Chemokines in arthritis: key molecules in pathogenesis and potential therapeutic targets

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An increasing wealth of evidence suggests an important role for chemokines and their receptors in the pathogenesis of inflammation. In this review, the authors explore the role of chemokines in inflammatory joint diseases, with particular emphasis on rheumatoid arthritis. Recent progress aimed at modulating the chemokine and chemokine receptor system for therapy will also be discussed.

Rheumatoid arthritis (RA) is the most common inflammatory joint disease [1], with an unknown etiology that results in the persistent inflammation of synovial tissue (ST) in the joints of affected patients. The synovitis that ensues results in cartilage and bone destruction and is linked with considerable morbidity [2]. Thus overall, RA is known to be associated with increased mortality [2,3]. Considerable work suggests that an autoimmune process drives the synovitis, which is characterized pathologically by proliferation and thickening of the lining layer, the phenomena of neoangiogenesis and a mononuclear cell infiltrate. This includes monocyte macrophages, B and T lymphocytes, dendritic cells, plasma cells (in some instances organized in typical lymphoid-like structures) and mast cells. The processes that result in these pathological appearances are the consequence of an orchestrated action of a multitude of soluble chemoattractants, in particular chemokines (CKs) and cell adhesion molecules that enable hematopoietic blood-borne cells to localize and be retained within the joint. CKs are chemotactic cytokines that play a crucial role in angiogenesis and leukocyte recruitment and exert chemotactic activity towards a variety of cell types. Therefore, CKs and CK receptors (CK-Rs) have recently become the focus of concerted efforts to understand their mechanistic role in the pathogenesis of inflammatory arthritis. Understanding their role and the pathways involved may allow the possibility of targeting them for therapy in RA.

Keywords: cell migration, chemokines, inflammation, lymphoid neogenesis, rheumatoid arthritis

Chemokines & chemokine receptors

CKs are low molecular weight (8–14 Kd), structurally-related, secreted or membrane-bound proteins [4] that function as cell attractants. More than 50 human CKs have been characterized so far, along with 18 receptors [5–12]. They share a tertiary structure, stabilized by four cysteine residues that form disulfide bonds, distinguishing them from the classical chemoattractant molecules (e.g., complement fragment peptides C3a and C5a and lipid molecules such as leukotriene B4 and platelet activating factor). They have been subdivided into four families (Table 1) on the basis of the arrangement of their cysteine residues. The two major subfamilies have the cysteine residues adjacent to each other (cysteine-cysteine [CC] CK) or separated by one amino acid (cysteine-x-cysteine [CXC] CK) [4]. The other two families consist of first the CX3C CK, fractalkine/CX3CL1 [13], with three amino acids separating the cysteine residues, and second the cysteine (C) CK group, lymphotactin/XCL1 and SCM-1β/XCL2, with two instead of four conserved cysteines [14] (see Table 1 for abbreviation definitions).

All CKs act via seven transmembrane domain CK-Rs that are coupled to guanosine–triphosphate-binding proteins. The binding of a CK to its cognate receptor can induce a wide spectrum of effects, including the arrest and firm adhesion of blood-borne cells on endothelial surfaces and transendothelial migration into tissues. These events involve CKs on the surface of endothelial cells interacting with their cognate receptors on leukocytes triggering intracellular signals. This leads to integrin clustering (increasing integrin avidity) and enhanced integrin affinity (through conformational changes) [15], which is followed by firm adhesion to the endothelium and leukocyte extravasation [16]. Once localized in the tissues, the final positioning of various leukocyte subsets is also driven by further gradients of CKs. Therefore, CKs allow leukocytes to circulate from the bloodstream to specific organs and move inside these tissues in a programmed fashion through spatial and temporal successions of

CK gradients [17,18]. However, the activity of CKs is not restricted simply to cell mobilization, as they can participate in cell activation, inducing the release of the content of cytoplasmic storage granules, or by upregulating the expression of soluble- and membrane-bound molecules. Examples of the latter being the induction of the expression of lymphotoxin (LT)β on B cells via BCA-1 (BLC)/CXCL13 [19], and of the former, the release of proteases from neutrophils and monocytes, which potentiate inflammation [20]. CKs are also intimately involved in angiogenesis [21]. Genetic analyses have shown that many CKs may have arisen from the reduplication of ancestral genes [4] and many are clustered in certain chromosomal locations. Two main clusters have been recognized: CXC CKs clustering at chromosome 4q12–13 and many CC CKs located in another cluster at 17q11.2 [4].

Table 1. Human CXC, C, CC and CX3C chemokines and cognate receptors

Common name ligand	Systematic name	Chemokine receptors
C family		
Lymphotactin/SCM-1α/ATAC	XCL1	XCR1
SCM-1β	XCL2	XCR1
CC family		
I-309	CCL1	CCR8
MCP-1/MCAF/TDCF	CCL2	CCR2
MIP-1 α/LD78α	CCL3	CCR1,CCR5
LD78β	CCL3L1	CCR1,CCR5
MIP-1β	CCL4	CCR5
LAG-1 gene duplication	CCL4L1	
RANTES	CCL5	CCR1, CCR3, CCR5
(C-10/MRP-2)	CCL6	CCR1, CCR2, CCR3
MCP-3	CCL7	CCR1, CCR2, CCR3
MCP-2	CCL8	CCR2, CCR3,CCR5
(MIP-1γMRP-2)	CCL9/CCL10	CCR1
Eotaxin	CCL11	CCR3
(MCP-5)	CCL12	CCR2
MCP-4	CCL13	CCR1, CCR2, CCR3
HCC-1	CCL14	CCR1,CCR5
HCC-2/lkn-1/MIP-1δ	CCL15	CCR1,CCR3
HCC-4/LEC/LCC-1	CCL16	CCR1,CCR2
TARC	CCL17	CCR4
DC-CK1/PARC/AMAC-1	CCL18	Unknown
MIP-3β/ELC/exodus-3	CCL19	CCR7
MIP3α/LARC/exodus-1	CCL20	CCR6
6Ckine/SLC/exodus-2	CCL21	CCR7
MDC/STC-1	CCL22	CCR4
MPIF-1/CKβ8/CKβ8–1	CCL23	CCR1
Eotaxin-2/MPIF-2	CCL24	CCR3
TECK	CCL25	CCR9
Eotaxin-3	CCL26	CCR3
CTACK/ILC	CCL27	CCR10
MEC	CCL28	CCR3/CCR10

AMAC: Alternative macrophage activation-associated chemokine; ATAC: Activation-induced T-cell-derived and chemokine-related cytokine; BCA: B-cell attracting; BLC: B lymphocyte chemokine; BRAK: Breast and kidney-expressed chemokine; C: Cysteine; CC: Cysteine-cysteine; CXC: Cysteine-x-cysteine; CTACK: Cutaneous T-cell attracting chemokine; DC: Dendritic cell; ELC: Epstein–Barr virus-induced molecule 1 ligand chemokine; ENA: Epithelial cell-derived neutrophil-activating protein; GCP: Granulocyte chemotactic protein; GRO: Growth related; HCC: Hemofiltrate cysteine-cysteine; IL: Interleukin; ILC: IL-11Ra-locus chemokine; IP: Interferon-γ inducible protein; LAG: Lymphocyte activating gene; LARC: Liver and activation-regulated chemokine; LCC: Liver CC chemokine; LEC: Liver-expressed chemokine; MCAF: Monocyte chemotactic and activating factor; MCP: Monocyte chemoattractant protein; MDC: Macrophage-derived chemokine; MEC: Mammary-enriched chemokine; MGSA: Melanoma growth stimulatory activity; Mig: Monokine induced by IFN-γ; MIP: Macrophage inflammatory protein; MPIF: Macrophage procoagulant inducing factor; MRP: Multidrug resistance-associated protein; NAP: Neutrophil activating protein; PARC: Pulmonary and activation-regulated chemokine; PF: Platelet factor; RANTES: Regulated on activation, normal T-cell-expressed and secreted; SCM: S-carboxymethyl; SDF: Stromal-cell derived factor; SLC: Secondary lymphoid organ chemokine; STC: Stem hematopoietic cells; TAC: T-cell-α chemoattractant; TARC: Thymus and activation-regulated chemokine; TDCF: Tumor-derived chemotactic factor; TECK: Thymus-expressed chemokine.

Table 1. Human CXC, C, CC and CX3C chemokines and cognate receptors (cont.).

Common name ligand	*Systematic name*	*Chemokine receptors*
CX3C family		
Fractalkine	CX3CL1	CX3CR1
CXC chemokine/receptor family		
GRO-α/MGSA-α	CXCL1	CXCR2 > CXCR1
GRO-β/MGSA-β	CXCL2	CXCR2
GRO-γ/MGSA-γ	CXCL3	CXCR2
PF4	CXCL4	Unknown
ENA-78	CXCL5	CXCR2
GCP-2	CXCL6	CXCR1, CXCR2
NAP-2	CXCL7	CXCR2
IL-8	CXCL8	CXCR1, CXCR2
Mig	CXCL9	CXCR3
IP-10	CXCL10	CXCR3
I-TAC	CXCL11	CXCR3
SDF-1 α/β	CXCL12	CXCR4
BCA-1(BLC)	CXCL13	CXCR5
BRAK/bolekine	CXCL14	Unknown
(Lungkine)	CXCL15	Unknown
Small inducible cytokine B6	CXCL16	CXCR6

AMAC: Alternative macrophage activation-associated chemokine; ATAC: Activation-induced T-cell-derived and chemokine-related cytokine; BCA: B-cell attracting; BLC: B lymphocyte chemokine; BRAK: Breast and kidney-expressed chemokine; C: Cysteine; CC: Cysteine-cysteine; CXC: Cysteine-x-cysteine; CTACK: Cutaneous T-cell attracting chemokine; DC: Dendritic cell; ELC: Epstein–Barr virus-induced molecule 1 ligand chemokine; ENA: Epithelial cell-derived neutrophil-activating protein; GCP: Granulocyte chemotactic protein; GRO: Growth related; HCC: Hemofiltrate cysteine-cysteine; IL: Interleukin; ILC: IL-11Ra-locus chemokine; IP: Interferon-γ inducible protein; LAG: Lymphocyte activating gene; LARC: Liver and activation-regulated chemokine; LCC: Liver CC chemokine; LEC: Liver-expressed chemokine; MCAF: Monocyte chemotactic and activating factor; MCP: Monocyte chemoattractant protein; MDC: Macrophage-derived chemokine; MEC: Mammary-enriched chemokine; MGSA: Melanoma growth stimulatory activity; Mig: Monokine induced by IFN-γ; MIP: Macrophage inflammatory protein; MPIF: Macrophage procoagulant inducing factor; MRP: Multidrug resistance-associated protein; NAP: Neutrophil activating protein; PARC: Pulmonary and activation-regulated chemokine; PF: Platelet factor; RANTES: Regulated on activation, normal T-cell-expressed and secreted; SCM: S-carboxymethyl; SDF: Stromal-cell derived factor; SLC: Secondary lymphoid organ chemokine; STC: Stem hematopoietic cells; TAC: T-cell-α chemoattractant; TARC: Thymus and activation-regulated chemokine; TDCF: Tumor-derived chemotactic factor; TECK: Thymus-expressed chemokine.

However, the genes of the more recently discovered CC and CXC CKs, which act mainly on lymphocytes, tend to be located elsewhere in chromosomal locations, away from the major clusters. Interestingly, several chromosome-4 CXC CKs that turned out to be highly specific for T lymphocytes (i.e., Mig/CXCL9, IP-10/CXCL10 and I-TAC/CXCL11) are also located in a minicluster separate from the major CXC cluster, which is located in chromosome 4q12–13 [4]. It has been suggested that this diversification is likely to reflect functional specialization that has developed during the evolution of this superfamily [22].

Chemokine network in RA synovium

CKs are produced in two main ways: first, they are produced constitutively in organs where cell mobilization is required for the maintenance of local homeostasis, (e.g., hematopoiesis in bone marrow and the genesis of the immune response in secondary lymphoid organs). Second, CKs can be upregulated in the context of inflammatory reactions, behaving as proinflammatory mediators, enhancing leukocyte migration and favoring the recruitment, antigen encounter and cooperation of different leukocyte subsets [23]. Some CKs can have a dual homeostatic/inflammatory role depending on the tissue and circumstances under which they are produced [24–26].

Secondary lymphoid organs are among the best anatomical sites where the constitutive expression and role of CKs have been studied. Under homeostatic conditions, T and B lymphocytes circulate from the bloodstream to secondary lymphoid organs but only a selected few (mainly naïve lymphocytes) are allowed to extravasate into the lymphoid tissues via high endothelial venules (HEVs). This selectivity is due to the interaction between specific CK-Rs expressed by lymphoid homing cells and CKs expressed constitutively in secondary lymphoid organs (e.g., the CK and CK-R pairings CCR7-SLC/CCL21 and CCR7-ELC/CCL19) [27,28]. Lymphoid CKs have a central role both in the formation of embryonic lymphoid structures [29] and in the maintenance

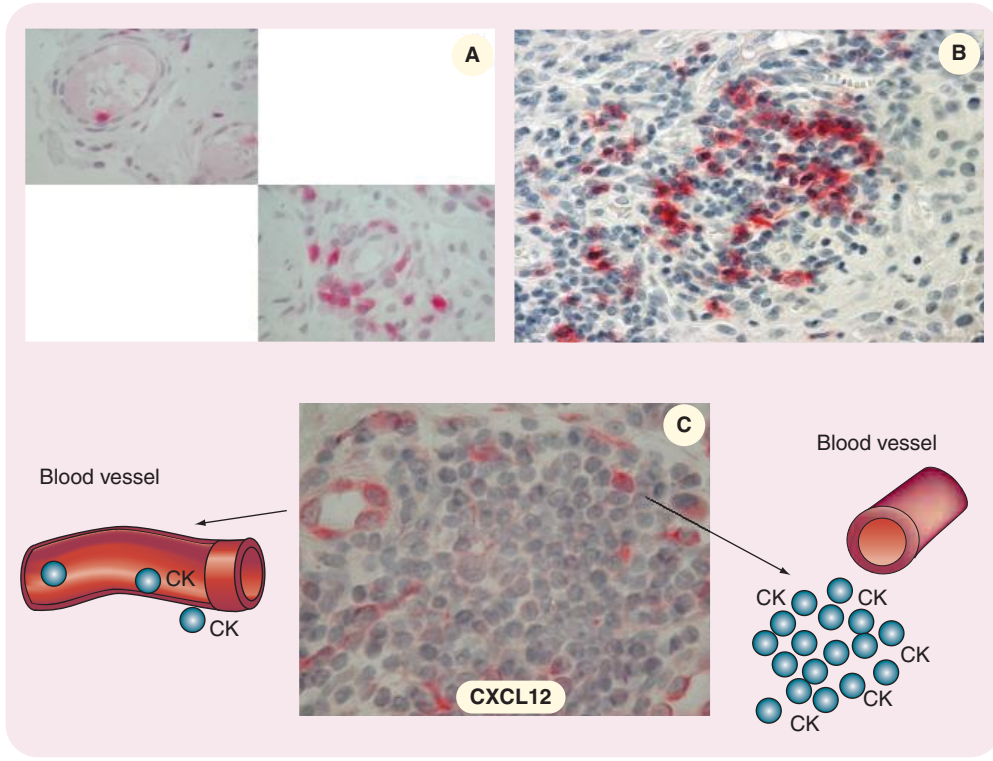
of the organizational structure of secondary lymphoid organs, directing specific leukocyte populations to discrete compartments and favoring interactions between distinct cell populations (e.g., dentritic cells [DCs] and T cells) [20,30].

The interest in lymphoid CKs and autoimmune diseases arises from the relationship observed between chronic inflammation and ectopic lymphoid neogenesis. In rheumatoid synovium, infiltrating cells show a tendency to form aggregates in approximately 40% of patients [31]. These lymphocytic aggregates vary in size, some resembling conventional germinal centres (~ 20%) in both appearance and function [32]. Recent observations have demonstrated that the synovial lymphoid neogenesis is associated with the ectopic production of lymphoid-constitutive CKs, such as CXCL13 and CCL21, which are produced constitutively in the B- and T-cell-rich areas of secondary lymphoid organs, respectively [31,33,34].

The authors have demonstrated, through *in situ* expression analyses, that in RA synovium CXCL13 and CCL21 can be produced in T/B cell aggregates with ectopic germinal centers. A similarity in the organizational distribution of these factors has also been shown in both secondary lymphoid tissue and RA synovial lymphoid aggregates. The overlapping expression patterns of these two CKs could indicate the preservation of their functional role at nonlymphoid as well lymphoid sites [34]. Importantly, lymphoid CKs are powerful lymphoid tissue morphogenetic factors that have been shown *in vivo* to have the capacity, when overexpressed in the pancreas of transgenic mice, to act upstream in the process of lymphoid neogenesis, and also at ectopic sites [35,36]. By dissecting progressive organizational phases of synovial lymphoid neogenesis, the authors have demonstrated that the production of CXCL13 and CCL21 does not require the stable environment of a fully formed germinal center, but can also be produced in minor and unstructured aggregates [34]. Similar results were obtained by dissecting ectopic sites of lymphoid neogenesis from salivary glands in Sjögren's syndrome [37]. This provides evidence for the possible expression of these molecules in the early phases of human ectopic lymphoid neogenesis and, by inference, their critical role in the process. These data are in agreement with work published by Takemura and colleagues, who have demonstrated by polymerase chain reaction (PCR) in homogenized tissues that these CKs (together with lymphotoxin LTβ) can predict the presence of different levels of organizational structures in RA patients [31]. Only *in vivo* studies in animals would demonstrate whether the expression of these factors is a consequence of the aggregational process or whether, as shown in mice, they can contribute as upstream triggers. Interestingly, recent data showed that CXCL13 and CCL21 can also be induced in the subchondral bone marrow of rheumatoid joints in association with local lymphoid aggregates. This process appears to be associated anatomically with areas of increased bone remodeling, suggesting the involvement of this compartment and of the local upregulation of chemoattractant factors in disease pathogenesis [38,39].

Other CKs produced constitutively in secondary lymphoid tissues, such as CCL19 and SDF-1/CXCL12, have been shown to be induced or upregulated in RA synovium. CCL19, together with CCL21, is involved in the recruitment and localization of $CCR7^+$ lymphocytes and mature DCs within secondary lymphoid tissue T-cell areas and has been found to be expressed at high levels by DCs of RA patients with active disease, with higher mRNA levels in ST from RA patients than those with osteoarthritis (OA) [40]. The participation of CXCL12 and its receptor (CXCR4) in the pathogenesis of RA has been suggested previously by several studies that have reported an upregulation of CXCL12 in RA synovium and a role for the ligand–receptor system in local monocyte and T-cell attraction [41,42]. An *in vivo* demonstration of these concepts has been provided by the authors' laboratory, showing that when CXCL12 was injected into human synovial tissue grafts implanted into severe combined immunodeficient (SCID) mice, it promoted U937 monocytoid cell recruitment from the bloodstream [41]. The same factors have also been shown to have a role in favoring B-lymphocyte accumulation and survival inside the tissue [43]. These data support both the concepts of CK participation at different steps in the inflammatory cascade and the multifunctional activity of a specific molecule that can be involved in both the process of leukocyte extravasation as well as in their perivascular clusterization (Figure 1). CXCL12 is a tumor necrosis factor (TNF)-α-independent CK [44] and its persistence in the synovia of patients treated with anti-TNF–α [45] suggests that it may be involved in alternative pathways mediating synovitis in RA.

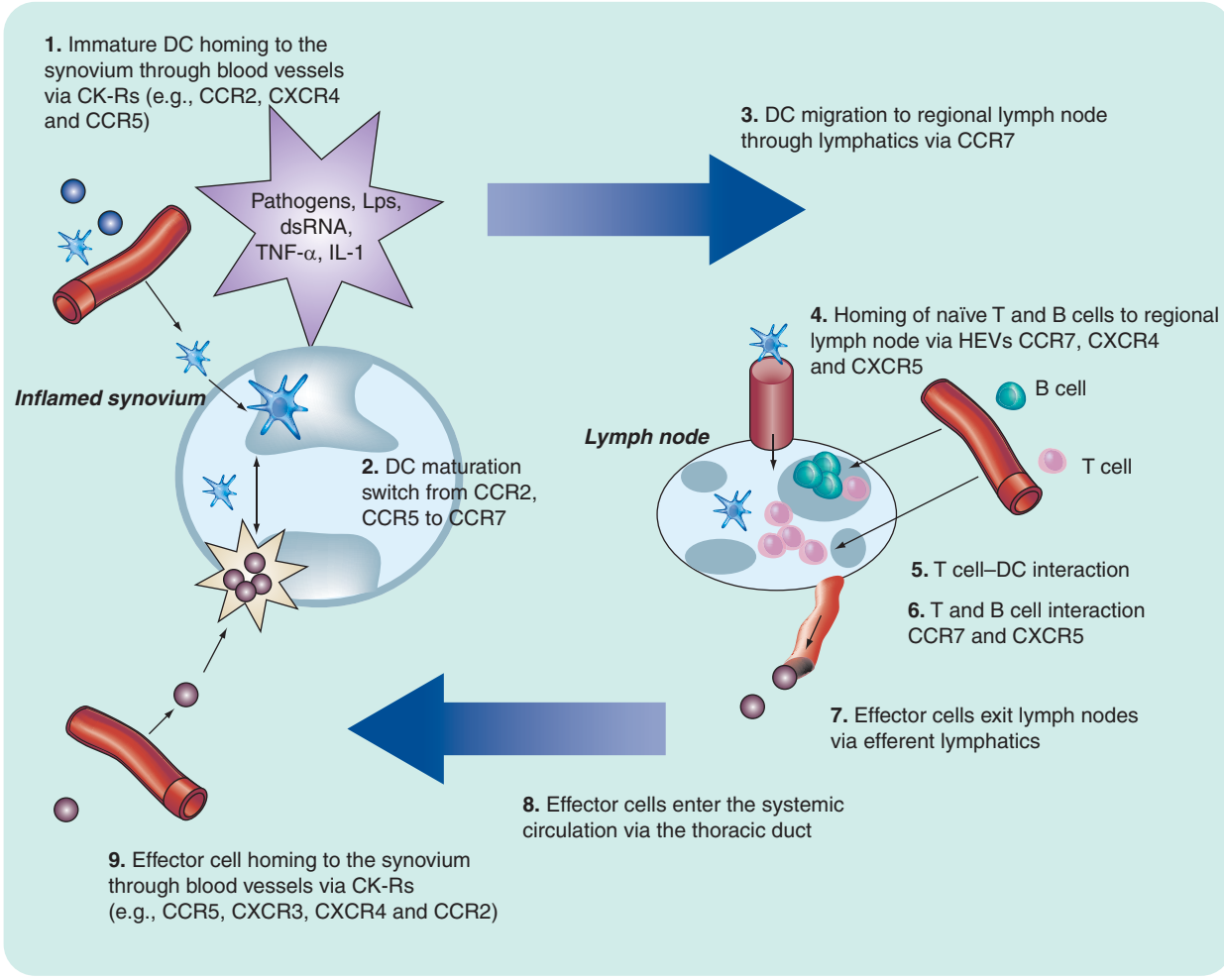
Figure 1. Role of CKs in the inflammatory process.



When expressed by the endothelium, CKs can mediate leukocyte homing from the bloodstream or, when expressed inside the tissue, can promote intratissue cell positioning, survival and activation.
A. Immunostaining capturing leukocytes inside a blood vessel and after endothelial transmigration (in red T cells). **B.** Leukocytes are shown in the phases of intratissue localization (in red B cells, immunohistochemistry). **C.** CKs, such as CXCL12 (in red, immunohistochemistry), are expressed by both the vascular endothelium and within cellular aggregates, suggesting that CKs are functionally involved in both the extravasation and clusterization of leukocytes perivascularly.
CK: Chemokine; CXC: Cysteine-x-cysteine.

RA synovium has been shown to contain a complex mixture of other inflammatory-inducible CKs, including MCP-1/CCL2 [46,47], MIP-1α/CCL3 [48,49], RANTES/CCL5 [48,50–52], CXCL10 [53,54], CXCL9 [55,56], GCP-2/CXCL6 [57,58] and ENA-78/CXCL5 [59], functioning as a chemoattractants of effector cell populations such as neutrophils, activated T cells and antigen presenting cells such as DCs. Experimental evidence in human synovium supports the implication of some of these factors in cell recruitment or retention. For example, phenotypic analysis of synovial T cells showed a marked enrichment of CXCR3 (CXCL9, CXCL10 receptor) and CCR5 (CCL3, CCL5 receptor) when compared with peripheral blood [60]. CKs and CK-Rs are also known to be involved in the migration of DCs, draining antigen (Ag) from peripheral tissues to secondary lymphoid organs where the priming and activation of naïve lymphocytes takes place [61]. A schematic model of putative recirculation pathways involving the synovium, lymphoid organs, blood and lymphatic vessels, is shown in Figure 2. Ag-presenting cells, such as immature DCs, can be recruited from the bloodstream to the inflamed joint. This process is made possible by the constitutive expression by these cells of CK-Rs (e.g., CCR2, CXCR4 and CCR5) for cognate CKs produced in the inflamed synovium [62]. Once in the joint they are exposed to an inflammatory milieu that allows for the maturation of DCs and an accompanying switch in their CK-Rs with upregulation of CK-Rs for homeostatic lymphoid CKs. Mature DCs then migrate to the regional lymph node, through lymphatics, where they can encounter naïve T and B cells constitutively expressing CK-Rs for lymphoid CKs expressed by HEVs. Ag-primed T cells proliferate under the control of mature DCs in the T-cell-rich area and activate B cells. T cells differentiate progressively into effector and memory T cells. Effector T cells downregulate lymphoid homing CK-Rs, allowing them to exit

Figure 2. Schematic model illustrating the potential role of chemokines and chemokine receptors in regulating systemic migratory routes and synovial homing of leukocytes.



CCR: Cysteine-cysteine receptor; CK-R: Chemokine receptor; CXCR: Cysteine-x-cysteine receptor; DC: Dentritic cell; HEV: High endothelial venule; Lps: Lipopolysaccharide; TNF: Tumor necrosis factor.

lymph nodes through efferent lymphatics and re-enter the systemic circulation via the thoracic duct. Effector cells express CK-Rs specific for ligands produced in inflamed tissues that favor their homing to the inflamed joints. It has not yet been defined whether synovial homing takes place by means of tissue-specific molecular mechanisms or by nonspecific, inflammation-mediated events. To date, the best characterized tissue specific T-cell populations are the skin and gut homing populations. Skin homing cells preferentially express CCR4 and gut homing lymphocytes CCR9, which facilitate adhesion and transmigration on CCL17/TARC- or CCL25/TECK-positive skin and intestinal microvasculature, respectively [63–66]. No such pairing has been identified so far for rheumatoid synovium.

While, as mentioned previously, CXCL12 appears to be produced independently from TNF-α, the production of many inducible CKs is regulated mostly by inflammatory cytokines such as interleukin (IL)-1 and TNF-α, known to play a critical role in synovial inflammation via processes (amongst others) of angiogenesis, integrin activation, chemotaxis and release of other mediators (e.g., MMPs).

CXCL8/IL-8 is one of the best-studied inflammatory CKs. It is produced by macrophages in the synovial compartment [67] and by fibroblasts following stimulation with IL-1α, IL-1β, TNF-α or lipopolysaccharide (LPS) [48]. CXCL8 has been shown to induce synovial inflammation in animal models [68] and to be upregulated in RA, specifically in affected

joints [69], with its levels in the sera of RA patients correlating with disease activity [70]. Furthermore, CXCL8 is a potent mediator of angiogenesis and was the first CK with a Glu-Leu-Arg (ELR) motif to be shown to have angiogenic properties, a function that has also been demonstrated in the RA joint [71]. Angiogenetic properties have been demonstrated for other CKs, such as CXCL12, CXCL5 and CX3CL1 [71–73]. ELR motifs were initially thought to confer angiogenic properties on the CXC CKs, with the absence of the motif resulting in inhibition of neovascuarization. This is now known not to be universally applicable [22].

As mentioned, several inflammatory CKs have been found to be upregulated in the serum, ST or synovial fluid (SF) of RA patients, when compared with normal or OA patients. However, given the nature of human studies (often capturing a snap shot), the exact dynamics of inflammatory CK production and their precise function has not yet been completely established in the context of RA. Some work in animal models of arthritis, however, has attempted to address the temporal relationship of CK expression. In rat adjuvant induced arthritis (AIA), higher levels of CCL3 were shown early in the course of the disease and higher levels of CCL2 predominantly in the later stages [74].

Other specific functions of inflammatory CKs have been elucidated. They include the role of CXCL1 in RA, which has recently been suggested as a potent stimulator for Blys release (a B-cell survival factor and member of the TNF-α family) from neutrophils, which may explain the high levels of Blys seen in SF of RA patients [75]. PF4/CXCL4 is known to play a role in angiogenesis and has been suggested as a marker of RA-associated vasculitis [76].

Other arthritides

The main volume of work on CK and arthritis focuses on RA, but limited studies have demonstrated a role for them in other arthritidies.

Osteoarthritis

Although not thought of as one of the inflammatory arthritidies, when the OA synovial membrane is examined it is not uncommon to see pathological evidence of inflammation [77], and for this reason the role of CK expression is beginning to be examined.

CXCL12 and CXCL13 have recently been investigated and shown to be involved directly in the remodeling process that occurs in the bone of OA patients by apparently inducing cellular proliferation and favoring new collagen Type I production [78]. CCL2 and CCL5 are both expressed in the SF and ST of OA patients [79] and may play a role by recruiting mononuclear leukocytes into the joint.

There is also evidence that some CKs and CK-Rs can be upregulated in OA as opposed to RA, such as CCL4/MIP-1β, which is upregulated to a higher level in OA rather than RA SF [80]. The level of expression of a selection of CK-Rs in cartilage (CCR1, CCR3, CCR5, CXCR1, CXCR2 and CXCR3), has been found to be low in inflammatory arthritis (both RA and psoriatic arthritis [PsA]) but normal-to-high in OA [81]. A hypothesis for this paradigm may be that CK-Rs are downregulated in inflammatory arthritis due to the increased production of metalloproteinases [81].

Crystal arthropathies

It has been demonstrated in murine macrophages that monosodium urate (MSU) crystals stimulate the production of CCL2, CCL3 and CCL4 mRNA [82].

Increased levels of CXCL8 and CCL2 have also been detected in the SF of patients suffering from gout [83,84] and CXCL8 secretion by neutrophils has been shown to be stimulated by both MSU and calcium pyrophosphate crystals [85]. CXCL8 has been investigated further in a rabbit model, where the induction of acute arthritis by intra-articular injection of MSU crystals was accompanied by a dramatic rise in the levels of CXCL8 in SF [86].

Psoriatic arthritis

Elevated concentrations of CCL2 have been found in PsA SF and correlated with memory T-cell numbers suggesting that CCL2-mediated chemotaxis is involved in the recruitment of T lymphocytes into the synovial compartment of patients with PsA [87].

Analogously to the above-described forms of arthritis, CXCL8 has been recognized in the ST of PsA patients, where it is expressed primarily by lining layer cells, lymphocytes and macrophages [88].

These data demonstrate the lack of disease specificity for the expression of some of the inflammatory CKs. Taken together, a potential role for CK modulation in all these forms of arthritis is suggested.

Biopathological studies in animal models

One of the features of the CK/CK-R system is the high degree of redundancy, which refers to the existence of multiple CKs functioning as ligands for the same CK-R and different CK-Rs binding the same CK.

The complex biochemical and biological interrelationship among CK and CK-Rs, probably far from being completely clarified, could imply a limitation in the therapeutic application of a specific CK/CK-R blockade in RA, as other CKs with related biological effects could compensate for the inactivation. However, recent loss-of-function experiments in animal models (both by gene knockout and by single–combined blockade of ligands and receptors), indicate that a specific CK/CK-R blockade can be efficacious in modulating the inflammatory cascade. This action suggests that redundancy in ligand–receptor binding may not necessarily be associated with full biological redundancy.

Both CKs and CK-Rs have been targeted *in vivo* in animal models. Specific inhibition of CXCL8 by therapy with a monoclonal antibody has been shown to be effective in inhibiting both leukocyte [86,89] and neutrophil [90] infiltration into the joints of LPS (or MSU)-induced arthritis in rabbits. An antagonist of the CXCL8 receptor, CXCR2, given to rabbits with arthritis induced by CXCL8, LPS and chronic Ag (ovalbumin) was shown to inhibit arthritis [91]. A CCL2 antagonist given to MRL/lpr autoimmune mice (a strain that spontaneously develops an arthritis similar to RA) prevented the development of arthritis, in contrast with control mice given CCL2, who had enhanced arthritis [92]. This work is supported by studies in rats with collagen-induced arthritis (CIA), who were given CCL2 antibodies, with a resultant improvement in disease both clinically and pathologically [93]. Receptor antagonists have been investigated in animal models. A modified form of CCL5 (met-RANTES), which functions as an antagonist for CCR1 and CCR5, resulted in the amelioration of rat AIA [94]. It has also been shown to act in the same way as in the mouse CIA model, resulting in lower disease severity in treated mice versus controls [95]. CCR2 blockade in CIA mice has been shown only to be effective during the initial phases of arthritis and not in established disease, when administration actually worsens arthritis [96]. This is explained by interference with $CCR2^{+}$ regulatory T cells that have anti-inflammatory properties crucial in the later stages of arthritis, and hence their down-regulation, allowing for worsening of arthritis [96]. This underlines the earlier message regarding the critical temporal relationship between CKs and may suggest a potential role for the delivery of different CK agonists/antagonists during different phases of disease in humans.

A specific CXCR4 antagonist, a T140 analog (T140 is a 14-mer peptide used as an agent in both HIV and oncological therapy), when used in CIA mice, significantly reduced clinical severity of the disease when administered continuously [97]. AMD3100, another different, specific antagonist of CXCR4, given by continuous infusion to interferon (IFN)-γ-deficient DBA/1 mice (used for the rapid onset of the arthritis compared with wild type) inhibited the onset of disease [98]. Antibodies have also been studied against specific CKs. Anti-CXCL13 antibodies administered to CIA mice resulted in the development of significantly less severe arthritis, with an associated reduction in follicular development in the synovium [99]. Interestingly, when a CXCL5 antibody is administered before the onset of disease in AIA animals, it is effective, but is ineffective in suppressing disease when arthritis is established [100]. Using anti-CXCL1 or anti-CCL3 antibodies before the onset of arthritis in the CIA mouse resulted in delayed onset and severity of disease [101].

Combination therapies have also been adopted and some efficacy has been shown. A combination of GRO-specific neutralizing antibodies and anti-CXCL8 was shown to reduce joint infiltration by leukocytes by 70% in LPS-induced rabbit arthritis, whereas each CK alone resulted in a 54% and 48% reduction, respectively [102]. Similarly, a combination of CCL2 and CXCL1 inhibition was more effective in reducing arthritis in MRL-Faslpr (a mouse model of spontaneous arthritis) AIA mice than when CCL2 was used alone [103].

A different approach is that of attempting to induce protective immunity. This has been tried using a naked DNA vaccine of CXCL10; when this was administered to AIA-prone rats, they failed to develop disease [104] and the antibodies produced could be used to transfer disease suppression. Specific knockout models have produced contrasting results, emphasizing the complexity of the biopathological role of CKs *in vivo*. A $CXCR2^{-/-}$ deficient mouse has shown less severe development of Lyme arthritis when compared with wild type, with the apparent inability of neutrophils to enter joints [105]. However, this result is difficult to extrapolate to humans, who express both CXCR1 and

CXCR2, whereas mice only express CXCR2. CCL3-null mice have also been examined for their response to collagen Type II and the development of CIA. They were found to have milder disease both clinically and histologically [106]. In contrast with these results, CCR5-deficient mice develop a disease phenotype indistinguishable from wild type [107]. Explanations for this are still being considered. In keeping with the blockade of CCR2 in established disease that results in a worsening of arthritis in CIA [96], CCR2-gene deficient mice do develop a severe form of CIA compared with wild type [107].

Potential therapeutic applications: human studies

Although there are many animal studies demonstrating the possibilities of manipulation of the CK system as therapy in RA, there has been relatively little progression from this to human pathology. This is because of several limitations, including the difficulty in using the prophylactic approach in arthritis prevention in humans, the fact that many CKs and receptors are species specific and the possibility that some ligands may act as agonists at one receptor and antagonists at another.

However, genetic analyses in humans have supported the concept of CKs and CK-Rs playing a pathological role in human disease. The CCR5 allele has received much interest and several groups have evaluated disease presence and severity in patients homozygous for the CCR5Δ32 mutation that encodes a defective CCR5 not expressed at the cell surface [108–110]. There is some dispute as to whether the gene deletion can predict the presence of RA [108,109], but the presence of the mutation predicts disease severity [111], and its absence in RA has been correlated with the absence of a rheumatoid factor in the serum of patients [108]. Recently, polymorphism of the promoter region of the *RANTES* gene was investigated and found to be associated with an increased susceptibility to RA [112].

Initial studies directly targeting CK/CK-Rs in humans have shown variable results. One Phase II study looked at a monoclonal antibody against CXCL8 in human RA, although a review article suggested lack of efficacy of the product, no formal results were published and so further conclusions cannot be drawn [113].

A small, Phase Ib, proof-of-concept study looked at an oral CCR1 antagonist in RA. A marked reduction in synovial inflammation (particularly macrophage numbers) 2 weeks following administration was shown, associated with an American College Of Rheumatology 20 response in 33% of the patients [114]. However, as reported by Ribeiro and colleagues, this promising result was not carried over into Phase II studies [115].

Executive summary

Chemokines & chemokine receptors

- Chemokines (CKs) are chemotactic cytokines that act not only to regulate cell movement but also participate in cell activation and angiogenesis and as such are intimately involved in the inflammation seen in the synovial membrane in rheumatoid arthritis (RA).
- More than 50 CKs and 18 CK receptors (CK-Rs) have been described so far.

The chemokine network in RA synovium

- CKs are both constitutively produced and upregulated at sites of inflammation.
- Lymphocyte trafficking from the bloodstream to secondary lymphoid organs is regulated closely by the expression of CKs and their receptors.
- Some patients with RA appear to develop sites of ectopic lymphoid neogenesis within the synovium. This process appears to be dependent on the expression of a specific pattern of CKs.
- A variety of other CKs and CK-Rs have been found to be expressed within synovial tissue (ST).

Other arthritides

- The majority of work surrounding CKs has focused on RA, but their importance in osteoarthritis, crystal arthropathies and seronegative spondyloarthropathies has been demonstrated by their expression in the ST of patients with these conditions.

Biopathological studies in animal models

- Redundancy of the CK system has not prevented therapeutic blockade in animal models.

Potential therapeutic applications: human studies

- Although there are many animal studies demonstrating the possibilities of manipulation of the CK system as therapy in RA, there has been relatively little progression from this to human pathology.

Conclusions

The CK/CK-R system is crucially involved in immune homeostasis, genesis of immune response and initiation and perpetuation of inflammation, hence the growing interest in targeting this system therapeutically in inflammatory arthritis. However, CKs/CK-Rs remain a complex, redundant system, that is only partially characterized. Although initial steps have been made in the development of therapeutic agents in humans, many aspects of CK biology *in vivo* need further clarification before safe and effective CK agents become available for treating patients. Nonetheless, the case for rational targeting of the CK/CK-R system has been made strongly and it is hoped that effective therapeutic agents will come into the clinic soon.

Future perspective

The next decade will see a rapid increase in our knowledge of the biological role of CKs and of their complex, inter-relationship *in vivo*. The authors believe that this will allow the targeting of the CK system in clinical practice, opening a new era in biological therapy in RA. It is highly likely that agents will be tailored to specific disease phenotypes (perhaps determined by ST analysis) and/or stage of disease progression.

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