EDITORIAL

Evil Humors Take Their Toll as Innate Immunity Makes Gouty Joints TREM-ble

Ru Liu-Bryan and Robert Terkeltaub

Acute gouty arthritis is a characteristically intense acute inflammatory reaction that erupts in response to articular tophaceous deposits of monosodium urate monohydrate (MSU) crystals. Full-blown acute gouty arthritis is dependent on the influx into the joint space of neutrophils, a cell type normally absent from this locus. Hence, the clinical term “gout,” classically described in the 5th century BC by Hippocrates, was derived in a remarkably prescient manner from the Latin word “gutta,” which literally means “drop.” Specifically, gout was named to reflect the notion of “evil humors” aberrantly seeping into affected joints. Recent advances have revealed the uncanny link between this ancient disease and innate immune inflammatory responses that are phylogenetically primeval.

Innate immunity provides a first line of defense against infection via primitive responses that are non-specific and broad in spectrum (1). Unlike adaptive immune responses, stereotypic innate immune “early induced” responses do not directly induce immunologic memory or lasting protective immunity, consistent in acute gout with the recurrent paroxysmal nature of the disease and the primary role in pathogenesis of “professional phagocytes.” Central to rapid innate immune inflammatory and killing responses is the remarkable capacity of all eukaryotic organisms to recognize the same constituents of microbes (termed pathogen-associated molecular patterns [PAMPs]). Seminal identification of Toll and Toll-like receptors (TLRs) that recognize PAMPs in Drosophila indicates that human innate immune mechanisms for discrimination of non-self PAMPs such as lipopolysaccharide (LPS) and peptidoglycan from self are phylogenetically primordial. The majority of encounters with microorganisms expressing PAMPs do not result in disease, but breaching of tissue barriers by pathogens can stimulate innate immune alternative complement pathway activation, the expression of chemokines and certain other inflammatory cytokines, and uptake of the pathogen by resident and recruited professional phagocytes in a rapid effort to eliminate the noxious agent.

Breaching of the remodeling tophi by MSU crystals

In gout, MSU crystals are deposited into tophaceous, granuloma-like synovial microenvironments that can remain quiescent before and in between flares of acute gout. Significantly, tophi are now recognized to continually recruit monocytes that differentiate to macrophages, which is consistent with ongoing remodeling (2). Recently, Yagnik and colleagues elegantly elucidated the capacity of the fully differentiated macrophage to exert antinflammatory effects on the uptake of MSU crystals (3–5). Many microscopic intrasynovial tophi appear to be walled-off by a ring of fibrinogen and other proteins (6). The predominant effect of serum protein binding to MSU crystals is physical suppression of crystal–cell interaction and consequent crystal inflammatory potential, mediated in large part by avid binding to the negatively charged MSU crystal surface of highly cationic apolipoprotein B (7,8). The tophus function as a macrophage-rich and fibroblast-rich holding tank for MSU crystals is not likely to be entirely antinflamma-
tory, given the direct association of tophi with chronic cartilage and bone erosion. However, the aforementioned observations collectively suggest that breaching of the remodeling tophus by naked MSU crystals is the fundamental trigger of gouty inflammation (Figure 1). Such changes in tophus containment of MSU crystals are likely stimulated by physical microtrauma to the joint, concurrent medical or surgical stress associated with systemic inflammatory cytokine release, or rapid rises and falls in ambient uric acid levels (e.g., due to changes in hydration, renal function, or diet, or to alcohol consumption or use of medications that markedly alter uric acid production or renal elimination).

**Figure 1.** Schematic representation of the innate immune early induced response in acute gouty arthritis, showing the central role of specific mediators of the innate immune response in triggering and amplifying acute gouty arthritis. MSU = monosodium urate monohydrate; TLR-2 = Toll-like receptor 2; MyD88 = myeloid differentiation factor 88; IL-1 = interleukin-1; TNFα = tumor necrosis factor α; TREM-1 = triggering receptor expressed on myeloid cells 1.

### Innate immune triggers for amplification of acute gout

The pathologic hallmark of gout is neutrophil influx into the synovium and joint fluid (6), and interruption of the vicious circle of neutrophil recruitment and activation appears central to the effectiveness of antiinflammatory therapies in acute gout. Neutrophil ingress into the gouty joint appears to be driven syner-

Innate immune recognition of the naked MSU crystal surface as a PAMP

In a series of recent studies, we implicated innate immune inflammatory responses to the naked crystal surface in the pathogenesis of acute gout (9–11). The process, set into motion partly via C5 cleavage catalyzed by the MSU crystal surface (12), involves terminal complement membrane attack complex formation that drives activation of the endothelium and generation of the major MSU crystal–induced neutrophil chemotaxin CXCL8 (interleukin-8 [IL-8]) (13,14) in vivo (9). We determined recognition of naked MSU crystals by TLR-2 in chondrocytes, and by TLR-2 and TLR-4 in macrophage lineage cells, to be critical for the respective capacities of inert MSU crystals to turn on resident mesenchymal lineage cells in the joint and induce macrophage lineage cell expression of proinflammatory cytokines (10,11). Particularly pivotal to MSU crystal–induced cell activation in vitro and acute MSU crystal–induced inflammation in vivo was myeloid differentiation factor 88 (MyD88) signaling, which transduces TLR-2 responses, some TLR-4 responses, as well as IL-1–induced cell activation (10,11).

In mediating MSU crystal recognition, TLR-2, TLR-4, and MyD88-dependent signaling play a key role in phagocytosis of MSU crystals by macrophages (11). TLRs are type I transmembrane molecules containing extracellular leucine-rich repeat motifs that recognize PAMPs (1). Recognition of ligands by TLRs facilitates TLR dimerization, and dimerization of TLRs triggers activation of signaling pathways. In this context, TLR-2 forms heterodimers with TLR-1 or TLR-6, but TLR-4 forms homodimers. The roles in recognizing MSU crystals of distinct TLR-2 heterodimers, or of the TLR-2 and TLR-4 accessory molecules CD14 and MD-2, are not yet known. Inert MSU crystals have a negatively charged, highly reactive surface that is known to nonspecifically bind many plasma proteins (8) and also engage integrins including CD11b/CD18 and the Fc receptor CD16 (15). Hence, it is not surprising that MSU crystals have the potential to nonspecifically engage TLR complexes with other proteins that synergistically recognize PAMPs. We speculate that in leukocytes, the formation of such functionally significant complexes, including TLR-2 or TLR-4, CD14, and β2 integrins (16–19), modulates dimerization of the TLRs and optimum recognition of and responsiveness to MSU crystals.
gistically by MSU crystal induction of IL-1β and tumor necrosis factor α (TNFα)–induced activation of the endothelium and E-selectin expression (20), and by critical chemotactic activities of CXCL8 and closely related chemokine ligands of CXCR2 (14,21). Neutrophil activation promoted by bathing in a broth of soluble inflammation mediators in the gouty joint promotes amplification of the acute flare. Typically, a small fraction of neutrophils in the joint space actively phagocytose MSU crystals. However, crystal uptake by neutrophils, a cardinal diagnostic finding in acute gouty synovial fluids, directly stimulates release of a variety of mediators that amplify the inflammatory reaction, including the closely related calgranulins S100A8 and S100A9 (22,23). S100A8 and S100A9 are low molecular weight calcium-binding proteins that are highly abundant in the cytosol of the resting neutrophil. S100A8 and S100A9 clearly represent the neutrophil-derived crystal-induced chemotactic factor identified and intensively studied ∼2–3 decades ago. Moreover, 2 non-TLR pattern recognition receptors also involved in innate immunity, specifically the scavenger receptor CD36 and multiligand receptor for advanced glycation end products, are the primary receptors for S100A8 and S100A9.

**Triggering receptor expressed on myeloid cells 1 (TREM-1) as a mediator of gouty inflammation**

In this issue of *Arthritis & Rheumatism*, Murakami and colleagues describe another potential mechanism for the “early induced” innate response to amplification of acute gouty inflammation (24). Specifically, they observed that MSU crystals induce expression of TREM-1 in phagocytes in vitro. Furthermore, in the mouse subcutaneous air-pouch model of acute MSU crystal–induced inflammation, TREM-1 expression is markedly up-regulated in infiltrating neutrophils (24). TREM-1 is a cell surface–expressed immunoglobulin superfamily member that associates with and signals through the adapter protein DAP12 (25–29). The natural ligands of TREM-1 are not yet known, but TREM-1 is up-regulated in response to a variety of microbial pathogens (25–28). In the study by Murakami and colleagues, engagement of monocytes with agonistic monoclonal antibodies of TREM-1 turned on phagocytes and amplified not only LPS-induced inflammation in vitro and in vivo (25,26) but also IL-1β and CCL2 (monocyte chemotactic protein 1) release in response to a low concentration of MSU crystals.

As summarized above and shown in Figure 1, a fundamental early induced innate immune response clearly triggers and amplifies acute experimental MSU crystal–induced inflammation. The natural human form of acute gouty arthritis also has the classic footprint of such an immune response. As such, TLR-2, TLR-4, and TREM-1 may provide novel therapeutic targets in acute gouty arthritis. However, it also is likely that TLRs and TREM-1 also play substantial roles in determining the outcome and phenotypic features of gouty inflammation. In this context, monocyte influx follows neutrophil influx into loci of gouty inflammation, and TLR-2, TLR-4, and MyD88 signaling mediate MSU crystal–induced expression by mononuclear phagocytes of transforming growth factor β (11), a central inflammation-limiting event in acute experimental gout (5,30). Furthermore, we have observed that TLR-2– and MyD88-dependent signaling are essential for MSU crystal–induced NF-κB activation in chondrocytes (10), which is particularly pertinent because NF-κB activation promotes not only triggering but also resolution of acute inflammation (31). Therefore, TLR-2, TLR-4, and MyD88 likely contribute to the remarkable, stereotypic self-resolution that spontaneously occurs in acute gout. Local shedding by activated monocytes and neutrophils of soluble TREM-1 (32) also has the theoretical potential to limit acute gouty inflammation.

**Final considerations for the proposed model**

The model presented in Figure 1 clearly has limitations for interpreting gouty arthritis triggering mechanisms in humans. Specifically, human gouty arthritis commonly erupts in joints already manifesting low-grade, subclinical inflammation related to preexisting tophi (33). In contrast, the above-described results from experimental mouse and rabbit acute gout models were derived by injection of relatively large amounts of MSU crystals into previously noninflamed air pouches or knee joints of animals naive to the crystals. Moreover, mice and rabbits, unlike humans, express uricase and are thereby able to rapidly degrade exogenous MSU crystals and in so doing alter the morphologic features of the crystals and generate the proinflammatory oxidant hydrogen peroxide. Despite these issues, we believe that further investigation of the full spectrum of inflammatory responses mediated by TLR-2, TLR-4, and TREM-1 in both acute and chronic phases of gouty arthritis will be of translational interest for prophylaxis and therapy of gouty inflammation.
386
receptor (TLR) dimerization reveals subcellular targeting of TLRs

17. Ogawa T, Asai Y, Hashimoto M, Uchida H. Bacterial fimbriae activate human peripheral blood monocytes utilizing TLR2, CD14

18. Monick MM, Powers L, Butler N, Yarovinsky T, Hunnighake GW. Interaction of matrix with integrin receptors is required for


monohydrate crystal-induced inflammation: in vitro and in vivo studies on the roles of tumor necrosis factor α and interleukin-1.

murine homolog of the interleukin-8 receptor CXCR2 is essential for
the occurrence of neutrophilic inflammation in the air pouch model of acute urate crystal-induced gouty synovitis. Arthritis

recruitment in response to monosodium urate monohydrate crystals in


25. Bouchon A, Facchetti F, Weigand MA, Colonna M. TREM-1 amplifies inflammation and is a crucial mediator of septic shock.


27. Aoki N, Zganajc A, Margetts P, Xing X. Differential regulation of
DAP12 and molecules associated with DAP12 during host responses to mycobacterial infection. Infect Immun 2004;72:
2477–83.


30. Lawrence T, Gilroy DW, Collville-Nash PR, Willoughby DA. Possible new role for NF-κB in the resolution of inflammation.

31. Lawrence T, Gilroy DW, Collville-Nash PR, Willoughby DA. Possible new role for NF-κB in the resolution of inflammation.

on myeloid cells-1 modulates the inflammatory response in murine

33. Pascual E, Castellano JA. Treatment with colchicine decreases white cell counts in synovial fluid of asymptomatic knees that