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Chemokine receptors in the human brain and their relationship to HIV infection

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Chemokine receptors have been recently identified as the important co-factors which in conjunction with CD4, mediate entry of HIV into its target cells. The brain is one of the most prominent targets of HIV infection, where it leads to HIV encephalitis (HIVE) and HIV-associated dementia. Knowledge of the distribution, physiology, and pathology of chemokines and chemokine receptors in the human brain is fundamental for understanding the pathogenesis of the interaction between HIV and the central nervous system (CNS). There is also increasing evidence that chemokine receptors expression in the CNS increases during pathological, especially inflammatory, conditions. The major co-factors for HIV infection, CCR5, CCR3, and CXCR4 have been detected in the human brain in a variety of cell types including microglia, astrocytes, neurons, and vascular endothelial cells. Furthermore, antibodies to chemokine receptors can also block HIV infectivity in cultured CNS cells. This indicates that chemokine receptors are likely to have a functional role in the pathogenesis of HIVE.

Keywords: chemokine receptors; cytokines; HIV; AIDS

Introduction

Nearly ten years ago, interleukin-8 (IL-8), was the first chemokine to be characterized. More than 30 human chemokines are known today, and the number is growing (Baggiolini *et al*, 1997). Chemokines have been implicated in physiologic functions ranging from development and cell growth, to angiogenesis and neoplasia, cellular migration, inflammatory regulation, in the response to injury, and in the interactions with pathogens, especially viruses (Baggiolini *et al*, 1997). The extent and diversity of involvement of the currently defined network of chemokines and their receptors in such varied biologic functions suggest that this important network may be considered as a separate biologic entity. Additional functions and consequences of chemokine/receptor interactions within various body compartments, including the CNS, are likely to be discovered in the near future.

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Chemokine structure

Chemokines constitute a large family of small cytokines; each molecule is composed of 92–125 amino acids with four conserved cysteines linked by disulfide bonds. Based on the positions of the amino-terminal cysteines, four subfamilies have been designated CC, CXC, C, and CX_3C. The prefix CC designate the chemokines with two adjacent cysteines while CXC designates chemokines that contain two cysteines separated by one amino acid. C-chemokines contain a single cysteine residue. The human chemokines genes are clustered on chromosome 4 (CXC chemokines) and 17 (CC chemokines). The three dimensional structure of the CXC chemokines PF4 and IL-8 have been determined (Clore and Gronenborn 1995). Their monomeric structure is similar, and it is comprised of an NH_2-terminal loop, three antiparallel β strands connected by loops, and a COOH-terminal α-helix. The structures of two other chemokines growth related protein α (GROα) and neutrophil activating peptide-2 (NAP-2), are similar to that of IL-8. The structure of two CC chemokines, macrophage inflammatory protein 1β (MIP-1β) and regulated-upon-activation, normal T expressed and secreted (RANTES) has also been revealed (Lodi *et al*, 1994; Chung *et al*, 1995). Nanomolar concentrations of the chemokines are often sufficient to elicit biological activities.

Chemokine receptors

The receptors to human chemokines are structurally related proteins within the superfamily of receptors that signal through heterotrimeric, GTP-binding, seven-transmembrane-domain (7TM) proteins (Baggiolini *et al*, 1997). They all contain two conserved cysteines, one in the NH_2 terminal domain and the other in the third extracellular loop; these are assumed to form a disulfide bond critical for the conformation of the ligand binding pocket. Following the chemokine classification, the receptors are divided into two major groups, CXC and CC. CXC chemokines have generally high affinity for single receptors (with at least one exception: IL-8 binds to CXCR1 and CXCR2) whereas CC chemokines frequently recognize two or more receptors.

Among the CXC chemokine receptors for neutrophils, the IL-8 receptor (IL-8R) (Holmes *et al*, 1991; Murphy and Tiffany 1991) is expressed as two subtypes: CXCR1 and CXCR2 (Baggiolini *et al*, 1997). CXCR1 recognizes only IL-8, while CXCR2 recognizes all other CXC neutrophil chemokines as well. Immunoflouresence studies have shown that CXCR1 and CXCR2 are expressed on neutrophils and monocytes, and in a small proportion of lymphocytes (Baggiolini *et al*, 1997). While the NH_2 terminal domain determines ligand selectivity, several other extracellular sites function as IL-8 binding and signaling domains (LaRosa *et al*, 1992; Gayle *et al*, 1993). CXCR3 which shares 36% homology with the IL8 receptors is a new chemokine receptor that recognizes interferon inducible protein 10 (IP10) and monokine induced by interferon γ (Mig). It is highly expressed in IL-2 activated T lymphocytes (including CD4+ and CD8+ cells), but not in resting T lymphocytes, B lymphocytes, monocytes, or granulocytes (Loetscher *et al*, 1996). CXCR4, formerly known as LESTR and Fusin (Federsppiel *et al*, 1993; Loetscher *et al*, 1994a) is now known as the ligand for SDF-1 (Bleul *et al*, 1996; Oberlin *et al*, 1996). It has a wide distribution in tissues including brain, heart, liver and colon (discussed below).

The CC chemokine receptors include CCR1 and CCR2, originally designated MIP-1α/RANTES and MCP-1 receptor, respectively, based on their ligands (Gao *et al*, 1993; Neote *et al*, 1993; Charo *et al*, 1994). CCR2 is divided into CCR2a and CCR2b which are RNA-splicing variants. CCR1 and CCR2 also recognize MCP-2 and MCP-3 (Baggiolini *et al*, 1997). CCR3, which is the eotaxin receptor, is prominently expressed in eosinophils and functions in the recruitment of these cells in allergic reactions (Daugherty *et al*, 1996; Ponath *et al*, 1996). CCR4 binds mainly to MIP-1α and RANTES while CCR5 recognizes MIP-1α, RANTES and MIP-1β (Power *et al*, 1995; Samson *et al*, 1996a).

Many 7TM receptor molecules with high similarity to chemokine receptors have been identified, but specific chemokine receptor functions have yet to be defined. These include BLR1 (Burkitt's lymphoma-derived receptor 1), MDR15 (monocyte derived receptor 15), EBI1 (Epstein Barr Virus infected lymphoma) (Birkenbach *et al*, 1993), and CMBRL1 (chemokine beta receptor like 1) (Combadiere *et al*, 1995). Several receptor molecules encoded by viruses that bind to chemokines have also been identified, including. US28 of cytomegalovirus (Gao and Murphy 1994) and ECRF3 of herpesvirus saimiri (Ahuja and Murphy 1993).

Functions of the chemokine/chemokine receptor system

The chemokine/chemokine receptors network forms a complex and sophisticated biochemical signaling system involved in the regulation of leukoeyte and lymphocyte trafficking that is necessary for functions of the host inflammatory defense responses, including homing, diapedesis, and disposal of inflammatory cells (Baggiolini *et al*, 1997). Although it was originally thought that the components of this system are distributed on hematopoietic cells, there is growing evidence that tissue chemokines and tissue chemokine receptors also exist.

MCP-1 was the first CC chemokine to be characterized biologically. It has been shown to attract monocytes but not neutrophils (Baggiolini *et al* 1997). MCP-2, MCP-3 and MCP-4 were discovered subsequently (Van Damme *et al*, 1992; Lodi *et al*, 1994; Uguccioni *et al*, 1996). MCP-1 today is known to attract monocytes, T lymphocytes and basophilic leukocytes. MCP-2, 3 and 4 also attract eosinophils. All four MCPs are potent attractants for activated T lymphocytes (Carr *et al*, 1994; Loetscher *et al*, 1994b; Roth *et al*, 1995). MCPs also enhance target cell lysis by NK cells (Taub *et al*, 1995). Eotaxin, a CC chemokine, has a powerful action on eosinophils and it is therefore considered to be a most relevant chemokine in the pathophysiology of allergic conditions including asthma (Jose *et al*, 1994). IP-10, a chemokine induced by IFN-γ was originally identified expressed in delayed type hypersensitivity reactions of the skin (Luster and Ravetch 1987; Taub *et al* 1993). Another IFN-γ-induced chemokine, Mig, was later described (Farber 1993; Liao *et al*, 1995). These two chemokines are involved in the regulation of lymphocytic infiltration observed in autoimmune lesions, delayed-type hypersensitivity responses, some viral infections, and certain tumors. Stromal cell derived factor-1 (SDF-1) was recently described as the ligand molecule for CXCR4 (Bleul *et al*, 1996; Oberlin *et al*, 1996). It stimulates monocytes, neutrophils. and peripheral blood lymphocytes. Mice that have been engineered not

to express the SDF-1 gene (knockouts) are not able to survive, indicating that this chemokine may have additional important functions (Nagasawa *et al*, 1996).

The role of chemokine receptors in HIV infection

Several members of the chemokine receptor family have been shown to serve as cofactors or co-receptors for HIV entry into CD4 positive cells, ending a long search for a molecule that confers species restriction to HIV infection (Feng *et al*, 1996). The first chemokine receptor identified as an HIV cofactor was CXCR4 (also called Fusin or Lestr), initially an orphan receptor, but now known to serve as receptor for SDF-1 a powerful leukocyte chemoattractant (Bleul *et al*, 1996; Oberlin *et al*, 1996). When co-expressed with CD4 in non-primate cells, CXCR4 allows infection with T-tropic HIV-strains, those strains that will also replicate in immortalized T-cell lines (Berson *et al*, 1996; Feng *et al*, 1996). CXCR4 mRNA is found in a variety of human cells susceptible to HIV infection and HIV-mediated fusion. including cell lines like HeLa and H9, and it is present in peripheral blood mononuclear cells (PBMCs). CXCR4 mRNA is also expressed abundantly in the CNS, and what is probably the bovine homologue of CXCR4 was originally cloned from the locus ceruleus in the brainstem (Rimland *et al*, 1991). Functional CXCR4 is expressed on rodent astrocytes and microglia (Heesen *et al*, 1996a,b; Tanabe *et al*, 1997a,b). CXCR4 mediated the entry of one laboratory-adapted HIV-2 isolate (HIV-2/vcp) into CD4 *negative* cells, indicating that in addition to its role as a cofactor it may also serve as a *primary* receptor for some HIV strains (Endres *et al*, 1996).

Other chemokine receptors like CCR5, CCR3 and CCR2b mediate fusion and infection with viruses obtained from macrophage tropic (M-tropic) strains (Alkhatib *et al*, 1996; Choe *et al*, 1996; Deng *et al*, 1996; Doranz *et al*, 1996; Dragic *et al* 1996). Recently, US28, the chemokine receptor encoded by MV has also been identified as a co-factor for HIV infection (Pleskoff *et al*, 1997).

A direct interaction between the gp120-CD4 complex and CXCR4 was recently demonstrated in immunoprecipitation experiments (Lapham *et al*, 1996), and a direct interaction between gp120 and CCR5 was inferred in studies in which labeled ligands MIP-1α, MIP-1β and RANTES were competed from binding to CCR5 by gp120 (Trkola *et al*, 1996; Wu *et al*, 1996). In those studies the CD4 molecule enhanced the binding between gp120 and CCR5, but CD4 was not absolutely necessary.

The events following attachment of HIV to both CD4 and a chemokine receptor are only beginning to be studied. Chemokines and HIV envelope glycoproteins from both T-tropic and M-tropic strains rapidly induce tyrosine phosphorylation of the protein tyrosine kinase Pyk2. The response requires CCR3 and CCR5 to be accessible on the cell surface. Thus, as a consequence of contact between HIV-1 and chemokine receptors, an activation of an intracellular signaling occurs which in turn can initiate multiple signaling pathways (Davis *et al*, 1997).

Studies of the ligand specificity domains in the chemokine receptor molecules determined that HIV M-tropic strains required either the amino-terminal domain or the first extracellular loop of CCR5. A CCR2b chimera containing the first 20 N-terminal residues of CCR5 supported M-tropic envelope protein fusion. Amino terminal truncations of CCR5/CCR2b chimeras indicated that residues 2–5 are important for M-tropic viruses, while 89.6 is dependent on residues 6–9. The identification of multiple functionally important regions in CCR5, coupled with differences in how CCR5 is used by M- and dual-tropic viruses, suggests that interactions between HIV-1 and entry cofactors are conformationally complex (Rucker *et al*, 1996).

Genetic polymorphism of chemokine receptors and its significance in HIV pathogenesis

Epidemiological studies concerning susceptibility to HIV revealed that rare individuals remain seronegative despite high risk life style for HIV infection. Others develop a more protracted course of disease (Levy 1993; Paxton *et al*, 1996). The link between chemokine receptors and HIV infection provided a potential explanation for the diversity in clinical phenotypes. A 32 base pair mutation in the CCR5 gene results in a truncated form of the polypeptide which falls to be expressed on the cell surface (Liu *et al*, 1996; Samson *et al*, 1996b). The absence of CCR5 on the cell surface prevents the co-receptor activity. Individuals who are homozygous for the Δ32 CCR5 are highly resistant to HIV infection while heterzygocity provides a slower progression of disease (Biti *et al*, 1997; O'Brien *et al*, 1997; Theodorou *et al*, 1997). Genetic polymorphism which affects disease progression was also found in the CXCR4 and CCR2 genes, however, mutations in those chemokine receptor genes does not affect transmission (Smith *et al*, 1997; Winkler *et al*, 1998). These discoveries support the hypothesis that M tropic viruses are responsible for virus transmission and only at later stages of the disease T tropic viruses which are associated with disease progression can emerge. Furthermore these discoveries not only provided further proof for the importance of chemokine receptors in HIV infection *in vivo*, but they also provide hope for utilization of this information in designing additional anti-HIV treatments.

Chemokines and chemokine receptors in the human brain

The major chemokine receptors that have been shown to serve as co-factors and co-receptors for HIV infection are CCR5, CCR3, CCR2b and CXCR4. There is evidence that all of these receptors (with the exception of CCR2b) are present in parenchymal brain cells. Other chemokine receptors are also present in the brain, however, their relevance to HIV infection is still unclear. The major target cells for HIV-1 infection of the CNS are microglia (Price *et al*, 1988; Watkins *et al*, 1990; Takahashi *et al*, 1996). Microglia express CCR3, CCR5, and small amounts of CXCR4 as detected by PCR and immunohistochemical analysis (He *et al*, 1997; Lavi *et al*, 1997; Tanabe *et al*, 1997a,b). In a series of experiments performed with fetal microglia, the CCR3 ligand, eotaxin, and a monoclonal antibody directed against CCR3 antibody inhibited HIV-1 infection of microglia, as did MIP-1β, which is a CCR5 ligand (He *et al*, 1997). In situ detection of HIV entry into microglia and astrocytes showed abundant infection (about 50% of infected cells in the culture) by M-tropic and dual tropic viruses into microglia, and a lower per cent of infected cells by T-tropic viruses which use CXCR4 (10%). Astrocyte cultures had only 2–3% of infected cells by viruses with the two types of tropisms. The study suggested that both CCR3 and CCR5 promote efficient infection of the CNS by HIV (He *et al* 1997).

In related experiments, using a combination of immunohistochemical staining and receptor binding studies. Hesselgesser *et al* showed that hNT cells, which are differentiated human neurons derived from the cell line NTera2, express functional chemokine receptors of the C-X-X and C-C types. These chemokine receptors include CXCR2, CXCR4, CCR1 and CCR5. He also demonstrated high-affinity binding of both types of chemokines to hNT neurons. The differentiated cells had a dose-dependent chemotactic responses to these chemokines, whereas the undifferentiated NTera2 cells did not. In addition, exposure of the NT2h cells to the envelope glycoprotein from the T lymphocyte cell (TCL) adapted strain HIV-1_{IIIB} inhibited binding of SDF-1 to the cells, indicating that the CXCR4 expressed by the neuronal cells can act as a ligand for gp120 (Hesselgesser *et al*, 1997).

We have recently shown that CXCR4 is present in the brain sections from HIV and non HIV patients. Immunohistochemistry and *in-situ* hybridization for CXCR4 and message localized the molecules to a variety of cell types including neurons, microglia, cerebral capillary endothelial cells, as well as occasional astrocytes (Lavi *et al*, 1997). Concomitantly, primary tissue culture of microglial cells, and differentiated NT2 cells also showed CXCR4 immunoreactivity. We also detected CXCR4 in the choroid plexus and ependyma (Lavi *et al*, 1997). Neuronal expression was the most surprising, and was localized to neurons in the hippocampus and other limbic regions rather than throughout the neuroaxis.

Neuronal expression of CXCR4 and other chemokine receptors that serve as HIV co-receptors may play a role in the pathogenesis of HIV dementia. For example, by mediating infection of these CD4 negative cells, or possibly by providing a mechanism for neuronal binding of gp120 (Hesselgesser *et al*, 1997). To determine whether the major chemokine receptors used as co-receptors by M-tropic HIV strains are also present in the brain we performed immunohistochemistry analysis of brain sections with antibodies for CCR5 and CCR3. Preliminary results suggest that both receptors are present in small glial cells (presumably microglia), however, CCR3 is also present in hippocampal neurons (Lavi *et al*, unpublished observation).

The mouse analogue of CXCR4 has also been shown to be present in the brain. Using degenerate PCR, the mouse homologue of human CXCR4 was cloned from a peritoneal exudate cell cDNA library; its predicted amino acid sequence is 91% identical to the human molecule. Twenty-eight of the 37 amino acid differences between mouse and human fusin are located in the ectodomains, suggesting that the intracytoplasmic components that mediate G protein binding and signaling are highly conserved. Northern blot analysis showed a message of 2.2 kb in thymus, spleen, neutrophils, and primary astrocyte cultures. Lymphoid and monocyte cell lines also expressed message for CXCR4 (Heesen *et al*, 1996a,b).

Other chemokine receptors not known to be associated with HIV infection are also present in the brain (Horuk *et al*, 1996; 1997). Archival tissues from various regions of the CNS were stained with specific mAbs to the Duffy receptor, CXCR1, which is the specific receptor for IL-8, and to CXCR2, which is a receptor shared by IL-8 and melanoma growth stimulatory activity. The Duffy receptor was expressed exclusively by Purkinje cells in the cerebellum. These inununohistochemical studies were confirmed with binding and radioligand cross-linking studies showing promiscuous chemokine receptor in the cerebellum. Although CXCR1 was not expressed in the CNS, CXCR2 was expressed at high levels by subsets of projection neurons in diverse regions of the brain and spinal cord. including the hippocampus, dentate nucleus, pontine nuclei, locus coeruleus, and paraventricular nucleus, and in the spinal cord (particularly anterior horn, interomediolateral cell column, and Clarke's column). Fibers that express CXCR2 included those in the superior cerebellar peduncle and the substantia gelatinosa. Immunohistochemical analysis of pathological tissues from patients with Alzheimer's disease showed expression of

CXCR2 in the neuritic portion of plaques surrounding deposits of amyloid (Horuk *et al*, 1996, 1997). RT–PCR and *in situ* hybridization were used to identify expression of the murine CCR1 MIP-1 alpha receptor on astrocytes (Tanabe *et al*, 1997a,b).

Tissue sections from normal human brain and active, chronic active, and chronic silent multiple sclerosis (MS) lesions were examined for the expression of the receptors for C5a, IL-8 and FMLP by immunohistochemistry. In normal brain tissue, there was low level expression of all three receptors in astrocytes and microglia. Immunohistochemical staining, was much more prominent in the foamy macrophages typical of acute lesions of multiple sclerosis. In addition, fibrous astrocytes stained intensely for the C5a receptor in chronic active multiple sclerosis. Receptor expression in chronic, inactive multiple sclerosis lesions was low, and was similar to that in normal brain, with staining confined to a few hypertrophic astrocytes and to occasional foamy macrophages. These were the first studies to demonstrate expression of these receptors in the CNS, and they suggested that chemotactic receptors may play a role in the inflammatory responses associated with multiple sclerosis lesions, and possibly in other CNS diseases (Muller-Ladner *et al*, 1996).

Several other molecules that have high homology to chemokine receptors have also been found in the CNS. A human cDNA encoding a putative G protein-coupled receptor designated chemokine beta receptor-like 1 (CMK-BRL1) was isolated from an eosinophilic leukemia library. Its deduced sequence is approximately 40% identical to previously cloned receptors for the beta chemokines like macrophage inflammatory protein-1 alpha (MIP-1 alpha). RANTES, and monocyte chemoattractant protein-1 (MCP-1), which are chemoattractants for blood leukocytes, and is 83% identical to the product of the orphan rat cDNA RBS 11. CMK-BRL1 is encoded by a small, single-copy gene that maps to chromosome 3p21 and is expressed in leukocytes. Its ligand is still unknown. CMKBRL1 mRNA was detectable by Northern blot hybridization in neutrophils and monocytes. but not eosinophils and was also found in eight solid organs that were tested with particularly high expression in brain (Combadiere *et al*, 1995).

Another putative chemokine receptor is RLCR1, a rat G-protein-coupled receptor with 43% homology to the Burkitt lymphoma chemokine receptor. Its mRNA was found to be highly expressed in the brain along with other tissues including spleen liver and heart. The distribution of this mRNA was determined by in situ hybridization to be in neurons and glial cells (Wong *et al*, 1996).

A series of cDNAs and a genomic clone (named RBS11) were isolated from a variety of rat brainstem, pituitary and/or spinal cord cDNA libraries and a genomic library by low-stringency hybridization screening with a rat angiotensin receptor cDNA. The RBS11 protein, as conceptualized from these DNAs, is a novel member of the rhodopsin family of the G-protein-coupled receptor (GCR) superfamily. Comparison of RBS11 to other members of the GCR superfamily suggests that the RBS11 protein might be a receptor for a peptide ligand in the chemokine family. Northern analysis indicates that the mRNA for RBS11 accumulates widely and unevenly in the adult rat, with the mRNA being most prominent in extracts of spinal cord, brain, kidney, gut, uterus and testes (Harrison *et al*, 1994).

In addition to chemokine receptors, chemokines are present in the CNS, and their expression increases in the presence of inflammation. CNS expression of two chemokine mRNAs, MCP-1 and IP-10, was shown to be closely related to the onset of clinical signs of murine experimental autoimmune encephalomyelitis (EAE) and correlated with the degree of inflammation. *In situ* hybridizations showed that astrocytes expressed the chemokine transcripts (Glabinski *et al*, 1995). Cultured 'rat brain macrophages' and astrocytes have been shown to express MCP-1. Expression of MCP-1 mRNA was up-regulated in the brain macrophage culture upon stimulation with lipopolysaccharide or pro-inflammatory cytokines such as IL-1β, TNFα and CSF-1 (Calvo *et al*, 1996).

In brain tissues from HIV-1 infected patients RT–PCR and RT *in situ*-PCR studies demonstrated elevated MIP-1α and MIP-1β mRNA expression in patients with dementia relative to comparable samples from HIV infected individuals without dementia. Cells expressing chemokines in HIV infected brains were identified morphologically as microglia and astrocytes (Schmidtmayerova *et al*, 1996). Another study on AIDS brains showed RT *in situ* PCR detection of MIP-1α and MIP-1β in viral negative cells suggesting that chemokine expression in the brain may be a concomitant transcriptional stimulation in neighboring uninfected cells (Nuovo and Alfieri 1996). In brains of macaque monkeys infected with simian immunodeficiency virus (SIV) up-regulation of chemokine expression was observed. The dominant intraparenchymal cells which were positive for chemokine expression were endothelial cells of capillaries and small blood vessels in addition to perivascular inflammatory cells and unidentified parenchymal cells. The detected chemokines included MIP1α and β, RANTES, MCP-3, and IP-10. Uninfected brains did not express any of these chemokines (Sasseville *et al*, 1996). Other viral infections of the brain have been associated with increased detection of chemokines in mice. These include mouse brains infected with lymphocytic choriomeningitis virus (Asensio and Campbell 1997), and mouse brains infected with mouse hepatitis virus (Lane *et al*, 1998). In both cases the predominant chemokine was Crg-2, the mouse analogue of IP-10.

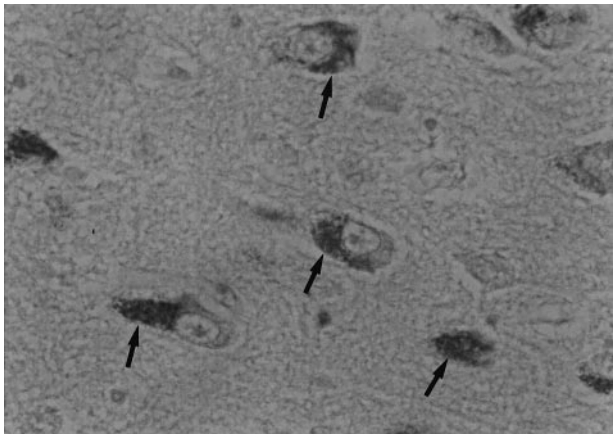

Figure 1 CXCR4 detected in hippocampal neurons. The anti CXCR4 monoclonal antibody 12G5, described in Endres et al. was used at a concentration of 17 μg/ml. Detection of 12G5 binding used the UltraProbe Universal Immunostaining Kit with avidin-biotin-complex reaction, alkaline phosphatase as the marker enzyme and Fast Red/Naphthol phosphate as the substrate-chromogen reagent. Secondary antibodies were prepared with the addition of 2% normal human serum for blocking of non specific reaction. Control experiments replacing the primary antibody with the same concentration of irrelevant immunoglobulins of the same (IgG2a) isotype were all negative (original magnification $\times$200).

Macrophage inflammatory protein (MIP)-1 alpha, also known as LD78, is a member of a family of chemokines that recruits leukocytes to sites of inflammation. Northern blot analyses showed that MIP-1 alpha mRNA is expressed in several human tissues, including brain. Glial cells in the white matter of brain tissues from four patients with schizophrenia and one with manic depressive illness were MIP-1 alpha positive by immunohistochemistry. Glial cells in the cortex from these patients were negative, except in one patient with schizophrenia in whom neurons as well as glial cells in the cortex stained positively for MIP-1 alpha. *In situ* hybridization showed that MIP-1 alpha mRNA was expressed in both neurons as well as glial cells in this patient (Ishizuka *et al*, 1997).

The time course and cellular source of MCP-1 in mouse brain after penetrating mechanical injury was analyzed, with particular focus on early time points before histologic detection of infiltrating mononuclear phagocytes. Glabinski *et al*, observed sharply increased steady state levels of MCP-1 mRNA within 3 h after nitrocellulose membrane stab or implant injury to the adult mouse brain, and MCP-1 protein elevations were documented at 12 h after injury. *In situ* hybridization combined with

Table 1 Chemokines and chemokine receptors in the brain

	CNC cell expression	*Reference*
Chemokines		
MCP-1	Astrocytes	Glabinski *et al*., 1995; Clavo *et al*, 1996
	Brain macrophages	Calvo *et al*, 1996
IP-10/CRG-2/Mig	Astrocytes	Glabinski *et al* 1995; Vanguri 1995; Lane *et al*, 1998
	Microglia	Vanguri 1995
	Ependyma, choroid plexus	Asensio and Campbell 1997
MIP-1α	Glial cells and neurons	Ishizuka *et al*, 1997
MIP-1β	Reactive astrocytes, macrophages	Ghirnikar *et al*, 1996
RANTES	Brain macrophages	Ghirnikar 1996
Nuerotactin	Microglia	Pan 1997
Chemokine receptors and putative receptors		
CCR1	hNT cultured neuronal cells	Hesselgesser *et al*, 1997
	Astocytes	Tanabe *et al*, 1997a,b
CCR2	Unknown	
CCR3	Microglia	He *et al*, 1997
	Neurons	Lavi, unpublished observation
CCR5	Microglia	He *et al*, 1997
	hNT cultured neuronal cells	Hesselgesser *et al*, 1997
CXCR1	Not expressed in the brain	Horuk *et al*, 1997
CXCR2	Neurons	Horuk *et al*, 1997; Xia *et al*, 1997
	hNT cultured neuronal cells	Hesselgesser *et al*, 1997
	Microglia, astocytes	Muller-Ladner *et al*, 1996
CXCR4	Neurons, microglia, endothelium	Lavi *et al*, 1997
	hNT cultured neuronal cells	Hesselgesser *et al*, 1997
	Microglia, astrocytes	Tanabe *et al*, 1997a,b
Duffy	Neurons (Purkinje cells)	Horuk *et al*, 1997
CMKBR21	Brain	Combadier *et al*, 1995
RBS11	Brain	Harrison *et al*, 1994
RLCR1	Neurons and glia	Wong *et al*, 1996
Apj	Brain	Matsumoto *et al*, 1996

immunohistochemistry for the glial fibrillary acidic protein astrocyte marker showed that astrocytes were the cellular source of MCP-1 mRNA at these early time points after mechanical brain injury. Stab injury to the neonatal brain evoked neither MCP-1 expression nor astroglilosis. These results demonstrated that chemokine gene expression comprises one component of the astrocyte activation program. The data are consistent with a role for MCP-1 in the central nervous system inflammatory response to trauma (Glabinski *et al*, 1996). Another study of experimental rat stab-wound brain injury found the expression of MIP-β and RANTES in macrophages surrounding the lesion and MIP-β also in reactive astrocytes (Ghirinkar *et al*, 1996).

Meningitis is accompanied by a differential immigration of leukocytes into the subarachnoid space. Since the mechanisms regulating leukocyte invasion are still incompletely understood, Sprenger *et al* studied the release of the neutrophil-attracting alpha-chemokines IL-8 and GRO-alpha and the mononuclear cell-attracting beta-chemokines MCP-1, MIP-1 alpha, and RANTES during meningitis. In 48 paired CSF and serum samples from patients hospitalized for meningitic symptoms, high levels of IL-8, GRO-alpha, and MCP-1 were detected in the CSF during bacterial and non bacterial meningitis. Elevated chemokine levels were not found in the blood serum samples taken in parallel. The release of MIP-1 alpha or RANTES was below detection limits. The IL-8 and GRO-alpha levels significantly correlated with the number of immigrated granulocytes in the CSF of patients with bacterial meningitis. A similar correlation was found when MCP-1 levels and the mononuclear cell count were analyzed in non bacterial meningitis. These findings suggest that the local production of the alpha-chemokines IL-8 and GRO-alpha and of the beta-chemokine MCP-1 represents the major chemoattractant stimulus for the differential recruitment of leukocytes into the subarachnoid space during meningitis (Sprenger *et al*, 1996).

Neurotactin is a newly described chemokine which belongs to a new delta-chemokine family. Unlike other chemokines, neurotactin has a unique cysteine pattern, Cys-X-X-X-Cys. It is predicted to be a type 1 membrane protein. and its gene is localized to human chromosome 16q. Neurotactin is chemotactic for neutrophils, both *in vitro* and may play a role in brain inflammation processes. Neurotactin mRNA is predominantly expressed in normal murine brain and its protein expression in activated brain microglia is up-regulated in mice with EAE, as well as in mice treated with lipopolysaccharide (Pan *et al*, 1997).

Under low stringency conditions, Northern blot analysis using the dopamin receptor D4 probe detected cross-hybridized mRNAs having a similar distributional profile to the D4 mRNA in human brain regions. Homology screening revealed one of the mRNAs to be an orphan seven-transmembrane receptor, APJ, abundantly expressed in the corpus callosum and spinal cord (Matsumoto *et al*, 1996).

Summary

Chemokines and chemokine receptors are emerging as important molecules in the CNS, expressed in normal brains and in various pathological conditions. Their anatomic distribution in the brain and the spectrum of pathological conditions in which expression of chemokine receptors is increased are only now beginning to be studied. Therefore, the questions that go beyond a simple descriptive analysis, have not yet been addressed. For example, what are the physiological functions of chemokine receptors in cells such as neurons that are not normally considered to be involved in inflammatory regulation? How do brain cells use chemokines and chemokine receptors in inflammatory and infectious conditions. How does the availability of chemokine receptors in brain cells and the pattern of use of the various co-receptors by HIV strains influence neurotropism and HIV infection of the brain? Are there non-inflammatory neurodegenerative conditions (like Alzheimer's disease) where chemokines and chemokine receptors also have a role in pathogenesis? Is the expression of specific chemokine receptors differentially up-regulated during pathological conditions? If so, is this phenomenon a significant pathogenic factor in inflammatory conditions of the brain or is it only an epiphenomenon?

Addressing these questions is likely to provide insights into those pathological processes of the CNS that involve inflammatory cells, and further our understanding of the relationships between the immune and the nervous systems.

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