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A Burgeoning Family of Biological Mediators: Chemokines and Chemokine Receptors

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Abstract. Chemokines are a superfamily of pro-inflammatory polypeptide cytokines that selectively attract and activate different cell types. Most of its members are small proteins that exhibit conserved cysteines in specific positions. Chemokines activate cells through their binding to shared or unique cell surface receptors which belong to the seven-transmembrane (STM), G-protein-coupled receptors (GPCRs). The large number of chemokines and chemokine receptors are indicative of the importance of these molecules in a variety of pathophysiological conditions.

Key words: chemokines; G protein-coupled receptor; leukocytes.

Introduction

Infiltration of leukocytes into inflammatory or injured tissues requires cell-associated and soluble factors which mediate the communications between circulating leukocytes and vascular cells. A superfamily of polypeptide chemoattractants known as chemokines (chemotactic cytokines), which comprises the largest family of cytokines identified so far, has been demonstrated to selectively induce rapid endothelial cell adhesion and transmigration of leukocyte subpopulations[3, 4, 38, 47]. Chemokines are characterized by their ability to induce directional migration and activation of leukocytes. They regulate leukocyte adhesion, trafficking, homing and angiogenesis, and contribute to lymphopoiesis and hematopoiesis. Chemokines are produced by a variety of cell types, including those of hematopoietic and non-hematopoietic origin, in response to antigens, polyclonal stimulants, cell irritants, as well as other cytokines. A number of chemokines are detectable locally in the course of a variety of disease states[3, 47, 59]. Chemokines bind and activate cell surface receptors that belong to the seven-transmembrane (STM), G protein-coupled receptor (GPCR) superfamily[45]. Several chemokine receptors have been identified as fusion co-factors for human immunodefficiency virus type 1(HIV-1)[41, 52]. *In vivo* studies using neutralizing antibodies, antagonists, or by deletion of chemokine and chemokine receptor

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genes have revealed that chemokines and their receptors play a pivotal role in host defense against microorganisms, including HIV-1, and in inflammatory or immunological conditions such as ischemic reperfusion injury, adult respiratory distress syndrome (ARDS), immune-complex-induced glomerulonephritis, atherosclerosis, autoimmune reactions and malignant tumors[24, 47]. This review intends to provide a brief summary of information about recent progress in chemokine and chemokine receptor research. Many excellent reviews are available detailing the role of chemokines and their receptors in particular patho-physiological conditions[26, 58, 61, 62].

Chemokines

Chemokines are 8- to 10-kDa proteins with 20 to 70% homology in amino acid sequences. Over 50 chemokines have been identified to date. They are now divided into 4 clearly defined families (CXC, CC, C and CX_3C) which are distinguished by the pattern of cysteine residues near the amino terminus[3–5, 29, 38, 47, 54, 71]. Their cell sources, inducers, target cells, as well as shared and unique receptors are listed in Tables 1 and 2. The CXC subfamily members have one amino acid (X) interrupting the first two of their 4 conserved cysteine residues and exihibit from 20 to 90% amino acid homology. Based on the presence or absence of an ELR amino acid motif (Glu-Leu-Arg) immediately preceding Cys-1 (ELR^+), the CXC chemokines are further divided into two subclasses. The ELR motif confers upon a CXC chemokine the ability to bind to the receptor CXCR2, and members of this largest group of CXC chemokines are generally neutrophil chemoattractants. The CXC chemokines lacking the ELR motif (ELR^-) are primarily lymphocyte chemoattractants and bind to either the receptors CXCR3 (MIG, IP-10, I-TAC), CXCR4 (SDF-1), or CXCR5 (BLC-1/BCA-1). However, a putative GPCR specific for the ELR^+-CXC chemokine platelet factor 4 (PF4) has not been identified. Interestingly, the expansion of the CXC chemokine family has been limited in comparison to the large number of novel CC chemokines recently described. The CC chemokines have no intervening amino acid between the first two of their four (or in the case of I-309 six) cysteine residues and exhibit 25–70% amino acid homology. In both CXC and CC subfamilies, disulfide bonds are formed between the first and third and between the second and fourth cysteines to establish a stable tertiary structure. Lymphotactin (Lptn) [31] is the

Table 1. Human CXC, C and CX_3C chemokines

Chemokine	Target cells[a]	Cell/tissue source[b]	Inducers	Receptor[c]
CXC				
ELR^+-CXC				
IL-8/NAP-1	N, T, MC, EC, NK, B	M, N, F, EC, K, NK, T, SMC, tumor cells	mitogen, inflammatory agents	CXCR1, CXCR2
GRO(α, β, γ)/MGSA	N, MC, EC, fresh T, melanocytes	M, N, T, K, F, EC, melanoma cells	LPS, IL-1, TNF	CXCR2 possibly CXCR1
NAP-2	N, EC	platelets, F, EC	platelet activation	CXCR2, CXCR1
ENA-78	N, EC	K, F, EC, SMC, Epi	IL-1, LPS, TNF	CXCR2, CXCR1
GCP-2	N, EC	osteosarcoma cells		CXCR2, CXCR1
ELR^--CXC				
PF4	F, EC	platelets	platelet activation	?
IP-10	T, NK, M, EC	M, T, F, EC, K	IFN, TNF, LPS	CXCR3
MIG	T, EC	M, hepatocytes	IFN-γ	CXCR3
I-TAC	Act. T	thymus, spleen, placenta, lung, liver, pancreas, etc.	IFN-β/γ, IL-1	CXCR3
BCA-1/BLC	B	lymphoid tissue	constitutive	CXCR5
SDF-1	virtully all somatic cells	bone marrow stromal cells	constitutive	CXCR4
C				
Lymphotactin	thymocytes, T, DC, NK	T, NK		XCR1
CX_3C				
Fractalkine/neurotactin	T, M, N	EC, M, microglial cells	TNF, IL-1	CX_3CR1

[a] The effect on EC includes induction of angiogenesis presumably due to migration and proliferation of EC in response to CXC chemokines with ELR motif, or the inhibition of angiogenesis by CXC chemokines lacking ELR motif.

[b] B – B lymphocytes, EC – endothelial cells, Epi – epithelial cells, F – fibroblasts, K – keratinocytes, M – monocytes, N – neutrophils, NK – natural killer cells, SMC – smooth muscle cells, T – T lymphocytes, Act. T – activated T lymphocytes.

[c] CXCR4 is a major fusion co-factor for T lymphocyte tropic HIV-1.

Table 2. Human CC chemokines

Chemokine	Main target cells	Main cell/tissue sources[a]	Major inducers	Receptor[b]
MCP-1	M, T, SMC, NK, Bas, DC	all cell types	LPS, mitogens, irritants, growth factors, micro-organisms, IL-1, TNF	CCR2b, CCR4, CCR9
MCP-2	M, T, NK, E, Eo, Bas, DC	osteosarcoma, M, F	IFN, IL-1, virus, mitogens, LPS	CCR1, CCR2b, CCR3, CCR5, CCR9
MCP-3	M, T, N, NK, E, Eo, Bas, DC	osteosarcoma, M,	IFN, mitogen, LPS	CCR1, CCR2b, CCR3, CCR9
MCP-4	M, Bas, Eo	M, Epi	inflammatory agents	CCR1, CCR2b, CCR3, CCR9, CXCR3
MCP-5	As MCP-1	M, SMC	inflammatory agents	CCR2, CCR3
ELC (MIP-3β)	M, T, DC	lymphatic tissue	inflammatory agents	CCR7
Eotaxin	Eo	EC, M, Epi, lung, Lym	inflammatory/allergic agents	CCR3, CCR9, CXCR3
Eotaxin-2 (MPIF-2)	Eo, Bas, resting T	Act.M, Act. T	GM-CSF, anti-CD-3 mAb	CCR3
I-309	T, M	M, Mas	inflammatory agents	CCR8
MIP-5 (HCC-2, LKN-1)	T, M, N (?), Eo (?)	liver, small intestine, colon	constitutive	CCR1, CCR3
LARC (MIP-3α)	M, immature DC	M, DC, liver/lung tissue	inflammatory agents	CCR6
MDC	DC, M, NK	Mac, thymus	constitutive	CCR4
MIP-1α	M, T, NK, DC, N, Eo, Stem cells	M, T, Mas, F	mitogens, inflammatory agents	CCR1, CCR3, CCR4, CCR5, CCR9
MIP-1β	M, T, NK, DC, Stem cells	M, T, F	inflammatory agents	CCR1, CCR5, CCR8, CCR9
RANTES	M, T, NK, DC, Eo, Bas	T, EC, platelets	inflammatory agents, mitogens	CCR1, CCR3, CCR4 CCR5
TARC	T	thymus, DC	PHA	CCR4, CCR8, CCR9
TECK	M, thymocytes, DC	thymus, small intestine	LPS, inflammatory agents	?
HCC-1 (MCIF, NCC-2)	M	spleen, liver, skeletal and heart muscle, gut, bone marrow	constitutive	CCR1, CCR9
HCC-4 (NCC-4, ILINCK, LEC, LMC)	M, lymphocytes	liver	IL-10	?
DC-CK-1 (PARC, MIP-4, AMAC-1)	T	lymphoid organs, DC, alveolar macrophage	IL-4, IL-10, IL-13	?
MPIF (CKβ-8, MIP-3)	M, resting T	M, lung, liver, placenta, bone marrow	IFN-β/γ	CCR1
6Ckine (SLC, Exodus-2, TCA-4)	T, B, NK, DC, mesengial cells (?)	lymphoid organs, gastrointestinal tract	constitutive	CCR7, CXCR3

[a] Bas – basophils, DC – dendritic cells, Eo – eosinophils, Epi – epithelial cells, Lym – lymphocytes, Mas – mast cells.
[b] CCR2b, CCR3, CCR5, CCR8 are fusion co-factors of various strains of HIV-1.

only member of the C chemokine subfamily with 16 kDa in molecular weight. CX_3C chemokine, known also as fractalkine or neurotactin, has three intervening amino acids between the N-terminal cysteines[6, 48]. Fractalkine or neurotactin has a molecular weight of 38 kDa, larger than any other known chemokine. Its 18 amino acid hydrophobic sequence at the C-terminus appears to be an endothelial cell membrane docking motif, and a chemokine component of 80 amino acids can be cleaved from its 240 amino acid mucin-like stalk. Fractalkine or neurotactin appears to function mostly in a cell-associated manner. Chemokines possess heparin binding capacity at their C-terminal end, which enables them to bind to glycosaminoglycan and other negatively charged sugar moieties on cell surfaces and matrix glycoproteins[66]. This property may result in the adsorption of chemokines onto endothelial cell lining of the blood vessels, connective tissues and cell matrices. Thus, chemokines immobilized on tissue or matrix surfaces may induce "haptotactic" migration of target cells[63].

As their amino acid sequences, the secondary and tertiary structures of chemokines are also similar. Several chemokine structures have been solved[10, 13, 23, 35] which reveal a relatively disordered N-terminus anchored to the rest of the molecule by disulfide bonds involving the two-N-terminal domain cysteines. This is followed by an extended loop that leads into three antiparallel β-pleated sheets (a so-called Greek key) which provide a flat base over which the C-terminal α-helix extends. At concentrations required for crystallization

or nuclear magnetic resonance (NMR) study, chemokines are generaly dimeric or tetrameric. However, at physiological (nanomolar) concentrations, chemokines are almost exclusively monomeric[39, 49]. An analogue of IL-8, chemically synthesized to be exclusively monomeric, was fully active and chemokines treated with acetonitrile to prevent multimer formation showed enhanced activity[8, 53]. The biological relevance of multimeric chemokine forms is not clear, but they may be important in the context of high local concentrations at sites of receptor binding and activation.

Several lines of evidence point to the importance of the N-terminal domain in the function of CXC and CC chemokines. The presence of the tripeptide ELR sequence near the N-terminus is essential for the activity of CXC chemokines that activate the receptors, CXCR1 or CXCR2[12, 25]. A further demonstration of the importance of this motif is that addition of ELR to the N-terminal domain of PF4 transforms it into a neutrophil chemoattractant that binds to IL-8 receptors[11]. However, the context in which ELR is placed must be important because ELR-modified IP-10 remains inactive on neutriophils. This suggests that other regions of CXC chemokines are important for receptor activation. In fact, it has recently been shown that there is a hydrophobic pocket in IL-8 involving the loop beyond the N-terminal cysteines that is essential for binding to one of the IL-8 receptors (CXCR1)[22, 37, 65]. Among CC chemokines, there is no amino acid sequence motif analogous to ELR that is associated with biologic effects on monocytes or T cells. Nonetheless, N-terminal regions of CC chemokines are clearly important for their activity, as demonstrated by deletion analysis and single amino acid substitutions[19, 69]. Similar to CXC chemokines, there are also data implicating the loop structure between the N-terminal cysteines and the first β sheet[69]. The C-terminal α-helices have also been shown to be important for imparting maximal biologic potency to chemokines. Part of this effect may be due to interactions between the α-helices and glycosaminoglycans. For example, IL-8's potency and efficacy are enhanced in the presence of heparan sulfate[64].

Many of the genes encoding chemokines have been maped and they cluster at specific loci. The genes for CXC chemokines are located on chromosome 4, with the exception of the stromal cell derived factor 1 (SDF-1), which is on chromosome 10. Most of the genes for CC chemokines are clustered on human chromosome 17q (except LARC on 2q, TARC on 16q, SLC on 9p and eotaxin-2 on 7q). The C and CX_3C chemokine genes map to human chromosomes 1q23 and 16, respectively. The clustering of chemokine genes suggests that these family members arose through gene duplication and subsequent divergence to achieve finely regulated physiological and pathophysiological effects.

Chemokine Receptors

Five receptors for CXC chemokines (CXCR1-5) and 9 for CC chemokines (CCR1-9) have been identified and molecularly cloned (Tables 1 and 2). The receptors for CX_3C chemokine fractalkine/neurotactin and lymphotactin (Lptn) have also been identified recently[30, 68]. Most chemokine receptors are expressed on different types of leukocytes. Some of them are restricted to certain cell subsets (e.g., CXCR1 is predominantly restricted to neutrophils), whereas others are more widely expressed (e.g., CCR2 is expressed on monocytes, T cells, natural killer cells, dendritic cells and basophils). In addition, chemokine receptors that are constitutively expressed on some cells can be inducible on other cell types. For instance, CCR1 and CCR2 are constitutively expressed on monocytes but are expressed on lymphocytes only after stimulation by interleukin 2[36]. Many chemokine receptors are solely expressed on cells undergoing activation and differentiation. Activated T lymphocytes of the T helper type 1 (Th1) phenotype express CXCR3, whereas CCR3, in addition to being expressed on eosinophils and basophils, is preferentially expressed on activated lymphocytes of the T helper type 2 (Th2) phenotype[56]. Thus, transient up-regulation of chemokine receptors on leukocytes allows selective amplification of either a Th1--type or a Th2-type allergic response. Some chemokine receptors are also expressed on nonhematopoietic cells, including neurons, astrocytes, epithelial cells and endothelial cells, suggesting that the chemokine and receptor system may have a broader homeostasis role in addition to leukocyte attraction and activation.

All chemokine receptors belong to the family of STM GPCR[45] and show various degrees of homology to GCPRs used by other leukocyte chemoattractants, such as platelet activating factor (PAF), leukotriene 4 (LTB4), activated complement component 5 (C5a) and formyl-methionyl-leucyl-phenylalanine (fMLP). Structurally, chemokine receptors are also related to the receptors for a number of neurotransmitters[45].

A chemokine receptor is composed of hydrophobic transmembrane domains, an N-terminus outside the cell surface, extracellular and intracellular loops as well as a C-terminus in the cytoplasmic compartment[45]. Chemokines bind with high-affinity to the N-terminus of the receptor and a secondary site on one of the ex-

tracellular loops. The C-terminus of the receptors contains a number of serine and threonine residues which, upon phosphorylation, are involved in signaling and receptor desensitization[40, 42–44, 67]. One of the intracellular loops of the chemokine receptors couples with heterotrimeric G proteins, and ligand binding to the receptor initiates a cascade of signal transduction events. Chemokine-induced receptor signaling is often sensitive to inhibition by pertussis toxin (PT), which deactivates selected G proteins[7, 58]. Activation of the receptors by chemokine ligands induces the exchange in the α subunit of the G proteins from the GDP to GTP bound state, dissociating the α from the β and γ G protein subunits. These subunits activate phospholipase (PL) Cβ1 and 2, followed by hydrolysis of phosphatidylinositol 4, 5-biphosphate (PIP_2), which leads to the formation of inositol triphosphate (IP_3) and diacylglycerol (DAG). By mobilizing intra- and/or extra-cellular Ca^{2+}, IP_3 mediates the capacity of chemokines to induce Ca^{2+} flux in leukocyte subsets[7, 58]. DAG activates protein kinase (PK) C which phosphorylates a number of effector molecules such as mitogen-activated protein kinases (MAPKs). The activation of isotypes of PLC and hydrolysis of PIP_2 vary among chemokine receptors and from cell type to cell type, which may account for the divergent cellular responses to a given chemokine. Chemokines, in addition, induce tyrosine phosphorylation in several cell types, involving the downstream MAPK cascade. Signals dependent on G proteins result in actin polymerization, reconstitution of adhesion molecules and other cellular components leading to cell migration, while tyrosine phosphorylation and DAG/PLD activation promote superoxide production. The signals resulting in the cell migration response can be dissociated from Ca^{2+} and PKC-dependent signaling pathways[7, 45, 58]. Other intracellular effectors include adenylcyclase and phospholipases A2, C and D[33]. After activation, chemokine receptors may become refractory to further stimulation with the same or other ligands[60]. This desensitization is usually complete with the same ligand and partial with other ligands and is probably crucial for maintaining the ability of the cell to "sense" a chemikine gradient.

There is a remarkable adaptation of chemokine receptors by micro-organisms to their own advantage. The Duffy antigen, known as DARC, was identified as a STM GPCR which binds several CXC and CC chemokines and is also used by *Plasmodium vivax* malaria parasites for invasion of human red blood cells[9]. DARC has been detected on post-capillary venule endothelial cells, Purkinje cells in the brain, and activated T lymphocytes, but it does not transduce chemokine signals. It has been suggested that DARC may act as a chemokine sink for clearance of excessive chemokines, or as a way to present chemokines to leukocytes[28]. A gene from human herpes virus 8 (HHV8) encoding a chemokine receptor has been detected in Kaposi's sarcoma of patients with acquired immune deficiency syndrome (AIDS)[21]. This receptor is highly homologous to receptors for CXC chemokine IL-8 (CXCR1 and CXCR2) and constitutively transduces signals. Thus, it may promote tumor angiogenesis due to an enhanced vascularization[2]. The open reading frame of cytomegalovirus (CMV) genome contains three STM GPCR sequences, one of which, US28, codes for a functional receptor for multiple CC chemokines[45]. Over the past 3 years, some chemokine receptors have been identified as major fusion co-receptors along with cellular CD4 for HIV-1[4, 41, 52]. CXCR4, which is a receptor for the CXC chemokine SDF-1, mediates the entry of T lymphocyte tropic HIV-1. Whereas CCR5, whose natural ligands are MIP-1α, MIP-1β, RANTES[41, 52] and MCP-2[20], is a fusion co-factor for monocyte tropic HIV-1 viruses. Other chemokine receptors, such as CCR2, CCR3[41, 52], CCR8[27] as well as CMV-US28[51] also have been reported to mediate cellular invasion by certain HIV-1 strains. Dualtropic HIV-1 strains may use both CXCR4 and CCR5[41, 52] or STRL33 on lymphocytes[34] for cell entry. In this context, chemokine ligands specific for these receptors (except for STRL33, whose ligand(s) is unknown), are able to competitively inhibit HIV-1 entry and replication in $CD4^+$ cells. Consequently, selected chemokines constitute a group of potent natural antagonists to HIV-1 infection of human cells[1, 14, 20, 27].

The pleiotropism and an apparent redundancy of chemokines and their receptors are based on the capacity of a given chemokine to use multiple receptors and the capacity of many of the receptors to respond to multiple chemokines. In addition, all leukocyte subsets express a multiplicity of chemokine receptors (Tables 1, 2). However, by using a gene-targeting approach, evidence is accumulating that each chemokine or receptor may have its unique place in various diseases. Disruption of CXC chemokine SDF-1 gene resulted in severely reduced B lymphcyte progenitors and prenatal death of the mice[46]. Disruption of the gene encoding the CC chemokine eotaxin partially reduces antigen-induced tissue eosinophilia[55], while deletion of MIP-1α gene protects mice from Coxsakie virus-associated myocarditis[15]. CCR1 is expressed on neutrophils, monocytes, eosinophils and lymphocytes and binds multiple chemokines such as MIP-1α, RANTES and MCP-3. Studies of mice with targeted gene disruption

of CCR1 provided evidence that CCR1 has non-redundant functions in hematopoiesis, host defence and inflammation. Disruption of the gene for CCR1 yielded mice resistant to pulmonary inflammation secondary to acute pancreatitis, possibly due to decreased production of TNF-α[18]. CCR1–/– mice also showed a disordered myeloid progenitor distribution, proliferation and trafficking. Neutrophils in CCR1–/– mice failed to respond to MIP-1α *in vitro* and *in vivo*, accompanied by accelerated mortality when the mice were challenged with *Aspergillus fumigatus*, a fungus principally controlled by neutrophils[17]. On the other hand, the CCR1–/– mice exhibited an increased Th1 response to *Schistosoma mansoni* egg injection, with reduced granuloma formation in the lung[17]. Deletion of another CC chemokine receptor CCR2 which has at least 5 ligands (MCP-1 through MCP-5) resulted in failure of macrophages to accumulate in inflammed peritoneum and the CCR2–/– mice failed to clear infection by the intracellular bacteria, *Listeria monocytogenes*[32]. Human subjects with a homozygous mutation in HIV-1 co-receptor CCR5 showed remarkably prolonged resistance to HIV-1 infection and the AIDS patients with heterozygous CCR5 mutation exhibit longer survival[16, 50]. Although the mice lacking CCR5 could develop normally in a pathogen--free environment[70], they showed reduced efficiency in clearance of *Listeria* infection, suggesting a defect in macrophage function. Interestingly, this reduced macrophage function might in fact be responsible for an enhanced resitance to LPS-induced endotoxemia. In addition, CCR5–/– mice had an enhanced delayed-type hypersensitivity as well as increased humoral responses to T cell--dependent antigen challenge, possibly due to an elevated production of cytokines of both Th1 and Th2 type. These observations suggest a fine-tuned chemokine and chemokine receptor network in the homeostasis in which one chemokine ligand or receptor plays a critical role.

Conclusions

Chemokines are distinct from other cytokines in their structure, cell surface receptors and restricted set of actions. The burgeoning number of chemokines and receptors has opened a new chapter in the research of pathophysiology. Chemokines possibly hold the key to selectively controlling the homing, recruitment and activation of highly specific cell types, thus playing important roles in the establishment, maintenance and clearance of many disease processes. Therefore, chemokines and chemokine receptors are important targets for the development of therapeutic agents.

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