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CHEMOKINES AND CHEMOKINE RECEPTORS IN AUTOIMMUNE ENCEPHALOMYELITIS AS A MODEL FOR CENTRAL NERVOUS SYSTEM INFLAMMATORY DISEASE REGULATION

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1. ABSTRACT

This article focuses on the distinct role of chemokines and chemokine receptors during CNS inflammation in experimental autoimmune encephalomyelitis (EAE) as an animal model for multiple sclerosis (MS). We review the evidence that chemokines and chemokine receptors have an intrinsic role in regulating and amplifying the inflammatory reactions in EAE or MS leading to disease outcome. A variety of studies examining temporal chemokine expression patterns, using chemokine and chemokine receptor knockout mice as well as administering passive anti-chemokine antibodies indicates that these molecules are critical regulatory components for leukocyte recruitment and/or leukocyte retention in the CNS. Therefore, chemokine and chemokine receptor expression is tightly interrelated to composition of inflammatory cells in CNS lesions and the onset of clinical diseases and provide viable targets for therapeutic intervention.

2. INTRODUCTION

Multiple Sclerosis (MS) is chronic or relapsing neuroinflammatory disease of the central nervous system (CNS) characterized by local inflammation and demyelination in the CNS as a result of the infiltration of activated or memory T cells and macrophages across the blood-brain barrier (BBB) (1). Pathogenicity of both MS and EAE involves: 1) activation of myelin-reactive T cells, 2) upregulated expression of chemokines and adhesion molecules, 3) focal T cells and macrophage infiltration into white matter in CNS, 4) demyelination and axonal injury and loss of neurological function (2). In the CNS lesions of MS patients and EAE animals, infiltrating immune cells are mostly monocytes, $CD4^+$ and $CD8^+$ T cells, rarely B cells, neutrophils or eosinophils (3). While it is accepted that MS (4) and EAE (5) are both CNS inflammatory diseases where neuroantigen-specific T cells are responsible for the induction of the process, onset of disease has been correlated with the influx of nonspecific inflammatory cells into CNS (6). Furthermore, endogenous CNS microglia have also been hypothesized to be important effector cells in the process of CNS inflammation leading to clinical paralysis (7).

EAE can be induced in susceptible mice by immunization with immunodominant peptides from myelin proteins such as myelin basic protein (MBP), proteolipid protein (PLP), and myelin oligodendrocytes glycoprotein (MOG) emulsified in complete Freund's adjuvant followed by injection of pertussis toxin as an additional adjuvant for certain mouse strains (8,9,10,11,12). Disease development is variable from strain to strain. For instance in SJL/J mice, PLP or MBP induce a relapsing-remitting progression, whereas C57BL/6 mice are resistant to MBP disease induction but develop a chronic form of disease induced by MOG. The pathogenesis of EAE is generally believed to begin with the activation and differentiation of Th1 cells (13,14,15,16) that leave the lymph nodes and traffic into the CNS via the cerebrovascular endothelium (17,18). However, the necessity for IFN-γ production by T cells is still a controversial subject in the pathogenesis of EAE (19,20). EAE also can be induced by transferring antigen specific $CD4^+$ T cells into recipient animals (21). Adoptively-transferred, activated T cells migrate to the CNS within 24 hours (22,23) and only neuroantigen-specific T cells remain (24).

Access of leukocytes, including activated T cells and macrophages, to CNS occurs through the intact blood brain barrier (BBB) regardless of CNS antigen specificity, which is coordinated by a series of molecular interactions. In the case of T cells, the first of these events appears to be an early rolling event mediated by P selectin as antibody treatment inhibited T cell migration into CNS (25). Cell surface expression of VLA-4 ($\alpha4\beta1$ integrin) by T cells (26) is necessary for entry of neuroantigen-specific T cells into the CNS as anti-VLA-4 treatment can inhibit T cell binding to cerebrovascular endothelium and inhibit the

development of histological and clinical EAE (27,28). There is additional evidence indicating that CD44 expression by migrating T cells is necessary for entrance into the CNS (18). A role for the matrix metalloproteinases (MMP7, 8 and 9) (29,30) has been postulated in the extravasation of T cells across cerebrovascular endothelium into the CNS. The adhesion molecule PECAM has been shown to be a negative regulator of disease as inhibition (31) or deletion (32) of the molecule results in exacerbation of symptoms. Less is known about the molecular requirements for macrophage migration into the CNS, however, the LFA-1/ICAM-1 receptor/counter receptor molecules have been postulated to be involved (33). According to the predicted model of leukocyte migration (34), chemokines are another molecular regulator of T cell and macrophage migration to the CNS.

3. CNS CHEMOKINE EXPRESSION

Chemokines are small molecular weight chemotactic cytokines that can be classified into four sub-families (CXC, CC, C, and CX_3C) based on the position of the conserved amino-terminal cysteines (35,36). The CxC chemokines are further categorized based on the presence or absence of a glutamate-leucine-arginine (ELR) motif in the amino terminus. Those chemokines (ligands for CXCR1 and CXCR2) which possess the ELR motif are chemotactic for neutrophils and are angiogenic while the non-ELR CxC chemokines (ligands for CXCR3) are chemotactic for activated T cells and are angiostatic (37). The CC family of chemokines are chemoattractant for a wide variety of cells types including monocytes/macrophages, T lymphocytes, basophils, eosinophils, and dendritic cells (38,39,40). The C family, lymphotactin, is chemotactic for T cells and NK cells (41) while the Cx_3C chemokine contains a chemokine domain attached to a membrane bound mucin chain which produces a soluble chemoattractant after proteolysis or mRNA processing (42). This chemokine is a chemoattractant for T cells, NK cells, and neutrophils (35). Overall promiscuity of chemokines and chemokine receptors can allow for redundant functions *in vivo.*

There are numerous reports in the literature that have demonstrated an association between chemokine mRNA or protein expression and appearance of clinical EAE. Hulkower *et al* .(43) demonstrated a correlation between CCL2 expression and EAE in the Lewis rat model. Using semi-quantitative RT-PCR and *in situ* hybridization Ransohoff *et al.* (44) demonstrated that CXCL10 and CCL2 were expressed in the spinal cord of SJL mice with relapsing EAE. Additional studies of relapsing EAE demonstrated up-regulation of mRNA chemokine expression for CCL5, CCL4, CCL3, CCL1, CXCL10, CCL2, CXCL1, and CCL7 just prior to the first appearance of clinical symptoms in a mouse model of EAE and that the chemokine levels remained elevated throughout the course of the disease (45).

One question that arises with respect to the idea that chemokines regulate migration of neuroantigens-specific T cells and macrophages into the CNS is whether chemokines are expressed by parenchymal cells prior to inflammation as opposed to infiltrating leukocytes after inflammation. It is important to realize that the existing evidence suggests CNS inducible chemokine mRNA expression correlates with histological signs of inflammation and is not detected in the absence of leukocyte infiltration (46,47). In support of this idea are studies that demonstrate co-localization of CCL3 and CCL5 mRNA with infiltrating leukocytes, while CXCL10 and CCL2 mRNA expression co-localized with astrocytes (48). In addition to the association between CNS mRNA levels and tissue-specific inflammation, CNS chemokine protein levels have been associated with differential phases of relapsing disease. CCL3 and CXCL10 protein levels have been shown to be elevated in the CNS following adoptive transfer of activated neuroantigens-specific T cells (49,50) and correlate with acute disease development while CCL2 levels increase with the development of the relapsing phase of disease (51). Therefore, it reasonable to speculate that there is a differential distribution of mononuclear cells during the disease course, with T cells migrating in response to CCL3 during the acute phase of disease and macrophages migrating in response to CCL2 during the relapsing phase of disease.

More recently parenchymal cells of the CNS have been shown to constitutively express a subset of chemokines that may be responsible for the initial T cell and/or macrophage migration into the CNS according to the multi-step paradigm (34). Cerebrovascular endothelial cells have been shown to express CCL19 and CCL21 (52), astrocytes have been shown to constitutively express CXCL12 (53) and neurons have been shown to express CX3CL1 (54). These examples support the idea that leukocytes may gain entry to the CNS via a chemokine-mediated mechanism where they in turn can express chemokines themselves and induce further chemokine expression by parenchymal cells such as astrocytes.

4. LEUKOCYTE CHEMOKINE RECEPTOR EXPRESSION

It has been hypothesized that chemokine receptors corresponding to specific ligands are expressed by CNS-infiltrating T cells and macrophages and that the level of CNS inflammation correlated with specific chemokine receptor expression. T cells and macrophages that gain entry to the CNS have been shown to express a wide variety of chemokine receptors, however, selective CCR1, CCR5 and CXCR3 expression has been documented for T cells that migrated to the CNS compared to those that remained in the periphery (55). The idea of disease-inducing Th1 cells specifically upregulating CCR1 and CCR5 expression has recently been corroborated (56,57). While CCR2 is expressed on both T cells and macrophages (55), the primary functionality for this receptor is related to macrophage migration to the CNS (58).

Studies of chemokine receptor expression by leukocytes in EAE have yielded strikingly similar results to what has been found in MS patients. CXCR3 expression

by circulating T cells was augmented in relapsing-remitting MS compared to control subjects and both CCR5 and CXCR3 expression was increased on T cells in peripheral blood during progressive MS (59). In cerebrospinal fluid (CSF) from patients with active MS $CXCR3^+$ T cells were enriched compared to peripheral blood (60). Additionally, $CCR5^+$ lymphocytes and macrophages were detected in the CSF of patients with active MS more frequently than peripheral blood (60). In a separate study of MS patients both $CD4^+$ and $CD8^+$ T cells in CSF at relapse were enriched for CXCR3 and CCR5 expression, and were reduced for CCR3 and CCR4 expression compared with those of the blood (61). Furthermore, CCR1 and CCR2 expression by T cells was increased in CSF and blood (61). Therefore, it appears that selective chemokine receptor expression is found on leukocytes that infiltrate into the CNS during different stages of demyelinating disease.

5. CHEMOKINE AND CHEMOKINE RECEPTOR KNOCKOUT

Chemokine and chemokine receptor knockout mice have been utilized to ask questions about the roles of those molecules in the development of EAE. Use of the CCL2-deficient mouse on the C57Bl/6 background in the study of EAE pathogenesis has demonstrated a requirement for this chemokine in the migration of macrophages to the CNS in order to effect acute clinical disease (62). In contrast, the CCL3-deficient mouse on a mixed C57Bl/6 and 129 background developed clinical EAE in a similar fashion compared to control animals (63).

A number of studies using mice genetically deficient for chemokine receptors have shown that CCR1 (64) and CCR2 (58) expression are biologically important for the development of acute EAE. In the CCR1 knockout mice there was approximately a 50% decrease in clinical disease severity, however, the mechanism behind disease attenuation is not known. Since both T cells and monocytes have been shown to express CCR1 (65), it is possible that CCR1 expression by either lymphocytes or monocytes or perhaps both is required for EAE development. In the CCR2 knockout mice, there was almost a total absence of disease due to a failure of monocytes to traffic to the CNS (58). These two examples are in contrast to EAE induction in CCR5 knockout mice where the same level of disease severity was seen compared to wild type control animals (63). One advance that has come from both the chemokine and chemokine receptor studies in EAE is development of small molecular weight antagonists to chemokine receptors. Indeed, a small molecular weight antagonist of CCR1 has shown efficacy in the inhibition of clinical EAE (66,67).

6. ANTI-CHEMOKINE TREATMENT STRATEGIES

The biological importance of CNS chemokine expression in EAE has also been explored and demonstrated by *in vivo* anti-chemokine antibody treatments strategies. Experiments have been designed to test the role of chemokines in acute and relapsing disease by varying the timing of anti-chemokine treatment relative to disease induction (68). In the SJL mouse EAE model anti-CCL3 (49) and anti-CXCL10 (50) treatment prevented acute clinical disease while anti-CCL2 treatment was shown to prevent relapsing disease (51). In the C57Bl/6 mouse EAE model anti-CCL2 and anti-CXCL10 treatment prevented acute clinical disease (68). More recently, CCL22 was shown to be an important chemokine in the pathogenesis of EAE (69) by regulating the early T cell activation events rather than effecting neuroantigen-specific T cell or macrophage migration into the CNS. A significant finding from the *in vivo* neutralization studies is that while a wide variety of chemokines may be expressed during inflammatory autoimmune disease, only a subset of chemokines actually plays a significant biologic role in disease pathogenesis, dispelling the idea of redundancy and promoting the concept of specific regulation.

7. PERSPECTIVE

Chemokines and their receptors are a family of inflammatory mediators that are associated with many tissue-specific inflammatory events including CNS diseases. One current view of chemokines is to regulate the migration of leukocytes at a particular tissue site for the general function of infection clearance and tissue repair. However, aberrant accumulation of leukocytes, including antigen-specific T cells and macrophages, can induce pathology and result in tissue-specific inflammatory disease. Our best understanding of the role of chemokines in CNS disorders comes from the EAE model where the temporal and spatial chemokine expression patterns appear to regulate mononuclear cell accumulation and subsequent disease development (70). In the case of autoimmune-mediated disease or bystander inflammatory tissue destruction, it would be beneficial to limit the biological effect of chemokine expression in order to control self-tissue damage. To this end small molecular weight chemokine receptor antagonists have been developed and are being evaluated for efficacy in a variety of human inflammatory diseases (71).

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