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Uric Acid-Driven Th17 Differentiation Requires Inflammasome-Derived IL-1 and IL-18

Cristina Conforti-Andreoni,* Roberto Spreafico,* Hong Liang Qian,* Nicolas Riteau,† Bernhard Ryffel,‡ Paola Ricciardi-Castagnoli,* and Alessandra Mortellaro*

Uric acid is released from damaged cells and serves as a danger signal that alerts the immune system to potential threats, even in the absence of microbial infection. Uric acid modulation of innate immune responses has been extensively studied, but the impact of this damage-associated molecular pattern on adaptive responses remains largely unknown. In this study, we report that, in the presence of NF-κB signaling, uric acid crystals were capable of stimulating dendritic cells to promote the release of cytokines associated with Th17 polarization. Accordingly, naive CD4+ T cells cocultured with uric acid-treated dendritic cells differentiated toward the Th17 lineage. Th17 differentiation required the inflammasome-dependent cytokines IL-1α/β and IL-18 in both in vitro and in vivo models, and the inflammasome adaptor protein ASC and caspase-1 were essential for Th17 responses. Collectively, our findings indicate a novel role for the danger signal uric acid, in cooperation with NF-κB activation, in driving proinflammatory Th17 differentiation. Our data indicate that sterile inflammation shapes adaptive immunity, in addition to influencing early innate responses. The Journal of Immunology, 2011, 187: 000–000.

Uric acid (UA) is a natural product of the catabolism of purines that can generate crystals of its sodium salt, monosodium urate (1). Among mammals, primates are unusual in that they lack a functional uricase enzyme, leading to high uric acid concentrations in body fluids that can reach supersaturation levels and form precipitates (2). Deposition of UA crystals in joints can cause gout, a painful disease characterized by joint swelling due to infiltration of leukocytes, especially neutrophils (3).

Although UA crystals have been linked with gout pathogenesis for more than a century, little was known about the immunological properties of this molecule until the last decade. Previous data have indicated that UA crystals can trigger the MAPK cascade and are ineffective activators of NF-κB signaling (4, 5), but only recently has the wider role played by UA in immune responses begun to be elucidated. First, Shi et al. (6) demonstrated that intracellular UA is abruptly released following necrotic cell death, resulting in a sudden increase in UA concentration in the local microenvironment, which promoted sterile inflammation. Specifically, UA was able to induce the upregulation of costimulatory molecules on dendritic cells (DCs), which in turn can drive the activation of CD8+ T cells. Second, upon NF-κB priming with LPS, UA crystals have been shown to activate the NLRP3 inflammasome (1). This results in a molecular platform that includes also the ASC adaptor protein and mediates the recognition of stress-related signals, ultimately leading to caspase-1 activation, which in turn is responsible for the processing and secretion of bioactive IL-1β and IL-18. Caspase-1 activation also promotes the release of IL-1α (7), which exerts biological activity even in an unprocessed state. Accordingly, interfering with NLRP3 activation upon stimulation with UA crystals results in impaired IL-1α/β and IL-18 secretion by macrophages and DCs, as well as a marked reduction in neutrophil influx in vivo (1). Collectively, these data suggest that NLRP3 may be responsible for many of the pathological properties of UA crystals in gout.

UA mediates the immunostimulatory effects of alum adjuvants by promoting APC activation and consequent Ag-specific CD4+ T cell proliferation (8), and alum alone can activate the NLRP3 inflammasome (9). The interaction of UA crystals with target cells is not mediated by a specific receptor on the cell surface, but instead depends on direct interactions with the cell membrane (especially cholesterol-rich lipid rafts). These interactions trigger Syk activation in a sustained fashion, since phagocytosis of large crystals is aborted (“frustrated”) by their prohibitive size and cannot be finalized (10). These data indicate that UA constitutes a potent endogenous danger signal, although the potential impact of UA crystals on Th cell polarization is unclear.

Previous data have described the effects of UA crystals on the innate immune system, but the functional consequences of UA generation for adaptive immunity remain largely undocumented. As one of the most ubiquitous sterile danger signals in mammals, and in view of the key role played by UA in alum adjuvanticity, we sought to determine how UA crystals shape adaptive immune responses. In the present study, we focused on adaptive CD4+ T cell polarization since adjuvants that are able to direct appropriately polarized responses will be key to the success of next-generation vaccination strategies. Unexpectedly, we found that UA crystals alone are poor stimulators of any (Th1, Th2, or Th17) Th cell response. However, when coupled with NF-κB activation (which “primes” inflammasomes and drives the expression of IL-1 and IL-18 precursors), UA crystals potently induced Th17 responses.
These effects were dependent on the inflammasome-related cytokines IL-1α/β and IL-18, as well as on ASC and caspase-1. Accordingly, NLRP3 deficiency significantly impaired Th17 polarization, at least in vitro.

**Materials and Methods**

**Mice**

C57BL/6 (B6) mice were purchased from the Biological Resource Center (BRC, Agency for Science, Technology, and Research [A*STAR], Singapore). IL-1R1–deficient mice and IL-18Rα–deficient mice were provided by J. Tschopp (University of Lausanne, Lausanne, Switzerland) (1), caspase-1–deficient mice were from R. Flavell (11), and IL-1α–deficient mice were provided by Y. Ikawa (University of Tokyo, Tokyo, Japan) (12). All experiments were conducted with age-matched mice, and all mutants were backcrossed to B6 background for at least 10 generations.

Animals (8–12 wk of age) were bred under specific pathogen-free conditions at the BRC (Singapore) or at the Institute Transgenose (Orleans, France). Experiments were performed under the approval of the Institutional Animal Care and Use Committee in compliance with the Law and Guidelines for Animal Experiments of the BRC (A*STAR).

**Generation and stimulation of bone marrow-derived DCs**

Bone marrow-derived DCs (BMDCs) were prepared as previously described (4), then cultured in GM-CSF–free complete medium in 96-well flat-bottom plates (Corning) and stimulated with either muramyl dipeptide (MDP, 10 μg/ml; InvivoGen), UA crystals (monosodium urate, 250 μg/ml; Alexis Biochemicals), or a combination of MDP and UA. All UA preparations were confirmed to be endotoxin-free using the Limulus amebocyte lysate test.

**Naive CD4+ T cell priming**

Splenic naive CD4+ T cells were isolated by depletion of CD11c+, CD49b+, CD8a+, CD19+, Ly6C-, and Ly6G+ cells using biotinylated Abs (BioLegend) and magnetic streptavidin-conjugated beads (Miltenyi Biotec), and followed by sorting of CD4+CD25+Foxp3+CD25-CDC25 cells with a FACSAria using PE-conjugated CD4 clone (clone RM4-5), FITC-conjugated CD62L clone (clone MEL-14), PE-Cy7–conjugated CD44 clone (clone IM7) BioLegend), and allophycocyanin-conjugated CD25 (clone PC61; BD Pharmingen) mAbs. Purified naive CD4+ T cells were cocultured with GM-CSF–derived BMDCs or with FLT3L-derived BMDCs (T cell/DC ratio, 1:1) and stimulated with UA crystals (250 μg/ml), MDP (10 μg/ml), TNF-α (20 ng/ml; R&D Systems), soluble CD40L (0.5 ng/ml; R&D Systems), or combinations of these reagents in the presence of 1 μg/ml anti-mouse CD3e Ab (clone 145-2C11; BioLegend) for 5 d in complete medium. For the GM-CSF stimulation condition only, cells were cocultured in conditioned medium from a GM-CSF–transduced B16 cell line to a final GM-CSF concentration of 30 ng/ml. In selected conditions, Th17 cells were also differentiated from naive CD4+ T cells using the recombiant cytokines TGFB-β (1 ng/ml) and IL-6 (20 ng/ml) in the presence of 1 μg/ml anti-CD3e and anti-CD28 Abs for 5 d. All reagents were checked with the Limulus amebocyte lysate test and were endotoxin-free.

**Immunizations**

Age- and sex-matched B6 mice were injected s.c. in their footpads with keyhole limpet hemocyanin (KLH; 100 μg) emulsified in IFA (1:1) in the presence of MDP (50 μg), UA crystals (350 μg), or a combination of MDP and UA. A separate group of mice received UA crystals in saline. Six days later, draining lymph nodes were collected and single-cell suspensions (0.5 × 10⁶ cells/well) were incubated with KLH (0.1 mg/ml) in 0.2 ml complete medium for 5 d.

**RNA isolation and quantitative real-time PCR**

Total RNA was isolated and retrotranscribed as previously described (4). Quantitative real-time PCR was performed using validated TaqMan primers and probes from Applied Biosystems: IL-17A (Mm00439619_m1), IL-17F (Mm00521423_m1), IL-21 (Mm00517640_m1), and IL-22 (Mm00444241_m1). Retinoic acid-related orphan receptor (ROR)γt expression was assayed by SYBR Green real-time PCR using the following primers: Rorc 5′-GCCAGGACAGGGAGCCAAGTT-3′ and 5′-GGATCCCAGATGACT-3′; HPRT, 5′-CAGGGCAAGACTTTGTTGAG-3′ and 5′-GGCTTTGTATTTGGCTTTTCC-3′. Amplification was performed using an Applied Biosystems 7500 real-time PCR system. The relative expression level of each gene was evaluated using the ΔΔCt method. The difference between the Ct of the target gene and the Ct of the GAPDH or HPRT housekeeping gene was normalized to the ΔΔCt of the untreated condition.

**ELISA**

IL-1α, IL-1β, IL-6, IFN-γ, and IL-4 were measured in cell culture supernatants using DuoSet ELISA development kits (R&D Systems). IL-17A and IL-23 were assayed using Ready-SET-Go! ELISA kits (eBioscience) following the manufacturer’s instructions. OD was measured at 450 nm using a Tecan M200 Infinite plate reader.

**Statistical analysis**

The data shown in each figure represent the mean of three or more independent experiments (error bars indicate SD). Data were analyzed using Prism 5 (GraphPad Software), and statistical significance was determined by one-way or two-way ANOVA followed by the Bonferroni posttest, or by an unpaired two-tailed Student t test, as indicated in the respective figure legends. The p values are indicated as *p < 0.05, **p < 0.01, and ***p < 0.001.

**Results**

**UA crystals promote Th17 polarization, but only in synergism with NF-kB activators**

UA crystals are endogenous danger signals that potently activate immune responses. To determine the effects of exposure to UA crystals on CD4+ Th cell responses, mice were immunized in their footpads with KLH alone or with KLH and UA crystals in saline or emulsified in IFA. Cytokines IFN-γ, IL-4, and IL-17A were then measured by ELISA upon restimulation of lymph node cells with KLH in vitro. Mice that had received KLH/UA exhibited only weak T cell differentiation comparable to the KLH alone control (Fig. 1). These data suggest that native protein Ags may not directly adsorb to UA crystals, and/or that UA alone may not give rise to the Ag depot necessary to induce effective vaccination responses. We therefore used IFA to emulsify the Ag/UA mixture, but this alternative approach also failed to induce differential T cell polarization when compared with sham control (IFA alone). These results indicate that UA crystals alone are not sufficient to drive Th cell responses.
IL-17A was measured by ELISA. Although coculture of CD4+ cultures were collected and the production of IFN-γ 5 d coculture, cell supernatants from MDP, UA, and UA/MDP only IFN-γ was undetectable (Fig. 2A). These data were confirmed by quantitative real-time PCR, which showed increased transcription of the Th17-associated cytokine genes IL-17A, IL-17F, IL-21, and IL-22 only when cocultures were stimulated with MDP and UA together (Fig. 2B). When analyzed by real-time PCR, expression of the Th17-specific transcription factor RORγt was significantly increased upon UA/MDP treatment in vitro, and it reached levels comparable with those detected in Th17 cells differentiated in the presence of TGF-β and IL-6 (Fig. 2C). Taken together, these data demonstrate that NF-κB priming is essential for UA-mediated Th17 polarization in vitro.

Microbe-derived MDP provided sufficient NF-κB activation to facilitate UA-driven Th17 polarization in vitro, but it was uncertain whether nonmicrobial, host-derived NF-κB activators could induce a similar polarization profile when combined with UA crystals. We therefore activated DCs with UA in combination with either CD40L, GM-CSF, or TNF-α as priming signals. After 5 d coculture with naïve CD4+ T cells, cytokine levels were assessed by ELISA. Only IFN-γ and IL-4 were detected in supernatants from cocultures stimulated with CD40L or TNF-α alone, whereas IL-17A was undetectable (Fig. 2D). In contrast, large amounts of IL-17A were produced by CD4+ T cells cocultured with UA-treated DCs in the presence of GM-CSF, CD40L, or TNF-α, regardless of the specific NF-κB activator used (Fig. 2D). These data demonstrate that Th17 differentiation as driven by UA crystals is independent of the mode of NF-κB priming in DCs. This finding was confirmed by intracellular cytokine staining, which identified CD4+ T cells as the source of IL-17A (data not shown).

UA crystals induce poor cytokine secretion by immune cells in vitro because they lack NF-κB-activating capability (1, 4, 13). To overcome this limitation, most previous in vitro studies have used UA crystals in combination with TLR agonists, primarily LPS (1, 14, 15). However, strong stimuli, such as LPS, induce profound immune modulation associated with substantial cytokine release, which could mask the specific contribution of UA crystals. We therefore tested the bacteria-derived molecule MDP, which provides NOD2-mediated NF-κB activation but only weak cytokine release (4, 16), to potentiate UA effects and induce CD4+ T cell differentiation. DCs were cultured in the presence of MDP alone, UA alone, or a combination of MDP and UA, together with splenic, naïve CD4+ T cells activated with an anti-CD3ε Ab. After 5 d coculture, cell supernatants from MDP, UA, and UA/MDP cultures were collected and the production of IFN-γ, IL-4, and IL-17A was measured by ELISA. Although coculture of CD4+ T cells with DCs exposed to UA or MDP alone failed to elicit significant cytokine production, combination treatment of DCs with UA/MDP induced significant production of IL-17A, which is not affected by any combination of UA and MDP treatment (Fig. 2A). These data were confirmed by quantitative real-time PCR, which showed increased transcription of the Th17-associated cytokine genes IL-17A, IL-17F, IL-21, and IL-22 only when cocultures were stimulated with MDP and UA together (Fig. 2B). When analyzed by real-time PCR, expression of the Th17-specific transcription factor RORγt was significantly increased upon UA/MDP treatment in vitro, and it reached levels comparable with those detected in Th17 cells differentiated in the presence of TGF-β and IL-6 (Fig. 2C). Taken together, these data demonstrate that NF-κB priming is essential for UA-mediated Th17 polarization in vitro.

To determine whether UA-driven Th17 polarization can be observed also in Ag-specific settings, DCs activated with UA alone, or by UA treatment in combination with MDP or CD40L, were cocultured with naïve OVA-specific CD4+ T cells isolated from DO11.10 TCR transgenic mice. Negligible amounts of IL-17A were detected in supernatants from cocultures treated with UA, MDP, or CD40L alone (Supplemental Fig. 1). However, OVA-specific IL-17A–producing CD4+ T cells were indeed induced by activation of DCs with UA/MDP or with UA/CD40L in combination (Supplemental Fig. 1). The BALB/c background of DO11.10 mice also served to confirm that the observed Th17 differentiation promoted by UA was not restricted to B6 mice alone. Taken together, these data demonstrate that DCs activated by UA crystals skew the differentiation of CD4+ T cells toward the Th17 lineage, but only in the presence of an NF-κB priming signal.

**FIGURE 1.** UA crystals alone are not sufficient to drive Th cell responses. B6 mice were immunized s.c. in the left rear footpad with KLH prepared in saline (saline), KLH emulsified in IFA (IFA), or were administered UA crystals in saline (UA) or UA prepared in IFA (UA/IFA). Six days later, draining lymph nodes were collected and total cells were restimulated in vitro with KLH for 5 d. Alternatively, right lymph nodes from saline-injected mice were collected as “uninjected” controls (uninj.). Quantities of IL-17A, IFN-γ, and IL-4 in supernatants were determined by ELISA. Each symbol represents the mean of quadruplicate culture stimulations from a single mouse lymph node. Statistical significance was determined by one-way ANOVA.

**Th17 polarization driven by UA/MDP-treated DCs depends on the NLRP3–IL-1/IL-18 axis in vitro**

UA crystals are potent activators of the NLRP3 inflammasome, a cytoplasmic sensor of cellular stress, which associates with the ASC adaptor to promote caspase-1–mediated processing and activation of IL-1 and IL-18 by macrophages and DCs (1, 17). To determine the mechanism underlying UA-induced Th17 polarization in the presence of a priming signal, we evaluated the profile of cytokines released by DCs. In the absence of MDP, DCs exposed to UA did not produce IL-1α/β or IL-18, supporting the two-signal model of inflammasome activation (Fig. 3A) (13). However, when primed with MDP, exposure to UA crystals elicited IL-1α, IL-1β, and IL-18 from wild-type (WT) DCs, but not from NLRP3-deficient (NLRP3−/−) DCs (Fig. 3A). Additionally, we confirmed that caspase-1 mediated not only the secretion but also the processing of IL-1β, as mature IL-1β (p17) was readily detectable in supernatants of DCs stimulated with UA/MDP, but not in cultures either unstimulated or stimulated with UA or MDP alone (Fig. 3B). NLRP3 deficiency impaired the release of mature IL-1β (Fig. 3B), but it did not affect the levels of IL-6 and IL-23 detected (Fig. 3A), as expected.

To assess whether NLRP3 activation might affect the ability of DCs to induce Th17 cell differentiation, WT and NLRP3-deficient DCs were cocultured with WT naive CD4+ T cells in the presence of UA, MDP, or a UA/MDP combination. We observed that Th17 cell polarization as induced by UA/MDP was impaired in cocultures of NLRP3−/− DCs when assessed by ELISA (Fig. 4A) or by intracellular staining (Fig. 4B). In contrast, NLRP3−/− DCs were unaffected in their ability to induce the secretion of IFN-γ and IL-4 by differentiating T cells (Fig. 4A, 4B). These data demonstrate that NLRP3 is required for DCs to promote in vitro induction of IL-17–producing T cells, but is dispensable for IFN-γ and IL-4 secretion.
To determine whether the inability of NLRP3−/− DCs to induce Th17 cells in vitro was due to impaired secretion of IL-1 and/or IL-18, WT DCs were cocultured with naive CD4+ T cells isolated from IL-1RI−/− or IL-18Rα−/− mice. IL-17A secretion by T cells upon UA/MDP stimulation was reduced in the absence of IL-1RI or IL-18R (Fig. 4C). The NLRP3 inflammasome-dependent cytokines IL-1 and IL-18 are thus required to drive Th17 polarization induced by UA/MDP. Interestingly, the impairment of Th17 polarization was counterbalanced by increased production of IFN-γ and IL-4, perhaps indicating that Th1/Th2 polarization is favored in the absence of IL-1 and IL-18.

DCs can be differentiated in vitro with GM-CSF or FLT3L (18), although each cytokine gives rise to distinct DC subsets. Whereas GM-CSF produces inflammatory DCs similar to human monocyte-derived DCs, FLT3L instead yields the in vitro counterpart of steady-state conventional DCs (18). The NLRP3 inflammasome-dependent cytokines IL-1 and IL-18 are thus required to drive Th17 polarization induced by UA/MDP. Interestingly, the impairment of Th17 polarization was counterbalanced by increased production of IFN-γ and IL-4, perhaps indicating that Th1/Th2 polarization is favored in the absence of IL-1 and IL-18.

DCs can be differentiated in vitro with GM-CSF or FLT3L (18), although each cytokine gives rise to distinct DC subsets. Whereas GM-CSF produces inflammatory DCs similar to human monocyte-derived DCs, FLT3L instead yields the in vitro counterpart of steady-state conventional DCs (18). Because the T cell polarizing activity of DCs may depend on the nature of their in vitro differentiation, we investigated whether UA-induced Th17 polarization by MDP-primed DCs was observed regardless of the cytokine used to first differentiate DCs. Cocultures of naive CD4+ T cells with UA/MDP-treated DCs exhibited comparable levels of NLRP3-dependent Th17 polarization irrespective of whether DCs were derived in FLT3L or GM-CSF (Supplemental Fig. 2A). Furthermore, a spleen-derived, growth factor-dependent, long-term DC line activated with UA/MDP also induced Th17 polarization, which was severely impaired by blocking IL-1RI signaling with IL-1R antagonist (Supplemental Fig. 2B).

Taken together, these data indicate that the NLRP3–IL-1/IL-18 axis, primed by an NF-κB activator and triggered by UA crystals, is crucial for in vitro Th17 polarization of CD4+ T cells by DCs, irrespective of the DC subset used.

**Ag immunization with UA/MDP results in IL-1– and IL-18–dependent Th17 polarization in vivo**

To determine whether UA/MDP cotreatment also elicited Th17 responses in vivo, WT B6 mice were immunized in their footpads with KLH emulsified in IFA in the presence of MDP, UA, or a combination of MDP and UA. Upon restimulation with KLH in vitro, lymph node cells from mice that had received KLH/UA/MDP treatment produced higher levels of IL-17A than did any other combination of stimuli, whereas no difference in IFN-γ was detected between any of the treatment regimens (Fig. 5A). Both intracel-
NF-κB–primed UA-activated DCs secrete cytokines favoring Th17 cell differentiation. WT or NLRP3−/− DCs were left untreated (UT) or stimulated for 24 h with either MDP, UA crystals, or combinations. Cell-free supernatants were assayed by (A) ELISA for IL-1α, IL-1β, IL-18, IL-6, and IL-23 or (B) Western blot for the mature form (p17) of IL-1β.

Discussion

In this study, we report a novel role for UA crystals in promoting DC-driven Th17 differentiation of naïve CD4+ T cells. Th17 polarizing cytokines IL-1α/β, IL-6, and IL-23 were produced exclusively by DCs upon combined stimulation through NF-κB, leading to differentiation of IL-17-producing T cells both in vitro and in vivo. Conversely, either NF-κB stimulation or UA crystals alone were unable to induce Th17 polarization under these conditions. These data indicate that cooperation between “danger” molecules and NF-κB priming signals can potently modulate adaptive immune responses, in addition to known influences on innate immunity (19).

Th17 cells are known to promote neutrophil-mediated inflammation, tissue remodeling, and repair by producing IL-17A, IL-21, and IL-22. Th17 responses are also responsible for immune protection against certain extracellular bacteria and fungi (20–22), and they exert critical roles in autoimmune pathologies in both animal models and human diseases (23, 24). Th17 differentiation is orchestrated by DCs, which can provide the necessary Ag presentation, costimulatory signals, and polarizing cytokines (to date, IL-6, TGF-β, and IL-23 in the mouse, and IL-1, IL-6, and IL-23 in humans) that are required to drive lineage commitment (25–28). Studies of sterile danger signals have previously focused on innate immunity, and few investigators have explored the relationship between nonmicrobial, sterile danger signals and Th cell responses (19, 29, 30). In the present study, we aimed to address this gap in our knowledge and observed that UA, an archetypal sterile danger signal, can cooperate with NF-κB signaling to potently induce Th17 differentiation.

In gouty arthritis, UA crystals stimulate synovial cells, monocytes/macrophages, and neutrophils, which infiltrate inflamed joints and secrete proinflammatory cytokines, including IL-1α, IL-18, TNFα, IL-6, and IL-23. This complex cytokine milieu induces and sustains the inflammatory response characteristic of gout, but similar cytokine profiles can also be observed in autoimmune diseases that affect joints, including rheumatoid arthritis and juvenile idiopathic arthritis. Interestingly, IL-17 concentrations in synovial fluids are significantly higher in gouty patients than in control patients suffering from osteoarthritis (31). Although a defined correlation between excess UA levels in blood (hyperuricemia) and Ag-specific immune responses has not yet been described, UA crystals (or other endogenous danger signals) released from dying cells in the tissues may well amplify and sustain the inflammatory response characteristic of gout, further exacerbating cytokine production. The additional roles of IL-1α/β in the inflammatory process are supported by our findings, where IL-1α/β is seen to augment the production of IL-17 in response to UA crystals.

In conclusion, this study demonstrates that UA crystals may play a critical role in the pathogenesis of gouty arthritis by potentiating Th17 differentiation, which may contribute to the development of joint inflammation and cartilage damage.
FIGURE 4. UA-driven Th17 polarization by primed DCs requires NLRP3, IL-1, and IL-18 in vitro. DCs differentiated from WT or NLRP3-deficient (NLRP3<sup>−/−</sup>) B6 mice were used to prime WT B6-derived naive CD4<sup>+</sup> T cells in the presence of MDP, UA crystals, or a combination of MDP and UA. On day 5, T cell polarization was assayed by measuring IL-17A, IFN-γ, and IL-4 by ELISA (A) or by intracellular staining (B). C, WT B6 DCs activated with MDP, UA, or MDP and UA were cocultured with naive CD4<sup>+</sup> T cells isolated from the spleen of WT, IL-1RI<sup>−/−</sup>, or IL-18Rα<sup>−/−</sup> B6 mice. Supernatants from cocultures were collected after 5 d for analysis of IL-17A, IFN-γ, and IL-4 by ELISA. Data were collected from three or more independent experiments. *<i>p</i> < 0.05, **<i>p</i> < 0.01, ***<i>p</i> < 0.001 by two-way ANOVA.
traditionally used to treat hyperuricemia in gout by lowering uric acid levels in the blood, has also shown efficacy in the treatment of other disorders, including autoimmune uveitis and inflammatory bowel diseases (35–37).

Using both in vitro and in vivo approaches, we have investigated the role of MDP and UA crystals in the induction of Th17 cells, revealing a critical role for both IL-1β and IL-18 signaling in this process. IL-1β is involved in the pathogenesis of many autoim-

![Graph](image-url)

**Figure 5.** Coimmunization with MDP and UA induces Ag-specific Th17 cells in vivo. B6 mice were immunized s.c. with KLH emulsified in IFA in the presence of saline, MDP, UA crystals, or MDP and UA. Six days later, draining lymph nodes were collected and total cells were restimulated in vitro with KLH for 5 d. A, Levels of IL-17A, IFN-γ, and IL-4 in supernatants were determined by ELISA. The data are pooled from four independent experiments and each symbol represents the mean of quadruplicate stimulations from a single mouse. *p < 0.05, **p < 0.01, ***p < 0.001 by one-way ANOVA. B, Lymph node cells were analyzed by intracellular staining for IL-17A, IFN-γ, and IL-4. Data show the proportion of IL-17+ (Th17), IFN-γ+ (Th1), and IL-4+ (Th2) cells among total CD4+ cells, from eight mice per group. C, RORγt expression in total lymph nodes isolated from B6 mice upon indicated treatments assessed by quantitative real-time PCR and normalized to levels measured in the saline control. *p < 0.05, **p < 0.01, ***p < 0.001 by two-tailed Student t test.

![Graph](image-url)

**Figure 6.** Inflammasome-dependent cytokines IL-1α/β and IL-18 drive UA-induced Th17 polarization of CD4+ T cells. Age- and gender-matched B6 mice were either WT, NLRP3−/− (A); ASC−/−, caspase-1−/−, IL-1α−/− (B); or IL-1RI−/− or IL-18Rα−/− (C). All animals were immunized s.c. with KLH emulsified in IFA in the presence of saline, MDP, UA crystals, or UA/MDP, as indicated. Draining lymph nodes were collected after 6 d, and total cells were restimulated in vitro with KLH for 5 d. IL-17A, IFN-γ, and IL-4 concentrations in supernatants were determined by ELISA. Data shown are mean ± SD of at least three to five mice per group and were collected from three independent experiments. *p < 0.05, ***p < 0.001 by two-way ANOVA.
mune diseases, and blocking IL-1 with neutralizing Abs or with receptor antagonists is an effective therapy across a broad range of indications (38). Studies in IL-1R1-deficient mice have demonstrated a crucial role for IL-1-mediated signaling in the Th17 polarization associated with experimental autoimmune encephalomyelitis (27, 39), yet the role played by IL-1 in murine Th17 differentiation is less clear than in humans (25). Together with recent data from other investigators (39), the present study sheds important light on the contribution of IL-1 to Th17-mediated immunity in mice. Despite IL-18 being better known to increase IFN-γ production by T cells to promote Th1-mediated responses (3), we found that IL-18 was also involved in the induction of UA-mediated Th17 differentiation in vivo, consistent with recent studies suggesting a link between IL-18 and Th17 polarization (40). In addition to IL-1β and IL-18, we found that IL-1α was also important for UA/MDP-induced Th17 immunity in vivo. Pro-IL-1α is not a substrate for caspase-1, but macrophages that lack either caspase-1 or NLRP3 are still impaired in their ability to secrete IL-1α (7, 41), as we observed in DCs in the present study (Fig. 3). These data indicate that IL-1R triggering by both IL-1α and IL-1β is crucial for UA-induced Th17 polarization of CD4+ T cells in the presence of a NF-κB priming signal.

In recent years, the ability of UA to activate the NLRP3 inflammasome has received increasing attention from investigators (1). In the present study, we tested whether UA/MDP-induced Th17 cell differentiation was mediated by similar activation of the NLRP3 inflammasome. Indeed, we observed that NLRP3-deficient DCs have a reduced capacity to induce Th17 cell polarization in vitro, and inflammasome components ASC and caspase-1 also played a crucial role in UA/MDP-driven Th17 immunity in vivo. The reduction in IL-17 secretion by CD4+ T cells in NLRP3−/− mice did not reach statistical significance, suggesting that NLRP3 may contribute to but is not critical for ASC/caspase-1-mediated processing of IL-1β and IL-18 in vivo in our settings. However, a role for an alternative inflammasome complex in driving IL-1β production through the shared adaptor ASC and caspase-1 cannot be excluded. It must be noted that in vitro findings seldom match in vivo data in the field of inflammasome research, even for very basic findings such as IL-1β release (42–44).

Indeed, the role of NLRP3 inflammasome in mediating specific adaptive responses in vivo is still controversial (45). In experimental autoimmune encephalomyelitis, NLRP3 is crucial for the onset of spinal cord inflammation by sustaining Th1 and Th17 responses (40), but another study attributed these effects to ASC rather than to NLRP3 (42). NLRP3 also appears dispensable for the onset of Ag-induced arthritis (46, 47). Similarly contradictory data describe the function of the NLRP3 inflammasome in mouse models of colitis and colitis-associated colon cancer (48, 49). Knock-in mouse models that carry human mutations identified in cryopyrin (7, 41), as we observed in DCs in the present study (Fig. 3). These data indicate that IL-1R triggering by both IL-1α and IL-1β is crucial for UA-induced Th17 polarization of CD4+ T cells in the presence of a NF-κB priming signal.

Indeed, the role of NLRP3 inflammasome in mediating specific adaptive responses in vivo is still controversial (45). In experimental autoimmune encephalomyelitis, NLRP3 is crucial for the onset of spinal cord inflammation by sustaining Th1 and Th17 responses (40), but another study attributed these effects to ASC rather than to NLRP3 (42). NLRP3 also appears dispensable for the onset of Ag-induced arthritis (46, 47). Similarly contradictory data describe the function of the NLRP3 inflammasome in mouse models of colitis and colitis-associated colon cancer (48, 49). Knock-in mouse models that carry human mutations identified in cryopyrin

## References


O'Connor, W., Jr., L. A. Zenewicz, and R. A. Flavell. 2010. The dual nature of
Evans, H. G., N. J. Gullick, S. Kelly, C. Pitzalis, G. M. Lord, B. W. Kirkham, and
Kuida, K., J. A. Lippke, G. Ka, M. W. Harding, D. J. Livingston, M. S. Sa, and