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REVIEW

Chemokines in homeostasis and diseases

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For the past twenty years, chemokines have emerged as a family of critical mediators of cell migration during immune surveillance, development, inflammation and cancer progression. Chemokines bind to seven transmembrane G protein-coupled receptors (GPCRs) that are expressed by a wide variety of cell types and cause conformational changes in trimeric G proteins that trigger the intracellular signaling pathways necessary for cell movement and activation. Although chemokines have evolved to benefit the host, inappropriate regulation or utilization of these small proteins may contribute to or even cause diseases. Therefore, understanding the role of chemokines and their GPCRs in the complex physiological and diseased microenvironment is important for the identification of novel therapeutic targets. This review introduces the functional array and signals of multiple chemokine GPCRs in guiding leukocyte trafficking as well as their roles in homeostasis, inflammation, immune responses and cancer.
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INTRODUCTION

Leukocyte accumulation is a hallmark of inflammation, immune responses and cancer progression and is mediated by a variety of small protein mediators named chemokines that bind to G protein-coupled receptors (GPCRs). Chemokine GPCRs are mainly expressed by leukocytes, and their major function was originally proposed to be guiding leukocyte trafficking and homing, activating integrins, generating superoxide anions and releasing of granule contents. These functions constitute the first-line host defense against invading microorganisms and tissue injury.[1] However, over the past decade, chemokines and their GPCRs have also been implicated in many essential pathophysiological processes, including development, angiogenesis, epithelial homeostasis and cancer progression.[2] This article aims to update the current understanding of the multifaceted functions of chemokines and their GPCRs to better position these molecules in the sphere of diseases for the identification of novel therapeutic targets. The readers are also advised to refer to excellent specific review articles for more in-depth information.[3–8]

CHEMOATTRACTANT GPCR AND LIGAND FAMILIES

GPCRs that mediate cell chemotaxis are categorized into classical and chemokine subfamilies according to the ligand source.[9] Classical GPCRs include formyl peptide receptors (FPR1, FPR2 and FPR3), platelet-activating factor receptor (PAFR), activated complement component 5 receptor (C5aR) and leukotriene B4 receptors (BLT1 and BLT2). Another classical chemoattractant GPCR is ChemR23, known as chemokine-like receptor 1 (CMKLR1). ChemR23 is mainly expressed in myeloid cells, such as human macrophages, immature dendriticc cells (DCs)[10] and plasmacytoid DCs (PDCs).[11] This receptor is detected in the thymus, bone marrow, spleen, fetal liver and lymphoid organs, suggesting its involvement in diverse pathophysiological processes. Structurally, CMKLR1 is more closely related to chemoattractant receptors, such as FPRs as well as the C3aR and C5aR, rather than to chemokine receptors.[12] The chemotactic ligands for ChemR23 include chemerin, the chemerin-derived peptide, C15 and the lipid resolving E1 peptide, Resolvin E1.[10,13] Chemerin is produced by cells of high endothelial venules in reactive lymph nodes (LNs) and in dermal blood

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vessels in autoimmune skin lesions. The ChemR23/chemerin pair has received increasing attention due to its involvement in many pathological conditions.[11] For example, in hemostasis, the distribution of PDCs is restricted to primary and secondary lymphoid organs. However, in autoimmune diseases (that is, lupus erythematosus disease, psoriasis and rheumatoid arthritis), allergy (that is, contact dermatitis and nasal mucosa polyps) and cancer, PDCs accumulate in non-lymphoid tissues as a result of the ChemR23/chemerin interaction.[11] ChemR23/chemerin mediates an important host defense against viral infection, as shown in mouse acute viral pneumonia, in which ChemR23 attenuates lung inflammation and enhances antiviral immunity. As a consequence, ChemR23$^{-/-}$ mice exhibit delayed viral clearance and increased mortality, which are associated with reduced PDC recruitment and production of type I interferon (IFN)[14] (Table 1).

Chemokine GPCRs are composed of four subfamilies based on the conserved N-terminal cysteine residues in their mature ligands, termed C-C cchemokine motif receptor (CCR), C-X-C cchemokine motif receptor (CXCR), CX3CR and XCR [15,16] (Table 2). A subset of GPCRs, recently termed atypical chemokine receptors, including ACKR1–ACKR6, have also been identified [17,18] (Table 3). A prominent feature of many chemoattractants and GPCRs is their promiscuity, that is, some chemoattractants bind more than one GPCR, and conversely, some GPCRs display overlapping ligand specificities with variable affinities and occasionally exhibit diverse functions.[19] This finding may represent a mechanism by which the host is able to mobilize cells with flexibility according to the changing microenvironment.

CHEMOKINES AND GPCRS IN HOMEOSTASIS

Homeostatic chemokines

Under normal conditions, homeostatic chemokines regulate cellular trafficking by directing cells that express corresponding GPCRs to specific locations. For example, in the skin, CCL27 recruits cutaneous leukocyte antigen (CLA)$^+$ T cells that express the receptor CCR10.[20] In the brain, CXCL12 mediates leukocyte homing via CXCR4[21]; in the small intestine, CCL25 recruits the homing of T cells that express α4β7 integrin and CCR9[22,23]; in the lungs, CXCL12 recruits stromal cell homing[24]; in LNs, CCL21 and CCL19 recruit CCR7$^+$ T cells, and CXCL13 recruits CXCR5$^+$ B and T cells; and in the bone marrow (BM), CXCL12 recruits CXCR4$^+$ hematopoietic stem cells (HSCs) and creates an HSC 'niche'.[25,26] Therefore, inhibition of the CXCL12–CXCR4 interaction 'liberates' HSCs from the BM niche into circulation (Table 4). Accumulating evidence indicates that homeostatic chemokines are critical for tissues and organs to maintain a consistent and dynamic leukocyte composition in preparation for immune responses triggered by insults.

Central nervous system (CNS)

A number of homeostatic chemokines are expressed in the vasculature of the blood–brain barrier, including CXCL12, CCL19, CCL20 and CCL21, to regulate the entry of leukocytes into the CNS for immunesurveillance. CXCL12 is also involved in the development of the CNS, including adult neurogenesis and neuronal survival, with opposing activity to the homeostatic chemokine CXCL14, which regulates synaptic inputs to neural precursors. CX3CL1, another homeostatic chemokine, promotes neuronal cell survival and communication with microglia, a process also partially regulated by CXCL12.[21]

Table 1 Classical chemoattractant GPCRs and ligands

Receptor	*Expression*	*Ligands*	*Functions*
FPR1	Myeloid cells, lymphocytes, tumor cells	Bacteria- and host-derived peptides	Leukocyte chemotaxis and activation, tumor growth, invasion, angiogenesis
FPR2	Myeloid cells, tumor cells	Bacteria- and host-derived peptides	Leukocyte chemotaxis and activation, antitumor defense, tumor invasion
FPR3	Monocytes, dendritic cells, tumor cells	Synthetic and host-derived peptides	Monocyte and DC chemotaxis and activation, tumor invasion
PAFR	Macrophages, granule leucocytes, various tissue cells, tumor cells	PAF	Leukocyte chemotaxis and activation, tumor growth and metastasis; angiogenesis
C5aR	Basophils, dendritic cells, mast cells, various nonimmune cells, tumor cells	C5a	Leukocyte chemotaxis and activation, tumor metastasis; oppositing function in angiogenesis
LTB4R (BLT1)	Neutrophils, macrophages, T-lymphocytes, tumor cells	LTB4	Leukocyte chemotaxis and activation, tumor growth
LTB4R (BLT2)	Most human tissues cells, leukocytes, tumor cells	LTB4	Leukocyte chemotaxis and activation, tumor growth and metastasis
CMKLR1 (ChemR23)	Mainly in myeloid cells	Chemerin, C15, RvE1	Migration of macrophages, DCs and PDCs

Abbreviations: CMKLR1, chemokine-like receptor 1; C5aR, activated complement component 5 receptor (C5aR), LTB4R, leukotriene B4 receptor; DC, dendritic cell; FPR, formyl peptide receptor; GPCR, G-protein coupled receptor; PAFR, platelet-activating factor receptor; PDC, plasmacytoid DC; RvE1, Resolvin E1.

Bone marrow

CXCL12 is constitutively produced by BM stromal cells, including vascular endothelial cells and osteoblasts.[25,28] CXCL12 interacts with CXCR4 to retain neutrophils in the BM. *CXCR4* with WHIM-associated mutations encodes a carboxyl terminal truncated receptor with impaired internalization and enhanced activation, resulting in abnormal neutrophil retention in the BM and neutropenia in the blood.[29–32] Conversely, deletion of CXCR4 in murine hematopoietic cells causes exacerbated neutrophil mobilization into circulation.[27,33–37] For example, mice carrying a myeloid-specific deletion of *Cxcr4* (myeloid-specific knock-out (MKO) mice), in which Cxcr4 in mature neutrophils is deleted in both the BM and blood, display a skewed redistribution of neutrophils from the BM to the blood and spleen.[37] Moreover, treatment of humans or mice with an antagonist of CXCR4 causes rapid neutrophil mobilization into circulation.[38,39] Similar to MKO mice, mice with a conditional deletion of *Cxcl12* display a significant increase in the number of circulating neutrophils.[40] Therefore, the CXCR4 and CXCL12 axis is critical for the retention of neutrophils in the BM, where granulopoiesis is controlled by granulocyte colony-stimulating factor.[25]

Table 2 Chemokine receptors and ligands

Receptor	*Ligands*
CCR1	CCL3, CCL4, CCL5, CCL7, CCL13, CCL14, CCL15, CCL16, CCL23
CCR2	CCL2, CCL7, CCL8, CCL12, CCL13, CCL16
CCR3	CCL5, CCL7, CCL8, CCL11, CCL13, CCL15, CCL16, CCL24, CCL26, CCL28
CCR4	CCL2, CCL3, CCL5, CCL7, CCL22
CCR5	CCL3, CCL4, CCL5, CCL8, CCL11, CCL14, CCL16
CCR6	CCL20
CCR7	CL19, CCL21
CCR8	CCL1, CCL4, CCL17
CCR9	CCL25
CCR10	CCL27, CCL28
CXCR1	CXCL6, CXCL8
CXCR2	CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8
CXCR3	CXCL9, CXCL10, CXCL11
CXCR4	CXCL12
CXCR5	CXCL13
CXCR6	CXCL16
CXCR7	CXCL11, CXCL12
CX3CR1	CX3CL1
XCR1	XCL1, XCL2

Abbreviations: CCL, C-C cchemokine motif ligand; CCR, C-C cchemokine motif receptor; CXCL, C-X-C cchemokine motif ligand; CXCR, C-X-C cchemokine motif receptor.

Secondary lymphoid organs (SLOs)

SLOs include the LNs, spleen and Peyer's patches. In mature SLOs, follicular DCs in B-cell follicles produce CXCL13, which promotes homeostatic recruitment of B cells via CXCR5. B cells in the marginal zone of the spleen depend on CXCR7 for physiological positioning. Treatment of mice with CXCR7 antagonists leads to a decrease of B-cell numbers in the splenic marginal zone.[41] In the T-cell area, fibroblastic reticular cells produce CCL19, CCL21 and CXCL12, which localize T cells

Table 3 Atypical chemokine receptors and ligands

Name	*Common aliases*	*Ligands*
ACKR1	DARC; Duffy	CCL2, CCL5, CCL11, CCL13, CCL14, CXCL1, CXCL2, CXCL3, CXCL7, CXCL8
ACKR2	D6, CCR9, CCR10	CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCL12, CCL13, CCL14, CCL17, CCL22
ACKR3	CXCR7; RDC1	CXCL11, CXCL12
ACKR4	CCRL1; CCX-CKR, CCBP2, CCR11	CCL19, CCL21, CCL25, CXCL13
ACKR5	CKRX, CRAM-A, L-CCR, CRAM-B, HCR, CCR11	CCL5, CCL19, chemerin
ACKR6	Nir1	CCL18

Abbreviations: CCL, C-C cchemokine motif ligand; CCR, C-C cchemokine motif receptor; CXCL, C-X-C cchemokine motif ligand; CXCR, C-X-C cchemokine motif receptor.

Table 4 Homeostatic chemokines

Location	*Chemokines*	*Receptors*	*Function*	*References*
Skin	CCL27	CCR10	Skin homing of T cells	20
Brain	CXCL12	CXCR4	Brain homing of leukocytes	21
Lung	CXCL12	CXCR4	Stromal cell homing	24
Spleen	CCL19, CCL21, CXCL13	CCR7, CXCR5	T cell, B cell and DC homing	27
Small intestine	CCL25	CCR9	Lymphocyte homing	8,9
Secondary lymphoid tissues	CCL21, CCL19, CCL13	CCR7, CXCR5	T cell, B cell and DC homing	22
Bone marrow	CXCL12	CXCR4	Neutrophil retention in BM	25,26

Abbreviations: CCL, C-C cchemokine motif ligand; CCR, C-C cchemokine motif receptor; CXCL, C-X-C cchemokine motif ligand; CXCR, C-X-C cchemokine motif receptor; DC, dendritic cell.

and DCs via CCR7 and CXCR4. Thus, even in the absence of an immune response, naive lymphocytes and antigen-presenting cells actively migrate to and remain in SLOs by chemokine and GPCR interactions.[4]

INFLAMMATORY CHEMOKINES

Unlike homeostatic chemokines, inflammatory chemokines are not constitutively expressed at high levels in the absence of tissue damage. Such chemokines are expressed by circulating leukocytes and other cell types only upon activation. Therefore, these molecules are also termed inducible chemokines, the expression of which is often triggered by pro-inflammatory mediators, such as tumor necrosis factor and IFN-γ, and microbial products, including lipopolysaccharide. Inflammatory chemokines are expressed by numerous cell types at almost any tissue location. This expression allows for the relatively transient attraction of inflammatory leukocytes to damaged tissues. Inflammatory chemokines include CCL1–5 and CXCL1–11. In general, CXCL1–8 regulate neutrophil migration and CXCL9–11 regulate activated T-cell migration. Most inflammatory CC chemokines induce chemotaxis of monocytes, macrophages and T cells. Almost all leukocytes express complex patterns of chemokine GPCRs that allow for responses to diverse chemokines that are induced under stress conditions.[42]

Neutrophil trafficking in inflammation

Neutrophil trafficking in inflammation is guided by multiple chemokine and classical chemoattractant GPCRs that are sequentially expressed by cells in a complex tissue microenvironment. For example, Fprs antecede CXCR2 to rapidly recruit the first wave of neutrophils into the liver of *Listeria*-infected mice in response to bacterial chemoattractant peptides.[43] In mouse skin wounds, the leukotriene B4 GPCR, BLT, is critical for neutrophil infiltration, followed by dynamic cell swarming, which requires the participation of the chemokine GPCR CXCR2 and a formyl peptide GPCR Fpr2.[44] For the normal healing of sterile skin wounds, coordinated neutrophil influx by Fprs and CXCR2 is needed.[45] Therefore, neutrophil trafficking in inflammatory responses is mediated by chemotactic cues that are established by pathogen-associated molecular patterns (chemotactic PAMPs) and damage-associated molecular patterns (DAMPs) via the cooperation of chemokines as well as classical chemoattractant GPCRs.

Monocytic cell recruitment in inflammation

Monocytes migrate out of the BM by following the CCL2 gradients produced by vascular endothelial cells in response to systemic PAMPs, such as Toll-like receptor (TLR) ligands. In mouse airway inflammation, production of the CCR2 ligand CCL2 and Fpr2 ligand CRAMP is increased in lung tissues. Tightly orchestrated trafficking of monocytic DCs is established by multiple chemoattractant GPCRs, in which CCL2/CCR2 mobilize Ly6C^{high} monocytic DC precursors from the BM into circulation,[46,47] where cells extravasate into the perivascular regions of the inflamed lung guided by CCL2 and become immature DCs upon exposure to the PAMPs and DAMPs present in the airway.[2] Immature DCs subsequently lose functional CCR2, but gain high-level expression of Fpr2, and migrate to peribronchiolar regions in response to the Fpr2 ligand, a host-derived chemotactic DAMP, CRAMP,[2] which not only forms a chemotactic gradient for DC recruitment but also promotes DC maturation in cooperation with TLR agonistic PAMPs.[48] A shift of chemoattractant GPCR expression occurs again as DCs mature when Fpr2 is downregulated, with highly elevated expression of the DC-homing chemokine GPCR CCR7, enabling mature DCs to be directed to lymphatic organs. Thus trafficking of monocytic DCs in the inflammatory airway consists of the finely tuned orchestration of chemoattractant GPCRs on the cell surface, initiating from CCR2, with Fpr2 as an intermediate and CCR7 as the final player, which accomplishes the last segment of homing.

CCR2 and CCL2 (MCP-1), as prototype chemokine GPCR and ligand partners, were discovered based on their capacity to mediate monocyte recruitment in inflammation and cancer.[9] Their participation is represented by a model of acute toxoplasmosis in which mouse resistance depends on the timely appearance of Ly6C^{hi} monocyte-derived effector macrophages via the CCR2/CCL2 interaction. Mice deficient in either the receptor CCR2 or ligand CCL2 were not able to control parasite replication due to a reduction in macrophage-produced interleukin-12 and reactive nitrogen intermediates.[49,50]

Trafficking of other immune cells

Mast cell precursors migrate from the BM to the periphery via CXCR2, BLT1 and/or CYSLT1, another chemoattractant GPCR, signaling. Eosinophils leave the BM via CCR3, while CCR7 is used by mature tissue-resident DCs to migrate into afferent lymphatic vessels through CCL21 gradients.[4] Thus, despite the vast number of chemokines and their GPCRs, little redundancy exists in complex pathophysiological processes.

Under normal conditions, CXCR5 mediates the homing of B cells into lymphoid organs. However, in systemic lupus erythematosus (SLE), CXCR4 guides B-cell migration from the dark to the light zone in lymphoid follicle germinal centers in response to locally produced CXCL12. In extrafollicular regions, B cells differentiate into plasma cells to produce anti-dsDNA antibodies. There is an increased CXCR4^{high}CXCR5$^{low/-}$ B-cell population in SLE patients with higher anti-dsDNA titers in circulation. Therefore, dysregulated CXCR4/CXCR5 expression on SLE B cells may skew the translocation of B cells to extrafollicular sites, presumably to allow cells to escape negative selection and apoptosis, leading to more active promotion of the development of SLE.[51] Such a shift in chemokine GPCR levels during disease progression may determine the final destiny of immune cells with the outcome of the disease at stake.

CHEMOKINES AND GPCRS IN HUMAN DISEASES

Chemokines and their GPCRs are associated with an extraordinary array of pathological conditions[52–55] (Table 5),

including autoimmune disorders[8,56–61] (for example, psoriasis, rheumatoid arthritis and multiple sclerosis); pulmonary diseases[62–65] (for example, asthma and chronic obstructive pulmonary disease); transplant rejection[70,71]; metabolic and vascular diseases[66–69,78] (for example, obesity, diabetes and atherosclerosis; as well as infectious diseases[72–77] (for example, HIV, sepsis and inflammatory bowel disease). The active participation of chemokines and GPCRs in disease progression makes these molecules the most prominent targets for drug development.

Table 5 Chemokine GPCRs in inflammation and immunity

Diseases	*Chemokine receptors*	*References*
Autoimmune disorder		
Psoriasis	CCR1, CXCR1, CXCR2, CXCR3	56–58
RA	CCR1, CCR2, CXCR1, CXCR2, CXCR3	56–60
MS	CCR1, CCR2, CXCR3	56,57,60,61
Pulmonary diseases		
Asthma	CCR2, CCR3, CCR4, CCR5, CCR6, CCR8, CXCR4, CX3CR1	62,63
COPD	CXCR1, CXCR2	64,65
Metabolic and vascular disease		
Atherosclerosis	CCR2, CX3CR1	66,67
Diabetes	CCR2, CX3CR1	56,68,69
Obesity	CCR2, CCR5, CXCR2	56,68
Transplant rejection	CCR1, CCR2, CCR5, CXCR3	70,71
Infectious diseases		
HIV	CCR5, CXCR4	56,72,73
Sepsis	CXCR1, CXCR2	74,75
IBD	CCR9	56,76,77

Abbreviations: CCR, C-C cchemokine motif receptor; COPD, chronic obstructive pulmonary disease; CXCL, C-X-C cchemokine motif ligand; CXCR, C-X-C cchemokine motif receptor; IBD, inflammatory bowel disease; GPCR, G-protein coupled receptor; HIV, human immunodeficiency virus; MS, multiple sclerosis; RA, rheumatoid arthritis.

CHEMOKINES AND GPCRS IN CANCER

One of the epoch-making discoveries of chemokines and their GPCRs was the discovery of the ability of these molecules to mediate tumor cell chemotaxis, thus increasing the cell motility required for metastasis. This phenomenon was first reported for the chemokine MCP-1 (CCL2), which mediates organ-specific dissemination of experimental murine lymphoma,[79,80] an example of tumor cells utilizing the fundamental properties of chemokines for cell progression. This principle was later supported by evidence that chemokine GPCRs mediate metastasis of human tumor cells to distant organs. Since then, chemokines and their GPCRs have been shown to have a key role in the initiation and progression of cancers of multiple organs, including the lungs, colon, liver, breast, cervix, prostate, bladder, ovary, esophagus, skin and lymphatics.[22,81–84] Many cancer cells express chemokine GPCRs that sense ligands as growth signals and are the driving force for a motile phenotype that enables invasion and metastasis (Table 6). CXCR4 is one of the most frequently identified chemokine GPCRs in cancer and has been implicated in >23 human cancers, including breast,[86–88] ovary,[89–91] prostate,[92,93] pancreas,[94–96] melanoma,[97,98] esophagus,[99] lung,[100] head and neck,[101] bladder,[102] colorectum[103] and stomach cancers [104] as well as osteosarcoma,[105] neuroblastoma,[106] acute lymphoblastic leukemia[85] and chronic lymphocytic leukemia.[107] Activation of the CXCR4–CXCL12 axis is correlated with angiogenesis,[90,120–124] proliferation[124–128] and metastasis of tumors[129–133] (Table 7). CCR7 is another GPCR that has been frequently implicated in the progression of cancers[113–118] (Table 8), including breast,[134] melanoma,[135,136] lung,[137] head and neck,[138] colorectum,[139] chronic lymphocytic leukemia,[140] stomach,[141] non-Hodgkin's lymphoma[142] and T-cell leukemia.[143] Other chemokine GPCRs, such as CXCR3 in childhood acute lymphoblastic leukemia,[85] CXCR5 in head and neck cancer[108] and CCR2 in prostate cancer, lymphoma, glioblastoma, ovarian cancer and breast cancer[109–112] have been described. CCR9 and CCR10[119] were also implicated in cancer invasion and melanoma metastasis (Table 6). Notably, some cancers may exploit the function of more than one chemokine GPCR to their advantage, demonstrating the

Table 6 Chemokine GPCRs in cancer

Chemokine receptors	*Cancers*	*References*
CXCR3	Acute lymphoblastic leukemia	85
CXCR4	Breast cancer, ovarian cancer, prostate cancer, pancreatic cancer, melanoma, oesophageal cancer, non-small cell lung cancer, head and neck cancer, bladder cancer, colorectal cancer, osteosarcoma, neuroblastoma, acute lymphoblastic leukemia, chronic lymphocytic leukemia, stomach cancer	85–107
CXCR5	Head and neck cancer	88,108
CCR2	Prostate cancer, glioblastoma, ovarian cancer, breast cancer	109–112
CCR7	Breast cancer, melanoma, non-small cell lung cancer, head and neck cancer, colorectal cancer, chronic lymphocytic leukemia, stomach cancer, non-Hodgkin's lymphoma, T-cell leukemia	113–118
CCR9	Melanoma	119
CCR10	Melanoma	119

Abbreviations: CCR, C-C cchemokine motif receptor; CXCR, C-X-C cchemokine motif receptor; GPCR, G-protein coupled receptor.

Table 7 CXCL12–CXCR4 axis in cancer

Function	*Mechanism*	*References*
Angiogenesis	Recruiting CXCR4$^+$ BM-derived monocytes to tumor; stimulating the formation of new tumor blood vessels	85,87,104,134,135
Proliferation	Cross-talking with EGFR in breast cancer	135–139
Metastasis	Promoting EMT by activating MEK/ERK, PI3K/AKT or Wnt/β-catenin pathway	140–143
Microenvironment	Attracting CXCR4$^+$ immune cells to tumor sites; stimulating the production of EGF, CCL2, CCL5, IL-1β and CXCL8 at tumor sites	85

Abbreviations: BM, bone marrow; CCL, C-C cchemokine motif ligand; CXCL, C-X-C cchemokine motif ligand; CXCR, C-X-C cchemokine motif receptor; EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; ERK, extracellular signal–regulated kinase; IL, interleukin; MEK, mitogen-activated extracellular signal-regulated kinase; PI3K, phosphoinositide-3 kinase.

Table 8 CCL21–CCR7 axis in cancer

Cancer	*Mechanism*	*References*
Colon cancer B-cell chronic leukemia T24 bladder cancer	Upregulating MMP-9	113,114
Breast cancer	Upregulating VEGF-C	118
Non-small cell lung cancer	Upregulation of VEGF-D	115
Esophageal squamous cell carcinoma	Stimulating MUC1 expression	116
Pancreatic cancer	Activating EMT and Erk/NF-κB pathway	117

Abbreviations: CCL, C-C cchemokine motif ligand; CCR, C-C cchemokine motif receptor; EMT, epithelial–mesenchymal transition; Erk, extracellular signal–regulated kinase; MMP, matrix metalloproteinase; MUC1, mucin 1; NF, nuclear factor; VEGF, vascular endothelial growth factor.

delicate evasion of cancer cells from host defense. The bright spot, in this respect, is in the experimental system, as targeting chemokines or their GPCRs has shown beneficial effects on cancer therapy.

THE FINE-TUNED ORCHESTRATION OF CHEMOKINE SYNERGY IN THE INFLAMMATORY RESPONSES

Directional migration and arrest of leukocytes during homeostasis, inflammatory responses and tumor development are delicately controlled by a multitude of chemokines. Immune cell trafficking to inflamed tissues depends on at least three distinct but closely related processes: (1) chemoattractant GPCR expression by migrating leukocytes; (2) gain or loss of GPCR responses regulated by environmental stimuli or homologous and heterologous receptor desensitization; and (3) presence of a dominant ligand gradient in a given anatomical compartment at a given time.[144–148]

Mounting evidence indicates that many GPCRs, including chemokine GPCRs (that is, CCR2, CCR5, CXCR1, CXCR2 and CXCR4), may function as homodimers or oligomers.[149] Heterodimers between CCR2 and CCR5 or CXCR4,[150–152] as well as between CXCR1 and CXCR2,[153] have been reported. The properties of chemokine GPCRs may enable cells to enhance their responsiveness to one or more ligands produced in the vicinity. However, chemokine ligand heterocomplexes may act either as receptor antagonists or exhibit synergy by simultaneously or sequentially activating more than one GPCR. This synergy is selective for chemokines that are present in the tissue environment, often at lower concentrations. For example, CCL21 and CCL19 may each form heterocomplexes with CXCL13 to activate CCR7 at lower ligand concentrations;[154] CCL22 and CXCL10 complexes more potently activate CXCR3 to enhance migration of CCR4$^+$ lymphocytes;[155] CCL19 and CCL21 may each interact with CCL7 to enhance monocyte chemotaxis and prevent CCL7 degradation mediated by atypical chemokine receptor 2;[156] and heterocomplexes of CXCL9 and CXCL12 enhance recruitment of CD8$^+$ T cells [157] (Table 9). Therefore, both chemokine GPCRs and their ligands have unique properties, acting singularly or in combinations, to exert functions that should benefit the host defense in complex disease states that call for the optimal and differential mobilization of subsets of leukocytes.

The biological activity of chemokines is also complemented by the pro-inflammatory molecule high-mobility group box 1 protein (HMGB1), which was initially demonstrated to interact with multiple cellular receptors, including TLR4 and receptor for advanced glycation end products. However, HMGB1 attenuates the chemotactic responses of mouse embryonic fibroblasts to the chemokine CXCL12 by blocking the receptor CXCR4 or neutralizing CXCL12, suggesting that the action of HMGB1 requires the expression of cell surface CXCR4 as well as the concomitant presence of CXCL12.[6,158,159] Interestingly, HMGB1 and CXCL12 form heterocomplexes and exhibit a monocyte chemotactic activity that is more potent than that of CCXL12 alone. Mechanistically, HMGB1 increases binding of CXCL12 to CXCR4 by fixing its N-terminal domain in a conformation that is better suited for receptor activation.[6,160]

Some chemokine ligands possess sugar moieties that bind lycosaminoglycans (GAGs), which immobilize chemokines on the endothelial surface to ensure the directionality of the chemotactic signals for leukocytes.[161–163] Chemokines that fail to be immobilized by endothelial GAGs may either be dispersed from the production site or diluted to concentrations below the receptor activating threshold, thereby reducing leukocyte extravasation into tissues. Thus microenvironment GAGs are pivotal to the spatial distribution of chemokine gradients that are necessary for proper leukocyte recruitment.[164]

Table 9 Fine tuning of leukocyte trafficking by chemokine complexes

Chemokine heterocomplexes	*Functions*	*References*
CCL21 or CCL19+CXCL13	Enhance binding and activating CCR7	145–147
CCL19+CCL21	Enhance the activity of CCR2 ligands and protection from degradation	145–147
CCL22+CCL4	Enhance CCR4 activation	153
CCL22+CXCL10	Enhance CXCL10 binding to CXCR3 or GAGs; stimulate the migration of CCR4-bearing lymphocytes	147
CCL21+CCL19+CCL7	Enhance monocyte migration and prevent CCL7 degradation	147
CXCL9+CXCL12	Enhance recruitment of $CD8^+$ T cells	155
CXCR4+CXCL12	Enhance cell migration in response to HMGB1	158
HMGB1+CXCL12	HMGB1 promotes CXCL12 binding and activation of CXCR4	156

Abbreviations: CCL, C-C cchemokine motif ligand; CXCL, C-X-C cchemokine motif ligand; CCR, C-C cchemokine motif receptor; CXCR, C-X-C cchemokine motif receptor; GAG, lycosaminoglycan; HMGB1, high-mobility group box 1 protein.

PERSPECTIVES

Chemokines and GPCRs are appreciated not only as important mediators for innate immune cell trafficking in response to acute inflammatory insults but also as central cellular fate determinants of adaptive immune responses. There has been rapid progress in the understanding of the biology of chemokines and their GPCRs in the initiation and progression of diseases. The discovery that selected chemokine GPCRs are also expressed on virtually all nonhemopoietic cell types, such as endothelial, epithelial and neuronal and muscular cells, has led to the recognition of the importance of these molecules in more pathophysiological processes, such as angiogenesis, organ development and tumorigenesis.

Despite the expanded knowledge of chemokines and GPCRs generated in past decades, questions remain concerning their involvement in each stage of human disease. Taking cancer as an example, CXCL12–CXCR4 is one of the chemokine/GPCR axes that attract intense attention to its therapeutic potential. Given the recent approval of CXCR4 antagonists for clinical use,[165] it is anticipated that some of these drug leads may soon be tested in cancer patients, particularly those with metastasis. However, owing to the diversity of cancers, it is unlikely that these findings will be able to be generalized. Therefore, more studies are needed to elucidate the whole picture of chemokines and their GPCRs in the growth of primary lesions and establishment of metastatic foci. In this context, it is possible that homeostatic chemokines may directly stimulate the viability and growth of nascent metastatic lesions. Another area in need of rigorous investigation is the biology of atypical chemokine GPCRs, such as D6 and DARC, in tumorigenesis and the metastatic potential of cancer cells. Therefore, recombinant chemokine decoy receptor proteins may have the potential to act as sinks to eliminate excessive chemokine ligands that may stimulate tumor growth and metastasis.

In summary, >20 years after the first discovery of chemokines and their GPCRs, the research field continues to attract a burgeoning army of participants with diverse interests in both basic and clinical research covering almost all aspects of inflammation, immunity, infection and cancer. Thus one should be extremely optimistic about the future benefit to human health derived from more rigorous studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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