorbid conditions. However, it is also possible that uric acid might contribute to comorbidities of gout in other ways, for instance by affecting redox potential, through direct effects on endothelium,[95,96,98] or by affecting lipid and/or cholesterol metabolism.[109] Further work is needed to understand the mechanisms underlying the relationships between uric acid and comorbidities of gout.

Implications for therapy

Presently, patients with gout are treated prophylactically with agents that reduce serum levels of uric acid by inhibiting its synthesis (xanthine oxidase inhibitors) or by increasing its excretion (uricosurics). Acute attacks of gout are treated with conventional anti-inflammatory agents (such as NSAIDS) or colchicine. These agents are reasonably effective in treating this disease; however, not all patients tolerate or respond to treatment. Moreover, patients with gout, even when asymptomatic, can continue to have subclinical, smoldering inflammation, the treatment of which might prevent joint erosion.[110,111] For most other crystal-based or particle-based diseases essentially no therapies are available to prevent or arrest disease.

The elucidation of the mechanisms by which urate and other particles cause disease is important not only for understanding disease pathogenesis but also for identifying potential new targets for therapy. One such target is the cytokine IL-1 and its receptor, IL-1R1. A number of biologic agents that block IL-1 pathways are approved for use in a number of indications, including rheumatoid arthritis and autoinflammatory diseases. These agents include a soluble IL-1 receptor antagonist (anakinra), a recombinant IL-1 receptor (IL-1 Trap; rilonacept) and an anti-IL-1β monoclonal antibody (canakinumab), and more agents are under development.[38,41]

Anakinra has been tested in a very small number of patients with gout and was found to rapidly ameliorate symptoms.[112] Similarly, canakinumab and rilonacept have shown efficacy in treating gout.[113–116] IL-1-blocking agents are currently being tested in the treatment of some of the comorbidities associated with gout, including diabetes mellitus and cardiovascular disease, and whether they are efficacious in other comorbidities as well as other sterile inflammatory diseases will be of interest to test.[38]

Anti-IL-1 biologic agents have some potential limitations. They are expensive, require injection and might confer an increased risk of infection. Therefore, the development of orally available small-molecule inhibitors of IL-1, as well as inhibitors more selective for sterile inflammation, would be of interest. The identification of the molecular mechanisms of inflammasome-dependent and independent pathways presents a number of potential drug targets, including the inflammasome itself and cathepsins.

As discussed, uric acid is one of the DAMPs that is released by dying cells and stimulates inflammation. This cell death-induced inflammation can cause further tissue injury and

contribute to disease, for example infarcts or other injuries. Depleting uric acid levels in animals reduces this kind of inflammation and, therefore, drugs that lower uric acid, (such as xanthine oxidase inhibitors) could be used to suppress these responses. Whether this lowering of serum uric acid to very low levels would increase the susceptibility of the host to infection or impair tumour immune surveillance is unknown, although such problems have not been apparent in patients with gout who are treated with these agents.

Conclusions

Uric acid becomes immunostimulatory when it undergoes a phase change by nucleating into crystals of MSU. This process occurs spontaneously in hyperuricemic patients that have supersaturating levels of uric acid in their biological fluids and might occur locally when cells die and release their intracellular stores of uric acid. Upon its release from dying cells, uric acid is believed to be one of the DAMPs that alert the immune system to injurious situations. In response to DAMPs, the immune system generates, among other responses, an inflammatory response that mobilizes innate soluble and cellular defenses in an attempt to deal with the injury. Therefore, gout is possibly a consequence of inappropriate generation of a physiological signal that leads to inflammation and disease. The innate immune responses that are triggered by urate crystals could theoretically contribute to comorbidities of gout, although this concept is speculative. Urate crystals cause inflammation by stimulating leukocytes to produce the proinflammatory cytokine IL-1β. This IL-1 production occurs when the MSU particles are ingested by phagocytes wherein they stimulate NLRP3 inflammasomes to activate the protease caspase-1; the activated caspase-1 then cleaves IL-1β into its active form. In addition, MSU crystals also stimulate cathepsin C-dependent proteases to generate bioactive IL-1β. The inflammasome-dependent and independent pathways of IL-1 production are similarly triggered by other irritant particles that cause disease. Therefore, common pathways exist through which many unrelated particles cause inflammation and disease. Recognition of these mechanisms could identify new molecular targets for therapeutics to potentially treat gout or other crystal-based diseases.

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Key points

1. Urate crystals function as a danger signal for both adaptive and innate immune responses
2. The crystallized form of uric acid, monosodium urate, causes the pathology of gout by activating the inflammatory responses
3. The NLRP3 inflammasome and its downstream effector, IL-1β, are activated by urate crystals; this pathway is important in sterile inflammation caused by irritant particles (including urate crystals in gout)
4. Inflammasome-independent mechanisms exist that contribute to particle-induced inflammatory responses
5. Comorbid conditions associated with hyperuricaemia and gout exist that might also be related to uric acid-induced mediators and inflammation

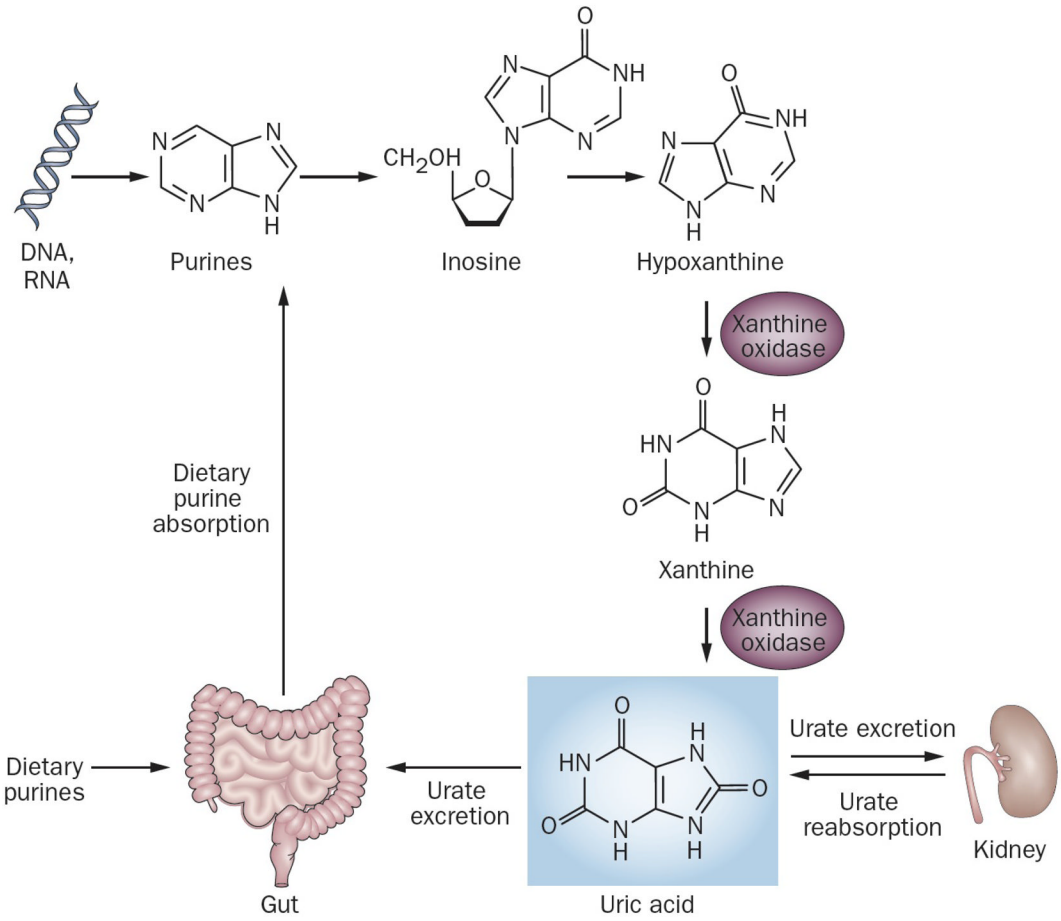

Figure 1. Biochemistry of uric acid and its homeostasis

Purines are absorbed from the diet through the gut, synthesized in the body and/or derived from the degradation of endogenous DNA and RNA. They are further oxidized by xanthine oxidase. Uric acid is the major end product of purine metabolism in humans. The majority of uric acid is excreted by the kidney and the rest in feces, although a substantial amount of uric acid is reabsorbed at the proximal tubule of the kidney

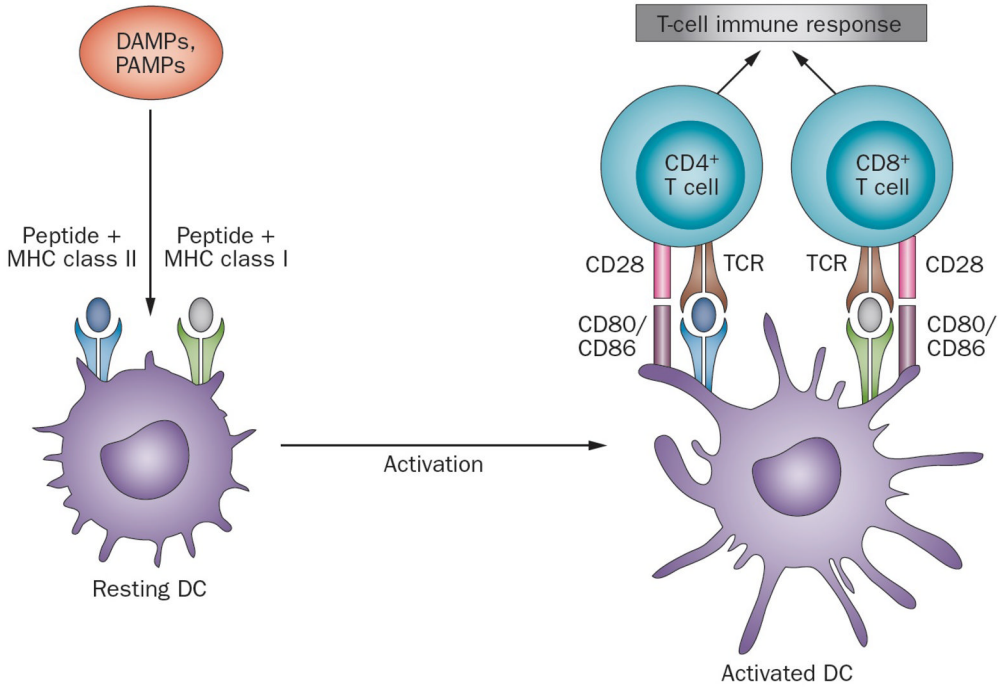

Figure 2. MHC molecules, antigen presentation and T-cell response

DCs detect and collect antigens and display them as peptides bound to MHC class I and class II molecules. In the presence of DAMPs, and/or PAMPs, DCs can be optimally activated with increased antigen presentation and the expression of costimulatory molecules such as CD80/CD86 (also known as B7.1 and B7.2 antigens to initiate a T-cell immune response. CD4 T cells are stimulated when they recognize peptide-MHC class II complexes plus co-stimulatory molecules and CD8 T cells are stimulated when they recognize peptide MHC class I complexes plus costimulatory molecules. Abbreviations: DAMP, damage-associated molecular pattern; DC, dendritic cell; PAMP, pathogen-associated molecular pattern; TCR, T-cell receptor.

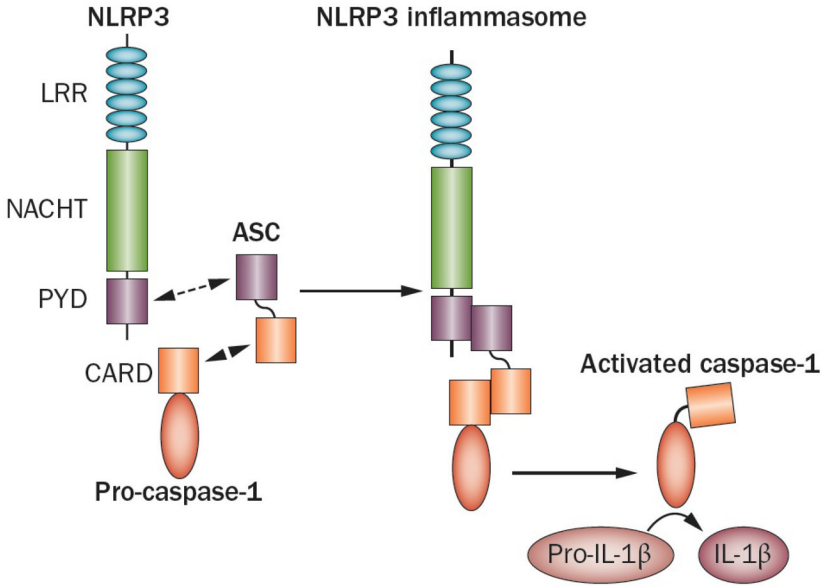

Figure 3. NLRP3 inflammasome activation

The NLRP3 inflammasome is made up of three components: NOD-like receptor protein (including LRRs, NACHT domain and PYD), ASC and pro-caspase-1. Assembly of these components to form inflammasomes leads to the cleavage and activation of caspase-1, which subsequently cleaves pro-IL-1β to form mature IL-1β. Abbreviations: ASC, apoptosis-associated speck-like protein containing a CARD; LRR, leucine-rich repeat region; NLRP3, NACHT, LRR and PYD domains-containing protein 3; PYD. pyrin domain.

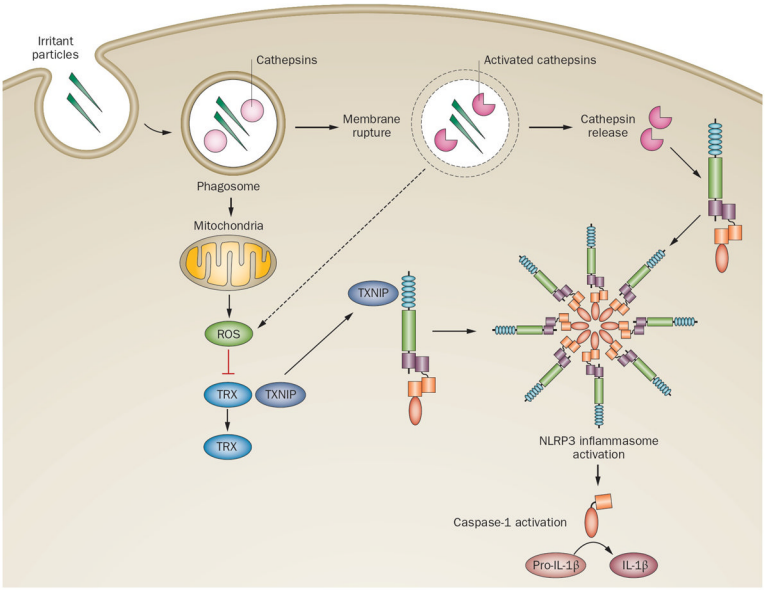

Figure 4. ROS and cathepsins involved in NLRP3 inflammasome activation

Internalized irritant particles, including MSU crystals, stimulate phagocytes and mitochondria to produce ROS, which leads to the release of TXNIP from TRX and subsequently to inflammasome activation. Irritant particles also stimulate NLRP3 inflammasomes with activated cathepsins released from membrane-destabilized lysosomes, although this mechanism has not yet been reported for MSU. A dashed line indicates a hypothetical pathway. Activated inflammasomes oligomerize and can form large macroscopic structures that are called specks. Abbreviations: ROS, reactive oxygen species; TRX, thioredoxin; TXNIP, thioredoxin- interacting protein.

Table 1

Irritant particles that cause IL-1-dependent inflammation

Particle	Disease	Stimulate IL-1	IL-1-dependent inflammation	NLRP3- dependence
Urate crystals	Gout	Ref. [36,59,61,120]	Ref. [36,59,121]	Ref. [59,61,120]
Calcium pyrophosphate	Pseudogout	Ref. [59]	Ref. [59,121]	Ref. [59]
Silica	Silicosis	Ref. [54,61,122]	Ref. [54]	Ref. [54,61,122]
Asbestos	Asbestosis	Ref. [61,122,123]		Ref. [61,122,123]
Cholesterol crystals	Atherosclerosis	Ref. [104,124]	Ref. [104]	Ref. [104,124]
Alum	None (adjuvant)	Ref. [54,58,92,125– 127]	Ref. [54]	Ref. [54,58,92,125– 127]
β-Amyloid aggregates	Alzheimer’s disease	Ref. [66]	Ref. [66]	Ref. [66]
Calcium phosphate/hydroxyapatite	Osteoarthritis	Ref. [128–130]	Ref. [129]	Ref. [128,130]
Titanium particles		Ref. [131]	Ref. [131]	Ref. [131]