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# Chemokine receptors and neural function

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**Numerous studies have demonstrated that chemokines play an integral role in diseases marked by inflammation. Recently, it has also been shown that chemokines and their receptors are widely expressed in the central nervous system by all types of cells, including neurons. The functions of neuronal chemokine receptors have yet to be fully defined. However, there are indications that neuronal chemokine receptors play an integral role in the development of the nervous system, in the regulation of neuronal excitability and in the signal transduction pathways that regulate neuronal survival. This review explores these topics and discusses the overall impact that chemokines may have on neuronal function.** *Journal of NeuroVirology* (2002) **8,** 573–584.

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## Introduction

Survival of the organism requires that the host response to injury, insult, and/or infection be swift and accurate. In vertebrates, that function is carried out by the immune system and initially characterized by the trafficking (i.e., circulation, homing, and extravasation) of the appropriate immune system cells to the site of damage. One of the most important classes of molecules involved in directing this process are the chemokines (CHEMOtaxic cytoKINES), a family of low-molecular-weight (8 to 10 kDa), basic proteins whose production can be up-regulated in the presence of inflammatory stimuli (Rollins, 1997; Baggiolini, 1998; Luster, 1998).

As local mediators of immune cell infiltration, chemokines are believed to play a prominent role in a variety of disease states characterized by inflammation. For example, arthritis (Barnes *et al*, 1998), asthma (Kita and Gleich, 1996; Lukacs *et al*, 1996; Griffiths-Johnson *et al*, 1997), atherosclerosis (Boring *et al*, 1998; Gu *et al*, 1998; Goslin *et al*, 1999), inflammatory bowel syndrome (Katsuta *et al*, 2000), and glomerulonephritis (Brown *et al*, 1996; Segerer *et al*, 2000) are all believed to be mediated in some part by chemokines and their effects on target cells. In addition, multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD), four neurological disorders that are discussed in this special issue, are all marked by inflammation at some point during disease onset or progression and thus are also very likely to include a chemokine-mediated component.

Chemokines and their receptors are also involved in certain virally mediated diseases, such as acquired immunodeficiency syndrome (AIDS) (Cocchi *et al*, 1995; Feng *et al*, 1996; Berger, 1997; Rucker *et al*, 1997). Studies have shown that two chemokine receptors, CCR5 and CXCR4, play a crucial role in human immunodeficiency virus (HIV)-1 infection (Alkhatib *et al*, 1996; Choe *et al*, 1996; Deng *et al*, 1996; Doranz *et al*, 1996; Dragic *et al*, 1996; Feng *et al*, 1996; Oberlin *et al*, 1996; Berson and Doms, 1998; Berger *et al*, 1999). This finding has been extended to studies of the central nervous system (CNS) and resulted in data implicating chemokine receptors in the neuropathogenesis of AIDS. For example, CCR5 expression has been found to be elevated in microglia of patients with HIV-1–associated dementia (HAD) (Rottman *et al*, 1997; Sanders *et al*, 1998;

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Vallat *et al*, 1998; Wang *et al*, 2002). In addition, chemokine receptor expression by cells in the CNS has been shown to facilitate infection by HIV-1 (Nuovo *et al*, 1994; Saito *et al*, 1994; Gabuzda *et al*, 1998; Brack-Werner, 1999; Sabri *et al*, 1999), a topic elaborated upon in this issue by Zheng and Benveniste. These reports, along with studies demonstrating that infected microglia and perivascular macrophages, the only types of cells in the CNS that allow for productive viral replication (Koenig *et al*, 1986; Wiley *et al*, 1986; Watkins *et al*, 1990; Lee *et al*, 1993; Takahashi *et al*, 1996), synthesize and secrete into the extracellular mileu soluble toxic products that elicit neuronal death, have led to the currently held belief that chemokine receptors play a major role in the molecular mechanisms by which HIV-1 induces neuronal loss (Giulian *et al*, 1990; Pulliam *et al*, 1991; Genis *et al*, 1992; Gelbard *et al*, 1994a, 1994b; Kaul *et al*, 2001). Indeed, such a mechanism of action could account for the neuropathological symptoms observed in AIDS (Sanders *et al*, 1998; Miller and Meucci, 1999; Zheng *et al*, 1999a, 1999b; Gabuzda and Wang, 2000; Pandey and Bolsover, 2000).

Although studies reporting the involvement of chemokines and their receptors in various disease states underscore their importance in the propagation of disease, the expression of chemokines and their cognate receptors in the CNS under normal conditions suggests that these molecules subserve physiological functions in the brain. This review will discuss that possibility, with particular emphasis on chemokine-mediated effects on neurons.

## Expression of chemokine receptors by neurons

It was initially believed that in the CNS, neurons were the sites of chemokine receptor expression, whereas astrocytes and microglia were the major sites of chemokine synthesis. However, numerous reports have made it increasingly clear that all of the major cell types in the brain are capable of expressing both chemokines and chemokine receptors, especially in the presence of proinflammatory stimuli (Lavi *et al*, 1998; Ascensio and Campbell, 1999; Hesselgesser and Horuk, 1999; Mennicken *et al*, 1999; Bajetto *et al*, 2001).

Chemokine receptors are members of the G-protein–coupled receptor (GPCR) superfamily (Murphy, 1994, 1996; Premack and Schall, 1996), a class of membrane receptors distinguished structurally by the presence of seven transmembrane domains and functionally by their ability to signal intracellularly through heterotrimeric GTP-binding proteins (Gether and Kobilka, 1998; Ji *et al*, 1998; Lefkowitz, 1998). Comparative analysis of the primary sequence of chemokine receptors has yielded several observations: (1) the total number of amino acids can range from 340 to 370; (2) the N-terminal segment is relatively short and acidic; (3) the presence of a cysteine at the N-terminus and in each of the four extracellular loops; (4) the conservation of the DRYLAIV sequence (or some slight variation thereof) in the second intracellular loop; and (5) the existence of numerous serines and threonines in the C-terminal tail that serve as targets for phosphorylation following receptor activation (Murphy, 1994, 1996; Baggiolini *et al*, 1997; Berson and Doms, 1998; Ward and Westwick, 1998; Bajetto *et al*, 2001). It has also been reported that chemokine receptors undergo dimerization upon ligand binding, and that such a consequence serves a pivotal role in transducing the signal within the cell (Rodriguez-Frade *et al*, 1999a, 2001; Vila-Coro *et al*, 1999).

At present, chemokine receptors are subdivided according to the chemokine ligands to which they bind. Because the $\alpha$ (CXC) and $\beta$ (CC) subfamilies comprise the largest groups of chemokine ligands, their cognate receptors also constitute the largest groups of chemokine receptors (Table 1). Noteably, a new classification system for chemokines and their receptors has been approved (Murphy *et al*, 2000; Zlotnik and Yoshie, 2000). However, for purposes of clarity and consistency with the large body of literature that was published prior to approval of the new classification system, this review will utilize the traditional nomenclature to describe chemokines and their receptors.

### *Neuronal expression of α chemokine receptors*

Expression of all of the $\alpha$ chemokine receptors by neurons has been reported. CXCR1, although originally believed to be lacking in the CNS, has been found at the level of mRNA in cholinergic septal neurons (Puma *et al*, 2001). CXCR2 expression, as revealed by immunohistochemistry, has been found on the surface of projection neurons in the hippocampus, dentate nucleus of the cerebellum, and

**Table 1** The $\alpha$ (CXC) and $\beta$ (CC) chemokines and their cognate receptors constitute the largest groups within the chemokine family

| *Chemokine receptors* | *Chemokine ligands* |
|---|---|
| CXCR-1 | IL-8, GCP-2 |
| CXCR-2 | IL-8, GRO-$\alpha$, -$\beta$, -$\gamma$, NAP-2, ENA-78, GCP-2 |
| CXCR-3 | IP-10, MIG, I-TAC |
| CXCR-4 | SDF-1$\alpha$, -$\beta$, -$\gamma$ |
| CXCR-5 | BLC/BCA-1 |
| CCR-1 | MIP-1$\alpha$, -1$\delta$, RANTES, MCP-2, -3, -4, HCC-1, MPIF-1 |
| CCR-2 | MCP-1, -2, -3, -4 |
| CCR-3 | Eotaxin-1, -2, -3, RANTES, MIP-3, MIP-1$\delta$, MCP-2, -3, -4 |
| CCR-4 | RANTES, MDC, TARC |
| CCR-5 | RANTES, MCP-2, MIP-1$\alpha$, -1$\beta$ |
| CCR-6 | LARC |
| CCR-7 | SLC, ELC |
| CCR-8 | I-309, TARC, MIP-1$\beta$ |
| CCR-9 | TECK |
| CCR-10 | ESkine |
| CCR-11 | MCP-1, -2, -4 |
| $CX_3CR$-1 | Fractalkine |
| XCR-1 | Lymphotactin $\alpha$, $\beta$ |

locus coeruleus (Horuk *et al*, 1997), as well as on hippocampal neurons from embryonic weeks 17 to 22 fetal brains (Hesselgesser *et al*, 1997). Studies of CXCR3 have revealed protein expression on neurons residing in the cortical and subcortical regions of an AD brain (Coughlan *et al*, 2000). Similar results have been found in studies of human fetal neurons and NT2.N neurons (Xia *et al*, 2000), a clonally derived cell line that possesses multiple features consistent with a neuronal phenotype, including glutamate receptors, all three classes of neurofilaments, acetylcholinesterase, muscarinic acetylcholine receptors, and polarized axonal and dendritic processes (Lee and Andrew, 1986; Pleasure *et al*, 1992; Llanes *et al*, 1995). Expression of CXCR4, a topic of particular interest given its role in brain development and AIDS-related neuropathology, has been reported at the level of mRNA and protein in various subpopulations of cortical, hippocampal, and cerebellar neurons in adult (Wong *et al*, 1996; Lavi *et al*, 1997; Westmoreland *et al*, 1998; Zhang *et al*, 1998) and embryonic (Jazin *et al*, 1997; Klein *et al*, 1999; McGrath *et al*, 1999; Boutet *et al*, 2001) brains. In addition, CXCR4 expression may follow a developmental pattern, as indicated by immunohistochemical and immunofluorescence data showing an increase in neuronal expression from birth to 9 months of age in rhesus macaque brain (Westmoreland *et al*, 2002). CXCR5 transcripts have been shown to be present in neurons localized to the granule and Purkinje cell layers of the cerebellum (Kaiser *et al*, 1993) as well as other brain regions and several neuronal cell lines (Kouba *et al*, 1993).

*Neuronal expression of $\beta$ chemokine receptors*

Reports of neuronal expression of $\beta$ chemokine receptors have focused mainly on CCR1, CCR2, CCR3, CCR4, and CCR5, with CCR3 and CCR5 being of particular interest due to their role in molecular mechanisms of HIV-1 infection. Expression of CCR5 has been detected at the protein level in human hippocampal and cerebellar neurons (Rottman *et al*, 1997), whereas CCR5 transcripts and protein have been reported in human embryonic neurons (Boutet *et al*, 2001). Human brain cell cultures have also been reported to express CCR5 and CCR3, as revealed by immunocytochemistry (Sanders *et al*, 1998). Studies in nonhuman primates have extended these observations to certain populations of neurons in the hippocampus, dentate gyrus, and cerebral cortex (Westmoreland *et al*, 1998, 2002; Zhang *et al*, 1998). These results were further elaborated upon in studies of normal brain that reported CCR3 staining in pyramidal neurons of the frontal cortex and entorhinal cortex as well as in large neurons of the dentate nucleus of the cerebellum (Sanders *et al*, 1998). Rat primary hippocampal neurons have also been reported to express transcripts of CCR1, CCR4, CCR5, CCR9, and CCR10 (Meucci *et al*, 1998). Interestingly, hNT neurons, a human cell line that has been reported to possess morphological, neurochemical, and physiological properties characteristic of mature neurons (Andrews, 1984; Pleasure and Lee, 1993; Hartley *et al*, 1999; Saporta *et al*, 2000), have been shown to express CXCR2, CXCR4, CCR1, and CCR5 (Hesselgesser *et al*, 1997). The $CX_3CR1$ receptor has been reported to be expressed by cultured hippocampal neurons and dorsal root ganglia neurons (Meucci *et al*, 1998; Oh *et al*, 2001). The C receptor has not yet been reported to be expressed on cells of the nervous system.

## Effects of chemokines on neurons

Much of the data reporting chemokine responsiveness by neurons indicate that they serve in a developmental and trophic capacity in the CNS and may also influence neuronal excitability and synaptic communication. Studies aimed at identifying the function of chemokines in the developing brain have reported that chemokines can influence the migration of several different populations of neurons and neuronal progenitors. For example, Released upon Activation Normal T-Cell Expressed and Secreted (RANTES) has been reported to induce migration of mouse dorsal root ganglion (DRG) neurons (Bolin *et al*, 1998). Other *in vitro* studies have demonstrated that stromal cell derived factor (SDF)-1 can elicit migration of cerebellar granule neurons (Bajetto *et al*, 1999) and mouse cerebellar granule precursor cells (Klein *et al*, 2001). Rat E15 neuronal progenitor cells have also been reported to exhibit the same response in a dose-dependent manner (Lazarini *et al*, 2000). These *in vitro* results were extended to the *in vivo* level in studies of mice lacking the genes for SDF-1 or CXCR4. Compared to wild-type animals, severe abnormalities in cerebellar development are observed in the brains of these animals. The specific defects reported included a marked distortion of the laminar structure, premature inward migration of external granule cells, and the absence of foliation. These results suggest strongly that chemokines are intimately involved in normal brain development (Ma *et al*, 1998; Zou *et al*, 1998). In addition, recent studies in CXCR4 knockout mice have revealed that other areas of brain development are also affected. The dentate gyrus of the hippocampus was found to be smaller than normal, and this effect was attributed not only to deficits in granule cell migration but also to a reduction in the number of dividing neuronal precursors (manuscript submitted).

The idea that chemokine receptors can affect the development and survival of neuronal precursors suggests possible trophic effects of chemokines. Indeed, studies exploring the effects of chemokines on cells of the immune system cells have yielded data indicating chemokines can promote survival and/or proliferation. These reports, combined with the mechanistic similarities of hematopoiesis and neuropoiesis (Mehler and Kessler, 1997; Quesenberry

*et al*, 1999; Terskikh *et al*, 2001), would suggest that an analogous effect of chemokines on cells in the CNS is very likely to exist. In support of this are several studies demonstrating that interleukin (IL)-8 can enhance the survival of cultured hippocampal pyramidal neurons (Araujo and Cotman, 1993; Horuk *et al*, 1997). Growth-related oncogene (GRO)-$\beta$ has also been shown to promote survival of cerebellar granule cells (Limatola *et al*, 2000a). Fractalkine, the sole member of the $\delta$ family of chemokines, has also been reported to promote survival of hippocampal pyramidal neurons in the presence of neurotoxic stimuli (Meucci *et al*, 2000). All of these reports serve as striking examples of how regulated expression of chemokines could provide survival promoting effects in the brain.

Although studies on IL-8 and fractalkine support a trophic role for chemokines in the CNS, there are reports of other chemokines, namely SDF-1$\alpha$, inducing the opposite effect. Several studies by independent investigators have reported that exposure of neurons to SDF-1$\alpha$ results in apoptotic death (Hesselgesser *et al*, 1998; Kaul and Lipton, 1999; Zheng *et al*, 1999a, 1999b). However, other investigators have reported that SDF-1$\alpha$ can actually promote neuronal survival (Meucci *et al*, 1998) and, in one case, proliferation of cerebellar granule precursor cells in the presence of the neuronal mitogen, sonic hedgehog (Klein *et al*, 2001). These contradictory results and similar observations of anti- (Suzuki *et al*, 2001) and proapoptotic (Colamussi *et al*, 2000) effects of SDF-1$\alpha$ in cells of the immune system suggest that the effect of SDF-1$\alpha$ on cell survival may be a context dependent one.

## Cellular effects of chemokines on neurons

One well-established event that follows chemokine receptor activation is the rapid dissociation of the respective heterotrimeric G-protein into the $\alpha$ and $\beta/\gamma$ subunits. In virtually every regard, this event is a critical one, for it provides a significant number of options for inducing cellular events (Gether, 2000; Dhanasekaran and Gutkind, 2001), including mobilization of intracellular calcium ($Ca^{2+}$) and modulation of ion channel activity (Miller, 1998). This has been demonstrated in studies of cells of the immune system known to be responsive to chemokines. For example, natural killer cells exhibit $Ca^{2+}$ transients following treatment with RANTES, SDF-1$\alpha$, and macrophage-derived chemokine (MDC) (Damaj *et al*, 1996). The same effect has been reported in neutrophils treated with IL-8 (Schorr *et al*, 1999). Other studies have shown that chloride channels and $Ca^{2+}$-activated potassium ($K^+$) channels in primary human macrophages open in the presence of SDF-1$\alpha$ and macrophage inflammatory protein (MIP)-1 (Liu *et al*, 2000). Eosinophils have also been reported to respond to chemokines in a similar manner, as demonstrated by the opening of $Ca^{2+}$-activated $K^+$ channels following RANTES exposure (Saito *et al*, 1996). Meanwhile, studies carried out in heterologous expression systems (e.g., *Xenopus laevis* oocytes) have shown that large currents due to the opening of G-protein–coupled inwardly rectifying $K^+$ channels (Kir 3.1) are observed following stimulation of CXCR4 and CCR5 (Madani *et al*, 1998). These results, especially given the important roles played by $Ca^{2+}$ and ion channels in neuronal function, have prompted investigators to look for similar effects in neurons.

Consistent with observations made of cells of the immune system, chemokine receptor activation in neurons has also been found to induce $Ca^{2+}$ transients and modulate ion channel activity. For example, mouse granule and Purkinje cells exhibit $Ca^{2+}$ transients following stimulation with IL-8 and GRO-$\alpha$ (Giovannelli *et al*, 1998). Similar effects have been observed in hippocampal neurons exposed to MDC-1, RANTES, SDF-1$\alpha$, and fractalkine (Meucci *et al*, 1998). The response of rat Purkinje neurons to chemokines appears to be quite varied, as $Ca^{2+}$ transients are observed in the presence of a wide variety of chemokines (Gillard *et al*, 2002). Interestingly, rat cerebellar granule cells exhibit responsiveness to a more narrow range of chemokines (Limatola *et al*, 2000b; Klein *et al*, 2001; Gillard *et al*, 2002). Studies on neuronal ion channels have reported that voltage-dependent calcium channels (VDCCs) are subject to inhibition by chemokines. For example, hippocampal pyramidal neurons in the presence of fractalkine exhibit a reduction in the voltage-sensitive $Ca^{2+}$ current (Meucci *et al*, 1998). Cholinergic septal neurons treated with IL-8 also respond in a similar manner (Puma *et al*, 2001). In addition, recordings of G1A1 cells (HEK293 cells stably expressing N-type VDCCs) treated with SDF-1$\alpha$ show a significant reduction in the $Ca^{2+}$ current (Oh *et al*, 2002). Interestingly, chemokines have also been shown to enhance the generation of action potentials in cultured rat dorsal root ganglia (Oh *et al*, 2001) as well as induce excitatory effects in rat Purkinje neurons (Gillard *et al*, 2002).

Another aspect of neuronal function that has been explored concerns the effects of chemokines on neurotransmitter release. Studies on granule cells and Purkinje cells in cerebellar slices have suggested that stimulation with SDF-1$\alpha$ probably results in the release of glutamate (Limatola *et al*, 2000b), whereas GRO-$\alpha$ may provoke the release of gamma-aminobutyric acid (GABA) (Giovannelli *et al*, 1998) at the synapse. Glutamate is the major excitatory neurotransmitter in the brain, and has been shown to play an important role in mediating synaptic plasticity (a topic which is discussed in this issue by Ragozzino). In addition, excess glutamate at the synapse can result in neurotoxicity (Choi and Rothman, 1990), an observation that may be consistent with the SDF-1$\alpha$–induced neuronal cell death previously described. Overall, these results suggest

that chemokines can regulate neuronal activity not only at the level of ion channel activity but also neurotransmitter release and synaptic communication.

## Molecular effects of chemokines on neurons

Because the response of neurons and other cell types to chemokines is the result of various intracellular events, many investigators have sought to examine the signaling pathways activated by chemokines. Most of these studies have been conducted in endothelial cells and in cells of the immune system and have reported the activation of a number of intracellular proteins. However, because attempts to undertake those same types of investigations in neurons are relatively recent, few reports are available regarding the effects of chemokines on intracellular signaling pathways in neurons. Nevertheless, the available data are consistent with the possibility that activation of signaling proteins in neurons may allow chemokines to regulate neuronal excitability and synaptic plasticity as well as the fate and development of neuronal progenitors, as described above.

Chemokines activate a number of signaling pathways, and virtually all of them have been found to involve $Ca^{2+}$. This property is consistent with the preceding discussion describing the $Ca^{2+}$ transients observed following chemokine receptor stimulation in neurons and other cell types. Molecules such as calcium/calmodulin-dependent kinase (CaMK) (Zheng *et al*, 1999a), protein kinase C (PKC) (Laudanna *et al*, 1998; Guinamard *et al*, 1999), and proline-rich nonreceptor tyrosine kinase 2 (Pyk2) (Del Corno *et al*, 2001) have all been shown to be activated by chemokines. Furthermore, the identification of Pyk2, a protein that has been shown to interact directly with c-Src (Dikic *et al*, 1996), has prompted investigators to examine members of the mitogen-activated protein kinase (MAPK) family as potential targets of chemokine receptor stimulation.

All three members of the MAPK family (e.g., extracellular signal–regulated kinases 1 and 2 [ERK1/2], c-Jun N-terminal kinase/stress-activated protein kinase [JNK/SAPK], and p38) have been observed to undergo phosphorylation following chemokine receptor activation in cells of the immune system. Phosphorylation of ERK1/2 has been reported following stimulation of CXCR1, CXCR2 (Venkatakrishnan *et al*, 2000), CXCR3 (Bonnachi *et al*, 2001), CXCR4 (Ganju *et al*, 1998), CCR1, CCR3 (Kampen *et al*, 2000), and CCR5 (Sato *et al*, 2001). Similarly, stimulation of CXCR3 with IP-10 and monokine induced by interferon gamma (MIG) in mouse cortical neurons results in the activation of ERK1/2 (Xia *et al*, 2000). The same effect has been observed following stimulation of CXCR2 in mouse primary cortical neurons (Xia and Hyman, 2002). Treatment of rat E15 neuronal progenitors and cortical neuronal cultures with SDF-1$\alpha$ also elicits the activation of ERK1/2 (Lazarini *et al*, 2000). The same observation has been made in hippocampal neurons stimulated with fractalkine (Meucci *et al*, 1998). Activation of JNK/SAPK has also been observed in immune system cells following exposure to RANTES, MIP-1$\beta$, and IL-8 (Yang *et al*, 2001) and as a result of open reading frame (ORF) 74 signaling (also known as HHV8 chemokine receptor, the constitutively active Kaposi's sarcoma–associated herpesvirus GPCR) (Bais *et al*, 1998). A similar effect has been reported in rat cerebellar granule neurons in which JNK1 activation was observed following stimulation of CXCR2 with GRO-$\beta$ (Limatola *et al*, 1999). Activation of p38 has been reported in immune system cells treated with RANTES (Sato *et al*, 2001; Wong *et al*, 2001) and GRO-$\alpha$ (Kampen *et al*, 2000) and suggested in a study of SDF-1–induced neuronal death (Kaul and Lipton, 1999). Signaling by ORF74 has also been reported to provoke activation of p38 (Bais *et al*, 1998). As indicated by these reports, MAPK family members are clearly targeted for activation following chemokine receptor stimulation in neurons.

Protein kinase B (PKB)/c-Akt, a serine/threonine kinase widely recognized for its involvement in cell survival, has also been identified as an intracellular target of chemokine receptor stimulation. Investigations of c-Akt in immune system cells have revealed that activation is observed following stimulation of CXCR3 (Bonnachi *et al*, 2001) and CXCR4 (Kijowski *et al*, 2001). The same effect has been observed in mouse primary cortical neurons treated with mouse homologue of human Gro-alpha (Gro-$\alpha$/KC) (Xia and Hyman, 2002) and in rat primary hippocampal neurons treated with fractalkine (Meucci *et al*, 2000). Consistent with this is the report that showed that phosphatidylinositol-3-kinase (PI3K), a key enzyme involved in the regulation of PKB/c-Akt, is activated following treatment with monocyte chemoattractant protein (MCP)-1 and RANTES in HEK293 cells expressing CCR2 and CCR5 (Mellado *et al*, 2001).

Studies of chemokine receptor activation in immune system cells have also reported the activation of a family of proteins known as janus kinases (JAKs) and signal transducers and activators of transcription (STATs). Investigations in immune cells have revealed that exposure to the $\beta$ chemokines RANTES and MIP-1$\alpha$ results in the activation of STAT1 and STAT3 (Wong and Fish, 1998). These results were further investigated with RANTES alone and led to the finding that the activation of both JAK2 and JAK3 accompany STAT1 and STAT3 in transducing the signal from CCR5 (Wong *et al*, 2001). In contrast, activation of other JAK/STAT family members, namely STAT5b and JAK1, was observed in the presence of RANTES in HEK293 cells engineered to expressed CCR5. This study also reported that the activation of STAT5b was found to depend heavily on its physical association with CCR5 (Rodriguez-Frade *et al*, 1999b). Another study has reported that T cells stimulated with the $\alpha$

chemokine SDF-1$\alpha$ results in activation of four members of the STAT family, STAT1, STAT2, STAT3, and STAT5b, and two members of the JAK family, JAK 1 and JAK2. Similar to the report on CCR5, activation of JAK1 and JAK2 was reported to be tightly associated with their physical association with CXCR4 (Vila-Coro *et al*, 1999). The activation of JAKs and STATs have yet to be reported in neurons exposed to chemokines.

Obviously, the activation of transcription factors such as STATs is an important end point in signaling pathways, because the proteins produced as a result of their activity significantly impact the cell's ability to respond appropriately to stimuli. Attempts to examine this concept in greater detail, particularly in the context of chemokine regulation of neurons, have led to the identification of several other well-studied transcription factors such as nuclear factor (NF)-$\kappa$B and cAMP response element binding (CREB) protein.

NF-$\kappa$B seems to be constitutively active in cells of the cortex and hippocampus, regions of the brain reported to be very active metabolically (Kaltschmidt *et al*, 1994). This observation is believed to indicate that activation of NF-$\kappa$B is associated with protective mechanisms (e.g., antioxidant production) operative in the presence of noxious stimuli (i.e., lipopolysaccharides, hypoxia, etc.) (Schutze *et al*, 1992; Kaltschmidt *et al*, 1994). In support of this are reports showing that NF-$\kappa$B activation can be induced in neurons following depolarization or treatment with glutamate (Guerrini *et al*, 1995), tumor necrosis factor-alpha (TNF-$\alpha$), or other proinflammatory cytokines (O'Neill and Kaltschmidt, 1997). Moreover, NF-$\kappa$B has been implicated in the regulation of neuronal survival and plasticity (Mattson *et al*, 2000) and transcriptional responses evoked by GPCRs (Ye, 2001). These reports, reminiscent of the effects of chemokines on immune cells and neurons, have led investigators to examine whether chemokine receptor stimulation could result in the activation of NF-$\kappa$B. This hypothesis has been confirmed by several recent reports. For example, ORF74 signaling has been reported to activate NF-$\kappa$B in endothelial cells (Pati *et al*, 2001). This same effect has been observed in hippocampal neurons treated with fractalkine (Meucci *et al*, 2000).

A third transcription factor that has been identified in studies of signal transduction pathways activated by chemokines is CREB protein. Transactivation of CREB occurs via phosphorylation at serine 133, a modification that has been shown to be inducible by increases in cAMP or $Ca^{2+}$ (Shaywitz and Greenberg, 1999). Although the former event is not observed in activation of chemokine receptors, the latter is, and hence investigators have explored the possibility that CREB is part of the signaling network targeted by chemokine receptor activation. Activation of CREB is observed in T cells treated with the CXC chemokine SDF-1$\alpha$ (Suzuki *et al*, 2001). The same effect has been reported in primary hippocampal neurons following treatment with SDF-1$\alpha$, RANTES, and fractalkine (Meucci *et al*, 1998). Interestingly, another study reported that although occupation of the calcium response element (CRE) was observed in human myeloid cells treated with MIP-1$\alpha$, it was not by CREB, but rather by a heterodimer composed of JunD and ATF-2. This was believed to result in part from the extremely close similarity of the consensus sequence of ATF-2 (5′-T(G/T)ACGTCA-3′) to that of CRE (5′-TGACGTCA-3′) as well as the report that ATF-2 DNA-binding affinity could be enhanced through phosphorylation by activated JNK, an event that was observed to occur in these cells following stimulation with MIP-1$\alpha$ (Yang *et al*, 2001).

As indicated by the preceding discussion, stimulation of chemokine receptors results in the activation of diverse proteins in the cell. These proteins are not always found within the same signaling pathways, and as a result, attributing the cellular response to the activation of a particular signal transduction pathway poses quite a challenge. One way investigators have attempted to explain how and why cells might experience such effects is by looking for "crosstalk" between pathways. More specifically, it is thought that activation of proteins conventionally associated with disparate pathways might be a consequence of enzymatic proteins (e.g., kinases) that interact with multiple substrates. For example, the classic, linear pathway leading to the activation of ERK1/2 is the Ras/Raf/MEK pathway. However, phosphorylation of ERK1/2 has also been reported to be a function of activated PKC (Ueda *et al*, 1996; Grammer and Blenis, 1997; Axmann *et al*, 1998). Interestingly, ERK1/2 can also serve as upstream regulators of CREB (Sato *et al*, 1997; Cammarota *et al*, 2001) and STAT5 phosphorylation (Pircher *et al*, 1997, 1999); yet STAT5 phosphorylation is believed to be mediated directly by JAK2 (Flores-Morales *et al*, 1998; Saharinen *et al*, 2000), a protein tyrosine kinase that can apparently activate NF-$\kappa$B indirectly by promoting its dissociation from I$\kappa$B (Digicaylioglu and Lipton, 2001). Another degree of complexity is added by the recent report that NF-$\kappa$B is likely to be a downstream target of PKC (McAllister-Lucas *et al*, 2001; Ruland *et al*, 2001), an enzyme that, depending on the particular isoform, can be regulated by both the production of diacylglycerol (DAG) and/or a rise in the level of intracellular $Ca^{2+}$ (Way *et al*, 2000). As evidenced by reports in the literature as well as within this review, all of these events occur following stimulation of chemokine receptors. Collectively then, these reports suggest that chemokines, in activating multiple signal transduction pathways, may foster the engagement of atypical affector:effector relationships such that "crosstalk" can occur in the generation of the cell's response.

Without dismissing the fact that the expression and function of proteins is a context sensitive issue, reports that certain proteins interact and regulate each other should be considered valuable information. The cell's response to extracellular stimuli is a

function of not a few but many proteins, and the identification of each of these proteins will help to develop a comprehensive explanation of how the cell utilizes different components of the intracellular machinery to achieve that response. In addition, because the controlled activity of proteins is the fundamental basis of signal propagation within the cell, individual proteins found to regulate the activity of other proteins can lead to the identification of "crosstalk" mechanisms utilized by the cell. Finally, as new findings are reported from studies linking signal to target, preexisting relationships between proteins will be referenced and evaluated for their relevance to and use in other cell systems. With respect to chemokine-mediated effects in the nervous system, this approach has been inarguably productive, for chemokine- (and cytokine)-mediated effects in the immune system have provided intriguing clues about parallel mechanisms operative in the CNS.

## Conclusion

The study of chemokines and their receptors has only recently been undertaken and as a result, knowledge of the details governing their existence and function are not yet complete. However, as investigations on chemokines continue to be reported, data from these studies will surely provide further insight about several issues that concern cell biology as whole. First, the ability of chemokines to induce directional migration of cells is an important phenomenon in normal and disease physiology, and the study of chemokines at the molecular and structural level will reveal how such a complex process can be controlled. Second, chemokines and their receptors have been reported to affect brain development, neuronal survival, and synaptic plasticity. However, the specific sequence of events and molecular mechanisms by which these effects are achieved are not yet known in their entirety. Admittedly, the data generated from further investigation of these topics will aid in the general understanding of development, apoptosis, and neuronal behavior. Third, most chemokines exhibit very low or no fidelity for a single receptor, and thus present an interesting model for how specificity can be achieved, both at the extracellular and intracellular level. With respect to the latter, the identification of signaling intermediates and transcription factors activated by chemokines should provide important information about how chemokines induce their cellular effects, and, by extention, provide leads for therapeutic agents in the treatment of diseases that are characterized by inflammation.

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