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Review

Chemokine and chemokine receptor expression in the central nervous system

Joseph Hesselgesser*,[1] and Richard Horuk[1]

[1]*Department of Immunology, Berlex Biosciences, 15049 San Pablo Avenue, Richmond, California 94806, USA*

A decade ago several new cytokines were described that orchestrated the activation and migration of immune cells. These newly described cytokines, of which interleukin-8 (IL-8) was a representative member, defined a novel group of molecules called chemokines (chemotactic cytokines). Chemokines are low molecular weight, 8–12 kDa, basic proteins that have been classified into four distinct families, CXC, CC, C and CX_3C, based on the position of their first two conserved cysteine residues. The expression and biological function of chemokines along with their cognate receptors have been well described on various subsets of leukocytes. Only more recently have these molecules been described on various cells within the central nervous system. These pro-inflammatory proteins have been implicated in a variety of diseases within the central nervous system from Multiple Sclerosis to AIDS dementia. While chemokines are likely to enhance the evolution of central nervous system inflammatory disorders they also have other roles in normal brain function and development. This review summarizes the role of chemokines and their receptors in the normal and pathophysiological brain.

Keywords: chemokine; multiple sclerosis; Alzheimer's disease; AIDS dementia; HIV; neuron; astrocyte

Introduction

In contrast to the old style Westerns where the heroes always wore white hats and the villains always wore black, chemokines, which are small, soluble, basic proteins that help to orchestrate the host immune response, can wear hats of either color depending on the circumstances. Normally, chemokines play a beneficial role in host defense by attracting and activating leukocytes to destroy cellular invasion by pathogenic organisms. However, sometimes chemokines can inappropriately activate immune cells leading to inflammation and host cell destruction that can culminate in autoimmune diseases such as multiple sclerosis.

Chemokines were originally defined as host defense proteins although now they are known to have a variety of other functions, such as growth regulatory and angiogenic properties, that extend beyond the traditional role in regulating leukocyte migration (Baggiolini, 1998; Luster, 1998). In addition, chemokine receptors can play an important role in the pathogenesis of several diseases including malaria (Barnwell *et al*, 1987; Horuk *et al*, 1993) and HIV-1 (Luster, 1998).

Chemokines have been classified into four groups dependent on the number and spacing of the first two conserved cysteine residues CXC, CX_3C, CC and C. The CXC chemokines include interleukin-8 (IL-8), melanoma growth stimulatory activity (MGSA), interferon-inducible protein-10 (IP-10) and stromal derived factor-1 (SDF-1), while the CC class includes RANTES, monocyte chemotactic protein-1 (MCP-1) and the macrophage inflammatory proteins-1 (MIP-1α,β). Fractalkine, which is chemotactic for T cells and monocytes, is the only member of the CX_3C chemokine group while lymphotactin, which is chemotactic for lymphocytes, is the sole C chemokine representative so far identified.

Each of the chemokines recognizes and induces the chemotaxis of a particular subset of leukocytes. For example, CXC chemokines, like IL-8 and MGSA, preferentially attract neutrophils and induce their activation by producing changes in neutrophil shape, transient increases in cellular calcium concentration and the upregulation of surface adhesion proteins (Baggiolini and Clark,

*Correspondence: J Hesselgesser
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1992). Several members of this family such as SDF-1 and IP-10 are important molecules involved in chronic inflammatory processes that involve activated T lymphocytes. In general the CC chemokines have no effects on neutrophils but can chemoattract other leukocytes. Eotaxin for example is a potent eosinophil chemoattractant and induces their degranulation, while RANTES and MCP-1 are chemoattractants for T cells and monocytes respectively (Schall, 1994).

The chemokines produce their biologic effects by interacting with specific receptors on the cell surface of their target cells (Horuk, 1994). Chemokine receptors are characterized by a heptahelical structure and belong to a superfamily of seven transmembrane domain proteins that are coupled to guanine nucleotide binding proteins (G-proteins) (Dohlman *et al*, 1991). So far 15 different chemokine receptors have been cloned (Luster, 1998).

While chemokines play an important role in host defense it has become abundantly clear that their expression is not solely restricted to immune cells. For instance under pro-inflammatory conditions many cell types, including endothelial cells, smooth muscle cells, tumor cells, astrocytes, microglia, and neurons, can produce chemokines or induce chemokine receptor expression. Once chemokines are induced they form solid phase gradients by binding to extracellular matrix proteins like glycosaminoglycans that decorate the cell surface of endothelial cells. These gradients then attract immune cells which undergo selectin mediated rolling along endothelial cells followed by firm adherence to chemokine-induced CD11/18 complexes. This process finally results in the diapedesis of leukocytes across the endothelial space into the tissues (Adams and Shaw, 1994; Springer, 1994). In the tissue spaces immune cells help to clear away cellular debris and release enzymes to destroy pathogens and begin the reparative process. Sometimes, however, the immune system is inappropriately activated inducing tissue damage that results in the release of host self antigens that are normally not seen by the immune system. These proteins are now seen as 'foreign' by the immune system and this results in the recruitment of another wave of immune cells. This vicious cycle of chronic immune responses results in further tissue damage that is a hallmark of autoimmune diseases like multiple sclerosis.

Endothelial cell production of chemokines and receptor expression

The human brain occasionally comes under attack by pathogens. The first line of defense is the blood brain barrier which consists of nonreactive endothelial cells that form tight junctions with astrocytic foot processes. Once activated by pro-inflammatory cytokines both the endothelial layer of the CNS and adjacent astrocytes can release a number of chemokines. For example, stimulation of endothelial cell cultures with IL-1α, IL-1β, TNF-α or IL-4 upregulates the expression of MCP-1 and MCP-4 (Rollins and Pober, 1991; Li *et al*, 1993; Garcia-Zepeda *et al*, 1996). IL-1 also upregulates the CC chemokines MIP-1α and RANTES (Chuluyan *et al*, 1995) and the CXC chemokine MGSA (Murakami *et al*, 1995). RANTES production by endothelial cells can be inhibited by IL-4 and IL-13 (Marfaing-Koka *et al*, 1995). Both IFN-γ and IL-4 can synergize with TNF-α and IL-1 to induce MCP-4 mRNA accumulation (Garcia-Zepeda *et al*, 1996). In addition, the Th1 cytokine IFN-γ induces the expression of the CXC chemokines IP-10 and MIG (Piali *et al*, 1998) and regulates the production of RANTES by endothelial cells ion response to TNF-α (Marfaing-Koka *et al*, 1995). A very recently identified chemokine from the new CX_3C branch is also expressed on endothelium (Bazan *et al*, 1997). This new chemokine, fractalkine (Bazan *et al*, 1997) or neurotactin (Pan *et al*, 1997), is expressed as a membrane bound chemokine. This chemokine is unique in that it appears to be 'presented' to immune cells on the end of a mucin stalk, most probably to gain better access to the circulation. Under other inflammatory conditions such as those observed in the monkey model of HIV, SIV infected macaque brain endothelium can produce MIP-1α,β, MCP-3 and RANTES (Sasseville *et al*, 1996). Once immune cells enter the brain parachyma they can further secrete cytokines to stimulate resident CNS cells and contribute to the pathologies of encephalitis.

Endothelial cells have also been shown to express chemokine receptors, notably DARC (Hadley *et al*, 1994; Peiper *et al*, 1995; Horuk *et al*, 1997), CXCR4 (Lavi *et al*, 1997; Gupta *et al*, 1998; Volin *et al*, 1998), and CCR5 (Edinger *et al*, 1997b; Rottman *et al*, 1997). Endothelial cells treated with IFN-γ downregulate CXCR4 expression while IL-1β, TNF-α and LPS induce transient changes in CXCR4 expression (Gupta *et al*, 1998). While the *in vivo* significance of chemokine receptor expression on endothelium has not been clarified it seems clear that CCR5 and CXCR4 expression could contribute to the spread of HIV into the CNS.

Endothelial cell produced chemokines help to induce the migration of primary immune response cells such as T lymphocytes, monocytes and neutrophils across the blood brain barrier and into the brain. In fact it has been shown that $CD4^+$ resting memory T-cells can extravasate through endothelial monolayers (Brezinschek *et al*, 1995). However, in the absence of an appropriate stimuli the newly migrated immune cells do not become activated. This has been most clearly demonstrated in transgenic animals that are induced to over express certain chemokines. For example overexpression of

the mouse neutrophil chemoattractant KC (the mouse equivalent of MGSA) in the CNS, under the control of the MBP promoter, leads to an increased migration of neutrophils within the brain (Tani *et al*, 1996a). Interestingly, these animals had little evidence of tissue damage since no 'foreign' antigen was presented. However, after chronic expression of KC many mice developed neurological symptoms with postural instability and rigidity. This neurological manifestation occured at 40 days of age long after maximal chemokine expression. Histopathology showed signs of microglial activation and blood-brain barrier disruption. Therefore after prolonged exposure to high levels of chemokines there was a breakdown of the blood brain barrier and immune exposure to CNS specific antigens with subsequent activation of resident microglia.

Astrocyte cell production of chemokines and receptor expression

The second critical cell type in maintaining the blood brain barrier is the astrocyte. Like endothelium, astroyctes seem to express a wide array of chemokines under various conditions. Astrocytes and astrocytoma cell lines can produce IL-8 (Aloisi *et al*, 1992; Desbaillets *et al*, 1997), RANTES (Barnes *et al*, 1996) and MCP-1 (Barna *et al*, 1994) in response to IL-1 or TNF-α. In a murine hepatitis virus-induced demyelinating model, astrocytes can specifically produce CRG-2 (murine IP-10) a potent T-cell chemoattractant (Lane *et al*, 1998). A number of glioblastoma cell lines (Desbaillets *et al*, 1994; Takeshima *et al*, 1994) as well as human malignant glioma surgical specimens (Takeshima *et al*, 1994) can express MCP-1. MIP-1α and MIP-1β mRNA expression has been detected in astrocytes from the cerebral cortex of individuals with HIV encephalitis brains (Schmidtmayerova *et al*, 1996). While astrocytes express a number of chemokines under pro-inflammatory conditions they also secrete low levels of IL-8 under normal circumstances (Aloisi *et al*, 1992; Desbaillets *et al*, 1997).

There are a number of reports that demonstrate astrocyte expression of chemokine receptors. For example, murine astrocytes express CCR1 and migrate in response to MIP-1α (Tanabe *et al*, 1997a). The same cells express functional CXCR4 receptors and migrate in response to SDF-1 (Tanabe *et al*, 1997b). Human fetal astrocytes in cell culture express low amounts of CXCR4 that can be dramatically upregulated upon exposure to IL-1β (Hesselgesser unpublished data). Human fetal astrocytes also express low amounts of CXCR2 (Hesselgesser *et al*, 1997). The HIV-1 coreceptor CCR5 has recently been shown to be expressed on astrocytes in the hippocampus and cerebellum (Rottman *et al*, 1997). Although the *in vivo* significance of chemokine receptor expression on astrocytes remains to be clarified it is possible that they could also contribute to the pathogenesis of HIV in the CNS.

Neuronal expression of chemokine receptors: a possible role in CNS development?

A variety of evidence suggests that cytokines play an important role as immunomodulators of the CNS (Cunningham *et al*, 1993). The best studied of these is IL-1 which appears to play a critical role in the modulation of neurohormone and neurotransmitter release as well as in the induction of neuronal sprouting following CNS injury (Cunningham and De Souza, 1993). These trophic effects of the cytokines are clearly dependent on their duration of exposure and concentration. With increased exposure and at higher concentrations these proteins become neurotoxic. A recent report suggests that IL-8 is a potential trophic factor (Araujo and Cotman, 1993). Treatment of hippocampal neurons with 1 μg/ml dose of IL-8 increased their survival in culture by 200% over control neurons. Our findings that CXCR2 receptors are present in hippocampal neurons (Figure 1) (Horuk *et al*, 1997) are compatible with the trophic effects of IL-8 described above and suggest that the effects of IL-8 are most likely mediated by specific binding to CXCR2.

Recently, other chemokine receptors such as CXCR4 have been shown to be expressed in pyramidal neurons of the hippocampus and the dentate gyrus (Lavi *et al*, 1997). Variable amounts of CXCR4 staining was also present in amygdala, cingulate gyrus, thalamous, and dentate nucleus of

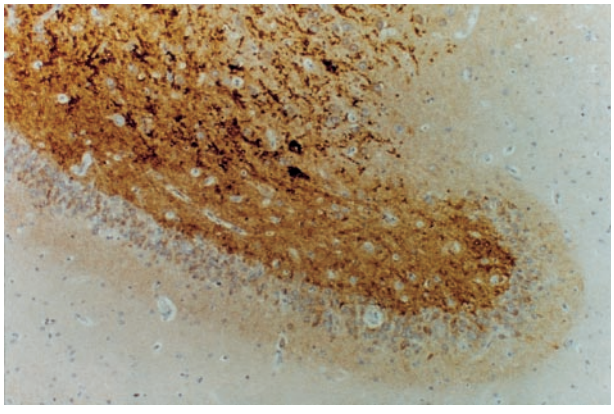


Figure 1 CXCR2 expression in the human hippocampus. Immunohistochemical detection of CXCR2 in dentate gyrus of human hippocampus. CXCR2 antibodies (Chuntharapai *et al*, 1994), mouse myeloma control antibodies, tissue preparation and immunohistochemistry were as previously described (Horuk *et al*, 1997). Intense staining of pyramidal neurons within the polymorphic layer. Granule cell bodies and their corresponding dendrites within the molecular layer are lightly positive for CXCR2 expression. (From Peiper *et al*, unpublished data).

the cerebellum (Lavi *et al*, 1997). Neurons in similar regions of the hippocampus and cerebellum are also positive for CCR5 expression (Rottman *et al*, 1997). The observations in the human brain have also been confirmed in nonhuman primates where certain populations of pyramidal neurons of the hippocampus, dentate gyrus and cerebral cortex are also positive for CXCR4, CCR3 and CCR5 in macaque brains (Westmoreland *et al*, 1998). A second report has also demonstrated CXCR4 and slight CCR3 staining of neurons in the brains of rhesus macaques but no CCR5 expression (Zhang *et al*, 1998).

In contrast to the studies described above, which were all carried out in adult CNS, there have now been several reports that suggest that chemokine receptors are present in fetal neurons suggesting a role in CNS development. Using standard neuronal culture techniques it was shown that neurons from E17–22 week fetal brains expressed CXCR2 but not CXCR1 or DARC (Hesselgesser *et al*, 1997). In addition hNT neurons, which are similar to immature post-mitotic cholinergic neurons that have numerous neuronal characteristics also express a number of chemokine receptors including CXCR2, CXCR4, CCR1 and CCR5 (Hesselgesser *et al*, 1997). These neuronal chemokine receptors bind their chemokine ligands with high affinity and based on chemotaxis assays are also biologically functional (Hesselgesser *et al*, 1997). Interestingly, data from several groups have shown that hNT cells can become established into the rat CNS and integrate to form neural networks with existing neurons further intimating that these cells are similar to immature human neurons (Kleppner *et al*, 1995; Borlongan *et al*, 1997).

Given that chemokine receptors are expressed on immature neurons and that chemokines are secreted by astrocytes it is tempting to speculate that these molecules might play a role in neuronal development. Considerable evidence to support this idea was recently provided from studies with CXCR4 knockout mice. Comparison of the brains from normal and CXCR4-deficient mice revealed abnormalities in the architecture of the cerebellum compared to normal mice (Ma *et al*, 1998; Zou *et al*, 1998). Neurons from the external granule layer (EGL) prematurely migrated toward the internal granule layer (IGL) and were positioned beneath the Purkinje cell layer or interspersed within the layer, a normally postnatal process (Ma *et al*, 1998; Zou *et al*, 1998). During normal development, neurons in the EGL migrate from the germinal matrix surface of the cerebellum through the molecular and Purkinje cell layers to form the IGL. The development of precisely formed neural networks, or neuronal patterning, is important for the proper function of the CNS ensuring neuronal cell to cell communication through a system of correctly formed synapses. In the CXCR4-deficient mice this orderly process is severely disrupted by the premature migration and abnormal positional clustering of neurons despite the presence of intact radial glia. Consistent with these studies SDF-1 gene knockout mice also showed a similar pattern of abnormal development of the cerebellum (Ma *et al*, 1998).

Thus, the work of Zou *et al* (1998) and Ma *et al* (1998) builds upon previous studies that have demonstrated neuronal expression of CXCR4 and suggests an important role for this receptor ligand pair in CNS development. What could this role be? Many possibilities suggest themselves. For example, there is compelling evidence for cross talk between chemoattractant receptors, i.e. signals from one receptor could induce the phosphorylation state of other receptors thus potentiating or inhibiting the normal function of other receptors. Further, CXCR4 could regulate EGL neuronal migration by inhibiting the ability of cells to respond to other chemoattractant signals. In fact, it has been previously shown that neurons migrate in response to chemokines (Hesselgesser *et al*, 1997; Bolin *et al*, 1998). Alternatively, recent studies have demonstrated that SDF-1 can induce apoptosis in a human neuronal cell line via CXCR4 (Hesselgesser *et al*, 1998). Thus, CXCR4 could aide in the apoptotic elimination of cells that have undergone incorrect migration in the CNS thus helping to ensure correct neuronal patterning.

Oligodendrocyte expression of chemokine receptors

In all previous studies looking at chemokine receptor expression of the CNS there has been no identification of specific staining of oligodendrocytes *in vitro* or *in vivo*. However, Robinson *et al* have recently reported that MGSA, and its rodent equivalent KC can, in conjunction with PDGF, act synergistically as growth factors for oligodendrocyte progenitor cells *in vitro* (Ransohoff, personal communication). Further, work from the same group has shown that the chemokine MGSA/KC is expressed in astrocytes within the spinal cord in a pattern similar to that of proliferating oligodendrocyte precursor cells. If these observations are confirmed by *in vivo* studies in the brain it would lend further credence to the idea that chemokines play important roles in the developing CNS.

Microglial expression of chemokines and chemokine receptors

It has been postulated that circulating HIV-infected immune cells transmit HIV into the CNS, presumably by interaction with and infection of microglia cells. Given that chemokine receptors are coreceptors for HIV and that microglia play an important

role in CNS inflammation these cells have come under close scrutiny in studies to determine the mechanism of HIV spread in the CNS. Messanger RNA for a number of chemokines including MIP-1α and MIP-1β have been detected in microglia from the cerebral cortex of individuals with HIV encephalitis (Schmidtmayerova *et al*, 1996). *In vitro* studies indicate that fetal brain microglial cultures stimulated with IL-1β, TNF-α, and LPS can produce MIP-1α, and MIP-1β (McManus *et al*, 1998). Activated microglia can also produce the CX_3C chemokine fractalkine/neurotactin in response to LPS (Pan *et al*, 1997). Microglia from SIV infected macaques can produce both CXC and CC chemokines including IP-10, MIP-1α,β, RANTES and MCP-3 (Sasseville *et al*, 1996).

In addition to chemokines migroglia from macaques also express a number of chemokine receptors including CXCR4, CCR3 and CCR5 (Westmoreland *et al*, 1998). Immunohistochemical staining of human brain sections has revealed that the T-tropic HIV coreceptor CXCR4 is expressed on microglia (Lavi *et al*, 1997; Vallat *et al*, 1998) and fetal microglia cultures (Vallat *et al*, 1998). The M-tropic HIV coreceptors CCR3 and CCR5 are also expressed on fetal microglia cultures (He *et al*, 1997). Interestingly a combination of antibodies and ligands to CCR3 and CCR5 have not been able to abrogate M-tropic HIV infectivity of these cells suggesting a possible role of other coreceptors (Ghorpade *et al*, 1998). Another candidate coreceptor CCR8 has been shown to be an HIV coreceptor *in vitro* (Horuk *et al*, 1998) and brain derived HIV isolates appear to use CCR8 efficiently for HIV entry (Jinno *et al*, 1998). However, the *in vivo* significance of these findings cannot be addressed until there is a direct demonstration of CCR8 expression in CNS cells and blockade of HIV entry by appropriate CCR8 receptor ligand(s). A recent study has demonstrated that molecular clones of envelopes from HIV-1 isolates of infected human brains could use several known coreceptors mostly CCR5 but also CCR3 and CXCR4 as entry molecules and also replicate readily in microglia (Shieh *et al*, 1998).

Why are chemokine receptors expressed on glial cells? What is their possible function? Glial cells carry out numerous functions in the CNS and these could potentially be aided by chemokines. For example glial cells act as a mechanical support system for nerve cells and during development can serve to guide migrating neurons to form correctly defined networks. Chemokines could aid this process by helping glial cells to form the scaffolding that guides migrating nerve cells and outgrowing axons to their appropriate positions during neuronal development. Glial cells also have a phagocytic function in the CNS helping to clear away debris that arises during neuronal degeneration. Chemokines could aid and abet this scavenger-like role of glial cells in much the same way that they direct tissue macrophages to clean up dead cells and kill invading pathogenic organisms.

Chemokines and Alzheimers disease

To determine whether chemokines could be involved in pathologic processes and/or reparative responses in the CNS, brain obtained from patients with neurodegenerative disorders has been analyzed for chemokine receptor expression. Immunohistochemical analysis of involved brain tissues from patients with Alzheimer's disease (AD) revealed high level expression of CXCR2 receptor in the neuritic portion of plaques, surrounding deposits of amyloid (Horuk *et al*, 1997). This immunoreactivity was detected in regions of the diseased brain which under normal circumstances showed very low level expression (Figure 2). These findings were later confirmed by another group also showing hippocampal neuronal expression of CXCR2 in AD plaques co-localizing β-amyloid (βA) ploypeptides (Xia *et al*, 1997).

It is interesting to note that βA peptides can potentiate the IL-1β-induced secretion of IL-8 by astrocytes (Gitter *et al*, 1995). These data further suggest that IL-8, via activation of CXCR2 receptors, could be a potential trophic factor for hippocampal neurons and suggest the possibility that chemokines may play a role in reparative processes and that their cognate receptors may be induced under these circumstances. It is possible that under certain pathophysiologic conditions, such as are observed in Alzheimers disease, the over production of IL-8 by astrocytes leads to the activation of CXCR2 receptors in the CNS that could contribute

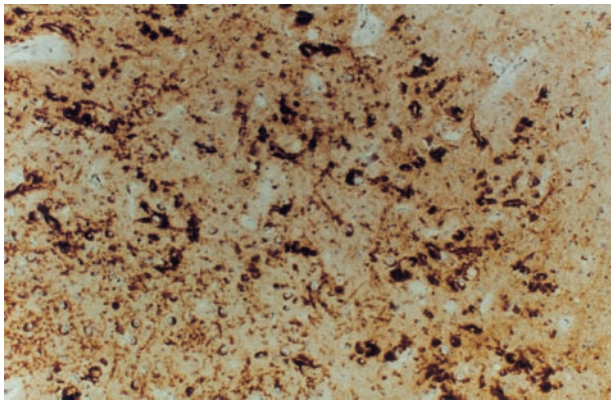


Figure 2 CXCR2 expression in Alzheimer's disease brain. CXCR2 antibodies (Chuntharapai *et al*, 1994), mouse myeloma control antibodies, tissue preparation and immunohistochemistry were as previously described (Horuk *et al*, 1997). Overexpression of CXCR2 within the hippocampus from AD brain section. (From Peiper *et al*, unpublished data).

to abnormal neuronal growth *in vivo*. While low levels of IL-8 may be necessary for cell to cell communication in the CNS, over production of this chemokine could lead to an abnormal physiology. But it has yet to be proven if CXCR2 expression in neuritic plaques of AD is a cause or a consequence of the disease. The study by Xia *et al* (1997) clearly stated that the number of CXCR2 positive plaques did not correlate to disease progression measured over several years.

Recently we found a marked increase in CCR1 expression in neurons of the entorhinal cortex and somatic staining of hippocampal neurons of the CA1 and CA3 region (Halks-Miller and Hesselgesser, unpublished data). However, we have not seen expression of CCR1 in cortical or hippocampal neurons of normal age matched brain tissue (Halks-Miller and Hesselgesser, unpublished data). CCR1 staining was also present in dystrophic processes around senile plaques. Confocal microscopy shows these processes to be neurofilament positive and CD68 negative (Halks-Miller and Hesselgesser, unpublished data). These findings suggest that CCR1 is upregulated in affected neurons in individuals with Alzheimers disease, perhaps in response to the disease process.

Chemokines and Multiple Sclerosis

Multiple sclerosis is an autoimmune disease mediated by T and B lymphocytes, and macrophages, which results in extensive inflammation and demyelination of the white matter (Ebers, 1986). Although the mechanisms responsible for causing this immunologic damage in the CNS are still unknown they are almost certainly mediated by infiltrating leukocytes. Initial interactions between invading T cells and monocytes in the CNS results in the production of cytokines such as TNF and IL-1. These cytokines induce a variety of effects including the upregulation of class II major histocompatability complex (MHC), cell adhesion glycoproteins, and the release of pro-inflammatory molecules by macrophages and activated microglia (Merrill, 1987). Although increased cell activation and upregulation of cell adhesion molecules are critical events in the pathogenesis of multiple sclerosis, recruitment of additional activated T cells and macrophages is also a significant feature of the disease. It is likely that a chemotactic gradient of immobilized chemokines, possibly bound to sulfated glycans (Strieter *et al*, 1989) on the subendothelial matrix (Huber *et al*, 1991), guides the directed flow of these blood leukocytes across the endothelium into the CNS.

A variety of evidence implicates chemokines in multiple sclerosis. For instance in an experimental allergic encephalitis (EAE) model of multiple sclerosis in the mouse mRNA levels for a number of chemokines including, KC, IP-10, MIP-1α, RANTES, MARC (murine MCP-3) and TCA-3 (murine I-309) are upregulated in spinal cord during the course of disease (Godiska *et al*, 1995). Chemokine transcript levels are induced several days prior to the onset of clinical disease and sustained throughout disease progression (Godiska *et al*, 1995). Co-localization studies demonstrated that MIP-1α and RANTES were produced exclusively by infiltrating leukocytes (Glabinski *et al*, 1997; Miyagishi *et al*, 1997) and that parenchymal neuroepithelial cells produced JE, IP-10 and KC, that co-localized with GFAP positive astrocytes (Glabinski *et al*, 1997). There is also data demonstrating that MCP-1 is upregulated in a rat EAE model (Hulkower *et al*, 1993) and that it is expressed in astrocytes during the acute phase of murine EAE (Ransohoff *et al*, 1993; Tani *et al*, 1996b).

The CX_3C chemokine, fractalkine/neurotactin is upregulated in activated microglia of murine EAE (Pan *et al*, 1997). The receptor for fractalkine/neurotactin, CX_3CR1, is expressed in NK cells, and monocytes and is upregulated during IL-2 expansion of $CD4^+$ and $CD8^+$ T lymphocytes (Imai *et al*, 1997). Expression of the chemokine TCA-3 (murine I-309) has been shown to correlate with the ability of proteolipid protein specific T-cell clones to mediate EAE (Kuchroo *et al*, 1993). In a recent study Karpus and Kennedy (1997) demonstrated that the chemokines MIP-1α and MCP-1 were differentially expressed depending on the severity or progress of disease. In this study MIP-1α levels correlated with acute onset of disease and MCP-1 production correlated with relapsing phases of the disease. Furthermore, antibodies to MIP-1α but not MCP-1 were able to inhibit development of acute disease but not relapsing EAE. While antibodies to MCP-1 did not inhibit the onset and acute phases of disease they were able to diminish the severity of relapsing disease. Studies from another group have also demonstrated that the chemokines JE (murine MCP-1), RANTES, MIP-1α, IP-10 and KC are also upregulated in the spinal cord and brain during the acute stages and chronic relapse of murine EAE (Glabinski *et al*, 1997).

While the role of each specific chemokine in human MS, is uncertain it is likely that these proteins probably work in concert to recruit immune cells into the CNS. The upregulation of MIP-1α, RANTES and MARC (murine MCP-3), the ligands for CCR1 (Neote *et al*, 1993; Combadiere *et al*, 1995) and CRG-2 (murine IP-10) (Lane *et al*, 1998) a ligand for CXCR3, probably serve to recruit T-cells and activate tissue macrophages that are critical to disease progression and development of pathology in the demyelinating mouse models.

Monocytes are likely to be attracted to MS lesions by MCP-1 and I-309, or in EAE by TCA-3 (murine I-309) and JE (murine MCP-1). I-309/TCA-3 is a specific ligand for CCR8 which is expressed on

monocytes (Roos *et al*, 1997; Tiffany *et al*, 1997). Recent *in vitro* studies show that TCA-3 can rescue thymic T-cells from apoptosis in response to dexamethasone (Van Snick *et al*, 1996). It is possible that overexpression of TCA-3, which is normally present at very low levels, in EAE (or MS) could protect T-cells normally undergoing apoptosis in the thymus. This would allow naive T-cells to become memory T-cells and contribute to CNS antigen spread as seen in MS.

During the onset of MS, specific T lymphocyte subsets and monocytes are recruited into the CNS by chemokines such as RANTES, IP-10 and MCP-1 which are produced by activated endothelial cells and astrocytes (Figure 3). A number of chemokines including MIP-1α, β and IP-10 are produced by $CD8^+$ T lymphocytes from MS patients that recognize proteolipid protein derived peptides presented by HLA class I molecules (Biddison *et al*, 1997; Honma *et al*, 1997). IP-10 is a potent chemoattractant for activated $CD4^+$ T lymphocytes and induces their migration and enhanced adhesion to the endothelium. This begins the cycle of chronic recruitment of myelin-specific T cells (Biddison *et al*, 1998) which enhances the activation of B lymphocytes to produce immunoglobulins that enhance the destruction of the blood brain barrier.

Monocytes, in response to MCP-1, also cross the blood brain barrier where they can be induced to differentiate into macrophages by certain cytokines. These tissue macrophages and resident microglia can become activated by MIP-1α to produce TNF-α and be induced to destroy and phagositize mature myelin by specific myelin antibodies. The local release of pro-inflammatory cytokines such as TNF from macrophages further accelerates the process of astrocyte and endothelial cell production of chemokines and adhesion molecules and contributes to the vicious cycle of chronic inflammation.

On the positive side it is also possible that chemokines could play a role in the remyelination or reparative processes of MS. For example MIP-1α could serve a beneficial function in recruiting monocytes and macrophages to clear myelin debris and thus allow oligodendrocyte progenitors to expand and differentiate. Since KC is also upregulated in EAE this chemokine may be working as a reparative factor to regenerate damaged neuronal processes and enhance oligodendrocyte proliferation and remyelination, perhaps via CXCR2.

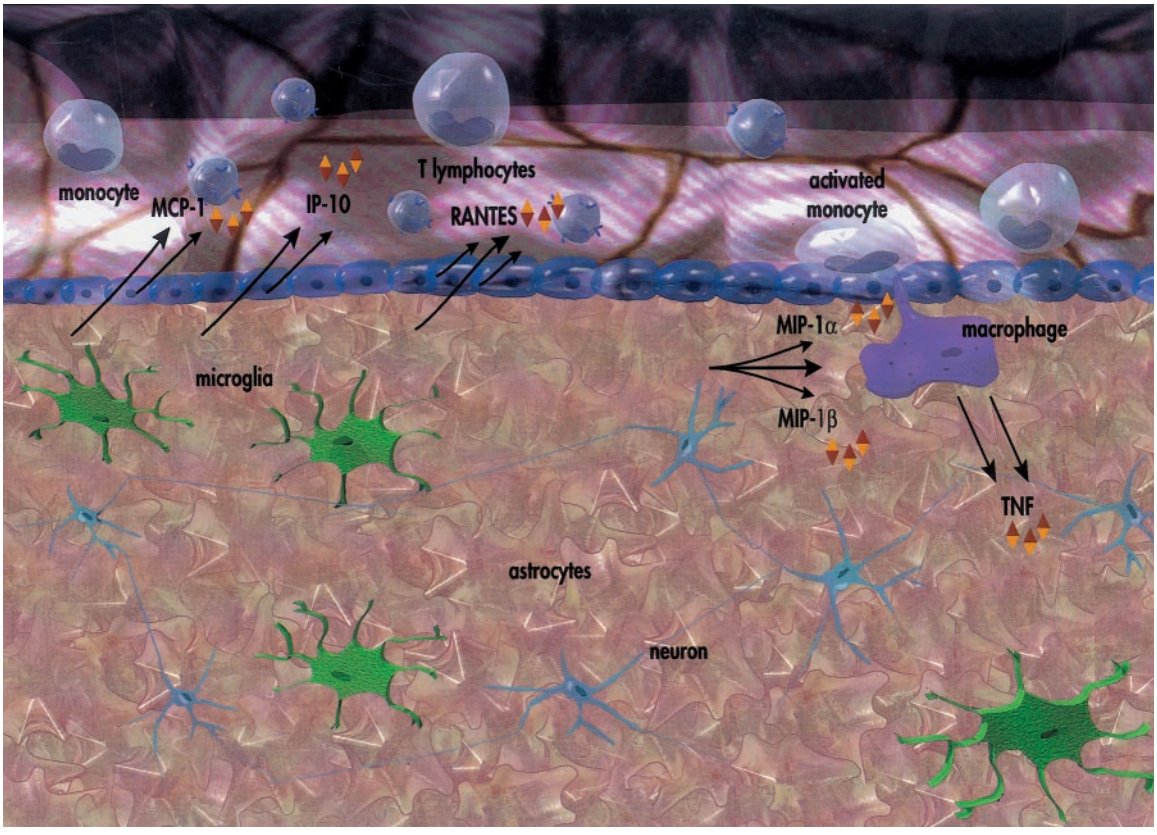


Figure 3 Schematic representation of chemokine action in MS brain. Several chemokines including IP-10 and RANTES are produced by astrocytes and activated endothelium to recruit T lymphocytes from the peripheral circulation to perivascular regions within the brain. MCP-1 secretion by endothelium and cytokine stimulated astrocytes chemoattract and activate monocytes from circulation. Monocytes eventually extravasate and mature to macrophages which are then stimulated by MIP-1α and MIP-β, produced by surrounding astrocytes, to secrete pro-inflammatory cytokines such as TNF and maintain the cycle of chronic inflammation.

Chemokine receptors in the CNS and HIV-1

Given the critical role of chemokines in host defense it is not surprising that chemokine receptors have themselves become portals of entry for pathogenic organisms. The human immunodeficiency virus HIV-1 which has been shown to require chemokine receptors as coreceptors for infection (D'Souza and Harden, 1996).

CD4 was initially identified as an entry factor for HIV-1. However, the fact that a small number of human cells and the vast majority of non human cells resisted infection by the virus (Ashorn *et al*, 1990; Dragic *et al*, 1992) raised the possibility that other accessory molecules were involved in HIV-1 infection and the search was on to find these elusive cofactors. The first clue to their identity was provided by Cocchi *et al* (1995) who showed that the chemokines MIP-1α, MIP-1β and RANTES could block viral infectivity *in vitro*. However, these chemokines were only able to block viral infectivity mediated by macrophage-tropic (M-tropic) viruses and were without effect on T-cell line-tropic (T-tropic) strains of virus. Shortly after these studies Feng *et al* (1996) demonstrated that a chemokine receptor now known as CXCR4, the SDF-1 receptor, was able to act as a cofactor for viral fusion of T-tropic but not M-tropic strains of HIV-1. Later, several reports flooded the literature with the discovery of a chemokine receptor, CCR5, specific for the chemokines MIP-1α, MIP-1β and RANTES (Samson *et al*, 1996) completed the circle and a number of investigators were able to simultaneously demonstrate that CCR5 was an entry cofactor for M-tropic isolates of HIV-1 (Alkhatib *et al*, 1996; Choe *et al*, 1996; Deng *et al*, 1996; Doranz *et al*, 1996; Dragic *et al*, 1996). Now several chemokine receptors have been described for infectivity by HIV-1 (He *et al*, 1997; Rucker *et al*, 1997; Horuk *et al*, 1998; Jinno *et al*, 1998) and the related viruses HIV-2 (Endres *et al*, 1996; Reeves *et al*, 1997), SIV (simian) (Deng *et al*, 1997; Edinger *et al*, 1997a, b; Farzan *et al*, 1997; Marcon *et al*, 1997) and FIV (feline) (Willett *et al*, 1997a, b; Hosie *et al*, 1998).

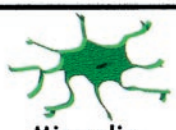
Circulating monocytes and T lymphocytes along with resident brain macrophages and microglia are believed to be one of the major routes of viral spread into the CNS and contribute to the pathologies seen in pediatric AIDS or AIDS dementia. Endothelial cells and microglia are the first line of defense for the CNS. As these cells become infected by HIV-1 from the circulation they contribute to further CNS expansion. Once virus replication has begun within the CNS, viral spread can occur by infection of reactive or fibrillary astrocytes and in restricted cases neurons. It is now accepted that primary isolates of HIV-1 are dual tropic in nature, i.e. they can infect cells via CCR5 mostly on macrophages (M-tropic) or non-syncytia-inducing (NSI) and CXCR4 on T-lymphocytes (T-tropic) or syncytia-inducing (SI) (D'Souza and Harden, 1996). However, as the number of CCR5 expressing cells in the body becomes low the virus tends to use CXCR4 (Connor *et al*, 1997; Scarlatti *et al*, 1997).

It has been recently reported that SI strains of HIV-1 will, slowly over time, up-regulate MIP-1α and RANTES (two of the ligands for CCR5) thus contributing to the growth of SI strains that use CXCR4 and induce a switch in viral tropism that is seen in the later stages of AIDS (Glushakova *et al*, 1997; Scarlatti *et al*, 1997). Some T-tropic strains of HIV-1 and HIV-2 have also evolved to use CXCR4 in the absence of CD4 (Hirka *et al*, 1991; Endres *et al*, 1996) and could infect cells such as vascular endothelial cells (Lavi *et al*, 1997; Moses *et al*, 1997), astrocytes and neurons (Hirka *et al*, 1991). Neurovirulent strains of SIV are also able to infect brain capillary endothelial cells via CCR5, but in the absence of CD4 (Edinger *et al*, 1997b).

Activated endothelial cells and astrocytes can produce a number of chemokines that can attract infected monocytes or T lymphocytes from circulation. These infected immune cells are able to cross the blood brain barrier in response to the chemotactic gradient and enter the perivascular regions of the brain. Once in the brain the HIV infected cells encounter resident microglia/macrophages. These resident immune cells express a number of chemokine receptors; CXCR4, CCR3, CCR5 (He *et al*, 1997; Lavi *et al*, 1997; Rottman *et al*, 1997). At this point the virus is able to use a number of chemokine receptors expressed on microglia. In most HIV encephalitis cases there is a restricted infectivity of endothelial cells, astrocytes and neurons (reviewed in Kolson and Pomerantz, 1996). Additionally granular staining of CXCR4 is seen in multinucleated giant cells of HIV infected brains (Lavi *et al*, 1997) a hallmark of ADC. It seems clear that the major source of continued viral replication within the CNS is from invading immune cells and resident microglia. However, other contributing factors to the pathology of ADC are due to disruptions in normal CNS functions of astroyctes and neurons. It is not necessary for these non-immune cell types to be directly infected by HIV; however viral proteins such as envelope, gp120, can induce abnormal cellular functions.

Neuronal apoptosis is a feature of HIV-1 infection within the brain (Adle-Biassette *et al*, 1995; Gelbard *et al*, 1995) although the exact mechanism(s) are not clearly understood. It is well documented that gp120 can cause abnormal functions of astrocytes inducing the release of a number of cellular metabolites and neurotoxic factors (Lipton *et al*, 1991; Meucci and Miller, 1996 and reviewed in Kolson and Pomerantz, 1996). Soluble gp120 can also be toxic to primary neurons *in vitro* (Bagetta *et al*, 1995; Aggoun-Zouaoui *et al*, 1996) and cause

Table 1 Summary of chemokine and chemokine receptor expression by cells of the central nervous system and expression in various disease states.

CNS Cell Type	Chemokine Production	Chemokine Receptor Expression	Disease Expression	Cytokine Regulation
 Endothelium	IL-8, MGSA IP-10, MIG, MCP-1,3,4 RANTES, MIP-1α Fractalkine	DARC CXCR4 CCR5	LPS Hypoxia MS (EAE) HIV (SIV)	IL-1β TNFα IFNγ IL-4 IL-13
 Astrocyte	IL-8 IP-10 MIP-1α,β RANTES MCP-1	CXCR2 CXCR4 CCR1 CCR5	MS (EAE) HIV AD HIV (SIV)	IL-1β TNFα
 Microglia	IP-10 RANTES MCP-3 MIP-1α,β Fractalkine	CXCR4 CCR3 CCR5	LPS MS (EAE) HIV (SIV)	IL-1β TNFα
 Neuron	???	DARC CXCR2 CXCR4 CCR1 CCR5	HIV AD	???
 Oligodendrocyte	???	CXCR2 (?)	MS (EAE) (?)	???

neuronal dysfunction and abnormal neuronal changes *in vivo* (Corboy *et al*, 1992; Toggas *et al*, 1994; Berrada *et al*, 1995). It has also been demonstrated that direct interactions between gp120 and neurons induces apoptosis even in the absence of macrophages/microglia (Bagetta *et al*, 1995; Aggoun-Zouaoui *et al*, 1996). A possible mechanism for this could involve gp120 binding to, and activation of, neuronal chemokine coreceptors.

Human neurons express the coreceptors CCR5 and CXCR4 (Lavi *et al*, 1997; Rottman *et al*, 1997). The neuronal cell line hNT also expresses functional CCR5 and CXCR4 receptors (Hesselgesser *et al*, 1997). Neurons are CD4 negative but can be infected by HIV in a very restricted manner, however, this does not readily lead to productive viral replication. T-tropic isolates of HIV-1 such as those that occur late in AIDS have been shown to infect hNT neurons in the absence of CD4 (Hirka *et al*, 1991). T-tropic gp120 can induce neuronal apoptosis in hNT cells by directly binding and activating CXCR4 receptors (Hesselgesser *et al*, 1997, 1998). In addition, the CXCR4 ligand SDF-1α is also able to induce apoptosis in these cells (Hesselgesser *et al*, 1998). Along a similar vein two recent studies have shown that HIV-1 and SIV envelope proteins can activate CXCR4 and CCR5 (Davis *et al*, 1997; Weissman *et al*, 1997). Taken together these studies suggest that soluble gp120 could mediate some of its CNS toxic effects directly, by binding to CXCR4 on neurons or even astrocytes. Direct activation of chemokine receptors on cells outside the immune system, specifically in the CNS could correlate to HIV CNS dysfunction and contribute to the pathology seen in CNS AIDS.

Conclusions

Although chemokines and their cognate receptors play an active part in regulating the trafficking of immune cells their expression throughout the CNS (Summary in Table 1) makes it abundantly clear that their role goes well beyond that. For example their effects on non-immune cells, such as neurons, and oligodendrocytes are likely to be very different and most probably include growth regulatory and

developmental effects. Thus, even though chemokines can induce the chemotaxis of neurons in a similar manner to that of leukocytes, their biological consequences are very different, i.e. chemokines play an important role in neuronal migration during fetal development. The role of chemokines and their receptors in the CNS are just beginning to be explored and given the complexities of the CNS it is quite probable that further discoveries regarding chemokine function in the brain still await to surprise us.

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