Semin Immunopathol
DOI 10.1007/s00281-013-0361-0

REVIEW

Neutrophils, IL-1β, and gout: is there a link?

Ioannis Mitroulis · Konstantinos Kambas · Konstantinos Ritis

Received: 6 October 2012 / Accepted: 9 January 2013

Abstract Gout is a prototype crystal-induced inflammatory disorder, characterized by neutrophil infiltration into inflamed joints. The identification of the role of NLRP3 inflammasome in the recognition of monosodium urate crystals and the subsequent release of IL-1β was a milestone in the elucidation of the pathogenesis of this disorder. IL-1β signaling is considered nowadays as the initiatory event that induces gouty inflammation and promotes the recruitment of vast numbers of neutrophils at the sites of inflammation. Crystal-induced neutrophil activation results in apoptosis inhibition, degranulation, superoxide production, cytokine release and, as recently described, formation of neutrophil extracellular traps, further amplifying the inflammatory process. Finally, neutrophil apoptosis and uptake of apoptotic material by macrophages drive the resolution of acute inflammation. In this review, we discuss the recent experimental data regarding the crosstalk between IL-1β and neutrophils in the pathogenesis of acute gout.

Keywords Gout · IL-1β · Neutrophil · Neutrophil extracellular traps · Autophagy

Introduction

Gout is the most common inflammatory arthritis in men older than 40 years, with an incidence of 1.4 in women and 4.0 in men per 1,000 person-years [1]. Hyperuricemia, resulting in the oversaturation of blood or synovial fluid with urate above the critical level for crystal formation, is the key metabolic process in this disease. The incidence of gout is directly correlated with the levels of serum urate. However, not all patients with hyperuricemia develop gout, as the 5-year risk for gout is 30.5 % in subjects with urate levels above 10 mg/dl [2]. The prevalence of the disease has risen within the last decades, rendering gout an important health care issue. This increase could be attributed in a number of factors including increase in longevity, lifestyle modifications, and increased use of loop and thiazide diuretics [3]. Despite the existence of longstanding effective treatment for acute attacks and the development of novel uric acid-lowering agents, the disease still represents a substantial health issue. For this reason, extensive investigation of the inflammatory mechanism implicated in the pathogenesis of gout has been conducted during the past years, leading in the development of effective biological agents for gouty attacks.

Acute gout is characterized by sudden onset, extremely painful, self-limited inflammatory attacks of mono- or oligoarticular arthritis. Disease flares last from days to weeks, while an increased frequency and duration of attacks is observed in patients inadequately treated. Except from this type of attacks, some patients develop a chronic, refractory to treatment arthritis, characterized by the formation of tophi. This type of arthritis often results in joint destruction, bone erosions, disfiguring, and disability [4].

The hallmark for the diagnosis of this disorder is the presence of needle-shaped monosodium urate (MSU) crystals in synovial fluid, often inside neutrophils, the predominant cell population in synovial fluid during acute attacks [5]. Crystallization of uric acid and the release of MSU crystals by soft tissue deposits are the initiatory pathogenic event in gout [6]. It is well recognized that the recognition of these crystals by innate immune cells results in the release of inflammatory cytokines and the progression towards inflammation. From the correlation of high uric acid levels with gout by Garrod and the identification of MSU crystals in the

Electronic supplementary material The online version of this article (doi:10.1007/s00281-013-0361-0) contains supplementary material, which is available to authorized users.

This article is a contribution to the special issue on Neutrophils - Guest Editors: Paul Hasler and Sinuhe Hahn.

I. Mitroulis · K. Kambas · K. Ritis (✉)
First Department of Internal Medicine, Democritus University of Thrace, University General Hospital of Alexandroupolis, Dragana Alexandroupoli, Greece
e-mail: ritis2@otenet.gr

I. Mitroulis
Department of Internal Medicine III, Division of Vascular Inflammation, Diabetes and Kidney, University Clinic Carl-Gustav-Carus, University of Dresden, Dresden, Germany

Published online: 24 January 2013

synovial fluid from patients with gout by McCarty and Hollander, several lines of evidence demonstrated the causative role of MSU crystal deposition at the affected sites in disease pathogenesis [7]. The characterization of MSU as a danger-associated molecular pattern and the description of intracellular pathways implicated in its recognition and the subsequent production of inflammatory cytokines by innate immune cells resulted in categorization of gout in the group of autoinflammatory disorders. During the past years, based on in vitro studies and in vivo animal models, gout has been identified as a prototype IL-1β-dependent autoinflammatory disease, an observation that has been also confirmed by clinical studies, addressing the therapeutic effect of IL-1 blockage [8, 9].

Herein, we seek to review novel data regarding the pathogenesis of gout with a special focus on the implication of neutrophils and IL-1β in disease pathogenesis. Experimental data regarding the role of IL-1β in the initiation and propagation of gouty attacks and the possible involvement of neutrophils as a key cell population in gouty inflammation are presented.

IL-1β

IL-1β is the most extensively studied member of the IL-1 family. It is a highly inflammatory cytokine, whose production and activity is tightly regulated in a multi-step process. Circulating blood monocytes, macrophages, and dendritic cells are considered as the main source of this cytokine [10]. Upon activation of Toll-like receptors (TLRs) or IL-1 signaling, IL-1β mRNA transcripts are rapidly expressed and the precursor of IL-1β, pro-IL-1β, is synthesized. Pro-IL-1β is biologically inactive and its processing to the active, secreted form takes places inside the cytoplasm or in specialized secretory lysosomes [10].

Pro-IL-1β needs a second signal in order to maturate to its active form. Several pathogen molecular patterns or endogenous danger-associated molecular patterns are recognized either directly or via reactive oxygen species (ROS) production or P2X7 activation by intracellular pattern recognition receptors. This process results in the assembly of macromolecular complexes termed inflammasomes and leads to caspace-1 activation, pro-IL-1β cleavage, and subsequent release of IL-1β [11–13]. NLRP3 inflammasome is the most studied one, implicated in a variety of disorders, including inherited autoinflammatory syndromes, type II diabetes mellitus, gout, and pseudogout [8, 9]. Among several endogenous danger signals, MSU crystals have been associated with NLRP3 inflammasome activation and IL-1β production, a key process in the initiation of gouty inflammatory attacks [9].

IL-1β exerts its biological functions through IL-1 type I receptor (IL-1RI) signaling. Binding of IL-1α or IL-1β to the extracellular domain of IL-1RI results in the recruitment of IL-1 receptor accessory protein (IL-1RAcP), forming a signaling complex [10, 14, 15]. Several intracellular adaptor molecules are recruited after the formation of this complex including myeloid differentiation primary response gene (88) (MYD88), TNF receptor-associated factor 6, or IL-1R-associated kinases, resulting in the induction of intracellular signaling, culminating in NF-kB-induced transcription of inflammatory genes [15].

Except from the aforementioned multi-step regulation of IL-1β production, the activity of this cytokine is also tightly regulated by several endogenous inhibitors. IL-1β binding to IL-1RI is antagonized by IL-1 receptor antagonist (IL-1Ra), which binds tightly to IL-1RI, abrogating the binding and subsequent signaling of both IL-1a and IL-1β [14, 16]. Additionally, IL-1β activity can be further suppressed by IL-1 receptor type II. This decoy receptor has been also found to interact with IL-1RAcP and IL-1, forming a non-signaling complex [14, 17]. It shows great affinity for IL-1β, while a soluble form of IL-RII (sIL-1RII) is also released by cells and binds with IL-1 in the extracellular environment, further diminishing active IL-1β levels in a paracrine manner [14, 15]. Single Ig IL-1 receptor (IL-R)-related molecule, a member of the IL-1R like family [18], and soluble IL-1RAcP (sIL-1RAcP) have been also identified as inhibitors of IL-1 signaling [19]. sIL-1RAcP has been suggested to interact with sIL-1RII, forming a dominant complex for the neutralization of IL-1β [14, 18].

IL-1β and MSU

Since the first description of crystal-induced IL-1 production by monocytes and macrophages about 30 years ago [20], extensive research has been performed to identify the intracellular pathways and cell populations responsible for IL-1β production in acute gout.

In vitro studies in human monocytes demonstrated that the phagocytosis of MSU crystals induces the release of several cytokines, including IL-1β, tumor necrosis factor-α (TNF-α), IL-8, and IL-6 [20–23]. It has been also demonstrated that the differentiation of these cells to macrophages after 3–5 days of incubation resulted in the abolishment of their ability for inflammatory cytokine release and the acquisition of a non-inflammatory phenotype [24]. These findings were supported by the inability of mouse macrophage cell lines to secrete TNF-α when incubated with MSU crystals [25]. It was concluded that infiltrating monocytes were responsible for the initiation of gouty inflammation, while their differentiation to macrophages could promote the resolution of inflammation by removing MSU crystals in a non-inflammatory manner. However,

these data were questioned by further studies which demonstrated the release of IL-1β by murine macrophages upon stimulation with MSU crystals. A study by Martin et al. reported 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 [26]. It was shown that resident macrophage depletion significantly inhibited neutrophil recruitment at the site of MSU injection and abrogated the production of pro-inflammatory cytokines, including IL-1β [26] (Fig. 1). Additionally, data derived from different experimental models have identified neutrophils as a crucial population in the delivery of active IL-1β during gouty inflammation [27, 29].

The pathways involved in the recognition of MSU crystals by phagocytes are another active research field. Signaling via TLR-2 and TLR-4 through the MyD88-dependent pathway and CD14, a co-receptor for TLR-4, has been proposed to be essential for MSU recognition and ingestion by murine macrophages [29, 30]. The implication of TLRs was challenged by Chen et al., demonstrating that MSU-dependent activation of macrophages was IL-1-MyD88-dependent but independent from TLR activation [31]. This study has further shown that other Toll/IL-1R adaptor molecules, including TIRAP/Mal, TRIF, and TRAM, and IL-18 receptor are not involved in MSU-dependent inflammatory process [32]. However, a recent study has shown that the engagement of TLR-2 by free fatty acids and not MSU is responsible for IL-1β production. Surprisingly, the authors reported that, in this model, IL-1β production was ASC- and caspase-1-dependent, but NLRP3-independent, which is not in accordance with previous reports that will be discussed below. The authors proposed that these findings provide a possible explanation for the triggering of gouty attacks after consumption of fatty meals or alcohol [32]. In consistent with this finding, Mylona et al. observed that the synergy between MSU crystals and TLR-2 agonists for IL-1β production is more prominent in peripheral blood monocytes from patients with gout [33].

A few years ago, Martinon et al. reported that NLRP3 inflammasome plays an essential role in sensing MSU crystals [34]. This report was a turning point in the investigation of intracellular pathways implicated in gouty inflammation. It was demonstrated that macrophages from mice deficient in inflammasome components, including NLRP3, caspase-1, and ASC, were not able to cleave pro-IL-1β upon phagocytosis of MSU crystals. Additionally, impaired neutrophil influx was observed after intraperitoneal crystal administration in mice deficient in these genes, providing a direct link between NLRP3 inflammasome and acute gouty attacks [34] (Fig. 1). Another group further demonstrated a few years later that lysosomal destabilization and rupture participates through cathepsin B release in NLRP3 inflammasome activation after crystal uptake by human monocytes and macrophage cell lines. It has been shown that this process is not specific for MSU crystals, and it is also implicated in NLRP3 activation by silica crystals and alum salts [35]. A recent study also demonstrated that 5-lipoxygenase-derived

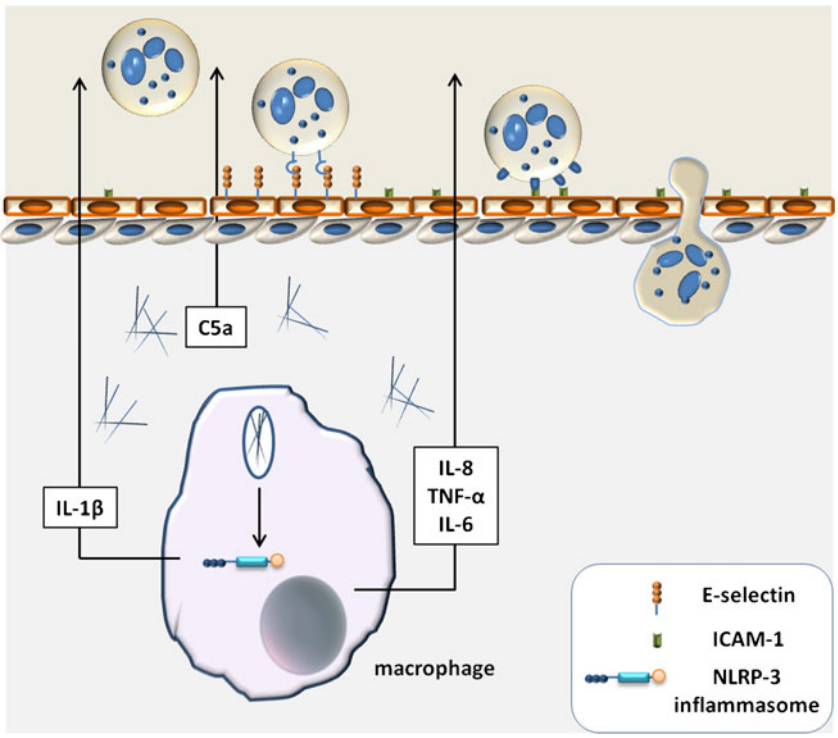

Fig. 1 Initiation of MSU crystal-dependent inflammation and neutrophil recruitment. Uptake of MSU crystals by resident macrophages induces the activation of NLRP3 inflammasome and the processing and release of IL-1β. Additionally, several other cytokines, including TNFα and IL-6, are produced by the cells. IL-1β, in co-ordination with these cytokines, promotes the recruitment of neutrophils at the inflamed joint through the up-regulation of E-selectin and ICAM-1 expression by the endothelial cells. Complement activation by MSU crystals is also suggested to contribute in neutrophil chemotaxis

leukotriene B_4 participates in MSU-driven NLRP3 inflammasome activation through ROS generation [36].

IL-1β in the pathogenesis of gout

Several lines of evidence suggest the critical regulatory role of IL-1β among a magnitude of pro-inflammatory cytokines produced during acute attacks in gout. High levels of IL-1β are detected in the synovial fluid from patients with acute gout during the initial phase of the attack [37]. Additionally, experimental data in murine models have demonstrated that IL-β signaling is indispensable for the induction of MSU-driven inflammation. As discussed in the previous section, mice deficient in components critical for IL-1β production or signaling are protected from inflammatory attacks and neutrophil influx at the sites of MSU injection [34, 36]. In a murine model, deficiency in IL-1 receptor significantly mitigated inflammation following MSU injection into the ankle joint. A similar effect was observed in wild-type mice treated with the IL-1 inhibitor IL-1 Trap (rilonacept). IL-1RI deficiency or IL-1 inhibition with rilonacept prevented hyperalgesia and attenuated cytokine and chemokine production, including IL-6, monocyte chemotactic protein-1 (CCL2), macrophage inhibitory protein-1 (CCL3), CXCL1 (KC), TNF-α, and granulocyte-colony stimulating factor [38].

These observations, derived from studies in animal models, are in accordance with the data from the clinical use of IL-1 inhibitors in gout. Treatment with a number of anti-IL-1 regimens, recombinant IL-1RA (anakinra), IL-1Trap (rilonacept), and anti-IL-1β monoclonal antibody (canakinumab), resulted in the resolution of acute gout attacks [8, 39–41]. Therapeutic strategies based on IL-1 inhibition are considered nowadays as an alternative treatment option, especially in patients with difficult-to-treat chronic gout [8]. These data further reinforce the proposed key role of IL-1β by the experimental models in the pathogenesis of this disorder.

Neutrophil in acute gout

Neutrophil identification in the synovial fluid and uptake of MSU crystals by these cells is the hallmark for the diagnosis of acute gout. In contrast to acute inflammatory arthritis, neutrophils are not present in the synovial fluid in normal joints. Moreover, these cells were rarely identified by immunochemistry in tophi derived from patients with chronic disease [42]. By the initiation of an inflammatory attack, neutrophils infiltrate into the synovial membrane and the synovial fluid in vast numbers, in a percentage greater than 80 % [37]. The number of infiltrating neutrophils is often extremely high, raising diagnostic problems with septic arthritis. Early studies in canine animal models of crystal-driven arthritis demonstrated the fundamental role of neutrophils in gouty inflammatory attacks. These studies indicated that neutrophil depletion significantly suppressed inflammation [43, 44]. Moreover, the restoration of MSU-dependent inflammation in neutropenic animals after perfusion with normal blood further reinforced the proposed implication of this cell population [44]. During the disease course, the number of neutrophils remains stable and declines during the resolution of the attack [37]. Neutrophil apoptosis and clearance of apoptotic cells by resident macrophages is a key step in this final phase of acute gouty inflammation [45].

Neutrophil recruitment and infiltration

Accumulation of neutrophils at the sites of tissue infection is an early event in immune response against invading pathogens [46, 47]. Additionally, neutrophils are recruited at the sites of sterile inflammation, promoting tissue injury. Inflammatory stimuli induce local neutrophil recruitment and also mobilize vast numbers of these cells from bone marrow, providing an adequate neutrophil supply and resulting in neutrophilia [46–48]. Several mediators contribute in neutrophil mobilization from bone marrow, including C5a, leukotriene B_4, and the CXC chemokine IL-8. However, studies in rodents demonstrated that CXCL1 and CXCL2 (MIP-2) are the critical molecules in this process [49, 50].

Neutrophil and endothelial cell interactions regulate the extravasation of the former cells. The initial event of this multi-step process depends on the expression of P- and E-selectins on the endothelial cells. Inflammatory stimuli induce the expression of these selectins on the endothelial surface, which promotes the rolling of flowing neutrophils along the endothelium [49]. Crawling neutrophils are exposed to chemokines derived from the endothelial cells, resulting in the activation of integrin adhesion receptors. Integrin activation and their binding to their endothelial ligands induce high-affinity interactions between neutrophils and endothelium. The most important integrins participating in this process are β2 integrins, macrophage antigen-1 (Mac-1; CD11b/CD18), and lymphocyte function-associated antigen 1 (LFA-1; CD11a/CD18), and integrin alpha4beta1 (Very Late Antigen-4; VLA-4). Mac-1 and LFA-1 mainly interact on endothelial surface with the intercellular adhesion molecules ICAM-1 and ICAM-2 and VLA-4 with vascular cell adhesion molecule-1 (VCAM-1) [49, 50]. Neutrophil firm arrest is followed by stable adhesion to endothelial cells. In the next step, neutrophils migrate mainly through inter-endothelial junctions. Neutrophil migration is mediated by several adhesive events including binding of LFA-1 to ICAM-1 and ICAM-2 [49, 50].

Neutrophil recruitment in gout

The impact of several neutrophil chemoattractants in the pathogenesis of acute gout has been extensively studied.

Limited data have linked complement activation with neutrophil recruitment in gout. First, high levels of complement split products have been identified in the synovial fluid from patients with active arthritis [51–53]. Data from in vitro studies further demonstrated the activation of complement proteins, including C1 [54], C2 [55], C3 [56, 57], C4, C5 [55], and factor B [56], in human serum treated with MSU crystals. Direct interactions between negatively charged crystal surface and complement proteins have been involved in complement activation, including assembly of a functional C5 convertase complex at the crystal surface resulting in the generation of active C5a and C5b [58]. The functional role of the complement system in acute gout inflammation has been studied in a rabbit model of knee synovitis. The authors described a significantly abrogated inflammatory response in C6-deficient rabbits after injection of MSU crystals, as demonstrated by attenuated joint swelling and markedly diminished neutrophil numbers in synovial fluid [59]. Additionally, reduced levels of IL-8 were detected in these rabbits. However, no further studies have been conducted to discriminate the role of complement split products in gout.

Early studies in animal models assessed the potential involvement of IL-8 in MSU-dependent arthritis. A study in a rabbit model of gouty arthritis demonstrated increased IL-8 levels in the synovial fluid at 12 h from crystal injection. Moreover, the intra-articular administration of monoclonal anti-IL-8 antibody resulted in the attenuation of inflammation at 12 h but failed to have any significant effect at earlier time points [60]. These data imply that IL-8 could play a significant role in MSU-dependent arthritis, not during the initiation, but for the evolution of inflammation. Another study in a murine model of crystal-induced synovitis further indicated the contribution of interleukin-8 receptor CXCR-2 in gouty inflammation [61].

A study by Ryckman et al. attempted to elucidate whether additional IL-8 chemotactic factors play a significant role in MSU-dependent neutrophil chemotaxis, using the air pouch model of gouty arthritis. Inhibition of CXCL1, CXCL2, CCL2, and CCL3 chemokine function by specific anti-murine antibodies had no effect on neutrophil migration. However, administration of antibodies against the myeloid-related peptides S100A8 and S100A9 attenuated neutrophil infiltration, suggesting that the release of these inflammatory factors contributes to disease pathogenesis [62].

Several lines of evidence have shown a clear-cut correlation between IL-1 signaling and neutrophil infiltration at the affected joints. It has been recently shown that NLRP3 inflammasome-dependent IL-1β production promoted neutrophil recruitment, which relied on the production of CXCR2-acting chemokines, mainly CXCL1 [36]. As it has been aforementioned, the implication of IL-1β signaling through IL-1RI has been clearly implicated in neutrophil recruitment. MyD88 was further identified as a critical downstream to IL-1RI molecule. Additionally, the authors sought to investigate the role of MyD88-dependent IL-1RI signaling in myeloid cells in gouty inflammation. However, chimeric mice deficient in IL-1R or MyD88 in bone marrow-derived cells alone were still prone to MSU-dependent inflammation [31]. These data suggest that secreted IL-1β exerts its function in non-myeloid cells. Endothelial cells have been proposed as the candidate cell population.

Indeed, IL-1β and TNF-α signaling in endothelial cells induces the over-expression of the leukocyte adhesion molecule E-selectin on cell surface [63]. In vitro and in vivo studies have linked E-selectin expression by endothelial cells with MSU-dependent inflammation. In vitro stimulation of endothelial cells with culture supernatants from MSU-treated monocytes induced the expression of E-selectin, ICAM-1, and VCAM-1 in an IL-1β and TNF-α manner [64] (Fig. 1). Using an in vivo model of MSU-driven monoarthritis, this group observed that treatment with anti-TNF-α antibody partially inhibited this effect, which is consistent with the in vitro findings [64]. Additionally, the expression of E-selectin by endothelial cells during acute inflammation has been shown in other animal models of MSU-dependent inflammation [65, 66]. Even though there are only scarce data regarding the role of IL-1β in promoting the interaction between neutrophils and endothelial cells in gout, it is well-established that neutrophil migration at the sites of gouty inflammation depends on IL-1β, as shown by studies in animal models with defected IL-1β production and signaling or using inhibitors of IL-1 signaling [34, 36, 38]. However, the intermediate steps in this process have not been elucidated yet. Fig. 1 depicts the continuum of events implicated in neutrophil recruitment in MSU crystal-dependent inflammation.

Neutrophil activation by MSU crystals

Neutrophils infiltrating the inflamed joints encounter and recognize MSU crystals, which results in their activation and the subsequent amplification of inflammation (Fig. 2). The ingestion of small-sized MSU crystals by neutrophils and their inclusion in phagosomes was an early finding [67]. However, whether the uptake of MSU crystals and the subsequent neutrophil activation are facilitated by crystal coating by immunoglobulins, complement components [68] or small polymeric size hyaluronate [69] has not been elucidated. Neutrophil activation by MSU crystals has been proposed to be mediated by FcgRIIIB and the b2-integrin CD11b receptors, as demonstrated by studies assessing the inhibitory effect of antibodies targeting these receptors on neutrophil functional responses [70]. On the other hand, the implication of TLR-2 or TLR-4 in the recognition of MSU crystals, as previously shown for monocyte/macrophage lineage cells, has not been assessed in neutrophils [29].

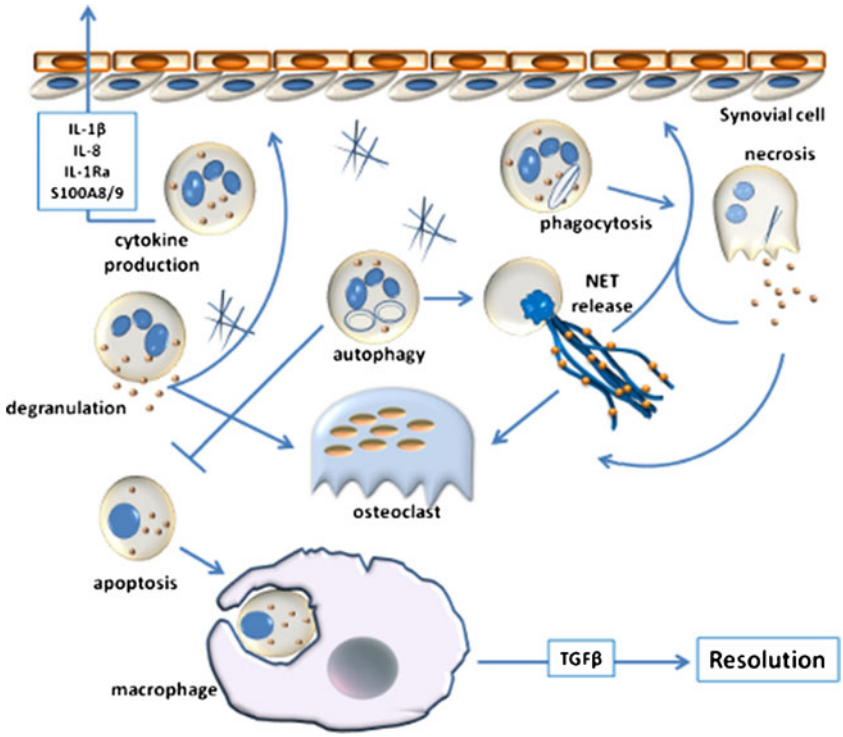

Fig. 2 Activation of neutrophils by MSU crystals. Crystal-induced neutrophil activation induces degranulation and release of proteolytic enzymes, as well as the secretion of cytokines and inflammatory mediators. Autophagy activation by MSU crystals is required for the formation of extracellular traps and the extracellular delivery of neutrophil granular enzymes. Neutrophil enzymes, like elastase, inflict tissue damage or may promote osteoclastogenesis. Autophagy induction could also be responsible for the observed delay in apoptosis in crystal-activated neutrophils. Phagocytosis of MSU crystals results in necrotic cell death, which fuels inflammation, while neutrophil apoptosis and clearance promotes the resolution of inflammation

In vitro studies assessed the intracellular pathways implicated in MSU-dependent neutrophil activation. Activation of tyrosine phosphorylation pathways is an essential signaling event in the activation of human neutrophils by MSU crystals. Src-family tyrosine kinase activation is an early event in this process [71, 72]. It is required for the stimulation of other kinases, including protein kinase C [73] and phosphatidylinositol 3-kinases [74]. However, we have recently demonstrated that inhibition of Src-tyrosine kinase signaling was not sufficient to inhibit the release of neutrophil extracellular traps (NETs) by MSU-treated human neutrophils [75].

Functional activity of neutrophils in response to MSU crystals

Infiltrating neutrophils at the sites of sterile inflammation play a crucial role in the modulation of the inflammatory process either by inflicting tissue injury or through interaction with other cell populations [48]. On the other hand, neutrophil apoptosis and clearance by macrophages is the hallmark of resolution of inflammation [47]. Neutrophils exert their detrimental role in sterile inflammation mainly through the extracellular release of a variety of mediators, including ROS, cytokines, chemokines, and granule constituents such as proteolytic enzymes and antimicrobial peptides [47, 48]. Activated neutrophils release both pro-inflammatory, including IL-1α, IL-1β, IL-6, or IL-17, and anti-inflammatory cytokines, including IL-1RA and TGF-β, while they release a variety of CXC- and CC-chemokines and members of the TNF family, including TNF-α and receptor activator of nuclear factor-κB ligand (RANK-L), enabling their crosstalk with immune and non-immune cells [76]. Recently, a novel function of neutrophils has been described: the release of NETs [77].

Several lines of evidence have demonstrated the function role of neutrophils in the amplification and maintenance of MSU crystal-dependent inflammatory attacks. This role is mediated by the release of a variety of mediators known to inflict tissue damage or promote inflammation, including ROS, antimicrobial peptides, the myeloid-related peptides S100A8 and S100A9, and cytokines/chemokines, including IL-1 and IL-8 [78] (Fig. 2).

Even though there are few data reporting a direct link between ROS and tissue injury in gout, a significant role for superoxide in the development of arthritis in several experimental animal models has been previously demonstrated [79, 80]. Neutrophils recruited at the sites of MSU crystal-driven inflammation actively produce superoxide in vivo [81], while in vitro incubation of neutrophils with MSU crystal stimulation induces ROS generation [81–83]. Neutrophil priming by TNF-α or granulocyte-macrophage colony-stimulating factor (GM-CSF) further amplified MSU-dependent ROS production. In consistency with these data, a recent study reported that neutrophils from patients with gout or asymptomatic hyperuricemia produced higher levels of superoxide when stimulated with MSU crystals [84]. Using conditioned media from monocytes stimulated with MSU crystals, this group further demonstrated the priming effect of IL-8 and, to a lesser extent, of IL-6 and

TNF-α in superoxide production [85]. These findings indicate that the inflammatory mediators in affected joints may increase the capacity of infiltrating neutrophils to release ROS.

The myeloid-related proteins, S100A8 and S100A9, are highly expressed in neutrophil cytoplasm and play a key role in neutrophil-dependent inflammation [86]. In vitro incubation of neutrophils with MSU crystals results in the release of S100A8 and S100A9 from neutrophils. Activation of CD11b, CD16, Src kinases, Syk, and tubulin polymerization has been implicated in this process [87]. As already mentioned, S100A8 and S100A9 were reported to participate in neutrophil recruitment in an animal model of MSU crystal-induced inflammation, while high levels of S100A8 and S100A9 were detected in synovial fluids of patients with gout [62]. IL-8 is an additional potent neutrophil chemoattractant, playing a key role in neutrophil recruitment in gout [60]. Cells of the monocytes/macrophage lineage are identified as the principal sources of IL-8 [88]. However, MSU crystals induce the production of this chemokine by neutrophils, suggesting a significant contribution of this cell population in the IL-8-dependent chemotactic activity [89]. These data suggest that the release of these chemotactic mediators by infiltrating neutrophils results in the amplification of inflammation.

Joint damage and bone erosions due to expanding tophi are important features of chronic gout, resulting in significant disability. Bone destruction is attributed to a dynamic interplay between several cell populations around and within tophus and MSU crystals [90]. Activation of osteoclasts is considered as the key pathogenic event in the development of bone erosions. Osteoclastogenesis is mainly regulated by interactions between osteoclast precursors and osteoblasts. Binding of receptor activator of RANK-L, which is produced by mature osteoblasts, other stromal cells, or activated T cells, to RANK expressed on osteoclast precursor cells mediates osteoclast maturation and bone resorption. On the other hand, osteoprotegerin (OPG) is a soluble decoy receptor for RANK and a negative regulator of osteoclastogenesis. OPG is secreted by osteoblasts and plays a controlled homeostatic role between bone formation and bone loss [91]. Recent data proposed a role for neutrophils in osteoclastogenesis in rheumatoid arthritis through the expression of RANK, RANK-L, and OPG [92]. However, this has not been studied in models of crystal-induced inflammation. Another study further demonstrated that the in vitro release of elastase by neutrophils treated with MSU crystals results in osteoblast retraction, allowing osteoclasts to resorb cell-free areas of bone matrix [93]. However, considering that neutrophils are present in low numbers in tophi, additional data are needed to support their involvement in bone erosions in gout.

Neutrophil apoptosis, autophagy, and NET release in gout

Enhanced neutrophil survival in acute septic and sterile inflammation has a fundamental role in the maintenance of high cell numbers both at the inflamed tissues and in the circulation [46, 47]. Additionally, the removal of these cells promotes the resolution of inflammation and the return to homeostasis. If dying neutrophils are not properly removed, they release their constituents further contributing in tissue destruction and inflammation [46, 47, 94].

Several types of cell death have been described for neutrophils. Apoptosis is a programmed cell death mediated by several pathways, including ROS, caspases, calpains, and cathepsins [94]. It is considered as a non-inflammatory process that results in neutrophil clearance through the uptake of apoptotic cells by macrophages. Delay of neutrophil apoptosis in acute inflammation, induced by signals, such as adhesion, transmigration, hypoxia, microbial products, and cytokines, is suggested to significantly participate in the pro-inflammatory effect of these cells [94]. Autophagy is an anti-apoptotic mechanism, which is activated during periods of cell stress in order to ensure cell survival. However, when autophagy induction is not able to preserve cell integrity, cells undergo cell death, which is characterized as autophagy-related cell death [95]. Activation of the autophagic machinery has been recently described in neutrophils stimulated with inflammatory mediators and has been implicated in neutrophil functions [96]. NETosis is a recently described cell death process, associated with the release of NETs, structures composed of chromatin and neutrophil granule and non-granule proteins [77]. Increasing data support the involvement of NET release in several non-infectious inflammatory disorders and the contribution of autophagy in this process [77]. However, a recent study provided evidence for the release of NETs as viable, which maintains their functional capacity [97]. Necrosis, in contrast to apoptosis, is a highly inflammatory cell death pathway [98]. During this process, cells lose the integrity of their plasma, which results in the extracellular release of inflammatory mediators. The role of other types of cell death, like oncosis or pyroptosis, has not been extensively investigated in neutrophils [98].

Apoptosis has been clearly implicated in the pathogenesis of acute gout (Fig. 2). Delayed spontaneous and TNF-α-dependent apoptosis was observed in neutrophils treated with MSU crystals [99, 100]. Additionally, neutrophil apoptosis and clearance of apoptotic cell by macrophages is a principal event during the resolution of inflammation [101]. Ingestion of apoptotic neutrophils results in the release of the anti-inflammatory cytokine TGF-β by these cells [102]. A recent study demonstrated that cannibalism of apoptotic neutrophils also induces TGF-β production, suggesting that neutrophils may have a significant contribution in the

resolution of acute gout through TGF-β release [103]. Despite the observed prolonged lifespan of neutrophils treated with MSU crystals, an earlier study reported that the uptake of crystals by neutrophils resulted in the destabilization of the phagosome and the leakage of lysosomal enzymes in the cytosol, which resulted in cell death [104]. Delayed apoptosis and release of lysosomal content in the extracellular environment are possibly involved in disease pathogenesis by maintaining high numbers of inflammatory cells and inflicting in tissue damage at the affected joints, respectively. These observations imply that cell death is induced in neutrophils that ingest crystals, while it is delayed in cells that do not uptake them.

Recent findings support the activation of autophagy in neutrophils in MSU crystal-induced inflammation. We have recently shown the accumulation of LC3-positive structures in human neutrophils treated with MSU crystals, suggesting the activation of autophagy [75]. We have also obtained preliminary data for the activation of autophagy in neutrophils incubated with synovial fluid from patients with acute gout (Fig. 3 and video clip in Electronic supplementary material). The possible involvement of IL-1β in the induction of this process by synovial fluid has to be shown, given the previously reported autophagy activation in neutrophil by this cytokine [96]. Moreover, the reported implication of autophagy in NET release [75, 105, 106] and in the delivery of intracellular proteins to NETs [106] further expands its involvement in MSU crystal-dependent inflammation. These observations and the reported anti-apoptotic effect of autophagy imply a significant role for autophagy in gout.

Crosstalk between IL-1β and neutrophils

The link between IL-1β and neutrophils in gout is bidirectional; neutrophils produce IL-1β, while IL-1β regulates neutrophil functions. Several lines of evidence suggest that neutrophils are an important source of IL-1β acute inflammatory disorders [107–109]. Recently, the secretion of IL-1β in a NLRP3 inflammasome-dependent has been shown [110]. However, previous reports indicated that neutrophil proteases are able for pro-IL-1β processing in an inflammasome-independent manner [107–109]. An intriguing study by Guma et al. in 2009 demonstrated that cleavage of pro-IL-1β by caspace-1 was not required for the development of MSU crystal-induced peritonitis [107]. This group reported that neutrophil recruitment and IL-1β levels in the peritoneal fluid were not attenuated in caspase-1-deficient mice. Neutrophil elastase has been identified as the culprit for the processing of pro-IL-1β. This was in consistency with a simultaneous publication, which indicated the substantial role of proteinase-3 in caspase-1-independent IL-1β production in a mouse of acute arthritis [108]. These studies imply that the recruitment of high numbers of activated neutrophils, which produce significant amounts of IL-1β, may be essential for acute IL-1β-driven inflammation, including gout. However, considering that neutrophils are not detected in the synovial fluid in non-inflamed joints or at tophi [42], the role of resident macrophages in the production of IL-1β at the initial phase of gout remains indispensable. The presence of an alternative to inflammasome pathways for IL-1β secretion may suggest that IL-1β targeting agents and not caspase-1 inhibitors are the optimal therapeutic strategies for acute IL-1β disorders, including difficult-to-treat gout. MSU crystal-dependent activation also results in the release of IL-1Ra [28]. The balance between IL-1β and IL-1Ra release by neutrophils during different phases of gout could participate in the progression of inflammation towards resolution.

Except from the indirect effect of IL-1β in the recruitment of neutrophils at the site of inflammation, there is experimental evidence that IL-1β is directly involved in neutrophil function. IL-1β signaling interferes with cell death pathways in neutrophils. IL-1β has been shown to

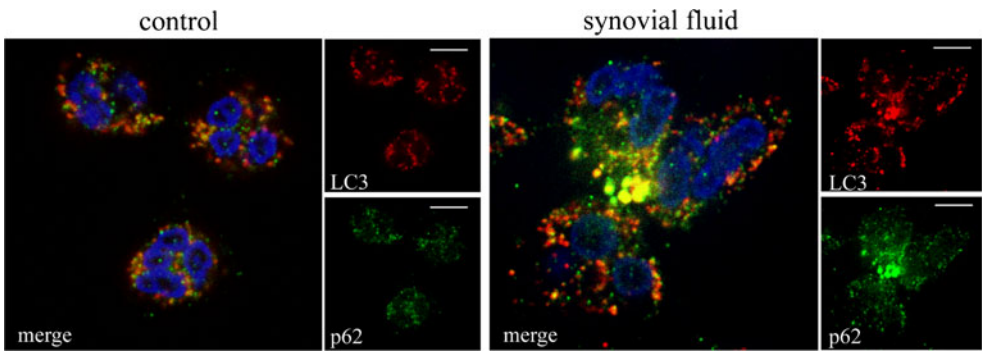

Fig. 3 Activation of autophagy in neutrophils treated with synovial fluid from patients with acute gout. Neutrophils were incubated for 30 min in the presence of synovial fluid in a final concentration of 10 %. The formation of LC3B (red) and p62 (green) double positive structures suggests the activation of autophagy. Neutrophils treated with normal serum served as control. Nuclear staining with DAPI. Single-plane analysis. Scale bars represent 5 μM

mediate the observed anti-apoptotic effect of LPS and GM-CSF. In this experimental model, treatment of neutrophils with blocking anti-IL-1β antibody or administration of IL-1R antagonist abolished the anti-apoptotic effect of these mediators [111]. Whether IL-1β participates in the prolongation of neutrophil lifespan in cells incubated with MSU crystal has to be demonstrated. IL-1β has been also shown to induce autophagy in human neutrophils [96], further implying a possible anti-apoptotic effect in IL-1β-related disorders.

NET release has been recently demonstrated in the context of gout. We have observed the formation of NETs by synovial fluid and peripheral neutrophils from patients with acute gouty arthritis. This was also observed in neutrophils treated with MSU crystals and synovial fluid or sera from such patients. IL-1β was partially involved in this process, while inhibition of autophagy with 3-MA and bafilomycin A1 attenuated this process [75]. These findings were confirmed by Schorn et al. [112]. The immobilization of MSU crystals by NET-releasing neutrophils has been recently demonstrated [113]. The authors proposed that the entrapment of crystals may act as a mechanism for the limitation of inflammation. However, this hypothesis needs further verification. Recent experimental data identified NETs as significant players in neutrophil-driven sterile inflammatory disorders. Except from their role in the exposure of intracellular neutrophil antigens, the extracellular delivery of chromatin and neutrophil proteins, including the serine proteases elastase, cathepsin G, and proteinase 3, promotes cellular interactions with several other cell populations, including platelets [114], lymphocytes [115], dendritic [116], epithelial, or endothelial cells [117]. Given that neutrophil serine proteases contribute in the cleavage of pro-IL-1β, the intra-articular production of NETs could be a significant participant in the propagation of gouty inflammation. The exact role of NET release in the propagation of inflammation of gout through possible interactions with synovial cells, osteoblasts, or osteoclasts has to be further assessed.

Concluding remarks

Recent clinical and experimental evidence highlight the significance of IL-1β-mediated inflammation in the pathogenesis of MSU crystal-induced inflammation, rendering gout a prototype IL-1β-mediated autoinflammatory syndrome. Recognition of MSU crystals by resident macrophages and generation of IL-1β by these cells is considered as the initiatory event in acute gouty inflammation. A consequent cascade of inflammatory mediators induces the recruitment of vast numbers of neutrophils at the inflamed joints. Neutrophils are the crucial cell population for the maintenance and amplification of inflammation through the release of cytokines, chemokines, ROS, proteases, or NETs. Neutrophil apoptotic cell death and clearance of apoptotic material by macrophages signifies the turn from active disease to the resolution of inflammation. Further experimental data are needed to evaluate the several steps that compose the pathway from IL-1β secretion by resident macrophages to neutrophil recruitment and activation, in order to enlighten the pathomechanism of several IL-1β-dependent autoinflammatory syndromes.

Acknowledgments This review was supported by the Hellenic Ministry of Education and General Secretariat for Research and Technology (ESPA project/No 898).

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