Chemokines and chemokine receptors

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The chemokines are a family of *chemo*tactic cyto*kines* that coordinate leukocyte trafficking and activation [in the immune system. Chemokines normally promote host defense and repair, but may also regulate non-immunological processes, such as organ development and angiogenesis. They can also be used to promote detrimental processes such as cancer and autoimmunity. Chemokines act by binding to G protein-coupled receptors, two of which, CCR5 and CXCR4, are exploited by HIV for target cell entry. This chapter describes the basic principles and clinical correlates of chemokine regulation of the immune system.

KEY CONCEPTS

Chemokine and chemokine receptor properties

- *Definition* Chemokines, the largest subgroup of cytokines, are defined by structure, not function; chemokine receptors are defined by function not structure.
- *Evolution* Chemokines and chemokine receptors arose in vertebrates, and have been copied or mimicked by many poxviruses, herpes viruses and retroviruses.
- *Ligand-receptor promiscuity* Chemokines usually bind more than one receptor subtype. Chemokine receptors usually bind more than one chemokine, but from a single chemokine subclass.
- *Cell biology* Chemokines coordinate leukocyte trafficking but may have prominent nontrafficking functions (e.g., lymphocyte proliferation/apoptosis/differentiation/activation, granulocyte degranulation/superoxide production, direct antimicrobial activity), as well as effects on other cell types in nonimmunologic contexts (e.g., development, cancer, angiogenesis).
- *Biology* Chemokines act redundantly or nonredundantly *in vivo*, depending on the context. Host chemokine receptors mediate antimicrobial defense, but certain pathogens (e.g. HIV) can exploit chemokine receptors to infect the host. Moreover, excessive or inappropriate chemokine expression may pathologically amplify immunologically mediated disease.

MOLECULAR ORGANIZATION OF THE CHEMOKINE SYSTEM

CHEMOKINES

Chemokines are found in vertebrates from teleost fishes to humans. Copies of vertebrate chemokine genes are also found in many herpes viruses and poxviruses. Defined by structure, not by function, there are at least 45 unique human chemokines, which makes them the largest family of cytokines. Most chemokines are 66–111 amino acids long. All occupy a common sector of sequence space bounded loosely by ~20% identity for any pairwise comparison. Tertiary structure is highly conserved, partly because the disulfide-bonded cysteines are conservatively spaced (Fig. 11.1). Chemokines are subclassified according to variations in cysteine number and location. All have at least two cysteines, and all but two have at least four (Tables 11.1 and 11.2). In the four-cysteine group, the first two are either adjacent (CC motif, n = 24) or separated by either one (CXC motif, n = 16) or three (CX3C motif, n = 1) non-conserved amino acids. The C chemokines (n = 2) have only two cysteines, corresponding to C-2 and C-4 in the other subgroups. Disulfide bonds link C-1 to C-3 and C-2 to C-4. The conserved chemokine fold contains three β sheets arranged in the shape of a Greek key, overlaid by a C-terminal α-helical domain and flanked by an N-terminal domain that lacks order.[1] Sequence identity is <30% between members of different groups, but ranges from ~30% to 99% among CC and CXC chemokines considered separately. The group names are used as roots, followed by the letter 'L' and a number (e.g., CXCL1) in a systematic nomenclature that was established to resolve competing aliases.[2]

CC and CXC chemokines can be subclassified by additional motifs. The seven CXC chemokines with Glu-Leu-Arg (ELR) N-terminal to C-1 are >40% identical, attract neutrophils, bind the receptor CXCR2, and are angiogenic (Table 11.1). Among CXC chemokines lacking ELR, only CXCL12 is angiogenic and attracts neutrophils. CXCL9–11 also are >40% identical and share a receptor (CXCR3), but are angiostatic rather than angiogenic.

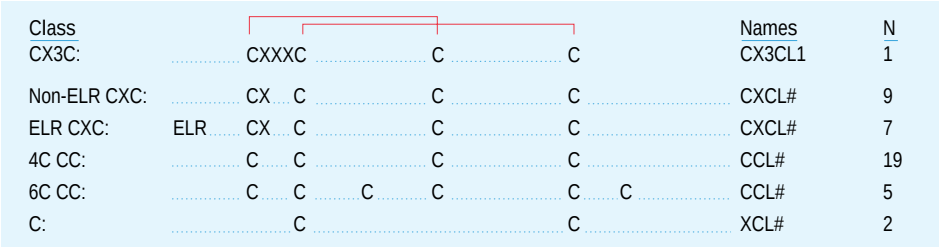

Fig. 11.1 Chemokine classification and nomenclature. Chemokine classes are defined by the number and arrangement of conserved cysteines, as shown. Brackets link cysteines that form disulfide bonds. ELR refers to the amino acids glu-leu-arg. X refers to an amino acid other than cysteine. The underscore is a spacer used to optimize the alignment. The N and C termini can vary considerably in length (not illustrated). For molecules with four cysteines, there are approximately 24 amino acids between Cys-2 and Cys-3 and 15 amino acids between Cys-3 and Cys-4. At right are listed the nomenclature system and the number of human chemokines known in each class (N).

Two cysteine-defined CC subgroups exist. Both have two additional cysteines (a total of six), with one in the C-terminal domain. They are distinguished by the location of the sixth cysteine, which can be found either in the C-terminal domain or between C-2 and C-3. CXCL16 and CX3CL1 cross classes to form a unique multimodular subgroup. Each has a chemokine domain, a mucin-like stalk, a transmembrane domain, and a C-terminal cytoplasmic module. Each can exist as either a membrane-bound or a shed form, enabling either direct cell–cell adhesion or chemotaxis, respectively.

Chemokine monomer, dimer and tetramer structures may occur. Complex quaternary structures bound to glycosaminoglycans (GAGs) on the surface of cells may also be important for function *in vivo*.[1] A native heterodimer composed of CCL3 and CCL4 subunits has been purified from activated human monocytes and peripheral blood lymphocytes.

CHEMOKINE RECEPTORS

Chemokine receptors are defined as mediators that activate cellular responses upon binding chemokines. All 19 known human subtypes are members of the seven-transmembrane (7TM) domain superfamily of G protein-coupled receptors.[3] Chemokine-binding, membrane-anchoring and signaling domains come from a single polypeptide chain. Homo- and heterodimers have been reported, but the physiologic form has not been clearly delineated.

The ligand–receptor relationship is typically promiscuous, but chemokine subgroup restricted (Table 11.3). A systematic receptor nomenclature formula exploits this as follows: receptor name = ligand subgroup root + R (for 'receptor') + number in order of discovery. An exception is the C chemokine receptor XCR1, where 'X' distinguishes it from CR1, the previously assigned name for complement receptor 1. For consistency, the XCR1 ligands are named XCL1 and XCL2.

Each chemokine has a unique receptor specificity profile. Conversely, each receptor has a unique chemokine specificity profile. Almost all chemokines are chemotactic agonists, and a few are both agonists at one receptor and antagonists at another. Differential receptor usage and differential regulation of expression may account for nonredundant function *in vivo* observed for chemokines acting at the same receptor.

KEY CONCEPTS

Immunologic classification of the chemokine system

- *Homeostatic system* Constitutively expressed ligands and receptors. Important in hematopoiesis and immune surveillance. Key receptors: CXCR4 on hematopoietic progenitors; CXCR5 on naïve B cells; CCR7 on mature dendritic cells and naïve T cells; and gut and skin-specific T-cell homing receptors CCR9 and CCR10, respectively.
- *Inflammatory system* In innate immunity, inducible ligands and constitutively expressed receptors (e.g., neutrophil CXCR2, macrophage CCR2, eosinophil CCR3, and NK cell CX3CR1). In adaptive immunity, inducible ligands and inducible receptors (e.g., CXCR3 and CCR4 on Th1 and Th2 CD4+ T cells, respectively).
- *Decoy receptors* Some membrane proteins that bind chemokines do not signal and act instead as scavengers/'decoy receptors' to limit chemokine action.

ATYPICAL CHEMOKINE SYSTEM COMPONENTS

Three human 7TM proteins (Duffy, D6 and CCX CKR) bind chemokines promiscuously but do not signal, and may function as scavengers.[4] Several endogenous nonchemokine ligands bind chemokine receptors, including aminoacyl tRNA synthetases and β defensin 2, possibly linking innate to adaptive immunity. In addition to chemokines, herpes viruses and pox viruses encode structurally related 7TM chemokine receptors, structurally unique chemokine-binding proteins (scavengers), and nonchemokine chemokine receptor ligands (agonists or antagonists). HIV also encodes chemokine mimics gp120 and tat. Viral chemokine elements may function to evade the immune system, to recruit new target cells, to reprogram gene expression for cell proliferation and angiogenesis, and for target cell entry.[5]

IMMUNOLOGIC CLASSIFICATION

Chemokines and chemokine receptors have differential leukocyte specificity, but together cover the full spectrum of leukocytes and populate two main subsystems, homeostatic and inflammatory (Table 11.4). Homeostatic

Table 11.1 The human CXC, CX3C and C chemokine families

ELR motif	Chemokine	Common aliases	Main source	Main immunologic roles	Chromosomal location
ELR+	CXCL1	GROα MGSA	Inducible in most hematopoietic and tissue cells Many tumors	Neutrophil trafficking	4q21.1
	CXCL2	GROβ			
	CXCL3	GROα			
ELR-	CXCL4	PF-4	Preformed in platelets	Procoagulant	
ELR+	CXCL5	ENA-78	Induced in epithelial cells of gut and lung; N, Mo, Plts, EC	Neutrophil trafficking	
	CXCL6	GCP-2	Induced in lung microvascular EC; Mo; alveolar epithelial cells, mesothelial cells, EC and Mφ		
	CXCL7	NAP-2	Preformed in platelets		
	CXCL8	IL-8	Induced in most cell types		
ELR-	CXCL9	Mig	Induced in PMN, Mφ, T cells, astrocytes, microglial cells, hepatocytes, EC, fibroblasts, keratinocytes, thymic stromal cells	Th1 response	
	CXCL10	IP-10	Induced in ECs, Mo, keratinocytes, respiratory & intestinal epithelial cells, astrocytes, microglia, mesangial cells, smooth muscle cells		
	CXCL11	I-TAC	ECs, Mo,		
	CXCL12	SDF-1, PBSF	Constitutive in bone marrow stromal cells; most tissues	Myelopoiesis HPC, neutrophil homing to BM B lymphopoiesis	10q11.21
	CXCL13	BCA-1	Constitutive in follicular HEV of secondary lymphoid tissue	Naïve B- and T-cell homing to follicles B1 cell homing to peritoneum Natural Ab production	4q21.1
	CXCL14	BRAK	Constitutive in most tissues, breast and kidney tumors	Macrophage migration	5q31.1
ELR+	(CXCL15)	(mouse only)	Constitutive in lung epithelial cells	Neutrophil trafficking	NA
ELR-	CXCL16	Sexckine	Constitutive in spleen; DCs of the T zone	T cell and DC homing to spleen	17p13
NA	CX3CL1	Fractalkine	EC, neurons, Mo, DC	NK, Monocyte, M and Th1 cell migration	16q13
	XCL1	Lymphotactin α	Epidermal T cells, NK, NK-T, activated CD8+ and Th1 CD4+ T cells	$CD62L^{lo}$ T effector cell migration	1q24.2
	XCL2	Lymphotactin β			

NA, not applicable. Mo, monocyte; PMN, neutrophil; DC, dendritic cell; EC, endothelial cell; HEV, high endothelial venule; MPC, myeloid progenitor cell; plt, platelet; MΦ, macrophage; GRO, growth-related oncogene; PF-4, platelet factor-4; GCP, granulocyte chemoattractant protein; ENA-78, 78 amino acid epithelial cell-derived neutrophil activator; NAP, neutrophil activating protein; IL-8, interleukin-8; Mig, monokine induced by IFN-γ; I-TAC; interferon-inducible T-cell α chemoattractant; SDF, stromal cell-derived factor; BCA, B cell-activating chemokine; BRAK, breast and kidney associated chemokine.

Table 11.2 The human CC chemokine family

Chromosomal location	Chemokine	Common aliases	Sources	Main immunologic roles
17q11-12	**CCL1**	I-309	Inducible in Mo and CD4+ and CD8+ αβ and CD4-CD8- γδ T cells	Th2 response
	CCL2	MCP-1	Inducible in Mo, fibroblasts, keratinocytes, EC, PMN, synoviocytes, mesangial cells, astrocytes, lung epithelial cells and MΦ. Constitutively made in splenic arteriolar lymphatic sheath and medullary region of lymph node, many tumors, and arterial plaque EC.	Innate immunity Th2 response CD4+ T cell differentiation
	CCL3	MIP-1α LD78α MIP-1αS	Inducible in Mo/MΦ, CD8+ T cells, B cells, plts, PMN, Eo, Ba, DC, NK, mast cells, keratinocytes, fibroblasts, mesangial cells, astrocytes, microglial cells, epith cells	Innate immunity Th1 response CD4 T cell differentiation
	CCL3L1	LD78β MIP-1αP	Similar to CCL3	Probably similar to CCL3
	CCL4	MIP-1β	Similar to CCL3	Innate immunity Th1 response
	CCL5	RANTES	--Inducible in EC, T cells, epithelial cells, Mo, fibroblasts, mesangial cells, NK cells --Constitutively expressed and stored in plt and Eo granules	Innate immunity Th1 and Th2 response
NA	**(CCL6)**	Mouse only	Inducible in bone marrow and peritoneal-derived MΦ	ND
17q11-12	**CCL7**	MCP-3	Inducible in Mo, plts, fibroblasts, EC, skin, bronchial epithelial cells, astrocytes	Th2 response
	CCL8	MCP-2	Inducible in fibroblasts, PMN, astrocytes Constitutively expressed in colon, small intestine, heart, lung, thymus, pancreas, spinal cord, ovary, placenta	Th2 response
NA	**(CCL9/10)**	Mouse only	Constitutively expressed in all mouse organs except brain; highest in lung, liver and thymus Induced in heart and lung	ND
17q11	**CCL11**	Eotaxin	Epithelial cells, EC, smooth muscle, cardiac muscle, Eo, dermal fibroblasts, mast cells, MΦ, Reed–Sternberg cells	Th2 response Eosinophil trafficking Mast cell trafficking Basophil trafficking, degranulation
NA	**(CCL12)**	Mouse only	Inducible in lung alveolar MΦ and smooth muscle cells; spinal cord. Constitutive expression in lymph node and thymic stromal cells	Allergic inflammation

NA, not applicable. Mo, monocyte; PMN, neutrophil; DC, dendritic cell; EC, endothelial cell; HEV, high endothelial venule; MPC, myeloid progenitor cell; plt, platelet; MΦ, macrophage; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; RANTES, regulated upon activation normal T-cell expressed and secreted; MRP, MIP-related protein; HCC, hemofiltrate CC chemokine; TARC, thymus and activation related chemokine; PARC, pulmonary and activation related chemokine; ELC, Epstein–Barr virus-induced receptor ligand chemokine; LARC, liver and activation related chemokine; SLC, secondary lymphoid tissue chemokine; MDC, macrophage-derived chemokine; MPIF, myeloid progenitor inhibitory factor; TECK, thymus expressed chemokine; CTACK, cutaneous T cell-associated chemokine; MEC, mucosa-associated epithelial cell chemokine; ND, not determined.

Continued

chemokines are differentially and constitutively expressed in specific microenvironments of primary and secondary immune organs, and recruit hematopoietic precursor cells, dendritic cells (DC) and naïve and memory lymphocyte subsets via constitutively expressed receptors. Noxious stimuli induce inflammatory chemokines in diverse tissue cells and leukocytes. Inflammatory chemokine receptors are constitutively expressed on myeloid and NK cells, but must be induced on activated effector lymphocytes. Dynamic shifts in receptor expression occur during DC and NK cell maturation and during lymphocyte maturation, activation and differentiation.[6,7]

Inflammatory CXC and CC chemokine genes are found in two main clusters on human chromosomes 4q12-q21 and 17q11-q21, respectively.

Table 11.2 The human CC chemokine family—cont'd

Chromosomal location	Chemokine	Common aliases	Sources	Main immunologic roles
17q11-12	**CCL13**	MCP-4	Inducible in nasal and bronchial epithelial cells; dermal fibroblasts; PBMCs; atherosclerotic plaque EC and MΦ Constitutively expressed in small intestine, colon, thymus, heart and placenta	Th2 response
	CCL14a	HCC-1	Constitutively expressed in most organs; high plasma levels	ND
	CCL14b	HCC-3	Same as CCL14b except absent from skeletal muscle and pancreas	ND
	CCL15	HCC-2; Lkn-1	Inducible in Mo and DC Constitutive RNA expression in liver, gut, heart and skeletal muscle, adrenal gland and lung leukocytes.	ND
	CCL16	HCC-4; LEC	Constitutively expressed in liver, possibly many other organs. Also, Mo, T cells and NK cells express mRNA.	ND
16q13	**CCL17**	TARC	Constitutive in normal DC and Reed–Sternberg cells of Hodgkin's disease	Th2 response
17q11.2	**CCL18**	DC-CK1, PARC	Constitutive in Mo/MΦ, germinal center DC	DC attraction of naïve T cells
9p13.3	**CCL19**	ELC, MIP-3β	Constitutive on interdigitating DC in secondary lymphoid tissue	Naïve and memory T cell and DC homing to lymph node
2q36.3	**CCL20**	LARC MIP-3α	Constitutive in lymph nodes, peripheral blood leukocytes, thymus, and appendix Inducible in PBMC, HUVEC	DC homing to Peyer's patch Humoral response
9p13.3	**CCL21**	SLC, 6Ckine	Constitutive in lymphatic EC, HEV and interdigitating DC in T areas of 2° lymphoid tissue, thymic medullary epithelial cells and EC	Naïve and memory T cell and DC homing to lymph node
16q13	**CCL22**	MDC	Constitutive in DC and MΦ Inducible in Mo, T and B cells	Th2 response
17q12	**CCL23**	MPIF-1	Constitutive in pancreas and skeletal muscle	ND
7q11.23	**CCL24**	Eotaxin-2	Inducible in Mo	Eosinophil migration
19p13.3	**CCL25**	TECK	Constitutive in thymic stromal cells and small intestine	Thymocyte migration Homing of memory T cells to gut
7q11.23	**CCL26**	Eotaxin-3	Constitutive in heart and ovary Inducible on dermal fibroblasts and EC	Th2 response
9p13.3	**CCL27**	CTACK, Eskine	Constitutive in placenta, keratinocytes, testis and brain	Homing of memory and effector T cells to skin
5p12	**CCL28**	MEC	Constitutive in epithelial cells of gut, airway	Homing of T cells to mucosal surfaces

However, homeostatic chemokine genes are on multiple chromosomes in small clusters. Thirteen of the 19 human chemokine receptor genes are clustered at 3p21–23, and *CXCR1* and *CXCR2* are adjacent at 2q34-q35. Chemokine/receptor gene repertoire may vary among closely related species. Gene copy number and sequence may also vary among individuals of a species. Such variation may affect the risk of disease.

CHEMOKINE PRESENTATION MECHANISMS

Chemokines act locally. They are probably presented tethered to matrix or to endothelial cells via glycosaminoglycans or transmembrane domains. The tethering cell may have produced the chemokine, or else imported it by transcytosis from neighbors. The ligand-binding site includes the receptor N terminus and one or more extracellular loops, which allow

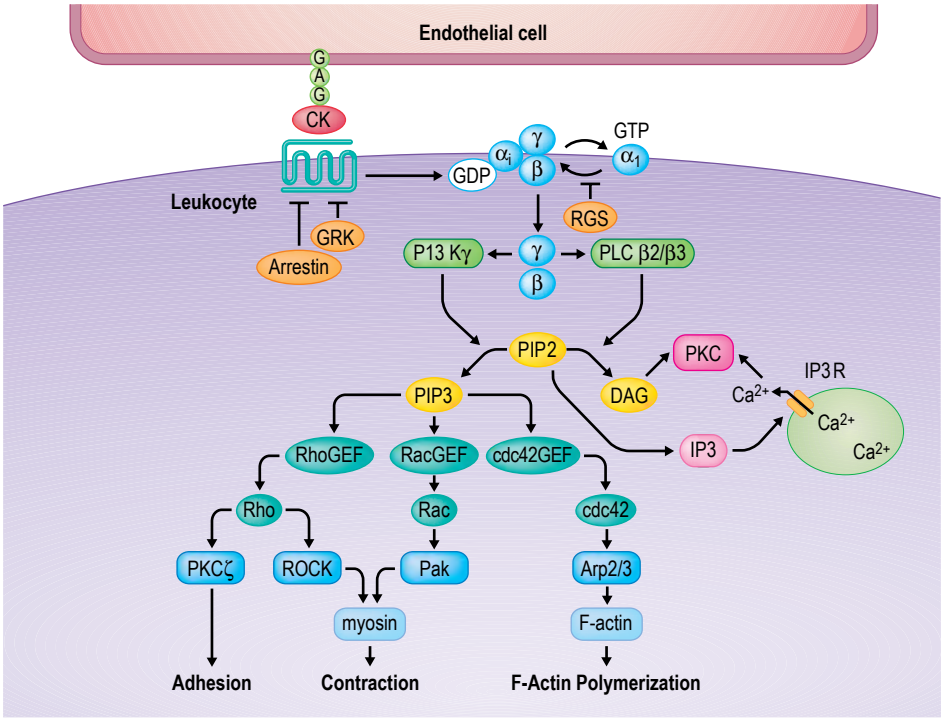

Fig. 11.2 Chemokine signal transduction in chemotaxis. Depicted are key steps in two of the main pathways induced by most chemokines. The PI3Kγ pathway is particularly important for cell migration. Chemokines are able to activate other pathways as well, including non-G_i-type G proteins, protein tyrosine kinases and MAP kinases. These pathways influence cell proliferation and activation. The model is modified from the Alliance for Cell Signaling (www.signaling-gateway.org). PLC, phospholipase C; PI3K, phosphatidylinositol-3-kinase; RGS, regulator of G protein signaling; DAG, diacylglycerol; IP3, inositol trisphosphate; PIP, phosphatidylinsol phosphate; GAG, glycosaminoglycan; CK, chemokine; PKC, protein kinase C; GRK, G protein-coupled receptor kinase; GEF, guanine nucleotide exchange factor.

docking of the chemokine N-loop domain and 7TM domains, which accept the chemokine's N terminus and are critical for triggering. Tyrosine sulfation on the receptor N terminus facilitates ligand binding.

LEUKOCYTE RESPONSES TO CHEMOKINES

During inflammation, leukocytes undergo transendothelial migration, a multistep process. Chemokines regulate at least two of these steps.[8] In an initial chemokine-independent step, leukocytes roll on inflamed endothelium in a selectin-dependent manner. Next, chemokines posted on endothelium stimulate rolling leukocytes to express activated β_2 integrins, which mediate firm adhesion via endothelial ICAMs. Leukocytes sense chemokine gradients, polarize, and become poised to crawl.[9] Motion involves shear-dependent coordinated cytoskeletal remodeling, involving expansion of the leading edge (lamellipodium), myosin-based contraction at the trailing edge (uropod), release of the uropod from substrate, and membrane lipid movement. Navigation through tissue may require relays of chemokines and adhesion molecules.

Chemokines may also modulate cell proliferation and death pathways.[10] Inflammatory chemokines may induce mediator release (e.g. defensins, proteases, perforins, histamine, eicosanoids), resulting in cytotoxic or vasomotor responses. Nevertheless, when injected systemically chemokines are typically well tolerated. Some chemokines, e.g. CXCL9–11, have direct antibacterial activity.

CHEMOKINE SIGNALING PATHWAYS

Chemokines trigger chemokine receptors to act as guanine nucleotide exchange factors (GEF), mainly for G_i-type G proteins.[11,12] This results in G protein activation and dissociation into α and βγ subunits, which in turn leads to the activation of diverse G protein-dependent effectors, including phospholipases A2, C (subtypes β2 and β3) and D, phosphatidylinositol-3-kinase γ (PI3Kγ), protein tyrosine kinases (PTK) and phosphatases, low molecular weight GTPases, and mitogen-activated protein kinases (Fig. 11.2).

Cytosolic and calcium-independent PLA2 catalyze the formation of arachidonic acid from membrane phospholipids and have been shown to enhance chemokine activation of human monocyte chemotaxis. PLC hydrolyzes PI-4,5-bisphosphate (PIP_2) to form 1,2-diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). IP_3 induces Ca^{2+} release from intracellular stores which acts with DAG to activate protein kinase C (PKC). PI3Kγ phosphorylates PIP_2 to form PIP_3, which recruits proteins