

## Efficacy and Mechanism of Action of Turmeric Supplements in the Treatment of Experimental Arthritis

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**Objective.** Scientific evidence is lacking for the antiarthritic efficacy of turmeric dietary supplements that are being promoted for arthritis treatment. Therefore, we undertook studies to determine the antiarthritic efficacy and mechanism of action of a well-characterized turmeric extract using an animal model of rheumatoid arthritis (RA).

**Methods.** The composition of commercial turmeric dietary supplements was determined by high-performance liquid chromatography. A curcuminoid-containing turmeric extract similar in composition to these supplements was isolated and administered intraperitoneally to female Lewis rats prior to or after the onset of streptococcal cell wall-induced arthritis. Efficacy in preventing joint swelling and destruction was determined clinically, histologically, and by measurement of bone mineral density. Mechanism of action was elucidated by analysis of turmeric's effect on articular transcription factor activation, microarray analysis of articular gene expression, and verification of the physiologic effects of alterations in gene expression.

**Results.** A turmeric fraction depleted of essential oils profoundly inhibited joint inflammation and periarticular joint destruction in a dose-dependent manner. In vivo treatment prevented local activation of NF- $\kappa$ B and the subsequent expression of NF- $\kappa$ B-regulated genes mediating joint inflammation and destruction, including chemokines, cyclooxygenase 2, and RANKL. Consistent with these findings, inflammatory cell influx, joint levels of prostaglandin E<sub>2</sub>, and periarticular osteoclast formation were inhibited by turmeric extract treatment.

**Conclusion.** These translational studies demonstrate in vivo efficacy and identify a mechanism of action for a well-characterized turmeric extract that supports further clinical evaluation of turmeric dietary supplements in the treatment of RA.

The use of botanical remedies for arthritis treatment is promoted in the US by the lay press and high-profile medical practitioners (1,2). Interest in the use of nonpharmaceutical arthritis treatments has grown with the withdrawal of Food and Drug Administration-approved antiinflammatory drugs (3). However, scientific data are almost uniformly lacking concerning the antiarthritic efficacy and mechanism of action of popular botanical remedies (4,5). The rational medicinal use of botanical dietary supplements is further complicated by the fact that the composition of over-the-counter botanical dietary supplements is not strictly regulated (4,5). Unfortunately, in the medical literature, the chemical composition and biologic activity of botanicals that are tested for antiarthritic efficacy are frequently also not well characterized (6–9). Therefore, benchmarks are lacking for assessing the potential suitability of commercially available botanical supplements or phytomedicines.

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Turmeric is one such botanical supplement whose use against arthritis, supported almost exclusively by its traditional, centuries-old use as an antiinflammatory agent in Ayurvedic medicine, has been heavily promoted (1,2). Turmeric, an underground stem (rhizome), is also used as a spice and is part of curry. Popular wisdom suggests that curcumin, 1 of 3 major phenolic curcuminoids that constitute 3–5% of turmeric, is the active antiinflammatory ingredient in turmeric (10). Indeed, an antiarthritic effect of curcumin has been reported in 1 small clinical study of rheumatoid arthritis (RA) and in 3 small descriptive studies of arthritis in animals (6–9). However, our research group has more recently demonstrated 1) that other, noncurcumin components of turmeric are also antiinflammatory, as measured by *in vitro* inhibition of prostaglandin production (11), and 2) that these components may act synergistically with each other and/or curcumin to block inflammation (11).

Because the antiarthritic efficacy of turmeric extracts analogous to dietary supplements (versus that of curcumin) had not previously been described, our laboratory isolated, chemically characterized, and determined the *in vivo* antiarthritic efficacy of a complex turmeric extract using streptococcal cell wall (SCW)-induced arthritis, an animal model of RA. In these initial studies, we reported that turmeric can indeed prevent joint inflammation (12). We have now extended these studies 1) to compare the chemical composition of our experimental turmeric extract with that of commercial turmeric dietary supplements available for over-the-counter use, 2) to examine the dose-dependent effect of our experimental turmeric extract on joint inflammation and actual joint destruction, and 3) to determine the effect of our experimental turmeric extract on systemic markers of inflammation. Last, and most important, because the *in vivo* antiarthritic mechanism of action of neither curcumin nor turmeric has previously been documented, we determined the mechanism of the joint-protective effect of turmeric in SCW-induced arthritis.

## MATERIALS AND METHODS

**Experimental turmeric extract preparation.** Turmeric powder (*Curcuma longa* L., Zingiberaceae) was purchased from San Francisco Herb and Natural Food Company, Wholesale Herbs, Spices and Teas (Fremont, CA). A crude methanolic turmeric extract was prepared from the ground rhizome as previously described (yield 9.5%) (12). A turmeric fraction devoid of essential oils was prepared by methanol

extraction of the marc obtained from an initial hexane extraction of ground rhizome, as previously described (yield 3.1%) (12).

**Chemical and biologic analyses of experimental and commercial turmeric.** Chemical analyses of extracts and commercial samples were performed as previously described using an 1100 series high-performance liquid chromatography (HPLC) system (Agilent, Palo Alto, CA) and stock solutions of pure curcumin, demethoxycurcumin, and *bis*-demethoxycurcumin (12). *In vitro* screening for antiinflammatory activity of extracts, as determined by inhibition of lipopolysaccharide (LPS)-induced prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) secretion from phorbol myristate acetate-differentiated U937 cells, was conducted before use in animals to ensure reproducibility of the extract preparation, as previously described (12).

**Animal procedures.** Female Lewis rats (Harlan, Indianapolis, IN) were administered a single intraperitoneal (IP) injection of vehicle (saline) or peptidoglycan-polysaccharides (25 µg rhamnose/gm body weight) isolated from the sonicated cell wall of group A *Streptococcus pyogenes* (Lee Laboratories, Grayson, GA) (12–14). At the indicated times, animals received an IP injection of turmeric extract or vehicle alone (0.5–1.0 µl/gm DMSO). Joint inflammation in each distal limb was scored daily in a blinded manner using standard criteria and an arthritis index scale of 0–4 per limb (12–14). Circulating white blood cell counts were determined on day 28 using an Hemavet 880 analyzer (CDC Technologies, Oxford, CT), and cell differentials were determined by manual counting. Serum creatinine and alanine aminotransferase (ALT) levels were measured on day 28 using an Endocheck Plus Chemistry Analyzer (Hemagen Diagnostics, Columbia, MD), and weights were recorded daily.

**RNA isolation.** At each time point (day 3 or day 28), rats were killed, and hind ankle joints were quickly stripped of skin and connective tissue, flash-frozen in liquid nitrogen, and stored at –70°C. Frozen samples were ground into a fine powder using a continuously cooled tissue Biopulverizer (Bio-Spec, Bartlesville, OK). Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA) followed by 2.5M lithium chloride precipitation. RNA purity and integrity were determined using a 2100 Bioanalyzer (Agilent), and only samples with an RNA integrity number of ≥7 were used for further analysis. Equal amounts of RNA were combined from 3 (nonarthritic) or 4 (arthritic) joints per treatment group to make 1 sample. Three such pooled RNA samples per treatment group (i.e., a total of 9–12 joints analyzed per group) were used for gene expression microarray analysis and real-time reverse transcription-polymerase chain reaction (RT-PCR).

**Microarray analysis of joint gene expression.** Pooled total RNA samples, as described above, were subsequently processed according to the manufacturer's recommendations (Expression Analysis Technical Manual; Affymetrix, Santa Clara, CA) to yield biotinylated complementary RNA (cRNA). Biotinylated cRNA was then fragmented, hybridization cocktails were prepared with 10 µg cRNA, and GeneChip Rat Genome 230 2.0 arrays (Affymetrix) were hybridized using standard Affymetrix protocols. The Rat Genome 230 2.0 arrays comprise more than 31,000 probe sets, analyzing more than 30,000 transcripts and variants from more than 28,000 well-substantiated rat genes. Chips were immediately washed and

**Table 1.** Chemical analysis of experimental turmeric extracts and commercial turmeric dietary supplements\*

Sample type, identification	Sample description [content per capsule]†	Sample amount, mg‡	Content of 3 major curcuminoids in samples, %§				Content, mg/capsule	Essential oils
			C	DMC	BDMC	Total		
Experimental								
Crude turmeric	Methanol extract of turmeric ground rhizome	-	21.4 ± 0.05	7.09 ± 0.13	5.14 ± 0.07	33.62 ± 0.16	-	Yes
Turmeric fraction	Methanol extract of hexane-washed turmeric ground rhizome	-	25.7 ± 0.16	8.68 ± 0.17	6.19 ± 0.09	40.52 ± 0.25	-	No
Commercial								
1	Turmeric rhizome [400 mg]	483.2 ± 6	1 ± 0.05	0.4 ± 0.02	0.4 ± 0.2	1.8 ± 0.21	8.70 ± 0.0012	Yes
2	Turmeric (root extract), guaranteed 95% (285 mg) curcumin [300 mg]	342.9 ± 11.1	13.6 ± 1.5	16.3 ± 0.21	3.8 ± 0.06	33.7 ± 1.52	115.56 ± 0.0006	No
3	Turmeric, dried extract (root), standardized to 95% curcuminoids [450 mg]; turmeric (root) [50 mg]	881.8 ± 18.9	8.2 ± 0.22	9.2 ± 0.26	1.85 ± 0.06	19.25 ± 0.35	169.75 ± 0.0003	No
4	Turmeric rhizome extract 95%, yielding 380 mg curcumin [400 mg]; turmeric rhizome [50 mg]	1,097.3 ± 4	4.4 ± 0.06	2.2 ± 0.11	3.5 ± 0.39	10.1 ± 0.41	110.83 ± 0.0004	No
5	Turmeric, dried extract (root), 95% curcuminoids [450 mg]; turmeric (root) [50 mg]	877.6 ± 20.1	8.3 ± 1.1	8.2 ± 0.4	1.95 ± 0.09	18.45 ± 1.17	161.92 ± 0.0007	No
6	Turmeric ( <i>Curcuma longa</i> rhizome), standardized 95% (380 mg) curcumin [400 mg]	474.3 ± 47.7	10.7 ± 1.3	13 ± 1.22	4.97 ± 0.53	28.67 ± 1.86	135.98 ± 0.0012	No
7	Turmeric rhizome supercritical extract (45% turmerones—36 mg) [80 mg]; turmeric rhizome post-supercritical ethanolic extract (11% curcumins—35.2 mg) [320 mg]	623.3 ± 23	3.1 ± 0.51	1.3 ± 0.14	1.3 ± 0.16	5.7 ± 0.55	35.53 ± 0.0010	Yes
8	Turmeric root extract, certified potency (standardized for 95% curcumins) [300 mg]; turmeric root [125 mg]	479.3 ± 16	13.7 ± 1.5	10.4 ± 0.21	3.1 ± 0.03	27.2 ± 1.51	130.37 ± 0.0006	No

\* Values are the mean ± SD. C = curcumin; DMC = demethoxycurcumin; BDMC = bis-demethoxycurcumin.

† Commercial samples from different brands, with description listed as indicated in supplement facts portion of label, were chosen at random from local stores in Tucson, AZ for analysis.

‡ Average weight of 3 capsules.

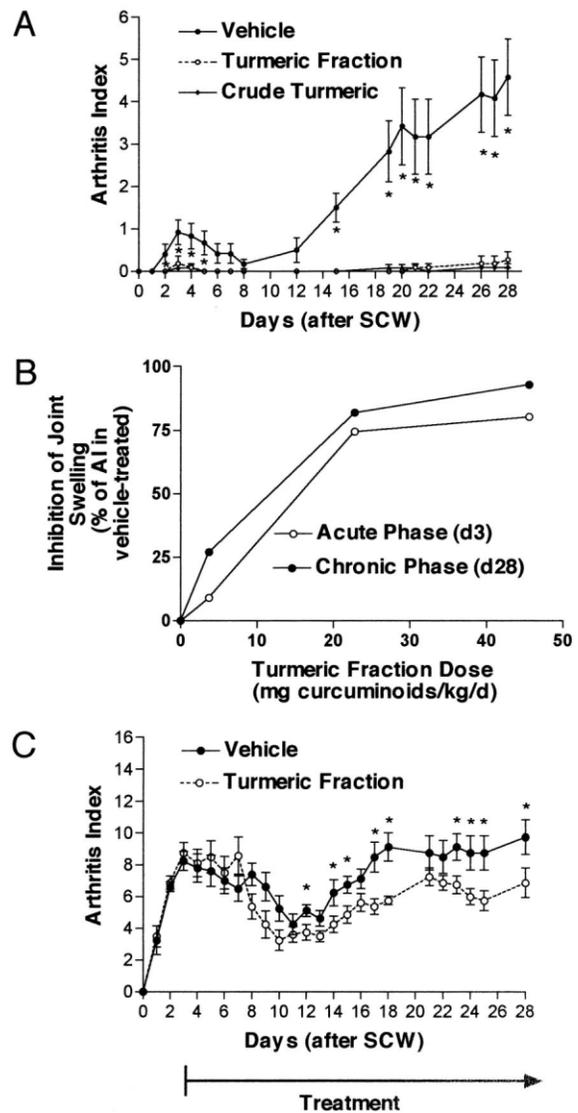
§ For each sample, three 10- $\mu$ l samples were subjected to high-performance liquid chromatography analysis, and the average result is shown (for commercial samples, results were also averaged over 3 capsules). In all 8 commercial samples, the average content of curcumin was 7.88 ± 1.68%, the average content of demethoxycurcumin was 7.63 ± 2.05%, the average content of bis-demethoxycurcumin was 2.61 ± 0.53%, and the average total content of the 3 major curcuminoids was 18.11 ± 4.06%.

stained using the GeneChip Fluidics Station 400 (EukGE-WS2v5 fluidics protocol; Affymetrix) and scanned with the GeneChip Scanner 3000 (Affymetrix).

Data were subsequently exported for analysis to GeneSpring version 7.0 (Silicon Genetics, Redwood City, CA). Stringent empirical and statistical analyses were used to compare gene expression profiles between rats in different treatment groups, with a cross-gene error model based on replicates. Normalized data (per gene, per chip, and per sample, with vehicle-treated controls serving as a reference point) were serially filtered in the following order to identify genes up- or down-regulated at least 2-fold in arthritic joints whose expression was modified by turmeric extract treatment: 1) genes that were present on at least 3 of our 12 chips per experimental time point, 2) genes whose expression was altered at least 2-fold in joints of untreated rats with SCW-induced arthritis compared with joints of control rats, 3) genes whose expression was statistically changed (as determined by analysis of variance [ANOVA]) between all of the treatment groups, and 4) genes whose expression was significantly ( $P < 0.05$ ) altered in joints of untreated rats with SCW-induced arthritis compared with joints of turmeric fraction-treated rats with SCW-induced arthritis (by Student-Newman-Keuls post hoc testing). All statistical analyses were performed with correction for multiple testing utilizing the Benjamini and Hochberg false discovery rate criterion as a method of choice to reduce the number of false-positive results.

**Real-time RT-PCR.** Changes in expression levels of selected physiologically important genes from the gene arrays were verified by TaqMan real-time RT-PCR analysis using the same 3 samples/group. Total RNA (250  $\mu\text{g}$ ) was reverse transcribed (iScript; Bio-Rad, Hercules, CA). Rat-specific primers for interleukin-1 $\beta$  (IL-1 $\beta$ ) (Rn00580432\_m1), cyclooxygenase 2 (COX-2) (Rn00568225\_m1), RANKL (Rn00589289\_m1), mannan-binding lectin serine peptidase 1 (Rn00434830\_m1), properdin factor B (Rn01526084\_g1), growth-related oncogene/keratinocyte chemoattractant (GRO/KC) (Rn00578225\_m1), monocyte chemoattractant protein 1 (MCP-1) (Rn00580555\_m1), and an 18S primer as an internal control (Hs99999901\_s1) were obtained from Applied Biosystems (Foster City, CA). Data were analyzed using the comparative cycle threshold ( $C_t$ ) method as a means of relative quantitation of gene expression, normalized to the endogenous reference (18S RNA) and relative to a calibrator (normalized  $C_t$  value obtained from control rats) and expressed as  $2^{-\Delta\Delta C_t}$ , as described by the manufacturer (Applied Biosystems).

**Histology.** All tissue specimens were fixed in 10% formalin; joints were subsequently decalcified in 10% EDTA (pH 7.0), and tissues were embedded in paraffin. Osteoclasts, identified by tartrate-resistant acid phosphatase (TRAP) staining, were counted in hind limb distal tibial growth plates 28 days after injection of SCW or vehicle, as previously described (13). An index of articular cartilage destruction in hind joint distal tibias on day 28 was determined using hematoxylin and eosin (H&E)-stained sections (0 = normal; 1 = minimal destruction; 2 = at least 50% destroyed; 3 = entirely destroyed) as previously described (13). Use of H&E (versus toluidine blue) staining for assessment of cartilage integrity has been previously verified in this model, since loss of proteoglycan matrix does not appear to occur in SCW-induced arthritis in the absence of cartilage invasion by synovium (13). Granuloma formation on day 28 was assessed in H&E-stained liver and spleen sections using standard criteria (13,15). Neutrophils



**Figure 1.** Effect of turmeric extracts on joint inflammation. Female Lewis rats were injected on day 0 with peptidoglycan-polysaccharides for streptococcal cell wall-induced arthritis (SCW; 25  $\mu\text{g}/\text{gm}$  body weight) or with vehicle. Joint swelling was assessed daily by calculating the mean  $\pm$  SEM arthritis index (AI), and statistical significance was determined by Student's *t*-test as described in Materials and Methods. **A**, Intraperitoneal (IP) injections of crude turmeric extract or essential oil-depleted turmeric fraction (both normalized to 46 mg curcuminoids/kg/day) or vehicle alone were begun 4 days prior to SCW administration ( $n = 11$ –12 animals/group) and were continued on a daily basis until 10 days after SCW injection, at which time the treatment frequency was decreased to 5 days/week. \* =  $P < 0.05$  versus crude turmeric extract or essential oil-depleted turmeric fraction. **B**, Indicated doses of the turmeric fraction or vehicle alone were administered IP as described in **A**. Shown is the arthritis index on day 3 (d3; acute phase) and day 28 (d28; chronic phase) ( $n = 11$ –53 animals/group). **C**, Delayed IP injection with turmeric fraction (23 mg curcuminoids/kg/day) or vehicle alone ( $n = 8$  animals/group) was begun after attainment of maximal joint swelling (day 3 post SCW injection) and continued on a daily basis until 10 days after SCW injection, at which time the treatment frequency was decreased to 5 days/week. \* =  $P < 0.05$  versus turmeric fraction.

**Table 2.** Toxicity, joint destruction, and bone marrow parameters in rats treated or not treated with turmeric fraction\*

Parameter	Vehicle	Turmeric fraction	SCW	SCW plus turmeric fraction
<b>Toxicity monitoring</b>				
Mortality, % (no. died/total tested)	0 (0/29)	7 (2/29)	0 (0/57)	5 (3/58)
ALT, units/liter	13.7 ± 0.9	17.3 ± 2.8	12.4 ± 1.0	14.6 ± 1.7
Creatinine, mg/dl	0.2 ± 0.02	0.2 ± 0.07	0.2 ± 0.01	0.2 ± 0.01
WBC count, ×10 <sup>3</sup> /μl	7.1 ± 0.4	7.9 ± 0.6	33.0 ± 3.4†	20.8 ± 3.2‡§
Hematocrit, %	38.7 ± 0.6	36.7 ± 1.0	27.8 ± 1.3†	32.6 ± 1.6‡§
<b>Joint destruction</b>				
Cartilage destruction index, 0–3 scale	0.13 ± 0.13	0.0 ± 0.0	2.40 ± 0.15†	0.82 ± 0.13§¶
BMD, gm/cm <sup>2</sup>	0.200 ± 0.005	0.185 ± 0.004	0.156 ± 0.006†	0.181 ± 0.004§
<b>Bone marrow</b>				
Periarticular osteoclasts, cells/mm <sup>2</sup>	44.5 ± 4.7	33.6 ± 3.6	73.6 ± 5.8†	38.2 ± 3.1#
Osteoclast formation, cells/well	168.8 ± 8.4	117.5 ± 8.2‡	149.5 ± 18.4	98.8 ± 6.8#
TNFα, pg/ml	42.01 ± 0.57	66.5 ± 6.0	355.2 ± 31.6†	48.8 ± 1.6#

\* Values are the mean ± SEM. Female Lewis rats were injected on day 0 with peptidoglycan–polysaccharides for streptococcal cell wall–induced arthritis (SCW; 25 μg/gm body weight) or with vehicle (saline). Intraperitoneal injections of turmeric fraction (23 and/or 46 mg curcuminoids/kg/day) or vehicle were begun 4 days prior to SCW administration and were continued on a daily basis until 10 days after SCW injection, at which time the treatment frequency was decreased to 5 days/week. Blood samples for measurement of alanine aminotransferase (ALT) or creatinine or a complete blood cell count (n = 29–58 animals/group starting the botanical 4 days before or 8 days after SCW injection, with a subset of 9–23 animals/group for the complete blood cell count) were obtained 28 days after SCW injection. Hind ankle joints and femurs were obtained on day 28 for histologic analysis of cartilage destruction (n = 6–20 joints/group), tartrate-resistant acid phosphatase staining of osteoclasts in distal tibial growth plates, and ex vivo measurement of bone mineral density (BMD) of the distal 25% of the femur (n = 8–16 femurs/group). Bone marrow on day 28 was isolated and combined from 3 tibias/group receiving 23 mg curcuminoids/kg/day for ex vivo culture (n = 4 wells/group) to determine macrophage colony-stimulating factor– and RANK-activating antibody–stimulated osteoclast formation (3 days after SCW injection) or lipopolysaccharide-stimulated release of tumor necrosis factor α (TNFα). WBC = white blood cell.

† P < 0.001 versus vehicle-treated rats, by analysis of variance (ANOVA) with post hoc testing.

‡ P < 0.05 versus vehicle-treated rats, by ANOVA with post hoc testing.

§ P < 0.01 versus SCW-injected rats not treated with turmeric fraction, by ANOVA with post hoc testing.

¶ P < 0.01 versus vehicle-treated rats, by ANOVA with post hoc testing.

# P < 0.05 versus SCW-injected rats not treated with turmeric fraction, by ANOVA with post hoc testing.

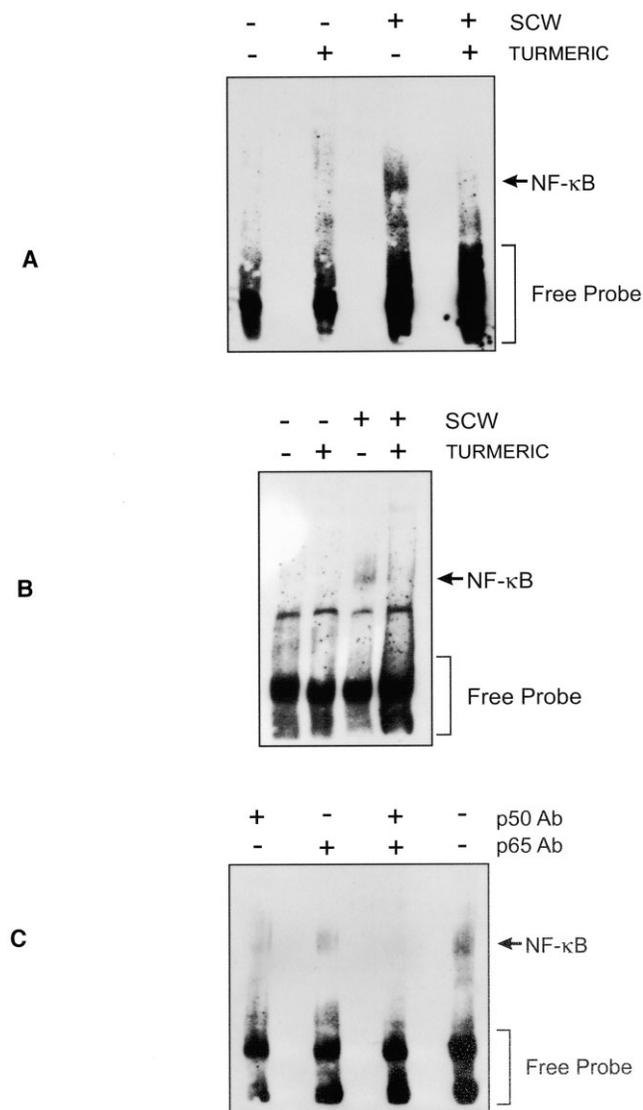
in joint effusions and synovium were identified in tibiotarsal joint sections by naphthol AS-D chloroacetate esterase staining, and macrophages were identified using ED1 antibody (versus IgG negative control) and standard immunohistochemical staining techniques as previously described (13,16). All histologic analyses were performed in a blinded manner.

**Bone mineral density (BMD).** BMD of the distal 25% of excised hind femurs was determined using a Piximus densitometer (GE Lunar, Madison, WI) as previously described (13).

**Activation of NF-κB in arthritic joints.** Hind ankle joints were isolated and biopulverized as described for RNA isolation. Nuclear proteins were then isolated using a standard extraction buffer and protocol (Nuclear Extraction kit; Panomics, Redwood City, CA). Equal amounts of protein (Protein Assay; Bio-Rad) from 3 joints per treatment group were pooled to create one 5-μg sample per treatment group. Electrophoretic mobility shift assay (EMSA) of unbound versus protein-bound DNA was performed by incubating nuclear extracts with a biotinylated DNA probe specific for NF-κB (AY1030 probe and EMSA Kit; Panomics). Specificity of reaction was determined either by incubation with excess unlabeled probe or by antibodies directed against p50 and p65, two subunits of NF-κB (SC-114 and SC-109, respectively; Santa Cruz Biotechnology, Santa Cruz, CA). Samples were electrophoresed on 6% polyacrylamide gels, transferred to a Biotodyne B Membrane (Pall, East Hills, NY), and incubated with streptavidin–horseradish peroxidase and a chemilumines-

cent substrate as described by the manufacturer (Panomics). Chemiluminescence was recorded on Hyperfilm ECL (Amersham, Piscataway, NJ).

**Cytokine and chemokine production.** MCP-1, GRO/KC, and tumor necrosis factor α (TNFα) levels in cell supernatants obtained from ex vivo culture of splenocytes or bone marrow cells isolated from in vivo–treated nonarthritic or arthritic animals were determined by rat multiplex enzyme-linked immunosorbent assay (ELISA; Linco Systems, St. Charles, MO) using a Luminex 100 system (Luminex, Austin, TX). Spleen cells were isolated using standard methods (16), and splenocytes from 3 spleens per treatment group were combined and plated at a density of 2 × 10<sup>6</sup> nucleated cells/well in 24-well plates (n = 4 wells per group) with 700 μl of Dulbecco's modified Eagle's medium (DMEM)/10% fetal calf serum (FCS). Supernatants were isolated after 48 hours and stored at –70°C for later analysis. Bone marrow cells were isolated by sterile flushing and combining of marrow from 3 tibias per treatment group. Nucleated cells were plated at 1 × 10<sup>6</sup>/well in 24-well plates (n = 4 wells/treatment group) with 700 μl of DMEM/10% FCS containing 1 μg/ml LPS. Cell supernatants were isolated after 24 hours and stored at –70°C for later analysis. Commercial ELISAs were used to determine joint levels of IL-1 (R&D Systems, Minneapolis, MN), MCP-1 (Assay Designs, Ann Arbor, MI), GRO/KC (R&D Systems), and RANKL (R&D Systems) using cytosolic tissue homogenates obtained during the isolation of nuclear proteins, as described above.



**Figure 2.** Effect of turmeric fraction on NF- $\kappa$ B activation. Nuclear proteins from the hind paws of animals treated with vehicle alone, turmeric extract (23 mg/kg/day), peptidoglycan-polysaccharides for streptococcal cell wall-induced arthritis (SCW), or SCW plus turmeric extract were isolated 3 days (A) or 28 days (B and C) after SCW injection from 3 paws per group and combined in equal amounts for analysis. Electrophoretic mobility shift assay of an unbound biotinylated DNA probe versus a protein-bound biotinylated DNA probe specific for NF- $\kappa$ B was performed using 1 combined sample per treatment group. Supershifts to verify band specificity and the presence of heterodimers or homodimers (C) were performed by incubating nuclear extracts with antibodies (Ab) directed against the p50 and p65 subunits of NF- $\kappa$ B. Specificity of the NF- $\kappa$ B band was also verified by competition with an excess of unlabeled NF- $\kappa$ B probe (results not shown).

**Osteoclastogenesis assay.** As previously described (17), subsequent to sterile flushing and combining of marrow from 3 tibias isolated from each treatment group, nucleated

cells were plated at  $2 \times 10^5$ /well in 24-well plates with 800  $\mu$ l of  $\alpha$ -minimum essential medium/15% FCS containing 50 ng/ml macrophage colony-stimulating factor (M-CSF; Intergen, Purchase, NY) plus 300 ng/ml RANK-activating antibody (R&D Systems). One-half of the media was replaced with fresh M-CSF- and RANK-activating antibody-containing media after 2 days. On day 5, the number of TRAP-positive (Acid Phosphatase Leukocyte TRAP kit no. 387-A; Sigma, St. Louis, MO) cells containing more than 3 nuclei was counted in each well (4 wells per treatment group).

**PGE<sub>2</sub> production in arthritic joints.** Hind ankle joints were isolated and biopulverized as previously described. A known amount of tissue ( $\leq 0.5$  gm) was homogenized using a Polytron in 1 ml of homogenization buffer (0.1M potassium phosphate [pH 7.4], 1 mM EDTA, and 10  $\mu$ M indomethacin). Samples were adjusted to 5 times the sample volume with acetone. Following centrifugation to remove precipitate, the supernatant was evaporated to dryness. Pellets were resuspended in 500  $\mu$ l of UltraPure water (Cayman Chemical, Ann Arbor, MI) and an equal volume of column buffer before loading on PGE<sub>2</sub> affinity purification columns (1 fresh column per joint to ensure uniform extraction) in accordance with the manufacturer's instructions (Cayman Chemical). Vacuum-dried samples ( $n = 5-7$  per treatment group) were reconstituted in sample buffer and assayed using a commercial PGE<sub>2</sub> ELISA (R&D Systems). Values are expressed as total ng PGE<sub>2</sub> per joint (18).

**Statistical analysis.** Values are presented as the mean  $\pm$  SEM except where indicated. Statistical significance was determined by ANOVA with post hoc testing, Student's *t*-test or by Fisher's exact test, as appropriate, using InStat software (GraphPad Software, San Diego, CA).

## RESULTS

**Results of chemical and biologic analysis of experimental turmeric extracts.** From ground turmeric rhizome, 2 extracts were isolated: 1) a crude extract containing essential oils and 34% (by weight) of the 3 major curcuminoids (curcumin, demethoxycurcumin, and *bis*-demethoxycurcumin) (Table 1) that inhibited in vitro LPS-stimulated PGE<sub>2</sub> production with a 50% inhibitory concentration (IC<sub>50</sub>) of 0.13  $\mu$ g/ml, and 2) an essential oil-depleted fraction containing 41% curcuminoids (Table 1) with an IC<sub>50</sub> of 0.48  $\mu$ g/ml for in vitro PGE<sub>2</sub> inhibition. The composition of each of the extracts was extremely complex, since  $>50\%$  of their weight consists of a complex mixture of compounds that either lack a chromophore or are present in quantities undetectable by HPLC. Identification of these other components is currently under way in one of the authors' laboratories (18).

**Results of chemical analysis of commercial turmeric dietary supplements.** The majority of over-the-counter turmeric dietary supplements tested were free of essential oil components (Table 1). As with our experimentally prepared turmeric fraction, the 3 major

**Table 3.** Gene expression in rats treated or not treated with turmeric fraction\*

Gene type, accession no.	Gene description	Acute phase (day 3)		Chronic phase (day 28)	
		SCW	SCW + turmeric	SCW	SCW + turmeric
<b>Chemokines</b>					
NM_031530	<b>Chemokine (C-C motif) ligand 2 (MCP-1)</b>	11.9	4.8	3.0	1.6
U22414	Chemokine (C-C motif) ligand 3 (MIP-1 $\alpha$ )	4.9	2.3	2.4	1.1
AF053312	Chemokine (C-C motif) ligand 20 (MIP-3 $\alpha$ )	42.6	15.8	32.0	17.0
NM_030845	<b>Chemokine (C-X-C motif) ligand 1 (GRO/KC)</b>	25.4	8.0	43.0	11.9
BF419899	Chemotactic protein 3	28.9	9.8		
NM_022214	CXC chemokine LIX	13.7	3.7	13.2	1.0
NM_016994	Complement component 3	9.1	5.3	8.1	3.1
NM_017334	cAMP response element modulator	2.7	1.1		
NM_013016	Protein tyrosine phosphatase, nonreceptor type substrate 1	2.0	1.5		
<b>Chemokine receptors</b>					
NM_053619	Complement component 5, receptor 1	2.1	1.5		
NM_133542	Immunoglobulin superfamily, member 6	4.6	2.5		
NM_020103	Ly6-C antigen	3.9	2.0		
NM_020542	MIP-1 $\alpha$ receptor gene	2.1	1.1	2.4	1.2
<b>Cell adhesion proteins</b>					
X73371	Fc $\gamma$ receptor, IgG, low affinity IIb	6.3	3.4		
BI296880	$\beta$ 3 integrin			2.8	1.5
NM_013180	$\beta$ 4 integrin			0.5	0.7
AF003598	$\beta$ 7 integrin	2.4	1.6		
BG668993	$\beta$ 8 integrin (predicted)			0.2	0.7
NM_012587	Integrin-binding sialoprotein			2.4	1.0
L25527	Selectin, endothelial cell	3.6	1.9		
BI296054	Selectin, platelet	3.2	1.9	4.1	1.9
<b>Interleukin-1 signaling</b>					
NM_031512	<b>Interleukin-1<math>\beta</math></b>	4.6	3.0	4.1	1.7
BF391914	Interleukin-1 receptor accessory protein	2.6	1.8		
NM_022194	Interleukin-1 receptor antagonist	31.3	6.3		
NM_053953	Interleukin-1 receptor, type II	4.2	1.6	3.4	1.7
NM_013037	Interleukin-1 receptor-like 1	3.8	2.0		
AA799471	Toll interacting protein (predicted)			0.4	0.9
AI070419	Toll-like receptor 1 (predicted)	3.0	1.6	2.3	1.2
<b>Arachidonic acid metabolic pathways</b>					
U03389	<b>COX-2</b>	3.4	1.6	3.9	1.3
AB048730	Prostaglandin E synthase	2.1	1.4		
NM_031557	Prostaglandin I <sub>2</sub> (prostacyclin) synthase			0.4	0.7
NM_053639	Leukotriene C <sub>4</sub> synthase	0.5	0.7		
AI411541	Prostaglandin E receptor 3 (subtype EP3)			0.3	0.6
NM_013115	Prostaglandin F receptor			2.4	0.7
NM_019243	Prostaglandin F <sub>2</sub> receptor negative regulator			2.2	1.0
<b>Complement pathway</b>					
AI639117	<b>Properdin factor B</b>	21.6	11.9	5.7	2.1
AI169829	<b>Mannan-binding lectin serine peptidase 1</b>	4.7	2.5	4.9	1.6
NM_016994	Complement component 3	9.1	5.3	8.1	3.1
NM_053619	Complement component 5, receptor 1	2.1	1.5		
AI045191	Complement component 6			0.4	0.8
NM_024157	Complement factor I			2.3	1.3

curcuminoids contributed <50% of product weight, with a total curcuminoid content of 1.8–33.7% (Table 1). This can be contrasted to the total curcuminoid content of curcumin sold by scientific and wholesale botanical suppliers (the usual source of uncharacterized curcumin

products reported in the literature [6–9,19–21]), which ranges from 82% to 94% (n = 6 randomly selected products) and again represents an essential oil-free mixture of all 3 of the major curcuminoids in varying ratios (data not shown).

Table 3. Cont'd

Gene type, accession no.	Gene description	Acute phase (day 3)		Chronic phase (day 28)	
		SCW	SCW + turmeric	SCW	SCW + turmeric
<b>Wnt signaling pathway stimulators</b>					
AA944349	Frizzled homolog 1 ( <i>Drosophila</i> )	2.6	1.6		
L02530	Frizzled homolog 2 ( <i>Drosophila</i> )			2.4	1.4
AI072892	Frizzled-related protein (predicted)			3.3	1.1
BF396545	Secreted Frizzled-related protein 2			7.5	1.5
<b>Wnt signaling pathway inhibitors</b>					
NM_053738	Wnt inhibitory factor 1	0.5	0.6		
BI288833	Dapper homolog 2, antagonist of $\beta$ -catenin ( <i>Xenopus</i> ) (predicted)	0.4	0.6	0.4	0.8
<b>Bone and cartilage destruction stimulators</b>					
NM_057149	<b>RANKL</b>	2.6	0.9	4.6	1.5
AA858815	Cathepsin C	3.1	1.6	2.7	0.9
NM_017320	Cathepsin S	2.3	1.4		
NM_031560	Cathepsin K			3.0	1.2
NM_013153	Hyaluronan synthase 2	7.9	3.1	2.1	1.3
NM_053963	Matrix metalloproteinase 12	8.0	3.7	9.5	1.8
NM_133523	Matrix metalloproteinase 3	10.9	7.2		
BI294977	Matrix metalloproteinase 19 (predicted)	2.3	1.4	3.8	1.5
M60616	Matrix metalloproteinase 13			3.3	1.6
U65656	Matrix metalloproteinase 2			2.4	1.4
NM_031055	Matrix metalloproteinase 9			5.0	1.3
<b>Bone and cartilage destruction inhibitors</b>					
NM_053819	Tissue inhibitor of metalloproteinases 1			6.2	2.4
NM_012870	Osteoprotegerin (OPG)			0.4	0.8
<b>Bone and cartilage formation</b>					
AA899303	Procollagen, type II, $\alpha$ 1	0.2	0.3		
BM391350	Procollagen, type XI, $\alpha$ 2	0.4	0.6		
AA819747	Procollagen, type XIII, $\alpha$ 1 (predicted)	0.5	0.7		
AI176393	Procollagen, type IV, $\alpha$ 1 (predicted)			2.0	1.1
BE128699	Procollagen, type VIII, $\alpha$ 1 (predicted)			2.0	1.2
AI230238	Procollagen, type X, $\alpha$ 1			0.1	0.9
BM389291	Procollagen, type XI, $\alpha$ 1			2.5	0.9
AA958001	Collagen triple-helix repeat-containing 1			3.9	1.4
NM_134452	Collagen, type V, $\alpha$ 1			2.2	1.3
NM_021760	Collagen, type V, $\alpha$ 3			2.1	1.1
AI101782	Collagen, type XVIII, $\alpha$ 1			3.0	1.6
BF392901	Collagen, type XXVII, $\alpha$ 1			2.5	1.4
NM_012827	Bone morphogenetic protein 4			0.5	0.7
AI230985	Bone morphogenetic protein 6			0.5	0.9
BM389026	Periostin, osteoblast-specific factor (predicted)			3.9	2.0
AF072892	Chondroitin sulfate proteoglycan 2	7.6	3.7	3.4	2.2

\* Values are the fold change from values in vehicle (saline)-treated animals. Female Lewis rats were injected on day 0 with peptidoglycan-polysaccharides for streptococcal cell wall-induced arthritis (SCW; 25  $\mu$ g/gm body weight) or with vehicle. Intraperitoneal injections of turmeric fraction (23 mg curcuminoids/kg/day) or vehicle were begun 4 days prior to SCW administration and were continued on a daily basis for 14 days, after which the treatment frequency was decreased to 5 days/week. RNA was isolated from joints obtained 3 days or 28 days after SCW injection and processed for analysis of gene expression using Affymetrix GeneChip Rat Genome 230 2.0 arrays. Normalized data are only listed for SCW-injected or SCW-injected and turmeric fraction-treated animals if the values for SCW injection plus turmeric fraction treatment were significantly different from those for SCW injection alone at a given time point ( $P < 0.05$ ), as determined by analysis of variance and Student-Newman-Keuls post hoc testing using GeneSpring software. For genes shown in boldface, regulation by turmeric fraction treatment was confirmed by real-time reverse transcription-polymerase chain reaction. MCP-1 = monocyte chemoattractant protein 1; MIP-1 $\alpha$  = macrophage inflammatory protein 1 $\alpha$ ; GRO/KC = growth-related oncogene/keratinocyte chemoattractant; COX-2 = cyclooxygenase 2.

**Effect of turmeric fraction on local and systemic inflammation in the SCW-induced arthritis model.** An initial experiment comparing the antiarthritic efficacy of the essential oil-free turmeric fraction with that of the

crude turmeric extract (dose normalized to 46 mg of the 3 major curcuminoids/kg/day) demonstrated a profound inhibition of SCW-induced arthritis that is rarely seen in this model when administration was begun 4 days prior

to SCW injection (Figure 1A). All subsequent experiments were conducted using the essential oil-free turmeric fraction, since its chemical composition most closely matched those of commercial dietary turmeric supplements purchased by the public. Turmeric fraction prevented acute and chronic arthritis with an  $IC_{50}$  of 12–16 mg curcuminoids/kg/day when administered IP (Figure 1B). Delayed treatment with turmeric fraction was also effective in preventing chronic arthritis (Figure 1C). An increase in mortality (6%) of unknown cause occurred in animals (normal or SCW injected) treated IP with turmeric fraction (Table 2). However, surviving turmeric fraction-treated animals had no signs of toxicity, as determined by measurement of ALT (Table 2), creatinine (Table 2), leukocyte counts (Table 2), hematocrit (Table 2), or daily weight gain (data not shown). Indeed, leukocytosis and anemia, 2 systemic signs of inflammation associated with SCW treatment (15), were significantly inhibited by the turmeric fraction (Table 2).

#### **Effect of turmeric fraction on joint destruction.**

Turmeric fraction significantly inhibited SCW-induced destruction of articular cartilage and periarticular bone, as measured by a histologic index of proximal tibia cartilage destruction (66% inhibition) and proximal femur BMD (57% inhibition), respectively (Table 2).

**Effect of turmeric fraction on NF- $\kappa$ B activation in joints.** Turmeric fraction inhibited NF- $\kappa$ B activation as early as day 3 in joints of SCW-injected animals (Figure 2A) and also during actual joint destruction (day 28) (Figure 2B). Confirmation of the identity of NF- $\kappa$ B was obtained by incubation with excess unlabeled probe (data not shown) and by supershift assay (Figure 2C), which suggested that both homodimers and heterodimers of the p50 and p65 subunits of NF- $\kappa$ B may be activated in joints of SCW-injected animals.

**Effect of turmeric fraction on gene expression in arthritic joints.** Turmeric fraction had little effect on gene expression in normal joints as determined by microarray analysis of more than 28,000 genes, altering the expression of only 28 or 6 known genes after 7 or 32 days of treatment, respectively (data not shown). In contrast, in arthritic joints, turmeric fraction significantly altered the expression of 351 genes (200 genes of known function) during acute joint swelling and 979 genes (498 of known function) during chronic joint destruction.

Turmeric treatment targeted at least 42 NF- $\kappa$ B-regulated genes, as identified by data mining software (Pathway Assist version 3.0; Stratagene, La Jolla, CA), including key regulators of joint inflammation and destruction in arthritis such as GRO/KC, MCP-1, IL-1 $\beta$ ,

COX-2, and RANKL (Table 3). Real-time RT-PCR was used to confirm the turmeric-induced suppressed expression of selected physiologically significant NF- $\kappa$ B-regulated genes (expressed as the mean  $\pm$  SEM fold change from control in joints of SCW-injected rats versus joints of SCW-injected and turmeric-treated rats), including GRO/KC ( $121.3 \pm 17.1$  versus  $39.4 \pm 9.4$  on day 3;  $P < 0.01$ ), MCP-1 ( $32.2 \pm 11.7$  versus  $9.7 \pm 2.5$  on day 3;  $P < 0.05$ ), IL-1 $\beta$  ( $6.0 \pm 0.9$  versus  $2.5 \pm 0.4$  on day 3;  $P < 0.01$ ), COX-2 ( $3.9 \pm 0.8$  versus  $1.5 \pm 0.1$  on day 3;  $P < 0.01$ ), and RANKL ( $3.6 \pm 0.2$  versus  $1.1 \pm 0.1$  on day 28;  $P < 0.001$ ), as well as components of the complement cascade (properdin factor B [ $36.8 \pm 1.3$  versus  $21.8 \pm 4.1$  on day 3;  $P < 0.05$ ] and mannan-binding lectin serine peptidase 1 [ $7.3 \pm 0.3$  versus  $4.8 \pm 0.5$  on day 3;  $P < 0.05$ ]).

Moreover, the expression of genes controlled by NF- $\kappa$ B-regulated gene products was also affected by turmeric treatment. For example, the expression of 70 IL-1-regulated genes, identified by Pathway Assist software, was normalized by turmeric treatment (data not shown), consistent with the inhibition of articular IL-1 $\beta$  gene expression as demonstrated by microarray analysis, real-time RT-PCR, and measurement of IL-1 $\beta$  protein levels in joints of SCW-injected rats versus joints of SCW-injected and turmeric-treated rats ( $403.8 \pm 135.9$  pg/mg total protein versus  $17.8 \pm 14.4$  pg/mg total protein;  $P < 0.05$ ).

Chemokines, including neutrophil chemokines (e.g., CXC chemokine LIX and GRO/KC) and monocyte chemokines (e.g., chemotactic protein 3 and MCP-1), comprised the majority of genes inhibited by turmeric fraction treatment whose expression was induced more than 10-fold in arthritic joints, as demonstrated by microarray analysis (Table 3). In joints of SCW-injected rats versus joints of SCW-injected and turmeric-treated rats, measurement of protein levels of MCP-1 ( $780.0 \pm 147.5$  pg/mg total protein versus  $57.0 \pm 29.8$  pg/mg total protein;  $P < 0.05$ ) and GRO/KC ( $23.8 \pm 4.3$  pg/mg total protein versus  $1.6 \pm 0.1$  pg/mg total protein;  $P < 0.05$ ), which were not detectable in control joints, confirmed the inhibitory effect of turmeric on chemokine expression. Adhesion factors that facilitate inflammatory cell recruitment to the joint were also targets of turmeric fraction treatment (Table 3).

In arthritic joints, turmeric fraction also suppressed gene expression that favored signaling by the IL-1 receptor superfamily (Table 3) (22), while increased expression of TNF $\alpha$  and IL-6 were unaltered by turmeric fraction treatment (data not shown). Increased PGE<sub>2</sub> synthetic pathways, complement activation, and Wnt signaling in arthritic joints were also blocked by

**Table 4.** Effect of turmeric fraction on inflammation\*

	Acute phase (day 3)				Chronic phase (day 28)			
	Vehicle	Turmeric fraction	SCW	SCW plus turmeric fraction	Vehicle	Turmeric fraction	SCW	SCW plus turmeric fraction
Inflammatory cells in joints, /mm <sup>2</sup> of tissue								
Neutrophils								
Effusions	0.0 ± 0.0	0.0 ± 0.0	685 ± 142†	298 ± 66‡§	0.0 ± 0.0	0.0 ± 0.0	1,134 ± 168†	6.3 ± 0.5§
Synovium	48.5 ± 16	18.2 ± 18	1,359 ± 332¶	1,006 ± 158¶	0.0 ± 0.0	0.0 ± 0.0	1,814 ± 289†	90.4 ± 27.2§
Monocyte/macrophages								
Synovium	0 ± 0	191 ± 191	3,494 ± 320†	961 ± 94‡§	196 ± 33	125 ± 27	1,453 ± 84†	316 ± 28§
Ex vivo splenocyte chemokine secretion, fold change from that in vehicle								
GRO	1.0 ± 0.2	0.6 ± 0.2	9.8 ± 1.7†	6.8 ± 0.9†#	1.0 ± 0.0	0.4 ± 0.1¶	4.2 ± 0.1†	0.7 ± 0.1‡§
MCP-1	1.0 ± 0.0	1.0 ± 0.0	155.8 ± 12.1†	110.8 ± 10.7†**	1.0 ± 0.2	0.8 ± 0.2	40.9 ± 9.9†	13.5 ± 2.5**
Joint PGE <sub>2</sub> , ng/paw	3.1 ± 0.8	6.4 ± 1.9	25.0 ± 7.6‡	13.9 ± 2.6	10.6 ± 2.7	4.6 ± 0.8	175.2 ± 44.1†	37.5 ± 25.2**

\* Values are the mean ± SEM. Female Lewis rats were injected on day 0 with peptidoglycan-polysaccharides for streptococcal cell wall-induced arthritis (SCW; 25 µg/gm body weight) or with vehicle. Intraperitoneal injections of turmeric fraction (23 mg curcuminoids/kg/day, except where indicated) or vehicle were begun 4 days prior to SCW administration and were continued on a daily basis for 14 days, after which the treatment frequency was decreased to 5 days/week. Joints were isolated for histologic analysis of cellular infiltrates on day 3 (n = 4–8 joints/group) or day 28 (n = 4–12 joints/group receiving 46 mg curcuminoids/kg/day). Constitutive chemokine secretion after 48 hours of ex vivo culture (n = 4 wells/group) was measured by enzyme-linked immunosorbent assay from splenocytes isolated and combined from 3 animals/group. Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) levels in joint extracts were measured as described in Materials and Methods on day 3 (acute phase) or day 28 (chronic phase) after SCW injection (n = 5–7/treatment group). See Table 3 for other definitions.

† P < 0.001 versus vehicle-treated rats, by analysis of variance (ANOVA) with post hoc testing.

‡ P < 0.05 versus vehicle-treated rats, by ANOVA with post hoc testing.

§ P < 0.001 versus SCW-injected rats not treated with turmeric fraction, by ANOVA with post hoc testing.

¶ P < 0.01 versus vehicle-treated rats, by ANOVA with post hoc testing.

# P < 0.05 versus SCW-injected rats not treated with turmeric fraction, by ANOVA with post hoc testing.

\*\* P < 0.01 versus SCW-injected rats not treated with turmeric fraction, by ANOVA with post hoc testing.

turmeric treatment (Table 3). Increased expression of cartilage-destroying matrix metalloproteinases and bone-destructive RANKL was also inhibited by turmeric fraction treatment (Table 3). Conversely, turmeric fraction normalized the suppressed expression of joint-protective and/or essentially anabolic gene products (Table 3). For example, expression of RANKL, the biologic gatekeeper for bone resorption (23,24), was increased 4.6-fold in arthritic joints on day 28, while expression of osteoprotegerin (OPG), an inhibitory RANK-decoy receptor (23,24), was suppressed to 40% of normal (RANKL:OPG expression ratio, an index of bone resorption normalized to control joints, of 11.5) (Table 3). In contrast, turmeric fraction almost normalized the RANKL:OPG ratio in SCW-injected animals on day 28 (1.9 in joints of SCW-injected and turmeric-treated rats versus 1.0 in joints of vehicle-treated rats) (Table 3). Assay of soluble RANKL protein levels in joints of SCW-injected rats versus joints of SCW-injected and turmeric-treated rats confirmed the inhibitory effect of turmeric on RANKL gene expression (200.6 ± 29.0 pg/mg total protein versus 7.1 ± 0.1 pg/mg total protein; P < 0.01).

**Effect of turmeric fraction on inflammatory cell influx in arthritic joints.** Turmeric treatment inhibited neutrophil and monocyte influx on both day 3 and day 28 in the joints of SCW-injected animals (Table 4).

**Effect of turmeric fraction on hepatic and splenic granuloma formation.** The turmeric fraction significantly inhibited granuloma formation in the liver (87% incidence in SCW-injected animals versus 36% incidence in SCW-injected and turmeric fraction-treated animals; P < 0.001) and spleen (73% incidence versus 23% incidence; P < 0.001). Increased neutrophil (GRO/KC) and monocyte (MCP-1) chemokine secretion from splenocytes isolated from SCW-injected animals was also inhibited by in vivo turmeric fraction treatment (Table 4).

**Effect of turmeric fraction on PGE<sub>2</sub> production in joints.** PGE<sub>2</sub> levels in the talotibial joints of SCW-injected animals were statistically elevated on day 3, but peaked during the chronic destructive phase of joint swelling (day 28) (Table 4). Turmeric fraction prevented 83% of the major increase in PGE<sub>2</sub> on day 28, while a 50% reduction in PGE<sub>2</sub> levels on day 3 did not achieve statistical significance (Table 4).

**Effect of turmeric fraction on osteoclast formation.** An increase in periarticular osteoclasts at sites of bone destruction, such as the tibial epiphysis and metaphysis, in SCW-injected animals was prevented by turmeric treatment, while osteoclast numbers in normal animals were unchanged (Table 2). In vivo turmeric treatment prevented the almost 10-fold increase in ex vivo LPS-stimulated TNF secretion from tibial bone marrow cells isolated from vehicle-treated SCW-injected animals (Table 2). In vivo turmeric fraction treatment also inhibited osteoclastogenesis induced ex vivo by M-CSF and a RANK-stimulating antibody in bone marrow cells isolated from SCW-injected and normal animals (Table 2).

## DISCUSSION

Complementary and alternative medicine use, including dietary supplements, is self-reported in 42% of persons with arthritis in US studies, with 72% using complementary and alternative medicines for disease treatment (25). In contrast, scientific data supporting dietary supplement use, which has increased since the passage of the Dietary Supplement Health and Education Act by Congress in 1994 (4), is frequently lacking not only in quantity, but also in quality, since researchers often do not appreciate the need to identify and describe test material (4,5). This is particularly true in the study of chemically complex botanicals. The results reported here are therefore significant in that they provide, to our knowledge, 1) the first documentation of the chemical composition of a curcumin-containing extract tested in vivo for antiarthritic efficacy, 2) the first evidence of antiarthritic efficacy of a complex turmeric extract analogous in composition to turmeric dietary supplements (versus uncharacterized curcumin products), and 3) the first in vivo documentation of mechanisms of action of curcumin-containing extracts in arthritis treatment. It is interesting to note that the enhanced efficacy of turmeric fraction in the prevention of arthritis compared with treatment of existing arthritis (82% versus 34% inhibition, respectively, for 23 mg/kg/day) is analogous to the protective effects of specific inhibitors of NF- $\kappa$ B (60% versus 33%, respectively [26]) or currently marketed inhibitors of TNF (65% versus 37%, respectively [27]) in animal models of RA.

Because turmeric is used as a dietary supplement, we documented its efficacy with both IP and oral administration in our initial SCW-induced arthritis studies (12). Because of known effects of gastrointestinal adsorption and metabolism on curcumin delivery, IP

dosing was subsequently used here in mechanistic studies to ensure uniform botanical delivery. A turmeric fraction  $IC_{50}$  of 15 mg curcuminoids/kg/day for IP administration, which approximates the 23 mg/kg/day dose used in our mechanistic studies, would correspond to a daily oral dose of  $\sim 1.5$  gm of the 3 major curcuminoids in humans if 1) one assumes that all of the biologic effect of the turmeric fraction is due to its curcuminoid content, 2) one assumes that curcuminoids have an oral bioavailability of 10% in humans (10), and 3) one additionally corrects for the surface area of rodents compared with that of humans (28,29). A dose of 1.5 gm/day is well below the 8 gm/day of uncharacterized curcumin product reported to be well tolerated and nontoxic in humans (30) and greater than the dose of 1 gm/day used in the only clinical study of a curcumin-containing product in RA of which we are aware, a study lacking a placebo arm that reported some clinical efficacy in a double-blind crossover trial of an uncharacterized, proprietary curcumin product and phenylbutazone (6).

The cause of mortality in 5 of 87 animals, which occurred after at least 17 days of daily IP administration of turmeric fraction, could not be elucidated by evaluation of complete blood cell counts, screening of liver or kidney function, or necropsy of surviving animals. It should be noted that no increased mortality was seen in our previous turmeric trials using oral or IP administration of a more "purified" extract, more than 90% of which consisted of the 3 major curcuminoids (12), suggesting the possibility that the other components (18) and/or IP dosing of the turmeric extract used here contributed to mortality.

Our demonstration of turmeric's in vivo inhibition of articular NF- $\kappa$ B, a transcription factor activated in vascular endothelium and synovial cells in RA joints (31), and of key inflammatory genes directly or indirectly activated by NF- $\kappa$ B suggests that NF- $\kappa$ B inhibition may be a critical mechanism of turmeric's protective antiarthritic effect. Results of previous in vitro studies demonstrating inhibition of NF- $\kappa$ B activation by blockade of upstream pathways by curcumin products (i.e., inactivation of the I $\kappa$ B kinase complex [32]) are consistent with this postulate. Thus, it would appear that turmeric dietary supplements share the same mechanism of action as antiarthritic pharmaceuticals currently under development that target NF- $\kappa$ B (33,34). Given the critical role of NF- $\kappa$ B as the "master switch" in innate immunity (35), these in vivo experiments also provide proof-of-concept for the use of this botanical in other diseases triggered by inappropriate activation

of NF- $\kappa$ B-regulated inflammatory pathways, including inflammatory bowel disease, asthma, and multiple sclerosis (33).

The data presented here do not exclude the possibility that turmeric also directly blocks inflammatory pathways that parallel, or are distal to, NF- $\kappa$ B. Moreover, the chemical complexity and *in vitro* antiinflammatory activity of noncurcuminoid subfractions of the turmeric extract would support this postulate (11). Clearly, blockade of chemokine production and inflammatory cell infiltration, whether due to inhibition of NF- $\kappa$ B or to other direct or indirect effects of turmeric, appears to be central to the *in vivo* antiinflammatory effect of turmeric, contributing to the prevention of both synovitis and granulomatous inflammation. Moreover, the fact that delayed turmeric treatment prevents arthritis when treatment is started 3 days, but not 8 days, after SCW injection (12) is also consistent with the hypothesis that blockade of early inflammatory cell influx is central to the protective antiinflammatory effect of turmeric. Turmeric's inhibition of other pathways whose role in arthritis and other inflammatory diseases is just being recognized, including canonical Wnt signaling and complement activation (36–39), further supports the postulate that its *in vivo* antiarthritic and, indeed, antiinflammatory effects are multifactorial.

Turmeric dietary supplements are often recommended to the public as alternatives to COX inhibitors (3). Results from our *in vivo* studies, however, suggest that turmeric inhibits PGE<sub>2</sub> production at sites of inflammation by preventing a local induction of COX-2 expression. Moreover, this conclusion is further supported by *in vitro* assays by our research group that reveal no effect of the turmeric fraction or of >90% curcuminoid extracts on COX-1 or COX-2 enzyme activity (Lantz RC, et al: unpublished observations).

In addition to the prevention of joint inflammation in the SCW-induced arthritis model, the effects of turmeric on osteoclast-mediated joint destruction are also noteworthy. *In vitro*, curcumin products can block RANKL-mediated osteoclast activation via direct effects on osteoclasts (19,20). However, our data provide the first *in vivo* documentation of an antiresorptive effect of a curcumin-containing turmeric product. Moreover, our results suggest that, in addition to possible direct effects on osteoclasts, turmeric also blocks the production of local inflammatory stimulators of osteoclasts, including normalization of the local RANKL:OPG ratio. Given the importance of IL-1 in mediating bone destruction in arthritis (40) and the resorptive effects of TNF $\alpha$  (41), it

is also interesting to note that turmeric suppressed the expression of IL-1, but not TNF $\alpha$ , in the joints during both the acute and chronic phases of SCW-induced arthritis.

In summary, just as the willow bark provided relief for arthritis patients before the advent of aspirin, it would appear that the underground stem (rhizome) of a tropical plant may also hold promise for the treatment of joint inflammation and destruction. Clearly, however, additional preclinical and clinical trials must be conducted before the use of turmeric for arthritis can be recommended. For example, contrary to the popular view that complex botanicals may offer advantages over the use of single compounds with respect to efficacy and toxicity, additional trials in our laboratory suggest that a more highly purified curcuminoid-containing extract of turmeric may be more potent and less toxic than the turmeric sample used in the studies reported here (12). In addition, the results of trials such as these that reveal remarkable antiinflammatory effects of botanicals can only be translated to clinical use if adequate and accurate information is available regarding the chemical content and biologic activity of commercial botanical supplements available for use. Finally, before turmeric supplements can be recommended for medicinal use, clinical trials are clearly needed to verify/determine whether treatment with adequate doses of well-characterized turmeric extracts can indeed prevent/suppress disease flares in RA patients, as well as to explore any potential benefits of turmeric dietary supplements in the prevention or treatment of more common forms of arthritis in the general population.

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