Chemokines and Chemokine Receptors in Neurological Disease: Raise, Retain, or Reduce?

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Summary: Chemokines and chemokine receptors comprise a large number of molecules implicated in a wide range of physiological and pathological functions. Numerous studies have demonstrated the roles of chemokines and chemokine receptors: 1) during development, by regulating hematopoiesis, cardiogenesis, and vascular and cerebellar development; 2) during tumor biology, by controlling cell proliferation, angiogenesis, and metastasis; and 3), especially during leukocyte migration, by acting on firm adhesion, locomotion, diapedesis, and chemotaxis. This review focuses on chemokine and chemokine receptor involvement in diverse neurological diseases and their therapeutic potentials. Because of its induction or upregulation during CNS pathologies, members of the chemokine system can be used as biological markers. CXCR4 and CXCL12, by the correlation between their expression and the glioblastoma tumor progression, could be a marker to grade this type of CNS tumor. CCR1, by virtue of specific expression in Aβ plaques, may be a marker for Alzheimer pathology. Downregulation of CCL2 in cerebrospinal fluid may be a candidate to characterize multiple sclerosis (MS), but needs additional investigation. Moreover, chemokines and chemokine receptors represent interesting therapeutic targets. Using chemokine receptor antagonists, several studies provided exciting findings for potential neurological disease treatment. Chemokine receptor antagonists reduce disease severity in animal models of MS. In glioblastoma, a CXCR4 antagonist (AMD3100) showed an inhibition of tumor growth. Inhibition of chemokine receptor signaling is not the only therapeutic strategy: for example, CXCR4–CXCL12 has anti-inflammatory properties and CX3CL1–CX3CR1 controls neurotoxicity. Thus, chemokine biology suggests several approaches for treating neurological disease. **Key Words:** Chemokines, chemokine receptors, neurological disease, cell trafficking, marker, antagonist.

INTRODUCTION

Chemokines—the term is a contraction of *chemotactic cytokines*—comprise a large family of small (8–14 kDa) basic proteins that display a wide variety of biological and pathological functions. *In vitro*, the signature assay for chemokines involves stimulation of leukocyte chemotaxis in a concentration-dependent manner. The first chemokine to be described was IL8 (CXCL8), identified in 1987 as a molecule with selective neutrophil chemoattractant properties.[1] Since then, the chemokine family steadily expanded, now including more than 50 molecules. Chemokines act by binding to G-protein-coupled cell-surface receptors on target cells. The first chemokine receptor (IL8–CXCL8 receptor) was discovered in 1991.[2] In parallel with their ligands, the interest in chemokine receptors has grown and now nearly 20 chemokine receptors have been described. Chemokine receptors are defined by selective, high-affinity ligand binding coupled with demonstrable biological activity (usually chemotaxis or calcium mobilization).

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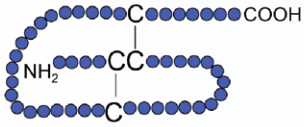
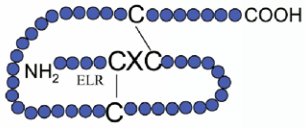
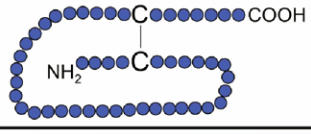
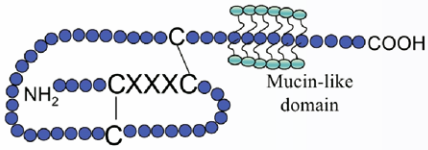
Chemokines

The complexity of the chemokine family is due to the large number of component molecules. Moreover, adding confusion to complexity, rapid discovery of new chemokines resulted in various research groups calling the same molecule by different names. This unmanageable situation motivated a consortium, at the Keystone Symposium on Chemokine and Chemokine Receptors in 1999, to create a systematic nomenclature.[3]

Chemokines are classified into four subfamilies according to the configuration of two positionally conserved cysteine residues near the NH_2 terminus. These include the CXC; CC; C; and CX3C subfamilies[4,5] (FIG. 1) (http://cytokine.medic.kumamoto-u.ac.jp/CFC/CK/Chemokine.html).

The CXC and CC chemokines are the two major sub-

Subfamily	Act on	Sub-group	Nomenclature name	Common name
CC Chemokines 	Monocytes Eosinophils Basophils T lymphocytes NK cells Dendritic cells	MCP/Eotaxin (allergenic)	CCL1	I-309
			CCL2	MCP-1
			CCL7	MCP-3
			CCL8	MCP-2
			CCL11	Eotaxin
			CCL12	MCP-5#
			CCL13	MCP-4*
			CCL24	Eotaxin-2
			CCL26	Eotaxin-3*
		Inflammatory	CCL3	MIP-1α
			CCL4	MIP-1β
			CCL5	RANTES
			CCL6	C10#
			CCL9	MIP-1γ#
			CCL10	CCL10#
			CCL18	MIP-4*
		HCC	CCL14	CC-1*
			CCL15	Leukotactin-1*
			CCL16	LEC
			CCL23	MPIF-1*
		Devlop-mental	CCL17	TARC
			CCL22	MDC
			CCL25	TECK
		Homeo-static	CCL19	ELC
			CCL20	LARC
			CCL21	SLC
		Others	CCL27	ESkine
			CCL28	MEC
CXC Chemokines 	Neutrophils Lymphocytes Monocytes	ELR+	CXCL1	GROα
			CXCL2	GROβ
			CXCL3	GROγ
			CXCL5	ENA-78
			CXCL6	GCP-2
			CXCL7	NAP-2
			CXCL8	IL-8
			CXCL15	Lungkine
		ELR-	CXCL4	PF-4
			CXCL9	MIG
			CXCL10	IP-10
			CXCL11	I-TAC
			CXCL12	SDF-1
			CXCL13	BCA-1
			CXCL14	BRAK
			CXCL16	CXCL16
C Chemokines 	Lymphocytes		XCL1	Lymphotactin α
			XCL2	Lymphotactin β*
CX3C Chemokines 	T lymphocytes Monocytes NK cells		CX3CL1	Fractalkine

* human only, # mouse only

FIG. 1. Chemokine nomenclature.

families. The largest consists of CC chemokines, which are characterized by the adjacent position of the first two cysteine residues. CC subfamily members have a large spectrum of action and can attract monocytes, eosinophils, basophils, T lymphocytes, natural killer (NK) cells, and dendritic cells. This heterogeneity also extends to their protein sequences and chromosome localization which allow for an informal categorization of this subfamily into various groups, including allergenic (or MCP–eotaxin), inflammatory, HCC (hemofiltrate CC chemokine), developmental, and homeostatic subgroups.[5] The MCP–eotaxin subgroup includes CCL2 (MCP1), the most extensively studied CC chemokine[6] (FIG. 1).

The CXC chemokines are characterized by the interposition of a single amino acid (X) between their first two cysteine residues. This CXC subfamily can be subclassified into two other groups, depending on the presence or absence of the sequence motif glutamic acid–leucine–arginine (ELR) near the N-terminus (FIG. 1). This structural characteristic of CXC chemokines provides a functional correlation: those containing the ELR motif bind and activate CXCR2, providing specificity for neutrophils and other CXCR2-positive cells, whereas those without the ELR motif have poor chemotactic ability for neutrophils and act primarily on lymphocytes and monocytes.

Unlike these two major subfamilies, the C and CX3C chemokines contain two members and one member, respectively. The C chemokines, which comprise XCL1 and XCL2, are distinguished from the other chemokine subfamilies by the presence of only two of the four conserved cysteine residues.[7] C chemokines can act on lymphocytes, but not on neutrophils or monocytes.

The sole CX3C chemokine is CX3CL1 (fractalkine). CX3CL1 is characterized by the presence of three amino acids between the first two cysteine residues and also by an extended C-terminal sequence including a mucin-like domain and transmembrane and cytoplasmic regions. According to these structural features, CX3CL1 can be soluble as well as membrane-bound[8] and acts as an adhesion molecule or a chemoattractant for T cells, NK cells, and mononuclear phagocytes.

In parallel to this conventional nomenclature, many chemokines can be broadly classified into two functional groups. The first group comprises the homeostatic chemokines, which are expressed constitutively and generally involved in lymphoid organ development and maintenance, as well as immune-surveillance cell trafficking. The second group is the inflammatory chemokines, which are induced by stimuli such as pathogens or inflammatory cytokines and involved in the mobilization of effector cells to sites of inflammation.

Chemokine receptors

Chemokines exert their biological functions by binding to seven-transmembrane-domain G-protein-coupled receptors on target cells. The chemokine and chemokine receptor nomenclatures are correlated, in that receptors that bind CC chemokines (for example) are termed CC, followed by 'R' for receptor and a number that denotes the order of cloning. Thus, the chemokine receptor family comprises the CC (CCR1–10), CXC (CXCR1–7), XCR1 and CX3CR1 receptors (FIG. 2). Chemokine specificity is largely restricted to receptors belonging to the same subgroup. In each subgroup, however, individual chemokines can bind more than one chemokine receptor just as single chemokine receptors can be activated by diverse chemokines. There are isolated instances of monogamous chemokine–chemokine receptor pairs: CXCL13–CXCR5, CXCL16–CXCR6, CCL1–CCR8, CCL25–CCR9, and CX3CL1–CX3CR1[9] (FIG. 2).

The expression of chemokine receptors is heterogeneous and is not restricted to hematopoietic cells. As with their ligands, chemokine receptor expression can be constitutive or inducible, but also downregulated by exposure to ligand or to activating and differentiating stimuli (FIG. 3). Moreover, some chemokine receptors are widely expressed, whereas others are restricted to certain specific cells or by specific activation or differentiation states.[4]

The activation of chemokine receptors is induced by the recognition and binding of their ligands. Based partly on analogy with other peptide ligands for G-protein-coupled receptors, the initial recognition between chemokines and their receptors implicates exposed loops between the β-strands of the chemokine fold and the chemokine receptor extracellular protruding regions. Next, the N terminal region of the chemokine initiates the activation of the receptor,[10] which is followed by the internalization of the complex. G proteins are then activated, driving dissociation of their heterotrimers into α and $\beta\gamma$ subunits. Next, various signaling cascade effectors are activated, including phospholipase C (PLC), MAP kinases, or phosphatidyl inositol-3OH kinase (PI-3K),[11,12] which leads to functional outcomes induced by chemokine receptor signaling (FIG. 3).

Chemokine receptor activation and signaling are strictly controlled by desensitization, which prevents overstimulation of cells and inappropriate response[12,13] (FIG. 3). Chemokine receptor desensitization implies a multistep process and a complex of proteins, including G-protein-coupled receptor kinases (GRKs) and β-arrestins. This process starts with the phosphorylation of the chemokine receptor C-terminal tail by GRKs, which increases the receptor affinity for β-arrestin proteins. The binding of β-arrestins to chemokine receptors prevents any other interaction between the receptor and G proteins. Then, the GRK–β-arrestin complex promotes the

	Receptor	Express on		Ligands			
		Granulocyte cells	Mononuclear cells	Affinity			
CC subfamily	**CCR1**	Neutrophils Eosinophils Basophils	Macrophage Immature DC T lymphocytes NK cells	CCL3 CCL5 CCL7 CCL13	CCL14 CCL15 CCL23	CCL8	CCL4 CCL2
	CCR2	Neutrophils	Macrophage T lymphocytes B lymphocytes NK cells	CCL2 CCL7 CCL8	CCL12 CCL13		
	CCR3	Eosinophils Basophils	T lymphocytes	CCL11 CCL13	CCL15 CCL24	CCL5 CCL7 CCL8 CCL26	
	CCR4		Immature DC T lymphocytes NK cells	CCL17 CCL22			
	CCR5		Macrophages DC T lymphocytes B lymphocytes	CCL3 CCL4	CCL5 CCL8		CCL7 CC11 CC13
	CCR6		Immature DC T lymphocytes B lymphocytes	CCL20			
	CCR7		Mature DC T lymphocytes B lymphocytes	CCL19 CCL21			
	CCR8		Macrophages T lymphocytes B lymphocytes	CCL1			
	CCR9		T lymphocytes	CCL25			
	CCR10		T / B lymphocytes	CCL27	CCL28		
CXC subfamily	**CXCR1**	Neutrophils	Macrophages	CXCL8		CXCL6	CXCL5
	CXCR2	Neutrophils Eosinophils	Macrophages	CXCL1 CXCL2 CXCL3	CXCL5 CXCL7 CXCL8	CXCL15	
	CXCR3		T lymphocytes B lymphocytes	CXCL9 CXCL10 CXCL11			
	CXCR4	Neutrophils	Macrophages DC T lymphocytes B lymphocytes	CXCL12			
	CXCR5		T lymphocytes B lymphocytes	CXCL13			
	CXCR6		T lymphocytes	CXCL16			
	CXCR7			CXCL11	CXCL12		
C subf.	**XCR1**		T lymphocytes NK cells	XCL1 XCL2			
CX3C subf.	**CX3CR1**		Macrophages T lymphocytes NK cells	CX3CL1			

FIG. 2. Chemokine receptor nomenclature.

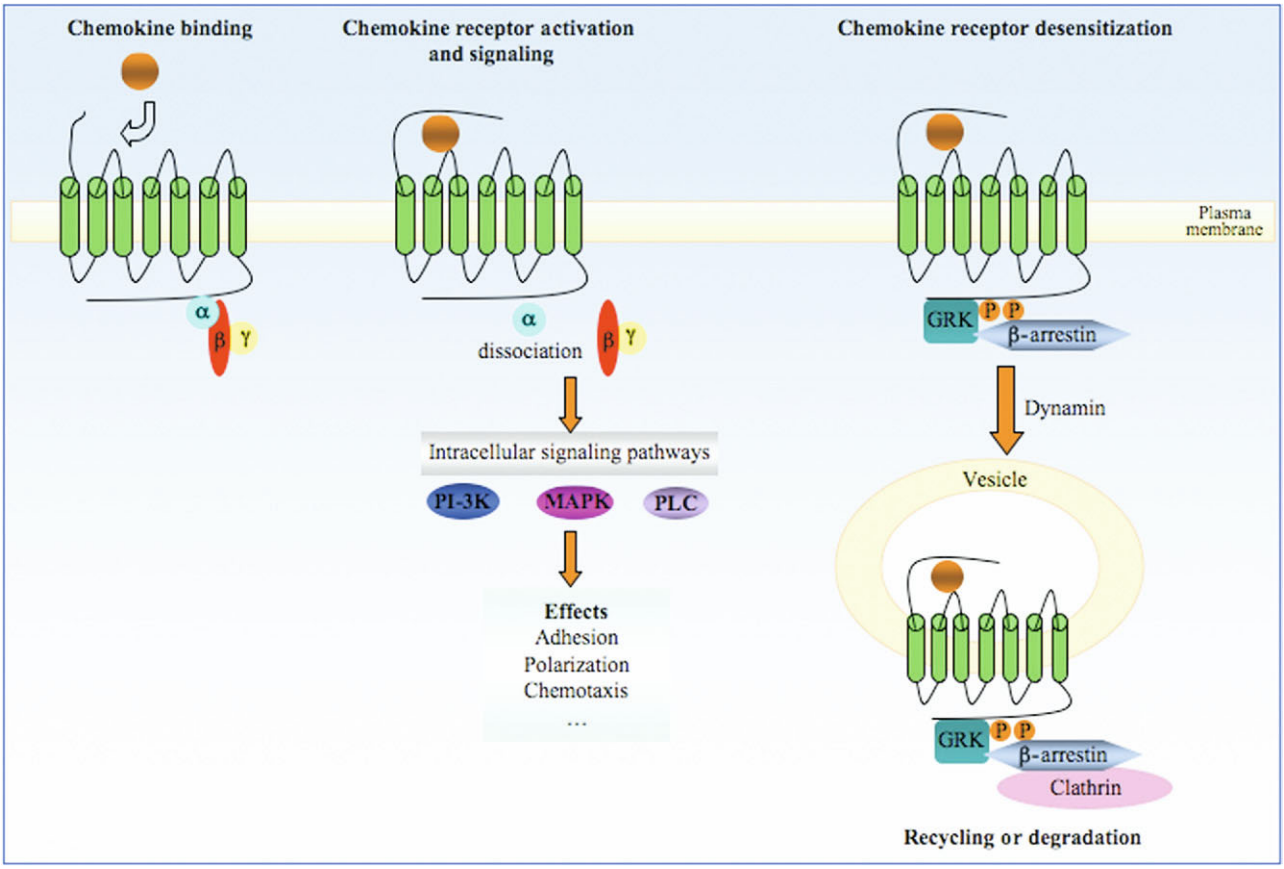


FIG. 3. Chemokine receptor signaling. Following ligand recognition and binding, chemokine receptor signaling starts with G protein activation, characterized by the dissociation of their heterotrimers into α and βγ subunits. Downstream effectors include MAPK, PI-3K, and PLC. This signaling cascade leads to varied functional outcomes, such as adhesion, polarization, chemotaxis, and the like. Desensitization starts with the C-terminal chemokine receptor tail phosphorylation, which increases the affinity of β-arrestin proteins for the receptor and prevents further interaction between chemokine receptors and G proteins. Clathrin-mediated internalization of the ligated chemokine receptor into vesicles is promoted by the GRKs–β-arrestin complex and requires the GTPase activity of dynamin. The internalized chemokine receptor is then degraded or recycled. *Abbreviations:* GRKs, G protein-coupled receptors kinases; MAPK, mitogen activated protein kinase; PI-3K, phosphatidyl inositol-3OH kinase; PLC, phospholipase C.

internalization of the chemokine receptor into vesicular compartments for degradation or recycling. In addition, several studies have suggested that GRKs and β-arrestins could also modulate chemokine receptor signaling by acting as adaptors for effectors such as PI-3K or MAP kinases.[13]

PLEIOTROPIC FUNCTIONS OF CHEMOKINES AND CHEMOKINE RECEPTORS

Initially studied because of their roles during inflammation, chemokines and chemokine receptors are now often studied in the broader contexts of leukocyte trafficking from circulation to tissues during development, immune surveillance, and inflammation. This leukocyte migration across the endothelium and the basement membrane is highly controlled and includes multiple steps: tethering, rolling, activation, firm adhesion, and diapedesis.[14,15] Molecules such as selectins, integrins, and chemokines are involved in the dialogue between leukocytes and endothelial cells.

Specifically, chemokines affect the firm adhesion of leukocytes under flow conditions by integrin activation, which leads to conformational changes of the integrins that increases their affinity for their endothelial receptors and paves the way for leukocyte extravasation.[16] Chemokines also regulate leukocyte–endothelial interactions at the levels of locomotion and diapedesis. Indeed, apical chemokines can promote locomotion of leukocytes to interendothelial junctions. Then, under fluid shear forces, morphological deformations of these leukocytes occur, resulting in the extension of chemokine receptor–enriched processes through junctions.[17] These morphological changes facilitate leukocyte exposure to abluminal chemokines and mediate diapedesis along a chemoattractant gradient.

In addition to their implication in leukocyte firm arrest, locomotion, and diapedesis, chemokines direct

cell migration in a concentration-dependent manner. Under physiological conditions, leukocyte chemotaxis is implicated in the permanent cell trafficking among bone marrow, blood, tissues, and lymphoid organs. Mature dendritic cell (DC), T cell, and B cell homing and recirculation are regulated by CCL19, CCL21, and CXCL13 expressed variously in lymphatic vessels, high endothelial venules (HEVs), and secondary lymphoid organs.[3,9,18,19] Thus, after CCR7 acquisition during maturation, DC are able to migrate into the T-cell zones of draining lymph nodes in response to CCL19 and CCL21 produced by lymphatic vessels. In the same way, naïve T cells, characterized by the expression of CCR7, move to lymph nodes in response to CCL19 and CCL21 through HEVs.[20] In parallel, the migration of B cells, which express CXCR5, to lymphoid organs is driven by CXCL13, produced by follicular stromal cells.[21]

Conjointly with these homeostatic functions, chemokines are implicated in leukocyte chemotaxis during a wide range of diseases, especially those with inflammatory components. Thus, chemokines are responsible for the accumulation and activation of leukocytes in tissues. The infiltrated cell type depends on the specificity of chemokine production and chemokine receptors present on nearby cells. For example, rheumatoid arthritis is characterized by monocyte and T cell infiltration into synovial tissues in response to CCL2, CCL3, and CCL5.[9] During obesity-induced diabetes, the involvement of CCL2 in the impairment of insulin-dependent glucose uptake in adipocytes via macrophage recruitment, as well as the implication of CCL3, has been demonstrated.[22,23] CXCL9, CXCL10, and CXCL11 have been implicated in type 1 T helper cell recruitment to inflamed skin during psoriasis and dermatitis.[24]

These are only three examples of diseases with chemokine-mediated cell recruitment and inflammation, but chemokines and chemokine receptors are involved in a large variety of pathologies, such as atherosclerosis, asthma, Crohn's disease, bacterial pneumonia, acute respiratory distress syndrome, bacterial or viral meningitis, sarcoidosis, and tuberculoid leprosy, as well as a wide variety of neurological diseases (addressed later in this review).

Other studies have also identified roles for chemokines during development, especially the critical role of CXCR4. Based on gene-targeted mice, varied studies have implicated CXCR4 in the survival of the embryo, earliest stage of B lymphopoiesis, hematopoiesis, vascular development, cardiogenesis, and cerebellar development.[25,26]

In addition, chemokines are involved in tumor biology by acting on cell proliferation, tumorigenesis, angiogenesis and metastasis. Several chemokines regulate cell proliferation. CXCL1, CXCL2, CXCL3, CXCL8, and CXCL12 can act as autocrine growth factors in various cancers such as melanoma, adenocarcinoma, glioblastoma, leukemia, and colon, gastric, hepatic, and pancreatic cancers.[27] Burger et al.[28] have also shown the potential implication of chemokine receptors, especially CXCR2, in the malignant transformation process. Other research groups also have demonstrated that chemokine and chemokine receptor expression could be involved in metastasis. For example, preliminary data regarding the involvement of CXCL8 in invasive melanoma or prostate cancers has been reported,[29,30] as well as CXCR4 in breast cancer[31] or CXCR3 in colon cancer.[32] Finally, another essential role of chemokines in tumor biology is their implication in angiogenesis, required for tumor growth. CXCL1, CXCL2, CXCL3, CXCL5, CXCL8, and CXCL12 have variously been implicated in the induction of angiogenesis of several tumors. In contrast, some chemokines (e.g., CXCL4, CXCL10, and CXCL9) may inhibit this process.[18]

CHEMOKINES AND CHEMOKINE RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

Because of their involvement in diverse neurological diseases, interest in chemokines and chemokine receptors in the CNS has been rapidly increasing. Nonetheless, the implication of the chemokine system in the physiological or pathological conditions of the CNS has only begun to be clarified.

We have already noted the extensive involvement of chemokines and chemokine receptor in CNS development. CXCL12–CXCR4 signaling controls the migration and survival of neural precursors.[33] CXCL1–CXCR2 have also been implicated in the migration and proliferation of oligodendrocyte progenitors.[34] A recent study,[35] using a CXCR2 knockout model, reported the importance of this chemokine receptor in the maintenance of oligodendrocyte lineage, myelination, and white matter in the CNS. In parallel to this implication in CNS patterning and developmental positioning, chemokines and chemokine receptors act as physiological neuromodulators. Several studies have demonstrated that chemokines CXCL1, CXCL8, or CXCL12 regulate neurotransmitter release or modulate ion channel activity at both the presynaptic and postsynaptic levels.[36,37] Moreover, a recent study has reported that CX3CL1, a chemokine constitutively expressed in the CNS (along with CXCL12 and CXCL14), is a potent neuromodulator of evoked excitatory synaptic transmission.[38]

Beyond their role in the CNS under physiological conditions, chemokines and chemokine receptors are studied primarily as mediators of CNS pathologies, especially those with an inflammatory component such as multiple sclerosis (MS). During neurological diseases, the expression of chemokines can be selectively induced or upregulated in a wide range of cells, including microglia, astrocytes, neurons, and endothelial cells.[9,39] These

molecules—both chemokines and chemokine receptors—represent potential therapeutic targets. In the next sections, we discuss several illustrative CNS therapeutic targets within the chemokine system.

CHEMOKINES AND CHEMOKINE RECEPTORS AS BIOLOGICAL MARKERS OF NEUROLOGICAL DISEASES

CNS tumors: the case of CXCL12–CXCR4

As we have noted, chemokines and chemokine receptors are involved in tumor biology by regulating cell proliferation, angiogenesis and metastasis. Several studies have demonstrated the important role of CXCR4–CXCL12 in the biology of the most aggressive type of primary brain tumor, glioblastoma multiforme (GBM) (also known as grade 4 astrocytoma). Expression of CXCR4 has been shown in the endothelial cells of neovessels, with a high expression of its ligand in tumor cells adjacent to these neovessels, suggesting a role of CXCL12 in promoting angiogenesis.[40] A correlation between the CXCR4 expression and the invasiveness of tumor cells has been reported in a wide range of cancers (e.g., breast cancer,[41] melanoma,[42] or prostate cancer[43]), in addition to GBMs.[44] CXCL12 has also been involved in the survival of glioma cells by activating the Akt pathway.[45]

These findings identify CXCR4–CXCL12 as potential prognostic biomarkers for GBMs, which display heterogeneity in regard to invasiveness, angiogenesis, and extent of necrosis. Studies analyzing the cellular and genetic changes which occur during the genesis and progression of human gliomas have demonstrated the overexpression of CXCR4 in GBM tissue as compared with normal brain tissue.[46,47] Rempel et al.[40] demonstrated a correlation between tumor grade and the expression of CXCR4 and its ligand CXCL12. Using immunohistochemistry, they found low level expression of CXCL12 and CXCR4 in lower grade GBM tumors and higher level expression of CXCR4 and CXCL12 in higher grade GBMs, which are characterized by large regions of angiogenesis and necrosis. These data suggest that CXCL12 and CXCR4 expression could be a useful marker for grading GBMs. In addition, a more recent article[48] reported a relation between the expression of CXCL12 and a significantly shorter time to tumor progression in low-grade glioma, suggesting a potential role of CXCL12 as a marker of early disease progression.

Alzheimer's disease: the case of CCR1

Alzheimer's disease (AD), the most commonly diagnosed dementia, is characterized by neuronal loss in cortical and subcortical regions, β-amyloid (Aβ) peptide plaque deposits, and neurofibrillary tangles. AD pathology is associated with inflammation in the form of microglial and astroglial reaction. Descriptive studies have demonstrated the presence of chemokines and their receptors in AD tissues. One study revealed elevated expression of CCR3 and CCR5 on reactive microglia, associated with amyloid deposits.[49] CCR5 ligands CCL3 and CCL4 were detected also in neurons and a subpopulation of reactive astrocytes.[49] CXCR3 was detected on neurons, and its ligand, CXCL10, was increased in astrocytes in AD brain tissues.[50] Like CXCR3, CXCR2 was expressed on neurons, with its expression strongly upregulated in a subpopulation of neuritic plaques.[51] CCL2 was found in mature, senile plaques and reactive microglia of AD brain tissues.[52] Moreover, *in vitro* studies suggest that Aβ peptides stimulate chemokine production by cultured microglia.[53]

Halks-Miller et al.[54] reported a specific expression of CCR1 in dystrophic neurites and neurons in AD lesions associated with amyloid plaques—this expression being undetectable in control brain or normal-appearing brain parenchyma of AD patients. Furthermore, the expression of CCR1 was observed at a very early time point in the disease and increased with progression of severity. These results suggest that CCR1 may be an early marker of AD-associated Aβ1-42 containing plaques. This study (on 86 autopsy-derived brains, including 40 cases of AD) presented novel and promising results—which need, however, to be confirmed in an additional cohort.

As already noted, Aβ peptide plaque deposits characterized AD pathology. Positron emission tomography (PET), using radiotracers with high affinity for Aβ peptide plaque deposits, is a noninvasive and promising technique for AD diagnosis (from other form of dementia) and for study of disease progression and therapeutic efficiency. At present, several radiotracers have demonstrated their relevance by their retention in senile plaques of AD patients.[55–57] Incorporation of radioactive tracer into small-molecule compounds that bind CCR1 with high affinity could lead to PET ligands, which would offer the novel possibility of detecting CCR1 in association with dystrophic neurites and complement the amyloid-binding compounds.

Multiple sclerosis: a jumble of chemokines and chemokine receptors

MS is an inflammatory, demyelinating disorder of the CNS. The roles of chemokines and chemokine receptors in MS pathogenesis have been widely investigated using blood cells, brain sections, cerebrospinal fluid (CSF) samples, or experimental autoimmune encephalomyelitis (EAE), an animal model of MS-associated inflammation. Analyses of the expression of chemokines and their receptors in MS have highlighted the complexity of this field, in that a large number of these molecules have been found to be involved in the trafficking of leukocytes.[58]

Several studies have investigated the expression of chemokines and chemokine receptors in the blood of MS patients. Significant increase of the CCR5 and CXCR3 expression on T lymphocytes in MS patients compared with controls has been reported,[59] as well as a higher secretion of CXCL8 from peripheral mononuclear cells, especially monocytes.[60] CCR7 and CXCR3 are expressed in the CSF by virtually all T lymphocytes. Apparent enrichment for CCR5 on CSF T cells merely reflects selective accumulation of memory cells in this compartment.[9,61] In MS patients and controls, CSF monocytes express both CCR5 and CCR1, but only a small minority of blood monocytes express CCR5.[62] Sorensen et al.[63] found elevated expression of CXCL10 and CCL5 in the CSF of MS patients, whereas CCL2 level was significantly decreased. Interestingly, this selective downregulation of CCL2 in the CSF of MS patients is not observed in noninflammatory neurological disorders, nor in other acute or chronic neuroinflammatory diseases, including stroke and HIV-1-associated encephalopathy. CSF CCL2 was also reduced (compared to non-neurological controls) in chronic neuroinflammatory disorders like HTLV-1-associated myelopathy.

An *in vitro* study suggested that the decrease of CCL2 in MS CSF could be a consequence of CCL2 consumption by CCR2-positive migrating cells, which then downregulate the expression of their receptors as they cross the blood–brain barrier in response to CCL2.[64] Studies of CNS tissues revealed that the vast majority of perivascular lymphocytes express CXCR3.[59,63] In parallel, CXCL10 (the appropriate ligand for CXCR3) is expressed by astrocytes and macrophages in MS lesions of the brain.[59] Moreover, in MS lesions, mononuclear phagocytes have been described to express CCR1 and CCR5 (as already noted). The expression of chemokines CCL2, CCL3, CCL4, CCL5, CCL7, and CCL8 was also demonstrated in MS lesions.[58,63]

By studying two of the four patterns of demyelination in active MS lesions,[65] Mahad et al.[66] showed that the number of infiltrating monocytes expressing CCR1 is decreased and the number expressing CCR5 is increased in late active demyelinating regions of pattern II lesions. Conversely, the number of cells expressing CCR1 and CCR5 are similar in all regions of pattern III lesions. Another study suggests that the expression of CX3CR1 by NK cells is associated with disease activity.[67] For now, however, none of the chemokine system molecules have been characterized as a specific marker of MS physiopathology.

Additional information has been provided by EAE studies. Using monophasic or relapsing EAE models, functional roles for CXCL1, CXCL10, CCR1, and CCR2 were observed during the acute phase; CCL2, CCR2, CCL20, and CCR6 were associated with relapses.[39]

In addition, some studies have reported a possible correlation between susceptibility, age of onset, or severity of disease in patients who display heterozygosity for the CCR5Δ32 mutation.[68–70] The findings are contradictory, however, and more recent work using a cohort of 221 MS patients failed to detect an association between CCR5Δ32 mutation and disease severity or age of onset.[71]

Modulating the chemokine system: consequences for cell trafficking

Chemokines and chemokine receptors are promising potential therapeutic targets. At the same time, because of their many functions and their complex interactions (a large number of molecules with different temporal and spatial expression patterns), using the chemokine system as a therapeutic target is challenging: Which elements to target? how to do so? and when to apply these therapeutics?

Several approaches are available for modulating chemokines and chemokine receptors, of which small-molecule, peptide, and neutralizing-antibody chemokine receptor antagonists represent the most highly developed. The identification of appropriate targets for MS has followed descriptive tissue analysis and research using gene targeting or antagonist-mediated blockade in mice with EAE.

For one example, tissue studies showed a large number of CCR1-positive mononuclear phagocytic cells associated with demyelinating plaques. Using myelin oligodendrocyte glycoprotein (MOG)-induced EAE and $CCR1^{-/-}$ mice, an important role for CCR1 was demonstrated in EAE pathogenesis.[72] Moreover, treatment with the CCR1 antagonist BX-471[73] produced positive effects on clinical and histological scores in a rat EAE model, supporting its therapeutic potential in MS. However, a phase I/II clinical trial of 105 relapsing–remitting MS patients who received oral CCR1 antagonist BX-471 or placebo gave negative results, in that numbers and sizes of acutely inflamed MS brain lesions (detected by gadolinium-enhanced magnetic resonance imaging (Gd^+ MRI) were equivalent in patients receiving the CCR1 antagonist or inactive placebo.[74]

The underlying reason for the failure of this widely anticipated trial is uncertain. Simplistically, it may be possible that CCR1 is not a suitable therapeutic target for MS treatment, in which case trial design would be irrelevant. Alternatively, it is plausible that the trial design failed to address the role of CCR1 in the pathogenesis of MS. One red flag is that EAE models used in the preclinical testing for CCR1 were all monophasic, so that disease pathogenesis more nearly resembled acute disseminated encephalomyelitis (ADEM) than MS. The distribution of $CCR1^+$ cells within MS lesions (at the borders of actively demyelinating lesions) may suggest a role for this receptor in generating tissue injury in these

lesions, rather than in leukocyte recruitment. If this hypothesis is correct, then imaging techniques that addressed lesion evolution[75] might be preferable for monitoring therapeutic effects of CCR1 blockade, as opposed to quantifying Gd^{+} MRI lesions. The distinction between these two possibilities can be made only by additional clinical trial endeavors, using either BX471 or other CCR1 antagonists.

The general take-home message is that clinical trial design for chemokine receptor blockade in MS patients must be developed in recognition that chemokine receptors are pleiotropic. Chemokine receptor functions beyond leukocyte chemoattraction may frequently play important roles in disease pathogenesis, and will require ingenious and individualized trial design strategies to capture these effects.

$CCR2^{-/-}$ mice were also shown to be resistant to MOG-induced EAE.[76] An oral antagonist to CCR2 (INCB3344)[77] was evaluated in a murine EAE model and inhibited macrophage accumulation in a dose-dependent manner and reduced disease severity. No study results have been reported in MS patients.

CCR5 has been intensively studied because of its implication in HIV infection and the unique genetic studies enabled by the presence of a common null allele in humans.[78] The potential role of CCR5 as a therapeutic target for MS has been reduced both by clinical and experimental observations. In particular, $CCR5^{-/-}$ mice exhibit the same susceptibility as wild-type mice to MOG-induced EAE.[79] Furthermore, using an N-terminal modified human CCL5 molecule (Met-RANTES) as antagonist of CCR1 and CCR5, Matsui et al.[80] reported that Met-RANTES did not alter the susceptibility to EAE, the clinical score during the acute phase and chronic–relapsing phase or leukocyte trafficking. However, Met-RANTES modestly reduced neurological disability during the chronic–plateau phase of EAE.[81]

As described previously, CXCR3 is present on virtually all perivascular lymphocytes in MS lesions, suggesting an important role for this chemokine receptor in directing T lymphocytes to sites of neuroinflammation. CXCR3 appeared to represent an exciting therapeutic target for MS disease, whose blockade might restrict the infiltration of pathogenic leukocytes into the CNS. Studies of EAE in $CXCR3^{-/-}$ mice,[82] however, failed to show any alteration in numbers or lineage of CNS-infiltrating leukocytes. Moreover, an exaggerated disease severity associated with an increase of the blood–brain barrier disruption and a reduction of T-cell IFNγ production were demonstrated in CNS tissues of $CXCR3^{-/-}$ mice with EAE.

As already noted, a critical role of CXCL12–CXCR4 has been proposed for brain tumor pathogenesis. *In vitro* and *in vivo* (xenograft mouse model) studies using the CXCR4 antagonist AMD3100 supported this concept by showing an inhibition of glioblastoma growth via increased apoptosis and decreased proliferation.[83] Studies using AMD3100 have also been conducted in the EAE model.[84] Animals treated with this CXCR4 antagonist displayed worsened clinical disease and extensive demyelination, although numbers of mononuclear cells were similar in CNS tissues and in vehicle-treated mice. The AMD3100-treated mice, however, showed an increase in microglial activation and remarkably dispersed intraparenchymal lymphocyte infiltrates. These observations suggest that CXCL12–CXCR4 retained mononuclear cells in the perivascular space and limited intraparenchymal inflammation. Because of this potential anti-inflammatory role, CXCR4 antagonists, which appear potentially applicable for treating glial tumors, may not represent appropriate therapeutics for CNS autoimmune disease.

The possible anti-inflammatory roles of CXCL12–CXCR4 suggest that increased expression could be a potential strategy for limiting CNS inflammation. A protective and anti-inflammatory role of CX3CL1 has also been suggested in AD.[85,86] Cardona et al.,[87] using three different *in vivo* models, also showed that the inhibition of CX3CR1 dysregulates microglial responses resulting in neurotoxicity. These data suggest preferentially a protective role of CX3CL1–CX3CR1 signaling, and raise concerns that CNS penetration by CX3CR1 antagonists might increase neuronal vulnerability. EAE in $CX3CR1^{-/-}$ mice showed exaggerated disease severity, associated with an impairment of the migration of regulatory NK cells to the CNS.[88] These results, and corollary studies, suggest a protective role of NK cells during EAE, and showed further that CX3CL1–CX3CR1 govern the migration of these cells to the CNS, but not to the liver.

Taken together, these data show the significant and daunting complexity of the chemokine system, posing challenges to the use of chemokine research for identifying therapeutic targets for neurological diseases. In specific diseases, some receptors exert pathogenic effects and require therapeutic blockade, whereas others are beneficial and could be upregulated. Moreover, one chemokine is often capable of binding multiple receptors, and individual receptors may be expressed on varied cell types. Finally, chemokines display pleiotropic functions. Thus, blocking one chemokine receptor to treat neurological disease could present unexpected results. Furthermore, following on evidence of an antagonist effect in an animal model, difficulties frequently occur in validating the same molecule in humans. The chemokine system is not strictly orthologous between humans and rodents, and many disease models are imperfect.

In spite of the many difficulties, however, several molecules are undergoing testing in clinical trials.[89] And, despite the magnitude of the challenge, the promise of translating chemokine biology to practice sustains our efforts to comprehend the implication of chemokines and their receptors in the pathogenesis of neurological disease.

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