Uric acid is a danger signal of increasing risk for osteoarthritis through inflammasome activation

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Uric acid (UA) is known to activate the NLRP3 (Nacht, leucine-rich repeat and pyrin domain containing protein 3) inflammasome. When activated, the NLRP3 (also known as NALP3) inflammasome leads to the production of IL-18 and IL-1β. In this cohort of subjects with knee osteoarthritis (OA), synovial fluid uric acid was strongly correlated with synovial fluid IL-18 and IL-1β. Synovial fluid uric acid and IL-18 were strongly and positively associated with OA severity as measured by both radiograph and bone scintigraphy, and synovial fluid IL-1β was associated with OA severity but only by radiograph. Furthermore, synovial fluid IL-18 was associated with a 3-y change in OA severity, on the basis of the radiograph. We conclude that synovial fluid uric acid is a marker of knee OA severity. The correlation of synovial fluid uric acid with the two cytokines (IL-18 and IL-1β) known to be produced by uric acid-activated inflammasomes and the association of synovial fluid IL-18 with OA progression, lend strong support to the potential involvement of the innate immune system in OA pathology and OA progression.

arthritis | inflammation | interleukin-18 | interleukin-1β | tumor necrosis factor alpha

Uric acid (UA) is constitutively present in normal cells, increased in concentration when cells are injured, and released from dying cells (1). On the basis of a theory proposed by Matzinger, the products of cell stress and tissue damage may represent "danger signals" that function as endogenous adjuvants recognized by the immune system (2). Matzinger proposed that immunity is controlled by an internal conversation between tissues and the cells of the immune system (3). This proposal introduced a new immunological model of an immune system capable of sensing cellular stress and tissue damage (4). Shi subsequently identified uric acid as one of these principal endogenous danger signals released from injured cells and mediating the immune response to antigens associated with injured cells (1). The molecular mechanism of this innate immune response to uric acid was further shown to be the result of the activation of the NALP3 inflammasome, a cytosolic, multiprotein complex that mediates caspase activation by uric acid crystals, leading to the production of the active forms of IL-1β and IL-18 (5). Recently, Kono et al. demonstrated in an in vivo hepatoxicity mouse model that uric acid is a physiological regulator of the inflammation induced by tissue injury (6). These data form the basis for our hypothesis that synovial fluid uric acid is a factor regulating tissue inflammation, disease severity, and progression in osteoarthritis (OA).

Uric acid is best known for its role in gout. When uric acid concentrations exceed the limit of solubility (~6.8 mg/dL or even lower under conditions of low pH or temperature), crystal formation can ensue, which is capable of activating the NALP3 inflammasome (5) and triggering the acute severe attacks of joint inflammation characteristic of gout (7). Several studies have previously posited an association of uric acid and OA. These include a study of hip replacement patients wherein elevated serum uric acid concentrations were associated with the presence of multijoint hand OA (8). A second study noted the apparent colocalization of gout attacks and radiographic OA at a multitude of joint sites (big toe, midfoot, knee, and distal finger joints) and suggested that OA may facilitate the localized deposition of gout (monosodium urate or MSU) crystals (9). To our knowledge, no previous study has assessed synovial fluid uric acid concentration with regard to radiographic and scintigraphic OA features and severity. In the Prediction of Osteoarthritis Progression (POP) study, we had a unique resource of paired serum and synovial fluid samples with which to evaluate the potential biological association and pathogenic role for uric acid in OA.

Results

Although this cohort was recruited on the basis of knee OA and absence of gout history, a surprising total of 39% of this cohort had serum uric acid concentrations above their respective reference ranges of 7.2 mg/dL for men and 6.0 mg/dL for women (10). The synovial fluid analyses were limited to 69 study participants (49 women and 20 men) with sufficient synovial fluid volumes for uric acid analyses. The mean ± SD age of this subset was 64.5 ± 10.1 y (68.9 ± 7.17 y for men, and 62.7 ± 10.6 y for women). The mean ± SD body mass index was 32.4 ± 7.1 kg/m^2 and was similar between the sexes. Knee OA ranged from 1 to 4 in severity (23.1, 14.6, 49.2, and 13.1% for each Kellgren-Lawrence grade).

Uric acid was quantified in both knees of 63 participants and single knees of 6 participants, and their paired sera. The mean serum uric acid concentrations were significantly higher than their paired synovial fluid uric acid concentrations ($P < 0.0001$) (Fig. 1). Of the 132 synovial fluid samples, matched serum exceeded synovial fluid uric acid concentrations for 113 (85.6%), whereas only 19 (14.4%) had greater synovial fluid than serum uric acid concentrations. The difference between the serum and synovial fluid ranged from +8.27 to −4.12 mg/dL (median difference +1.15 mg/dL). As serum uric acid levels increased, the difference between the serum and synovial fluid uric acid also increased significantly ($r^2 = 0.1978$, $P < 0.0001$) (Fig. 2*A*). Serum uric acid correlated strongly only with synovial fluid uric acid ($P < 0.0001$), whereas synovial fluid UA, IL-18, and IL-1β correlated strongly with each other ($P < 0.0001$) (Fig. 2 *B–D*). A statistically significant correlation was found between the serum-synovial fluid UA gradient and synovial fluid IL-18 ($r^2 = 0.176$, $P < 0.0001$) as well as synovial fluid IL-1β ($r^2 = 0.236$, $P = 0.0005$) (Fig. S1). Additionally, a statistically significant correlation was also found between synovial fluid TNFα and synovial fluid IL-18 ($r^2 = 0.241$, $P < 0.0001$) as well as synovial fluid IL-1β ($r^2 = 0.061$, $P = 0.002$) (Fig. S2). No correlation was found for synovial fluid chondroitin sulfate (CS), allantoin, or pyrophosphate (PPi) with each other or with any of the other synovial

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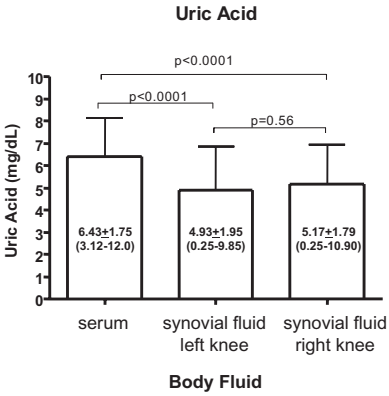

Fig. 1. Blood and synovial fluid uric acid concentrations for paired samples. Mean serum (n = 69) uric acid concentrations were significantly higher than their paired synovial fluid uric acid concentrations for left (n = 67) and right (n = 65) knees ($P < 0.0001$). Mean synovial fluid uric acid concentrations from left and right knees were not significantly different (P = 0.22) (paired t test). Error bars represent the SD. The numeric values depicted on the bars are mean ± SD (concentration range).

fluid measures (UA, IL-18, IL-1β, TNFα) or with the serum-synovial fluid UA gradient (Figs. S3–S5).

The mean ± SD (range) synovial fluid concentrations of the cytokines were as follows: IL-18 127.1 ± 66.5 pg/mL (8.8–299.5), n = 130; IL-1β 0.375 ± 0.446 pg/mL (0–1.589), n = 48; and TNFα 3.79 ± 2.21 pg/mL (0.76–16.33), n = 123. The mean ± SD (range) synovial fluid concentration of CS was 60 ± 24 μg/mL (7.4–141), n = 118. The mean ± SD (range) synovial fluid concentration of allantoin was 0.37 ± 0.31 μg/mL (0.03–1.52), n = 111. The mean ± SD (range) synovial fluid concentration of PPi was 2.78 ± 0.63 μmol/L (0.91–6.76), n = 123. Knee dominance had no effect on synovial fluid analyte concentration and all our analyses were knee, not individual based, controlling for within-subject correlation.

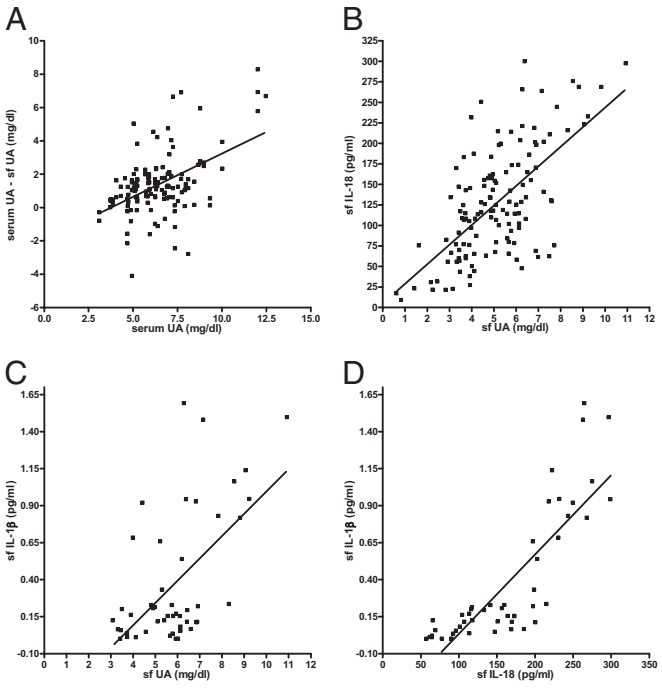

Fig. 2. Synovial fluid uric acid and cytokine correlations. (*A*) Bland-Altman analysis showed a significant association of increased uric acid gradient between the blood and synovial fluid with increasing serum uric acid concentration (R^2 = 0.20, $P < 0.0001$). (*B–D*) Strong correlations were found between synovial fluid uric acid, IL-18, and IL1β. Linear regression was used to provide unadjusted r^2 and general estimating equations (GEEs), with adjustments for age, sex, BMI, and chondrocalcinosis, were used for P values with the following results: (*B*) IL-18 and UA (r^2 = 0.41, $P < 0.0001$); (*C*) IL-1β and UA (r^2 = 0.34, $P < 0.0001$); and (*D*) IL-18 and IL-1β (r^2 = 0.69, $P < 0.0001$).

Three-year follow-up bone scans, radiographs, and pain scores were available for 37, 31, and 38 knees, respectively. Models were run using this 3-y follow-up subset to determine whether the baseline concentrations of synovial fluid measures were associated with changes in knee OA severity and pain over time (Table 1). Adjusting for age, sex, body mass index, and baseline OA severity, baseline IL-18 and TNFα were associated with a 3-y change in osteophyte scores.

Discussion

Although still of uncertain etiology, there are many clinical factors that contribute to the risk and progression of OA, including obesity, joint malalignment, trauma, age, and female sex (13). The strong association demonstrated here, between synovial fluid uric acid and OA severity, quantified radiographically and scintigraphically, demonstrates that uric acid is a marker of disease severity and raises the strong possibility that uric acid may be a factor promoting the pathological process of OA through activation of the inflammasome. This was supported further by a strong association of synovial fluid IL-18 and IL-1β with OA severity and longitudinal data demonstrating an association of baseline synovial fluid IL-18 with radiographic OA progression over 3 y.

Chondrocytes appear normally to be in a state of autophagy thought necessary for maintaining cellular integrity, function, and survival (14). The majority of chondrocytes in OA cartilage, however, are severely degenerated (15) and many are undergoing cell death or "chondroptosis" (16). It was recently demonstrated that OA cartilage is associated with a reduction and loss of regulators of the autophagy pathway (ULK, Beclin-1, and LC3 expression) and an increase in cell death (characterized by increased expression of PARP p85) (14). The extent to which uric acid is released during autophagy is currently unknown, but cell death could result in local supersaturation of uric acid as cytolysis generates large quantities of purines from RNA and DNA (17). As suggested by Matzinger's theories, the release of uric acid could provide an inflammatory danger signal responsible for activating a chronic immune inflammatory response. This response to uric acid has recently been further elucidated as being the result of activation of the NALP3 inflammasome with subsequent production of the active forms of IL-18 and IL-1β (5). The mean concentration of IL-18 we measured in synovial fluid was 1.4 times higher than previously reported for OA synovial fluid, 3.5 times higher than previously reported for normal synovial fluid, but still 2 to 3 times lower than typically seen in rheumatoid arthritis (RA) (18, 19). IL-1β is not typically detected in normal synovial fluid (20, 21) and is very low (3.91 ± 4.66 pg/mL) or undetectable (22) in OA synovial fluid. Our measured concentrations of synovial

Table 1. Associations of analytes with knee OA severity and progression of knee OA using individual models (adjusted for age, sex, BMI, and chondrocalcinosis)

	Parameter estimate (95% CIs) *P* value*							
	Baseline knee OA severity status				Change (Δ) in knee OA severity status†			
	Bone scan	JSN	OST	Pain	ΔBone scan	ΔJSN	ΔOST	ΔPain
SF Uric Acid	0.12 (0.05/0.19)	0.06 (−0.003/0.12)	0.14 (0.07/0.21)	0.02 (−0.03/0.07)	0.04 (−0.10/0.18)	0.001 (−0.03/0.03)	−0.02 (−0.18/0.13)	−0.07 (−0.15/0.0001)
	0.001	0.06	<0.0001	0.41	0.58	0.97	0.77	0.05
SF ln IL-18	0.57 (0.32/0.82)	0.21 (0.01/0.40)	0.45 (0.18/0.73)	0.14 (0.02/0.25)	0.23 (−0.14/0.59)	0.04 (−0.05 /0.13)	0.46 (0.06/0.86)	−0.02 (−0.21/0.17)
	<0.0001	0.04	0.001	0.02	0.22	0.40	0.02	0.84
SF ln IL-1β	0.09 (−0.002/0.19)	0.16 (0.004/0.31)	0.25 (0.11/0.40)	0.05 (−0.04/0.13)	0.10 (−0.24/0.45)	0.04 (−0.02/0.09)	−0.37 (−0.89/0.15)	−0.02 (−0.18/0.13)
	0.06	0.045	0.001	0.28	0.55	0.18	0.16	0.78
SF ln TNFα	0.44 (0.31/0.78)	0.16 (0.04/0.45)	0.29 (−0.04/0.62)	0.12 (−0.01/0.26)	0.04 (−0.57/0.65)	0.06 (−0.05/0.16)	0.70 (0.11/1.29)	−0.02 (−0.28/0.24)
	<0.0001	0.02	0.08	0.07	0.90	0.30	0.02	0.87

JSN, joint space narrowing; OST, osteophyte; BMI, body mass index; Δ, change over 3 y in knee OA status; SF, synovial fluid; ln, natural log transformed.
**P* values adjusted by GLM (generalized linear modeling to control for within-subject correlation of knee data).
†Additional adjustment for baseline OA status.

fluid IL-1β were similarly low or undetectable despite the use of a high sensitivity assay. Because the production of mature active IL-18 and IL-1β requires inflammasome activation (23), these results (high concentrations of synovial fluid IL-18, with correlation between synovial fluid UA and synovial fluid IL-18 and IL-1β) provide strong evidence of a potential role for uric acid in stimulating the production of these cytokines in OA, most likely from resident macrophages via inflammasome activation.

It is already believed that macrophages play a role in osteoarthritis. It was recently demonstrated that both inflammatory and destructive responses generated by osteoarthritis synovium are dependent largely on macrophages. These effects are cytokine driven through a combination of IL-1 and TNFα (24). In gout, monocytes and immature macrophages act to stimulate an acute gout attack triggered as TLR2 and TLR4, which in conjunction with the cytosolic TLR adapter protein myeloid differentiation factor 88 (MyD88), induce the ingestion of MSU crystals by phagocytes and activate the production of proinflammatory molecules, TNFα, IL-1β, IL-6, IL-18, and IL-8, and endothelial activation (5, 25–29). We hypothesize a similar but subacute process in the OA joint.

IL-18 is a proinflammatory cytokine that plays a unique and influential role in both the innate and adaptive immune responses (30, 31). IL-18 has been shown to increase production of the potent inflammatory effector TNFα in a dose-dependent manner, with even small amounts of IL-18 (1 pg/mL) inducing TNFα production (31–33). Indeed, the principal effect of IL-18 in the RA joint seems to be the direct promotion of TNFα production via binding with macrophage IL-18R (33). That synovial fluid TNFα was expressed in response to IL-18 stimulation in this study is further supported by the study of Martinon who observed a delayed release of TNFα in response to uric acid and speculated that it was in response to inflammasome activation (5). The strong correlation we found between synovial fluid IL-18 and TNFα is consistent with these reports. The concentration of synovial fluid TNFα we found in our subjects was similar to previous reports for OA synovial fluid and approximately sixfold lower than has been reported for RA (34, 35). Although we found a strong correlation of TNFα with uric acid concentration and OA severity, the lower levels of TNFα when compared with RA, along with the correspondingly lower IL-18, suggest a less acute inflammatory process driven by IL-18 as part of an innate immune response.

Although the concentration of IL-1β we found was not particularly high, its correlation with OA severity is not surprising. IL-1β action is under tight regulation with the most important control being the balance between the expression of the IL-1RI active receptor and the IL-1RII inactive or decoy receptor (36, 37). It is well established that in the OA joint there is imbalance between the expression of these two receptors with an up-regulation of the active receptor (IL-1RI) and little or no expression of the decoy receptor (IL-1RII), thereby significantly increasing the potency of even small amounts of IL-1β (37–39).

To our knowledge, no previous study reported an association of IL-18 with pain in OA. Recent animal studies have revealed a role for IL-18 in pain. In a study of mechanisms of antigen-induced inflammation in mice, IL-18 was shown to play a significant role in hypernociception via the production of IFN-γ, ET-1, and PGE_2 (40). In a recent tibial fracture model in rats, IL-18 up-regulation via inflammasome activation was shown to play a contributory role in complex regional pain syndrome (41). Our data would suggest a similar role for IL-18 in humans.

One recent study of cartilage degeneration in the ankle revealed that MSU surface crystal deposits correlated strongly with cartilage lesions in the talus (42). This study suggested that MSU crystals may contribute both to the initiation and propagation of cartilage degradation. Muehleman proposed that metabolic changes and cartilage fibrillation or fissures enhance the nucleation of MSU crystals, which in turn contribute to mechanically induced damage (42). A process similar to the one that occurs in the ankle likely occurs in the knee, characterized by loss of pro-

teoglycan from the cartilage into the synovial fluid, facilitating the nucleation of uric acid MSU crystals that could precipitate onto the surface of cartilage and act as a nidus for inflammation and mechanical degradation (43). In this scenario, the amount of uric acid in the synovial fluid sufficient for contributing to the cartilage degradation process is less than the concentration typically associated with clinical gout and gout flares (>6.8 mg/dL). Accordingly, the amount of uric acid in the synovial fluid and not in the serum would correlate with radiographic and scintigraphic measures of OA severity, as demonstrated in this study. Unlike previous reports that found no increase in serum uric acid for those with knee OA, serum uric acid concentrations for many of our subjects were higher than established reference ranges (8, 10, 44). Synovial fluid uric acid was also higher in our study compared with a previous report (45).

One limitation of this study is that we were unable to assess the presence or absence of MSU crystals in the joint because all of the synovial fluid samples had been centrifuged and stored as supernatants. Crystalline uric acid, as little as 1.0 mg/dL, and not soluble uric acid, is thought capable of activating dendritic cells and macrophages and priming the T cell response (1, 5, 46). Interestingly, the median drop we found in soluble uric acid from serum to synovial fluid was 1.15 mg/dL. We wonder whether the serum/synovial fluid gradient represents precipitated uric acid. Shi proposed that a chemical phase transition could be the key event that transformed the normal autologous uric acid component into a danger signal. However, as suggested in Muehlemann's study of MSU crystals and cartilage degradation in the ankle, uric acid in the synovial fluid likely adopts both forms—crystalline and soluble—depending on the chemical environment (42). Laurent and Burt et al. both reported, and we confirmed (summarized in *Materials and Methods*), that uric acid in vitro was less soluble with increasing amounts of chondroitin sulfate (47, 48), a common constituent of OA synovial fluid. We found no correlation between synovial fluid CS and uric acid, or CS and the serum-synovial fluid UA gradient. However, other components of OA synovial fluid have been reported to facilitate uric acid crystal formation (43, 48, 49), so CS may not act alone and therefore would not alone correlate with inflammasome-activated cytokines. For several decades the variation in the protein coat of MSU crystals has been suggested to regulate immune responses to them. Terkeltaub showed that the inflammatory response to MSU crystals could be inhibited by apo B lipoprotein (50). Kanevets et al. have recently demonstrated substantial amounts of uric acid-binding antibodies in unimmunized mice (51), and Ig-coated MSU crystals have been observed in gout specimens (summarized in Kanevets, ref. 51). Uric acid-binding antibodies facilitated urate crystal formation but did not block uric acid-mediated activation of dendritic cells (51). Further work is indicated to evaluate for the presence of uric acid-binding antibodies in OA synovial fluids as another possible potentiator of crystal formation.

Soluble uric acid is also suggested to exert an antiinflammatory effect, possibly due to its role as an antioxidant, on distant sites of inflammation (46, 52). The action of various reactive oxygen species upon uric acid leads to multiple oxidation products with the most abundant being allantoin (53). The synovial fluid allantoin concentrations we observed were similar to those previously reported in RA synovial fluid (54), and just as in that study, as well as other studies of plasma in healthy adults (55), we found no correlation between allantoin and uric acid concentrations. More importantly, we found no correlation between allantoin and the difference in uric acid concentrations from serum to synovial fluid. Thus, oxidative consumption of uric acid within the joint would likely not account for the lower concentrations of uric acid in the joint fluid relative to the serum.

Calcium pyrophosphate dihydrate (CPPD) crystals have also been shown to activate the NALP3 inflammasome although the amount of CPPD required for activation is considerably higher (10 times) than for MSU (5). CPPD is considered the etiologic agent in pseudogout as well as chondrocalcinosis (56). When CPPD is found in OA synovial fluid in conjunction with MSU, the concentration of CPPD is always very low (57). In our study we would have excluded subjects with pseudogout on the basis of exclusion of individuals with gout-like attacks, and controlled for the presence of radiographic chondrocalcinosis (CC) during the analysis. CPPD crystals are thought to form when excess inorganic pyrophosphate (PPi) complexes with calcium to form crystals (58). The concentration of synovial fluid PPi we measured was similar to what has been previously reported for OA synovial fluid (59) and was not associated with any of the synovial fluid cytokines or OA severity. These data decrease the likelihood that CPPD is the source of inflammasome activation in our subjects.

In summary, we compared serum and synovial fluid uric acid levels in patients with knee OA but with no clinical evidence or self-report of gout. We found a significant difference in uric acid levels between matched sera and synovial fluid despite the fact that synovial fluid is a dialysate of blood. Synovial fluid uric acid correlated with radiographic and scintigraphic measure of OA severity and synovial fluid IL-18 and IL-1β, two cytokines known to be produced by uric acid-activated inflammasomes. The precarious balance between soluble uric acid and MSU crystals within

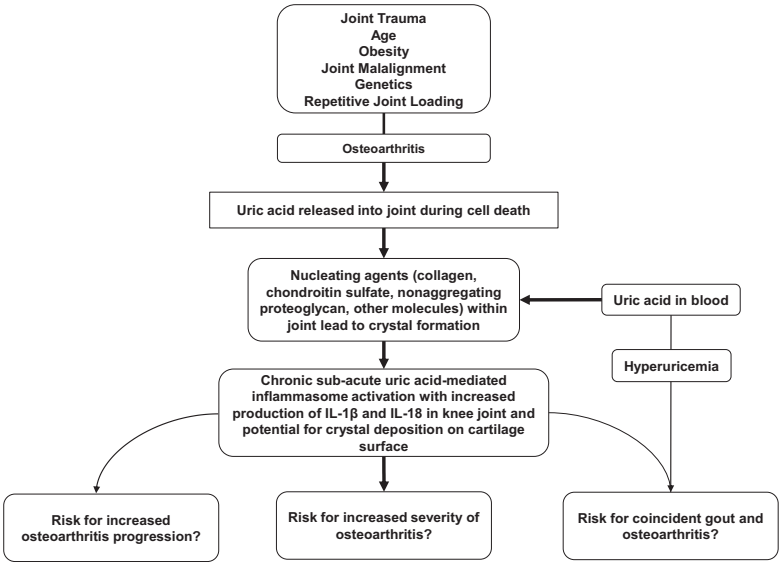

Fig. 3. Schematic depicting the interaction of OA and uric acid. According to this model, osteoarthritis leads to the release of nucleating agents for urate crystallization. The development of osteoarthritis also leads to increased cell death and the release of uric acid. Taken together with uric acid diffused into the joint fluid from the blood, these factors could contribute to constitutive subacute inflammation and progression of osteoarthritic joints via inflammasome activation.

the joint, the prevalence of serum uric acid concentrations in the crystal-forming range in this knee cohort, and possibly uric acid released from injured or dying chondrocytes, may, in the presence of nucleating agents such as nonaggregated proteoglycans, collagen, and chondroitin sulfate (43, 47–49), tip the balance toward MSU crystallization and the proinflammatory state. A model integrating these concepts is proposed in Fig. 3. These results lend strong support to the potential involvement of the innate immune system in OA pathology. It will be important to better understand the physical chemical properties and forms of uric acid in the OA joint and to further validate synovial fluid uric acid as a risk factor for OA progression as this would have important clinical implications given the availability of well-established pharmacological means of systemically lowering uric acid.

Materials and Methods

Study Population. A total of 159 participants (118 female, 41 male) were enrolled in the National Institutes of Health sponsored POP study. A total of 138 participants returned for follow-up 3 y later to assess progression of knee OA. The study was conducted from January 2003 until March 2008 and was approved by the Duke University Institutional Review Board with informed consent being obtained from all subjects. Participants met radiographic criteria for knee OA on the basis of a Kellgren-Lawrence grade of 1–4 (60) and American College of Rheumatology criteria (61) for symptomatic OA of at least one knee. OA exclusion criteria included: exposure to a corticosteroid (either parenteral or oral) within 3 mo before the study evaluation; knee arthroscopic surgery within 6 mo before the study evaluation; known history of avascular necrosis, inflammatory arthritis, Paget's disease, joint infection, periarticular fracture, neuropathic arthropathy, pseudogout, or reactive arthritis. None of the participants had a history of gout in the knee, and none were taking traditional gout medications (allopurinol or colchicine) or anticoagulants. One patient had a remote history of symptoms suggestive of podagra.

Radiographic Imaging. Posteroanterior fixed-flexion knee radiographs were obtained with the SynaFlexer lower limb positioning frame (Synarc) and were read by the consensus of two graders, blinded to the results of any of the other measures. In addition to Kellgren-Lawrence grade (60), knee radiographs were scored for individual radiographic features of OA of the weight-bearing tibiofemoral joint for joint space narrowing (JSN) and osteophyte (OST) scored semiquantitatively (0–3 scale) using the standardized OARSI atlas (62). Maximum possible scores were 6 for JSN and 12 for OST. Intrarater reliability of the X-ray readings by intraclass correlation coefficients were 0.69 for KL grade, 0.84 for JSN, and 0.81 for OST as described previously (11).

Scintigraphic Imaging. Scintigraphic images of the knee were obtained at 2.5 h (late phase) after injection of ^{99m}Tc methylene diphosphonate. Four views of the knee (anteroposterior, mediolateral, lateromedial, and posteroanterior) were obtained to precisely localize the site of uptake within each knee. The intensity of bone scintigraphic radiotracer uptake was scored semiquantitatively (0–3: 0 = normal, 1 = mild, 2 = moderate, and 3 = intense) by consensus of an experienced nuclear medicine physician (REC) and a senior nuclear medicine resident blinded to the other data. Each compartment of the tibiofemoral knee joint was scored (medial and lateral) for a maximum possible score of 6. A random 20% of the knee bone scans were rescored, blinded to the original scores; intraclass correlation coefficients ranged from 0.86 to 0.89 for the lateral and medial knee compartments as previously described (11).

Serum and Synovial Fluid Samples. Serum and knee synovial fluid samples were obtained within 10 min of each other after a 2-h fast in the mid-afternoon. All synovial fluid samples were centrifuged and cell free supernatants, along with sera, were frozen at −80 °C. Uric acid concentrations were measured on baseline sera of all 159 participants. Sufficient volumes of synovial fluid were available for quantification of uric acid from 132 knees representing 69 individuals. A total of 97 of the synovial fluid samples were obtained directly (neat, no dilution); 35 synovial fluid samples were collected by small volume (10 mL) lavage with the precise correction factor to account for the dilutional effects of lavage determined by the urea correction method as previously described (63). Of these 132 synovial fluid samples, remaining sample volumes were adequate for measurement of IL-18 from 130 knees, IL-1β from 48 knees, chondroitin sulfate (CS) from 118 knees, TNFα and PPi from 123 knees, and allantoin from 111 knees. See *SI Materials and Methods* for all biochemical analyses of these samples.

Pain Scores. Knee symptoms were ascertained by the NHANES I criterion (64) of pain, aching, or stiffness on most days of any 1 month in the last year; for subjects answering yes, symptoms were quantified as mild, moderate, or severe, yielding a total score of 0–3 for each knee.

Statistical Analysis. To meet assumptions of normality by the D'Agostino and Pearson omnibus test (GraphPad Software), all synovial fluid analytes, other than uric acid, were natural log transformed. The proportion of individuals with serum uric acid concentrations greater than a well-established reference range (>7.2 mg/dL for males and >6 mg/dL for females) (10) was calculated for the total sample (n = 159) and the subsample (n = 69) of individuals with paired sera and synovial fluid. The values were compared by paired t test. When lavaged synovial fluid samples that had been corrected for dilution were analyzed separately from neat samples, no effect of dilution was seen, so those results were pooled together. Descriptive statistics and univariate analyses were performed using Graphpad Prism software. Relationships between synovial fluid analytes and OA were analyzed using the GenMOD procedure, to control for within-subject correlation of knee data, with the addition of a repeated statement to control for age, sex, BMI, and chondrocalcinosis (GLM, SAS Enterprise Guide). This procedure is equivalent to using general estimating equations (GEE). Because r^2 values are not provided with GLM/GEE, linear regressions were also performed to provide unadjusted r^2 values (Graphpad Prism).

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