

OPEN ACCESS

Repository of the Max Delbrück Center for Molecular Medicine (MDC)  
Berlin (Germany)  
<http://edoc.mdc-berlin.de/9652/>

## Therapeutic targeting of chemokine signaling in Multiple Sclerosis

---

*Isabell Hamann, Frauke Zipp, and Carmen Infante-Duarte*

Published in final edited form as:  
Journal of the Neurological Sciences. 2008 Nov 15 ; 274: 31-38  
doi: [10.1016/j.jns.2008.07.005](https://doi.org/10.1016/j.jns.2008.07.005)  
Elsevier (The Netherlands) ►

# Therapeutic targeting of chemokine signaling in Multiple Sclerosis

Isabell Hamann<sup>1,2</sup>, Frauke Zipp<sup>1,2</sup>, and Carmen Infante-Duarte<sup>1,2</sup>

<sup>1</sup> Cecilie Vogt Clinic for Neurology in the Helios-Klinikum Berlin-Buch (HKBB), Charité - Universitätsmedizin Berlin, Germany

<sup>2</sup> Max Delbrueck Center for Molecular Medicine, Charitéplatz 1, 10117 Berlin, Germany

**ABSTRACT** | Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that is initiated and maintained by continuous migration of inflammatory immune cells from the periphery into the target organ. However, in autoimmunity, migration of immune cells is not only involved in the pathogenesis but also in the down-modulation of the autoimmune attack, which is probably mediated by the infiltration of certain regulatory immune cell populations inside the affected organs. The migratory activity of both proinflammatory and regulatory leucocytes is controlled by chemokines and their receptors. Thus, targeting the directed migration of immune cells and regulating leukocyte trafficking across the blood-brain barrier (BBB) by means of modulation of chemokine signaling receptors might open up new therapeutic avenues not only for MS but also for other autoimmune diseases. In this review we summarize the chemotactic signaling pathways known to be involved in neuroinflammation to date and the viability of these pathways as targets for therapeutic strategies.

**KEYWORDS** | Multiple Sclerosis; Chemokines; EAE; T Cells; Blood-Brain Barrier.

## 1. Introduction

In Multiple Sclerosis (MS), inflammatory immune cells are repeatedly recruited from the periphery, reinforcing the local inflammatory reaction in the brain [1]. This process results in local demyelination, damage to oligodendrocytes, axonal injury and neuronal apoptosis [2-5]. In the context of inflammation, the recruitment of activated leukocytes into the target tissue is controlled by a heterogeneous family of chemotactic cytokines, the chemokines [6] and [7]. To date, approximately 50 human and mouse chemokines and 18 chemokine receptors have been identified. Chemokines are classified into four subgroups based on the position of their terminal cysteine residues and are referred as C, CC, CXC and CX3C chemokines [8] (see Table 1). They bind specifically to the chemokine receptors, members of the superfamily of seven-transmembrane G protein-coupled receptors that not only mediate cell migration and adhesion, but also influence other processes such as T cell differentiation [9], angiogenesis, and maturation of T, B and dendritic cells (DC) [6]. Although specific, the interactions between ligand and receptors are characterized by their promiscuity and redundancy, the former because one given chemokine can bind to numerous receptors within its subgroup and a particular receptor may have several ligands [10-12], and the latter because different chemokines can mediate the same effects.

In recent years, numerous publications have reported on the role of chemokines and chemokine receptors in the pathogenesis of MS and its widely accepted animal model, the experimental autoimmune encephalomyelitis (EAE). EAE can be induced not only in mice and rats but also in guinea pigs, rabbits, or primates [13]. In mice, EAE can be actively initiated by immunizing the animal with peptides derived from myelin proteins such as myelin basic protein (MBP), proteolipid protein (PLP) or myelin oligodendrocyte glycoprotein (MOG) [14]. The disease can also be induced passively by adoptive transfer of *in vitro*-generated, myelin-specific T cells in recipient mice [15]. This passive model highlights, in particular, the importance of the migration of myelin-specific T cells into the CNS for inducing neuroinflammation. In EAE and MS, infiltration of T cells and, in general, of leukocytes is a crucial step in the disease pathogenesis. Therefore, the

possible modulation of this inflammatory event by targeting specific chemokine/chemokine receptor systems represents an obvious and very promising therapeutic goal. The chemokine system can be interfered with by, for instance, binding chemokines to glycosaminoglycans, inhibiting intracellular chemokine-activated signaling, or decreasing the translation of chemokine mRNA. Neutralizing antibodies against either chemokines or chemokine receptors is also a feasible approach to targeting the chemokine/chemokine receptor system. However, so far, the most promising method for blocking chemokine signaling is using specific receptor antagonists [16-18].

## 2. The challenge of blocking chemokine signaling in MS

Chemokines are evolutionary preserved molecules that are responsible for cell movement beyond immune cell trafficking, as they also regulate other processes, such as hematopoiesis [19], angiogenesis [20], or organogenesis, including CNS formation [21]. Therefore, although modulating immune cell migration into the CNS may represent an ideal way of combating neuroinflammation, accurately determining which other processes may be regulated by a given target chemokine is crucial. The best means of investigating the actual function of chemokines and their receptors is probably generating gene-manipulated (transgenic or deficient) mice. For instance, in two studies, both CXCL12- and CXCR4-deficient mice died perinatally [22,23] and thereby clarified the non-redundant character of this signaling pathway and its pivotal role during development. Thus, even if the attempt to translate knowledge from the animal to the human situation is justly criticized, these models have rapidly led to an understanding of chemokine signaling pathways and have provided the basis for their use as therapeutic targets. Nevertheless, to precisely elucidate the functions and involvement of certain chemokine systems in disease, in general, and in neuroinflammation, in particular, more appropriate animal models are needed, such as conditional or tissue-specific “knockout” mice, which would allow the depletion of a given signaling pathway during EAE or in certain cell types only.

Our recent data on the use of CCR1 antagonist in MS highlights the difficulty of this animal-to-human translation. This antagonist was pioneer in targeting chemokine signaling and, although the data obtained in animal models were really promising, CCR1 blockade did not show any significant beneficial effect in a phase II clinical trial in patients with relapsing-remitting MS (RRMS) [18]. In general, one important difficulty with drugs that selectively target one molecule is a situation of redundancy, in which the organism will find a means of compensating for deleted target molecules [24]. This problem is even more drastic when the target molecules are redundant by nature, as is the case with chemokines. Moreover, these compensatory mechanisms could vary between different species, making it difficult to predict what the outcome of a blockade tested in animal models will be in humans. Additionally, although most of the chemokines are homologue in humans and rodents, there are several exceptions, such as CXCL8, that have no structural and/or functional homologue in rodents. Finally, the major obstacle in therapeutically blocking chemokine signaling in MS is the disease itself. In contrast to EAE, MS is a very heterogeneous disorder – clinically and, probably, also pathologically [24,25]. In such a disease, one could speculate that chemokine antagonists that showed very promising results in animal models and pilot studies in patients, are ineffective in a phase II trial simply because the treatment was not administered at the right time or to the right patients. Nevertheless, in spite of these difficulties, pharmaceutical companies still consider chemokine receptors as promising therapeutic targets. Almost every major company has a row of potential blockers in clinical development for different indications, including MS. The outcome of this wager will become clear over the next decade of clinical research.

In the next section of this review, we illustrate the most relevant chemokines and chemokine receptors involved in the pathology of MS and EAE, which, thus, represent potential targets in the treatment of chronic neuroinflammation (see Table 2).

### 3. Chemokines and chemokine receptors in Multiple Sclerosis and EAE

#### 3.1. The CC family of chemokines

##### 3.1.1. CCR1 and CCR5 and their ligands

A huge body of evidence highlights the role of CCR1 and CCR5 in the pathogenesis of MS and EAE. CCR1 and CCR5 are expressed by different cell types, including T-lymphocytes, monocytes/macrophages and immature dendritic cells (iDCs) [26,27]. CCR1 and CCR5 are classic examples of very promiscuous receptors that are able to bind and also share different ligands, namely CCL3 (MIP-1 $\alpha$ ), CCL5 (RANTES) and CCL7 (MCP-3). In MS patients, CCR1-/CCR5-positive cells were reported to accumulate within perivascular elements [28] and, in the cerebrospinal fluid (CSF), levels of CCL5 have been found to be elevated [29]. Furthermore, the expression of both CCL3 and its receptor CCR5 (primarily expressed on T cells) was increased in MS lesions [30]. However, CCR5 expression on peripheral blood cells did not correlate with MRI activity in patients [31].

In mice, expression of CCR1 and CCR5, as well as of their ligands, CCL3 and CCL5, was increased in the CNS during EAE [32-35]. Interestingly, although the blockade

of this pathway during passive EAE using anti-CCL3 antibodies prevented the disease [35-37], mice deficient for CCL3 or CCR5 are not resistant or less susceptible to EAE [38]. In line with that, administration of anti-CCL3 during remission had no effect on acute or relapsing EAE [35], indicating that blockade of CCR5 signaling is not sufficient for preventing EAE and that targeting CCL3 is only beneficial during the initial disease phase. In contrast, treatment of mice with Met-RANTES (a CCR1 and CCR5 antagonist [39]) did not affect the course of monophasic EAE but attenuated the severity of chronic-relapsing EAE [40]. Using CCR1-deficient mice, Rottman et al. reported a milder disease course in the absence of the receptor [41] and, in line with that, Liang et al. demonstrated that a specific CCR1 antagonist, BX471, was able to ameliorate EAE in rats [42]. All these findings highlighted the key role of CCR1-mediated signaling in the development of neuroinflammation. Furthermore, the fact that BX471 showed a high bioavailability in dogs treated orally with the drug [42] led us to consider CCR1 as a promising target for MS, a disease that, to date, can only be treated with parenterally administered immunomodulatory drugs as a first-line therapy. Consequently, a multicenter, randomized, double-blind, placebo-controlled trial was designed to evaluate the safety, tolerability and effects of BX 471 on MS disease activity. Unfortunately, although blockade of CCR1 was safe and well-tolerated, it was not able to reduce the cumulative number of newly active lesions in patients with MS. However, we observed a trend towards a reduction of T2 lesion-volume accrual in treated patients, suggesting a possible impact of CCR1 inhibition on tissue injury rather than on initial leukocyte infiltration in MS pathology [18].

##### 3.1.2. CCR2 and CCL2

Similarly to CCR1, CCR2 is also involved in the recruitment of inflammatory cells, mainly T cells and monocytes, to target organs [43,44]. In EAE, it has been shown that CCR2 expression is increased during the initial attack and spontaneous relapse of chronic-relapsing EAE [32] and that the expression of the ligand correlated with disease severity in active EAE [34]. In line with that, CCR2-deficient mice are resistant to EAE [45] or developed a milder disease [46], and CCL2-deficient mice show a delayed onset of active EAE with reduced clinical signs and are also resistant to passive EAE [47]. Interestingly, administration of anti-CCL2 had no effect on disease onset but on the course of established EAE [35]. Considering these data as a whole, the CCR2/CCL2 system could conceivably represent an excellent target for the treatment of MS. However, it has also been demonstrated that transgenic expression of CCL2 in the CNS may be beneficial for the EAE, probably because the constitutive expression of CCL2 suppresses Th1-like cytokine expression [48] and, in relapsing-remitting MS (RRMS), no substantial role of CCR2 or CCL2 has been described [31,49]. However, in a multicenter phase II clinical trial the CCR2 antagonist MLN1202 has been associated with reductions of MRI activity in RRMS patients [50]. Another CCR2 antagonist, CCX915, has been shown to be safe and efficient in a phase I study and a phase II clinical trial with MS patients is planned ([www.chemocentry.com](http://www.chemocentry.com)). Additionally, a multicenter phase II clinical trial with the CCR2 antagonist MK-0812 in RRMS was initiated in 2005 ([www.clinicaltrials.gov](http://www.clinicaltrials.gov); study #NCT00239655); no data on this study have been published to date (see Table 3).

### 3.1.3. CCR7 and its ligands

Although originally CCR7 was exclusively related to homing of naïve cells and DCs to secondary lymphoid organs and, thus, to initiation of adaptive immune responses, in recent years it has been shown that this system is also involved in migration of effector cells and DCs to the inflamed tissue and in maintenance of immune tolerance [51]. CCL21 has been shown to be constitutively expressed in the murine brain regardless of whether inflammation exists [52]. The expression of CCR7 [53,54], as well as of its ligands CCL19 and CCL21 [54], is up-regulated in the inflamed CNS during EAE. In contrast, CCL19 is constitutively expressed in the human brain, and in MS patients, CCL19 but not CCL21 is over-expressed in both active and chronic inactive lesions, as well as in the CSF [55]. In humans, CCR7 is mainly expressed on maturing DCs within inflammatory MS lesions and DCs in the CSF [56]. Therefore, CCR7 and particularly its ligand CCL19 have been implicated in the immunosurveillance of the CNS during normal conditions. Additionally, recent reports in mice indicate that CCR7 expression on regulatory T cells is essential for their suppressive function *in vivo* but not for priming [57] and [58]. Similarly, in the EAE, CCR7-deficient mice show similar disease intensity compared to wild-type (WT) animals [59], indicating that, also in this case, the absence of CCR7 do not impede priming of encephalitogenic T cells. Whether CCR7 is also essential for regulation in EAE and MS remains unclear.

### 3.1.4. CCR4 and CCR8, and their ligands

CCR4 and CCR8 have also been shown to be expressed on CD4/CD25-positive regulatory T cells [60], and to mediate migration of regulatory cells to both, target tissues [61-63] and lymph nodes [64]. In autoimmune neuroinflammation, the ligands for CCR4, CCL17 and CCL22 are up-regulated. It has also been shown that CCL17 is over-expressed in MS [65] and that the expression of CCL22 correlates with disease activity and neuroinflammation in EAE [66]. In a model of relapsing-remitting EAE, expression of CCL22 and CCR4 was increased during the initial attacks and the subsequent relapses, while expression dwindled during phases of remission. In chronic-relapsing EAE, CCL22 and CCR4 were weakly expressed at the onset but were up-regulated during progression of the disease [66]. Thus, the elevated production of CCR4 ligands during the disease appears to be responsible for recruitment of regulatory cells and disease amelioration. Both the CCR4 ligands and the ligand for CCR8, CCL1 have been shown to be up-regulated in the CNS after therapeutic IL-4 gene delivery at EAE onset. In this study, the beneficial effects of the therapy seemed to be mediated by recruiting of CCR1- and CCR8-positive regulatory cells into the brain [67].

However, CCR8 seems to be implicated not only in regulation but also in disease pathology. Apart from its expression on regulatory cells, CCR8 is expressed by endothelial cells and monocytes [68]. In humans, Trebst et al. showed CCR8 expression on microglia and phagocytic macrophages in actively demyelinating MS lesions, in the periplaque and in the normal-appearing white matter [69]. During EAE, CCR8 was up-regulated in the brain during the first attack of chronic-relapsing disease and in the spinal cord during all phases of the disease [53], while its receptor CCL1 was expressed in

the spinal cord of EAE mice 1–2 days before clinical onset [70]. This, together with the fact that the induction of EAE in CCR8-deficient mice led to a later onset of the disease and to milder symptoms compared to control mice [71], suggests that CCR8 signaling is involved in disease pathology by mediating the recruitment of inflammatory monocytes to the MS/EAE brain. Although a functional antagonist of CCR8 has been already synthesized [68], data on its application in EAE have not yet been published.

## 3.2. The CXC family of chemokines

### 3.2.1. CXCR1 and CXCR2, and their ligands

As in the case of CCR1 and CCR5, the receptors CXCR1 and CXCR2 are very promiscuous and bind to and share different chemokines (see Table 1). In the CNS, both receptors are constitutively expressed at low levels on oligodendrocytes and are believed to play additional roles in the CNS, such as modulating synaptic transmission, neuronal chemotaxis and control of cell proliferation and survival [72,73]. The fact that the receptors are highly expressed on oligodendrocytes in MS tissue while the common ligands, CXCL1 (GRO- $\alpha$ ) and CXCL8 (IL-8), are not detectable in normal CNS tissue but on hypertrophic astrocytes in active MS lesion, led to the suggestion that this chemokine axis may be involved in attracting oligodendrocytes to the side of the lesion and, thus, controls repair processes during CNS inflammation [72-74].

However, in the murine model, this receptor–ligand system seems to be involved in disease development, rather than neuroregeneration. Glabinski et al. demonstrated that during EAE, the expression of CXCL1 correlated with disease intensity [33] and the CXCR2 expression in relapsing EAE was significantly increased in the spinal cord during the first and the second attack of the disease [75]. To complete this picture, Carlson et al. recently showed that CXCR2-deficient mice did not develop clinical signs of EAE, and that anti-CXCR2 treatment reduced EAE severity by preventing BBB breakdown, development of inflammatory infiltrates and up-regulation of proinflammatory cytokines [76]. Thus, this chemokine system is one example of animal-model data that cannot be translated directly to the human situation, probably due to the fact that CXCL8 is one of the few human chemokines that have no structurally identical homologue in rodents.

### 3.2.2. CXCR3 and its ligands

The chemokine receptor CXCR3 is preferentially expressed on activated Th1 T cells but also on activated CD8-positive T cells, natural killer (NK) cells, DCs, and microglia/monocytes. Its known ligands are CXCL9 (Mig), CXCL10 (IP-10) and CXCL11 [77,78]. Particularly the CXCR3/CXCL10 system has been implicated in inflammatory processes in the CNS. The receptor is expressed on astrocytes throughout the whole normal human brain and has been reported to be up-regulated in MS lesions [72,79,80]. Also the ligands CXCL10 and CXCL9 have been shown to be expressed on astrocytes [30,80]. In contrast, on oligodendrocytes, CXCR3 is not influenced by inflammation and it is expressed constitutively in low levels in the normal brain and also in MS [72]. In terms of the immune system, the percentage

of CXCR3-positive T cells is increased in blood of patients with RRMS [30] and CXCR3-positive T cells are found in the perivascular space of active lesions [80]. Additionally, the expression of CXCR3 on peripheral CD4-positive T cells has been reported to correlate with MRI in patients with MS [31]. However, no relation between CXCR3 expression and MRI activity was found in another study [81]. In the CSF of patients with MS, levels of CXCL10 [29,82] and CXCL9 [29] are significantly incremented, and correlate with MRI activity. Interestingly, in optic neuritis, CXCR3 expression in the CSF correlated with the increased expression of its ligand CXCL10 and with the presence of oligoclonal bands in CNS, which usually is indicative of MS [81].

Taken together, these findings suggest that the CXCR3/CXCL10 axis plays a role in the recruitment of, in particular, inflammatory T lymphocytes, but also other mononuclear cells into the CNS during MS. However, in EAE studies, this implication has not been confirmed or, rather, shows the opposite scenario. The first reports on the role of CXCL10 on EAE showed that administration of an anti-CXCL10 antibody after adoptive transfer EAE significantly attenuated clinical symptoms and decreased mononuclear cell infiltration into the CNS [83]. In line with this inflammatory implication of CXCL10, Carter et al. recently demonstrated that, in mice, both CXCL9 and CXCL10 can be induced by the Th1-related cytokine IFN-gamma [84]. However, the findings by Fife and colleagues conflicted with those of another study, in which administration of an anti-CXCL10 antibody after induction of active EAE in rats resulted in an exacerbation of the disease and an enhanced migration of CXCR3-positive T cells from the lymph nodes into the CNS [85]. At this stage, the inconsistent findings could be explained by the use of different animal models (passive EAE in mice vs. active EAE in rats). Later on, the induction of EAE in CXCL10-deficient mice indicated, unexpectedly, that these mice are more susceptible to EAE [86]. Two years later, and in concordance with this data, it was reported that also CXCR3-deficient mice suffered from a more severe EAE than WT animals. Interestingly, although, in this study, the number of CD4- and CD8-positive T cells in CNS infiltrates was similar in CXCR3-deficient and control mice, the production of IFN-gamma was reduced in the deficient animals [87]. These increased disease severity in CXCR3-deficient mice was confirmed recently by Müller and colleagues. In this study the authors presented evidence for the implication of CXCR3 signaling in controlling inflammation by retaining CD4-effector T cells and, at the same time, promoting regulatory T cell accumulation inside the perivascular space [77]. A study of PLP-induced EAE showed that mice treated with an agonistic anti-CXCR3 antibody one day before EAE induction, and on days 3 and 7 after EAE induction, experienced basically no clinical signs of EAE, with significant reduction of mononuclear cell infiltrates into the CNS [78]. However, blocking the receptor function with an anti-CXCR3 antibody during the effector phase of a PLP-induced EAE has been shown to significantly aggravate EAE [87]. In summary, it seems that the implication of this signaling pathway varies depending on the disease phase and at this stage of understanding we cannot draw any conclusions as to how exactly the CXCR3 signaling pathway is implicated in neuroinflammation.

### 3.2.3. CXCR4 and CXCL12

CXCL12 (SDF-1) is constitutively expressed by endothelial cells and to a smaller extent on astrocytes in normal human brain tissue, and attracts mainly lymphocytes and monocytes but not neutrophils [88-90]. Together with its only known receptor, CXCR4, this chemokine/receptor system has been suggested as playing a role in controlling lymphocyte migration into the CNS at the BBB. In lesions of active and chronic inactive MS lesions, CXCL12 is highly and widely expressed. In CSF of RRMS patients, the levels of CXCL12 are also increased [89,91]. Data by McCandless et al. showed that, during EAE, blockade of CXCR4 induced a more severe disease with increased infiltration of immune cells in the parenchyma. Additionally, in normal CNS and in the CNS of mice at EAE onset, CXCL12 was expressed at the basolateral side of endothelium, attracting the leukocytes to this area and restricting their migration into the tissue. During the subsequent disease peak, the expression changed. Thus, this perivascular up-regulation of the ligand during inflammation seems to control tissue damage [88]. Recently, the same research group confirmed this hypothesis in humans by immunohistochemical analysis of the expression of CXCL12 at the BBB in CNS tissues from MS and non-MS patients. In this study, they showed that MS was also associated with an altered CXCL12 expression [92]. Thus, the chemokine CXCL12 is probably crucial for retaining inflammatory CXCR4-positive cells in the perivascular space, and consequently, preventing them from migrating into the parenchyma and causing injury.

### 3.2.4. CXCR5 and CXCL13

Patients with MS show abnormal B cell activity within the brain, accompanied by accumulation of B cells in MS lesions and by the production of oligoclonal IgG [93]. The chemokine CXCL13, originally named B cell-attracting chemokine-1 (BCA-1), regulates B cell migration into lymphoid tissue and is hardly or not detectable in spinal cords of mice under normal conditions. During EAE, CXCL13 expression is up-regulated. Bagaeva et al. recently showed that CXCL13-deficient mice experience an attenuated course of EAE and flow cytometry analysis revealed fewer infiltrating mononuclear cells into the CNS [52]. Similar to the data obtained in mice, CXCL13 is undetectable in normal CNS whereas in MS the chemokine is detectable within active but not in chronic inactive lesions and the levels of CXCL13 are increased in CSF of RRMS [91]. The results suggest that the chemokine receptor has a pathogenic role in establishing and maintaining inflammatory infiltrates in the CNS during EAE and probably during MS.

## 3.3. The CX3C-family of chemokines

### 3.3.1. CX3CR1 and CX3CL1

The chemokine receptor CX3CR1 is the only member of the CX3C group, and CX3CL1 (fractalkine) is its only known ligand, which exists in soluble and membrane-bound form [94]. The receptor is expressed on CD8-positive T cells, monocytes and NK cells in the periphery [95], and is restricted to microglia in the CNS [96]. CX3CL1 is expressed mainly by endothelial cells [94] and on neurons [97]. Both CX3CR1 and CX3CL1 have been shown to be constitutively expressed in the rodent as well

as the human CNS [98-100]. In humans, the level of CX3CL1 is elevated in CSF of patients with various neurological inflammatory diseases. However, this ligand is increased in MS patients in sera alone [101]. In EAE the highest expression of CX3CR1 on microglia was detectable in areas of late active and complete inactive demyelination, and cells expressing mRNA for CX3CR1 accumulated quickly in the site of inflammation within the CNS, although, most of the cells were not lymphocyte-like cells [98,102]. In contrast to our expectations, CX3CR1-deficient mice developed a much more severe course of EAE, accompanied by an increased mortality and hemorrhagic inflammatory lesions. Interestingly, the number of NK cells was significantly reduced in the inflamed CNS of these mice, whereas T cells, monocytes/macrophages and NKT cells did not require the receptor to migrate into the CNS during EAE [103]. This is indicative of a possible regulatory, rather than pathological, function of NK cells and CX3CR1 during neuroinflammation. In line with this, we previously demonstrated a significantly lower expression of the chemokine receptor CX3CR1 exclusively on NK cells in RRMS patients compared to healthy individuals. Interestingly the frequency of CX3CR1-positive NK cells is increased in active patients [104], which led to the question of whether this "normal" expression may account for disease remission after the attack. Currently, we are in the process of clarifying the exact role of CX3CR1 in the pathology of MS.

#### 4. Summary

With the overarching aim of delineating known chemokines that may represent therapeutic targets in MS, we have summarized and discussed the current data on the members known to be involved in the physiology and/or pathology of the CNS. These chemokines can be categorized into three groups: (i) those that mediate trafficking of inflammatory cells in the CNS, (ii) those that probably mediate the migration of regulatory cells, (iii) and those that restrict the spreading of the inflammation. To the first group belongs a series of chemokines that bind to CCR1/CCR5 or CCR2, or to CXCR1/CXCR2, CXCR3 or CXCR5. Signaling through CXCR1/CXCR2 can be excluded at this point as potential targets because of the difficulties of translating data from animal models to humans; additionally, the exact implication of CXCR3 is still unclear and more data are needed on the role of CXCR5 signaling in neuroinflammation. In contrast, the CCR-chemokines offer better candidates. Our study with the CCR1 antagonist is, so far, the only published phase II trial that blocked a chemokine pathway in MS. Unfortunately, the treatment showed no beneficial effect, probably because of the functional redundancy of this group of chemokines in humans. In the last 3 years, however, companies have moved to the CCR2 pathway, the blockade of which has been already shown to be beneficial in EAE and, in the first pilot trials, in MS.

To the second group belong the chemokines that bind to CCR4, probably CCR7, and CX3CR1. These receptors appear to mediate trafficking of regulatory cells into the CNS, thus contributing to down-regulation of the autoimmune response. The role of CCR8 is still unclear. Although in EAE it appears to be involved in movement of regulatory cells, in humans, it seems to mediate the infiltration of inflammatory monocytes into the CNS.

Finally the CXCR4- and perhaps CXCR3-binding chemokines represent the third group. These chemokines may exert a control function by maintaining the inflammatory cell inside the perivascular spaces and impeding their migration to CNS parenchyma and consequent tissue damage. Obviously, the chemokine signaling pathways involved in regulation of the inflammatory cascade do not represent targets for blockade. It is crucial, however, to understand these specific regulatory mechanisms that, in the near future, may lead to the design of novel therapeutic strategies in MS.

In summary, we can conclude that despite the efforts by research institutes and companies, the only compounds that may currently offer some potential for MS treatment are the antagonists to CCR2. Over the next year we will learn whether this (and perhaps other novel antagonists) represents a real therapeutic option for patients with MS.

#### Acknowledgements

The CCR1 antagonist study was supported by Schering AG and the German Ministry of Science. The study on CX3CR1 was additionally funded by a grant from the Charité (Rahel Hirsch stipend) to CID. We would like to thank all patients who participated in the CCR1 antagonist study, as well as the patients who collaborated to the CX3CR1 investigations. We also thank Ari Liebkowsky for reading the manuscript as a native English-speaker.

#### Corresponding Author

Carmen Infante-Duarte, Cecilie Vogt Clinic for Neurology in the HKBB, Neuroscience Research Center, Charité — Universitätsmedizin, 10117 Berlin, Germany. Tel.: +49 30 450539028; fax: +49 30 450539906.

#### References

1. K.J. Kennedy and W.J. Karpus, Role of chemokines in the regulation of Th1/Th2 and autoimmune encephalomyelitis, *J Clin Immunol* 19 (5) (1999), pp. 273–279.
2. O. Aktas, A. Smorodchenko, S. Brocke, C. Infante-Duarte, T.o.p.h.o.f.f.U. Schulze and J. Vogt *et al.*, Neuronal damage in autoimmune neuroinflammation mediated by the death ligand TRAIL, *Neuron* 46 (3) (2005), pp. 421–432.
3. A. Diestel, O. Aktas, D. Hackel, I. Hake, S. Meier and C.S. Raine *et al.*, Activation of microglial poly(ADP-ribose)-polymerase-1 by cholesterol breakdown products during neuroinflammation: a link between demyelination and neuronal damage, *J Exp Med* 198 (11) (2003), pp. 1729–1740.
4. B.D. Trapp, J. Peterson, R.M. Ransohoff, R. Rudick, S. Mork and L. Bo, Axonal transection in the lesions of multiple sclerosis, *N Engl J Med* 338 (5) (1998), pp. 278–285.
5. J.W. Peterson, L. Bo, S. Mork, A. Chang and B.D. Trapp, Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions, *Ann Neurol* 50 (3) (2001), pp. 389–400.
6. D. Rossi and A. Zlotnik, The biology of chemokines and their receptors, *Annu Rev Immunol* (2000), pp. 18217–18242.
7. I.F. Charo and R.M. Ransohoff, The many roles of chemokines and chemokine receptors in inflammation, *N Engl J Med* 354 (6) (2006), pp. 610–621.

8. A. Zlotnik and O. Yoshie, Chemokines: a new classification system and their role in immunity, *Immunity* 12 (2) (2000), pp. 121–127.
9. S.A. Luther and J.G. Cyster, Chemokines as regulators of T cell differentiation, *Nat Immunol* 2 (2) (2001), pp. 102–107.
10. P. Kivisakk, C. Trebst, D.J. Eckstein, A.P. Kerza-Kwiatecki and R.M. Ransohoff, Chemokine-based therapies for MS: how do we get there from here?, *Trends Immunol* 22 (11) (2001), pp. 591–593.
11. D.M. Muller, M.P. Pender and J.M. Greer, Chemokines and chemokine receptors: potential therapeutic targets in multiple sclerosis, *Curr Drug Targets Inflamm Allergy* 3 (3) (2004), pp. 279–290.
12. L. Cartier, O. Hartley, M. Dubois-Dauphin and K.H. Krause, Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases, *Brain Res Brain Res Rev* 48 (1) (2005), pp. 16–42.
13. R. Gold, C. Lington and H. Lassmann, Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research, *Brain* 129 (Pt 8) (2006), pp. 1953–1971.
14. O. Aktas, S. Waiczies, A. Smorodchenko, J. Dorr, B. Seeger and T. Prozorovski *et al.*, Treatment of relapsing paralysis in experimental encephalomyelitis by targeting Th1 cells through atorvastatin, *J Exp Med* 197 (6) (2003), pp. 725–733.
15. A. Smorodchenko, J. Wuerfel, E.E. Pohl, J. Vogt, E. Tysiak and R. Glumm *et al.*, CNS-irrelevant T-cells enter the brain, cause blood-brain barrier disruption but no glial pathology, *Eur J Neurosci* 26 (6) (2007), pp. 1387–1398.
16. Z. Johnson, C.A. Power, C. Weiss, F. Rintelen, H. Ji and T. Ruckle *et al.*, Chemokine inhibition—why, when, where, which and how?, *Biochem Soc Trans* 32 (Pt 2) (2004), pp. 366–377.
17. E.E. Ubogu, M.B. Cossoy and R.M. Ransohoff, The expression and function of chemokines involved in CNS inflammation, *Trends Pharmacol Sci* 27 (1) (2006), pp. 48–55.
18. F. Zipp, H.P. Hartung, J. Hillert, S. Schimrigk, C. Trebst and M. Stangel *et al.*, Blockade of chemokine signaling in patients with multiple sclerosis, *Neurology* 67 (10) (2006), pp. 1880–1883.
19. H.E. Broxmeyer, Chemokines in hematopoiesis, *Curr Opin Hematol* 15 (1) (2008), pp. 49–58.
20. R. Benelli, G. Lorusso, A. Albini and D.M. Noonan, Cytokines and chemokines as regulators of angiogenesis in health and disease, *Curr Pharm Des* 12 (24) (2006), pp. 3101–3115.
21. A. Bajetto, R. Bonavia, S. Barbero, T. Florio and G. Schettini, Chemokines and their receptors in the central nervous system, *Front Neuroendocrinol* 22 (3) (2001), pp. 147–184.
22. T. Nagasawa, S. Hirota, K. Tachibana, N. Takakura, S. Nishikawa and Y. Kitamura *et al.*, Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1, *Nature* 382 (6592) (1996), pp. 635–638.
23. Q. Ma, D. Jones, P.R. Borghesani, R.A. Segal, T. Nagasawa and T. Kishimoto *et al.*, Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in C, *Proc Natl Acad Sci U S A* 95 (16) (1998), pp. 9448–9453.
24. C. Infante-Duarte, S. Waiczies, J. Wuerfel and F. Zipp, New developments in understanding and treating neuroinflammation, *J Mol Med* (2008) Jan 15. [Electronic publication ahead of print].
25. F. Zipp and O. Aktas, The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases, *Trends Neurosci* 29 (9) (2006), pp. 518–527.
26. K.J. Katschke Jr., J.B. Rottman, J.H. Ruth, S. Qin, L. Wu and G. LaRosa *et al.*, Differential expression of chemokine receptors on peripheral blood, synovial fluid, and synovial tissue monocytes/macrophages in rheumatoid arthritis, *Arthritis Rheum* 44 (5) (2001), pp. 1022–1032.
27. M. Oppermann, Chemokine receptor CCR5: insights into structure, function, and regulation, *Cell Signal* 16 (11) (2004), pp. 1201–1210.
28. C. Trebst, T.L. Sorensen, P. Kivisakk, M.K. Cathcart, J. Hesselgesser and R. Horuk *et al.*, CCR1+CCR5+ mononuclear phagocytes accumulate in the central nervous system of patients with multiple sclerosis, *Am J Pathol* 159 (5) (2001), pp. 1701–1710.
29. T.L. Sorensen, M. Tani, J. Jensen, V. Pierce, C. Lucchinetti and V.A. Folcik *et al.*, Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients, *J Clin Invest* 103 (6) (1999), pp. 807–815.
30. K.E. Balashov, J.B. Rottman, H.L. Weiner and W.W. Hancock, CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions, *Proc Natl Acad Sci U S A* 96 (12) (1999), pp. 6873–6878.
31. P. Putheti, M. Morris, L. Stawiarz, N. Teleshova, P. Kivisakk and M. Pashenkov *et al.*, Multiple sclerosis: a study of chemokine receptors and regulatory T cells in relation to MRI variables, *Eur J Neurol* 10 (5) (2003), pp. 529–535.
32. A.R. Glabinski, B. Bielecki, S. O'Bryant, K. Selmaj and R.M. Ransohoff, Experimental autoimmune encephalomyelitis: CC chemokine receptor expression by trafficking cells, *J Autoimmun* 19 (4) (2002), pp. 175–181.
33. A.R. Glabinski, V.K. Tuohy and R.M. Ransohoff, Expression of chemokines RANTES, MIP-1alpha and GRO-alpha correlates with inflammation in acute experimental autoimmune encephalomyelitis, *Neuroimmunomodulation* 5 (3–4) (1998), pp. 166–171.
34. A.R. Glabinski, B. Bielecki and R.M. Ransohoff, Chemokine upregulation follows cytokine expression in chronic relapsing experimental autoimmune encephalomyelitis, *Scand J Immunol* 58 (1) (2003), pp. 81–88.
35. K.J. Kennedy, R.M. Strieter, S.L. Kunkel, N.W. Lukacs and W.J. Karpus, Acute and relapsing experimental autoimmune encephalomyelitis are regulated by differential expression of the CC chemokines macrophage inflammatory protein-1alpha and monocyte chemoattractant protein-1, *J Neuroimmunol* 92 (1–2) (1998), pp. 98–108.
36. B.T. Fife, M.C. Paniagua, N.W. Lukacs, S.L. Kunkel and W.J. Karpus, Selective CC chemokine receptor expression by central nervous system-infiltrating encephalitogenic T cells during experimental autoimmune encephalomyelitis, *J Neurosci Res* 66 (4) (2001), pp. 705–714.
37. W.J. Karpus, N.W. Lukacs, B.L. McRae, R.M. Strieter, S.L. Kunkel and S.D. Miller, An important role for the chemokine macrophage inflammatory protein-1 alpha in the pathogenesis of the T cell-mediated autoimmune disease, experimental autoimmune encephalomyelitis, *J Immunol* 155 (10) (1995), pp. 5003–5010.
38. E.H. Tran, W.A. Kuziel and T. Owens, Induction of experimental autoimmune encephalomyelitis in C57BL/6 mice deficient in either the chemokine macrophage inflammatory protein-1alpha or its CCR5 receptor, *Eur J Immunol* 30 (5) (2000), pp. 1410–1415.
39. A.E. Proudfoot, R. Buser, F. Borlat, S. Alouani, D. Soler and R.E. Offord *et al.*, Amino-terminally modified RANTES

- analogues demonstrate differential effects on RANTES receptors, *J Biol Chem* 274 (45) (1999), pp. 32478–32485.
40. M. Matsui, J. Weaver, A.E. Proudfoot, J.R. Wujek, T. Wei and E. Richer *et al.*, Treatment of experimental autoimmune encephalomyelitis with the chemokine receptor antagonist Met-RANTES, *J Neuroimmunol* 128 (1–2) (2002), pp. 16–22.
  41. J.B. Rottman, A.J. Slavin, R. Silva, H.L. Weiner, C.G. Gerard and W.W. Hancock, Leukocyte recruitment during onset of experimental allergic encephalomyelitis is CCR1 dependent, *Eur J Immunol* 30 (8) (2000), pp. 2372–2377.
  42. M. Liang, C. Mallari, M. Rosser, H.P. Ng, K. May and S. Monahan *et al.*, Identification and characterization of a potent, selective, and orally active antagonist of the CC chemokine receptor-1, *J Biol Chem* 275 (25) (2000), pp. 19000–19008.
  43. M. Mack, J. Cihak, C. Simonis, B. Luckow, A.E. Proudfoot and J. Plachy *et al.*, Expression and characterization of the chemokine receptors CCR2 and CCR5 in mice, *J Immunol* 166 (7) (2001), pp. 4697–4704.
  44. D.J. Mahad and R.M. Ransohoff, The role of MCP-1 (CCL2) and CCR2 in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE), *Semin Immunol* 15 (1) (2003), pp. 23–32.
  45. L. Izikson, R.S. Klein, I.F. Charo, H.L. Weiner and A.D. Luster, Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2, *J Exp Med* 192 (7) (2000), pp. 1075–1080.
  46. S. Gaupp, D. Pitt, W.A. Kuziel, B. Cannella and C.S. Raine, Experimental autoimmune encephalomyelitis (EAE) in CCR2(–/–) mice: susceptibility in multiple strains, *Am J Pathol* 162 (1) (2003), pp. 139–150.
  47. D.R. Huang, J. Wang, P. Kivisakk, B.J. Rollins and R.M. Ransohoff, Absence of monocyte chemoattractant protein 1 in mice leads to decreased local macrophage recruitment and antigen-specific T helper cell type 1 immune response in experimental autoimmune encephalomyelitis, *J Exp Med* 193 (6) (2001), pp. 713–726.
  48. A. Elhofy, J. Wang, M. Tani, B.T. Fife, K.J. Kennedy and J. Bennett *et al.*, Transgenic expression of CCL2 in the central nervous system prevents experimental autoimmune encephalomyelitis, *J Leukoc Biol* 77 (2) (2005), pp. 229–237.
  49. T.L. Sorensen, R.M. Ransohoff, R.M. Strieter and F. Sellebjerg, Chemokine CCL2 and chemokine receptor CCR2 in early active multiple sclerosis, *Eur J Neurol* 11 (7) (2004), pp. 445–449.
  50. B. Sharrack, T. Leach, E. Jacobson, D.D. Donaldson, X. Xu and M. Hu, Frequent MRI study of a novel CCR2 antagonist in relapsing–remitting multiple sclerosis, *Ann Neurol* 62 (S11) (2007), pp. S74–S75.
  51. T. Worbs and R. Forster, A key role for CCR7 in establishing central and peripheral tolerance, *Trends Immunol* 28 (6) (2007), pp. 274–280.
  52. L.V. Bagaeva, P. Rao, J.M. Powers and B.M. Segal, CXC chemokine ligand 13 plays a role in experimental autoimmune encephalomyelitis, *J Immunol* 176 (12) (2006), pp. 7676–7685.
  53. B. Bielecki, A. Mazurek, P. Wolinski and A. Glabinski, Expression of chemokine receptors CCR7 and CCR8 in the CNS during ChREAE, *Scand J Immunol* 66 (4) (2007), pp. 383–392.
  54. S. Columba-Cabezas, B. Serafini, E. Ambrosini and F. Aloisi, Lymphoid chemokines CCL19 and CCL21 are expressed in the central nervous system during experimental autoimmune encephalomyelitis: implications for the maintenance of chronic neuroinflammation, *Brain Pathol* 13 (1) (2003), pp. 38–51.
  55. M. Krumbholz, D. Theil, F. Steinmeyer, S. Cepok, B. Hemmer and M. Hofbauer *et al.*, CCL19 is constitutively expressed in the CNS, up-regulated in neuroinflammation, active and also inactive multiple sclerosis lesions, *J Neuroimmunol* 190 (1–2) (2007), pp. 72–79.
  56. P. Kivisakk, D.J. Mahad, M.K. Callahan, K. Sikora, C. Trebst and B. Tucky *et al.*, Expression of CCR7 in multiple sclerosis: implications for CNS immunity, *Ann Neurol* 55 (5) (2004), pp. 627–638.
  57. M.A. Schneider, J.G. Meingassner, M. Lipp, H.D. Moore and A. Rot, CCR7 is required for the *in vivo* function of CD4 + CD25 + regulatory T cells, *J Exp Med* 204 (4) (2007), pp. 735–745.
  58. A. Menning, U.E. Hopken, K. Siegmund, M. Lipp, A. Hamann and J. Huehn, Distinctive role of CCR7 in migration and functional activity of naive- and effector/memory-like Treg subsets, *Eur J Immunol* 37 (6) (2007), pp. 1575–1583.
  59. A. Pahuja, R.A. Maki, P.A. Hevezi, A. Chen, G.M. Verge and S.M. Lechner *et al.*, Experimental autoimmune encephalomyelitis develops in CC chemokine receptor 7-deficient mice with altered T-cell responses, *Scand J Immunol* 64 (4) (2006), pp. 361–369.
  60. A. Iellem, M. Mariani, R. Lang, H. Recalde, P. Panina-Bordignon and F. Sinigaglia *et al.*, Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by CD4(+)CD25(+) regulatory T cells, *J Exp Med* 194 (6) (2001), pp. 847–853.
  61. C.R. Cardoso, G.P. Garlet, A.P. Moreira, W.M. Junior, M.A. Rossi and J.S. Silva, Characterization of CD4 + CD25 + natural regulatory T cells in the inflammatory infiltrate of human chronic periodontitis, *J Leukoc Biol* 84 (1) (2008), pp. 311–318.
  62. Y. Mizukami, K. Kono, Y. Kawaguchi, H. Akaike, K. Kamimura and H. Sugai *et al.*, CCL17 and CCL22 chemokines within tumor microenvironment are related to accumulation of Foxp3 + regulatory T cells in gastric cancer, *Int J Cancer* 122 (10) (2008), pp. 2286–2293.
  63. J. Haas, L. Schopp, B. Storch-Hagenlocher, B. Fritzsching, C. Jacobi and L. Milkova *et al.*, Specific recruitment of regulatory T cells into the CSF in lymphomatous and carcinomatous meningitis, *Blood* 111 (2) (2008), pp. 761–766.
  64. Q. Yuan, S.K. Bromley, T.K. Means, K.J. Jones, F. Hayashi and A.K. Bhan *et al.*, D. CCR4-dependent regulatory T cell function in inflammatory bowel disease, *J Exp Med* 204 (6) (2007), pp. 1327–1334.
  65. K. Narikawa, T. Misu, K. Fujihara, I. Nakashima, S. Sato and Y. Itoyama, CSF chemokine levels in relapsing neuromyelitis optica and multiple sclerosis, *J Neuroimmunol* 149 (1–2) (2004), pp. 182–186.
  66. S. Columba-Cabezas, B. Serafini, E. Ambrosini, M. Sanchez, G. Penna and L. Adorini *et al.*, Induction of macrophage-derived chemokine/CCL22 expression in experimental autoimmune encephalomyelitis and cultured microglia: implications for disease regulation, *J Neuroimmunol* 130 (1–2) (2002), pp. 10–21.
  67. E. Butti, A. Bergami, A. Recchia, E. Brambilla, U. Del Carro and S. Amadio *et al.*, IL4 gene delivery to the CNS recruits regulatory T cells and induces clinical recovery in mouse models of multiple sclerosis, *Gene Ther* 15 (7) (2008), pp. 504–515.
  68. S. Ghosh, A. Elder, J. Guo, U. Mani, M. Patane and K. Carson *et al.*, Design, synthesis, and progress toward optimization of potent small molecule antagonists of CC chemokine receptor 8 (CCR8), *J Med Chem* 49 (9) (2006), pp. 2669–2672.

69. C. Trebst, S.M. Staugaitis, P. Kivisakk, D. Mahad, M.K. Cathcart and B. Tucky *et al.*, CC chemokine receptor 8 in the central nervous system is associated with phagocytic macrophages, *Am J Pathol* 162 (2) (2003), pp. 427–438.
70. R. Godiska, D. Chantry, G.N. Dietsch and P.W. Gray, Chemokine expression in murine experimental allergic encephalomyelitis, *J Neuroimmunol* 58 (2) (1995), pp. 167–176.
71. C.A. Murphy, R.M. Hoek, M.T. Wiekowski, S.A. Lira and J.D. Sedgwick, Interactions between hemopoietically derived TNF and central nervous system-resident glial chemokines underlie initiation of autoimmune inflammation in the brain, *J Immunol* 169 (12) (2002), pp. 7054–7062.
72. K.M. Omari, G.R. John, S.C. Sealfon and C.S. Raine, CXC chemokine receptors on human oligodendrocytes: implications for multiple sclerosis, *Brain* 128 (Pt 5) (2005), pp. 1003–1015.
73. D. Nguyen and M. Stangel, Expression of the chemokine receptors CXCR1 and CXCR2 in rat oligodendroglial cells, *Brain Res Dev Brain Res* 128 (1) (2001), pp. 77–81.
74. K.M. Omari, G. John, R. Lango and C.S. Raine, Role for CXCR2 and CXCL1 on glia in multiple sclerosis, *Glia* 53 (1) (2006), pp. 24–31.
75. A.R. Glabinski, S. O'Bryant, K. Selmaj and R.M. Ransohoff, CXC chemokine receptors expression during chronic relapsing experimental autoimmune encephalomyelitis, *Ann N Y Acad Sci* (2000), pp. 917135–917144.
76. T. Carlson, M. Kroenke, P. Rao, T.E. Lane and B. Segal, The Th17-ELR + CXC chemokine pathway is essential for the development of central nervous system autoimmune disease, *J Exp Med* 205 (4) (2008), pp. 811–823.
77. M. Muller, S.L. Carter, M.J. Hofer, P. Manders, D.R. Getts and M.T. Getts *et al.*, CXCR3 signaling reduces the severity of experimental autoimmune encephalomyelitis by controlling the parenchymal distribution of effector and regulatory T cells in the central nervous system, *J Immunol* 179 (5) (2007), pp. 2774–2786.
78. S. Arimilli, W. Ferlin, N. Solvason, S. Deshpande, M. Howard and S. Mocchi, Chemokines in autoimmune diseases, *Immunol Rev* (2000), pp. 17743–17751.
79. S.H. Goldberg, M.e.e.r.P. van der, J. Hesselgesser, S. Jaffer, D.L. Kolson and A.V. Albright *et al.*, CXCR3 expression in human central nervous system diseases, *Neuropathol Appl Neurobiol* 27 (2) (2001), pp. 127–138.
80. J.E. Simpson, J. Newcombe, M.L. Cuzner and M.N. Woodroffe, Expression of the interferon-gamma-inducible chemokines IP-10 and Mig and their receptor, CXCR3, in multiple sclerosis lesions, *Neuropathol Appl Neurobiol* 26 (2) (2000), pp. 133–142.
81. T.L. Sorensen, H. Roed and F. Sellebjerg, Optic neuritis: chemokine receptor CXCR3 and its ligands, *Br J Ophthalmol* 88 (9) (2004), pp. 1146–1148.
82. E. Scarpini, D. Galimberti, P. Baron, R. Clerici, M. Ronzoni and G. Conti *et al.*, IP-10 and MCP-1 levels in CSF and serum from multiple sclerosis patients with different clinical subtypes of the disease, *J Neurol Sci* 195 (1) (2002), pp. 41–46.
83. B.T. Fife, K.J. Kennedy, M.C. Paniagua, N.W. Lukacs, S.L. Kunkel and A.D. Luster *et al.*, CXCL10 (IFN-gamma-inducible protein-10) control of encephalitogenic CD4+ T cell accumulation in the central nervous system during experimental autoimmune encephalomyelitis, *J Immunol* 166 (12) (2001), pp. 7617–7624.
84. S.L. Carter, M. Muller, P.M. Manders and I.L. Campbell, Induction of the genes for Cxcl9 and Cxcl10 is dependent on IFN-gamma but shows differential cellular expression in experimental autoimmune encephalomyelitis and by astrocytes and microglia *in vitro*, *Glia* 55 (16) (2007), pp. 1728–1739.
85. S. Narumi, T. Kaburaki, H. Yoneyama, H. Iwamura, Y. Kobayashi and K. Matsushima, Neutralization of IFN-inducible protein 10/CXCL10 exacerbates experimental autoimmune encephalomyelitis, *Eur J Immunol* 32 (6) (2002), pp. 1784–1791.
86. R.S. Klein, L. Izikson, T. Means, H.D. Gibson, E. Lin and R.A. Sobel *et al.*, IFN-inducible protein 10/CXC chemokine ligand 10-independent induction of experimental autoimmune encephalomyelitis, *J Immunol* 172 (1) (2004), pp. 550–559.
87. L. Liu, D. Huang, M. Matsui, T.T. He, T. Hu and J. Demartino *et al.*, Severe disease, unaltered leukocyte migration, and reduced IFN-gamma production in CXCR3<sup>-/-</sup> mice with experimental autoimmune encephalomyelitis, *J Immunol* 176 (7) (2006), pp. 4399–4409.
88. E.E. McCandless, Q. Wang, B.M. Woerner, J.M. Harper and R.S. Klein, CXCL12 limits inflammation by localizing mononuclear infiltrates to the perivascular space during experimental autoimmune encephalomyelitis, *J Immunol* 177 (11) (2006), pp. 8053–8064.
89. T.M. Calderon, E.A. Eugenin, L. Lopez, S.S. Kumar, J. Hesselgesser and C.S. Raine *et al.*, A role for CXCL12 (SDF-1alpha) in the pathogenesis of multiple sclerosis: regulation of CXCL12 expression in astrocytes by soluble myelin basic protein, *J Neuroimmunol* 177 (1–2) (2006), pp. 27–39.
90. M. Li and R.M. Ransohoff, Multiple roles of chemokine CXCL12 in the central nervous system: a migration from immunology to neurobiology, *Prog Neurobiol* 84 (2) (2008), pp. 116–131.
91. M. Krumbholz, D. Theil, S. Cepok, B. Hemmer, P. Kivisakk and R.M. Ransohoff *et al.*, Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment, *Brain* 129 (Pt 1) (2006), pp. 200–211.
92. E.E. McCandless, L. Piccio, B.M. Woerner, R.E. Schmidt, J.B. Rubin and A.H. Cross *et al.*, Pathological expression of CXCL12 at the blood-brain barrier correlates with severity of multiple sclerosis, *Am J Pathol* 172 (3) (2008), pp. 799–808.
93. R. Magliozzi, S. Columba-Cabezas, B. Serafini and F. Aloisi, Intracerebral expression of CXCL13 and BAFF is accompanied by formation of lymphoid follicle-like structures in the meninges of mice with relapsing experimental autoimmune encephalomyelitis, *J Neuroimmunol* 148 (1–2) (2004), pp. 11–23.
94. J.F. Bazan, K.B. Bacon, G. Hardiman, W. Wang, K. Soo and D. Rossi *et al.*, A new class of membrane-bound chemokine with a CX3C motif, *Nature* 385 (6617) (1997), pp. 640–644.
95. P. Fraticelli, M. Sironi, G. Bianchi, D. D'Ambrosio, C. Albanesi and A. Stoppacciaro *et al.*, Fractalkine (CX3CL1) as an amplification circuit of polarized Th1 responses, *J Clin Invest* 107 (9) (2001), pp. 1173–1181.
96. A.E. Cardona, E.P. Pioro, M.E. Sasse, V. Kostenko, S.M. Cardona and I.M. Dijkstra *et al.*, Control of microglial neurotoxicity by the fractalkine receptor, *Nat Neurosci* 9 (7) (2006), pp. 917–924.
97. J.K. Harrison, Y. Jiang, S. Chen, Y. Xia, D. Maciejewski and R.K. McNamara *et al.*, Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia, *Proc Natl Acad Sci U S A* 95 (18) (1998), pp. 10896–10901.
98. D. Sunnemark, S. Eltayeb, M. Nilsson, E. Wallstrom, H. Lassmann and T. Olsson *et al.*, CX3CL1 (fractalkine) and CX3CR1 expression in myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis:

- kinetics and cellular origin, *J Neuroinflammation* (2005), p. 217.
99. W.J. Schwaeble, C.M. Stover, T.J. Schall, D.J. Dairaghi, P.K. Trinder and C. Linington *et al.*, Neuronal expression of fractalkine in the presence and absence of inflammation, *FEBS Lett* 439 (3) (1998), pp. 203–207.
  100. S. Hulshof, E.S. van Haastert, H.F. Kuipers, P.J. van den Elsen, C.J. De Groot and V. van der *et al.*, CX3CL1 and CX3CR1 expression in human brain tissue: noninflammatory control versus multiple sclerosis, *J Neuropathol Exp Neurol* 62 (9) (2003), pp. 899–907.
  101. S. Kastenbauer, U. Koedel, M. Wick, B.C. Kieseier, H.P. Hartung and H.W. Pfister, CSF and serum levels of soluble fractalkine (CX3CL1) in inflammatory diseases of the nervous system, *J Neuroimmunol* 137 (1–2) (2003), pp. 210–217.
  102. D. Sunnemark, S. Eltayeb, E. Wallstrom, L. Appelsved, A. Malmberg and H. Lassmann *et al.*, Differential expression of the chemokine receptors CX3CR1 and CCR1 by microglia and macrophages in myelin-oligodendrocyte-glycoprotein-induced experimental autoimmune encephalomyelitis, *Brain Pathol* 13 (4) (2003), pp. 617–629.
  103. D. Huang, F.D. Shi, S. Jung, G.C. Pien, J. Wang and T.P. Salazar-Mather *et al.*, The neuronal chemokine CX3CL1/fractalkine selectively recruits NK cells that modify experimental autoimmune encephalomyelitis within the central nervous system, *FASEB J* 20 (7) (2006), pp. 896–905.
  104. C. Infante-Duarte, A. Weber, J. Kratzschmar, T. Prozorovski, S. Pikol and I. Hamann *et al.*, Frequency of blood CX3CR1-positive natural killer cells correlates with disease activity in multiple sclerosis patients, *FASEB J* 19 (13) (2005), pp. 1902–1904.

Table 1: Chemokines and chemokine receptors

Receptor	Chemokine	Old nomenclature
<i>C family</i>		
XCR1	XCL1	Lymphotactin
XCR2	XCL2	SCM-1beta
<i>CC family</i>		
CCR1	CCL3	MIP-1alpha
	CCL3L1	LD78beta
	CCL5	RANTES
	CCL7	MCP-3
	CCL14	HCC-1
	CCL15	HCC-2, Lkn-1, MIP-1alpha
	CCL16	HCC-4
	CCL23	MPIF-1, Ckbeta8
CCR2	CCL2	MCP-1
	CCL7	MCP-3
	CCL8	MCP-2
CCR3	CCL5	RANTES
	CCL7	MCP-3
	CCL8	MCP-2
	CCL11	Eotaxin-1
	CCL13	MCP-4
	CCL15	HCC-2, Lkn-1, MIP-1delta
	CCL24	Eotaxin-2, MPIF-2
	CCL26	Eotaxin-3
	CCL28	MEC
CCR4	CCL17	TARC
	CCL22	MDC, STCP-1
CCR5	CCL3	MIP-1alpha
	CCL3L1	LD78beta
	CCL4	MIP-1beta
	CCL5	RANTES
	CCL14	HCC-1
CCR6	CCL20	MIP-3alpha, exodus-1
CCR7	CCL19	MIP-3beta, exodus-3
	CCL21	SLC, exodus-2
CCR8	CCL1	I-309
CCR9	CCL25	TECK
CCR10	CCL27	CTACK, ILC
	CCL28	MEC
Unknown	CCL18	PARC, DC-CK1, AMAC1
<i>CXC family</i>		
CXCR1	CXCL1	GRO-alpha
	CXCL6	GCP-2
	CXCL8	IL-8
CXCR2	CXCL1	GRO-alpha
	CXCL2	GRO-beta
	CXCL3	GRO-gamma
	CXCL5	ENA-78
	CXCL6	GCP-2
	CXCL7	NAP-2
	CXCL8	IL-8
CXCR3	CXCL4	PF4
	CXCL9	Mig
	CXCL10	IP-10
	CXCL11	I-TAC
CXCR4	CXCL12	SDF-1
CXCR5	CXCL13	BLC/BCA-1
CXCR6	CXCL16	
Unknown	CXCL14	BRAK
<i>CX3C family</i>		
CX3CR1	CX3CL1	Fractalkine

Table 2: Chemokine receptors and chemokines in MS and EAE

Chemokine receptor	Ligand	Description in MS and EAE	Reference	
CCR1	CCL3	• CCR1 <sup>+</sup> cells accumulated in perivascular elements in patients	[28]	
	CCL3L1	• Elevated levels of CCL5 in CSF and of CCL3 in lesions of MS patients	[29,30]	
	CCL5	• Increased CNS expression of chemokines and receptor during EAE	[32-35]	
	CCL7	• Prevention of EAE after anti-CCL3 treatment	[35-37]	
	CCL14-16 CCL23	• CCL3-deficient mice: no resistance or less susceptibility to EAE • No effect of antagonist Met-RANTES in monophasic but slightly attenuated effects in chEAE • Milder severity of EAE in CCR1-deficient mice • Amelioration of EAE in rats with CCR1 antagonist BX471 • No effect of lesion number in MS patients after treatment with BX471	[38] [40] [41] [42] [18]	
CCR2	CCL2	• Increased CCR2 expression during initial attacks and spontaneous relapse in chEAE	[32]	
	CCL7	• Correlation of CCL2 correlated with EAE severity	[34]	
	CCL8	• CCR2 <sup>-/-</sup> mice: resistant to EAE • CCL2 <sup>-/-</sup> : delayed onset in active and resistance to passive EAE • Anti-CCL2: no effect at time of adoptive transfer but ameliorated effects during remission of EAE • CCL2-transgenic expression of low levels of in the CNS: milder form of active EAE, though similar number of CNS infiltrates	[45] [47] [35] [48]	
		• No substantial role of CCR2 in MS, no correlation with MRI activity • Reduced MRI activity in patients treated with CCR2 antagonist	[31,49] [50]	
		• Up-regulation of ligands during neuroinflammation	[61,63]	
CCR4	CCL17 CCL22	• CCR4 expression correlates with disease activity	[61]	
CCR5	CCL3	• Increased level of CCR5 in MS lesions	[30]	
	CCL3L1 CCL4 CCL5 CCL14	• No correlation of CCR5 expression and MRI activity in MS patients • Increased CNS expression of CCL3/CCL5 and receptor during EAE • Prevention of EAE after anti-CCL3 treatment • No resistance or less susceptibility to EAE in CCR5-deficient mice • No effect of antagonist Met-RANTES in monophasic but slightly attenuated effects in chEAE	[31] [32-35] [35-37] [38] [40]	
CCR7	CCL19	• Constitutive expression of CCL21 regardless of inflammation	[52]	
	CCL21	• Up-regulation of receptor and ligands during neuroinflammation in EAE • Constitutive expression CCL19 in human brain, up-regulation during MS • CCR7 expression associated with DCs in MS lesions and in CSF • CCR7 <sup>-/-</sup> : similar EAE intensity compared to wild-type mice	[53,54] [55] [56] [59]	
CCR8	CCL1	• CCR8 expression on microglia and phagocytes in actively demyelinating MS lesions • CCR8 up-regulation during EAE • CCL1 expression before clinical onset of EAE in spinal cord • Later onset and milder symptoms of EAE in CCR8-deficient mice	[66] [53] [67] [68]	
			[69]	
CXCR1	CXCL1	• Constitutive expression of the receptor on oligodendrocytes	[69]	
	CXCL6 CXCL8	• Increased expression of CXCL8 on oligodendrocytes, no detection of ligands in MS tissue • Correlation of CXCL1 expression with EAE intensity	[69,71] [33]	
CXCR2	CXCL1-3 CXCL5-8	• CXCL2 expression increased in spinal cord during first and second attack of EAE • No clinical sign of EAE in CXCR2-deficient mice • Reduced disease activity in EAE after anti-CXCR2 treatment	[72] [73] [73]	
	CXCR3	• Constitutive expression of receptor on astrocytes in normal brain and up-regulation in MS lesions	[69,76,77]	
CXCR3	CXCL9-11	• No difference in receptor expression on oligodendrocytes in MS compared to normal brain • Increased expression of CXCR3 <sup>+</sup> T cells in blood of MS patients • Accumulation of CXCR3 <sup>+</sup> T cells in perivascular space of MS lesions • Correlation of CXCR3 expression on blood T cells and MRI activity in MS patients • No correlation of CXCR3 expression and MRI activity in MS patients • CXCL9 and CXCL10 increased in CSF of MS patients and correlate with MRI activity • Anti-CXCL10 treatment: decreased clinical and pathological severity of passive EAE • Exacerbation of active EAE in rats after anti-CXCL10 administration • Higher susceptibility to EAE in CXCL10-deficient mice • More severe EAE in CXCR3-deficient mice • Reduced severity of active EAE after treatment with anti-CXCR3 antibody	[69] [30] [77] [31] [78] [29,79] [80] [82] [83] [74,84] [75]	
	CXCR4	CXCL12	• Anti-CXCR3 administration: aggravation of active EAE during effector phase • CXCL12 constitutively expressed by endothelial cells and astrocytes in normal human brain • CXCR4 expression in active and chronic inactive MS lesions, and increased levels of CXCL12 in CSF of MS patients • More severe EAE after blocking CXCR4 function	[84] [85-87] [86,88] [85]
				[85,89]
	CXCR5	CXCL13	• Altered expression pattern of CXCL12 associated with tissue damage in EAE and MS • Abnormal B cell activity in MS patients with accumulating B cells in lesion • Up-regulation of CXCL13 expression during EAE • Less severe EAE in CXCL13 deficient mice with fewer infiltrating mononuclear cells into the CNS • No detection of CXCL13 in normal human brain but in active MS lesions, and increased levels in CSF of MS patients	[90] [52] [52] [88]
				[88]
	CX3CR1	CX3CL1	• Constitutive expression of receptor and ligand in brain • Increased level of ligand in CSF of patients with neuroinflammation, but only in MS patients increased levels of ligand in sera • High expression of receptor in late active and inactive demyelination, and accumulation of CX3CR1 mRNA expressing cells at site of inflammation • CX3CR1 <sup>-/-</sup> mice develop more severe EAE with increased mortality and less infiltrating NK cells into the CNS • Lower expression of CX3CR1 on NK cells in MS patients	[95-97] [98] [95,99] [100] [101]

chEAE = chronic EAE.

Table 3: Clinical studies with receptor antagonists in MS

Receptor	Antagonist	Phase	Company
CCR1	AVE9897	Phase I completed	SanofiAventis
	MLN3897	Phase I completed	Millenium Pharmaceuticals
	BX471	Phase II completed	Schering AG
CCR2	CCX915	Phase II planned	ChemoCentryx
	INCB8696	Phase I ongoing	Incyte
	MK-0812	Phase II ongoing	Merck