REVIEW ARTICLE

The Immunological Basis in the Pathogenesis of Gout

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ABSTRACT

Gout is an inflammatory arthritis characterized by red, tender, hot and tumid joints. The development cause and process of gout is very sophisticated; recent studies, notwithstanding, have offered novel perspectives on the mechanism from an immunological viewpoint. The pathological process of gout involves both innate and adaptive immune responses. Other studies have demonstrated that gout development is associated with the presence of monosodium urate (MSU) crystals which serve as a "danger signal" affecting certain immune cells, cytokine production, and effector molecule expression, triggering both types of immune responses. Different cell subsets, cytokines, pattern recognition receptors (PRRs) and the inflammasome have had noticeable effects on the pathogenesis of gout. In the present review, we discuss the contributions of MSU-mediated immune responses in gout, which helps to better understand the mechanism of gout development.

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Keywords: Gout, Monosodium urate, Immune response, Innate, Adaptive

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INTRODUCTION

Gout is a chronic inflammatory disease characterized by high sustained levels of uric acid and the sedimentation of monosodium urate (MSU) crystals in articular and periarticular tissues (1,2). As an increasingly rampant arthritis disease, particularly in China, gout has become a health hazard to both men and women (3,4). MSU plays a crucial role as "a danger signal" that warns certain foreign bodies and convenes the immune cells in order to protect the body from injury, activating innate immune responses and triggering adaptive immune responses. MSU and the deposition of uric acid crystals create an "arch-criminal" that induces the immune response, rather than being merely a waste product of purine catabolism in the body (5). Although it has been well-known that gout is caused by MSU, the details in the inflammatory process are yet to be fully fathomed. The review at hand focuses on new insights as to the manner MSU triggers innate and adaptive immunological reactions and the role of such components as immune cells, cytokines, pattern recognition receptors (PRRs), and the inflammasome in gout.

1. The role of innate immune responses in gout

Innate immunity is the core of the long-term evolution of the human immune system (6), which is slowly but surely formed in the germ-line development. It is the innate immune defense bulwarking the body from external pathogenic microorganisms. It contains resident innate immune cells, such as macrophages, natural killer (NK) cells, neutrophils, mast cells, and so forth. Further involved in the innate immune system process are a series of PRRs, such as nucleotide oligomerization domain (NOD)-like receptors (NLRs) and Toll-like receptors (TLRs).

1.1 Innate immune cells in gout

Gout is an immune system reaction to the MSU crystals that damage tissues surrounding the joints. Innate immune cells (monocytes/macrophages, mast cells, and neutrophils) and NK cells (vital effector cells of innate immunity) involve in the pathogenesis of acute gouty inflammation (7).

Monocytes/macrophage cells are important members of the innate immune system that, in all stages of the immune response, participate in the process of arthritis (8). These cells strongly interact with MSU, despite its convoluted immunological implications (9), and they have a significant part in recognizing MSU crystals and initiating acute inflammation (10,11). Martin WJ *et al.* (12) found that local macrophages are activated in the early response triggered by MSU, followed by circulating monocytes being recruited, thereby infiltrating the inflamed sites. The activated monocytes/macrophages produce pro-inflammatory cytokines, such as interleukin (IL) -6, IL-8, tumor necrosis factor (TNF) –α, and IL-1β (13-15). It goes without question that IL-1β is produced by monocytes and macrophages around MSU crystals; however, IL-1β has only recently been identified as a pivotal cytokine in gouty inflammation. Recent data show that MSU engages in the caspase-1-activating NALP3 inflammasome, entailing the production of active IL-β (16) and neutrophil influx in the joint, both of which drive and sustain gouty inflammation (17,18).

When the neutrophils (normally existing in the joints) meet with uric acid crystals, they are activated. The activated neutrophils bring about neutrophil ingress and the paroxysm of gout inflammation episodes (19,20). Numerous neutrophils flow into joint fluid and gather the joints stimulated by MSU crystals in the early processes of gout formation (19). Further expressed are the nuclear factor (NF)-κB and AP-1 transcription factor-dependent genes including a series of inflammatory cytokines, such as IL-1β, TNF-α, IL-6, CXCL8, and cyclo-oxygenase 2 (6,19,20).

Among other cell types conducing to the initiation of gouty inflammation, mast cells deserve a mention. A murine model deficient in mast cells, induced by MSU crystals, significantly alleviated neutrophil granulocyte recruitment (21), yet increased numbers of mastocytes appeared early in the MSU air pouch model of human gout. In addition, mastocytes are capable of secreting IL-1β and activating the NLRP3 inflammasome (22).

Based on a myriad studies, NK cells, another ilk of innate immune cell, have been found to participate in acute gouty inflammation, though their role is still nebulous. Primarily, NK cells may interact with monocytes/macrophages, which is the first step in initiating gouty inflammation. Second, in the joint tissues of patients with gout, there exist more CD56+ NK cells which are major components of the immune system and can secrete a great number of inflammatory cytokines (23-25), hence the plausibility of the fact that NK cells are involved in gouty inflammation in innate immune responses (7).

1.2 Pattern recognition receptors and the inflammasome in gout

The innate immune system is composed of an array of receptors that can recognize pathogens or small molecular proteins, resulting in immune cell activation (6). Although receptors on the immune cells cannot directly interact with MSU crystals, it has been confirmed that PRRs are implicated in the innate immune responses triggered by MSU crystals (8).

TLRs, as part of the innate immune system, are crucial sensors located on leukocytes that can perceive “danger” signals (26). The role of TLRs has grown increasingly pellucid in the pathogenesis of inflammatory diseases (22). MSU, it has been reported, interacts with the PRRs, TLR2, TLR4, and cell surface adaptor CD14, which activates MyD88-dependent signaling (the downstream pathway) in order to promote the secretion of cytokines (such as IL-1β) and neutrophil infiltration (6,9,23-25). Liu-Bryan R *et al.* (27) found that TLR2 can recognize MSU crystals (28) and it has been thought that TLR2 is a dominant factor specifying the degree of inflammation and the development process of gouty arthritis (29).

NLRs, as key cytosolic innate immunity regulators, are pivotal to perceiving microbial protein structures and area signals (30). Similar to TLRs, NLRs can spot protein structures through their leucine-rich repeat domains. A myriad studies have demonstrated that NLRP3 is involved in MSU crystal-induced inflammation (5), generatingIL-1β, a process in which the NLRP3 inflammatory body is the core protein (6). A few studies have illustrated that MSU crystals in mice, trigger innate immune responses, mainly through the activation of NLRP3 and the production of IL-1β (11,16,31). In NLRP3-deficient mice, the macrophages were not capable of producing IL-1β and following the injection of MSU crystals, NLRP3 deficiency impaired neutrophil recruitment, indicating that the NLRP3 inflammasome constitutes an

important part of the innate immune response (32,33). Such findings suggest that the inflammasome can efficaciously control the inflammation in gout (33).
In general, the interaction among MSU crystals and innate immune cells (such as macrophages, (NK) cells, neutrophils, and mast cells) and PRRs (such as TLRs and NLRs) constitutes the innate inflammation in gout by the secretion of cytokines such as IL-1β, IL-6, IL-8, TNF-α, CXCL8, and cyclo-oxygenase, boosting the expression of transcription factor-dependent genes such as nuclear factor-κB and AP-1 (Figure 1).

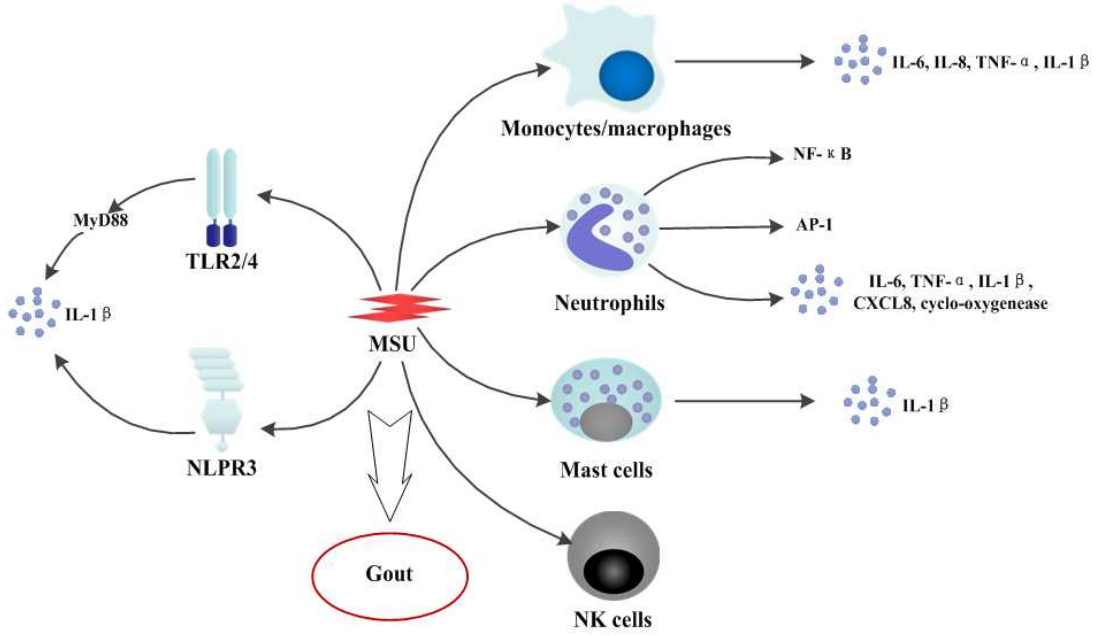

Figure 1. Innate immune responses in gout.

MSU crystals generate IL-1β through activating NLRP and TLR2/4-MyD88. In addition, the interaction between MSU crystals and the innate immune cells, such as macrophages, (NK) cells, neutrophils, and mast cells, entails the secretion of a high number of cytokines, such as IL-1β, IL-6, IL-8, TNF-α, CXCL8, and cyclo-oxygenase.

2. The role of adaptive immune responses in gout

Adaptive immunity, in contrast to innate immunity, is acquired by individuals, characterized by specificity, diversity, and memory, and composed of T and B lymphocytes. This immune response works through plasma cells secreting antibodies (humoral immunity) and effector T cells (cellular immunity) (34).

2.1 Cellular immunity in gout

It is a known fact that $CD4^+$T cells, $CD8^+$T cells, and γδ T cells are the main cells involved in adaptive immunity. Based on their surface molecular markers and functions, these T cells can be classified into several different subsets. CD4+T cells are classified into five different subtypes, helper T (Th)1, Th2, Th17, regulatory T (Treg) cells, and follicular helper T (Tfh) cells, each having their parts in particular type of the immune response, including promoting or suppressing immune responses. $CD8^+$T cells are categorized into two categories: cytotoxic T cells (CTLs) and $CD8^+$Treg cells, the former being the main cell killer bulwarking the host from infection, and the latter inhibiting immune responses to avoid overreaction. Furthermore, γδ T cells are a minor population of T cells implicated in both innate and adaptive immunity (35).

Webb R *et al.* (36) were the first to observe that MSU crystals are able to directly activate T cells in an antigen-independent manner in patients with gout, hence the fact that the deposition of MSU crystals in joint tissues directly results in synovitis and bone destruction. Kong *et al.* (37) reported that activated T cells differentiated osteoclasts by the NF-κB ligand (RANKL). Lee *et al.* (38) have recently found that the destruction of the bone in gout arthritis is partly due to the activated receptors of RANKL on T cells.

Th17 cell is a kind of Th cell identified by the secretion of IL-17 (35,36,39). A host researchers have demonstrate that IL-17A and/or IL-17F have key roles in rheumatoid arthritis (RA), psoriasis, juvenile idiopathic arthritis (JIA), and other autoimmune diseases (40-42). Recent data have also pointed to the fact that MSU is capable of differentiating and activating Th17 in the presence of IL-1α/β and IL-18; furthermore, and inflammasome adaptor protein ASC and caspase-1 are essential for Th17 responses (43).

In addition, Shi Y *et al.* (44) found that MSU is further capable of stimulating CD8+ T cells via triggering intense phagocytosis, secreting inflammatory cytokines and upregulating co-stimulatory molecules. Furthermore, Dalbeth N *et al.* (45) determined that CD8+T cells are identified in both the corona and fibrovascular zones in all gouty uratoma samples. Moreover, MSU crystals can upregulate the expression of co-stimulatory molecules on antigen presenting cells (APCs), such as dendritic cells (DCs), which, in turn, activates $CD8^+$ T cells (43).

Bone damage in gouty arthritis mainly results from MSU crystals immersing into bone tissue, inducing granulomastous response and entailing erosions and bone destruction. Interleukin-1 plays a vital role in granuloma formation in gouty arthritis (46). A recent study has corroborated that osteoblasts contribute to bone erosion in gouty arthritis (47). On the other hand, the mechanism that links MSU crystals to increased numbers of osteoclasts, is yet to be elucidated. Interestingly, in contrast to effector T cells, Tregs suppress the differentiation of osteoclasts (48). $CD4^+CD25^+Foxp3^+$ Treg cells are a customized $CD4^+$T cell subset maintaining tolerance to self-components in order to preempt overresponse by suppressing and regulating effector T cells' activity (49). In recent years, several studies have specified that Treg cells attenuate bone resorption by osteoclastogenesis and restrain osteoclastogenesis by secreting IL-4, IL-10, and TGF-β1 (50-53).

2.2 Humoral immunity in gout

B cells are a crucial member of the immune system, essentially conducing to humoral immunity, a major branch of adaptive immunity. Immunoglobulins are a pivotal element in the host defense of humoral immunity and are exclusively generated by B cells following antigen-mediated activation of their innate B-cell receptors (54).
Natural MSU crystals bind with immunoglobulins and trigger crystal-induced inflammation, yet the mechanism is not entirely pellucid. Kaneko *et al.* (55) found that MSU crystal formation was influenced by many factors, such as globulin levels, in vitro. If one adds enough γ-globulin to a uric acid solution, the formation of MSU crystals is accelerated. Moreover, other studies have posited that patients with gout can also generate antibodies in the face of MSU crystals (56). Kanevets *et al.* (57) found further evidence confirming that IgM immunoglobulins adhere to MSU crystals and promote MSU crystallization in a dose-dependent manner. In humans, nonetheless, most immunoglobulins that bind with MSU are IgG (19).
Lai S *et al.* (58) showed that a large number of $CD20^{+}$B cells can be found around the tissue and hypertension is also associated with augmented $CD20^{+}$B cells. These data fully indicate that B-lymphocytes, as specific immune cells, are involved in the adaptive immune response in gouty arthritis.
As described, T cells participate in adaptive immune responses in gouty arthritis via promoting osteoclastogenesis by the expression of RANKL. On the contrary, Tregs thwart osteoclastgenesis by suppressing and regulating the activity of effector T cell and the secretion of the IL-4, IL-10, and TGF-β1 cytokines. What is more, MSU stimulates $CD8^{+}$ T cells and drives Th17 differentiation by secreting inflammatory cytokines and upregulating co-stimulatory molecules. B cells promote crystallization via the generation of immunoglobulins, which stick to MSU crystals and accelerate the adaptive immune responses in gout (Figure 2).

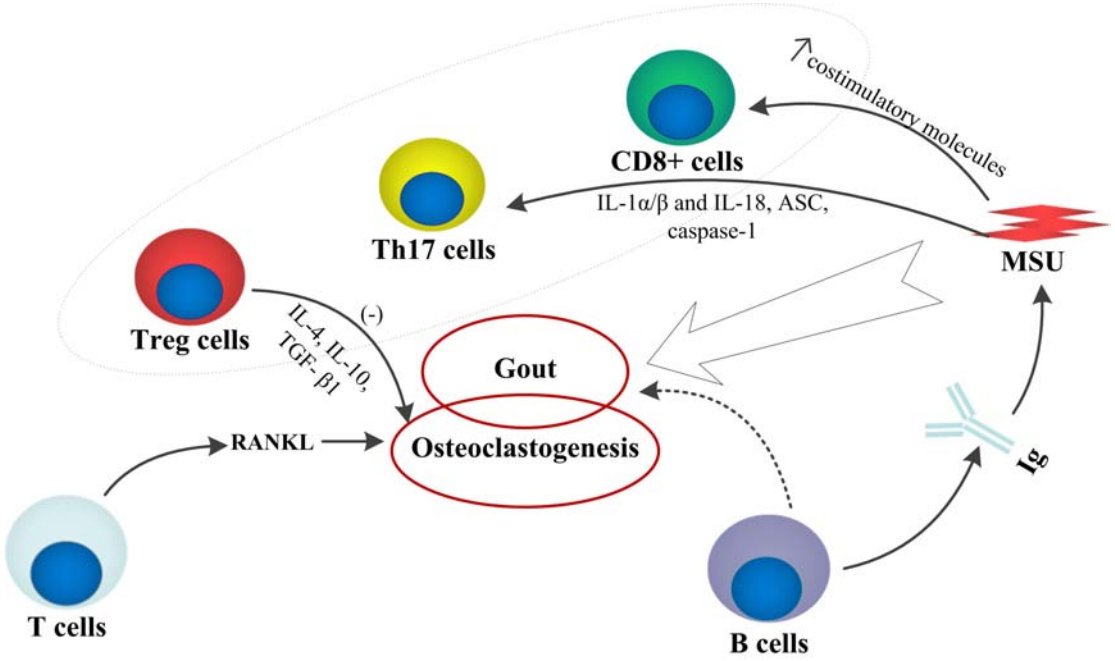

Figure 2. The adaptive immune responses in gout.

T cells might play important roles in the adaptive immune response in gouty arthritis via promoting osteoclastogenesis by the expression of RANKL. Tregs could suppress osteoclastogenesis in gouty arthritis. MSU can stimulate $CD8^+$ T cells and drive Th17 differentiation. B cells might promote crystallization via the generation of immunoglobulins.

In conclusion, regarding gout, MSU modulation in innate immune responses has been expansively investigated. The interactions among resident innate immune cells, PRRs, the inflammasome, and MSU are important in the pathogenesis of gout. Meanwhile, several studies have focused on the role of adaptive immune responses in gout. However, the impact of the damage-associated molecular pattern on adaptive responses remains mostly obscure, hence the fact that understanding these mechanisms is conducive to interfering with this inflammatory pathway and possibly develop effective therapies in the days to come.

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