Cutting Edge Commentary: Chemokine Regulation of Experimental Autoimmune Encephalomyelitis: Temporal and Spatial Expression Patterns Govern Disease Pathogenesis

William J. Karpus and Richard M. Ransohoff

*J Immunol* 1998; 161:2667-2671; http://www.jimmunol.org/content/161/6/2667

**References**

This article cites 37 articles, 14 of which you can access for free at: [http://www.jimmunol.org/content/161/6/2667.full#ref-list-1](http://www.jimmunol.org/content/161/6/2667.full#ref-list-1)

**Why The JI?** Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: [http://jimmunol.org/subscription](http://jimmunol.org/subscription)

**Permissions**

Submit copyright permission requests at: [http://www.aai.org/About/Publications/JI/copyright.html](http://www.aai.org/About/Publications/JI/copyright.html)

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: [http://jimmunol.org/alerts](http://jimmunol.org/alerts)
Experimental autoimmune encephalomyelitis (EAE) is a CD4+ Th1-mediated demyelinating disease of the central nervous system that serves as a model for multiple sclerosis (MS). There are several considerations that suggest a role for chemokines in the disease process. First, chemokines are highly expressed in the central nervous system with a tight temporal relationship to disease activity. Second, in vivo neutralization studies showed a distinct role for specific chemokines in the evolution of the process. Third, the selective and differential expression of chemokines in differing models of EAE bears a close relationship to the patterns of inflammatory pathology. Fourth, the spatial distribution of chemokine expression could plausibly contribute to lesion architecture.

Finally, preliminary observations in MS material suggest that chemokine expression observed in EAE may provide useful information regarding the pathogenesis of inflammation in MS. We propose that temporal and spatial expression of chemokines are crucial factors, complementing adhesion molecule up-regulation, that regulate EAE disease activity. The Journal of Immunology, 1998, 161: 2667–2671.

Chemokines are low m.w. chemotactic peptides that can be divided into four distinct subfamilies: C-x-C, C-C, C, and C-x-C, based on the position of the first two cysteines in the amino terminus as well as functional and genetic considerations. The C-x-C family members containing an receptor-binding glutamate-leucine-arginine (ELR) motif are primarily chemotactic for neutrophils and for endothelial cells (8). C-x-C chemokines that lack this motif (“non-ELR C-x-C”) are chemoattractant for activated T cells and block angiogenic effects of the ELR-bearing family members. The C-C family members are primarily chemotactic for monocytes/macrophages, T lymphocytes, basophils, and eosinophils (9–11). The C family contains one member, lymphotactin, that is chemotactic for T cells and NK cells (12). The sole member of the C-x-C family (neurotactin/fractalkine) consists of a chemokine domain tethered to a mucin stalk; a soluble chemokine can be generated by proteolysis or alternative processing of the mRNA precursor (13). Although members of the chemokine subfamilies show considerable chemoattractant specificity for leukocyte subpopulations, there are many exceptions.

Studies of chemokines in EAE can be broadly separated into two categories: descriptive analysis of chemokine expression and interventional studies of chemokine function. Expression studies from numerous labs have demonstrated high levels of chemokine message and protein, with tight correlation to disease onset. Several characteristics of the disease model affect the observed chemokine expression...
patterns. In EAE induced by active immunization of SJL mice or Lewis rats, CNS expression of chemokines at onset includes a diversity of both C-X-C and C-C chemokines produced at time points that are inseparable from symptoms of neurologic disease (14–20). These findings suggest that the generation of chemokine expression in the CNS is due to localized stimulation through production of inflammatory cytokines by activated T cells and macrophages. It has not been shown that cytokines within the circulation can mediate intrathecal chemokine production. In SJL mice that are induced to develop EAE by adoptive transfer of encephalitogenic T cells, chemokine expression during the initial acute attack is characterized by more restricted array of cytokines that are likely to be elaborated in large part by transferred lymphocytes that accumulate in the CNS compartment (21, 22). The expression of chemokines during disease relapse at late time points after adoptive transfer is similar in mice that developed EAE after active immunization (17, 22). The chemokine expression patterns that have been described thus far following EAE induction in various animal models using various induction protocols are summarized in Table I.

Chemokine production by encephalitogenic T cells

Regardless of Ag specificity, activated T cells can enter the CNS perivascular space; however, only tissue Ag-specific T cells persist (4). In models of EAE induced by adoptive transfer of neuroantigen-specific T cells, the number of Ag-specific T cells in the CNS infiltrate has been reported to range from 1 to 12% (2, 23) (our unpublished data). These observations raise compelling questions concerning mechanisms for CNS accumulation of additional Ag-specific T cells, Ag-nonspecific T cells, and macrophages.

There are several plausible (and not mutually exclusive) mechanisms by which Ag-specific T cells might direct inflammatory cell recruitment to the CNS. The first is that neuroantigen-specific T cells enter the intrathecal perivascular space and recruit additional Ag-specific and Ag-nonspecific T cells and macrophages by secreting chemokines within the CNS target organ. Kuchroo et al. (24) demonstrated that encephalitogenic T cells up-regulated proinflammatory cytokines and chemokines, including MIP-1α, MIP-1β, and TCA-3, when restimulated in vitro. Godiska et al. (20) also showed that encephalitogenic T cells could up-regulate chemokines upon Ag stimulation.

A second, indirect mechanism for encephalitogenic T cells to induce leukocyte accumulation in the CNS would entail induction of chemokine expression by cellular elements associated with the cerebral vasculature and blood-brain barrier (BBB). These cells include astrocytes, perivascular macrophages, and pericytes as well as endothelial and smooth muscle cells. Cells of these varied lineage and differentiation groups can all secrete chemokines in vitro after appropriate inflammatory stimuli (25–27).

Chemokines in acute EAE

The first demonstration that chemokine expression was associated with acute EAE came from Hulkower et al. (28). Using a rat model of acute EAE in which animals develop a single disease episode followed by spontaneous remission, these workers demonstrated that MCP-1 mRNA was expressed in the CNS of rats with close temporal relation to symptom onset. Furthermore, they noted that when the animals entered remission, MCP-1 mRNA could no longer be detected. In both the active and adoptive SJL mouse models of EAE Godiska et al. (20) demonstrated CNS chemokine mRNA expression (MIP-1α, MIP-1β, RANTES, TCA-3, IP-10, MCP-1, and stromal cell-derived factor) before the onset of clinical disease and showed that levels remained elevated throughout the course of acute clinical disease.

The timing of CNS parenchymal chemokine expression (preceding or following the initial influx of encephalitogenic T cells) was addressed by sensitive chemokine mRNA detection. Chemokine expression was never detected in the absence of inflammatory infiltrates (15). Therefore, it appears that activated neuroantigen-specific T cells migrate to CNS perivascular sites and secrete chemokines that act to amplify the subsequent inflammatory process, including accumulation of mononuclear cells. It is reasonable to speculate that the encephalitogenic T cells secrete proinflammatory cytokines such as TNF-α and IFN-γ that have the capability to induce astrocytes to express RANTES, IP-10, MIP-1α, and MCP-1 (29), resulting in the recruitment of additional mononuclear cells.

We demonstrated the biologic importance of chemokine expression in the pathogenesis of acute EAE by in vivo Ab treatment experiments. CNS MIP-1α, but not MCP-1, protein expression was shown to correlate with increasing EAE severity and anti-MIP-1α, but not anti-MCP-1 nor anti-RANTES, treatment prevented acute clinical EAE (21) (our unpublished observations). Furthermore, in vivo anti-MIP-1α treatment reduced accumulation of mononuclear cells in the CNS (21). Our experiments demonstrated that MIP-1α is an important factor in the pathogenesis of acute EAE by virtue of its ability to induce CNS mononuclear cell accumulation.

### Table I. Summary of CNS chemokine expression patterns in EAE

<table>
<thead>
<tr>
<th>Strain</th>
<th>Antigen</th>
<th>Initial Attack</th>
<th>Remission</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJL</td>
<td>PLP139-151/CFA</td>
<td>+ + + + + + + +</td>
<td>+ + + + + + + +</td>
<td>20,37</td>
</tr>
<tr>
<td></td>
<td>PLP139-151 T cells</td>
<td>± + + + ND</td>
<td>± + ND</td>
<td>21,22</td>
</tr>
<tr>
<td>SW×J</td>
<td>PLP139-151/CFA</td>
<td>+ + + + + + + + +</td>
<td>+ + + + + + + +</td>
<td>14-16,18,19,38</td>
</tr>
<tr>
<td>BALB/GKO</td>
<td>MBP/CFA</td>
<td>+ + ND ND ND</td>
<td>ND ND ND</td>
<td>RMR; unpublished</td>
</tr>
<tr>
<td>PL/J</td>
<td>PLP43-64/CFA</td>
<td>+ + ND + + + ND</td>
<td>ND + + ND</td>
<td>Nonrelapsing disease model</td>
</tr>
<tr>
<td>Lewis</td>
<td>MBP/CFA</td>
<td>+ + ND ND ND</td>
<td>ND ND ND</td>
<td>Nonrelapsing disease model</td>
</tr>
<tr>
<td></td>
<td>MBP T cells</td>
<td>+ + + + + + + ND</td>
<td>ND ND ND</td>
<td>Nonrelapsing disease model</td>
</tr>
<tr>
<td></td>
<td>S100b T cells</td>
<td>+ + + + + ND</td>
<td>ND ND ND</td>
<td>Nonrelapsing disease model</td>
</tr>
</tbody>
</table>

*SW×J (SWR × SJL/J; BALB/GKO, IFN-γ−/− mice on BALB/c background.*

**MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein-1α; IP-10, IFN-γ inducible protein 10; MBP, myelin basic protein; S100b, astrocyte calcium binding protein.*

### References

2668 CUTTING EDGE COMMENTARY
Chemokines in relapsing EAE

The clinically relevant question arises as to what chemokines are produced in the CNS during the relapses of EAE. Does the CNS expression pattern remain the same or change with evolving disease? To begin to address this question, we analyzed the CNS of SJL × SWR/F₁ mice with chronic relapsing EAE for the expression of both C-x-C and C-C chemokines (14). There was a dramatic increase in MCP-1 mRNA and protein expression in the brain and an increase in MCP-1 mRNA expression in the spinal cord during the relapsing phase of disease. MIP-1α mRNA expression in the spinal cord remained elevated from the acute EAE episode through the relapsing phase of disease. Furthermore, MCP-1 expression was localized to astrocytes, whereas MIP-1α expression was localized to the perivascular mononuclear cell infiltrate. That MCP-1 expression plays a biologically relevant role in the relapsing EAE disease process was very recently demonstrated by the ability to ameliorate relapsing clinical disease with anti-MCP-1 treatment (our unpublished observations). Inhibition of relapsing EAE was associated with a decreased influx of macrophages.

Chemokine expression influences EAE lesion composition and architecture

We postulate 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 target tissue. These ideas are summarized in Figure 1. Figure 1A shows a cerebral microvessel, with intact BBB; the solid arrow indicates direction of flow. An activated T cell (solid circle) extravasates across BBB (dotted arrow) and undergoes restimulation (dotted arrow) by Ag (solid diamond) and APC (open oval) in the perivascular space. Reactivated T cells persist in CNS tissue compartment. Figure 1B shows an activated T cell/APC complex in the perivascular space producing inflammatory cytokines (arrow) that stimulates endothelial production of cellular adhesion molecules (hatched area of blood vessel) and degrade BBB function. Activated T cells and APC also express chemokines, including MIP-1α and RANTES. Figure 1C demonstrates that the simultaneous presence of chemoattractants and focal endothelial activation results in accumulation of mononuclear inflammatory cells within perivascular space. Expression of MIP-1α and RANTES in the perivascular space serves to focus the inflammatory infiltrate toward the perivascular rather than parenchymal area. However, additional cytokine products of these mononuclear cells (including IL-1, TNF-α, and IFN-γ) stimulate nearby astrocytes to express chemokines such as IP-10 and MCP-1. Figure 1D shows consequences of the tissue distribution of chemokines in EAE lesions. Once a significant inflammatory infiltrate has accumulated and activated astrocyte production of chemokines, macrophages begin to laminate at the outer border of the lesion and migrate into parenchyma along gradients of MCP-1 and related chemokines. Activated T cells invade parenchyma toward higher concentrations of IP-10. Many T cells remain in the perivascular space near higher levels of RANTES and MIP-1α. MCP-1 and IP-10 expression by activated astrocytes is a later factor involved in the induction of further mononuclear cell infiltration including Ag-specific T cells responsible for epitope spreading and episodes of relapsing disease as well as additional monocytes/macrophages. It is not unlikely that T cells and macrophages can respond sequentially to more than one chemotactic signal (30, 31). In our model T cells would first respond to MIP-1α and/or RANTES and later migrate into parenchyma in response to delayed MCP-1 expression.

Adoptive transfer studies in Lewis rats have been informative regarding differential chemokine expression in disease models, with distinct patterns of histologic inflammation. One model utilized T cells from myelin basic protein (MBP)-immunized rats that developed typical signs of EAE including highly inflammatory tissue injury in the CNS with prominent neurologic symptoms. The lesion architecture was characterized by a predominance of macrophages (90% of total cells invading the parenchyma) (32). Rats that received equal numbers of T cells from S100β-primed animals developed mild clinical experimental autoimmune panencephalitis (EAP) with exuberant perivascular inflammatory infiltrates that

FIGURE 1. Temporal and spatial distribution of chemokine expression in murine EAE influences CNS lesion composition and architecture. Symbols: dark circle, T lymphocyte; hatched circle, monocyte/macrophage; solid diamond, neuroantigen; open oval, perivascular APC, star, astrocyte; cross-hatching on vessel wall, focal region of endothelial cell activation including up-regulation of cellular adhesion molecules and disrupted BBB function.
were approximately 50% macrophages with very little infiltration of the parenchyma (33). CNS chemokine expression differs in the two models (17). MBP-specific T cells elicit CNS chemokine expression at very high levels, with predominant expression of the macrophage-directed products MCP-1 and MIP-1α. RANTES, which attracts both monocytes and T cells, is expressed at lower levels and with a delayed appearance. EAP rats demonstrated CNS levels of RANTES equivalent in amount and more persistent to those observed in rats with MBP-directed disease. However, CNS MCP-1 and MIP-1α levels in rats with EAP were approximately 10-fold reduced by comparison to the rats with EAE. The cellular localization of Ag was not a determining factor in the intensity and spatial and temporal expression patterns of chemokines during 14 C-labeled cell during acute, chronic, and relapsing experimental allergic encephalomyelitis. Lab. Invest. 58:167.


References


7. Krakowski, M. L., and T. Owens. 1997. Role of chemokines in the pathogenesis of MS has not been well established. An early study demonstrated elevated MIP-1α expression in the cerebrospinal fluid (CSF) of MS patients compared with control patients with other neurologic diseases and the increased levels correlated with increased CSF leukocyte counts (34). More recently, we examined the expression of chemokines in the CSF of MS patients showing new onset MS and clinically definite MS compared with control neurologic patients (our unpublished data). IP-10 and RANTES CSF levels were elevated in MS patients compared with controls, and the levels of IP-10 correlated with increased CSF leukocyte counts. Because both IP-10 and RANTES are potent T cell chemoattractants, it is reasonable to postulate that the elevated levels of these chemokines during active episodes of MS induce accumulation of T cells into the CNS. Recent findings in MS support the relevance of chemokine tissue distribution, as demonstrated in EAE (35). CNS expression of RANTES in MS brain was shown to predominate at the edge of active plaques in T cell-rich areas of the lesion (36). RANTES is a chemoattractant for both T cells and macrophages and could be a key pro-inflammatory factor in the pathogenesis of MS. MCP-1 was demonstrated in parenchymal astrocytes where its expression may be related to macrophage invasion of the CNS.

Conclusion

Chemokines are important inflammatory mediators involved in the regulation of autoimmune diseases. Discovering the sources and spatial and temporal expression patterns of chemokines during CNS autoimmune demyelinating disease opens up new potential targets for therapeutic intervention.

Acknowledgments

We thank the Williams Family Fund for Multiple Sclerosis Research and Dr. Marie Tani for helpful comments on the manuscript. We also thank Drs. N. Lukacs, S. Kunkel, and R. Strieter from the University of Michigan for their support.

References


