Multiple sclerosis is an autoimmune demyelinating disease of the human central nervous system with an unknown etiology. Crucial to its pathogenesis is the accumulation and activation of mononuclear cells in the central nervous system. Chemokines and their receptors are proposed to play a major role in the inflammatory recruitment of leukocytes. Besides analyses of relationships between chemokine or chemokine receptor gene polymorphisms and multiple sclerosis susceptibility and severity, analyses of chemokines and their receptors in patients with multiple sclerosis remain descriptive. In clinical material, chemokines and chemokine receptors can be examined in body fluids (blood and cerebrospinal fluid) and in brain tissues obtained via biopsy or autopsy. Research results will be summarized in this review, and a general model of leukocyte migration into the central nervous system under normal and inflammatory conditions will be proposed. Furthermore, opportunities and challenges for future investigations will be identified.

Multiple sclerosis (MS) is an autoimmune disorder of the human central nervous system (CNS) with an unknown etiology. Mononuclear cell accumulation and activation in the CNS are crucial steps in the pathogenic cascade, which frequently leads to irreversible injury to myelin and axons. Chemokines and their receptors have been associated with migration of lymphocytes, monocytes, eosinophils, basophils, and neutrophils under pathological and physiological conditions and have emerged as salient targets for investigation.1,2 Acting through specific high-affinity receptors on the surface of target leukocyte subsets, chemokines reversibly activate leukointegrins, consistent with a role in modulating leukocyte-endothelial interactions.4 In vitro and in vivo, chemokines also attract leukocytes to migrate along concentration gradients.5,6 Thus, chemokines are proposed to function critically to define the cellular composition of inflammatory infiltrates.7 Central nervous system inflammatory responses are also shaped by the unique anatomic character of the blood-brain barrier and, therefore, impose special challenges for leukocyte trafficking (and for investigators trying to elucidate these processes).

Besides analyses of relationships between chemokine and chemokine receptor gene polymorphisms and MS susceptibility and severity, analyses of chemokines and their receptors in patients with MS remain descriptive. In clinical material, chemokines can be investigated in body fluids (blood and cerebrospinal fluid [CSF]) of patients with MS and in brain tissues obtained via biopsy or autopsy. Chemokine receptors can also be examined on inflammatory cells in the blood, CSF, or parenchymal compartment (Figure 1). The allure of chemokine research is enhanced by the fact that the receptors are susceptible to selective blockade by small-molecule antagonists. This review will summarize research results to date. Future directions and challenges will also be outlined.

GENETIC STUDIES

Polymorphisms in genes for chemokines and chemokine receptors have been described. They could either lead, as in the

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case of the CCL3 gene, to a more potent chemokine ligand or, as in the case of the Δ32 mutation in the CCR5 gene, to a nonfunctional receptor, so that homozygotes for this mutation are “functional” knockouts for CCR5.

There are preliminary indications that chemokine or chemokine receptor gene polymorphisms might be related to either the susceptibility or the severity of MS. Considerable attention has been focused on the Δ32 CCR5 gene mutation. Individuals homozygous for the Δ32 CCR5 genotype were not protected from MS. However, individuals heterozygous for the Δ32 mutation experienced prolonged disease-free intervals, compared with individuals with a fully functional CCR5. In familial MS cases, Δ32-heterozygous individuals exhibited a mean 3-year delay in MS onset. Fitgen and colleagues reported that the risk of developing MS was decreased in individuals who possessed specific microsatellite polymorphisms in the CCR5 gene. Interestingly, the β-chemokine gene cluster was identified by Teuscher and colleagues as eae7, a locus that regulated susceptibility to experimental autoimmune encephalomyelitis in mice. Common polymorphisms in other genes, such as CCR2, CX3CR1, or CCL3, have been identified and associated with varied disorders, including the acquired immunodeficiency syndrome, hepatitis C virus infection, and atherosclerosis. Their relationship to MS susceptibility and severity awaits further investigation.

**STUDIES OF CHEMOKINES AND THEIR RECEPTORS IN BLOOD AND CSF OF PATIENTS WITH MS**

**Chemokine Receptor Expression on Circulating Cells**

Several investigators have used flow cytometry to address whether patients with MS show preferential expression of chemokine receptors on circulating cells. The expression of CCR5 on circulating T cells was found to be significantly increased in patients with MS compared with controls. In addition, CXCR3 expression on circulating T cells was significantly higher in patients with MS compared with controls in some, but not all, studies. One group reported a modestly increased migratory rate of T cells toward CCL3 and CCL5, arguing in favor of the functional significance of CCR5 on T cells.

Little is known about the association between numbers of chemokine receptor–bearing circulating cells and disease activity or disease course. Misu and coworkers compared the number of CCR5+ circulating CD4+ cells during relapse and 3 weeks later during remission in 6 patients with relapsing-remitting MS. CCR5 expression was increased during relapse, compared with control individuals. During remission, CCR5 values decreased, suggesting an association of CCR5+ T cells with disease activity. These results await further confirmation and extension in long-term longitudinal studies.

**Table 1. Chemokine Receptors Described in Patients With MS**

<table>
<thead>
<tr>
<th>Chemokine Receptor</th>
<th>Ligands</th>
<th>Description in Patients With MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR1</td>
<td>CCLs 3, 5, 7, 14, 15, 16, and 23</td>
<td>CSF: expressed on the majority of monocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lesion: expressed on newly infiltrating monocytes</td>
</tr>
<tr>
<td>CCR2</td>
<td>CCLs 2, 7, and 13</td>
<td>Lesion: expressed on macrophages</td>
</tr>
<tr>
<td>CCR3</td>
<td>CCLs 5, 7, 8, 11, 13, 15, 24, and 26</td>
<td>Lesion: expressed on macrophages</td>
</tr>
<tr>
<td>CCR5</td>
<td>CCLs 3, 4, and 5</td>
<td>Blood: elevated on circulating T cells in patients with MS compared with controls</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CSF: enriched on T cells, compared with blood, in patients with MS, and expressed by the majority of monocytes</td>
</tr>
<tr>
<td>CXCR3</td>
<td>CXCLs 9, 10, and 11</td>
<td>Blood: elevated on T cells in patients with MS compared with controls</td>
</tr>
</tbody>
</table>

*MS indicates multiple sclerosis; CCR, CC chemokine receptor; CCL, CC chemokine ligand; CSF, cerebrospinal fluid; CXCR, CXC chemokine receptor; and CXCL, CXC chemokine ligand.*
Of additional interest are the effects of standard treatments, such as interferon beta-1a or -1b and glatiramer acetate, on the expression of chemokine receptors. Zang and coworkers\textsuperscript{24} showed that in vitro exposure of T cells to interferon beta-1a selectively inhibited messenger RNA expression for CCL3, CCL5, and CCR5. T cell expression of CCR5 was significantly reduced in patients with MS who were treated with interferon beta-1a compared with untreated patients with MS. Furthermore, reduction of CCR5 surface expression was correlated with decreased T cell migration toward CCL3 and CCL5. However, in a recent study by Wandinger et al,\textsuperscript{25} using DNA microarrays, CCR5 gene expression was found to be up-regulated in peripheral blood mononuclear cells cultured for 24 hours in the presence of interferon beta-1b. In addition, peripheral blood mononuclear cells of patients with MS who were treated with interferon beta-1b for 6 months showed an up-regulation of CCR5 gene expression compared with baseline expression.\textsuperscript{25}

In summary, patients with MS exhibit a higher percentage of circulating CCR5\textsuperscript{*} cells than controls, and the number of CCR5-expressing cells in patients with MS is associated with disease activity. The effects of interferon beta-1a or -1b treatment on chemokine receptor expression are controversial and remain uncertain. All reported findings await confirmation in larger patient populations.

Little is known about the expression on circulating cells of chemokine receptors other than CCR5 and CXCR3. This informational lacuna is somewhat surprising, because CCR1 and CCR2 have emerged as crucial players in the experimental autoimmune encephalomyelitis model: gene-targeted mice (knockouts) for both CCR1 and CCR2 were shown to be protected from EAE.\textsuperscript{26-29} This informational lacuna is somewhat surprising, because CCR1 and CCR2 have emerged as crucial players in the experimental autoimmune encephalomyelitis model: gene-targeted mice (knockouts) for both CCR1 and CCR2 were shown to be protected from EAE.\textsuperscript{26-29}

### Chemokines in the CSF

There have been limited reports concerning chemokine levels in the CSF of patients with MS. Sørensen and coworkers\textsuperscript{20} reported similar observations and, in addition, showed that during remission, CCR5 expression, but not CXCR3, was significantly up-regulated in the CSF of patients with MS compared with control CSF. Interestingly, CCL2 was significantly decreased. Other investigators\textsuperscript{30-33} have independently confirmed these findings with regard to CXCL10 and CCL2 (Table 2). The sources of chemokines in the CSF remain to be further elucidated. In a study by Kivisakk and coworkers,\textsuperscript{32} the production of CCL2 and CCL5 by CSF mononuclear cells was similar in patients with MS and in other inflammatory controls, consistent with production of chemokines by CNS parenchymal cells during inflammation. It remains to be further clarified if altered CSF chemokine levels reflect MS disease activity or MS disease state. In addition, the effects of standard treatment and correlation with disease activity markers (such as magnetic resonance imaging) await further studies.

### Expression of Chemokine Receptors on CSF Mononuclear Cells

Investigations of chemokine receptor expression on mononuclear cells in the CSF compartment are of special interest for understanding cell trafficking into the CNS. Sørensen and colleagues\textsuperscript{32} addressed this question by comparing chemokine receptor expression on circulating and CSF cells using flow cytometry: more than 90% of the T cells in the CSF of patients with MS expressed CXCR3, whereas only about 40% of circulating T cells were CXCR3\textsuperscript{+}. However, controls without neurological disease exhibited identical high levels of CXCR3 expression on CSF T cells (T. L. Sørensen, MD, C.T., R.M.R., and F. Sellebjerg, MD, PhD, unpublished data, 2001). Therefore, it can be concluded that T cells capable of entering the CSF compartment express CXCR3, irrespective of the presence of inflammation in the CNS. CCR5-expressing T cells were also enriched in the CSF, but constituted only a minority of total CSF cells. In controls, a similar fraction of CSF T cells was CCR5\textsuperscript{+}, indicating that the presence of CCR5\textsuperscript{+} T cells in the CSF compartment was independent of CNS inflammation (T. L. Sørensen, MD, C.T., R.M.R., and F. Sellebjerg, MD, PhD, unpublished data, 2000). Misu and coworkers\textsuperscript{20} reported similar observations and, in addition, showed that during remission, CCR5 expression, but not CXCR3 expression, was reduced on CSF T cells compared with expression during relapse (Table 1).

### Table 2. Chemokines Described in Patients With MS\textsuperscript{*}

<table>
<thead>
<tr>
<th>Systematic Name</th>
<th>Old Name</th>
<th>Description in Patients With MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL2</td>
<td>MCP-1</td>
<td>CSF: decreased in patients with MS compared with controls\textsuperscript{12,115-117}</td>
</tr>
<tr>
<td>CCL3</td>
<td>MIP-1\textsuperscript{α}</td>
<td>Lesion: expressed by macrophages and microglial cells\textsuperscript{14,35,38}</td>
</tr>
<tr>
<td>CCL4</td>
<td>MIP-1\textsuperscript{β}</td>
<td>Lesion: expressed by inflammatory cells and activated neuroglial cells\textsuperscript{14,35,38}</td>
</tr>
<tr>
<td>CCL5</td>
<td>RANTES</td>
<td>CSF: elevated in patients with MS compared with controls\textsuperscript{15}</td>
</tr>
<tr>
<td>CCL7</td>
<td>MCP-2</td>
<td>Lesion: expressed by astrocytes and inflammatory cells\textsuperscript{14,35,37}</td>
</tr>
<tr>
<td>CCL8</td>
<td>MCP-1</td>
<td>Lesion: expressed by astrocytes and inflammatory cells\textsuperscript{14,35,37}</td>
</tr>
<tr>
<td>CXCL10</td>
<td>IP-10</td>
<td>CSF: elevated in patients with MS compared with controls\textsuperscript{15,33,31}</td>
</tr>
</tbody>
</table>

*MS indicates multiple sclerosis; CCL, CC chemokine ligand; MCP, monocyte chemoattractant protein; CSF, cerebrospinal fluid; MIP, macrophage inflammatory protein; RANTES, regulated upon activation, normal T cell expressed and secreted; CXCL, CXC chemokine ligand; and IP-10, interferon-inducible protein 10.
Conclusions from the previously discussed studies can only be drawn with some caution. There is little known about the effects of transmigration through an endothelial monolayer (or more specifically through a blood-brain barrier model) on chemokine receptor expression. If expression of CXCR3, for example, by T cells in the CSF compartment results from preferential accumulation of cells expressing this chemokine receptor, then this receptor may provide a therapeutic target. Alternatively, if CXCR3 up-regulation was caused by the process of trafficking into the CSF compartment (or mediated by conditions within the CSF microenvironment), the interpretation of the data would be quite different.

Less information is available about the expression of chemokine receptors on monocytes in the blood and CSF of patients with MS. In 6 patients with MS, Sellebjerg and coworkers found that the majority of CSF monocytes (76%) expressed CCR5, compared with only 4% in the circulation. These observations have subsequently been confirmed in a larger study including patients with MS, with optic neuritis, and without neurological disease. The majority of CSF monocytes expressed CCR1 and CCR5, while CCR1+/CCR5+ monocytes constituted a minority of monocytes in the circulating pool. 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 chemokine receptors on cells in the 2 compartments. It remains to be clarified if specific receptor-ligand pairs are used in pathological conditions such as MS.

STUDIES OF CHEMOKINES AND CHEMOKINE RECEPTORS IN THE BRAIN: TISSUE OF PATIENTS WITH MS

Mononuclear cells that have crossed the blood-brain barrier and, therefore, represent newly arrived hematogenous cells in the CNS are localized in the perivascular space of the CNS white matter. To invade into the CNS parenchyma, these cells cross the perivascular glia limitans, formed by astrocyte and microglial processes.

Immunohistochemical studies have described CXCR3 expression on the majority and CCR5 expression on a subset of perivascular lymphocytes in autopsy material from patients with MS (Table 1). These observations reflect findings of CXCR3 and CCR5 expression on CSF lymphocytes. These CXCR3+ and CCR5+ perivascular cell accumulations are rarely observed in control brain specimens (C.T. and R.M.R., unpublished data, 2001), despite the abundant presence of CXCR3 and CCR5 on lymphocytes in controls. It is, therefore, proposed that the retention of CXCR3+ T cells in patients with MS is due to the presence of appropriate ligands. CCL10, for example, is expressed by astrocytes in lesions of patients with MS. It is proposed that CNS-infiltrating CXCR3+ cells, in the absence of ligand, recirculate. Our hypothesis concerning the trafficking of mononuclear cells into the CNS is illustrated in Figure 2.

Chemokine receptor expression on mononuclear phagocytes in lesions of patients with MS has been described. These studies reported expression of CCR1, CCR5, and, to a lesser extent, CCR2 and CCR3 on mononuclear phagocytes (Table 1). In a recent study, the expression of CCR1 and CCR5 on mononuclear phagocytes was examined in relation to demyelinating activity and spatial distribution in active lesions of patients with MS. Newly infiltrating CCR1+CCR5+ hematogenous monocytes were identified in the perivascular space. Although enrichment of CCR1+CCR5+ monocytes in the CSF compartment was observed independent of CNS inflammation, CCR1+CCR5+ perivascular cell accumulations were not detected in noninflamed brain sections. We, therefore, propose that CCR1+/CCR5+ monocytes are only retained in the CNS perivascular space in the presence of appropriate ligands (Figure 2). Such ligands include the β-chemokines CCL3, CCL4, and CCL5. Several groups have also focused on the expression of these chemokines within lesions of patients with MS, with generally concordant findings: CCL3 and CCL4 expression is found on parenchymal inflammatory cells (macrophages and microglia), and CCL3 is found in addition on activated neuroglial cells. CCL5 expression is associated with perivascular inflammatory cells and, to a lesser extent, with astrocytes. In addition, expression of CCL2, CCL7, and CCL8 was described on astrocytes and inflammatory cells within lesions of patients with MS.

Conclusions from the previously discussed studies remain to be elucidated if this heterogeneity is also reflected in the differential expression of chemokines and chemokine receptors. A first hint in this direction is provided by a flow cytometry study by Wu and colleagues, examining the expression of CCR5 and other lymphocyte determinants on circulating T cells in Japanese patients with Asian (opticospinal) or Western variants of MS. Patients with the Asian variant of MS showed a significant increase of CCR5 expression on circulating T cells during relapse and remission, compared with controls, while patients with the Western variant of MS showed a significant increase only during relapse. Further studies are needed to confirm and extend these findings, which might have major impacts on the identification and treatment of patients with different MS subtypes.

OPPORTUNITIES AND CHALLENGES

Since the discovery of the chemokines and their receptors, considerable attention has been devoted to understanding their roles in leukocyte accumulation and activation during inflammatory processes of the human...
CNS. As summarized in this review, many contributions have identified potential ligand and receptor pairs (e.g., CXCL10 and CXCR3; CCL3 or CCL5 and CCR1; CCL3, CCL4, or CCL5 and CCR5; and CCL2 and CCR2) as critical elements in directing leukocyte subsets to the CNS in patients with MS. Further challenges for investigators in this field will lie in identifying the particular roles of each ligand/receptor pair by relating temporal and spatial expression to the multistep process of MS pathogenesis. Ultimately, this effort is directed toward identifying viable targets for therapeutic interventions. Of particular interest are small-molecule chemokine receptor antagonists, which are under development and in phase 1 clinical trials. Use of these new therapeutic strategies will not only open hopeful new dimensions to the treatment of MS but will also add to our understanding of this devastating disease.

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Figure 2. Leukocyte trafficking into the central nervous system (CNS): proposed functions of chemokines and their receptors. Cerebrospinal fluid (CSF) leukocytes, in reports presented to date, display a distinct profile of chemokine receptor expression that appears to be independent of the presence of neuroinflammatory disease. We suggest that leukocyte migration from the bloodstream into the CSF compartment is a chemokine- and chemokine receptor–mediated process that supports physiological leukocyte trafficking to the CNS. A. In the uninflamed situation, leukocytes, in the absence of appropriate ligand, recirculate either directly (as shown) or via lymphatics (not shown). B. In the presence of CNS inflammation, leukocytes are retained in the CSF compartment by virtue of the presence of appropriate ligands. Furthermore, chemokines produced under inflamed conditions in the brain parenchyma form a chemoattractant gradient to direct leukocyte trafficking from the CSF/perivascular space into the CNS parenchyma.

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