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# ROLE OF CHEMOKINES AND THEIR RECEPTORS IN THE PATHOGENESIS OF MULTIPLE SCLEROSIS

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## 1. ABSTRACT

Multiple sclerosis (MS) is an autoimmune disease of the human central nervous system (CNS) of unknown etiology that causes demyelination and associated tissue injury. Trafficking of inflammatory T cells into the CNS is a crucial event in the pathogenesis of MS, a process in which chemokines and their receptors have been demonstrated to play an important role. Chemokines are key mediators of inflammation and have major effects on migration of cells to the sites of inflammation as well as activation of recruited and resident CNS cells. This paper summarizes recent and new information about the expression and function of elements of the chemokine system in MS and its animal model experimental allergic encephalomyelitis. Analysis of the chemokine system provides insights into mechanisms of CNS inflammatory reactions and may lead to new targets of therapeutic intervention in MS.

## 2. INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disease of the human central nervous system that affects approximately 2.5 million people worldwide. Typically, onset occurs between ages 20-40 with a peak at age 30 (1). The disease does not cause mortality, but in the majority of the affected individuals it causes significant chronic morbidity and it is the leading cause of neurological disability in young adults (2). In most patients, MS starts as a disease with episodes of neurological dysfunction followed by substantial improvement (relapsing remitting MS). After an individual different long disease duration recovery is often less complete and a gradual progression of disability may develop (secondary progressive MS). The remaining 10-15% of patients experience slow neurodegeneration from onset, which is termed primary progressive MS (3, 4).

The cause of the disease is unknown (5). Many immune abnormalities have been described in MS, which indicates that the immune system plays a central role in its pathogenesis (6). The current consensus is that MS pathogenesis comprises an initial autoimmune inflammatory phase, followed by a phase of selective demyelination and a neurodegenerative phase with axonal pathology (7). Based on molecular, genetic and epidemiological data, it is believed that MS occurs in genetically susceptible individuals that are exposed to certain environmental triggers (8, 9). It is widely accepted that viral infection can provide such a trigger (1). Individuals with such genetically determined susceptibility to MS harbor T cells that react with central nervous system (CNS) myelin autoantigens. At some point they are activated in the periphery by exogenous triggers, probably by molecular mimicry (10). This enables them to migrate through the blood-brain barrier to the brain and spinal cord. Reactivated in the CNS, these cells of either $CD4^+$ helper or $CD8^+$ cytotoxic phenotype release pro-inflammatory Th1 cytokines and orchestrate the destruction of the myelin sheath by various types of immune cells (11). Specifically, the typical MS lesion contains different perivascular hematogenous T cells and the myelin degradation can be shown to be associated with accumulation of highly activated macrophages (12). Recent studies indicate that the disease is not pathologically homogeneous, so some cases may feature a dissociation of inflammation and tissue injury (13). Destruction follows one of the four pathological patterns: 1. T cell- and macrophage- mediated demyelination; 2. Antibody-mediated demyelination that involves complement activation; 3. Distal oligodendrogliopathy and oligodendrocyte apoptosis, and 4. Primary oligodendrocyte degeneration. In most of the cases the intensity of inflammation is directly related to the extent of tissue injury. Tissue injury in MS extends far beyond demyelination. Axonal loss, which causes irreversible disability, occurs early in the course of the disease (14). But it is unclear whether axonal damage is the consequence of a primary active destructive process, or of increased vulnerability through chronic demyelination (15). Taken together, the recruitment of macrophages and T lymphocytes into the CNS is a crucial event in the pathogenesis of MS, which frequently leads to irreversible injury to myelin and axons.

Chemokines constitute a large family of small chemoattractant cytokines that share important structural

features and the ability to attract leucocytes. The regulated interactions of chemokines with their respective cell surface receptors mediate the recruitment of specific leucocyte subpopulations to the sites of inflammation (16). Considerable attention has recently focused to understand leukocyte trafficking during inflammatory processes that affect the CNS like MS. Characteristics of CNS inflammation are distinct from those in other organs because of the presence of the blood brain barrier (BBB), which affords a partial isolation from the circulating cellular and molecular elements of the immune system (17). The process of leukocyte recruitment into the CNS involves several steps beginning with weak adhesion and rolling on the endothelium of the BBB, followed by firm arrest on the luminal side of the endothelium and subsequent diapedesis across the BBB (18). Chemokines are involved in several of these steps: Induction and activation of leukocyte adhesion molecules that mediate firm adhesion to the endothelium, establishment of a chemotactic concentration gradient resulting in recruitment of the cell across the endothelial monolayer, and the induction of proteolytic enzymes involved in the breakdown of extracellular matrix proteins (19, 20). Migration of inflammatory cells within the CNS parenchyma is poorly understood but may be directed by chemotactic gradients created by chemokines that diffuses from sites of production at foci of inflammation (21).

It is hypothesized that chemokines and their receptors are involved in the pathogenesis of MS, and interventions targeted to the chemokine system might be feasible for modulating the course of the disease. This review summarizes research results about the expression and function of elements of the chemokine system in the pathogenesis of MS. Future directions and challenges will be outlined. Since many current ideas about MS pathophysiology stem from data obtained in various forms of experimental autoimmune encephalomyelitis (EAE) this will be discussed as well.

## 3. CHEMOKINES AND CHEMOKINE RECEPTORS

Chemokines are a group of small (8-14 kDa), structurally related molecules released by a variety of cell types. More than 40 chemokines have now been identified in humans. They have been associated with migration of lymphocytes, monocytes, eosinophils, basophils, and neutrophils under pathological and physiological conditions. Their role in the development and regulation of autoimmunity has become one of the foci of exponentially growing interest in recent years. Furthermore they have a wide range of effects on many different cell types beyond the immune system, including, for example, various cells of the CNS or endothelial cells, where they result in either angiogenic or angiostatic effects (22, 23).

Chemokines are subgrouped into two major and two additional chemokine subfamilies based on the position of their terminal cysteine residues and their main functional properties: CXC (also called the α family), CC (ß), C (lymphotactin) and CX3C (fractalkine) (24). In CXC chemokines the corresponding cysteine residues are separated by an additional amino acid. The corresponding genes in humans are clustered on chromosome 4. Structural differences in the CXC chemokines carry functional implications. The CXC chemokines are further divided by the presence of a specific amino-acid sequence, called the ELR-motif (glutamic acid-leucine-arginine) located near the N-terminus. These CXC chemokines are chemoattractant for neutrophils. The non-ELR-CXC chemokines are inert to neutrophils but are potent chemoattractants for activated T lymphocytes. Typical members are CXCL8 (old name IL-8) and CXCL10 (IP-10). In CC chemokines the first two cysteine residues are adjacent near the N-terminus. Most of the CC chemokines are clustered on chromosome 17 in humans, and are chemotactic for monocytes/macrophages, T lymphocytes, eosinophils and basophils. Typical members are CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1ß) and CCL5 (RANTES). C and CX3C chemokines predominantly stimulate migration of mononuclear inflammatory cells. Although chemoattractant specificities for subfamilies of leukocytes have been described, there are several exceptions for individual chemokines.

Chemokines function through specific interaction with chemokine receptors, which are members of the seven-transmembrane G protein-coupled receptor family that mediate a variety of leukocyte responses including chemotaxis and immune activation (25). The proposed chemokine nomenclature is based on the chemokine receptor nomenclature currently in use, which uses CXC, CC, XC, or CX3C followed by R (for receptor) and then a number. Corresponding to the chemokine families we have six CXC-receptors (CXCR1-6), 10 CC-receptors (CCR1-10), one C-receptor 1 (XCR1), and one CX3C-receptor (CX3CR1, the fractaline receptor). Some chemokine receptors can bind more than one ligand, but chemokine receptors usually do not cross subfamily barriers (25). A number of chemokine receptors are preferentially, but not exclusively, expressed on T cells in association with the Th1 and Th2 phenotype. CCR5 and CXCR3 have associated with the Th1 phenotype, while CCR3, CCR4, and CCR8 have been associated with the Th2 phenotype (26).

## 4. CHEMOKINES IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

Experimental allergic encephalomyelitis (EAE) is a T cell-mediated demyelinating disorder of the CNS, which serves as an animal model for MS to study the clinical, pathological, and immunological features of autoimmune demyelinating disease. Data obtained from this model helped to define the role of chemokines and served to generate hypotheses that guided MS research.

In the relapsing model of EAE upregulation of mRNA chemokine expression for CCL5 (RANTES), CCL4 (MIP-1ß), CCL3 (MIP-1α), CCL1 (TCA-3), CCL10 (IP-10) and CCL2 (MCP-1) was demonstrated one or two days before onset of clinical symptoms (27). These mRNAs were still expressed, albeit at lower levels, even during clinical remission of the disease. Administration of *in vivo*

anti MIP-1α and anti IP-10 treatment prevents the development of acute clinical onset and also ameliorates the severity of ongoing clinical disease, providing evidence that these chemokines play an important role in EAE (28, 29).

Chemokines were detected in the presence of inflammatory infiltrates and CNS expression of chemokine mRNA correlated with histological signs of inflammation (30, 31). Colocalization experiments have shown that MIP-1α and RANTES were expressed by infiltrating mononuclear cells, whereas IP-10 and MCP-1 were expressed by astrocytes (32). Inflammatory cytokines (IFNγ, TNFα and IL-1) produced by activated T cells and macrophages can stimulate cells to produce chemokines (33). IP-10 expression was not detected in gamma-interferon knockout mice after EAE induction, suggesting that cytokine release is a prerequisite for the expression of certain chemokines (34). Furthermore it was shown that infiltrating inflammatory leukocytes express chemokine receptors that are appropriate for binding the chemokines that are produced in the affected CNS tissue (35).

Elhofy *et al.* postulated that differential spatial and temporal chemokine production by specific cell types serve as an important regulatory mechanism in the pathogenesis of EAE by directing mononuclear cell infiltration and trafficking within the CNS (36): Expression of MIP-1α, MCP-1, and RANTES in the perivascular space focus the inflammatory infiltrate initially toward the perivascular area. Additional cytokine products of these inflammatory cells (IFNγ, TNFα and IL-1) stimulate nearby astrocytes at the glial limitans to express chemokines such as IP-10 and MCP-1 which would direct the migration of leukocytes toward the parenchyma. Macrophages migrate into parenchyma along gradients of MCP-1 and activated T cells invade parenchyma toward higher concentrations of IP-10. MCP-1 and IP-10 expression by activated astrocytes is postulated to be a later factor involved in the induction of further mononuclear cell infiltration including antigen specific T cells responsible for epitope spreading as well as monocytes/macrophages that are the end stage cells responsible for the demelination of axons and direct axonal transaction.

In addition to effects on leukocyte trafficking, chemokines may also play a role in T cell differentiation and regulating Th1/Th2 cytokine profiles. Cells incubated in the presence of MIP-1α showed enhanced IFNγ production (Th1 cytokine), whereas cells incubated in the presence of MCP-1 showed enhanced IL-4 (Th2 cytokine) production (37). While MIP-1α drove Th0 cells to differentiate to Th1, MCP-1 drove Th0 cells to differentiate to Th2 (38). Administration of anti-MIP-1α, but not of anti-MCP-1, prevents the development of both acute and relapsing EAE as well as infiltration of leukocytes into the CNS initiated by the adoptive transfer of autoantigen-specific T cells (28). Since Th1 cytokines promote, while Th2 cytokines reverse EAE and MS, the effect of chemokines on cytokine profiles may influence the clinical course of the diseases by several distinct, but related mechanisms.

## 5. EXAMINING CHEMOKINES AND CHEMOKINE RECEPTORS IN PATIENTS WITH MULTIPLE SCLEROSIS

### 5.1. Brain parenchyma

The first step of the MS lesion development in the brain parenchyma is the active transmigration of mononuclear cells across the blood-brain barrier (BBB). To invade the CNS parenchyma, these cells further cross the perivascular glia limitans, formed by astrocyte and microglial processes. Cerebral endothelial cells, which represent the major component of the BBB, produce RANTES, IP-10, MIG and MCP-1 (39). Immunohistochemical studies of autopsy brain sections containing active MS lesions have described CXCR3 expression on the majority of perivascular lymphocytes (40, 41, 42). These CXCR3 positive perivascular cell infiltrates are rarely observed in control brain specimens (43). It was suggested that the retention of CXCR3+ T cells in patients with MS is due to the presence of its ligand (IP-10) and CXCR3 cells, in the absence of ligand, recirculate (43). IP-10 positive cells were also detected in large numbers in active MS lesions and IP-10 was expressed by astrocytes in MS lesions (40,41, 42). These results suggested that interactions between CXCR3 and its ligand IP-10 might mediate trafficking and retention of T cells in active MS-lesions. A significant increase of CXCR3 cells were found when comparing early and late stages of MS lesions, suggesting a continous accumulation of CXCR3+ T cells in active MS lesions (43). It implies that the continous migration of CXCR3+ T cells into the CNS parenchyma may be targeted not only at early stages of lesion development. Furthermore, CXCR3-positive subpopulations of T cells from MS patients synthesized more IFN-γ than those from controls (41). This proinflammatory Th1 associated cytokine has been directly implicated as activating the inflammatory disease process based on the observation that when given to MS patients, systemic IFN-γ increases the frequency of clinical relapses (44). These results suggested that CXCR3 and IP-10 might be pertinent for the pathogenesis of acute relapses in relapsing remitting MS.

Concerning CCR5, immunohistochemistry of lesion material indicated that relatively few perivascular T cells were CCR5 positive but a large population of activated phagocytotic macrophages expressed CCR1/CCR5 (45). In this study, the expression of CCR1 and CCR5 on mononuclear phagocytes was examined in relation to demyelinating activity and spatial distribution in active MS lesions. Newly infiltrating CCR1+/CCR5+ hematogenous monocytes were found in perivascular cell cuffs and at the demyelinating edges of evolving lesions, which were not found in non-inflamed brain sections. During further activation in lesions, infiltrating monocytes downregulate CCR1 but not CCR5, whereas microglia upregulate CCR5. Trebst *et al.* proposed that CCR5+ monocytes are only retained in the CNS perivascular space in the presence of appropriate ligands (46). Such ligands include the ß-chemokines CCL3, CCL4, and CCL5, which were also detected within active lesions of MS patients: CCL3 and CCL4 expression was found on macrophages and microglia, and CCL5 expression was associated with perivascular inflammatory cells and, to a lesser extent, with astrocytes (47, 48).

**Table 1.** Chemokines and their related receptors, for which an association with multiple sclerosis has been described

| Chemokine Systematic name | Human Ligand (old name) | Cellular source | Chemokine Receptors |
|---|---|---|---|
| CCL2 | MCP-1. Monocyte chemoattractant protein1 | Cerebral endothelial cell, astrocyte, T cell | CCR2 |
| CCL3 | MIP-1α. Macrophage inflammatory protein-1 | Macrophage, T cell, microglia | CCR1, CCR5 |
| CCL4 | MIP-1ß. Macrophage inflammatory protein-2 | Macrophage, T cell, microglia | CCR5 |
| CCL5 | RANTES. Regulated upon activation normal, T cell expressed and secreted | T cell, macrophage, Cerebral endothelial cell, astrocyte | CCR1, CCR3, CCR5 |
| CCL7 | MCP-3. Monocyte chemoattractant protein-3 | Astrocyte | CCR1, CCR2, CCR3 |
| CCL8 | MCP-2. Monocyte chemoattractant protein-2 | Astrocyte | CCR3 |
| CXCL10 | IP-10. Gamma interferon-inducible protein | T cell, macrophage, Cerebral endothelial cell, astrocyte | CXCR3 |

In summary, it appears likely that the conceptual scheme of leukocyte trafficking into active MS lesions, that was derived from EAE animal model, may be applicable to the human disease MS. Further studies have to clarify the relationship between ligand expression and corresponding chemokine receptors and the correlation of these components with demyelination and axonal damage in lesions of patients with MS.

### 5.2. Blood and cerebrospinal fluid

We evaluated chemokine CXCL10 concentrations and its receptor CXCR3 expression on T-cells in the CSF and peripheral blood (PB) to investigate mechanisms of inflammatory MS pathogenesis during acute relapses *in vivo*, as measured by the occurrence of active MRI lesions (49). First we used flow cytometry to address whether patients with RR-MS show preferential expression of CXCR3 on circulating and CSF T-cells. In accordance with several other reports (40, 50, 51, 52) CXCR3-positive T-cells were enriched in the CSF compared with the PB and more than 75% of the T-cells in the CSF expressed CXCR3 receptor. Second we investigated CXCL10 levels by ELISA in the serum and CSF from patients with RR-MS. CXCL10 was found in up to 10 fold higher concentrations in CSF compared to serum in RR-MS patients. The BBB function was largely intact in more than 90% of all patients in this study as detected by a normal Qalb. Therefore, the elevated CSF concentrations reflect strong intrathecal CXCL10 release rather than diffusion from blood. The differential distribution of CXCR3-receptors on T-cells and CXCL10 concentrations in the 2 compartments may be mainly caused by trafficking of activated T-cells from the circulation into the CSF-compartment and may be also mediated by conditions within the CNS microenvironment. This is supported by the finding that even patients without inflammatory neurological diseases showed a differential distribution of CXCR3 expression and CXCL10 concentration in the 2 compartments (own unpublished data). Little is known about the association between chemokines and their receptors and MS disease activity. In our study expression of CXCR3 receptors on CSF T-cells was significantly higher in RR-MS patients undergoing acute attacks, as illustrated by Gd-enhancing lesions on MRI, compared to RR-MS patients without enhancing lesions (49). A possible explanation for the accumulation of CXCR3 positve T-cells in the CSF during acute MS-attacks could be the preferential migration of activated CXCR3 positive T cells from the circulation into the CNS. This is further supported by our observation of decreased numbers of CXCR3 positive circulating T-cells in RR-MS patients compared to healthy controls. In another study increased CXCL10 concentrations in the CSF were associated with relapses in MS (52). These results await further confirmation and extension in long-term longitudinal studies.

In contrast to CXCL10, it was shown that CCL2 levels are reduced in the CSF of MS patients compared with control patients with non-inflammatory neurological disease and CSF concentration of CCL2 significantly increased in RR-MS patients treated with methylprednisolone (53). Furthermore, we found that CSF and serum levels of CCL2 were reduced in RR-MS patients undergoing acute attacks, as illustrated by Gd-enhancing lesions on MRI, compared to RR-MS patients without enhancing lesions (54). It has been proposed that MS relapses are triggered by predominance of Th1 and depression of Th2 cytokines. In T cell cultures CCL2 drove Th0 cells to differentiate to Th2 cells, and CCL2 expression enhanced IL-4 production, the cardinal Th2 cytokine (38, 55). Therefore, the decreased CCL2 levels in CSF in our study might reflect increased Th1 activity during acute attacks.

Less information is available about the expression of chemokine receptors on monocytes in the blood and CSF of patients with MS. The majority of CNS monocytes expressed CCR1 and CCR5, while CCR1+/CCR5+ monocytes are a minority of monocytes in the peripheral blood (45). The presence of CCR1+/CCR5+ monocytes in the CSF was independent of CNS inflammation.

In summary, trafficking of mononuclear cells from the circulation into the CSF compartment appears to be a chemokine and chemokine receptor mediated process, as reflected by the differential distribution of soluble chemokines and chemokine receptors on cells in the blood and CSF compartment. Further ß-chemokines are differentially released in the CSF during acute attacks of RR-MS, which might reflect different immunregulatory roles in RR-MS (Table 1).

## 6. PERSPECTIVES AND THERAPEUTIC STRATEGIES

Based on the results from EAE and human MS, a hypothetical model for the role of chemokines is suggested

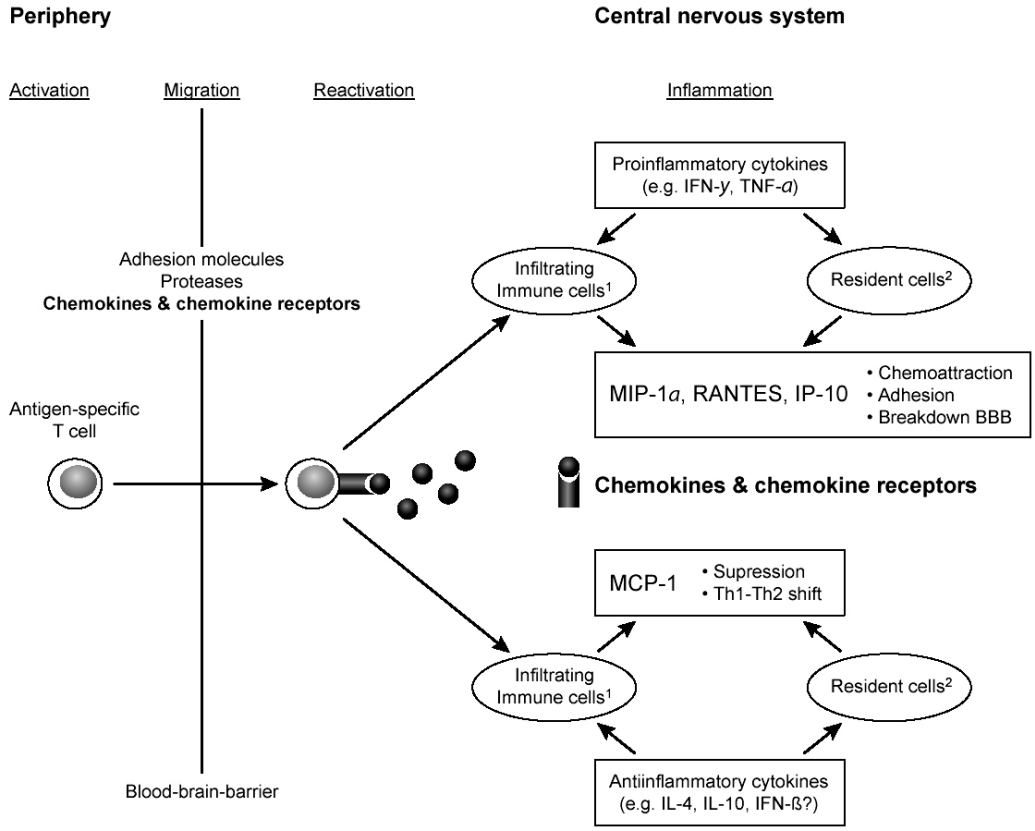


**Figure 1.** Proposed functions of chemokines and chemokine receptors in the pathogenesis of multiple sclerosis. Migration of antigen – specific T cells from the bloodstream into the central nervous system (CNS) is a chemokine- and chemokine receptor mediated process. In the presence of CNS inflammation infiltrating immune cells (T cells, macrophages) are retained and reactivated in the CNS. Immune cells and resident CNS cells (endothelial cells, astrocytes and microglia) release chemokines and other proinflammatory cytokines, which accelerate adhesion, chemoattraction and blood-brain-barrier (BBB) breakdown. This stimulates more immune cells to cross the BBB and to migrate into the CNS to sites of inflammation. Other chemokines like MCP-1 may exhibit immunosuppressive functions, controlling the chronic inflammation in MS-plaques.

(Figure 1). During active inflammatory demyelination in experimental animals and humans, there is an explosion of chemokine production by invading leukocytes, such as T lymphocytes and macrophages and by resident glia. These chemokines and their related receptors are actively involved in the pathogenesis of EAE and MS by their capacity to attract leukocytes into the CNS and by other immunoregulatory functions (e.g. maintaining the Th1/Th2 balance). Thus, both chemokines and chemokine receptors are potential targets for immune-based therapy for MS.

First, the development of small molecule antagonists to chemokine receptors may yield interesting results (20). For example, a novel non-peptide CCR1 antagonist that showed high affinity to the CCR1 receptor was identified, permitting efficient blocking of MIP-1α and RANTES. A CCR1 antagonist is being tested in a phase II clinical trial of MS. Second, vaccination with naked DNA encoding CC chemokines may be another interesting approach for therapeutic interventions in RR-MS. Vaccination with naked DNAs of MCP-1 and MIP-1α from CNS samples of EAE rats has been proved successful in preventing subsequently induced EAE (56). This suppression lasted even if disease was induced 2 months after administration of naked DNA vaccines. The mechanism of this approach could be the induction of significant humoral responses against target chemokines. Third, blocking chemokine activity by specific monoclonal antibodies, which has been shown successful in EAE (28, 29), may also be a useful therapeutic target in MS. But it remains unclear to what extent studies of target validation in animal models can be adapted to the human situation. A recent trial with TNF-α neutralization, which was active in EAE, failed to demonstrate clinical benefits in MS, but showed enhanced relapse frequency and increased gadolinium-enhancing lesions on MRI. This effect might have been related to the complex cytokine interaction, where cytokines and chemokines exert multiple functions depending on the stage of lesion development (57). At present, a clear pathogenic role for a particular chemokine or receptor in the pathogenesis of MS has not been shown. Before specific MS therapies become available for human use, we must learn more about the particular roles of each chemokine and its receptor by relating temporal and spatial expression to the multistep progress of MS pathogenesis. In addition, it needs to be carefully considered which

chemokine and chemokine receptor antagonists might be effective in different disease patterns or phases.

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