INFLAMMATORY MICROCRYSTALS STIMULATE INTERLEUKIN-6 PRODUCTION AND SECRETION BY HUMAN MONOCYTES AND SYNOVIOCYTES

PIERRE-ANDRÉ GUERNE, ROBERT TERKELTAUB, BRUCE ZURAW, and MARTIN LOTZ

Crystal-related joint diseases are often associated with systemic inflammatory manifestations, including increased levels of acute-phase proteins, leukocytosis, and fever. Recently, interleukin-6 (IL-6) has been identified as a pluripotent mediator of inflammatory and immunologic responses and the major hepatocyte-stimulating factor. In this study, we demonstrated that monosodium urate (MSU) and calcium pyrophosphate dihydrate (CPPD) crystals, and to a lesser extent, hydroxyapatite crystals, increased IL-6 production by synoviocytes and monocytes in vitro. Immunoprecipitation experiments showed that MSU and CPPD crystals, but not hydroxyapatite crystals, were able to increase the release of newly synthesized IL-6. Crystal-induced IL-6 stimulated acute-phase protein synthesis, immunoglobulin production, and hybridoma cell proliferation, which was neutralized by a specific antibody to IL-6. High levels of IL-6 were found in synovial fluid from patients with gout and pseudogout. These results demonstrate that MSU and CPPD crystals can induce IL-6 production in synoviocytes and monocytes, and that synovial fluid from patients with gout and pseudogout contains high levels of IL-6. Crystal-induced IL-6 is likely to be an important mediator of inflammatory responses in acute gout and pseudogout.

Acute gout and pseudogout are often associated with systemic inflammatory manifestations, including fever, leukocytosis, and elevated erythrocyte sedimentation rate with increased levels of acute-phase proteins (1,2). Interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) are secreted by monocytes exposed to monosodium urate (MSU) crystals and may cause some of these systemic manifestations. The secretion of IL-1 and TNFα by monocytes and synoviocytes that are exposed to calcium pyrophosphate dihydrate (CPPD) and hydroxyapatite (HA) crystals is relatively low compared with that induced by MSU crystals (3–7).

IL-1 and TNFα play only a limited role in the induction of the acute-phase response (8–10). In contrast, IL-6 is the major hepatocyte-stimulating factor (HSF), and it also displays many other functions, including pyrogenicity and the stimulation of hematopoietic colony formation, in which it synergizes with IL-3 (11). In addition, it modulates the growth and differentiation of B lymphocytes (12,13), T lymphocytes (14–16), and chondrocytes (Guerne P-A, Lotz M: unpublished observations). We have demonstrated

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previously that this cytokine is present in the synovial fluid (SF) of patients with diverse arthropathies (17). It is constitutively secreted by synoviocytes and monocytes in the presence of fetal calf serum (FCS), and its synthesis is up-regulated by IL-1, TNFα, and lymphotoxin (17). Because IL-6 has been detected in joint fluids from patients with acute pseudogout (17), we extended our analyses to include SF from patients with other forms of crystal-associated synovitis, and we determined the effects of MSU, CPPD, and HA crystals on the synthesis and release of IL-6 by human synoviocytes and peripheral blood monocytes.

MATERIALS AND METHODS

Monocyte isolation and culture. Peripheral blood mononuclear cells were obtained by Histopaque gradient sedimentation, and monocytes were isolated by adherence as described (18).

Synoviocyte isolation and culture. Synoviocytes were isolated from surgical specimens freshly removed from patients undergoing knee or hip joint replacement for avascular necrosis or osteoarthritis. Collagenase digestion and culture were performed as described previously (19). Synoviocytes were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with L-glutamine, penicillin, streptomycin, and 10% FCS. At confluence, the cells were trypsinized and recultured. The cell lines were used for the experiments between passages 1 and 7. Mycoplasma contamination was inhibited by negative hybridization with a specific probe (Genprobe, San Diego, CA).

Cell stimulation. Freshly isolated blood monocytes were cultured with the various stimuli in 96- or 48-well plates containing DMEM supplemented with 1% FCS (if not otherwise indicated) at a concentration of 4×10^5/ml. The supernatants were collected at the time points indicated, centrifuged to remove crystals and cell debris, and stored at −20°C until they were tested for the presence of IL-6. Synoviocytes were transferred to 24-well plates (10^5/well) containing DMEM supplemented with 10% FCS. At confluence, usually after 1 day, the cells were washed and stimulated in the same manner as the monocytes. Cultures were performed in the presence of polymyxin B (12.5 μg/ml) to remove endotoxins.

IL-6 bioassays. *Hybridoma growth factor (HGF) assay.* The B9.9 murine hybridoma (20) (kindly provided by Dr. R. Nordan, NIH) was maintained in RPMI 1640 supplemented with penicillin, streptomycin, L-glutamine, 10% FCS, and 0.2% (volume/volume) supernatant from poly(rI): poly(rC)-stimulated human skin fibroblasts, as a source of IL-6. For the Il-6 assays, cells were washed 5 times, and 10,000 cells per well were placed in 96-well, flat-bottom plates and were supplemented with serial dilutions of the samples to be tested in a final volume of 150 μl RPMI 1640 containing L-glutamine, penicillin, streptomycin, and 5% FCS. Assays were performed in triplicate. After 48 hours, 1 μCi of tritiated thymidine (^{3}H-TdR) was added to each well, and the cells were incubated for 4 more hours before harvesting onto glass fiber filters. Radioactivity was quantified by liquid scintillation counting.

Alternatively, proliferation was assessed according to the colorimetric method described by Mossmann (21). In each assay, a standard curve using recombinant B cell stimulatory factor 2 (rBSF-2) (13,22) was included. One unit of IL-6 in culture supernatants was defined as the reciprocal of the dilution giving half-maximal stimulation. In this method, rBSF-2 is typically detected at 0.08 pg/ml, and the specific activity of the preparation used (13) is 125 units/ng.

BSF-2 assay. BSF-2 activity was quantified as the induction of IgG secretion by the human B lymphoblastoid cell line CESS (23). The CESS cells were maintained in RPMI 1640 supplemented with penicillin, streptomycin, L-glutamine, and 10% FCS. For the assay, cells were washed and cultured (5,000/well in 96-well, flat-bottom plates) in the presence of various concentrations of test samples at a total volume of 150 μl/well, in RPMI 1640 containing 5% FCS, in triplicate experiments. After 5 days, the supernatants were collected. The concentration of IgG was then determined by enzyme-linked immunosorbent assay (ELISA) (24).

Assay for HSF activity. We have demonstrated that IL-6 is a potent inducer of C1 esterase inhibitor (C1INH) in the human hepatoma cell line Hep-G2 (Lotz M, Zuraw B: unpublished observations). These cells were maintained in RPMI 1640 supplemented with penicillin, streptomycin, L-glutamine, and 10% FCS. For the assays, Hep-G2 cells were trypsinized and plated in microtiter wells (96-well, flat-bottom plates) at 10,000 cells/well. At confluence, the medium was removed and replaced with 150-μl aliquots of fresh RPMI 1640 supplemented with 5% FCS and aliquots of the supernatants to be tested, at a final concentration of 1:25. After 4 days, the plates were centrifuged and the Hep-G2 supernatants were removed and stored at −20°C until their content of C1INH was assayed by ELISA (18).

Antibody neutralization studies. Aliquots of culture supernatant were preincubated for 2 hours at 37°C with rabbit antiserum to BSF-2 (25) or with preimmune rabbit IgG (1–10 μg/ml) before being added to the cells used in the IL-6 assays.

Metabolic labeling and immunoprecipitation. Confluent synoviocyte cultures in 24-well plates were washed 3 times with Hanks' balanced salt solution, and were cultured for 24 hours in 500 μl methionine-free RPMI 1640 medium containing 1% FCS, 20 μCi/ml ^{35}S-methionine (ICN Biochemicals, Irvine, CA), and the various stimuli to be tested. After 24 hours, the supernatants were collected, centrifuged, and stored at −20°C until analyzed. The supernatants were either concentrated to 50 μl with ultraspin concentrators (Ultracent; Bio-Rad, Richmond, CA) or used unconcentrated. Concentrated supernatants (50 μl) were then precleared by a 1-hour incubation at 4°C with 50 μl of 10% protein A–agarose (Zymed, San Francisco, CA) in Net2 buffer (50 m*M* Tris [pH 7.4], 0.5% N-P40, 150 m*M* NaCl, 5 m*M* EDTA, 0.02% sodium azide, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS]) and protease inhibitors (2 mg/ml soybean trypsin inhibitor, 5 m*M* leupeptin, 5 m*M* antipain, 0.1 m*M* phenylmethylsulfonyl fluoride). The incubation was done with gentle shaking.

Unconcentrated samples in 200-μl aliquots were pre-

cleared with 50 μl of 20% protein A–agarose. The supernatants were recovered after centrifugation and were incubated overnight at 4°C with 3 μl of anti–IL-6 (1 mg/ml) or the equivalent concentration of a control antibody, with gentle shaking. The same volume of 10% or 20% protein A–agarose in Net2 buffer was then added, and incubation was continued for an additional 3 hours. The precipitates were washed 7 times in Net2 at 4°C, resuspended in 50 μl of sample buffer (0.0625*M* Tris [pH 6.8], 2% SDS, 2% 2-mercaptoethanol, and 15% glycerol containing 0.025% bromphenol blue), and boiled for 1–5 minutes. The agarose beads were pelleted by centrifugation, and the supernatants were separated on 15% SDS–polyacrylamide gels according to the method of Laemmli (26). Before autoradiography, the gels were dehydrated by washing twice in dimethyl sulfoxide and were dried and impregnated for 1 hour with PPO–DMSO (Du Pont, Boston, MA).

Synovial fluids. The SF samples were collected from patients undergoing diagnostic and therapeutic arthrocentesis at the San Diego VA Medical Center and Scripps Clinic. In addition, SF samples from 2 patients with hydroxyapatite crystal–associated shoulder arthropathy were a generous gift of Dr. P. Halverson, Medical College of Wisconsin, Milwaukee, WI. Fluids were centrifuged at 1,200*g* for 10 minutes to remove the cells. Some fluids had been digested with hyaluronidase. Acute gout and pseudogout were defined as inflammatory effusions containing leukocytes with intracellular crystals.

Reagents. MSU crystals were prepared under pyrogen-free conditions as previously described (27). CPPD and HA crystals were the generous gift of Dr. N. Mandel, Medical College of Wisconsin. Recombinant human IL-1β was donated by Dr. C. A. Dinarello, Tufts University Medical School, Boston, MA; rBSF-2 (13) and the polyclonal rabbit anti–BSF-2 (IgG fraction, 1 mg/ml) were generously provided by Drs. T. Hirano and T. Kishimoto, Institute for Molecular and Cellular Biology, Osaka University, Osaka, Japan. The endotoxin content of the concentrated stocks of the lymphokine preparations was less than 0.006 ng/ml (*Limulus* amoebocyte lysate assay). Purified endotoxin (lipopolysaccharide), from *Escherichia coli*, was purchased from Sigma (St. Louis, MO). Polymyxin B was purchased from Burroughs Wellcome (Research Triangle Park, NC). The ELISA kit for IL-1β measurement was purchased from Cistron (Cambridge, MA).

Statistical analyses. The statistical analyses were performed with the program Statview II (Abacus Concept Inc., Berkeley, CA) and a Macintosh SE computer (Apple Computer, Cupertino, CA). *P* values were defined based on the log of the IL-6 titers, using the unpaired, 2-tailed *t*-test.

RESULTS

Monocyte IL-6 secretion induced by microcrystals. Peripheral blood monocytes cultured in medium containing 1% FCS spontaneously released IL-6 activity, as assessed by the B9.9 HGF assay, a specific and highly sensitive assay for IL-6. When the cells were stimulated with various concentrations of crystals, the secreted IL-6 activity was significantly increased. The effects of the crystals on IL-6 production were dose dependent, with different maximal IL-6 levels induced by the 3 types of crystals (Figure 1). IL-6 production induced by MSU crystals was detectable with doses as low as 1.4 μg/ml, and the concentration of these crystals that effected optimal production of IL-6 was 12 μg/ml. Exposure of cells to concentrations of MSU crystals >111 μg/ml was associated with less IL-6 production. For the CPPD crystals, the optimal concentration and the minimal effective stimulatory dose were higher (333 μg/ml and 12 μg/ml, respectively). The optimal concentration of HA crystals was also 333 μg/ml. With concentrations above 333 μg/ml, there was a decline toward the baseline level of IL-6 production. IL-6 induction by HA crystals was generally weaker and more variable than with the other 2 crystals (values consistently between 2,000 units/ml and 8,000 units/ml).

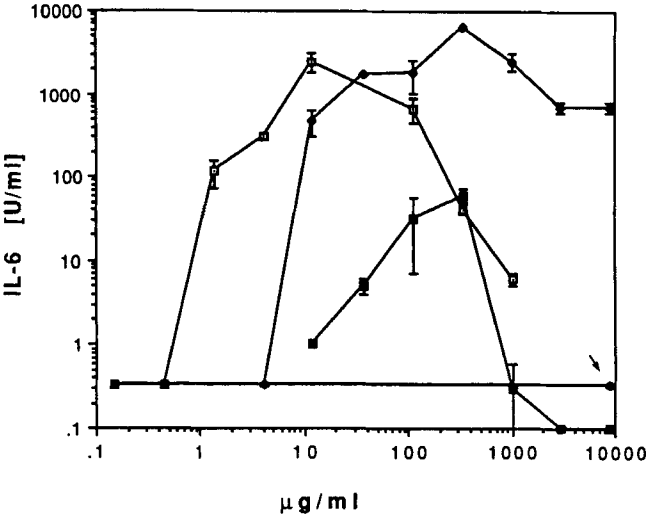

Figure 1. Interleukin-6 (IL-6) induction by various concentrations of crystals in monocytes. Freshly isolated peripheral blood monocytes were cultured in medium supplemented with 1% fetal calf serum alone (control) (**arrow**) or with monosodium urate (MSU) (□), calcium pyrophosphate dihydrate (CPPD) (◆), or hydroxapatite (HA) (■) crystals at the indicated concentrations. After 48 hours, the supernatants were collected. Duplicate supernatants of 2 different cultures were then pooled and tested for IL-6 activity by the B9.9 hybridoma cell proliferation assay. Results are the mean ± SEM of triplicate determinations.

Synoviocyte IL-6 secretion induced by microcrystals. We assessed the ability of the MSU, CPPD, and HA crystals to stimulate synoviocytes, which are known to be an intraarticular source of IL-6 (17). As

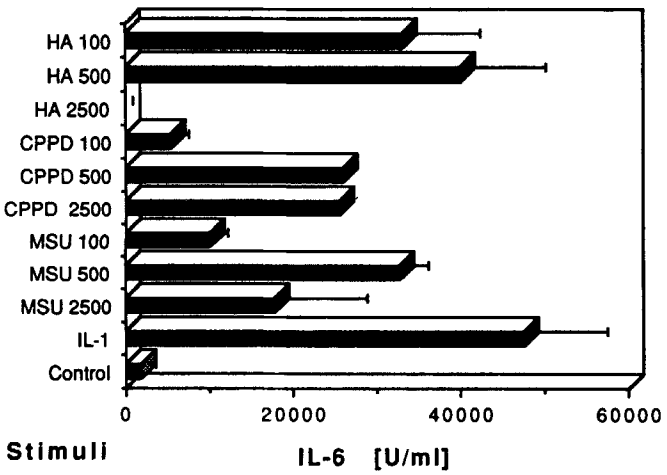

Figure 2. IL-6 induction by crystals in synoviocytes. Synoviocytes from a patient with avascular necrosis, at the fifth passage, were cultured in medium supplemented with 1% fetal calf serum and 12.5 μg/ml polymyxin B alone (control), in the presence of IL-1β (20 ng/ml), or with the different crystals at the concentrations (in μg/ml) indicated. At 56 hours, the supernatants were harvested and tested for IL-6 activity by the B9.9 hybridoma proliferation assay. Results are the mean and SEM of triplicate determinations. See Figure 1 for definitions.

demonstrated in Figure 2, in synoviocytes (derived from a joint removed for avascular necrosis), the crystals induced IL-6 activity at higher titers than did those in peripheral blood monocytes. However, the optimal stimulatory concentration of all crystals, particularly MSU, appeared higher in synoviocytes than in monocytes (~500 μg/ml for the 3 crystals). Similar results were obtained with synoviocytes from osteoarthritis patients (results not shown).

Kinetics of IL-6 induction. Evidence of IL-6 appeared early after stimulation of monocytes with the 3 types of crystals (Figure 3). At 6 hours, the IL-6 activity in the supernatants was already close to maximum for MSU and half-maximum for CPPD crystals. HA crystals appeared to induce IL-6 production more slowly. In synoviocytes, IL-6 induction by HA was even slower, and levels that were significantly higher than in control cultures were apparent only after 56 hours.

Influence of crystals on cell viability. To rule out the possibility that the action of the crystals was due to release of preformed IL-6 after cell lysis, we assessed

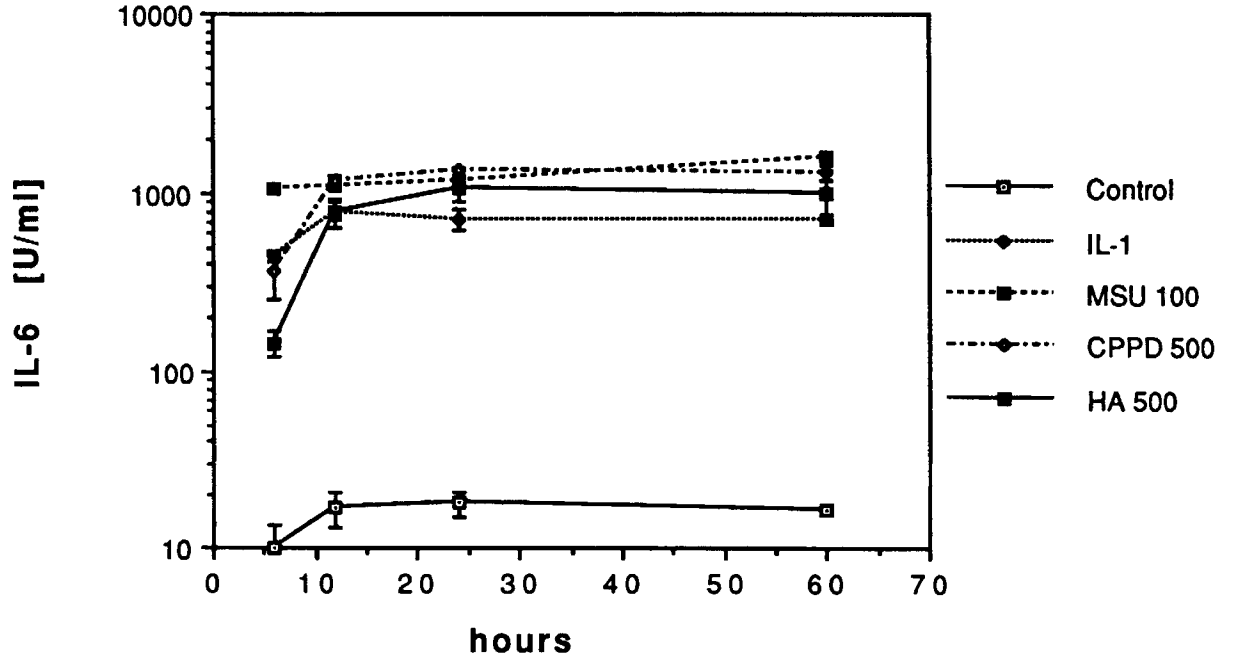

Figure 3. Kinetics of IL-6 production by crystal-stimulated monocytes. Monocytes were cultured in medium supplemented with 1% fetal calf serum alone, with IL-1β (20 ng/ml), or with the different crystals at the concentrations (in μg/ml) indicated. At the time points indicated, 20 μl of each culture was harvested. Supernatants of 2 different cultures were pooled and tested for IL-6 activity by the B9.9 hybridoma proliferation assay. Results are the mean ± SEM of triplicate determinations. See Figure 1 for definitions.

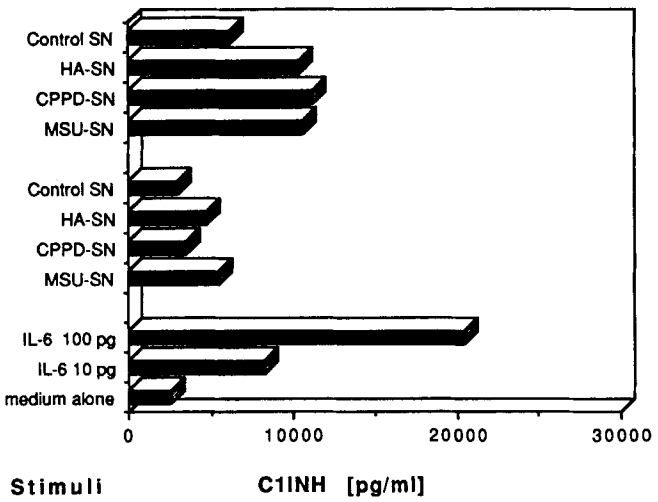

Figure 4. Induction of the acute-phase protein C1 esterase inhibitor (C1INH) by supernatants (SN) of crystal-activated monocytes or synoviocytes, as determined by the IL-6 hepatocyte-stimulating factor assay. SN of unstimulated cells (control) or cells stimulated with optimal doses of crystals were obtained as described in Figures 1 and 2 and tested for C1INH induction in the hepatoma cell line Hep-G2. C1INH was determined by enzyme-linked immunosorbent assay. The 4 upper bars represent synoviocyte SN, and the next 4 bars represent samples from monocytes. Results are the mean of triplicate C1INH determinations of 3 pooled SN. For comparison, the 3 lower bars represent C1INH produced by Hep-G2 cells in medium alone or stimulated with recombinant human IL-6 at the concentrations indicated. See Figure 1 for other definitions.

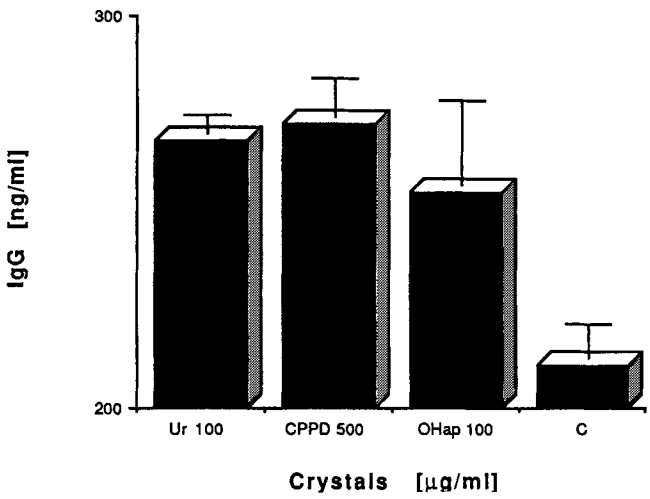

Figure 5. IgG production induced by supernatants from crystal-activated monocytes, as determined by an interleukin-6 B cell stimulatory factor 2 assay. Supernatants from unstimulated monocytes (C) or monocytes stimulated with monosodium urate (Ur), calcium pyrophosphate dihydrate (CPPD), or hydroxyapatite (OHap) crystals at the concentrations indicated were obtained as described in Figure 1 and tested for IgG induction in the human lymphoblastoid cell line CESS. IgG levels were determined by enzyme-linked immunosorbent assay. Results are the mean and SEM of triplicate IgG determinations of 2 pooled monocyte supernatants.

the viability of crystal-exposed monocytes by erythrocin dye exclusion and of crystal-exposed myelomonocytic U937 cells by ^{3}H-TdR incorporation. None of the 3 types of crystal induced any detectable cell lysis or diminution of ^{3}H-TdR incorporation at the concentrations used to obtain optimal IL-6 release (data not shown).

Functional characterization of crystal-induced IL-6. To determine if the material derived from crystal-stimulated cells also exhibited the other IL-6 activities relevant to the systemic manifestations associated with crystal-associated arthritis, supernatants were tested for their ability to enhance production of acute-phase proteins (HSF activity) and of IgG (BSF-2 activity). Figure 4 shows that synoviocytes or monocytes stimulated by the 3 types of crystals released an activity that increased the synthesis of C1INH, an acute-phase protein specifically induced by IL-6 in the human hepatoma cell line Hep-G2 (Lotz M, Zuraw B: unpublished observations). Crystal-induced monocyte supernatants were also able to enhance IgG production by the human lymphoblastoid cell line CESS, as shown in Figure 5.

Antibody neutralization. To confirm that the activity induction by the crystals was indeed a function of IL-6, we performed an antibody neutralization in the monocyte supernatants, using a polyclonal rabbit antibody to BSF-2. This antibody inhibited the

Table 1. Antibody neutralization of crystal-induced interleukin-6 (IL-6) in monocytes, using a polyclonal rabbit anti–IL-6 antiserum*

Crystal (concentration)	Concentration of antiserum		
	0	1 μg/ml	10 μg/ml
Control	238 ± 24	28 ± 28	0 ± 0
MSU (0.1 mg/ml)	4,836 ± 1,485	961 ± 106	0 ± 0
CPPD (0.5 mg/ml)	1,893 ± 555	279 ± 48	0 ± 0
HA (0.5 mg/ml)	983 ± 132	33 ± 33	0 ± 0

* Monocytes were cultured in 1% fetal calf serum–supplemented medium in the absence (control) or presence of monosodium urate (MSU), calcium pyrophosphate dihydrate (CPPD), or hydroxapatite (HA) crystals at an optimal concentration. After 48 hours, the supernatants were collected. Duplicates of 2 cultures were pooled and incubated for 1 hour at 37°C with a polyclonal antibody to recombinant human IL-6 at the concentrations indicated, prior to testing for IL-6 activity in the B9.9 hybridoma proliferation assay. Results are expressed in units/ml and are the mean ± SEM of triplicate determinations.

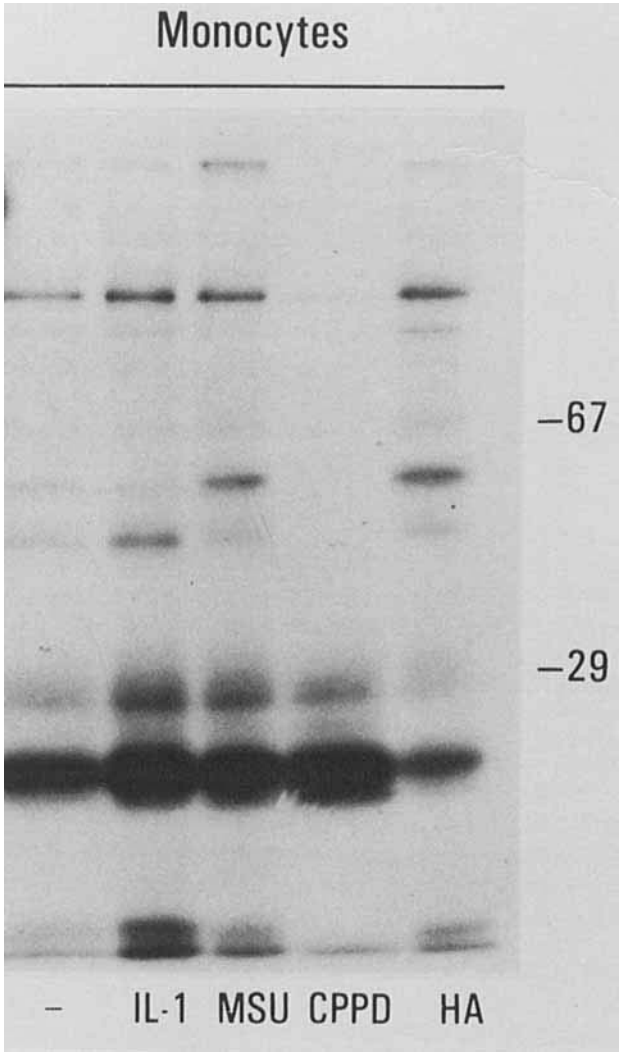

Figure 6. Immunoprecipitation of IL-6 produced by crystal-induced monocytes. Supernatants were obtained by culturing 8×10^5 cells/ml in methionine-free medium containing 5% fetal calf serum and ^{35}S-methionine in the presence of IL-1β, 20 ng/ml (lane 2), MSU, 100 μg/ml (lane 3), CPPD, 500 μg/ml (lane 4), HA, 500 μg/ml (lane 5), or without stimulus (lane 1). The labeled proteins were immunoprecipitated with a polyclonal antibody to IL-6 and protein A–agarose. Proteins were then separated on a 12.5% sodium dodecyl sulfate–polyacrylamide gel. Autoradiography was performed after impregnation in PPO–DMSO. Numbers on the right represent molecular weights (kd) of synthesized proteins. See Figure 1 for other definitions.

IL-6 activity induced by the crystals in a dose-dependent manner (Table 1): Partial neutralization occurred at a concentration of 1 μg/ml, and complete inhibition occurred at 10 μg/ml. A preimmune rabbit serum did not significantly reduce the IL-6 activity (data not shown).

Table 2. Comparison of IL-6 and IL-1 levels in supernatants from crystal-stimulated synoviocytes and monocytes*

	HGF (units/ml)	HSF (C1INH induction, pg/ml)	ELISA (pg/ml)
Monocyte			
Control	14	2,932	<20
MSU	834	5,466	1,377
CPPD	1,010	3,497	376
HA	834	4,720	442
Synoviocyte			
Control	12,086	6,166	<20
MSU	57,924	10,603	<20
CPPD	62,657	11,179	<20
HA	47,279	10,372	<20

* Peripheral blood monocytes and synoviocytes (synoviocytes from patients with avascular necrosis, at the fifth passage) were cultured in the absence (control) or presence of crystals at the optimal concentrations, as expressed above, for 72 hours. Supernatants were divided into aliquots and tested for IL-6 activity by the hybridoma growth factor (HGF) and hepatocyte-stimulating factor (HSF) assays and for their IL-1 content by enzyme-linked immunosorbent assay (ELISA). Results are the mean of triplicate determinations. C1INH = C1 esterase inhibitor; see Table 1 for other definitions.

Immunoprecipitation of crystal-induced IL-6. To characterize the crystal-induced IL-6 proteins and to determine if the activity found in the supernatants was due to de novo protein synthesis or merely to a release of already synthesized protein, we performed an immunoprecipitation analysis of ^{35}S-methionine–labeled monocyte supernatants. Figure 6 shows that mono-

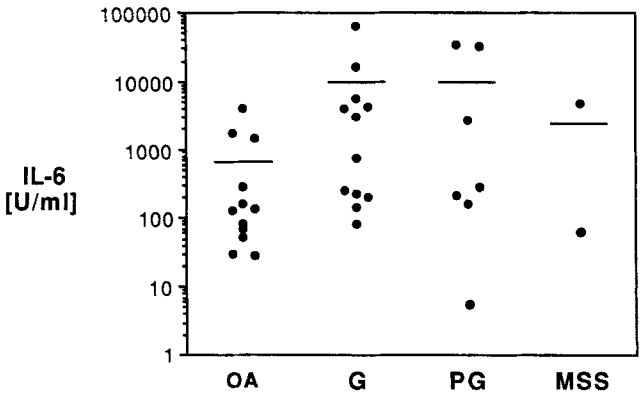

Figure 7. Interleukin-6 (IL-6) activity in synovial fluids. Synovial fluids were collected from patients with various crystal-associated arthropathies: osteoarthritis (OA), acute gout (G), pseudogout (PG), and Milwaukee shoulder syndrome (MSS). The fluids were serially diluted and tested for IL-6 by the B9.9 hybridoma proliferation assay. Each point represents a different patient. Results are the mean of triplicate determinations (SEM <15%). They are compared with an IL-6 standard (recombinant B cell stimulatory factor 2). Units of IL-6 are shown on a $\log_{10}$ scale.

cytes stimulated by crystals, as well as by IL-1β, synthesized proteins that migrated as a doublet between 23 kd and 30 kd in 12.5% SDS–polyacrylamide gels, a finding that is consistent with the known molecular mass of IL-6. Additional proteins of larger molecular weight were precipitated by the antiserum to IL-6. These may represent larger molecular weight isoforms of IL-6 and seem to be induced by the stimuli used in these experiments. The IL-6 proteins immunoprecipitated from the MSU- and CPPD-induced supernatants were clearly more abundant than those from the unstimulated supernatants. In contrast, HA crystals did not appear to increase the IL-6 immunoprecipitates over background intensity. The results indicate that MSU and CPPD crystals augment IL-6 synthesis, and that this is probably not the case with HA crystals. Similar results were obtained with immunoprecipitated synoviocyte supernatants (not shown).

Comparison of IL-6 and IL-1 levels in supernatants from synoviocytes and monocytes. Because IL-1 is known to enhance the production of IL-6 by different cell types, including synoviocytes, we compared the IL-1 content of a set of culture supernatants with their IL-6 activity to determine if IL-6 production was dependent on IL-1 release. The results, shown in Table 2, indicate that there is no correlation between the levels of the 2 cytokines. In monocytes, the 3 types of crystals induced comparable levels of IL-6 activity, as assessed by the HGF and HSF assays, whereas the levels of IL-1 induced by CPPD and HA crystals were significantly lower than those induced by MSU. In the synoviocyte cultures, the differences were even more clear-cut. These cultures contained even higher levels of IL-6, but IL-1 was not detectable by the radioimmunoassay, which has a sensitivity of 20 pg/ml.

IL-6 activity in synovial fluids. To correlate these in vitro results with in vivo effects of the crystals, we examined the IL-6 content of a series of SF samples from patients with crystal-related arthropathies. High levels of IL-6 activity could be detected in all of the samples from gout patients (mean 9,754 units/ml) (Figure 7); substantial levels were also found in fluids from patients with acute pseudogout (mean 9,960 units/ml). The mean values in both gout and pseudogout samples were considerably higher than those in osteoarthritis (OA) fluids (mean 673 units/ml) ($P = 0.019$ and $P = 0.21$, respectively), although some of the values in the gout and pseudogout samples were in the range of the OA values. Only 2 samples were available from patients with Milwaukee shoulder syndrome; 1 of them demonstrated very high titer of IL-6 activity. No apparent differences were noted between fluids that were treated with hyaluronidase and the untreated samples.

DISCUSSION

The results of this study demonstrate that microcrystals associated with inflammatory joint diseases are potent inducers of de novo IL-6 synthesis and/or release from monocytes and synoviocytes. Correspondingly, substantial levels of IL-6 are found in synovial fluids from patients with various crystal-induced arthropathies.

MSU and CPPD crystals are known to activate neutrophils and monocytes, and the resultant release of inflammatory mediators is believed to be important in specific aspects of the pathogenesis of these diseases (28). For example, MSU and CPPD crystals can stimulate monocyte release of IL-1 and TNFα (3–7). However, IL-1 and TNFα, though potentially involved in the induction of the systemic inflammatory changes seen in acute crystal-induced arthritis, including the hepatic acute-phase response, leukocytosis, and fever, are likely only partially responsible for these changes. This is because of the biologic inactivity of IL-1 when it is bound to carriers such as α_2-macroglobulin, a binding protein for various cytokines (29–32). Consistent with this finding, circulating IL-1 bioactivity and immunoreactivity can only be detected after fractionation of plasma or serum (33,34). In addition, the spectrum of hepatic acute-phase proteins directly induced by IL-1 or TNF in cultures of hepatocytes or hepatoma cells is limited (8–10) and does not include proteins that change dramatically during crystal-induced inflammation in vivo, such as C-reactive protein, fibrinogen, and serum amyloid A protein (35). In contrast, Il-6 has recently been identified as a cytokine that can mediate these changes.

Like TNFα and IL-1, IL-6 is produced by monocytes, but IL-6 can also be produced by a large number of different tissues (17,36–42). Interestingly, in most cell types, IL-1 and TNFα are potent inducers of IL-6 (17,43), which may be the mediator of some IL-1 or TNFα effects. This is certainly the case with the hepatic acute-phase response, since IL-6 has been shown to stimulate production of most acute-phase proteins in vitro and in vivo (44,45).

IL-6 appears to be a systemically active mediator. Its activity can be readily detected in plasma from patients with various inflammatory diseases or

after endotoxin injection (46). Circulating levels of IL-6 increase very early following injury, preceding the hepatic acute-phase response (47). The levels of IL-6 in serum or plasma are far greater than those of IL-1 in similar specimens and correlate closely with the erythrocyte sedimentation rate (Lotz M: unpublished observations), which is the test most frequently used to determine disease activity in crystal-induced arthritis, and in contrast to IL-1, IL-6 retains its activity when bound to α_2-macroglobulin (48). As a pyrogen and colony-stimulating factor that promotes proliferation of granulocyte-monocyte progenitors, IL-6 is a likely contributing factor in the leukocytosis and fever seen in crystal-induced arthritis. At present, no definitive role has been established for IL-6 in the intraarticular processes associated with crystal-induced arthritis. It may be involved in the regulation of chondrocyte function (Guerne P-A, Carson D, Lotz M: unpublished observations), but it does not appear to stimulate connective tissue catabolism in synoviocytes (17), which probably constitutes a major function of IL-1 and TNFα in the pathogenesis of crystal-induced arthritis.

Our results provide direct support for the role of IL-6 in the systemic inflammatory manifestations in crystal-induced arthropathies. Very high levels of IL-6 are present in some synovial fluids from patients with gout and pseudogout, representing the highest levels of any of the 3 monokines found in this body fluid. It should be noted that not all SF from gout and pseudogout patients had significantly higher IL-6 levels than the SF from OA patients. This is consistent with the varying degrees of systemic inflammatory manifestations seen in patients with these arthropathies. The high concentration of IL-6 in gout and pseudogout patient SF is most likely the consequence of the ability of low concentrations of MSU and CPPD crystals to directly stimulate IL-6 synthesis and release. However, the induction of IL-6 by exposure of monocytes to C5a (Guerne P-A, Lotz M: unpublished observations) that is generated by crystal-induced complement activation (28) is also possible.

It is of interest that HA crystals have a relatively weak ability to induce production of IL-6, compared with MSU and CPPD crystals. This correlates with the general lack of prominent systemic inflammatory manifestations, such as those seen in acute gout and pseudogout, in patients with joint diseases associated with intraarticular HA crystals (49).

The 2 cell types examined in this study are probably important sources of crystal-induced IL-6 in synovia. Because MSU, CPPD, and HA crystals deposited in cartilage and chondrocytes can readily produce IL-6 (Guerne P-A, Lotz M: unpublished observations), it will be of interest to see whether chondrocytes have the same relative sensitivity to the 3 types of crystals as do monocytes and synoviocytes. Among the inflammatory cells infiltrating the joint in crystal-induced arthritis, monocytes are presumably the most prominent IL-6 producer, since neutrophils do not appear to release significant levels of IL-6 (Terkeltaub R, Lotz M: unpublished observations).

Analysis of the same set of culture supernatants for IL-1 and IL-6 showed that the levels of the 2 cytokines did not correlate (Table 2). Synoviocyte cultures contained high levels of IL-6, but IL-1 was not detectable with an ELISA having a sensitivity of 20 pg/ml. At present, the mechanism by which crystals induce IL-6 production is not defined. It is possible that it is associated with crystal-induced release of IL-1. It is also possible that crystals have a direct effect on IL-6 production, since it has already been shown that IL-6 gene transcription can be induced via mobilization of calcium and activation of protein kinase C (50), and both of these activation events can be triggered by microcrystals (51,52). In addition, crystal-induced TNFα may play a role in stimulating IL-6. Further experiments are in progress to generate definitive information on the mechanism of crystal induction of IL-6.

In summary, MSU and CPPD crystals can trigger monocytes and synoviocytes to synthesize and release substantial amounts of IL-6, which we propose to be an important systemic mediator of inflammatory manifestations in crystal-induced arthritis.

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