Gouty Arthritis: New Targets for Future Therapies

Batista NV, Teixeira MM, and Amaral FA*
Department of Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Brazil

INTRODUCTION

Gout is the most common form of arthritis worldwide [1]. It is a consequence of the deposition of monosodium urate (MSU) crystals in the joints, mainly in individuals with chronic hyperuricemia. In these individuals, there may be oversaturation of urate and precipitation of crystals predominantly in peripheral joints and surrounding tissues. Acute gout attacks are extremely painful and lead to joint disability, although with self-limited inflammation. However, continuing and prolonged deposition of MSU crystals can result in irreversible joint damage, bone erosion, development of disfiguring subcutaneous tophi and permanent disability [2]. Moreover, gout is associated with several other conditions that affect longevity and well-being of patients, including the metabolic syndrome [3], and cardiovascular [4-6], and renal diseases [7], evidencing the complexity and severity of this inflammatory condition.

Multiple risk factors are associated with the incidence of gout, including sustained hyperuricemia due to genetic factors [8], or medication [9], that impair renal excretion of uric acid; purine-rich diet and alcohol consumption [10], age and sex, with higher prevalence in elderly individuals and men [11], comorbid conditions, such as obesity and chronic renal disorder [9], and local tissue characteristics, exemplified by the increased propensity for nucleation and growth of MSU crystals in osteoarthritis joints [12]. Here, we focus our attention on the main mechanisms associated to joint inflammation in gout, exploring the strategies for experimental studies and the new possibilities for the treatment of acute gout.

Pathogenesis of acute gout: mechanisms based on IL-1β production

The deposition of MSU crystals in the joints and surrounding tissues is the initial event for the gout attack, since the crystals are rapidly recognized by innate immune cells culminating in the release of several inflammatory mediators and chemoattractant molecules. MSU crystals-induced inflammation is driven mainly by the release of the mature form of the cytokine interleukin (IL)-1β by synoviocytes (fibroblasts and macrophages) [13]. In in vivo conditions, these cells store pro-IL-1β (inactive form) in their cytoplasm and the active form is processed and released after the phagocytosis of MSU crystals. Importantly, the investigation of MSU crystals-induced IL-1β release in in vitro studies normally needs previously de novo synthesis of pro-IL-1β by the cells through Pattern Recognition Receptors (PRR) activation, such as lipopolysaccharides from Gram-negative bacteria binding to Toll-like receptor (TLR)-4 (priming process). Recently, we demonstrated that the cytokines TNF-α and MIF have important contribution to MSU crystals-induced inflammation through the production of pro-IL-1β both in vitro and in vivo [14,15]. Furthermore, the production of mature form of IL-1β by human fibroblasts was dependent on previous priming with serum amyloid A protein upon MSU crystals stimulation [16].

The most important machinery for the cleavage of pro-IL-1β is the assembly of the cytoplasmic macromolecular complex termed inflammasome. In gout, the oligomerization of the inflammasome NLRP3 (nucleotide binding domain and leucine rich repeat containing proteins – prototype NLRP3) occurs after the phagocytosis of MSU crystals, culminating in the recruitment of the adaptor protein ASC (apoptosis-associated speck-like protein containing CARD), which lead to the cleavage and activation of caspase-1 and subsequently the cleavage of pro-IL-1β [17,18]. Experimentally, macrophages obtained from mice deficient in components of NLRP3 inflammasome (NLRP3, ASC, or caspase-1) were unable to process and release active IL-1β in response to MSU crystals [19]. In addition, the injection of MSU
crystals in different compartments (peritoneal and joint cavities) in these knockout mice caused reduced inflammatory responses, including decreased IL-1β release, neutrophil recruitment, tissue damage and pain when compared to wild-type mice [19,20]. Regarding the importance of NLRP3 in the human context, recent studies suggest that NLRP3 mutations contribute to the development of auto inflammatory diseases, including hereditary periodic fever syndrome. NLRP3 polymorphisms could be related to more susceptibility for gouty arthritis or even be genetic markers for gout. In accordance, there is an association between the variant rs3806268 in NLRP3 gene and the risk of primary gout in a Chinese population [21]. However, other studies have not shown any association between genetic variants in NLRP3 polymorphisms and the susceptibility to gouty arthritis [22,23].

The precise mechanisms for NLRP3 activation by crystalized structures, including MSU crystals, are still under investigation. However, it has been proposed that NLRP3 is a sensor for common cellular changes downstream of triggering events (phagocytosis of MSU crystals) such as reactive oxygen species (ROS) production [24], potassium efflux [25], and release of cathepsin B from the lysosome [26]. Once released, IL-1β stimulates the synthesis of several other inflammatory molecules, including chemokines responsible for neutrophil recruitment [27,28]. Experimentally, the depletion of resident macrophages decreased the accumulation of neutrophils in murine peritoneal cavity following MSU crystals stimulation [30]. Alternative to NLRP3/caspase-1-dependent mechanism, neutrophil-derived serine proteases (cathepsin G, elastase, and proteinase 3) or mast cell-derived serine proteases (granzyme A and chymase) can cleave pro-IL-1β into a secreted and biologically active form of IL-1β [31-33]. Mast cells contribute to the early stages of MSU crystals-induced inflammation. Depletion of endogenous mast cells in mice has been found to significantly inhibit neutrophil influx in MSU crystals-induced peritonitis [34]. Furthermore, the presence of resident mast cells is associated to increased acute tissue swelling following intra-articular injection of MSU crystals in mice in a mechanism dependent on IL-1β production [35]. However, although mast cells are able to produce IL-1β in a NLRP3-dependent way through lipopolysaccharide stimulation [36], is not clear if the production of this cytokine in gout is also NLRP3-dependent or occurs through specific proteases [33].

Neutrophils quickly migrate to the joint following stimulation of MSU crystals in a mechanism dependent on the production of IL-1β. Neutrophil swarm to the joint occurs through the production of several chemoattractants, including CXCR1/2 binding chemokines, leukotriene B4, platelet-activating factor and calcium-binding proteins (S100A8 and S100A9) [37-42]. The importance of neutrophils to the pathogenesis of acute gout has been demonstrated experimentally by depletion of neutrophils or targeting their chemoattractants agents, resulting in reduced tissue inflammation [43-45]. Moreover, needle-shaped MSU crystals are found inside neutrophils [46]. Once migrated to the joint, neutrophils could contribute to the additional production of IL-1β [47-49]. However, the production of IL-1β by these cells seems not to be entirely dependent on inflammasome/caspase-1 activation, since other proteases compensated the loss of caspase-1 in vivo [49]. Recently, it was demonstrated in vitro that murine neutrophils can indeed signal via the NLRP3 inflammasome, although only under soluble agonist’s stimulation and not by particulate/crystalline molecules [50]. This last finding has been validated in vivo, once neutrophils did not significantly contribute to alum-induced IL-1β production in mice. On the other hand, it was proposed that resident peritoneal macrophages, and not infiltrating neutrophils or monocytes, are the cell population responsible for IL-1β release at the initial stages of experimental gouty inflammation [30]. Thus, neutrophils contribute to acute joint dysfunction and damage, although further studies are needed to stablish the contribution of neutrophils as a relevant source of IL-1β and for chronic joint dysfunction and damage.

**Experimental models of gout: basic research and pre-clinical studies**

Experimental animal models are extremely useful to complement human investigations. In the context of gout, there are some models with important pathophysiological characteristics of gout, such as the activation of NLRP3 inflammasome for IL-1β production, recruitment of neutrophils, joint swelling and pain. Investigators have used distinct compartments in animals to inject MSU crystals, elucidating air pouches, peritoneum, and joint. These compartments may yield different responses and may be studied in different species, although the majority of experiments have been conducted in mice.

Considering that gout attacks occurs in the joints, the closest models of gout consist on the injection of MSU crystals direct into the joint, either into tibiotarsal joint (ankle) [51], or into tibiofemoral (knee) joints [14,20,52,53]. In these models, there is a possibility to recover cells, especially neutrophils, from the joints and also the quantification of inflammatory molecules in surrounding tissue or even into the synovial cavity (although more difficult to obtain). Moreover, the clinical analysis can be performed through histopathology and joint pain binoception assays, such as thermal (Hargreaves’ test) or mechanical (von Frey filaments) tests [54]. However, the uses of other compartments (air pouch and peritonitis) are also useful, mainly considering the higher exudate volume recovered for the analysis of inflammatory parameters.

In addition to the identification of new mechanisms associated to gouty arthritis, these proof-of-concept models are very useful for the studies of new targets for the treatment of gout, varying from NLRP3 inhibitors to blockers of neutrophil recruitment to the joint. In this regard, basic researchers need to improve the quality of these models. For instance, in vivo experimental systems only model acute aspects of gout, while the most aggressive form of the disease occurs when it becomes chronic, leading to permanent tissue damage and pain. Since mice produce uricase, an enzyme that cleaves uric acid, its blockage or not by particulate/crystalline molecules [50]. This last finding has been validated in vivo, once neutrophils did not significantly contribute to alum-induced IL-1β production in mice. On the other hand, it was proposed that resident peritoneal macrophages, and not infiltrating neutrophils or monocytes, are the cell population responsible for IL-1β release at the initial stages of experimental gouty inflammation [30]. Thus, neutrophils contribute to acute joint dysfunction and damage, although further studies are needed to stablish the contribution of neutrophils as a relevant source of IL-1β and for chronic joint dysfunction and damage.

**Experimental models of gout: basic research and pre-clinical studies**

Experimental animal models are extremely useful to complement human investigations. In the context of gout, there are some models with important pathophysiological characteristics of gout, such as the activation of NLRP3 inflammasome for IL-1β production, recruitment of neutrophils, joint swelling and pain. Investigators have used distinct compartments in animals to inject MSU crystals, elucidating air pouches, peritoneum, and joint. These compartments may yield different responses and may be studied in different species, although the majority of experiments have been conducted in mice.

Considering that gout attacks occurs in the joints, the closest models of gout consist on the injection of MSU crystals direct into the joint, either into tibiotarsal joint (ankle) [51], or into tibiofemoral (knee) joints [14,20,52,53]. In these models, there is a possibility to recover cells, especially neutrophils, from the joints and also the quantification of inflammatory molecules in surrounding tissue or even into the synovial cavity (although more difficult to obtain). Moreover, the clinical analysis can be performed through histopathology and joint pain binoception assays, such as thermal (Hargreaves’ test) or mechanical (von Frey filaments) tests [54]. However, the uses of other compartments (air pouch and peritonitis) are also useful, mainly considering the higher exudate volume recovered for the analysis of inflammatory parameters.

In addition to the identification of new mechanisms associated to gouty arthritis, these proof-of-concept models are very useful for the studies of new targets for the treatment of gout, varying from NLRP3 inhibitors to blockers of neutrophil recruitment to the joint. In this regard, basic researchers need to improve the quality of these models. For instance, in vivo experimental systems only model acute aspects of gout, while the most aggressive form of the disease occurs when it becomes chronic, leading to permanent tissue damage and pain. Since mice produce uricase, an enzyme that cleaves uric acid, its blockage or not by particulate/crystalline molecules [50]. This last finding has been validated in vivo, once neutrophils did not significantly contribute to alum-induced IL-1β production in mice. On the other hand, it was proposed that resident peritoneal macrophages, and not infiltrating neutrophils or monocytes, are the cell population responsible for IL-1β release at the initial stages of experimental gouty inflammation [30]. Thus, neutrophils contribute to acute joint dysfunction and damage, although further studies are needed to stablish the contribution of neutrophils as a relevant source of IL-1β and for chronic joint dysfunction and damage.

**Experimental models of gout: basic research and pre-clinical studies**

Experimental animal models are extremely useful to complement human investigations. In the context of gout, there are some models with important pathophysiological characteristics of gout, such as the activation of NLRP3 inflammasome for IL-1β production, recruitment of neutrophils, joint swelling and pain. Investigators have used distinct compartments in animals to inject MSU crystals, elucidating air pouches, peritoneum, and joint. These compartments may yield different responses and may be studied in different species, although the majority of experiments have been conducted in mice.

Considering that gout attacks occurs in the joints, the closest models of gout consist on the injection of MSU crystals direct into the joint, either into tibiotarsal joint (ankle) [51], or into tibiofemoral (knee) joints [14,20,52,53]. In these models, there is a possibility to recover cells, especially neutrophils, from the joints and also the quantification of inflammatory molecules in surrounding tissue or even into the synovial cavity (although more difficult to obtain). Moreover, the clinical analysis can be performed through histopathology and joint pain binoception assays, such as thermal (Hargreaves’ test) or mechanical (von Frey filaments) tests [54]. However, the uses of other compartments (air pouch and peritonitis) are also useful, mainly considering the higher exudate volume recovered for the analysis of inflammatory parameters.

In addition to the identification of new mechanisms associated to gouty arthritis, these proof-of-concept models are very useful for the studies of new targets for the treatment of gout, varying from NLRP3 inhibitors to blockers of neutrophil recruitment to the joint. In this regard, basic researchers need to improve the quality of these models. For instance, in vivo experimental systems only model acute aspects of gout, while the most aggressive form of the disease occurs when it becomes chronic, leading to permanent tissue damage and pain. Since mice produce uricase, an enzyme that cleaves uric acid, its blockage or not by particulate/crystalline molecules [50]. This last finding has been validated in vivo, once neutrophils did not significantly contribute to alum-induced IL-1β production in mice. On the other hand, it was proposed that resident peritoneal macrophages, and not infiltrating neutrophils or monocytes, are the cell population responsible for IL-1β release at the initial stages of experimental gouty inflammation [30]. Thus, neutrophils contribute to acute joint dysfunction and damage, although further studies are needed to stablish the contribution of neutrophils as a relevant source of IL-1β and for chronic joint dysfunction and damage.
Perspectives for future therapies for gout

The current treatment for gout consist in decreasing joint inflammation and pain during acute flares and reducing hyperuricemia mainly during inter critical periods of gout. The therapeutic choices for the treatment of acute gout attacks include non-steroidal anti-inflammatory drugs (NSAIDs), colchicine and corticosteroids [56]. In the inter critical period, strategies aim to reduce or maintain serum uric acid levels below the saturation point, to dissolve existing crystals or prevent further crystal formation. The most common drugs for urate-lowering therapy are xanthine oxidase inhibitors, which reduce endogenous production of uric acid through inhibiting the conversion of hypoxanthine to xanthine and further xanthine to uric acid (allopurinol and febuxostat). Furthermore, uricosuric agents (benzbromarone, probenecid and sulfinpyrazone) prevent reuptake of uric acid at the proximal renal tubule and increase its renal excretion [57]. For most patients, the current therapies for acute gout is effective. However, many patients with chronic gout remaining unresponsive or do not tolerate these therapies. This is due to different factors including age, comorbidities, potential drug-drug interactions and risks of upper gastrointestinal ulceration and acute renal failure. Therefore, the development of alternative treatment strategies is highly necessary.

As mentioned, the NLRP3/caspase-1/IL-1β axis has a major role during the initial events of acute gout attacks, as evidenced experimentally using cell cultures, animal models or through clinical studies. Thus, it is reasonable to speculate that targeting those molecules may be useful for the treatment of gout when standard drugs are contraindicated or impractical. Among them, the most investigated target has been the neutralization of IL-1β with biologic drugs [58-63]. A number of therapeutic biological IL-1β inhibitors have been developed tested in different clinical trials and are currently clinically available. These include the dimeric fusion protein rilonacept (Arcalyst®) and a human monoclonal antibody called canakinumab (Ilaris®), which neutralise IL-1β directly. In addition, recombinant anakinra (Kineret®), an IL-1 receptor antagonist developed for the treatment of Rheumatoid Arthritis [64,65], is another option. Canakinumab has been recently licenced for the treatment of gout in Europe, but not in the USA. However, rilonacept, a drug initially used for the treatment of Cryopyrin-Associated Periodic Syndromes (CAPS), syndromes associated with NLRP3 mutations, was recently rejected for gout treatment for FDA approval due to safety concerns [66].

Numerous promising inhibitors of NLRP3 inflammasome activation have been characterized. Although they could be useful for the treatment of gout, most studies still need further investigation before use. Probenecid is a uricosuric drug primarily used for the treatment of gout and other diseases associated with hyperuricemia [67]. Although this drug increases the rate of renal excretion of uric acid, it was discovered that probenecid also inhibits pannexin 1 channels (important for potassium efflux and consequently NLRP3 activation) [68]. MCC950, a diarylsulfonylurea-containing compound has been demonstrated to impair caspase-1-dependent processing of IL-1β through inhibiting both canonical and non-canonical activation of the NLRP3 inflammasome [69]. It was demonstrated that MCC950 can block ATP-, nigericin-, MSU crystals- and silica-induced IL-1β release from bone-marrow-derived macrophages, human monocyte-derived macrophages and peripheral blood mononuclear cells [70].

Parthenolide and Bay 11-7082, both NF-κB pathway inhibitors, have also been described as inhibitors of ATP-, nigericin- and MSU crystals-induced NLRP3 inflammasome activation in human THP-1 macrophages. Parthenolide can inhibit caspase-1 activation in response to NLRP3 stimulation or directly block NLRP3 by inhibiting its ATPase activity, a pathway required ATP-induced NLRP3 activation [71]. β-Hydroxybutyrate (BHB), a ketone body produced during fasting state, inhibits caspase-1 activation and IL-1β release in the presence of a large number of NLRP3 agonists, including ATP, nigericin, MSU crystals, and silica particles. In vivo, BHB attenuates caspase-1 activation and IL-1β secretion in a mouse model of urate-crystal-induced peritonitis [72]. Finally, it was demonstrated that omega-3 fatty acid, through G protein-coupled receptor 120 (GP1R120) and GP40, also prevents NLRP3 inflammasome-dependent inflammation. LPS-primed BMDMs incubated with docosahexaenoic acid had decreased caspase-1 cleavage and IL-1β secretion following the injection of miRNA-223 mimics reduced brain oedema and improved neurological functions in NLRP3-dependent mouse models of acute gout. These results suggest that these molecules in the inhibition of MSU crystals-induced NLRP3 activation, although further studies are necessary to establish the efficacy of these therapeutic strategies in the gout context.

Parthenolide and Bay 11-7082, both NF-κB pathway inhibitors, have also been described as inhibitors of ATP-, nigericin- and MSU crystals-induced NLRP3 inflammasome activation in human THP-1 macrophages. Parthenolide can inhibit caspase-1 activation in response to NLRP3 stimulation or directly block NLRP3 by inhibiting its ATPase activity, a pathway required ATP-induced NLRP3 activation [71]. β-Hydroxybutyrate (BHB), a ketone body produced during fasting state, inhibits caspase-1 activation and IL-1β release in the presence of a large number of NLRP3 agonists, including ATP, nigericin, MSU crystals, and silica particles. In vivo, BHB attenuates caspase-1 activation and IL-1β secretion in a mouse model of urate-crystal-induced peritonitis [72]. Finally, it was demonstrated that omega-3 fatty acid, through G protein-coupled receptor 120 (GP1R120) and GP40, also prevents NLRP3 inflammasome-dependent inflammation. LPS-primed BMDMs incubated with docosahexaenoic acid had decreased caspase-1 cleavage and IL-1β secretion following the injection of miRNA-223 mimics reduced brain oedema and improved neurological functions in NLRP3-dependent mouse models of acute gout. These results suggest that these molecules in the inhibition of MSU crystals-induced NLRP3 activation, although further studies are necessary to establish the efficacy of these therapeutic strategies in the gout context.

The identification of endogenous mechanisms that control NLRP3 inflammasome activation or its protein synthesis open a new window for the development of new targets for the treatment of gout and other related diseases. MicroRNAs (miRNAs) are noncoding RNAs responsible for post-transcriptional regulation of gene expression and have been intensively studied for therapeutic purposes [74]. The miRNA-223 has been showed to suppress NLRP3 inflammasome activity by control its protein expression through a conserved binding site within the 3’-UTR of NLRP3 transcript [75]. Although the latter study did not use MSU crystals as stimuli, the over expression of miRNA-223 in macrophages decreased the secretion of IL-1β following the activation of NLRP3 by ATP and nigericin [75]. Interestingly, the injection of miRNA-223 mimics reduced brain oedema and improved neurological functions in NLRP3-dependent rat model of intracerebral haemorrhage [76]. Downstream of NLRP3 activation, caspase-1 is also a target for miRNA. miRNA-135b can directly prevents caspase-1 protein expression in vitro and in vivo using a NLRP3-dependent model of smoking in mice [77]. Interestingly, the production of miR-135b in the cigarette-exposed mice is dependent on IL-1 receptor activation, demonstrating a negative feedback role of miRNA-135b to control lung inflammation.

CONCLUSION

Recent studies have clearly established canonical intracellular signalling pathways activated by MSU crystals in gout, especially mechanisms of activation of the NLRP3 inflammasome platform and subsequent maturation and release of IL-1β. This knowledge has opened new opportunities for the development of novel treatments for gout. However, it is still necessary that these are validated in gouty patients and, especially, whether they may
be beneficial in chronic gout. Meanwhile, further information about the function of different cell types in gouty arthritis, their contribution to the initial and resolving phases of inflammation, and the improvement of current experimental models of gout certainly help to translate pre-clinical findings in to novel medications for patients.

ACKNOWLEDGEMENTS

This work was supported by grants from Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq), Fundacao de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) and Coordenadoria de Aperfeicoamento do Pessoal de Nivel Superior (CAPES)

REFERENCES


Amaral et al. (2016)
Email: famaral@icb.ufmg.br


