

Chemokines in and out of the central nervous system: much more than chemotaxis and inflammation

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Abstract: Actions of chemokines and the interaction with specific receptors go beyond their original, defined role of recruiting leukocytes to inflamed tissues. Chemokine receptor expression in peripheral elements and resident cells of the central nervous system (CNS) represents a relevant communication system during neuroinflammatory conditions. The following examples are described in this review: Chemokine receptors play important homeostatic properties by regulating levels of specific ligands in blood and tissues during healthy and pathological conditions; chemokines and their receptors are clearly involved in leukocyte extravasation and recruitment to the CNS, and current studies are directed toward understanding the interaction between chemokine receptors and matrix metalloproteinases in the process of blood brain barrier breakdown. We also propose novel functions of chemokine receptors during demyelination/remyelination, and developmental processes. *J. Leukoc. Biol.* 84: 587–594; 2008.

Key Words: demyelination · matrix metalloproteinases (MMPs) · blood brain barrier (BBB) · experimental autoimmune encephalomyelitis (EAE) · coronavirus · development

INTRODUCTION

The central nervous system (CNS) is characterized by a relatively immunosuppressive environment as a result of the lack of lymphatic drainage, resident dendritic cells (DC), and MHC expression [1]. Moreover, the blood brain barrier (BBB) restricts inflammatory leukocyte trafficking into the CNS [2, 3]. However, the CNS has the potential of developing efficient innate, adaptive, and regulatory immune responses. During neuroinflammatory disorders, such as multiple sclerosis (MS), the maintenance of this specialized environment is disturbed, and the loss of the BBB integrity is a critical event in disease pathogenesis. BBB disruption allows leukocytes to traverse the vessel wall, accumulate within perivascular spaces, and then progress across the glia limitans into the parenchyma to initiate a destructive inflammatory response [4]. These events are strictly regulated by adhesion molecules, chemokines, and matrix metalloproteinases (MMPs) [5–7].

The chemokine system is comprised of ~50 molecules and 20 receptors in humans, with orthologs in other mammalian species [8, 9]. The chemokine ligand superfamily is divided into subgroups, of which the largest are the CC chemokines (28 members) and the CXC chemokines (16 members). Chemokine subgroup members, encoded in multigene arrays, are functionally related and signal to corresponding families of chemokine receptors, which are G-protein-coupled receptors (GPCRs) and act specifically through *Pertussis* toxin-sensitive $G_{\alpha i}$ components. GPCRs are drug targets [10], and the biotech/pharmaceutical industry has mounted substantial efforts to modulate chemokine receptor activity, heightening the medical importance of chemokine-mediated regulation of homeostatic and inflammatory processes. First identified by their ability to mediate leukocyte chemoattraction in vitro, chemokines are now recognized to govern a wide array of functions during inflammation and immunity.

Over the past decade, chemokine receptors have been localized to various cell types other than blood leukocytes [11, 12]. Of particular interest in the CNS, specific chemokine receptors have been detected on microglia, astrocytes, oligodendrocytes, neurons, and brain microvasculature. As an important corollary statement, the use of immunohistochemistry needs always to be controlled by appropriate studies in receptor-deficient knockout mice where available, given the notorious lack of specificity with these reagents. Further, in situ hybridization also needs to be used to confirm findings. It is important to define the roles of chemokines and their receptors in healthy mice and in models of neural pathology. We will describe experimental approaches we use to study chemokine biology in vivo during normal and neuroinflammatory conditions and report our observations regarding the roles of chemokine receptors in ligand homeostasis; demyelination/remyelination; their connection to MMPs and BBB breakdown; and their participation during developmental processes (**Fig. 1**).

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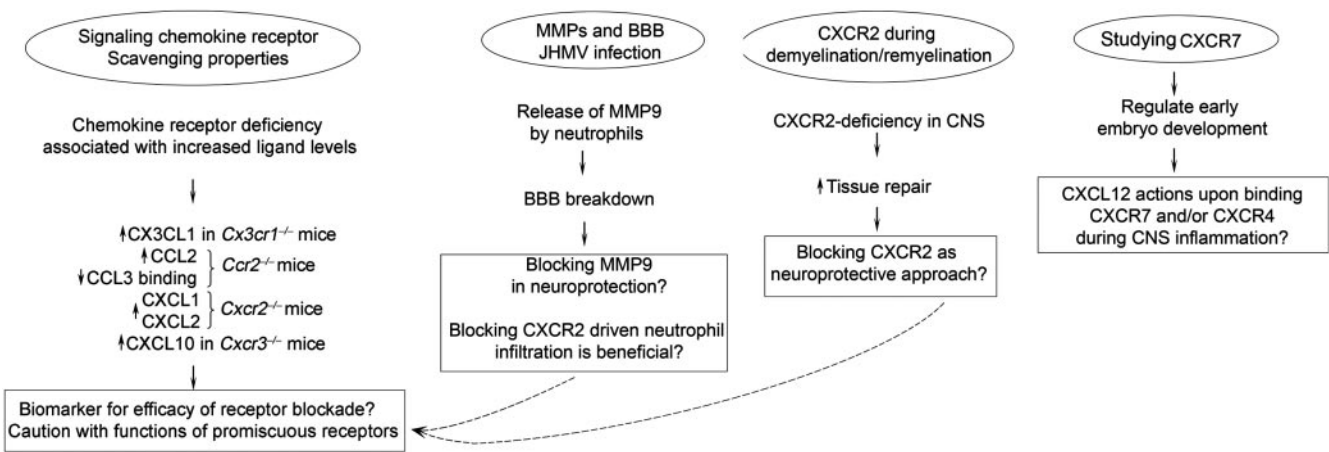


Fig. 1. Understanding the biology of chemokine receptors. The focus of our research is schematically presented, and the four different areas integrate some complex observations that suggest key regulatory functions of chemokine receptors. Our observations suggest that signaling chemokine receptors play a role in clearance of specific ligands with important implications for receptors that bind to more than one ligand, and where blockade approaches are proposed. CXCR2 is explored in the context of demyelinating models, and preliminary data show that the absence of CXCR2 in the CNS is associated with enhanced tissue repair. Also, CXCR2 is presented as an important chemoattractant for neutrophils during JHM strain of mouse hepatitis virus (JHMV) infection, and we are currently studying the role of neutrophil-derived MMP9 in BBB integrity. Developmental studies are used to examine the role of CXCR7 during neurogenesis.

CHEMOKINE SYSTEM AND ITS ROLE IN HOMEOSTATIC FUNCTIONS

Chemokine-binding molecules (a.k.a., nonsignaling or “decoy” receptors) and chemokine sequestration

Three chemokine receptor-like molecules—D6, the Duffy antigen receptor for chemokines (DARC), and ChemoCentryx-chemokine receptor (CCX-CKR; also known as CCRL1)—have the capacity to bind chemokines without evoking the prototypical cellular responses [13, 14] such as chemotaxis or activation. These atypical receptors sequester chemokines and regulate their bioavailability and therefore, are critical regulators of inflammation [15].

The Duffy blood group antigen was described over 50 years ago and later recognized as the entry receptor on erythrocytes for some malarial parasites [16]. The Duffy antigen was named DARC after it was shown that it has the unusual capability to bind a broad spectrum of chemokines of CC and CXC subfamilies [17, 18]. DARC is expressed by endothelial cells [19, 20] and is accordingly involved in transferring chemokines across the endothelium, and as a result of the abundance red blood cells (RBCs), DARC is seen as a receptor involved in clearance of chemokines in the blood. D6 is an extremely promiscuous receptor [21], binding to at least 12 chemokines of the CC subfamily, all of which are inflammatory chemokines [13, 21]. Mice lacking D6 exhibited exaggerated, cutaneous, inflammatory responses and an increased susceptibility to the development of skin cancer [22]. Unexpectedly, D6-deficient mice were also resistant to induction of experimental autoimmune encephalomyelitis (EAE) as a result of impaired encephalitogenic responses [23]. Analyses of skin tissues at the immunization site in *D6*^{-/-} mice showed CD11c⁺ aggregates, suggesting that DC might be trapped at the site of antigen injection, therefore limiting antigen presentation [23].

Contrasting D6-binding properties, CCX-CKR binds the homeostatic chemokines CCL19, CCL21, CCL25, and human CXCL13 [24, 25]. CCX-CKR is predicted to modulate homeostatic lymphocyte and DC trafficking, key migratory events in acquired immune responses that are directed by CCX-CKR-binding chemokines, which reveal functional and biochemical diversity within the chemokine receptor family and provide insights into novel mechanisms of chemokine regulation.

Signaling chemokine receptors and their potential scavenging functions

For the past 5 years, we have been studying the role of the fractalkine receptor CX3CR1 during CNS inflammation [26]. Fractalkine is a unique CNS chemokine present on neuronal membranes and capable of being released as a soluble protein by constitutive or stress-activated a disintegrin and metalloproteinase-family protease activity [27–29]. Fractalkine exerts its functions by binding to CX3CR1 on microglial cells. Although they are mainly produced in the CNS, fractalkine and CX3CR1 also have a distinctive peripheral pattern of expression. Fractalkine is found at low levels in endothelial and some epithelial cells of selected tissues such as kidney, lung, prostate, and heart but not spleen or liver [30]. CNS endothelial cells do not express fractalkine. Circulating monocytes and NK cells express CX3CR1 [31]. In our studies, we determined the levels of soluble fractalkine in the normal, healthy mouse brain, and we found that the levels of soluble fractalkine in wild-type and *Cx3cr1*^{-/-} mice were dramatically different. Native, CX3CR1-deficient mice exhibited over 50 times more soluble fractalkine in the brain and in the serum compared with normal, wild-type mice [32]. These observations led us to hypothesize that signaling chemokine receptors are required for ligand homeostasis. We continued our studies by determining the levels of ligands in various chemokine receptor-defi-

cient strains (Fig. 1). We analyzed mouse lines lacking chemokine receptors, including members from the CC and CXC receptor family, such as CCR2, CXCR2, and CXCR3, in addition to CX3CR1/CCR2 double-deficient mice. These chemokine receptor-deficient lines allowed us to determine the levels of constitutive ligands such as CX3CL1 and inflammatory ligands such as CCL2, CCL3, CXCL1, and CXCL10 in circulation (serum) and tissues during healthy conditions.

Our results indicated that deficiency of chemokine receptors is associated with increased levels of the corresponding ligands [32]. *Ccr2*^{-/-} mice exhibited approximately three times more circulating CCL2 when compared with wild-type. *Cxcr2*^{-/-} mice showed levels of circulating CXCL1 and CXCL2 that were more than 30-fold increased when compared with wild-type mice. Circulating levels of CXCL10 in naïve, wild-type and *Cxcr3*^{-/-} mice were undetectable. However, after inducing EAE, we found that *Cxcr3*^{-/-} mice exhibited significantly higher CXCL10 levels. Examination of chemokine levels in CNS tissues, where CX3CL1 and CXCL1 are expressed, also revealed that these ligands were significantly higher in *Cx3cr1*^{-/-} and *Cxcr2*^{-/-} mice, respectively, when compared with wild-type mice [32]. Expression analysis showed that CX3CL1 mRNA levels were comparable in *Cx3cr1*^{-/-} and wild-type mice, indicating that increased CX3CL1 was not caused by accumulation of mRNA. Reconstitution of CX3CR1- and CXCR2-deficient mice with wild-type bone marrow substantially restored chemokine homeostasis, supporting the notion that chemokines are cleared from the periphery and in some instances, from CNS tissue by cells expressing specific receptors [32]. The differences observed in ligand concentration in the various chemokine receptor-deficient mice analyzed are not clear but might be a result of the degree of promiscuity of the receptor or the relative expression levels of ligand and alternate receptors.

It is well documented that chemokine functions can be suppressed by chemokine receptor desensitization and internalization upon ligand binding [33, 34]. Therefore, we examined whether ligands present at high concentrations could alter the expression of an alternate receptor when the cardinal receptor is absent. We performed a CCL3-binding assay by flow cytometry in PBMC and resident peritoneal macrophages from *Ccr2*^{-/-} mice to analyze the functional availability of CCR1. We found the proportion of CCL3-bound cells was reduced significantly in *Ccr2*^{-/-} mice when compared with wild-type mice [32]. Therefore, excess CCR2 ligands, such as CCL7, which bind CCR1, can down-regulate this receptor.

Involvement of chemokine receptor in homeostasis of specific ligands came from studies that show rapid use of CCL2 by wild-type macrophages and increased amounts of CCL2 in *Ccr2*^{-/-} mice in response to alloantigen [35]. Moreover, our group reported that CCL2 is consumed by CCR2+ migrating PBMC in a human BBB model [36]. These observations support an important biological role of signaling chemokine receptors as scavengers of specific ligands (Fig. 1).

CHEMOKINES AND MMPs DURING BBB DISRUPTION

MMPs and brain inflammation

MMPs, a large family of proteolytic enzymes, are involved in the degradation of protein components of the extracellular matrix, such as collagen, elastin, fibronectin, and laminin [37, 38]. These highly regulated enzymes are implicated in a variety of physiological (angiogenesis, bone remodeling, etc.) and pathological processes, in particular, in the pathogenesis of CNS inflammatory disorders [37]. Increased expression of several MMPs has been found in the serum, cerebrospinal fluid, and CNS of MS patients [39]. Various MMPs are also increased in the CNS during experimental MS mouse models [40, 41]. In addition, intracerebral injection of MMPs to healthy mice induces BBB breakdown and leukocyte recruitment [42], whereas MMP inhibition blocks the onset or the development of EAE and even reverses the disease [43, 44]. Among the MMPs, MMP9 is of particular interest, as it is increased in the serum of MS patients, especially during clinical relapse, and correlates with disease activity on gadolinium-enhanced brain magnetic resonance imaging [45, 46]. Furthermore, young, MMP9-deficient mice are resistant to EAE [47]. These findings implicate MMP9 in BBB disruption.

Role of MMP9 in BBB disruption during JHMV infection

In collaboration with Drs. Cornelia Bergmann and Steve Stohlman, we are evaluating the role of MMP9 in BBB disruption using the murine model of CNS demyelination induced by neurotropic coronavirus (JHMV) infection [48]. JHMV infection along with Theiler's murine encephalomyelitis virus (TMEV) represent excellent viral models to study mechanisms involved in demyelinating diseases, such as MS. CNS infection with the neurotropic strain of mouse hepatitis virus (MHV) induces an acute inflammatory response, followed by a chronic CNS infection with ongoing demyelination. In contrast to TMEV infection, infectious MHV remains undetectable during the chronic phase of JHMV infection, although viral antigens and RNA are present. Furthermore, chemokine and MMP expression during JHMV infection [48, 49] is well characterized and provides the basis to study individual factors contributing to parenchymal CNS infiltration by leukocytes. It is known that MMP9 protein levels are increased during JHMV infection [50], and this correlates with neutrophil recruitment. However, MMP9 mRNA is not altered, unlike MMP3 and MMP12 mRNA, which appear up-regulated [49].

Neutrophils store MMP9 within their granules and are abundant, inflammatory cells entering the CNS early during infection, and their infiltration correlates with a rapid decline in BBB integrity. To study the role of neutrophils and MMP9 in BBB disruption during JHMV infection, neutrophils were depleted using anti-Gr1 antibody (RB6-8C5) [51]. Neutrophil depletion resulted in reduction of leukocytes in the brain, lessened BBB disruption, and absence of MMP9 activity, suggesting a role of this enzyme in BBB breakdown. However, a direct implication of MMP9 in mediating the loss of BBB integrity was not demonstrated. To address this question,

MMP9-deficient mice were infected with JHMV, and preliminary data revealed decreased leukocyte infiltration in infected *Mmp9*^{-/-} mice compared with wild-type controls (C. Savarin, Cornelia Bergmann, Stephan Stohlgan, unpublished results). Analyses of BBB permeability need to be performed to confirm the direct role of MMP9 in BBB disruption.

We are interested in characterizing the cell type(s) responsible for MMP9 release during JHMV infection. The anti-Gr1 antibody RB6-8C5, classically used for neutrophil depletion, recognizes Ly-6G and Ly-6C, two distinct surface antigens expressed by neutrophils (Ly6G^{high}) and immature bone marrow-derived macrophages (Ly6C^{high}) [52], suggesting that anti-Gr1 antibody treatment depletes neutrophils and recruited monocytes. Importantly, macrophages constitute a second population present in the CNS at the peak of neutrophil infiltration following JHMV infection [48] and represent a potential source of MMP9 [39, 40].

To assess the specific contribution of MMP9 release by neutrophils and/or macrophages in BBB disruption, JHMV pathogenesis will be studied in CXCR2- and CCL2-deficient mice. Using *Cxcr2*^{-/-} mice, a chemokine receptor that binds CXCL1 and CXCL2 [53], the two major neutrophil chemoattractants in JHMV-induced CNS inflammation, we will be able to confirm neutrophil involvement in BBB disruption during JHMV infection. Previous studies [54] showed that JHMV infection in CCR2-deficient mice was associated with increased mortality and absence of T cell infiltration, which are the primary effectors in viral clearance of infected CNS resident cells [55, 56]. The CCR2 ligand, CCL2, has also been implicated in chemoattraction of T cells to the inflamed CNS [57]. However, our preliminary results showed that migration of CD4 and CD8 T cells into the CNS of CCL2-deficient mice was similar to control mice, suggesting that CCL2 is not implicated in CNS T cell recruitment during JHMV infection. By contrast, a significant reduction of F4/80⁺ macrophage infiltration was observed, indicating an important contribution of CCL2 in macrophage migration within the CNS following JHMV infection. These observations confirmed the relevance of using CCL2-deficient mice to study the implication of macrophages in MMP9 release and BBB disruption.

CXCR2 AND ITS ROLE IN LEUKOCYTE INFILTRATION AND OLIGODENDROCYTE-MEDIATED TISSUE REPAIR DURING DEMYELINATION AND REMYELINATION

CXCR2/CXCL1 in oligodendrocyte development

CXCR2 is expressed by peripheral monocytes and neutrophils and in the CNS, is present on oligodendrocyte progenitor cells (OPCs) in the brain and spinal cord [58]. One of the most studied ligands for CXCR2, CXCL1 is expressed by spinal astrocytes and acts as a potent promoter of OPC proliferation. CXCR2 mediates monocyte arrest under flow conditions [59] and in the CNS, governs migratory arrest of oligodendrocyte precursors and proliferative responses during development [58, 60]. Therefore, in addition to the properties of chemokines in migration described originally, CXCR2/CXCL1 interactions

control the positioning of OPCs in the developing spinal cord by arresting their migration.

Global lack of CXCR2 is associated with relative resistance to demyelination

We are interested in defining the function of CXCR2 during myelin repair. For this research, we use *Cxcr2*^{-/-} mice, initially generated in 1994 on a BALB/c background, to study myelopoiesis [61]. We crossed *Cxcr2*^{-/-} mice for eight generations to the SWXJ (H2q/s) and C57BL/6 background to determine the role of CXCR2 during demyelination/remyelination processes in vivo using the mouse models of EAE, cuprizone intoxication, and focal demyelination by lyssolecithin (LPC) injection (L. Liu, Abdelmadjid Belkadi, Taofung Hu, K. Choi, Lindsey A. Darnall, Robert H. Miller, R. M. Ransohoff, unpublished data). We found that *Cxcr2*^{-/-} mice were relatively resistant to induction of EAE. Disease onset, kinetics, mortality, and peak day of disease severity in the rare *Cxcr2*^{-/-} mice that developed signs of EAE were equivalent to that seen in *Cxcr2*^{+/+} littermates. Interestingly, recovery from the initial attack of EAE was faster and more robust in *Cxcr2*^{-/-} mice, and disease scores in the resolution phase were significantly lower than in *Cxcr2*^{+/+} mice. Six weeks after cuprizone feeding, a significant difference in the tissue area affected by demyelination was observed in wild-type mice (60% demyelinated tissue) versus *Cxcr2*^{-/-} mice (10% demyelinated tissue). Similar observations were obtained 7 days after lesion induction by LPC injection, and wild-type mice exhibited a 30% demyelinated area compared with 10% in *Cxcr2*^{-/-} mice.

Lack of CXCR2 in the CNS is associated with improved lesion repair

We proposed the hypothesis that CXCR2 mediates pathogenic effects during EAE at two separate levels. First, CXCR2 acts as an “arrest receptor” for myeloid cells promoting accumulation of inflammatory effectors in the CNS, as reported for atherosclerosis models. Second, the CXCR2 ligand CXCL1 expressed by reactive astrocytes at lesion edges [62–64] may arrest OPCs and block the remyelination process by precluding OPC entry into the lesions, thereby suppressing lesion repair. We proposed that EAE lesions might resolve more efficiently in mice lacking CXCR2 in the OPCs. We addressed this issue by constructing radiation bone marrow chimeras. Following sublethal irradiation, bone marrow from heterozygous mice was used for reconstitution, giving rise to chimeric *Cxcr2*^{+/-} → *Cxcr2*^{+/+} and *Cxcr2*^{+/-} → *Cxcr2*^{-/-} mice. Both of these chimeric mice express CXCR2 on hematopoietic cells, but *Cxcr2*^{+/-} → *Cxcr2*^{-/-} mice selectively lack CXCR2 on OPCs (and other radio-resistant cells). We found strong evidence that absence of CXCR2 in the CNS was associated with improved lesion repair in EAE. *Cxcr2*^{+/-} → *Cxcr2*^{+/+} and *Cxcr2*^{+/-} → *Cxcr2*^{-/-} mice had comparable incidence of EAE, indicating that loss of function of CXCR2 on leukocytes caused reduced incidence of EAE in *Cxcr2*^{-/-} mice. The day of onset, peak disease (reflecting kinetics of neurological impairment), and severity of EAE were all equivalent in both groups of mice. However, as seen previously in *Cxcr2*^{-/-} mice, the timing and extent of recovery were accelerated in mice lacking CXCR2 in

the CNS. This result suggested that the absence of CXCR2 in the CNS led to improved repair of EAE lesions. This notion was supported by the observation that lesion size in *Cxcr2*^{+/-} → *Cxcr2*^{+/+} and *Cxcr2*^{+/-} → *Cxcr2*^{-/-} mice was comparable at the peak of EAE, correlating to the equivalent disease scores at this stage. However, during the recovery phase (10 days after onset of neurological signs), we found significantly smaller lesions in *Cxcr2*^{+/-} → *Cxcr2*^{-/-} mice. Furthermore, after cuprizone intoxication, we found equal demyelination in *Cxcr2*^{+/-} → *Cxcr2*^{-/-} and *Cxcr2*^{+/-} → *Cxcr2*^{+/+} mice at 4, 5, and 6 weeks after cuprizone treatment. However, *Cxcr2*^{+/-} → *Cxcr2*^{-/-} mice exhibited enhanced remyelination 7 days after removing cuprizone compared with *Cxcr2*^{+/-} → *Cxcr2*^{+/+} mice. A similar result was found after LPC injection (L. Liu, A. Belkadi, T. Hu, K. Choi, L. Darnall, R. H. Miller, R. M. Ransohoff, unpublished data). Therefore, blocking CXCR2 might confer neuroprotection and anti-inflammatory effects.

ON BEYOND IMMUNITY: CHEMOKINES REGULATE DEVELOPMENTAL AND PHYSIOLOGICAL PROCESS OF THE PERIPHERAL AND CNS

Signaling pathways by CXCL12 binding to CXCR4 and CXCR7

CXCL12 and CXCR4 represent a chemokine receptor pair that plays multiple functions during developmental and pathological processes. CXCR4 was the first known receptor for CXCL12. Previous studies [65, 66] demonstrated abnormal migration of cerebellar neurons in CXCR4- or CXCL12-deficient mice, suggesting a role of the CXCL12-CXCR4 axis in CNS development. Binding of CXCL12 to CXCR4 causes the activation of a series of downstream signaling pathways that regulates cell proliferation, migration, and survival in the CNS [67]. Recently, an orphan receptor, previously termed RDC1, was shown to be the second receptor for CXCL12, according to the gene structure, chromosomal location, and its high affinity to CXCL12 [68, 69]. This new chemokine receptor is now termed CXCR7. Interestingly, CXCL11, another CXC chemokine formerly known as IFN-inducible T cell α chemoattractant, can also bind CXCR7 with high affinity. Some of our efforts are concentrated in understanding the role of CXCR7 in the CNS in relationship to the various CXC ligands identified so far.

Although CXCR7 is expressed in various cell types of peripheral tissues as well as the CNS [69], its role in peripheral tissues is poorly understood. Binding of CXCL12 to CXCR7 provides cultured, receptor-bearing cells with a growth and survival advantage and increased adhesive properties [69, 70]. However, signaling pathways triggered by binding of CXCL12 to CXCR7 remain controversial with a number of mutually contradictory published results [68–73].

In cancer biology, CXCR7 may be envisioned as an interesting target, as its expression at the mRNA level is increased in malignant gliomas compared with the normal brain tissues. By in situ hybridization, it was confirmed that CXCR7 expression is confined to vessels of gliomas but not normal CNS

tissue. Furthermore, it was demonstrated that CXCR7 is also expressed on tumor-associated vasculature in breast and lung tumors. Enhanced expression of CXCR7 in tumor cells not only promotes tumor growth but also enhances the progression of metastases. It is plausible that CXCR7 works by sequestering CXCL11 or CXCL12 [74], making CXCR7 and CXCR4 potential targets for intervention in tumor biology. Of relevance, specific small molecular compounds for CXCR7 inhibited the growth of human tumor cells in mouse tumor models [69]. Therefore, blockade approaches to inhibit CXCR7, and its effects on metastatic spreading are a major area of research.

Role of CXCR7 during development

Consistent with previous observations [69], our mRNA expression studies have demonstrated that CXCR7 is expressed during the early development of CNS [71]. However, we did not observe significant differences in the distribution of major CNS cell populations between *Cxcr7*^{-/-} and wild-type mice. Hippocampus, dentate gyrus, and cerebellum of *Cxcr7*^{-/-} mice appeared normal. In the developing spinal cord and dorsal root ganglia at Embryonic Day 15.5 (E15.5), normal neurogenesis occurs, and expression of CXCR4 is maintained in the *Cxcr7*^{-/-} neural tube (E9.0–E12.5). These observations indicate that CXCR7 is not directly involved in neurogenesis. It is important to note that there are many developmental defects associated with CXCR4 deficiency; such defects include, among others, distorted laminar structure and abnormal migration of cells from the proliferative, external granule layer in the developing cerebellum, with establishment of proliferating aggregates and absence of foliation [65].

In Zebrafish, an important role for CXCR7 has been revealed recently in the development of the lateral line [75, 76], a mechanosensory system comprised of sensory organ neuromasts. All neuromasts originate from a migrating primordium, which moves from head to tail during the development of a lateral line. CXCR7 is expressed in the posterior-lateral line, and a complementary expression pattern between CXCR7 and CXCR4 was described. In particular, expression of CXCR7 is strong in the posterior part but absent in the leading half of primordium, and expression of CXCR4 is mainly detected in the leading part of primordium [75, 76]. Morpholino knockdown of CXCR7 leads to the deficient migration of primordium that eventually affects the distribution of neuromasts. Other studies also support this observation by showing that antisense knockdown of CXCR7 specifically affects the migration of trailing cells, resulting in tissue stretching [76]. Further studies are needed to delineate in detail the downstream mechanisms of CXCR7 during mammalian development.

CONCLUSION AND FUTURE PERSPECTIVES

Understanding the mechanisms that govern the expression and regulation of chemokines and their receptors is crucial to define the consequences of chemokine receptor blockade for therapeutic purposes. Chemokine receptor-deficient murine models are instrumental in defining the roles of chemokines during in vivo inflammatory conditions. Our results implicate

signaling chemokine receptors in consumption and clearance of specific ligands. For some receptors, the promiscuity of ligand binding can alter their expression if alternate ligands are present in high levels. The observation of increased ligand levels in chemokine receptor-deficient mice carries relevance for therapeutic application of chemokine biology [77]. In particular, chemokine elevation may represent a valuable biomarker for the efficacy of blocking molecules, and more importantly, this phenomenon needs to be taken into account where unexpected (although not necessarily detrimental) consequences might arise.

Our studies show that global deficiency of CXCR2 is associated with relative resistance to demyelination. However, using three different in vivo models, we found that reconstitution of CXCR2 on hematopoietic cells “rescued” demyelination. Blockade of CXCR2 might promote remyelination in MS lesions, as well as reducing inflammation. Therefore, blocking CXCR2 might confer neuroprotection and anti-inflammatory effects. Among therapeutic strategies under investigation for treatment of MS, several groups focus on preventing leukocyte infiltration within the CNS. MMP9, by its implication in BBB disruption, constitutes a potential and promising target for MS treatment (Fig. 1). Moreover, treatment of EAE with MMP inhibitors showed interesting findings, but some of these inhibitors were then abandoned because of side-effects as a result of the lack of inhibitor selectivity [78–80]. Indeed, MMPs can also display beneficial roles, particularly in tissue repair [81]. Regarding these data, it is important to know the exact role and source of MMPs during MS pathogenesis, before using MMP inhibitor treatment. Our data, by demonstrating a direct effect of MMP9 in BBB disruption and identifying which cell type is implicated in MMP9 release, would bring additional information essential for potential MS therapeutic strategies. CXCR7 and CXCR4 are also targets for inhibition for clinical intervention, particularly in cancer biology. Our efforts are concentrated in defining the specific actions of chemokine receptors in the brain and their linkage with peripheral elements during neuroinflammatory conditions with the hope that successful clinical, therapeutic approaches might be developed.

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