

Review

Chemokines and Chemokine Receptors as Novel Therapeutic Targets in Rheumatoid Arthritis (RA): Inhibitory Effects of Traditional Chinese Medicinal Components

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Chemokines belong to a large family of inflammatory cytokines responsible for migration and accumulation of leukocytes at inflammatory sites. Over the past decade, accumulating evidence indicated a crucial role for chemokines and chemokine receptors in the pathophysiology of rheumatoid arthritis (RA). RA is a chronic autoimmune disease in which the synovial tissue is heavily infiltrated by leukocytes. Chemokines play an important role in the infiltration, localization, retention of infiltrating leukocytes and generation of ectopic germinal centers in the inflamed synovium. Recent evidence also suggests that identification of inhibitors directly targeting chemokines or their receptors may provide a novel therapeutic strategy in RA. Traditional Chinese medicinals (TCMs) have a long history in the treatment of inflammatory joint disease. The basis for the clinical benefits of TCM remains largely unclear. Our studies have led to the identification of numerous novel chemokine/chemokine receptor inhibitors present in anti-inflammatory TCMs. All of these inhibitors were previously reported by other researchers to have anti-arthritis effect, which may be attributable, at least in part, to their inhibitory effect on chemokine and/or chemokine receptor. Therefore, identification of agents capable of targeting chemokine/chemokine receptor interactions has suggested a mechanism of action for several TCM components and provided a means of identifying additional anti-RA TCM. Thus, this approach may lead to the discovery of new inhibitors of chemokines or chemokine receptors that can be used to treat diseases associated with inappropriately overactive chemokine mediated inflammatory reactions. *Cellular & Molecular Immunology*. 2004;1(5):336-342.

Key Words: chemokine, receptor, rheumatoid arthritis, traditional Chinese medicine

Introduction

Chemokines are chemoattractant cytokines that direct the migration of leukocytes, and are induced by inflammatory cytokines, growth factors and pathogenic stimuli (1). Chemokines are highly basic proteins with molecular weights ranging from 6-14 kD. Chemokine proteins are classified into four highly conserved groups — CXC, CC, C and CX3C — based on the position of the first two cysteines that are adjacent to the amino terminus (1). More

than 50 chemokines have been discovered so far and there are at least 18 human seven-transmembrane-domain chemokine receptors. Interaction of chemokines with these specific G-protein coupled receptors on target cells produces their biological effects, which includes promoting cell adhesion, invasion, mobilization, interactions with the extracellular matrix (ECM) and survival (2). Further, by binding to their receptors, chemokines produce additional biological functions such as directing the traffic of phagocytic leukocyte, and activation of leukocyte to generate superoxide anions and release of granule contents, thus enabling leukocytes to serve as the first-line host defense against invading microorganisms (3). However, leukocyte accumulation and activation also can cause tissue damage, which can culminate in inflammation, autoimmunity and graft rejection.

There is growing evidence of the involvement of chemokines and their receptors in a broad range of physiological and pathological processes, and those discoveries have led to new insights concerning the therapeutic potential of these proteins. Recent studies have provided irrefutable evidence that chemokines and their receptors are pivotal mediators in the pathophysiology of inflammatory joint diseases, since they have been found to be critical for the recruitment, localization and retention of inflammatory cells in the inflamed synovium and play an

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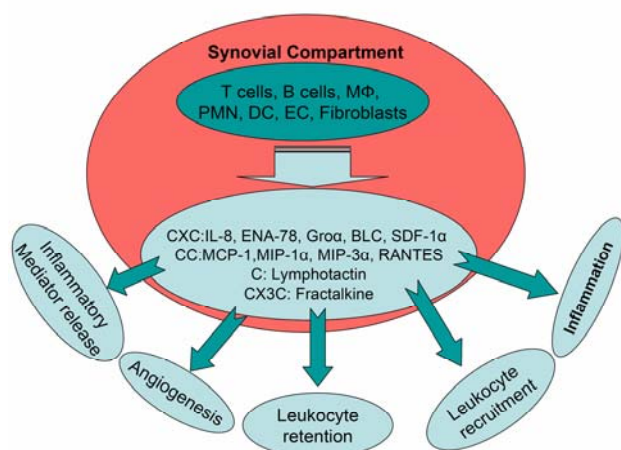


Figure 1. Chemokines produced in inflamed synovium and their roles.

important part in synovitis and tissue destruction (4). Blockade of chemokine and chemokine receptor interactions has emerged as a novel therapeutic strategy in RA (5).

Traditional Chinese medicine (TCM) has a long history of successfully treating chronic inflammatory diseases, including RA (6, 7), however the mechanism remains to be elucidated. Since chemokines and chemokine receptors play such a crucial role in the pathophysiology of RA, therefore, inhibition of chemokine activity or blockade of chemokine receptor function should be evaluated in the study TCM with anti-RA activity. Furthermore, identification of inhibitors for chemokines or chemokine receptors from TCM may yield novel lead chemical entities for the new drug development.

In this review, we will briefly summarize the evidence that chemokines and chemokine receptors play a crucial role in the sustaining the chronic inflammation in the RA synovium. Additionally we will provide evidence showing that directly targeting chemokines or chemokine receptors by pharmacological approach is a valuable therapeutic strategy.

Chemokines and their receptors in RA pathogenesis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation and destruction of the cartilage and bone in the joints (8). The characteristic features of the disease are synovial lining hyperplasia and chronic infiltration of inflammatory cells including T lymphocytes (mainly of Th1 cells), B lymphocytes, monocytes/macrophages and granulocytes. The hyperplastic synovial lining is mainly composed of highly activated macrophage-like cells and proliferating fibroblast-like cells. The infiltrating lymphocytes are localized in the tissue just below the synovium and are found around the blood vessels and in the stroma. Frequently, lymph-node-like aggregates are observed. It is generally accepted that chemokines and their receptors are critical for the

recruitment and localization of cells in the inflamed synovium (4, 9).

It is not surprising that inflammatory chemokines are abundantly expressed in the inflamed RA synovium, since these proinflammatory cytokines inducers, including tumor necrosis factor- α and IL-1 β , are well known to play a crucial role in the pathogenesis of RA (4). Over the years, a significant body of evidence has been generated to support a role for chemokines and their receptors in the pathogenesis of RA.

Activated synovial fibroblasts and monocytes/macrophages are the main producers of chemokines, such as IL-8 (CXCL8) (10), epithelial-neutrophil activating protein-78 (ENA-78 or CXCL5), monocyte-chemoattractant protein-1 (MCP-1 or CCL2), regulating upon activation, normal T-cell expressed and secreted RANTES or CCL5 and macrophage inflammatory protein-1 α (MIP-1 α or CCL3) in RA synovial tissue, and elevated levels of these chemokines are also detected in RA synovial fluids (11). IL-8 and ENA-78, which act on granulocytes, and MCP-1, RANTES and MIP-1 α , which act primarily on monocytes and lymphocytes, are involved in the selective recruitment and activation of these cells. Recent studies have focused on the analysis of chemokine receptor expression in RA synovial T lymphocytes. The expression of chemokine receptors CXCR3, CXCR6 and CCR5 is upregulated on memory CD4⁺ T lymphocytes, which represents the predominant infiltrate in the inflamed synovium (12-17). These inflammatory chemokine receptors are prominently expressed on effector T lymphocytes (Th1 cells) that mediate the Th1 mediated inflammation such as in RA. It is also pertinent that the RA synovial memory CD4⁺ T lymphocytes express high levels of the chemokine receptor CXCR4, and that its ligand stromal cell-derived factor 1 (SDF-1 or CXCL12) is produced by synovial fibroblasts and endothelial cells (18, 19). Since CXCR4 expression is enhanced by IL-15 and transforming growth factor- β , which are present in the inflamed joint, interaction of SDF-1 with CXCR4 has been suggested to be important in retaining the cells at this site (18, 19).

In about 20% of patients with RA, infiltrating T and B lymphocytes accumulate underneath the synovial lining layer and organize into lymphocyte aggregates with germinal centers (GCs) (20-22). These ectopic lymphoid structures share many features with secondary lymphoid tissue and are thought to contribute to the pathogenesis of the disease (23). Some homing chemokines, which are mainly expressed in lymphoid tissues, have been implicated in the formation of lymphoid structures (24, 25). The first indication of their involvement came from studies of CXCR5, which is highly selective for the single chemokine B-cell-activating chemokine-1 (BCA-1 or CXCL13). BCA-1/CXCL13 is expressed in the RA synovium and has been reported to be critical in the formation of RA-associated follicular aggregates (26, 27). Intriguingly, a direct correlation was found between the occurrence of follicular dendritic cells (FDCs) and GC-positive follicles in rheumatoid synovitis (27). GCs were observed only when FDCs, which produce BCA-1, were present in the follicle. Whether the FDCs develop locally from precursors or whether they are recruited to this area is not known. In either case, they are critical for

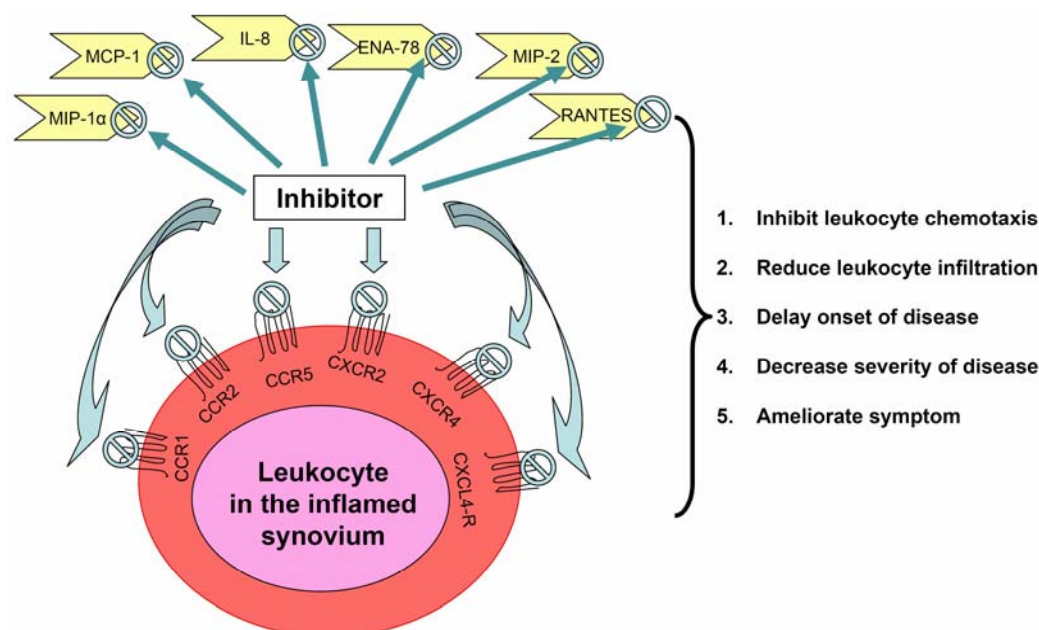


Figure 2. Directly targeting chemokines and chemokine receptors is a novel strategy in RA therapy.

lymphoid neogenesis in the synovium. Furthermore, the strict requirement of FDCs suggests that antigen recognition events play a major role in the development of tertiary lymphoid tissue.

Recently, it was reported that FPRL1 mRNA was also expressed by fibroblast-like synoviocytes (FLS), macrophages, and endothelial cells isolated from the synovial tissue of patients with RA and other categories of inflammatory arthritis. This observation suggests that ligands for FPRL1 may also participate in the pathophysiology of inflammatory arthritis (28).

Directly targeting chemokines or chemokine receptors in RA therapy

It was reported that mice, which have only one of two receptors for neutrophil chemoattractants (IL-8) have fewer neutrophils recruited to an artificial air-pouch during acute urate crystal-induced gouty synovitis (29). Furthermore individuals carrying the $\Delta 32$ -CCR5 allele, which encodes a mutated nonfunctional CCR5, exhibit a reduced RA incidence and severity of disease (30, 31). These studies suggested that inhibition of chemokine receptor interactions might be useful for RA treatment.

Chemokine production in the RA joint could be targeted indirectly through the inhibition of cytokine/chemokine production. For example, infliximab (an anti-TNF- α mAb) reduced synovial expression of IL-8/CXCL8 and MCP-1/CCL2 in RA patient (32). In this review we will only focus on the approaches which directly target chemokines and their receptors as potential RA therapeutics.

A small molecular weight CCR1 antagonist (CP-481, 715) was reported to inhibit 90% of the monocyte chemotactic activity present in 11/15 rheumatoid arthritis synovial fluid samples (33). In a recent study performed on

human RA patients, a CCR1 antagonist was found to significantly reduce the level of CCR1 expressing cells in the synovial joint. Furthermore, RA patients who were given the CCR1 antagonist showed a trend towards clinical improvement (34). In a MRL-lps mouse model of arthritis, daily injection of a MCP-1 receptor antagonist [MCP-1 (9-76), a 67 amino acid sequence of MCP-1], prevented the onset of arthritis as monitored by joint swelling and by histopathological evaluation of the joints. In contrast, mice treated with native MCP-1 show enhanced arthritic symptoms (35). Administration of Met-RANTES, a CCR1/CCR5 antagonist or a CCL5/RANTES antibody, was also effective at reducing the severity of the disease in mice (36) and rats (37). One study showed that by targeting the chemokine receptor CCR5 with the TAK-779, a CCR5 and CXCR3 inhibitor (38), both the incidence and severity of collagen II-induced arthritis in DAB/1 mice were reduced. Closer examination showed that cellular infiltration in the antagonist treated group was reduced (39). AMD3100, a potent CXCR4 specific antagonist, was reported to reduce the severity of autoimmune collagen-induced arthritis (CIA) (40). Another CXCR4 antagonist, 4F-benzoyl-TN14003 (an analogue of 14-mer peptide T140), significantly ameliorated the clinical severity of CIA in a mouse model (41). A bioactive synthetic peptide derived from the angiogenesis inhibitor chemokine PF4/CXCL4 also suppressed murine CIA (42).

In regard to direct targeting of chemokines, anti-chemokine antibodies have demonstrated therapeutic effect in arthritis models. Anti-human ENA-78/CXCL5 antibody administered before the onset of the disease modified the severity of RA in rats, while administration of anti-ENA-78/CXCL5 antibody after clinical onset of RA did not modify the disease (43). Antibody for IL-8/CXCL8 was shown to attenuate joint swelling and neutrophil infiltration that occurred in monosodium urate crystal-induced arthritis in rabbits (44). CIA mice passively

immunized with antibodies directed against either MIP-1 α /CCL3 or MIP-2/CXCL-2 demonstrated a delay in the onset of arthritis and a reduction in the severity of arthritis (45). In a rat CIA model, injection of neutralizing mAb for MCP-1/CCL2 significantly decreased the number of infiltrating macrophage in the lesion and reduced the ankle swelling (46). Based on these results, a phase II clinical trial of the humanized IL-8/CXCL8 antibody ABX-IL-8 for use in the treatment of RA was initiated (Abgenix Inc., California, USA) (47).

Taken together, these studies demonstrate increasing evidence that directly targeting chemokine receptors (CCR1, CCR2, CCR5, CXCR2, CXCR4 and PF4/CXCL4 receptor) and chemokine ligands (MCP-1/CCL2, MIP-1 α /CCL3, RANTES/CCL5, MIP-2/CXCL-2, ENA-78/CXCL5, IL-8/CXCL8) provides a promising and intriguing approach to RA treatment (Figure 2).

Inhibitory effects of TCM components on chemokine and chemokine receptor

There is now compelling evidenced that inappropriate activation of the chemokine network is associated with many diseases, therefore these proteins and their receptors are attractive targets for new therapeutic development. Unlike cytokines, which have pleiotropic effects, chemokines target specific leukocyte subsets and, in some settings, may only attract these cells without activating them. Antagonism of a single chemokine ligand or receptor would be expected to have a relatively circumscribed effect, thereby endowing the antagonist with a profile of limited side effects. The challenge now is to develop small-molecule antagonists with good bioavailability (48), and to pursue this purpose, a considerable effort has been made by academic community as well as by pharmaceutical industry (49). Traditional Chinese medicines (TCMs) have a long history in the management of various diseases and have recently been shown to target G-protein coupled receptors (50). Some TCMs have been shown to possess potent anti-inflammatory efficacy, including anti-RA activity (7). A previous study demonstrated that an anti-arthritic herbal medicine inhibited leukocyte (mast cell) migration (51), presumably by targeting the interaction of chemokine and chemokine receptor. We, therefore, hypothesized that inhibition of chemokine or chemokine receptor may be a fundamental mechanism underlying the anti-inflammatory action of some TCM. Furthermore, those TCM may contain chemokine or chemokine receptor antagonistic component(s) which may be used as lead chemical compounds in the development of new therapeutics. Our research approach has resulted in the discovery of numerous novel chemokine/chemokine receptor antagonists from anti-inflammatory TCMs. In addition, inhibition of the production of proinflammatory cytokines and chemokines might also be a fundamental action of anti-inflammatory TCM (52), however, this topic is not included in this review.

Bile acid

Niu Huang (also termed as calculus bovis, bezaor bovin, ox gallstone), a biliary product, which is supposed to remove

heat and toxic substance in TCM, has been reported to suppress immune response (53). Bile acids (deoxycholic acid, DCA and cholic acid, CA) are the major active components of traditional Chinese drugs and formulae in which biliary products are an ingredient. One of our previous studies indicated that QKL (Qing Kai Ling), a multi-component herbal preparation, inhibited a number of chemokine and classic chemoattractant-induced leukocyte migration, including blockade of fMLP-induced leukocyte migration (54). DCA is a component of QKL which contains Niu Huang. Our subsequent observations revealed that DCA significantly inhibited fMLP-induced monocyte and neutrophil chemotaxis and calcium mobilization. DCA also blocked the binding of [³H]fMLP and anti-formyl peptide receptor (FPR) monoclonal antibodies (mAb) to the cells. The inhibitory effects of DCA on calcium mobilization and anti-FPR-mAb binding to the receptor could be abrogated by washing DCA out of the cell suspension, suggesting that DCA blocked fMLP receptors *via* a steric hindrance mechanism, not *via* receptor internalization. DCA had no significant inhibitory effects on MCP-1, SDF-1 α , or C5a-induced monocyte function, or C5a- or IL-8-induced neutrophil function. Our experimental results suggest that blockade of fMLP receptors may contribute to the anti-inflammatory effects of traditional medicine containing DCA (55). Additional studies in our lab discovered that chenodeoxycholic acid (CDCA), and ursodeoxycholic acid (UDCA) although less potent than DCA, also selectively blocked the function of FPR and FPRL1 (56).

Bile acids (including CDCA, UDCA) have been demonstrated to be effective in the treatment of rheumatoid arthritis patient (57-59). The anti-RA activity of bile acids may be partially attributable to their blockade of FPRL1, since the FPRL1 gene is upregulated in inflamed synovial tissue, suggesting that the therapeutic targeting of FPRL1 may inhibit pathophysiologic pathways leading to progressive inflammatory arthritis (28).

Tannic acid

Our initial observation demonstrated that SHHL (Shuang-huanglian, an injectable multiple TCM preparation) inhibited certain chemokine-induced chemotaxis (54). We identified the compound(s) should responsible for this activity by testing aqueous extracts of three ingredients of SHHL. An extract of Lianqiao (dried fruit of Forsythia suspense) potently inhibited radiolabeled SDF-1 α binding to cells. Monitored by the binding assay, we sequentially purified the active component in extract of Lianqiao by chromatography procedures. The result indicated that a tannic acid might be responsible for the activity. Tannic acid, at nontoxic concentrations, specifically inhibited SDF-1 α /CXCL12-induced human monocyte migration (IC₅₀, 7.5 μ g/ml) but did not inhibit MCP-1/CCL2, MIP-1 α /CCL3, RANTES/CCL5, fMLP, or C5a-induced migration. The compound markedly blocked SDF-1 α /CXCL12 binding to THP-1 cells (IC₅₀, 0.36 μ g/ml). Tannic acid also inhibited SDF-1 α /CXCL12-induced, but not epidermal growth factor (EGF)-induced, migration of MDA 231 breast tumor cells. Additionally, 0.5 μ g/ml of tannic acid selectively inhibited SDF-1 α /CXCL12-mediated, but not

Table 1. Inhibitor of chemokines and chemokine receptors identified from traditional Chinese medicine.

Compound	Chinese medicine	Activity	Reference
Bile acids (DCA, CDCA, UDCA)	Biliary products (ox gallstone, bear bile...)	Selectively blocked FPR and FPRL1 function	(55, 56)
Tannic acid	Lianqiao (dried fruit of Forsythia suspense)	Selectively blocked interaction of SDF-1 α and CXCR4	(60)
Shikonin	Zicao (dried root of Lithospermum erythrorhizon)	Non-selectively inhibited function of chemokine receptors	(65)
Baicalin	Huangqin (Dried root of Scutellaria baicalensis)	Directly interfered with chemokine ligands	(67, 68)

basic fibroblast growth factor (bFGF)- or endothelial cell growth supplement-mediated, bovine aorta endothelial cell (BAEC) capillary tube formation (60). Thus the tannic acid inhibits both the chemotactic and proangiogenic consequences of SDF-1 α /CXCR4 interactions.

Tannic acid has been reported to inhibit adjuvant-induced polyarthritis in rat and to inhibit leukocyte migration to the inflammatory site (61). SDF-1 α /CXCR4 is a key chemokine/chemokine receptor in retention of inflammatory cells in the synovium and angiogenesis (18, 19). Blockade of SDF-1 α /CXCR4 by various antagonists has been demonstrated to suppress RA (40, 41). Therefore, blockade of SDF-1 α /CXCR4 interaction may be one of mechanisms underlying anti-arthritis action of tannic acid. Since tannic acid is abundant in the plant kingdom, the impact of tannic acid in the daily dietary vegetables and grains on the RA needs to be evaluated in the future studies.

Shikonin

Shikonin, a naphthoquinone pigment isolated from the Chinese herbal-Zicao (dried root of Lithospermum erythrorhizon), is a potent anti-inflammatory agent (62). Shikonin derivatives had been reported to inhibit complete Freund's adjuvant-induced chronic arthritis in rat (63). We found that shikonin selectively blocked RANTES/CCL5 and MIP-1 α /CCL3 binding to CCR1 and consequently inhibited RANTES-induced, but not EGF-induced, CCR1/HEK cells migration (64). Furthermore, we observed that shikonin, at nanomolar concentration, inhibited monocyte chemotaxis and calcium flux in response to a wide variety of CC chemokines (MCP-1/CCL2, MIP-1 α /CCL3, and RANTES/CCL5), CXC chemokine (SDF-1 α /CXCL12), and classic chemoattractants (fMLP and complement fraction C5a). Presumably, this effect of shikonin is based on interference with signal transduction by these receptors. Shikonin down-regulated surface expression of CCR5, a primary HIV-1 co-receptor, on macrophages to a greater degree than the other receptors (CCR1, CCR2, CXCR4, and the formyl peptide receptor). CCR5 mRNA expression was also down-regulated by the compound. Additionally, shikonin inhibited the replication of a multidrug-resistant strain and pediatric clinical isolates of HIV in human peripheral blood mononuclear cells. Our results suggest that the anti-HIV and anti-inflammatory activities of shikonin may be related to its inhibition of chemokine receptor expression and function (65). RA pathogenesis

involves multiple chemokine receptors. The property of shikonin as a pan chemokine receptor inhibitor, may contribute to its reported anti-arthritis action.

Baicalin

Baicalin (BA, 7-glucuronic acid, 5, 6-dihydroxyflavone) is a flavonoid compound purified from the medicinal plant *Scutellaria baicalensis* Georgi, which has been used for treatment of various inflammatory diseases including arthritis (66). Our laboratory demonstrated that BA inhibited the binding of a number of chemokines to human leukocytes or cells transfected to express specific chemokine receptors (67, 68). This was associated with a reduced capacity of the chemokines to induce cell migration. Co-injection of BA with CXC chemokine interleukin-8 (IL-8) into rat skin significantly inhibited IL-8 elicited neutrophil infiltration. BA did not directly compete with chemokines for binding to receptors, but rather acted through its selective binding to chemokine ligands. This conclusion was supported by the fact that BA cross-linked to oxime resin bound chemokines of the CXC (SDF-1 α , IL-8), CC (MIP-1 β , MCP-2), and C (lymphotactin (Ltn)) subfamilies. BA did not interact with CX3C chemokine fractalkine/neurotactin or cytokines, such as TNF- α and IFN- γ , indicating that its action is selective. These results suggest that one possible anti-inflammatory mechanism of BA is to bind to a variety of chemokines and limit their biological function (67).

In summary, our studies of the anti-inflammatory TCMs have led the identification of selective chemokine receptor antagonists (bile acids, tannic acid), a pan chemokine receptor inhibitor (shikonin) as well as a chemokine ligand inhibitor (baicalin) (Table 1). Interestingly, all these compounds have been reported by other researchers to suppress the arthritis. The relationship of the capacity of these TCM components to block interactions of chemokines with chemokine receptors and their anti-arthritis activities needs to be defined by additional studies. These compounds can also be used to generate chemical variants with greater anti-chemotactic and presumably more potent anti-inflammatory effects.

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