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Contribution of Mast Cell–Derived Interleukin-1β to Uric Acid Crystal–Induced Acute Arthritis in Mice

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Objective. **Gouty arthritis is caused by the precipitation of monosodium urate monohydrate (MSU) crystals in the joints. While it has been reported that mast cells (MCs) infiltrate gouty tophi, little is known about the actual roles of MCs during acute attacks of gout. This study was undertaken to assess the role of MCs in a mouse model of MSU crystal–induced acute arthritis.**

Methods. **We assessed the effects of intraarticular (IA) injection of MSU crystals in various strains of mice with constitutive or inducible MC deficiency or in mice lacking interleukin-1β (IL-1β) or other elements of innate immunity. We also assessed the response to IA injection of MSU crystals in genetically MC-deficient mice after IA engraftment of wild-type or IL-1β$^{-/-}$ bone marrow–derived cultured MCs.**

Results. **MCs were found to augment acute tissue swelling following IA injection of MSU crystals in mice. IL-1β production by MCs contributed importantly to MSU crystal–induced tissue swelling, particularly during its early stages. Selective depletion of synovial MCs was able to diminish MSU crystal–induced acute inflammation in the joints.**

Conclusion. **Our findings identify a previously unrecognized role of MCs and MC-derived IL-1β in the early stages of MSU crystal–induced acute arthritis in mice.**

Acute attacks of gout are initiated by the precipitation of crystals of monosodium urate monohydrate (MSU) in joints. The prevalence of gout has increased recently, with ~6.1 million people with a history of gout in the US alone (1). While several lines of evidence support the importance of interleukin-1β (IL-1β) in gout (2,3), less is known about the extent to which different populations of innate immune cells contribute to IL-1β production in this disorder.

Mast cells (MCs) are sentinels of innate immunity that occur in virtually all vascularized tissue (4). Traditionally regarded primarily as effector cells in IgE-dependent acquired immune responses, MCs are now emerging as key players, together with dendritic cells and monocytes, in first defense against invading pathogens and in interactions with environmental stimuli and external toxins (4). Upon activation, MCs can secrete a large spectrum of mediators, including stored products such as histamine and tryptase, as well as many cytokines, including IL-1β (5).

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Dr. Schwartz is inventor on a patent for a tryptase assay, which Virginia Commonwealth University has licensed to Thermo Fisher and for which Virginia Commonwealth University shares the royalties with the inventor.

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Because many patients with gout respond clinically to treatment with inhibitors of IL-1 (6) and because MCs represent a source of IL-1 in a mouse model of antibody-mediated arthritis (5), we hypothesized that MCs can contribute to the early stages of acute arthritis in response to uric acid crystals through the production of IL-1β. We report herein evidence that strongly supports that hypothesis.

MATERIALS AND METHODS

Mice. WBB6F$_1$-*Kit$^{W/W\text{-}v}$* (Kit$^{W/W\text{-}v}$) mice (and the corresponding control WBB6F$_1$-Kit$^{+/+}$ [Kit$^{+/+}$] mice), B6.129S7-*Il1rI^{tm1Imx}*/J (IL-1RI$^{-/-}$) mice, B6.129P2-*Il18^{tm1Aki}*/J (IL-18$^{-/-}$) mice, and C57BL/6-*Gt(ROSA)26Sor$^{tm1(HBEGF)Awai}$*/J (iDTR$^{fl/fl}$) mice were purchased from The Jackson Laboratory. C57BL/6J (wild-type [WT]) mice were obtained from The Jackson Laboratory and either were bred at the Stanford University Research Animal Facility or were maintained there for at least 2 weeks before being used in experiments. C57BL/6-*Kit$^{W\text{-}sh/W\text{-}sh}$* (Kit$^{W\text{-}sh/W\text{-}sh}$) mice were originally provided by Peter Besmer (Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY); we backcrossed these mice to C57BL/6J mice for more than 11 generations (7). Mcpt8$^{DTR/+}$ (and the corresponding control Mcpt8$^{+/+}$) (8), IL-1$\alpha^{-/-}$ (9), IL-1$\beta^{-/-}$ (9), TNF$^{-/-}$ (10), Cpa3-Cre;Mcl-1$^{fl/fl}$ (and the corresponding control Cpa3-Cre;Mcl-1$^{+/+}$) (11), and Cpa3-Cre;iDTR (generated by crossing Cpa3-Cre mice [11] with iDTR$^{fl/fl}$) mice were all on the C57BL/6 background and were bred and maintained at the Stanford University Research Animal Facility. We used age-matched male mice for all experiments. All animal care and experimentation were conducted in compliance with the guidelines of the National Institutes of Health and with the specific approval of the Institutional Animal Care and Use Committee of Stanford University.

Human serum and synovial fluid samples. We studied human synovial fluid samples under protocols that were approved by the Stanford University Institutional Review Board and included the informed consent of the subjects. Samples of synovial fluid from actively inflamed large or medium joints were obtained by needle aspiration performed by a board certified rheumatologist (JS) at the VA Hospital (Palo Alto, CA). Grossly bloody fluid was excluded from analysis. Synovial fluid was centrifuged at 1,000*g* for 10 minutes, and supernatants were removed and frozen at −80°C until used in the experiments described below. The diagnosis of gout was confirmed by identification of negatively birefringent intracellular needle-shaped crystals on microscopic examination of synovial fluid under polarizing light microscopy. The diagnosis of rheumatoid arthritis (RA) was made as defined by the American College of Rheumatology 1987 revised criteria for the disease (12).

Serum levels of histamine were measured by a competitive enzyme-linked immunosorbent assay (ELISA) using a kit from Beckman Coulter. IL-1β levels were measured using a high-sensitivity ELISA (lower detection limit 0.16 pg/ml; eBioscience). Total tryptase levels were measured using an immunocapture assay (ImmunoCAP; Phadia Diagnostics). Levels of mature tryptase were measured by ELISA as described elsewhere (13). Assays for both total and mature tryptase were performed in parallel at Virginia Commonwealth University by individuals who were not aware of the identity of individual specimens.

Preparation and intraarticular (IA) injection of MSU crystals. MSU crystals were prepared as described previously (2). One gram of uric acid (Sigma) in 180 ml of 0.01*M* NaOH was heated to 70°C. NaOH was added as required to maintain the pH between 7.1 and 7.2, and the solution was filtered and incubated at room temperature, with slow and continuous stirring, for 24 hours. MSU crystals were kept sterile, washed with ethanol, dried, autoclaved, and resuspended in phosphate buffered saline (PBS) by sonication. MSU crystals contained <0.005 endotoxin units/ml of endotoxin (*Limulus* amebocyte lysate endotoxin assay; GenScript).

In most experiments (and unless stated otherwise), 0.5 mg of MSU crystals in 10 μl of PBS was injected intraarticularly in one ankle joint, and PBS alone was injected in the contralateral ankle joint. We used Microliter #705 syringes (Hamilton) with 27-gauge needles for all IA injections. Injections were performed with the mice under isoflurane anesthesia, and the quality of IA injection was controlled by assessing the location of MSU crystal deposits histologically on ankle tissue collected 24 hours after the injection. In some experiments, we used MC-deficient mice engrafted with bone marrow–derived cultured MCs (BMCMCs) from WT mice in one ankle and BMCMCs from IL-1$\beta^{-/-}$ mice in the contralateral ankle, and we injected these mice with MSU crystals in both ankles as described below. We also injected diphtheria toxin (DT)–treated Cpa3-Cre;iDTR mice with MSU crystals in both ankles (see below). Ankle swelling was measured at different time points using precision calipers (Fisherbrand Traceable Digital Calipers; Fisher Scientific).

Culture and adoptive transfer of MCs. BMCMCs were obtained by culturing bone marrow cells from C57BL/6J WT mice or from C57BL/6-IL-1$\beta^{-/-}$ mice in 20% WEHI-3 conditioned medium (containing IL-3) for 6 weeks, at which time cells were >98% c-Kit+FcεRIα+. BMCMCs were transferred by IA injection (2 injections, each consisting of 10^6 cells in 10 μl of PBS). Experiments were performed 6 weeks after transfer of BMCMCs.

DT-mediated ablation of MCs or basophils. For MC ablation, Cpa3-Cre^{+};iDTR$^{fl/+}$ and Cpa3-Cre^{-};iDTR$^{fl/+}$ littermates received 2 IA injections 1 week apart, each consisting of 50 ng of DT in 20 μl of PBS, in one ankle joint, and PBS alone was injected in the contralateral ankle joint. Mice were injected with MSU crystals in both ankles 1 week after the last DT injection. In preliminary experiments, we also assessed whether MCs were depleted 2 days after a single intraperitoneal (IP) injection of 500 ng of DT (data available online at http://med.stanford.edu/gallilab/Figures.html). For basophil depletion, Mcpt8$^{DTR/+}$ and Mcpt8$^{+/+}$ littermates received a single IP injection of 500 ng of DT 2 days before IA injection with MSU crystals.

Antibodies and flow cytometry. We used flow cytometry to identify and enumerate blood basophils (CD49b+IgE+), monocytes (Gr-1^{low}CD11b+Siglec-F−), neutrophils (Gr-1^{high}CD11b+Siglec-F−), and eosinophils (SSC^{high}Siglec-F+), as well as peritoneal MCs (c-Kit+IgE+). Briefly, blood cells were lysed by treatment with ACK lysis buffer 2 times for

5 minutes each. Cells were blocked with unconjugated anti-CD16/CD32 antibodies on ice for 5 minutes and then stained with a combination of the following antibodies on ice for 30 minutes: for blood leukocyte analysis, phycoerythrin (PE)–labeled Siglec-F (E50-2440; BD Biosciences), eFluor 450–labeled CD11b (M1/70; eBioscience), allophycocyanin (APC)–labeled CD49b (DX5; eBioscience), biotin-labeled IgE (23G3; eBioscience), and fluorescein isothiocyanate (FITC)–labeled Gr-1 (RB6-8C5; eBioscience); and for peritoneal MC analysis, APC-labeled c-Kit (ACK2; eBioscience) and biotin-labeled IgE. Cells were then incubated for 15 minutes with PE–Texas Red–streptavidin (BD PharMingen). Data were acquired with LSRII and Accuri C6 flow cytometers (BD Biosciences) and analyzed with FlowJo software (Tree Star).

Histologic analysis. Joints were fixed in 10% formalin, decalcified for 10 days in 0.5*M* EDTA, pH 8, embedded in paraffin, and 4-μm sections were prepared and stained with 0.1% toluidine blue (for histologic examination of MCs) or with hematoxylin and eosin (for histologic examination of leukocytes). Images were captured with an Olympus BX60 microscope using a Retiga-2000R QImaging camera run by Image-Pro Plus Version 6.3 software (Media Cybernetics).

Statistical analysis. A nonparametric Mann-Whitney test (2-tailed) was used for statistical analysis of tryptase, histamine, and IL-1β levels in human synovial fluid samples. Differences between groups were assessed for statistical significance by analysis of variance (for ankle swelling) or Student's unpaired *t*-test (for comparison of only 2 sets of data). *P* values less than 0.05 were considered statistically significant. Except where indicated otherwise, all data are presented as the mean ± SEM.

RESULTS

Contribution of MCs to MSU crystal–induced ankle swelling in mice. To investigate the importance of MCs in acute gouty arthritis, we developed a mouse model consisting of performing IA injections of MSU crystals into the ankle joints of mice (Figures 1A–C). Injection of MSU crystals induced ankle swelling that was maximal at 24 hours (Figures 1A and B), a time at which acute inflammatory infiltrates were observed histologically (Figure 1C).

We found that MC- and basophil-deficient Cpa3-Cre$^+$;Mcl-1$^{fl/fl}$ mice (11) had reduced ankle swelling compared to their littermate controls in this model, especially during the first 3 hours, during which little or no response above that induced by PBS was observed in the Cpa3-Cre$^+$;Mcl-1$^{fl/fl}$ mice (Figure 2A). However, substantial ankle swelling (reaching 59% of that seen in the MSU crystal–injected joints of Cpa3-Cre$^+$;Mcl-1$^{+/+}$ mice), as well as leukocyte infiltration, was observed at 24 hours in the Cpa3-Cre$^+$;Mcl-1$^{fl/fl}$ mice (Figure 2B). These results indicate that MCs and/or basophils contribute importantly to the early stages of inflammation in this

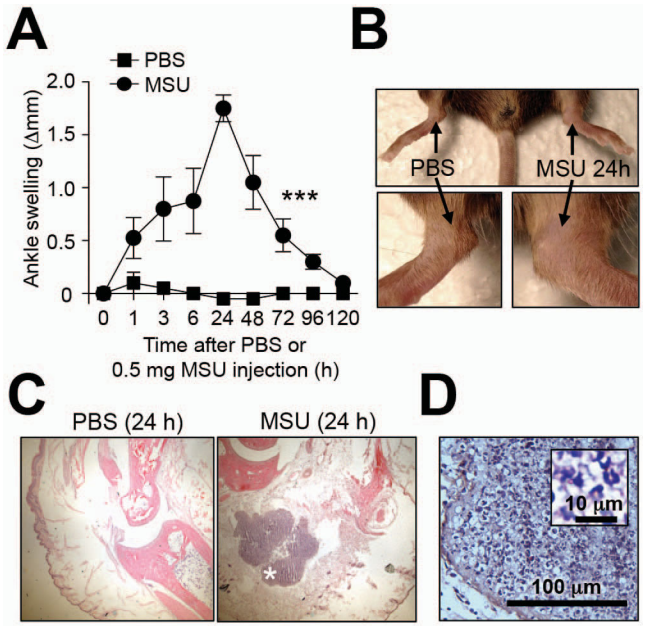

Figure 1. Mouse model of monosodium urate monohydrate (MSU) crystal–induced acute arthritis. C57BL/6J mice were injected intraarticularly with MSU crystals (0.5 mg in 10 μl) in one ankle joint and vehicle (10 μl of phosphate buffered saline [PBS]) in the contralateral ankle joint. **A,** Time course of changes in MSU crystal–induced ankle swelling. Values are the mean ± SEM of 2 independent experiments. *** = $P < 0.001$ versus controls, by analysis of variance. **B,** Representative photographs of MSU crystal–induced ankle swelling obtained at 24 hours. Images at the bottom are magnified views of the areas indicated by the **arrows** in the top image. **C,** Photomicrographs of hematoxylin and eosin–stained sections of ankle joints obtained at 24 hours. Original magnification × 40. **D,** Higher-magnification view of the area marked with an **asterisk** in the MSU crystal–treated mouse joint section shown in **C**. **Inset,** Enlargement of the leukocyte infiltrate.

model and that other cell types also contribute to MSU crystal–induced tissue swelling and leukocyte infiltration, particularly at later intervals after MSU crystal injection.

Because Cpa3-Cre$^+$;Mcl-1$^{fl/fl}$ mice are markedly deficient in both MCs and basophils, we next assessed the relative contribution of these 2 cell populations in this model of acute gout. Basophils can be selectively ablated by injection of DT into Mcpt8$^{DTR/+}$ mice (8), which express the DT receptor (DTR) only in basophils. DT-mediated depletion of basophils in Mcpt8$^{DTR/+}$ mice did not affect MSU crystal–induced ankle swelling (Figure 2C), suggesting that basophils do not importantly contribute to the acute response to MSU crystals.

In contrast, IA engraftment of Cpa3-Cre$^+$;Mcl-1$^{fl/fl}$ mice with BMCMCs from C57BL/6J (WT) mice restored MSU crystal–induced ankle swelling to levels observed in Cpa3-Cre$^+$;Mcl-1$^{+/+}$ littermate controls,

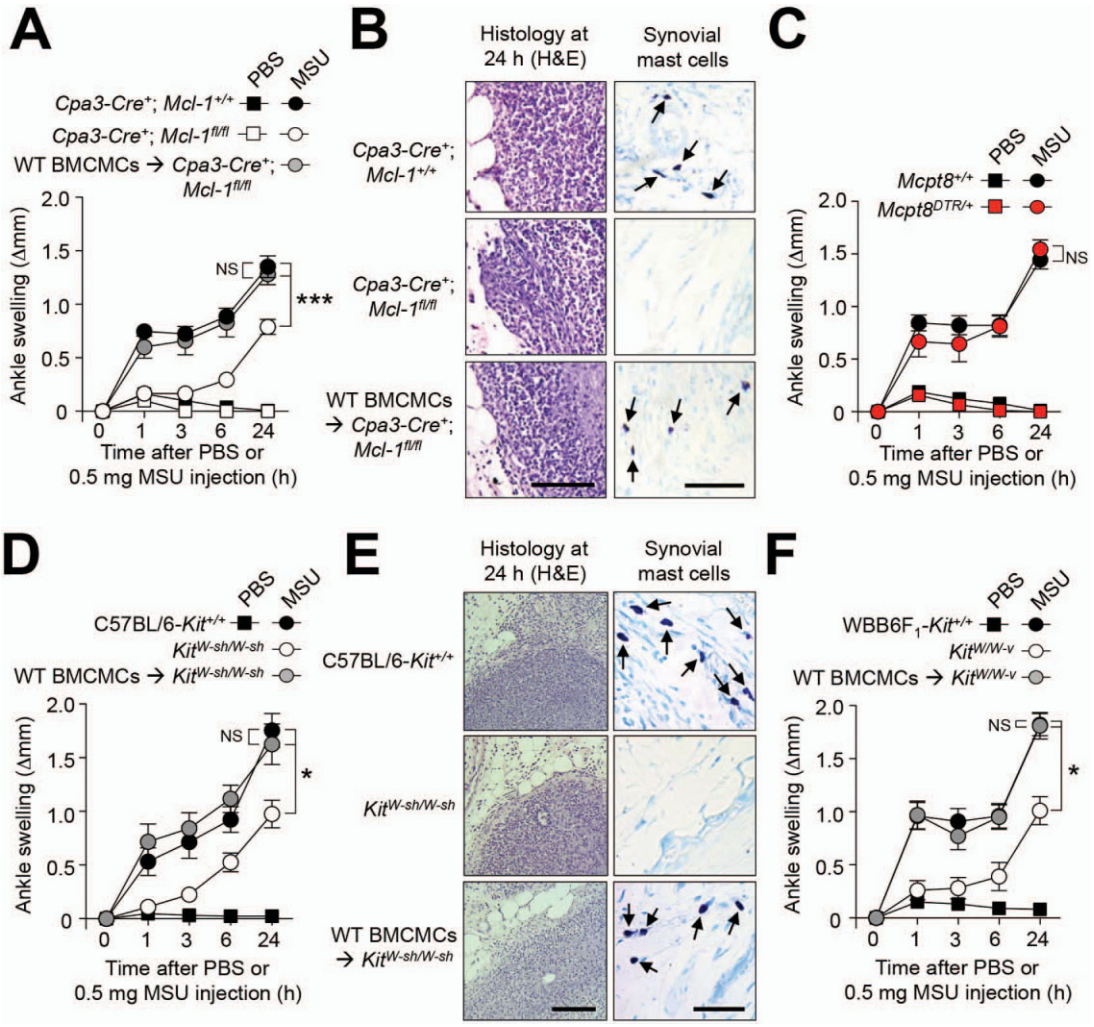

Figure 2. Mast cell (MC) amplification of monosodium urate monohydrate (MSU) crystal–induced ankle swelling. **A, C, D,** and **F,** Changes in ankle thickness after intraarticular (IA) injection of 0.5 mg of MSU crystals or phosphate buffered saline (PBS) in the following groups: MC- and basophil-deficient Cpa3-Cre^{+};Mcl-1$^{fl/fl}$ mice (n = 17) and their Cpa3-Cre^{+};Mcl-1$^{+/+}$ littermates (n = 20) and Cpa3-Cre^{+};Mcl-1$^{fl/fl}$ mice engrafted IA (→) with C57BL/6J (wild-type [WT]) bone marrow–derived cultured MCs (BMCMCs) (n = 10) (**A**); diphtheria toxin–treated, basophil-deficient Mcpt8$^{DTR/+}$ mice (n = 9) and their Mcpt8$^{+/+}$ littermates (n = 9) (**C**); C57BL/6-Kit$^{+/+}$ mice (n = 13), MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice (n = 12), and Kit$^{W\text{-}sh/W\text{-}sh}$ mice engrafted IA with C57BL/6J (WT) BMCMCs (n = 12) (**D**); and WBB6F$_1$-Kit$^{+/+}$ (WT) mice (n = 10), MC-deficient WBB6F$_1$-Kit$^{W/W\text{-}v}$ mice (n = 10), and WBB6F$_1$-Kit$^{+/+}$ mice engrafted IA with WBB6F$_1$-Kit$^{W/W\text{-}v}$ (WT) BMCMCs (n = 10) (**F**). Values are the mean ± SEM of 3 (**C**, **D**, and **F**) or 3–5 (**A**) independent experiments. * = $P < 0.05$; *** = $P < 0.001$ by analysis of variance. NS = not significant. **B** and **E,** Photomicrographs of hematoxylin and eosin (H&E)–stained (for leukocytes) and toluidine blue–stained (for MCs) sections of ankle joints obtained at 24 hours from the mouse groups shown in the left panel of **A** (**B**) and **D** (**E**). **Arrows** indicate MCs. Bars = 100 μm.

demonstrating an important contribution of MCs (Figure 2A). IA engraftment with BMCMCs, which was performed 6 weeks before injection of MSU crystals, restored MC populations locally in the ankle synovium (to ~50% of the levels observed in WT mice), but no MCs were observed in the contralateral ankle joint or at other locations, such as the ear pinna or the spleen (data available online at http://med.stanford.edu/gallilab/Figures.html). Thus, our results show that local activation of synovial MCs contributes importantly to ankle swelling in this model of acute gout.

MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice also had significantly diminished ankle swelling compared to C57BL/6-Kit$^{+/+}$ (WT) mice at 24 hours following IA injection of MSU crystals, with the difference from the response in the corresponding WT mice being especially notable at

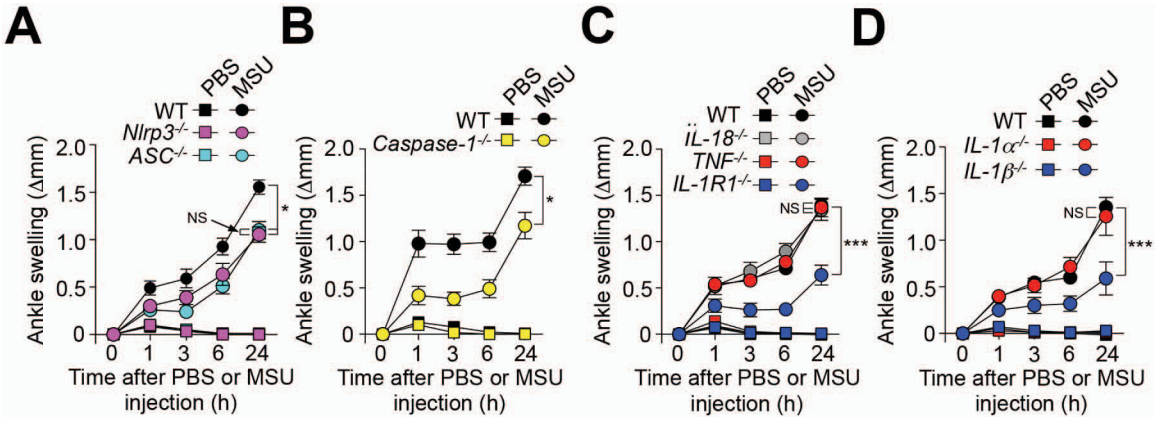

Figure 3. Contributions of the NLRP3 inflammasome, interleukin-1 receptor type I (IL-1RI), and IL-1β to MSU crystal–induced ankle swelling. Changes in ankle thickness after intraarticular injection of 0.5 mg of MSU crystals or PBS were determined in all mouse groups. **A,** C57BL/6J (WT) mice (n = 11), NLRP3$^{-/-}$ mice (n = 9), and ASC$^{-/-}$ mice (n = 10). **B,** C57BL/6J (WT) mice (n = 14) and caspase 1$^{-/-}$ mice (n = 11). **C,** C57BL/6J (WT) mice (n = 16), IL-18$^{-/-}$ mice (n = 10), TNF$^{-/-}$ mice (n = 12), and IL-1RI$^{-/-}$ mice (n = 13). **D,** C57BL/6J (WT) mice (n = 9), IL-1$\alpha^{-/-}$ mice (n = 7), and IL-1$\beta^{-/-}$ mice (n = 10). Values are the mean ± SEM. Differences in swelling between MSU crystal–injected ankle joints and the corresponding PBS-injected ankle joints were significant at each time point ($P < 0.05$ by Student's unpaired *t*-test) for all groups of mice. * = $P < 0.05$; *** = $P < 0.001$ by analysis of variance. See Figure 2 for other definitions.

early intervals after MSU crystal injection (Figure 2D). For example, at 1 or 3 hours after IA injection of MSU crystals, ankle swelling in WT mice was 4.9 times (at 1 hour) or 3.2 times (at 3 hours) the corresponding levels in MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice. In contrast, by 24 hours after MSU crystal injection, the corresponding reactions in the WT mice were ~1.8 times those in the MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice. MSU crystals induced statistically indistinguishable levels of ankle swelling in Kit$^{W\text{-}sh/W\text{-}sh}$ mice and WT mice engrafted IA with WT BMCMCs, further confirming that differences in responses between Kit$^{W\text{-}sh/W\text{-}sh}$ mice and WT mice were due to the lack of MCs in the Kit$^{W\text{-}sh/W\text{-}sh}$ mice, as opposed to other c-*kit*–related abnormalities (14,15) (Figures 2D and E).

Similar to the results we obtained with the Cpa3-Cre^{+};Mcl-1$^{fl/fl}$ mice, IA engraftment of Kit$^{W\text{-}sh/W\text{-}sh}$ mice with WT (C57BL/6J) BMCMCs restored the MC population locally in the ankle synovium (to ~60% of the levels observed in the corresponding C57BL/6-Kit$^{+/+}$ mice), but no MCs were observed in the contralateral ankle or in the ear pinna. However, we observed some MCs in the spleen in 3 of the 9 IA BMCMC–engrafted Kit$^{W\text{-}sh/W\text{-}sh}$ mice analyzed, albeit at much lower levels than those observed when such mice are engrafted intravenously with BMCMCs (16–18). Consistent with our findings in Cpa3-Cre^{+};Mcl-1$^{fl/fl}$ mice, MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice also developed substantial leukocyte infiltration at 24 hours after injection of MSU crystals (Figure 2E).

We obtained very similar results using c-*kit* mutant WBB6F$_1$-Kit$^{W/W\text{-}v}$ mice, the corresponding WBB6F$_1$-Kit$^{+/+}$ (WT) mice, and MC-deficient WBB6F$_1$-Kit$^{W/W\text{-}v}$ mice engrafted IA with WBB6F$_1$-Kit$^{+/+}$ BMCMCs (Figure 2F). Taken together, these results demonstrate that MCs can contribute significantly to the acute tissue swelling response to IA injection of MSU crystals in mice, especially at early intervals after challenge with MSU crystals.

Role of the NLRP3 inflammasome, IL-1 receptor type I (IL-1RI), and IL-1β in MSU crystal–induced ankle swelling in mice. We then analyzed in more detail the mechanism by which MSU crystals induce ankle swelling in mice. The NLRP3 inflammasome (composed of NLRP3, ASC, and caspase 1) can convert proIL-1β and proIL-18 into their active forms and is thought to play a central role in gout through the production of IL-1β (19,20). We found that NLRP3$^{-/-}$, ASC$^{-/-}$, and caspase 1$^{-/-}$ mice each had diminished ankle swelling in this model as compared to WT mice, especially at early intervals after injection of MSU crystals (Figures 3A and B), but they still developed both substantial ankle swelling (Figures 3A and B) and acute inflammatory infiltrates (data not shown) by 24 hours. Thus, our results show that both NLRP3 inflammasome–dependent and NLRP3 inflammasome–independent pathways likely mediate the acute arthritis in this mouse model.

Using mice deficient in IL-1RI or IL-18, we found that IL-1RI, but not IL-18, contributes to MSU

crystal–induced acute ankle swelling (Figure 3C). However, similar to mice deficient in components of the NLRP3 inflammasome, IL-1RI$^{-/-}$ mice developed substantial tissue swelling (Figure 3C) and acute inflammatory infiltrates (data not shown) by 24 hours after injection of MSU crystals. Although tumor necrosis factor (TNF) is not a product of the NLRP3 inflammasome, because of the importance of TNF in other models of MC-dependent inflammation (4), we also assessed the potential role of this cytokine in MSU crystal–induced inflammation. However, we observed similar MSU crystal–induced ankle swelling in WT mice and TNF$^{-/-}$ mice (Figure 3C).

IL-1RI is the receptor for both IL-1α and IL-1β. We did not detect any significant difference between WT and IL-1$\alpha^{-/-}$ mice in this model (Figure 3D). In contrast, we found a clear role of IL-1β in the acute response to IA injection of MSU crystals (Figure 3D).

MC-derived IL-1β contribution to MSU crystal–induced ankle swelling. Because IL-1β can be derived from many different cell types, we assessed the importance of MCs as a source of IL-1β in this model, using MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice engrafted IA with C57BL/6J (WT) BMCMCs in one ankle joint and C57BL/6 IL-1$\beta^{-/-}$ BMCMCs in the contralateral ankle. Six weeks after MC engraftment, we injected MSU crystals into both ankle joints. We found that MSU crystal–induced swelling in the ankle engrafted with WT BMCMCs was very similar to that observed in C57BL/6-Kit$^{+/+}$ (WT) mice, whereas swelling in the ankle engrafted with IL-1$\beta^{-/-}$ BMCMCs was significantly diminished and statistically indistinguishable from levels of swelling in MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice not engrafted with BMCMCs (Figure 4A). We observed very similar anatomic distributions and numbers of MCs in the ankles of Kit$^{W\text{-}sh/W\text{-}sh}$ mice engrafted with WT or IL-1$\beta^{-/-}$ BMCMCs (data available online at http://med.stanford.edu/gallilab/Figures.html), indicating that the observed differences in MSU crystal–induced ankle swelling did not simply reflect differences in MC numbers or distribution between such ankles.

We obtained very similar results when we tested MC-deficient WBB6F$_1$-Kit$^{W/W\text{-}v}$ mice, the corresponding WBB6F$_1$-Kit$^{+/+}$ WT mice, and MC-deficient WBB6F$_1$-Kit$^{W/W\text{-}v}$ mice engrafted with C57BL/6J WT BMCMCs or C57BL/6 IL-1$\beta^{-/-}$ BMCMCs (Figure 4B). Taken together, our results support an important role of MC-derived IL-1β in the early stages of the tissue swelling response to IA injection of MSU crystals.

Reduced MSU crystal–induced ankle swelling following local ablation of MCs. We next designed experiments to evaluate the potential therapeutic benefit of targeting MCs in gout. Because drugs that solely and specifically suppress MC activation have not yet been reported, we developed an alternative experimental strategy to selectively deplete MCs. We mated *Cpa3-Cre*–transgenic mice (which express Cre under the control of the MC-associated carboxypeptidase A3 [Cpa3] promoter) (11,14) to iDTR$^{fl/fl}$ mice, which bear a Cre-inducible DTR. We performed local (IA) injection of low doses of DT in an attempt to achieve selective ablation of synovial MCs. Such treatment resulted in a marked depletion of MCs in the ankle joint of Cre^{+} mice but not Cre^{-} mice (Figure 5A) (additional data available online at http://med.stanford.edu/gallilab/Figures.html). The MC depletion was local and appeared to be specific for MCs, since IA injection of DT did not affect the numbers of MCs in the contralateral PBS-treated ankle joint (Figure 5A) (additional data available online at http://med.stanford.edu/gallilab/Figures.html) or ear pinna (Figure 5B), nor were blood basophils, monocytes, neutrophils, or eosinophils affected (Figures 5C–F). Using this approach, we found that local ablation of MCs can significantly reduce ankle swelling in the gout model (Figures 5G and H).

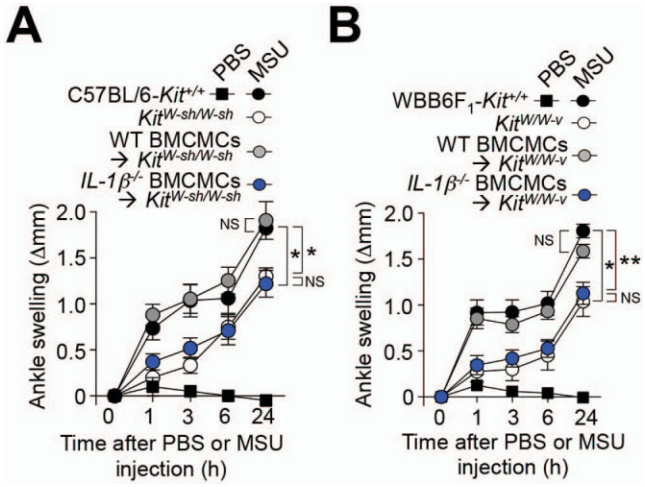

Figure 4. Contributions of MC-derived interleukin-1β (IL-1β) to MSU crystal–induced ankle swelling. Changes in ankle thickness following IA injection of 0.5 mg of MSU crystals or PBS were determined in all mouse groups. **A,** C57BL/6-Kit$^{+/+}$ mice (n = 8), MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice (n = 6), and Kit$^{W\text{-}sh/W\text{-}sh}$ mice engrafted IA (→) with either C57BL/6J (WT) (n = 11) or C57BL/6 IL-1$\beta^{-/-}$ (n = 10) BMCMCs. **B,** WBB6F$_1$-Kit$^{+/+}$ mice (n = 17), MC-deficient WBB6F$_1$-Kit$^{W/W\text{-}v}$ mice (n = 8), and WBB6F$_1$-Kit$^{W/W\text{-}v}$ mice engrafted IA with either C57BL/6J (WT) (n = 15) or C57BL/6 IL-1$\beta^{-/-}$ (n = 11) BMCMCs. Values are the mean ± SEM of 2 (for MC-deficient Kit$^{W\text{-}sh/W\text{-}sh}$ mice) or 3 (all other mice) independent experiments. * = $P < 0.05$; ** = $P < 0.01$ by analysis of variance. See Figure 2 for other definitions.

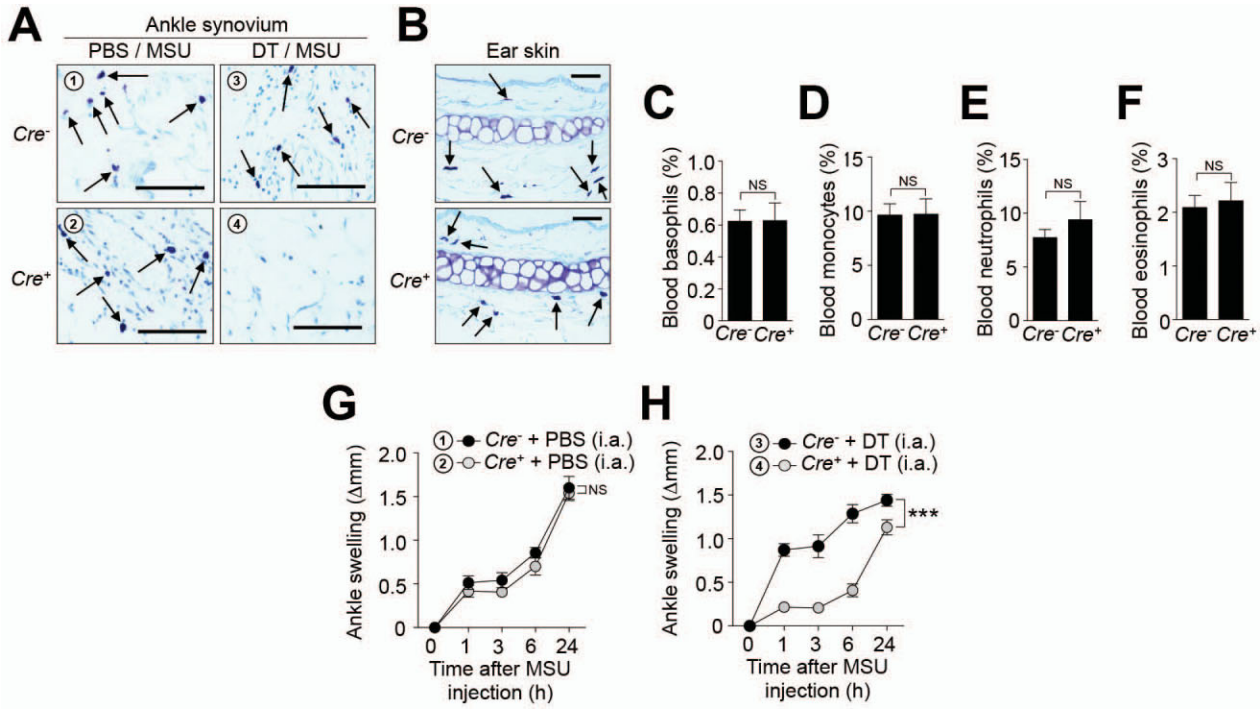

Figure 5. Reduced MSU crystal–induced ankle swelling following local and selective ablation of MCs. Cpa3-Cre$^+$;iDTR$^{fl/+}$ (Cre$^+$; n = 13) and Cpa3-Cre$^-$;iDTR$^{fl/+}$ (Cre$^-$; n = 7) mice were injected IA with DT (2 injections of 50 ng 1 week apart) in one ankle and vehicle (PBS) in the contralateral ankle. One week after the last DT injection, 0.5 mg of MSU crystals was injected into both ankles. **A** and **B,** Toluidine blue–stained tissue sections, showing ablation of synovial MCs **(arrows)** in the ankle joint after treatment with diphtheria toxin (DT) (but not PBS) in Cre$^+$ mice **(A)** and showing the presence of MCs **(arrows)** in the skin of the ear in Cre$^-$ and Cre$^+$ mice **(B)**. Bars = 50 μm. **C–F,** Percentage of basophils (CD49b+IgE+) **(C)**, monocytes (Gr-1^{low}CD11b+Siglec-F–) **(D)**, neutrophils (Gr-1^{high}CD11b+Siglec-F–) **(E)**, and eosinophils (SSC^{high}Siglec-F+) **(F)** in blood leukocytes isolated 1 hour before MSU crystal injection, analyzed by flow cytometry. Values are the mean ± SEM. None of the comparisons were statistically significant (NS) by Student's unpaired *t*-test. **G** and **H,** Changes in ankle thickness after IA injection of MSU crystals and either PBS or DT in the same mouse groups examined in **A**. Encircled numbers correspond to those shown in the upper left corner of the images shown in **A**. Values are the mean ± SEM of 2 (for Cre$^-$ mice) or 3 (for Cre$^+$ mice) independent experiments. *** = $P < 0.001$ by analysis of variance. See Figure 2 for other definitions.

Detection of tryptase, histamine, and IL-1β in synovial fluid samples from patients with gout. Finally, we searched for evidence of local activation of MCs during acute attacks of gout by measuring levels of tryptase and histamine (2 mediators stored in MC granules and released upon MC degranulation) in synovial fluid samples from patients who were undergoing joint aspiration for relief of a symptomatic flare of gout. Because obtaining biopsy specimens of synovial tissue in this setting is not clinically indicated, we were not able to directly analyze MCs in the joint synovium. We compared levels of tryptase, histamine, and IL-1β in synovial fluid samples from patients with acute gout to those in synovial fluid samples from patients with active RA, a disease known to be associated with MC activation (21).

Mature tryptase (retained by MCs until they are activated to degranulate) and total tryptase (comprised of mature tryptase and protryptase [spontaneously secreted by resting MCs]) (22), as well as histamine, were present in synovial fluid samples from patients with gout at levels similar to those in specimens from patients with RA (Figures 6A–C). These results support the conclusion that MCs are locally activated during acute attacks of gout in humans. In addition, synovial fluid samples from gout patients had significantly higher levels of IL-1β than did those from RA patients (Figure 6D), which is consistent with the known central role of this cytokine in gouty inflammation (6,23,24).

DISCUSSION

While it has been reported that MCs infiltrate gouty tophi (25), little is known about the actual roles of MCs either in that setting or during acute attacks of

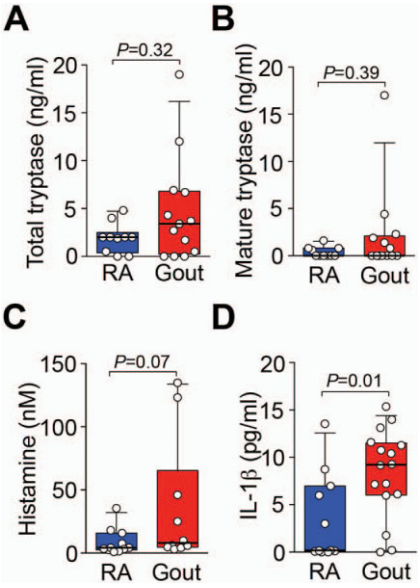

Figure 6. Tryptase, histamine, and interleukin-1β (IL-1β) levels in synovial fluid samples from patients with gout and patients with rheumatoid arthritis (RA). Levels of total (**A**) and mature (**B**) tryptase, histamine (**C**), and IL-1β (**D**) were measured by enzyme-linked immunosorbent assay in synovial fluid samples from patients with RA (n = 10–11) or gout (n = 10–16). Data are shown as box and whisker plots. Each box represents the 25th to 75th percentiles. Lines inside the boxes represent the median. Whiskers represent the 10th and 90th percentiles. Each circle represents an individual patient. *P* values were calculated by nonparametric Mann-Whitney test (2-tailed).

gout. Similarly, previous studies have linked MC activation and MSU crystal–induced acute inflammation in rat air pouches (26) or in the mouse peritoneal cavity (27), but there have been no previous studies analyzing the contributions of MCs to MSU crystal–induced acute arthritis. We therefore developed a mouse model of MSU crystal–induced acute arthritis and, with the use of that model, identified several lines of evidence supporting the conclusion that MC activation importantly contributes to the development of MSU crystal–induced acute arthritis.

Because studies performed using various models of antibody-dependent arthritis demonstrated conflicting results when tested in different strains of MC-deficient mice (15,28,29), we have suggested that, ideally, definitive investigation of the possible roles of MCs in mouse models of disease should be assessed using at least 2 different strains of MC-deficient mice, including one that lacks mutations affecting c-Kit structure or expression (14). Using this approach, we showed that MSU crystal–induced ankle swelling was significantly reduced in 2 types of c-*kit*–mutant MC-deficient mice ($Kit^{W/W\text{-}v}$ and $Kit^{W\text{-}sh/W\text{-}sh}$ mice), as well as in c-*kit*–independent MC- and basophil-deficient Cpa3-Cre;$Mcl\text{-}1^{fl/fl}$ mice (11,14), but not in basophil-deficient $Mcpt8^{DTR}$ mice (8). We also showed that engraftment of each of the 3 types of MC-deficient mice with wild-type MCs locally in the ankle joint was sufficient to restore WT levels of MSU crystal–induced acute ankle swelling.

It is now well established that MSU crystals activate the NLRP3 inflammasome in vitro, leading to the production of IL-1β and IL-18 (2), but results regarding the role of the NLRP3 inflammasome in inflammation induced by injections of MSU crystals in vivo have been the subject of controversy (2,30–33). While all reports are consistent concerning a significant role of ASC, caspase 1, and IL-1RI, some studies (30,33), but not others (31,32), support an important role of NLRP3. We found that, like MC-deficient mice, the $NLRP3^{-/-}$, $ASC^{-/-}$, caspase $1^{-/-}$, and $IL\text{-}1RI^{-/-}$ mice developed significantly lower levels of ankle swelling than those in WT mice at early intervals after IA injection of MSU crystals but still exhibited substantial tissue swelling and leukocyte infiltration by 24 hours after injection of the crystals. Thus, our results show that both inflammasome-dependent and inflammasome-independent pathways likely mediate tissue swelling in this model of MSU crystal–induced acute arthritis.

Previous studies have demonstrated roles of IL-1β in MSU crystal–induced inflammation in mice (30,31) and of IL-1α in mediating neutrophil recruitment after intraperitoneal (IP) injection of MSU crystals (32). We confirmed the latter finding using IP injection of MSU crystals in $IL\text{-}1\alpha^{-/-}$ mice (data not shown), but we did not detect any significant difference between WT and $IL\text{-}1\alpha^{-/-}$ mice in our model. In contrast, we found a clear role of IL-1β in the acute response to IA injection of MSU crystals.

Many cell types can produce IL-1β, including MCs (5), macrophages (34), dendritic cells (35), and neutrophils (36). MC-derived IL-1β was implicated in a model of antibody-dependent arthritis studied in $Kit^{W/W\text{-}v}$ mice that had been systemically engrafted with BMCMCs (5). In the present study, using local engraftment of the ankle with WT or $IL\text{-}1\beta^{-/-}$ BMCMCs in 2 types of c-*kit*–mutant MC-deficient mice ($Kit^{W/W\text{-}v}$ and $Kit^{W\text{-}sh/W\text{-}sh}$ mice), we show that MC-derived IL-1β can contribute importantly to MSU crystal–induced acute ankle swelling in this model.

Our results indicate that MCs contribute importantly to the early stages of inflammation in this model of acute gout but that other cell types also contribute to MSU crystal–induced tissue swelling and leukocyte in-

filtration, particularly at later intervals after MSU crystal injection. Among the potential resident inflammatory cells that could also mediate arthritis in this model, macrophages have been shown to produce IL-1β through activation of the NLRP3 inflammasome after stimulation with MSU crystals in vitro (2). Moreover, depletion of macrophages by pretreatment with clodronate liposomes reduces the inflammatory response induced by intraperitoneal injection of MSU crystals (37).

Previous studies have shown that human and mouse MCs also express components of the NLRP3 inflammasome and can produce IL-1β in response to costimulation with lipopolysaccharide (LPS) and ATP (38,39). However, we could not detect significant IL-1β release in either mouse BMCMCs or primary human peripheral blood–derived cultured MCs (40) when stimulated with MSU crystals, either alone or after overnight priming with LPS (data not shown). While important differences probably exist between such ex vivo–derived cultured MCs and the endogenous MCs present in synovial tissue, our results suggest that mouse synovial MCs in their natural microenvironment may be more responsive to MSU crystals than are ex vivo–derived mast cells, that synovial MCs are stimulated indirectly by another MSU crystal–sensitive cell, and/or that multiple stimuli are required to elicit MC activation and IL-1β secretion upon exposure to MSU crystals.

To assess the potential therapeutic benefit of targeting MCs in gout, we developed a new strain of mice, Cpa3-Cre;iDTR$^{fl/+}$ mice, in which local injection of DT results in selective ablation of MCs from the ankle joint. We showed that such local ablation of MCs significantly reduced ankle swelling in the model, validating the hypothesis that MCs represent an important therapeutic target in this model of MSU crystal–induced acute arthritis.

Finally, we searched for evidence of MC activation in humans with gout. MC-associated mediators, such as histamine and tryptase, have been detected in synovial fluid samples from RA patients, findings that have been interpreted as being consistent with MC activation in this setting (41,42). Both histamine and tryptase are stored in MC granules and can be released upon MC activation. MCs are the major source of histamine in tissue; however, several other cell types can also produce histamine, including basophils (43) and neutrophils (44). Tryptase is a more specific (and stable) marker of MC activation (45). We confirmed the presence of both histamine and tryptase in synovial fluid samples from RA patients and showed that similar levels of these MC-associated mediators are found in synovial fluid samples obtained during acute attacks of gout. These results suggest that local MC activation occurs during acute attacks of gout in humans. We also showed that synovial fluid samples from patients with acute gout contained significantly higher levels of IL-1β than did those from patients with RA, which is consistent with an important role of IL-1β in gout (46–48).

In summary, our findings indicate that MCs and MC-derived IL-1β contribute importantly to the tissue swelling observed at early intervals after intraarticular injection of MSU crystals. Although care should be taken in extrapolating to humans the results obtained in mice, our findings raise the possibility that even transient inhibition of MC activation may confer benefit in acute gout.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Galli had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Reber, Tsai, Galli.

Acquisition of data. Reber, Marichal, Sokolove, Starkl, Gaudenzio, Iwakura, Karasuyama, Schwartz.

Analysis and interpretation of data. Reber, Marichal, Sokolove, Starkl, Gaudenzio, Schwartz, Robinson, Tsai, Galli.

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Erratum

In the Reply letter by Golding et al published in the May 2014 issue of *Arthritis & Rheumatology* (pages 1403–1404), the institutional affiliation of the first author was listed incorrectly. The affiliation of Dr. Amit Golding should have read "Baltimore VA/VAMCHS, Baltimore, MD."

We regret the error.