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The fine balance of chemokines during disease: trafficking, inflammation and homeostasis

Sandra M. Cardona, Jenny A. Garcia, and Astrid E. Cardona

Summary

The action of chemokines (or ‘chemotactic cytokines’) is recognized as an integral part of inflammatory and regulatory processes. Leukocyte mobilization during physiological conditions, trafficking of various cell types during pathological conditions, cell activation and angiogenesis are among the target functions exerted by chemokines upon signaling via their specific receptors. Current research is focused in analyzing changes in chemokine/chemokine receptor patterns during various diseases with the aim to modulate pathological trafficking of cells, or to attract particular cell types to specific tissues. This review focuses on defining the role(s) of certain chemokine ligands and receptors in inflammatory neurological conditions such as multiple sclerosis. In addition, the role(s) of chemokines in neurodegenerative conditions such as Alzheimer’s disease and Parkinson’s disease are also described, as well as the contribution of chemokines to the pathogenesis of cancer, diabetes and cardiovascular disease.

Keywords

Chemokines; microglia; monocytes; Multiple Sclerosis; diabetes; cancer

1. Introduction

The chemokine family includes at least 46 human ligands that signal by binding to specific receptors. As implied by the name derived from *chemotactic cytokines*, these molecules are best known for their role in facilitating cellular migration. It is now recognized that chemokines are key players not only in inflammatory processes such as autoimmune disorders and infectious diseases, but also in cancer, particularly in the regulation of metastasis. Chemokines are also important for normal cell positioning and path finding during development, cellular activation and homeostatic cell trafficking under physiological conditions.

Most chemokines are small (8–14 KDa) proteins that interact with G-protein coupled receptors. These molecules have been characterized predominantly in mammals but are also found in chicken, teleost fish (1), and possible homologs are also present in nematodes. There are 18 functionally signaling chemokine receptors and four atypical chemokine receptors: Duffy Antigen Receptor for Chemokines (DARC), D6, CCX-CKR and CXCR7 which bind chemokines with high affinity but are unable to activate the signal transduction pathways that typical receptors do (2, 3). Therefore, atypical chemokine receptors modulate immune responses by removing ligands from inflammatory sites; scavenging, sequestration, buffering and intracellular transport best define their function (4).

Chemokines are divided into CC, CXC, CX3C and XC groups according to their structure, arrangement and spacing of two conserved cysteine residues. The CXC family has a nonconserved intervening amino acid residue in between the two cysteine residues, the CC family has two cysteine residues in juxtaposition, whereas in the CX3C family there are three intervening amino acids in between the two cysteine residues. The CXC family can be

further subdivided into two categories based on the presence of the sequence glutamic acid-leucine-arginine (or ELR) at the NH₂ terminus immediately before the first cysteine of the CXC motif (5). The ELR motif dictates chemokine specificity for binding to their cognate receptors. Similar to chemokines, chemokine receptors adopt a matching nomenclature, for example the chemokine fractalkine or CX3CL1 (L for ligand) binds to CX3CR1 (R for receptor).

Based on their expression patterns and functions, chemokines can also be grouped as: inflammatory (e.g. CCL1-13, CCL23, 24, CXCL1-3, CXCL5-11), involved in leukocyte mobilization during inflammation or homeostatic (e.g. CCL14-16, CCL25, CCL27, CXCL12, 13) (6–8) implying involvement in leukocyte trafficking during normal physiological states. In addition, analyses of chemokine ligands and their receptors families show patterns of genomic organization attributable to evolutionary pressures (8). Genomic organization further classifies chemokines into those whose genes are located in large clusters ('major-cluster' chemokines) and the 'non-cluster' or 'mini-cluster' chemokines whose genes are located separately in unique chromosomal locations (8). In the human and mouse genomes, there are two major clusters of CC chemokine genes and two clusters of CXC genes, plus numerous non-clustered or mini-cluster genes of both types. Mouse models have provided significant knowledge of chemokine function under normal and pathophysiological conditions. However, as we extrapolate data from mice to humans, it is important to take into account the particular chemokine gene under study and the significant differences in the chemokine superfamily across mammalian species. We will focus this review on the impact of chemokine biology and research in a chronic neuroinflammatory disorder such as multiple sclerosis (MS), neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD), and in the pathology of diseases ranked as the leading causes of death including, cancer, diabetes and cardiovascular disease.

2. Chemokines in Health and Disease

2.1 Chemokines and Central Nervous System Disorders

Under normal physiological conditions, the central nervous system (CNS) effectively controls and limits hematogenous leukocyte entry into the brain and spinal cord parenchyma. Multiple sclerosis is a disabling, neurological disorder that affects the CNS, causing loss of sensory and motor function. The etiology of MS is unknown. MS is considered a multifactorial or polygenic disease, whereby multiple genes in combination with lifestyle and environmental factors contribute to disease development and progression. Inflammation, myelin loss, axonal degeneration and progressive neurological dysfunction are the hallmarks of this disease (9–11).

In MS, allelic variation in the major histocompatibility class (MHC) class II regions exerts the single strongest effect on genetic risk, especially those containing human leukocyte antigen (HLA)-DRB1*150 (12, 13). Modestly powered genome-wide association studies have enabled the identification of more than 50 additional risk loci and multiple variants appear to exert modest individual effects in disease susceptibility (14). Genes coding for cytokine pathways including the chemokine receptor CXCR5, and receptors for the cytokines interleukin (IL)-2, IL-7, IL-12 and tissue necrosis factor (TNF), costimulatory molecules (C37, CD40, CD58, CD80, CD86 and CLECL1), signal transduction molecules (CBLB, GPR65, MALTI, RGS1, ATAT3, TAGAP, TYK2), the adhesion molecule vascular cell adhesion molecule (VCAM) among others (15) have been identified.

Sunlight, specifically through its role in producing active vitamin D, has been proposed as a key environmental factor for MS (16). Studies of the vitamin D response elements (VDRE) and HLA-DRB1 haplotypes show that the most significant MHC-II risk haplotype for MS

includes a functional VDRE in the proximal promoter region of HLA-DRB (17, 18). The identified VDRE was found close to the highly conserved MHC-II specific regulatory modules SXY important for constitutive, and interferon (IFN)- γ induced expression of HLA class II genes coordinated by the MHC2TA transactivator (18). Although there is controversy whether MS is a primary autoimmune disorder, these genetic associations provide potential avenues to mechanistically determine relevance pathogenic interactions between susceptibility loci and environmental factors.

When considering how complex the inflammatory milieu is in MS with involvement of myeloid cells (e.g. monocytes, macrophages, dendritic cells and microglia), T cells (e.g. CD4, CD8, $\gamma\delta$ T cells and effector Th1, Th17 subsets among others), B cells, and natural killer (NK) cells, etc., the crucial role of chemokines in the timely trafficking of selected leukocyte subpopulations during disease progression requires emphasis. Blocking inflammatory cell influx to the CNS, in particular T cells (including the recently described subset of Th17 pathogenic T cells) and macrophages has been a major focus for therapeutic intervention in MS. However, due to the fact that most leukocyte populations express more than one chemokine receptor and often one ligand can bind to more than one receptor, applying chemokine receptor blockade to ameliorate MS has been a challenging task.

Studies using the most widely accepted experimental animal model of MS, experimental autoimmune encephalomyelitis (EAE), reveal that blocking recruitment of myeloid cells (e.g. inflammatory Ly6C+CCR2+ monocytes) into the CNS is protective as shown by decreased EAE severity in mice lacking CCR2 (19, 20). As for B cells, their accumulation is highly dependent on CXCL13 expression (21) and the implications of modulating B cell trafficking are currently unclear due to the fact that B cells can contribute to the pathogenesis of EAE through the production of anti-myelin antibodies that at least in part, facilitates demyelination, as well as exert protective functions via regulation of effector T cell functions (22).

Several chemokine receptors mediate T cell trafficking. CD4⁺ CCR7⁺ memory T cells predominate in the CSF of MS patients. CXCR3 and CCR5 also play a role in T cell recruitment and expression of their chemokine ligands correlates with Th1 responses. Microglial activation also plays an important role in the pathology of MS and CX3CR1 was shown to inhibit microglial activation and neurotoxicity (23). Together with CCR2, CX3CR1 not only controls the trafficking of selected monocyte populations during EAE but exerts important regulatory roles on antigen presenting cells and therefore impacting effector T cells responses (24). Since blocking T cell and monocyte infiltration with chemokine receptor antagonists has been challenging thus far, chemokine receptors have also been used to target regulatory cells to specific organs. For instance, CCR5 has been utilized to home suppressive neural stem cells to the EAE brain and ameliorate inflammation (25).

In addition to chemoattraction, some chemokine receptors have been implicated in indirect modulation of effector function, such as CCR7 that acts on dendritic cells (DCs) to induce IL-23 production which in turns drives the generation of pathogenic Th17 cells (26). Another chemokine with dual roles in the CNS is CXCL8; it may drive recruitment of neural stem cells and oligodendrocyte progenitors to sites of demyelination, but in turn could also contribute to the failure of myelin repair (27). The complexity of the chemokine system in the CNS is further illustrated by CXCR4-CXCL12 interactions. CXCR4 is expressed by cells of immune system and the central nervous system. Signaling in response to CXCL12 binding triggers migration and recruitment of immune cells including T cells and monocytes to the MS brain and its experimental models (28, 29), but is also important for the migration of neurons and oligodendrocyte precursor cells (30). This ligand-receptor pair also plays a role in CNS development and organogenesis (31).

Alzheimer's and Parkinson's disease are the most common neurodegenerative disorders. Alzheimer's disease (AD) accounts for about two thirds of dementia cases, and inflammation is now recognized as an important component of its pathology. Parkinson's disease (PD) is characterized by the progressive loss of neurons in the substantia nigra; clinically manifesting with resting tremors, bradykinesia, rigidity and postural instability. The genetic mapping gene-association studies as part of the Human Genome Project have accelerated the rate of identification of genes involved in AD and PD. It is accepted that aggregates of amyloid beta (A β)₄₂ and α -synuclein are neurotoxic and contribute to AD and PD respectively. However, the expression profiles of chemokines has provided critical information with reference to biomarker identification, as well as ways to accelerate aggregate clearance. For instance, CXCR4 expression preceded the loss of dopamine neurons in the MPTP-model of PD, implying a possible molecular target for PD treatment (32). Also, CX3CL1 was neuroprotective in the 1-methyl-4-phenyl- tetrahydropyridine (MPTP) and 6-hydroxydopamine (OHDA) induced dopaminergic lesion models of PD (33, 34) via modulation of microglia activation.

Studies addressing the role of the CX3CL1 receptor, CX3CR1 in AD pathogenesis using CX3CR1-deficient mice have been somewhat discordant. The genetic model and analytical approaches used largely account for these differences. For instance, deleting CX3CR1 in Tau transgenic mice exacerbated Tau phosphorylation and aggregation as well as behavioral impairments (35). In contrast, CX3CR1-deficiency prevented neuronal loss and microglial migration without affecting amyloid deposition in the triple transgenic model of PD harboring three mutant gene: Presenilin-1 (PS1M146V), β -amyloid precursor protein (APPswe) and Tau protein (TauP301L) (36). Other studies showed that CX3CR1-deficiency attenuated amyloid deposition in AD models that develop high plaque deposition (37, 38). A more recent study showed that CX3CR1 deficiency exacerbates functional deficits in human amyloid precursor protein transgenic mice (hAPP), associated with elevated cytokine production and Tau pathology and removing CX3CR1 in hAPP mice worsened memory retention (39).

Although analyses of chemokines in serum of PD patients did not reveal significant differences when compared with healthy controls, five CSF proteins (A β peptide 1–42, Flt3 ligand, CX3CL1, DJ1 and α -synuclein) were identified as potential biomarkers that may correlate with disease severity and progression (40). Furthermore, early stage AD patients with the highest levels of CSF CCL2 exhibited a significantly faster cognitive decline and developed dementia within a shorter time period compared to those with lower CCL2 levels. Therefore, CCL2 has been proposed as a candidate predictive biomarker of future AD progression (41).

2.2 Cancer

It is now widely recognized that tumor cells have the capability to modulate chemokine(s) and chemokine receptor(s) expression (42). Chemokines can affect tumor spreading by promoting angiogenesis via interaction with their receptors on endothelial cells or indirectly via attracting inflammatory cells that release angiogenic factors such as fibroblast growth factor (bFGF) and vascular endothelial cells growth factor (VEGF) from the periphery to the tumor site. In addition, chemokines secreted by tumor cells have autocrine effects and induce tumor growth by interacting with chemokine receptors on tumor cells. Stromal cells within the tumor such as fibroblasts, DCs, lymphocytes, macrophages and neutrophils, also participate in the disease process, as they can become activated through direct cell-cell interactions, or by cytokines and chemokines they produce creating a positive feedback in tumor growth (43). The CXC chemokine subfamily significantly contributes to the body's immune response to tumors, as well as tumor pathology. *In vivo* data indicate that certain

chemokines can serve as tissue-specific attractant molecules for tumor cells. Particularly, ELR⁺ CXC members are increased in a variety of tumors, and correlate with a more invasive (metastatic) and more vascularized (angiogenic) tumor phenotype. Therefore, the ELR⁺ CXC chemokines can promote neovascularization, while ELR⁻ CXC chemokines can inhibit tumor angiogenesis acting on tumor growth and repression, respectively (5).

CXCR4 has also been implicated in the formation of metastases. CXCR4 is the chemokine receptor most commonly found in human and murine cancer cells, and has been implicated in the metastatic dissemination of tumors including: pancreatic cancers, astroglomas, myelomas, B cell lymphomas and chronic lymphocytic leukemias (44). Furthermore, CXCL12 and the two receptors it binds to, CXCR4 and CXCR7, are widely expressed in normal tissues and are involved in fetal development, mobilization of hematopoietic stem cells, and trafficking of naïve lymphocytes. CXCR4-CXCL12 interactions and down-stream signaling, often partially regulated by Akt phosphorylation, promote the growth and survival of tumor cells allowing them to proliferate at distant and less favorable sites from the primary tumor. This phenomenon results from a mixed array of pathogenic responses such as increased cell migration, actin polymerization, and increased expression of angiogenic mediators such as VEGF. Chemokines and chemokine receptors may also exert indirect roles during tumor spreading; CXCL12 and CXCL10 act as activators of matrix metalloproteinase (MMP)-9 secretion; a molecule shown to be an important mediator of colorectal cancer invasion and metastasis (45).

Chemokines such as CXCL1, CXCL2, CXCL3, and CXCL8, have been studied in melanoma and shown to directly contribute to tumor cell growth, angiogenesis, and protection of tumor cells against apoptosis (46). CXCL8 may facilitate tumor metastasis through its autocrine growth-promoting function as well as its induction of MMP activity, promoting the transmigration of tumor cells through basement membranes (47). CXCL8 was also associated with increased invasiveness through the transcriptional activation of the MMP-2 gene, augmenting collagenase activity in human melanoma cells (48). Similarly, in prostate cancer CXCL8 over-expression induced the expression of MMP-9 that in turn increased tumor cell invasiveness and metastatic potential in nude mice (49).

Inflammatory cytokines such as CXCL1 and CXCL8 can enhance tumor cell proliferation and have effects on angiogenesis. The production of these cytokines can be induced by the mutated human cancer oncogene *Ras* and oncogenic components of the EGFR-Ras-Raf signaling pathway (50, 51). On the other hand, *Myc*, a gene involved in an array of cellular functions is found over-expressed in many tumor cells where it maintains key aspects of the tumor phenotype including tumor cell autonomous proliferation and remodeling of the extracellular microenvironment. Moreover, studies in a pancreatic islet tumor model showed that *Myc*-activated cells produced chemokines with subsequent recruitment of mast cells capable of inducing angiogenesis and tumor growth (52, 53). Based on the information above, chemokine gradients play their role by attracting tumor-promoting cells, but it is important to consider that such gradients can be modified by malignant cells in a manner that the absence of specific chemokines such as CXCL4, prevents the recruitment of circulating dendritic cell precursors and their *in situ* differentiation, which results in an advantageous environment for the tumor cells (54).

There is evidence that interactions between chemokine receptors expressed in cancer cells and the corresponding chemokines secreted within the target organs mediate organ-specific metastasis (44). Chemokines expressed by specific organs promote tumor cell adhesion to microvessel walls, facilitating extravasation into target tissues and leading to tumor cell migration (55). CXCR4 has been implicated in the organ-specific metastatic spreading of colorectal cancer to the liver (56–60). It was also reported that intestinal metastasis only

occurs in patients whose original biopsies had CCR9+ malignant cells (61). CX3CR1 is expressed in tumors of neural origin such as gliomas and neuroblastomas where it participates in adhesion, transendothelial migration and mobilization of the malignant cells (62). High levels of CX3CR1 are also expressed in pancreatic cancer where is involved in invasion of cells along pancreatic nerves. Similarly, other types of epithelial cancer cells, such as breast and prostate express high levels of CX3CR1(63).

It is well established that certain chronic inflammatory diseases such as prostatitis, hepatitis and pancreatitis predispose to cancer. The development of cancer under these inflammatory conditions is thought to be related to a 'built-in' chemokine microenvironment caused by the tissue damage (64). Therefore, the interplay between inflammation and cancer is supported by *de novo* production of inflammatory mediators and certain chemokines, as well as their receptors, which are downstream of an oncogenic change (65). CC chemokines, particularly CCL2 and CCL5, may recruit macrophage precursors into the tumor microenvironment contributing to the local inflammatory reaction (65–68).

As described above, CXC chemokines are key players at multiple stages of tumor progression and metastasis. This favors the recognition of chemokines and their receptors and downstream signaling pathways for treating malignant tumors. Monoclonal antibodies against chemokine receptors and neutralizing monoclonal antibodies against chemokines have been used successfully to inhibit the growth and migration of malignant tumor cells in experimental settings. Antibodies to CCL2 significantly reduce tumor burden in mice bearing prostate cancer (69) and reduced metastasis and prolonged survival in a mouse model of breast cancer (70). Blockade of the CXCL12-CXCR4 signaling pathway can suppress cancer cell migration, invasion, angiogenesis and metastases formation (71, 72). The use of a newly developed specific inhibitor of the CXCR4 receptor (Plerixafor) was successful to mobilize hematopoietic stem cell to peripheral blood and is FDA-approved in adult patients with non-Hodgkin lymphoma or multiple myeloma (73, 74). Anti-angiogenic properties of CXCL9 and CXCL10 have been used as therapeutic controllers of tumor growth. Administration of CXCL10 or CXCL9 has shown to decrease tumor growth and angiogenesis in mice with adenocarcinomas (75) and non-small cell lung cancer (76). The therapeutic modulation of chemokine ligand-receptor signaling in cancers needs to be approached with extreme caution as chemokines have pivotal roles in healthy tissues.

2.3 Diabetes and Cardiovascular disease

According to the American Diabetes Association (ADA), there are approximately 25.8 million children and adults diagnosed with diabetes throughout United States of America, accounting for about 8.3% of the population. Type I Diabetes Mellitus (T1DM), formerly known as juvenile diabetes, prevails in children and young adults. T1DM is classified as an immune-mediated disease secondary to the destruction of beta cells of the pancreas responsible for insulin production, resulting in hyperglycemia. Type II Diabetes Mellitus (T2DM) is the most common form of diabetes, accounting for about 90% of cases and is highly associated with obesity. In T2DM either the body does not produce enough insulin or target organs are insensitive to it. Common complications of diabetes include cardiovascular disease, stroke, diabetic retinopathy, nephropathy and peripheral neuropathy.

Cytokines and chemokine expression in T2DM patients is associated with activation of the immune system and has conferred an important role to inflammation in its pathogenesis, either as a consequence of oxidative stress (77, 78), endoplasmic reticulum stress (79), pancreatic amyloid deposition (80), lipid deposition and lipotoxicity (81, 82) and glucotoxicity (83, 84) or as a consequence of all of above pathologic processes (85–89). Inflammatory mediators upregulated in the obese state are more significantly produced by adipose tissue macrophages as opposed to adipocytes (90, 91). The observation that

macrophage cell counts are increased in obese individuals (90) suggests a role for these cells in normal adipocyte function. The phenotype of adipose tissue macrophages remains to be studied, along with their specific role(s) in insulin resistance.

Components of the adaptive immune system are also found in adipose tissue in T2DM. These include T cells (CD4+ and CD8+), CD4+ T helper cells (Th1 and Th2 phenotypes), along with regulatory T cells (Treg) (92), associated with elevated levels of cytokines and chemokines. CCL2 is found at higher levels in the adipose tissue and circulation of obese patients compared to lean controls (93, 94). Importantly, CCL2 and CXCL10 levels were found significantly higher in epithelial membranes and vitreous samples of patients suffering from diabetic retinopathy, suggesting a role these chemokines in the recruitment of macrophages and monocytes to the diabetic eye (95–98). These findings are consistent with studies showing a decreased number of macrophages in the adipose tissue of mice deficient in CCL2 or its receptor, CCR2 (99). *In vitro* studies revealed increased production of CXCL8 and CCL3 by islet cells treated with high concentrations of glucose (100). Similarly, rodent models of T2DM showed an increased production of CXCL1, CCL2 and CCL3 chemokines from isolated islets (101).

Histopathological examination of pancreas from diabetic organ donors showed significant upregulation of the chemokines CCL5, CCL8, CCL22, CXCL9, CXCL10 and CX3CL1 (102). In experimental models, CCR5 was shown to contribute to monocyte infiltration to diabetic retinas (103) and adipose tissue and CCR5-deficient bone marrow conferred protection against impaired glucose tolerance (104). In contrast, CXCR3-deficient NOD mice exhibited an accelerated development of diabetes attributed to the lack of Tregs required to effectively migrate into and regulate Th1 T-cell mediated inflammation within the pancreatic islets (105).

Analyses of a large cohort of German Caucasians, and studies from Turkey and China reported a decreased prevalence of T2DM in populations with the CCL2 G-2518 gene variant (106). It was also reported that insulin infusion can exert an anti-inflammatory effect by suppressing plasma concentrations of key chemokines involved in monocyte trafficking including CCL2 and CCL5 and their respective receptors CCR2 and CCR5 (107). Due to the importance of monocytes/macrophages in the pathology of diabetes, anti- mouse CCL2 drugs (108) and CCR2 antagonists (109) have been developed to treat T2DM diabetic nephropathy and other associated complications. The validation and applicability to T2DM patients is still uncertain. CX3CL1 was also identified at higher levels in the subcutaneous adipose tissues of obese diabetic patients when compared to lean patients and this ligand was shown to modulate monocyte adhesion to adipocytes. Therefore, CX3CL1 was associated with obesity, insulin resistance and T2DM and has been suggested as a diagnostic and therapeutic target in cardiometabolic disease (110).

CX3CL1 is accepted by many in the field as an adipose-expressed chemokine that best links T2DM and one of its major complications, heart disease (111). The association between polymorphisms in its receptor, CX3CR1 and heart disease has been a topic of interest over the last 10 years. In humans, two single nucleotide polymorphisms give rise to four allelic receptor variants. The most studied forms are CX3CR1^{V249/T280} (also considered as the wild type receptor), and the variant alleles CX3CR1^{I249/T280} CX3CR1^{I249/M280} present in 20–30% of the population. These changes decrease fractalkine affinity and correlate with enhanced susceptibility to age-related macular degeneration (112, 113) and protection from atherosclerosis (114–116). CX3CR1 genotyping was performed in a cohort of 339 Caucasian individuals who underwent cardiac catheterization. An association was observed between CX3CR1 I249 allele expression and reduced prevalence of coronary artery disease

(CAD). Furthermore, endothelium-dependent vasodilation was greater in these individuals compared to individuals heterozygous for the CX3CR1-V249 allele (116).

Another study investigated the association of the CX3CR1 alleles M280 and I249 with CAD and with acute coronary syndrome (ACS). Although the study found no association between expression of the I249 or the M280 allele with the occurrence of CAD, I249 and M280 appeared to have opposite effects on the occurrence of ACS, with allele I249 conferring an elevated risk (117). The protective or detrimental effect of the CX3CR1 polymorphic variants was also investigated in early-onset and late-onset CAD patients. The I249 mutation was associated with multiple coronary vessel disease in older patients while V249 homozygosity was associated with the early onset CAD (115). In the Ludwigshafen Risk and Cardiovascular Health cohort, no relationship was found between CX3CR1 genotypes or corresponding haplotypes and the prevalence of CAD or myocardial infarction (118). Although the role of CX3CR1 human variants in heart disease is controversial, the role of CX3CL1 in heart disease is further supported by significantly increased serum levels in unstable angina pectoris patients with plaque rupture compared with patients in other groups.

3. Concluding Remarks

There has been significant recent interest in modulating the interactions between chemokines and their receptors as novel anti-inflammatory drugs. Chemokine receptors have proved difficult to antagonize, largely due to their promiscuity and the compensatory loops available within the chemokine subfamilies. Clinical trials for CCR1, CCR2, CCR3, CCR5, CCR9, CXCR1, CXCR2, CXCR3 and CXCR4 are ongoing (111, 119, 120) for treatment of arthritis, multiple sclerosis, T2DM, HIV, inflammatory bowel disease, chronic obstructive pulmonary disease, psoriasis, and cancer. Plerixafor (CXCR4 blocker) has been in the market since 2008 and proven to be effective to mobilize hematopoietic stem cells in combination the granulocyte-colony stimulating factor. Therefore, the common features of these G-protein coupled receptors may continue providing avenues to modulate common chemokine-dependent second messenger signaling pathways. With the advanced technologies available and the rapid progress in our understanding leukocyte trafficking to sites of inflammation and tissue, the interest to target specific chemokine receptors still continues and efforts geared towards developing efficacious chemokine receptor antagonists to treat neuroinflammatory disorders, as well systemic and peripheral-tissue restricted inflammatory diseases.

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