Research Article

Modified Huo-Luo-Xiao-Ling Dan Suppresses Adjuvant Arthritis by Inhibiting Chemokines and Matrix-Degrading Enzymes

Siddaraju M. Nanjundaiah, 1 David Y.-W. Lee, 2 Zhongze Ma, 2 Harry H. S. Fong, 3 Lixing Lao, 4 Brian M. Berman, 4 and Kamal D. Moudgil 1

1 Department of Microbiology and Immunology, University of Maryland School of Medicine, HSF-1, Suite 380, 685 West Baltimore Street, Baltimore, MD 21201, USA
2 Mailman Research Center, McLean Hospital, Harvard Medical School, Belmont, MA 02478, USA
3 Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60612, USA
4 Center for Integrative Medicine, University of Maryland School of Medicine, East Hall, 520 W. Lombard Street, Baltimore, MD 21201, USA

Correspondence should be addressed to Kamal D. Moudgil, kmoud001@umaryland.edu

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Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting the joints that can lead to deformities and disability. The prolonged use of conventionally used drugs is associated with severe adverse reactions. Therefore, safer and less expensive therapeutic products are continually being sought. Huo-Luo-Xiao-Ling dan (HLXL), a traditional Chinese herbal mixture, and its modified versions possess anti-arthritic activity. In this paper, we examined the influence of modified HLXL on two of the key mediators of arthritic inflammation and tissue damage, namely, chemokines and matrix-metalloproteinases (MMPs) in the rat adjuvant-induced arthritis (AA) model of RA. We treated arthritic Lewis rats with HLXL (2.3 g/kg) by daily gavage beginning at the onset of AA. The control rats received the vehicle. At the peak phase of AA, rats were sacrificed and their draining lymph node cells (LNC) and spleen adherent cells (SAC) were tested. The HLXL-treated rats showed a significant reduction in the levels of chemokines (RANTES, MCP-1, MIP-1α, and GRO/KC), MMPs (MMP 2 and 9), as well as cytokines (IL-6 and IL-17) that induce them, compared to the control vehicle-treated rats. Thus, HLXL controls arthritis in part by suppressing the mediators of immune pathology, and it might offer a promising alternative/adjunct treatment for RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic debilitating autoimmune disease that affects over 1 percent of the population worldwide [1]. The commonly used drugs such as nonsteroidal anti-inflammatory drugs (NSAIDS) and biologics (e.g., antitumor necrosis-α antibody) are effective in alleviating the symptoms of the disease. However, the prolonged use of these drugs is associated with severe adverse reactions [2, 3]. In addition, these drugs are expensive, and not all patients respond well to them. In view of these limitations, it is essential to continue the search for safer and less expensive alternatives to the conventionally used drugs [4, 5]. Natural plant products represent a promising group of therapeutic agents for arthritis. However, one of the major concerns in seriously considering these products for therapeutic purposes is that the mechanisms of action of many of them are poorly defined, if at all.

RA primarily targets the joints, and is characterized by inflammatory synovitis mediated by leukocytes and the proinflammatory cytokines secreted by them [1, 6]. The migration of leukocytes from the peripheral blood into the joints is directed by chemotactic cytokines (chemokines) [7]. Furthermore, severe arthritis is associated with cartilage and bone damage, which is mediated in part by the matrix-degrading enzymes, matrix metalloproteinases (MMPs) [8, 9]. Therefore, chemokines and MMPs are attractive targets for the treatment of arthritis [10, 11].
Chemokines are small, biologically active molecules that attract specific populations of inflammatory cells and regulate their trafficking to the site of inflammation. Among the chemokines that play an important role in inflammation, including RA, are regulated upon activation, normal T cell expressed, and secreted (RANTES), also known as chemokine C-C motif ligand 5 (CCL5); monocyte chemotactic protein-1 (MCP-1), or CCL2; macrophage inflammatory protein-1α (MIP-1α), or CCL3; and growth regulated oncogene-keratinocyte chemoattractant (GRO/KC), or chemokine C-X-C motif ligand 1 (CXCL1) [12–15]. The chemokines gene-keratinocyte chemoattractant (GRO/KC), or chemok-protein-1 (MCP-1), or CCL2; macrophage inflammatory expressed, and secreted (RANTES), also known as chemok- including RA, are regulated upon activation, normal T cell

 Evidence-Based Complementary and Alternative Medicine

2. Materials and Methods

2.1. Animals. Five- to six-week-old male Lewis (LEW/Hsd) (RT1;1) rats were purchased from Harlan Sprague-Dawley (HSD) (Indianapolis, IN, USA) and then maintained in the animal care facility of the University of Maryland School of Medicine, Baltimore. All experimental procedures performed on these rats were in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC).

2.2. Composition and Characteristics of HLXL. The traditional Huo-Luo-Xiao-Ling Dan (HLXL) consists of four herbs, namely, Danggui (Angelica sinensis (Oliv.) Diels), Danshen (Salvia miltiorrhiza Bge.), Ruxiang (Boswellia carterii Birdw.), and Moyao (Commiphora myrrha Engl.). We have previously reported [21–23] the rationale and the nature of modification of this original formula, and the modified HLXL is a defined mixture of 11 herbs, namely, Ruxiang (Boswellia carterii Birdw.), Qianghuo (Notopterygium incisum Ting ex H.T. Chang), Danggui (Angelica sinensis (Oliv.) Diels), Baishao (Paonia lactiflora Pall.), Gancao (Glycyrrhiza uralensis Fisch.), Yanhusuo (Corydalis yanhusuo W.T. Wang.), Danshen (Salvia miltiorrhiza Bge.), Chuanxiong (Ligusticum chuanxiong S.H. Qiu.), Qin jiao (Gentiana macrophylla Pall.), Guizhi (Cinnamomum cassia Presl.), and Duhuo (Angelica pubescens Maxim). The compounds isolated from HLXL include steroids, terpenes, alkaloids, flavonoids, glycosides, and acids [21]. The methods for the preparation of HLXL, for the characteristics of its component herbs, and for the assessment of its toxicity have been described in detail elsewhere [22, 23]. As in our earlier studies [23, 24] the batch of HLXL tested in this study was thoroughly characterized by HPLC fingerprinting, which were characterized by the peak shapes, numbers, intensities, and retention times of all individual compounds (data not shown). Moreover, the marker compounds, swer-tiamarin (from Gentiana macrophylla), osthol (Angelica pubescens), paeoniflorin (Paonia lactiflora), iso-imperatorin (Notopterygium incisum), liquiritin (Glycyrrhiza uralensis), columbianadin (Angelica pubescens), liquiritigenin (Glycyr rhiza uralensis), falcarnidol (Notopterygium incisum), angelol B (Angelica pubescens), cryptotanshinone (Salvia miltiorrhiza), bergapten (Angelica pubescens), tanshinone IIA (Salvia miltiorrhiza), senkyunolide A (Ligusticum chuan- xiong), ostruthin (Notopterygium incisum), phenethyl trans- ferulate (N. incisum), and anhydrotoptol (N. incisum), served as references for quality control purposes. In a recent study [21], we reported that some of the above-mentioned herbs other than the 4 traditionally used in classical HLXL, possessed compounds that served as ligands for the enzyme cyclo-oxygenase 2 (COX-2). This further reinforces in part the rationale for the modification of the original HLXL formula.

2.3. Treatment of Arthritic Rats with HLXL. Lewis rats were immunized subcutaneously (s.c.) with 1 mg/rat heat-killed M. tuberculosis H37Ra (Mtb) (Difco, Detroit, MI) in 200 μL of mineral oil (Sigma-Aldrich) at the base of the tail. Following the onset of arthritis, these rats were randomly divided into two groups (experimental and control). HLXL was finely powdered and suspended in 200 mL of water using a pestle and mortar, and it was fed (2.3 g/kg) to the experimental group of rats using a gavage needle (FNC-16-3, Kant Scientific Corporation, Torrington, CT, US) beginning on the day of onset of arthritis (d 10) and then continued
up to the peak phase of AA (d 18). On the corresponding
days, the control group of rats received water (the vehicle)
by gavage. All rats were examined and graded regularly for
the severity of arthritis as described earlier [24, 26]. The test
samples were collected from rats when the disease reached
the peak phase (d 18) in controls.

2.6. Determination of Matrix Metalloproteinase (MMP)/
Cytokine mRNA Expression by qRT-PCR. RNA was isolated
from these SAC as described below. A single cell suspension
of LNC was prepared in HL-1 serum free medium (Ventrex
Laboratories, Portland, ME) supplemented with 2 mM L-
glutamine, 100 U/mL penicillin G sodium, and 100 μg/mL
streptomycin sulfate. In each experiment, LNC from 3
rats were pooled for testing. These LNC were cultured (8
× 10^6 cells/well) in a 6-well plate (Corning Incorporated
Corning, NY) for 24 h at 37°C in an atmosphere of 95% air
and 5% CO_2. Sonicated Mtb (10 μg/mL) was used for res-
timulation of LNC. Keyhole limpet hemocyanin (KLH) was
used as the control antigen. Total RNA was prepared from
these LNC for testing by quantitative real-time polymerase
chain reaction (qRT-PCR) as described below. Alternatively,
the LNC culture supernatant was harvested for testing by a
Multiplex assay as elaborated below.

2.7. Determination of Chemokine/Cytokine Protein Expression
by Multiplex Suspension Bead Array Immunoassay. Multiplex
assays were performed at the Cytokine Core Facility (Univer-
sity of Maryland, Baltimore) using the Luminex 100 analyzer
(Luminex Corp., Austin, TX) and the level of cytokines (as
protein) in 24 h culture supernatant of LNC and SAC were
measured. The Rat chemokine 4-plex kit (Millipore) was
used to measure RANTES, MCP-1, MIP-1α, and GRO/KC and
the Rat cytokine 2-plex kit was used to measure IL-6 and
IL-17.

2.8. Measurement of MMP Activity Using a Zymogram Assay.
The MMP activity in the culture supernatants of LNC and
SAC stimulated with or without Mtb was determined using a
zymogram assay as described previously [26, 28]. Briefly,
culture supernatant was loaded onto a gelatin-coated, pre-
cast polyacrylamide gel (Bio-Rad). Electrophoresis was
carried out under SDS-nonreducing conditions at constant
temperature. The gel was incubated with 2.5% Triton X-100
at room temperature for 1-2 h to remove SDS. The gel
was then washed 3-4 times with water to remove Triton
X-100 and incubated overnight at 37°C in a developing
buffer (Tris-HCl, pH 7.4) containing 5 mM CaCl_2, 0.2 M
NaCl, and 0.02% [Brij 35. Thereafter, the gel was stained
with Coomassie Brilliant Blue R-250. Standard MMP-2 and
MMP-9 (Sigma) were used as positive controls. The MMP
activity was visualized and scanned after destaining.
Thereafter, the intensity of the bands was quantitated by
densitometry using Image J software.

2.9. Statistical Analysis. The data were expressed as mean ±
SEM. Student’s t-test and ANOVA were used to assess the
significance of differences using GraphPad Prism version 4.0.
A P value of <0.05 was considered significant.

3. Results

As reported earlier [24], we observed in this study that HLXL

treatment of arthritic Lewis rats reduced the severity of AA.
The mean arthritic score on d 18 (peak phase of AA), was
2.2 for HLXL-treated group compared to 4.6 for the control
water-treated group, and this difference was statistically
significant (P < 0.02). We then determined the effect of
HLXL on specific chemokines, MMPs and cytokines on d
18 of arthritis and compared the results with those obtained
from the control rats. The results are presented below.

3.1. HLXL Treatment Downmodulates Chemokine Production
in Arthritic Lewis Rats. Chemokines and their receptors
coordinate the movement of cells of the immune system and
direct these cells to the site of inflammation. The antigen-
draining lymph nodes are the site of initial cellular activation
and interactions. In this context, we tested the effect of
HLXL treatment on chemokines produced by the draining
LNC. Specifically, we tested for RANTES, MCP-1, MIP-
1α, and GRO/KC. These chemokines were measured in
culture supernatants of the draining LNC restimulated with
sonicated Mtb for 24 h (Figure 1). We observed a 4.4-fold
3.2. HLXL Suppresses MMP-9 and MMP-2 Activity in Arthritic Rats. MMPs mediate the degradation of extracellular matrix macromolecules, and they play an important role in cartilage and bone damage in arthritic joints. Therefore, to further understand the mechanisms underlying the arthritis-protective effect of HLXL, we evaluated the mRNA expression and enzyme activity of MMPs in SAC that were harvested from HLXL-treated or control rats and then restimulated with sonicated Mtb for 24 h. Treatment with HLXL caused a 1.7-fold decrease in the expression of MMP-9 but no significant change in MMP-2 mRNA expression (see Supplementary Figure 1 in Supplementary material available online at doi: 10.1155/2012/589256). Furthermore, there was 2.3-fold suppression in the activity of MMP-9 and a 2.7-fold decrease in MMP-2 activity in HLXL-treated group compared to the controls (Figure 2).

3.3. HLXL Treatment Inhibits Antigen-Induced Pro-Inflammatory Cytokine Response in Arthritic Rats. IL-6 and IL-17 are proinflammatory cytokines that have a significant effect on different chemokines and MMPs involved in the arthritogenic processes. Therefore, we tested the levels of these two cytokines (as proteins) in the draining LNC that were harvested from HLXL-treated and control arthritic rats and then restimulated in vitro for 24 h with sonicated Mtb. There was a significant decrease in the level of IL-6 ($P < 0.0002$) and IL-17 ($P < 0.0001$) in HLXL-treated rats compared with water-treated rats (Figures 3(a) and 3(b)). HLXL-treated group
Evidence-Based Complementary and Alternative Medicine

Figure 2: The effect of HLXL on MMP activity in arthritic rats. Spleen adherent cells (SACs) harvested from rats fed with HLXL or the vehicle (water) were stimulated for 24 h with sonicated Mtb. The supernatants obtained from these cultured cells were analyzed for MMP-9 (a) and MMP-2 (b) activity using a zymogram assay. The results are representative of two independent experiments (Mtb = heat-killed M. tuberculosis H37Ra).

showed a 1.8-fold decrease in IL-6 and a 4.3-fold decrease in IL-17 compared to the controls. Furthermore, in SACs stimulated with sonicated Mtb, there was a 14 fold decrease in IL-6 and a 3.4 fold reduction in IL-17 in HLXL-treated rats compared to vehicle-treated rats (see Supplementary Figure 2). Thus, HLXL inhibited cytokine production in LNC as well as SAC, indicating a systemic effect.

4. Discussion

In this study based on the rat AA model of human RA, we observed that HLXL-treated rats had significantly reduced levels of chemokines (RANTES, MCP-1, MIP-1α, and GRO/KC) as well as matrix-degrading enzymes, MMP2 and MMP9. Also reduced were the levels of the proinflammatory cytokines IL-6 and IL-17.

4.1. The Involvement of Chemokines in the Pathogenesis of Arthritis and the Therapeutic Targeting of Chemokines. High levels of RANTES are found in the synovial fluid and serum obtained from patients with RA, and it promotes the migration of mononuclear cells (including lymphocytes) from blood vessels and synovial fluid into the synovium [12]. MCP-1 is chemotactic for monocytes and T lymphocytes [15] whereas GRO-α is a chemoattractant for neutrophils in RA [13]. Elevated levels of MIP-1α are found in sera and synovial fluid of RA patients suggesting that MIP-1α plays an important role in the progression of the disease [14]. Initial clinical trials on chemokine blockade in patients with RA suggest that targeting the chemokine and chemokine receptor family might provide a promising therapeutic approach for this deliberating disease [10]. In this context, the results of our study showing that the above-mentioned 4 chemokines are suppressed in HLXL-treated rats compared to the controls are of high significance.

In the AA model, high levels of RANTES are found in the peripheral blood at the onset of the disease, whereas MCP-1 is first detected in the synovial tissue and later detected in the peripheral blood on day 18 (peak phase of AA), when joint inflammation is already very active [25]. These results suggest that RANTES is essential for the initiation of the disease, whereas MCP-1 follows the disease onset. Blocking the receptor for MCP-1 before the onset of arthritis in AA affords protection against the disease [29]. In our study reported here, we observed a positive correlation between arthritic scores and MCP-1 levels, with both being reduced after HLXL treatment. Furthermore, our findings of suppression of MCP-1 and RANTES after HLXL treatment are supported by those of another study showing reduced serum levels of MCP-1 and RANTES in RA patients following treatment with formulations of dried encapsulated juice concentrate [30]. Similarly, these chemokines can be suppressed by green tea extract [31]. In regard to GRO/KC, it was shown in the CIA model that this chemokine is elevated in the synovial
Figure 3: Inhibition of proinflammatory cytokines in HLXL-treated arthritic rats. Lymph node cells (LNCs) harvested on d 18 (peak phase of arthritis) from HLXL-fed or water-fed rats were cultured for 24 h with or without sonicated Mtb. The culture supernatants were tested for IL-6 (a) and IL-17 (b) using a multiplex assay. The results were expressed as Δpg/mL. The results are representative of two independent experiments. (Mtb = heat-killed *M. tuberculosis* H37Ra).

Figure 4: A schematic representation of the diverse mechanisms involved in HLXL-induced suppression of AA. HLXL attenuated arthritis via suppressing the disease-regulating chemokines (regulated upon activation, normal T cell expressed and secreted: RANTES; monocyte chemotactic protein-1: MCP-1; macrophage inflammatory protein-1α: MIP-1α; growth regulated oncogene-keratinocyte chemoattractant: GRO/KC), matrix metalloproteinases (MMP-2 and MMP-9), and cytokines (IL-6 and IL-17). (S; spleen, L; lymph node, IJ; inflamed joint, Mtb; heat-killed *Mycobacterium tuberculosis* H37Ra).

fluid of arthritic rats and that its levels are reduced following suppression of arthritis by an inhibitor of spleen tyrosine kinase [32]. The results of our study in the AA model showed significant suppression of GRO/KC in HLXL-treated rats, which was associated with reduced severity of AA compared to the control rats.

4.2. The Induction and Regulation of MMPs by Pro-Inflammatory Cytokines and Chemokines, and the Control of MMP Activity for Therapeutic Purposes. Macrophages and fibroblasts synthesize and secrete several MMPs that participate in the degradation of ECM components and contribute significantly to the tissue damage in RA [9]. Furthermore, MMP-9 is believed to play a key role in directing the migration of macrophages and neutrophils in RA [8, 33]. Synovial fluid from RA patients contains high levels of MMP-2 and MMP-9 [16]. Therefore, MMP-2 and MMP-9 are attractive targets for the treatment of RA [11]. In this context, our results showing
that HLXL decreases MMP-9 and MMP-2 activity in AA are of direct relevance to RA.

Besides MMPs, the increased activity of the proinflammatory cytokines such as IL-6 and IL-17 is closely associated with the destruction of cartilage and bone in RA. IL-6 stimulates the production of MMP-2 and MMP-9 [17]. IL-17 also enhances MMP-2 and MMP-9 expression and is synergistic with IL-1β and TNF-α in inducing the expression of other proinflammatory cytokines and MMPs [8]. We observed that both IL-6 and IL-17 (as proteins) were significantly reduced after HLXL treatment. This is the first report on HLXL-mediated suppression of IL-6 in AA. In addition, our study revealed the inhibitory effect of HLXL on IL-17 at the protein level, validating our earlier finding on the reduction of IL-17 mRNA expression [24]. We suggest that the downregulation of IL-6 and IL-17 had a significant influence, either directly or indirectly, on the MMP activity, which was found to be decreased in HLXL-treated rats.

Chemokines also regulate the expression and activity of MMPs. For example, RANTES and MCP-1 activate monocytes to secrete TNF-α, and TNF-α in turn stimulates MCP-1, which then induces MMP-9 production by monocytes [34]. On the basis of our results, we suggest that HLXL-induced reduction in MCP-1 might have contributed in part to the reduced production of MMP-9.

4.3. The Bidirectional Interplay between Pro-Inflammatory Cytokines and Chemokines. Overproduction of IL-6 is a characteristic feature of RA, and inhibiting IL-6 activity is one of the therapeutic options for treating RA. Other investigators [35] have shown that RANTES and MCP-1 regulate IL-6 production by fibroblast-like synoviocytes in RA. These observations support our results of HLXL-induced concurrent decrease in the above-referenced chemokines as well as IL-6. The other prominent proinflammatory cytokine, IL-17, stimulates the production of multiple chemokines such as MCP-1 and GRO/KC [36]. In our study, we have shown the concurrent downregulation of chemokines and IL-17 in HLXL-treated rats compared to the control rats.

In conclusion, HLXL acts on different chemokines, MMPs, and cytokines and suppresses their expression, which in turn leads to attenuation of inflammatory arthritis (Figure 4). The precise mechanisms of inhibition of these mediators of inflammation are currently under investigation.

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References

[1] P. E. Lipsky, “Rheumatoid arthritis,” in Harrison’s Principles of Internal Medicine, A. Fauci, E. Braunwald, D. Kasper et al., Eds., pp. 2083–2092, McGraw Hil, New York, NY, USA, 17th edition, 2008.
[2] G. A. FitzGerald, “Coxibs and cardiovascular disease,” The New England Journal of Medicine, vol. 351, no. 17, pp. 1709–1711, 2004.
[3] J. M. Scheiman, “The impact of nonsteroidal anti-inflammatory drug-induced gastropathy,” The American Journal of Managed Care, vol. 7, no. 1, supplement, pp. S10–S14, 2001.
[4] D. M. Taibi and C. Bourguignon, “The role of complementary and alternative therapies in managing rheumatoid arthritis,” Family & Community Health, vol. 26, no. 1, pp. 41–52, 2003.
[5] S. H. Venkatesha, B. M. Berman, and K. D. Moudgil, “Herbal medicinal products target defined biochemical and molecular mediators of inflammatory autoimmune arthritis,” Bioorganic & Medicinal Chemistry, vol. 19, no. 1, pp. 21–29, 2011.
[6] P. P. Tak and B. Brennihan, “The pathogenesis and prevention of joint damage in rheumatoid arthritis: advances from synovial biopsy and tissue analysis,” Arthritis & Rheumatism, vol. 43, no. 12, pp. 2619–2633, 2000.
[7] P. Loetscher and B. Moser, “Homing chemokines in rheumatoid arthritis,” Arthritis Research, vol. 4, no. 4, pp. 233–236, 2002.
[8] D. V. Jovanovic, J. Martel-Pelletier, J. A. Di Battista et al., “Stimulation of 92-kd gelatinase (matrix metalloproteinase 9) production by interleukin-17 in human monocyte/macrophages: a possible role in rheumatoid arthritis,” Arthritis & Rheumatism, vol. 43, no. 5, pp. 1134–1144, 2000.
[9] P. S. Burrage, K. S. Mix, and C. E. Brinckerhoff, “Matrix metalloproteinases: role in arthritis,” Frontiers in Bioscience, vol. 11, no. 1, pp. 529–543, 2006.
[10] J. J. Haringman, R. L. Oostendorp, and P. P. Tak, “Targeting cellular adhesion molecules, chemokines and chemokine receptors in rheumatoid arthritis,” Expert Opinion on Emerging Drugs, vol. 10, no. 2, pp. 299–310, 2005.
[11] C. Jackson, M. Nguyen, J. Arkell, and P. Sambrook, “Selective matrix metalloproteinase (MMP) inhibition in rheumatoid arthritis—targeting gelatinase A activation,” Inflammation Research, vol. 50, no. 4, pp. 183–186, 2001.
[12] L. Boiardi, P. Macchioni, R. Meliconi, L. Pulsatelli, A. Facchini, and C. Salvarani, “Relationship between serum RANTES and CCL2 mRNA levels and radiological progression in rheumatoid arthritis patients treated with methotrexate,” Clinical and Experimental Rheumatology, vol. 17, no. 4, pp. 419–425, 1999.
[13] A. E. Koch, S. L. Kunkel, M. R. Shah et al., “Growth-related gene product α. A chemotactic cytokine for neutrophils in rheumatoid arthritis,” The Journal of Immunology, vol. 155, no. 7, pp. 3660–3666, 1995.
[14] Y. Hatano, T. Kasama, H. Iwabuchi et al., “Macrophage inflammatory protein 1 alpha expression by synovial fluid neutrophils in rheumatoid arthritis,” Annals of the Rheumatic Diseases, vol. 58, no. 5, pp. 297–302, 1999.
[15] S. Shahrara, S. R. Pickens, A. M. Mandelin et al., “IL-17—mediated monocyte migration occurs partially through CC chemokine ligand 2/monocyte chemoattractant protein-1 induction,” The Journal of Immunology, vol. 184, no. 8, pp. 4479–4487, 2010.
[16] Y. Yoshihara, H. Nakamura, K. Obata et al., “Matrix metalloproteinases and tissue inhibitors of metalloproteinases in
synovial fluids from patients with rheumatoid arthritis or osteoarthritis,” *Annals of the Rheumatic Diseases*, vol. 59, no. 6, pp. 455–461, 2000.

[17] A. E. Kossakowska, D. R. Edwards, C. Prusinkiewicz et al., “Interleukin-6 regulation of matrix metalloproteinase (MMP-2 and MMP-9) and tissue inhibitor of metalloproteinase (TIMP-1) expression in malignant non-Hodgkin’s lymphomas,” *Blood*, vol. 94, no. 6, pp. 2080–2089, 1999.

[18] B. G. Wan and B. H. Tao, “An 87 cases’ clinical observation, arthritis treated by modified Huo-Luo-Xiao-Ling Dan,” *Jiangxi Journal of Traditional Chinese Medicine*, vol. 33, article 12, 2002.

[19] Y. C. Wang, S. Y. Ji, and R. Wang, “The clinical application of Huo-Luo-Xiao-Ling Dan,” *Jiangxi Journal of Traditional Chinese Medicine*, vol. 30, article 53, 2002.

[20] D. Bernsky and R. Barolet, *Chinese Herbal Medicine: Formulas and Strategies*, Eastland, Seattle, Wash, USA, 1990.

[21] H. Cao, R. Yu, Y. Choi et al., “Discovery of cyclooxygenase inhibitors from medicinal plants used to treat inflammation,” *Pharmacological Research*, vol. 61, no. 6, pp. 519–524, 2010.

[22] L. Lao, A. Y. Fan, R. X. Zhang et al., “Anti-hyperalgesic and anti-inflammatory effects of the modified Chinese herbal formula Huo Luo Xiao Ling Dan (HLXL) in rats,” *The American Journal of Chinese Medicine*, vol. 34, no. 5, pp. 833–844, 2006.

[23] R. X. Zhang, A. Y. Fan, A. N. Zhou et al., “Extract of the Chinese herbal formula Huo Luo Xiao Ling Dan inhibited adjuvant arthritis in rats,” *Journal of Ethnopharmacology*, vol. 121, no. 3, pp. 366–371, 2009.

[24] Y. H. Yang, R. Rajaiah, D. Y. Lee et al., “Suppression of ongoing experimental arthritis by a Chinese herbal formula (Huo-Luo-Xiao-Ling Dan) involves changes in antigen-induced immunological and biochemical mediators of inflammation,” *Evidence-based Complementary and Alternative Medicine*, vol. 2011, Article ID 642027, 10 pages, 2011.

[25] Z. Szekanecz, M. M. Halloran, M. V. Volin et al., “Temporal expression of inflammatory cytokines and chemokines in rat adjuvant-induced arthritis,” *Arthritis & Rheumatism*, vol. 43, no. 6, pp. 1266–1277, 2000.

[26] R. Rajaiah, M. Puttabatyappa, S. K. Polumuri, and K. D. Moudgil, “Interleukin-27 and interferon-γ are involved in regulation of autoimmune arthritis,” *The Journal of Biological Chemistry*, vol. 286, no. 4, pp. 2817–2825, 2011.

[27] S. H. Venkatesha, H. Yu, R. Rajaiah, L. Tong, and K. D. Moudgil, “Celastrus-derived celastrol suppresses autoimmune arthritis by modulating antigen-induced cellular and humoral effector responses,” *The Journal of Biological Chemistry*, vol. 286, no. 17, pp. 15138–15146, 2011.

[28] S. A. Komeh-Nkrmah, S. M. Nanjundaiah, R. Rajaiah, H. Yu, and K. D. Moudgil, “Topical dermal application of essential oils attenuates the severity of adjuvant arthritis in lewis rats,” *Phytotherapy Research*, vol. 26, no. 1, pp. 54–59, 2012.

[29] C. M. Brodmerkel, R. Huber, M. Covington et al., “Discovery and pharmacological characterization of a novel rodent-active CCR2 antagonist, INCB3344,” *The Journal of Immunology*, vol. 175, no. 8, pp. 5370–5378, 2005.

[30] Y. Jin, X. Cui, U. P. Singh et al., “Systemic inflammatory load in humans is suppressed by consumption of two formulations of dried, encapsulated juice concentrate,” *Molecular Nutrition & Food Research*, vol. 54, no. 10, pp. 1506–1514, 2010.

[31] H. Marotte, J. H. Ruth, P. L. Campbell, A. E. Koch, and S. Ahmed, “Green tea extract inhibits chemokine production, but up-regulates chemokine receptor expression, in rheumatoid arthritis synovial fibroblasts and rat adjuvant-induced arthritis,” *Rheumatology*, vol. 49, no. 3, pp. 467–479, 2010.

[32] P. R. Pine, B. Chang, N. Schoettler et al., “Inflammation and bone erosion are suppressed in models of rheumatoid arthritis following treatment with a novel Syk inhibitor,” *Clinical Immunology*, vol. 124, no. 3, pp. 244–257, 2007.

[33] D. Ahrens, A. E. Koch, R. M. Pope, M. Stein-Picarella, and M. J. Niedbala, “Expression of matrix metalloproteinase 9 (96-kd gelatinase B) in human rheumatoid arthritis,” *Arthritis & Rheumatism*, vol. 39, no. 9, pp. 1576–1587, 1996.

[34] S. C. Robinson, K. A. Scott, and F. R. Balkwill, “Chemokine stimulation of monocyte matrix metalloproteinase-9 requires endogenous TNF-α,” *European Journal of Immunology*, vol. 32, no. 2, pp. 404–412, 2002.

[35] T. Nanki, K. Nagasaka, K. Hayashida, Y. Saita, and N. Miyasaka, “Chemokines regulate IL-6 and IL-8 production by fibroblast-like synoviocytes from patients with rheumatoid arthritis,” *The Journal of Immunology*, vol. 167, no. 9, pp. 5381–5385, 2001.

[36] M. J. Ruddy, F. Shen, J. B. Smith, A. Sharma, and S. L. Gaffen, “Interleukin-17 regulates expression of the CXC chemokine LIX/CXCL5 in osteoblasts: implications for inflammation and neutrophil recruitment,” *Journal of Leukocyte Biology*, vol. 76, no. 1, pp. 135–144, 2004.