Chemokines

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Naofumi Mukaida

Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University

Contact Information
Dr. Naofumi Mukaida
Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
Tel: +81-76-264-6735
Fax: +81-76-234-4520
E-mail: mukaida@staff.kanazawa-u.ac.jp

I. Introduction

Since the first description of CXCL8/interleukin-8 (IL-8) as a neutrophil chemotactic cytokine in 1987, a family of structurally related cytokines has been identified. Because most of them exhibit chemotactic activity against a limited spectrum of leukocytes, they are now called chemokines (*chemo*tactic cyto*kines*). Chemokines direct the movement of various types of cells, particularly leukocytes, along concentration gradient, by modulating expression and structure of adhesion molecules and cytoskeletal proteins of the target cells. Due to their molecular stability and target specificity, chemokines are presumed to be crucial in leukocyte infiltration and subsequent activation in inflammation. Moreover, chemokines participate in other various biological phenomena including immune reactions, angiogenesis, and organ development.

Chemokines are divided into four subgroups, CXC, CC, C, and CX3C (Table 1). CXC, CC, and CX3C chemokines have four cysteines, whereas C chemokines have only two, corresponding to the second and fourth cysteines in the other groups. CXC and CX3C chemokines are distinguished by the presence of one (CXC) and three amino acids (CX3C), while the first two cysteines are adjacent in CC chemokines. Chemokines exhibit 3-β sheets with α helix at the carboxyl terminal portion, due to the presence of two disulfide bridges formed between the first and the third, and between the second and the fourth cysteines. CXCL16 and CX3CL1 are expressed in a membrane-bound form but other chemokines are secreted proteins with a molecular weight about 10 kDa. They exhibit a basic nature and their α helix structure is responsible for preferential binding to proteoglycans on the vascular endothelial cells and to extracellular matrix proteins. Chemokine receptors comprise a large branch of the rhodopsin family of cell-surface G-protein-coupled receptors (GPCR) with seven-transmembrane domains. Functional binding to the target cells and subsequent signaling is mediated by these receptors.

A nomenclature system has been proposed for chemokines and their receptors. Systematic chemokine names, shown in Table 1 with their common names, are built from cysteine subclass roots, followed by “L” for “ligand”. The numbers correspond generally to the same number used in the corresponding gene nomenclature. Because most chemokine receptors are restricted to a single chemokine subclass, the nomenclature system of chemokine receptors is rooted by the chemokine subclass specificity, followed by “R” for “receptor” and the number (Table 2).

II. Chemokine receptors and their signal transduction mechanism

Until present, approximately 20 chemokine receptors have been identified (Table 2). Chemokine receptors are a G-protein coupled receptor (GPCR) with 7 transmembrane portions and are coupled with heterotrimeric Gαβγ proteins bound to intracellular loops (Figure 1). The Gα subunit contains a GTPase domain involved in binding and hydrolysis of GTP. In the inactive state, the Gα subunit binds GDP, and interacts directly with the intracellular loop of chemokine receptors and with Gβ subunit, which in turn forms a tight complex with Gγ subunit. A chemokine specifically recognizes and binds the receptor. Consequently, the amino-terminus of the chemokine interacts with the receptor, leading to the activation of receptor. Simultaneously, ligand binding induces internalization of the chemokine receptor by using the clarthrin-mediated pathway or the lipid rafts/caveole-dependent internalization routes. Internalized receptors are recycled and reappear on the cell surface quickly.

The ligand binding activates pertussis toxin-sensitive and receptor-coupled G-proteins, particularly Gαi proteins. G-proteins, upon conversion to the guanosine triphosphate (GTP)-bound form, dissociate into Gα and Gβγ subunits. Generated Gβγ activates phosphatidylinositol 4-phoshate kinase (PIP-K), phosphpolipase C (PLC)-β, and phosphosphatidyl-3-kinase-γ

(PI3Kγ). PIP-K and PLC-β generate inositol 1,4,5-trisphosphate (IP_3) and diacyl glycerol (DAG). IP_3 induces transient increase in intracellular Ca^{2+} through mobilization of intracellular Ca^{2+} store while DAG activates protein kinase C. These steps are required for superoxide production and granule release but not chemotaxis. The activation of PI3Kγ leads to the generation of PIP_3. PIP_3, in turn, activates protein kinase B (Akt) and small GTPases, resulting in chemotaxis and adherence. In addition, active Gα and Gβγ facilitate the polarization of the cells with the leading edge (pseudopodium) in the front and the formation of a trailing tail (uropod) at the back. PI3K and Rac accumulate at the leading edge to induce actin polymerization and F-actin formation. Simultaneously, Rho and its effector molecules accumulate at the trailing edge to facilitate actomyosin contraction and tail retraction, thereby leading to the migration of the cells. PIP_3 also activates Raf and mitogen-activated protein kinase (MAPK) pathway, leading to the transcription of various genes.

GPCR-mediated signals can be down-regulated by RGS (regulator of G-protein signaling) proteins. RGS proteins appear to enhance the endogenous GTPase activities and thus decrease the half-life of the active GTP-bound state of both trimeric G-proteins and small GTPases. RGS1, RGS3, and RGS4 reduce CXCL8-mediated migration and adherence together with attenuated CXCL8-induced MAPK activation.

The binding of a chemokine to its corresponding receptor expose the tyrosine residue in DRY motif in the second transmembrane region. This exposure allows access of Janus kinase, which activates the receptor by tyrosine phosphorylation. Simultaneous activation of Janus kinase leads to the recruitment of STAT (signal transducers and activators of transcription) and eventually STAT-mediated expression of the target genes (Figure 1). Moreover, this pathway requires ligand-induced homodimerization of chemokine receptors, as observed on other GPCRs that can frequently exist as dimers and/or high-order oligomers.

III. Chemokines in leukocyte trafficking and functions

Chemokines are functionally designated as homeostatic and inflammatory chemokines. Homeostatic chemokines are constitutively expressed and control basal leukocyte trafficking, such as lymphocyte homing to secondary lymphoid organs. On the contrary, the inflammatory chemokines are expressed in response to infection and tissue injury, and are responsible for the recruitment of effector leukocytes to the site of inflammation. Some chemokines exert both homeostatic and inflammatory funcitons. Representative inflammatory chemokines are CXC chemokines, which bind CXCR1 and/or CXCR2 and possess a 3-amino acid sequence, glutamic acid-leucine-arginine (the “ELR” motif), immediately preceding the CXC sequence. Human ELR-motif-positive CXC chemokine genes are present in cluster at chromosome 4q13.3. These chemokines regulate cooperatively neutrophil infiltration observed in acute inflammation. Another major inflammatory chemokine is CCL2, which have a crucial role in monocyte/macrophage infiltration in chronic inflammation. Moreover, CCR3-binding CC chemokines, particularly CCL11, CCL24, and CCL26, are major regulators of eosinophil infiltration.

Immune response consists of two phases; innate and adaptive immunity. Innate immune response is mediated by natural killer (NK) cells in addition to neutrophils and monocytes. NK cells migrate to lymph nodes mainly by utilizing CXCR3 and CCR7, while their migration to the inflamed tissues including tumor sites involves CCR1, CCR2, CCR5, CXCR3, and CX3CR1. Thus, the ligands for these receptors can regulate NK cell trafficking and augment their functions.

Adaptive immunity is mediated by the interaction between antigen-presenting cells and T and B lymphocytes. Dendritic cells (DCs) are professional antigen-presenting cells and widely distributed over peripheral tissues. DCs in peripheral tissues are in an immature state with a high capacity to

endocytose various materials and express various chemokine receptors including CCR1, CCR2, CCR4, CCR5, CCR6, CCR8, and CXCR4. The ligands for these receptors can attract immature DCs, which capture exogenous and endogenous antigens. When DCs capture antigens in the presence of inflammatory stimuli, they change to a mature state with a loss of endocytosis ability and start to migrate into the T cell areas of regional lymph nodes via afferent lymphatic venules under the guidance of CCR7. Mature DCs process the antigens into the peptides presented on MHC molecules, exhibit enhanced expression of co-stimulatory molecules, and induce primary immune responses through antigen presentation to T cells in the regional lymph node.

In addition to mature DCs, the ligands for CCR7, CCL19 and CCL21, induce the migration of naïve T and B lymphocytes towards the T cell zone of the secondary lymphoid organs. Moreover, CXCL13 induce B cell homing to follicle of the lymph node under homeostatic conditions. In the lymph nodes, DCs present antigen to T lymphocytes to make them effector cells. T lymphocytes are polarized into various typrs of effector cells including cytotoxic T lymphocytes, Th1, Th2, and regulatory T cells. Effector T lymphocytes express distinct sets of chemokine receptors (Table 2) and migrate to the periphery in response to the corresponding chemokine to conduct their functions.

IV. Chemokines and non-leukocytic cells

Neovascularization is crucial for tissue repair at inflammatory responses and tumor growth. ELR motif-positive CXC chemokines, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8 can directly promote the migration and proliferation of endothelial cells and eventually neovascularization, mainly interacting with CXCR2 but not CXCR1. CXCL12 is not an ELR-positive CXC chemokine but exhibits potent angiogenic effects. In addition, three CC chemokines, CCL2, CCL11, and CCL16 have also been implicated in tumor

neovascularization. Moreover, CCL2 can indirectly induce tumor neovascularization by recruiting tumor-associated macropahges, which can produce various angiogenic factors and can be incorporated into tumor vasculature. On the contrary, CXCL4 and interferon-inducible ELR motif-negative CXC chemokines such as CXCL9, CXCL10, and CXCL11 inhibit the angiogenesis and after binding a common receptor, CXCR3.

Chemokines have direct impacts on tumor cells. Oncogene-induced senescence (OIS) serves as a potent barrier against oncogenic transformation by suppressing the unscheduled proliferation of early neoplastic cells. The cells undergoing OIS secrete CXCR2-binding chemokines and IL-6 through the activation of 2 pro-inflammatory transcription factors, C/EBP-β and NF-κB. The secreted CXCR2-binding chemokines can promote the arrest of cellular growth and eventually delay the early phase of tumorigenesis. On the contrary, pro-tumorigenic gene expression and proliferation in tumor cells can be enhanced by various chemokines including CXCL8, CXCL12, CXCL16, CCL19, CCL20, CCL21, CCL27, and CCL28. Moreover, CXCL8 and CXCL12 have an important role in epithelial-mesenchymal transition (EMT), a crucial step for tumor invasion and metastasis. CXCL12 and CCL21 can further confer the survival advantage on tumor cells circulating in bloodstream. Furthermore, CXCR4, CCR7, CCR9, CXCR1, and CXCR2 are detected in tumor cells and their ligands can induce the chemotaxis of the corresponding receptor-expressing cells. As a consequence, specific chemokine receptor-expressing tumor cells can migrate to and survive in the organs with high expression levels of respective chemokines. Thus, chemokines may accelerate metastasis by promoting tumor cell proliferation or preventing tumor cell death.

These findings collectively suggest that chemokines can prevent tumorigenesis in the early phase by inducing cellular senescence and can also

promote invasion and metastasis in the later phase by enhancing the motility and survival of tumor cells.

V. Chemokines and virus infection

CCR5 and CXCR4 have been described as major co-receptors of human immunodeficiency virus (HIV)-1 entry. Viruses capable of exploiting CCR5 (R5-trpoic) are predominant during the asymptomatic phase of HIV infection, whereas viruses found in late-stage use preferentially CXCR4 as their co-receptor. Non-silent CXCR4 polymorphism has been identified but is not associated with progression to AIDS. The Δ32 mutation in the *CCR5* gene leads to the production of a truncated CCR5, due to a premature stop codon arising from a 32 bp deletion in the gene sequence. Individuals homozygous for the CCR5Δ32 (1 % of Caucasians) do not express CCR5 at the cell surface and therefore are resistant to the infection by R5-tropic HIV strains. The CCR5-m303A mutation also introduces a premature stop codon and confers resistance to HIV infection in vitro. Other chemokine rececptors, CCR2, CXCR6, and CX3CR1, can be utilized by several HIV strains.

Human cytomegalovirus, a β-herpesvirus, encodes two CXC chemokine homologs, UL146 and UL147 and one CC chemokine homolog, UL128. Cytomegalovirus further encodes four chemokine receptor homologs, US28, US27, UL33, and UL78, and one chemokine scavenging protein, UL21.5. These virus gene products are presumed to have an important role in the life cycle of cytomegalovirus. Among γ-herpesvirus, Epstein-Barr virus encodes one chemokine receptor homolog while Kaposi sarcoma-associated herpes virus encodes three CC chemokine homologs and one chemokine receptor homolog.

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Chemokine name	Other names for human chemokines	Chromosome	Main receptors
CXC			
CXCL1	groα, melanoma growth stimulating activity (MGSA)	4q13.3	CXCR2
CXCL2	groβ	4q13.3	CXCR2
CXCL3	groγ	4q13.3	CXCR2
CXCL4	platelet factor 4 (PF4)	4q13.3	CXCR3
CXCL5	endothelial derived neutrophil attractatant-78 (ENA-78)	4q13.3	CXCR2
CXCL6	granulocyte chemotactic protein-2 (GCP-2)	4q13.3	CXCR1, CXCR2
CXCL7	neutrophil activating protein-2 (NAP-2)	4q13.3	CXCR2
CXCL8	interleukin-8 (IL-8)	4q13.3	CXCR1, CXCR2
CXCL9	monokine induced by interferon γ (Mig)	4q21.1	CXCR3
CXCL10	interferon-inducible protein-10 (IP-10)	4q21.1	CXCR3
CXCL11	interferon-inducible T cell α chemoattractant (I-TAC)	4q21.1	CXCR3, CXCR7
CXCL12	stromal cell-derived factor-1 (SDF-1)α/β	10q11.21	CXCR4, CXCR7
CXCL13	B-lymphocyte chemoattractant (BLC)	4q21.1	CXCR5
CXCL14	breast and kidney (BRAK)	5q31.1	unknown
CXCL15	unidentified in human		
CXCL16	SR-PSOX	17p13.2	CXCR6
CXCL17	DMC	19q13.2	unknown
CC			
CCL1	I-309	17q11.2	CCR8
CCL2	monocyte chemoattractant protein (MCP)-1	17q11.2	CCR2
CCL3	macrophage inflammatory protein (MIP)-1α, LD78α	17q11.2	CCR1, CCR5
CCL3L1	MIP-1αP, LD78β	17q12	CCR1, CCR3, CCR5
CCL4	MIP-1β, small inducible cytokine A4	17q12	CCR5
CCL4L1	small inducible cytokine A4-like 1	17q12	CCR5
CCL5	RANTES	17q12	CCR5, CCR1, CCR3
CCL6	unidentified in human		
CCL7	MCP-3	17q11.2	CCR1, CCR2, CCR3
CCL8	MCP-2	17q11.2	CCR1, CCR2, CCR3, CCR5
CCL9	unidentified in human		
CCL10	unidentified in human		
CCL11	eotaxin	17q11.2	CCR3, CCR5
CCL12	unidentified in human		
CCL13	MCP-4	17q11.2	CCR1, CCR2, CCR3, CCR5
CCL14	human hemofiltrae CC chemokine (HCC)-1	17q12	CCR1
CCL15	HCC-2	17q12	CCR1, CCR3
CCL16	HCC-4	17q12	CCR1, CCR2, CCR5
CCL17	thymus and activation-regulated chemokine (TARC)	16q13	CCR4
CCL18	pulmonary and activation-regulated chemokine (PARC)	17q12	unknown
CCL19	EBI1-ligand chemokine (ELC), MIP-3β	9p13.3	CCR7
CCL20	MIP-3α, LARC	2q36.3	CCR6
CCL21	secondary lymphoid-tissue chemokine (SLC), 6Ckine	9p13.3	CCR7
CCL22	macrophage-derived chemokine (MDC)	16q13	CCR4
CCL23	myeloid progenitor inhibitory factor (MPIF)-1	17q12	CCR1
CCL24	eotaxin-2, MPIF-2	7q11.23	CCR3
CCL25	thymus-expressed chemokine (TECK)	19p13.2	CCR9
CCL26	eotaxin-3	7q11.23	CCR3
CCL27	interleukin-11 receptor α-locus chemokine (ILC)	9p13.3	CCR10
CCL28	mucosae-associated epithelial chemokine (MEC)	5p12	CCR10, CCR3
CX3C			
CX3CL1	fractalkine	16q13	CX3CR1
C			
XCL1	lymphotactin, single cysteine motif (SCM)-1α	1q24.2	XCR1
XCL2	SCM-1β	1q24.2	XCR1

Table 1. Human hemokines and their corresponding receptors

Receptor	Chemokines	Receptor expression in			
		Leukocytes	Epithelium	Endothelium	Fibroblast
CXC					
CXCR1	CXCL6, 8	PMN	+	-	-
CXCR2	CXCL1, 2, 3, 5, 6, 7, 8	PMN	+	+	-
CXCR3	CXCL4, 9, 10, 11	Th1, NK	-	+	-
CXCR4	CXCL12	Widespread	+	+	-
CXCR5	CXCL13	B	-	-	-
CXCR6	CXCL16	activated T	+	-	+
CXCR7	CXCL11, CXCL12	Widespread	+	+	-
CC					
CCR1	CCL3, 3L1, 5, 7, 8, 13, 14, 15, 16, 23	Mo, Mϕ, iDC, NK	+	+	-
CCR2	CCL2, 7, 8, 13, 16	Mo, Mϕ, iDC, NK, activated T, B	+	+	-
CCR3	CCL5, 7, 8, 11, 13, 15, 24, 26, 28	Eo, Ba, Th2	-	+	-
CCR4	CCL17, 22	iDC, Treg, Th2, NK, T, Mϕ	-	-	-
CCR5	CCL3, 3L1, 4, 4L1, 5, 8, 11, 13, 16	Mo, Mϕ, NK, Th1, activated T	+	-	+
CCR6	CCL20	iDC, activated T, B	+	-	-
CCR7	CCL19, 21	mDC, Mϕ, naïve T, activated T	+	-	-
CCR8	CCL1	Mo, iDC, Th2, Treg	-	-	-
CCR9	CCL25	T	+	-	-
CCR10	CCL27, 28	activated T, Treg	+	-	-
CX3C					
CX3CR1	CX3CL1	Mo, iDC, NK, Th1	+	-	-
XC					
XCR1	XCL1, 2	T, NK, DC	-	-	-

Table 2. The human chemokine system. Leukocyte anonyms are as follows: Ba, basophil; Eo, eosinophil; iDC, immature dendritic cell; mDC, mature dendritic cell; Mo, monocyte; Mϕ, macrophage; NK, natural killer cell; PMN, polymorphonuclear cell; Th1, type I helper T cell; Th2, type II helper T cell; Treg, regulatory T cell.

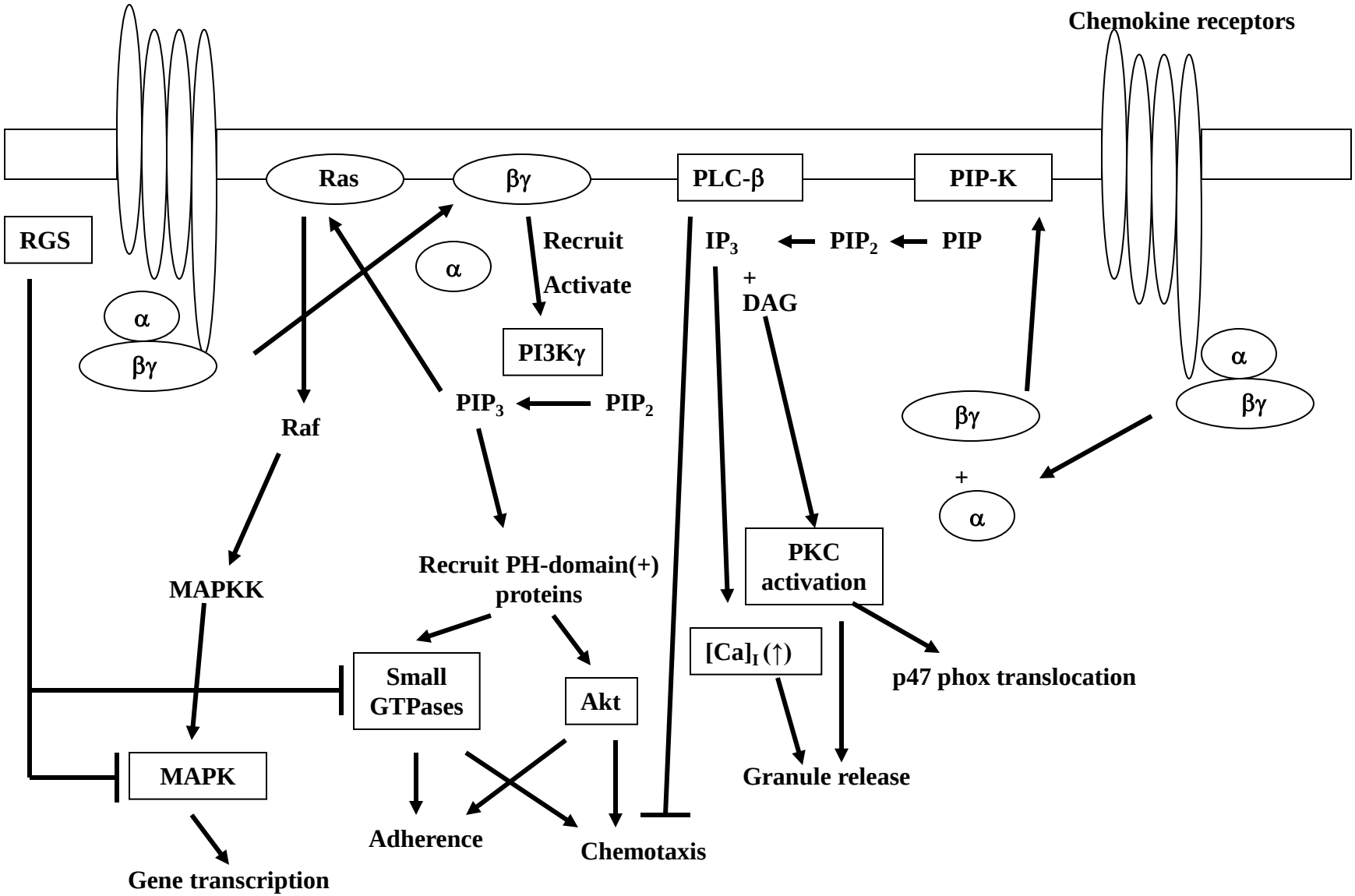

Figure. Major signaling pathways of chemokines