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CXC chemokine receptors in the central nervous system: Role in cerebellar neuromodulation and development

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Chemokines and their receptors are constitutively present in the central nervous system (CNS), expressed in neurons and glial cells. Much evidence suggests that, beyond their involvement in neuroinflammation, these proteins play a role in neurodevelopment and neurophysiological signaling. The goal of this review is to summarize recent information concerning expression, signaling, and function of CXC chemokine receptor in the CNS, with the main focus on the developmental and neuromodulatory actions of chemokines in the cerebellum. *Journal of NeuroVirology* (2002) **8,** 559–572.

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Introduction

Chemokines are molecules with chemoattractant properties whose main accepted role is leukocyte recruitment in inflammatory sites (Baggiolini *et al*, 1997). The discovery that chemokines and their receptors are up-regulated under various central nervous system (CNS) pathologies defined their potential role in neuroinflammation (Glabinski and Ransohoff, 1999). In the last few years, emerging evidence accumulated that expand the functional role of CNS chemokines from biological mediators of inflammatory responses to molecules playing a developmental and neuromodulatory role under physiological conditions. Evidence of chemokine implication in many biological activities has been obtained both *in vivo* and *in vitro* in neurons from several brain areas. Many excellent reviews have recently been published on the role of chemokines and chemokine receptors in the CNS (Asensio and Campbell, 1999; Miller and

Meucci, 1999; Mennicken *et al*, 1999; Glabinski and Ransohoff, 1999; Hesselgesser and Horuk, 1999; Xia and Hyman, 1999; Bacon and Harrison, 2000; Bajetto *et al*, 2001b). Thus, this review focuses on the effects of CXC chemokine receptors stimulation on central neurons from restricted brain regions, namely the cerebellum, where these receptors likely play a key functional role. From many respects, the characterization of chemokine receptor function in the cerebellum may serve as a paradigm for the entire CNS.

CXCR2

Like all the other chemokine receptors, CXCR2 belongs to the G-protein–coupled receptor family of proteins (reviewed by Baggiolini, 2001; Horuk, 2001). Ligands for CXCR2 comprise interleukin-8 (IL-8/CXCL8), the growth-related oncogene γ (GROγ/CXCL1), the growth-related oncogene γ (GROγ/CXCL2), and the granulocyte chemotactic protein-2 (GCP-2/CXCL6) (see Murphy *et al*, 2000, for review). Agonist-receptor interaction induces a conformational change in the receptor structure that causes the G-protein–dependent activation of phospholipase C γ enzymes, with the generation of the two intracellular second messengers, diacylglycerol and inositol 1,4,5-trisphosphate (IP_3) and with the mobilization of intracellular Ca^{2+} (Wu *et al*, 1993).

Table 1 CXC receptors expression○in the central nervous system

	Human	*Rodent*
CXCR1	Not detected[12,29]	mRNA in SeN,[24] OLP[21]
CXCR2	**Subset of neurons in brain and spinal cord,[12,29]** fetal neurons and hNT2,[5] As and MG,[14] oligodendroglioma[25]	**Developing brain,[18] PN,[7] GC[7,16]**; mRNA in **cerebellum**,[7] HiN,[20] SeN,[24] and OLP[21]
CXCR3	**PN[8,28,30] and granular layer,[30]** cortex, striatum, hippocampus, spinal cord,[30] fetal neurons and hNT2,[5] As[8,30]	mRNA in mouse brain[3] and MG[3]
CXCR4	Adult brain regions including **cerebellum**, hippocampus, cortex, thalamus, olfactory bulb[27]; adult neurons and MG,[15] fetal neurons,[4,5,10,31] hNT2,[5] and As[31]; not detected in embryonic brain[28]①	**GC,[1,17] PN,[6,17] and glial fibers,[17] IGL, and EGL in postnatal cerebellar cortex,[11]** CN,[1,13] NP,[13] DRG,[22] As,[1,13,26] MG,[26] CXCL12 binding in adult brain,[2] mRNA in the developing brain (**including cerebellum**),[9,19,32]②HiN,[20] CN,[23] As,[23] MG[23]

As, astrocytes; CN, cortical neurons; DRG, dorsal root ganglion cells; EGL, external granular layer; GC, granule cells; HiN, hippocampal neurons; hNT2, human teratocarcinoma cells; IGL, internal granular layer; MG, microglia; NP, neuronal progenitors; OLP, oligodendrocyte precursors; PN, Purkinje neurons; SeN, septal neurons.
○Protein expression is intended unless otherwise indicated; ①CXCR4 expression was detected in neurons and glial cells from fetal and postnatal macaque brain (Klein *et al*, 1999; Westmoreland *et al*, 2002); ②CXCR4 expression decreases during development (Jazin *et al*, 1997; Klein *et al*, 2001); **In bold**, CXC receptors expression in cerebellar cells.
References: [1]Bajetto *et al*,1999; [2]Banisadr *et al*, 2000; [3]Biber *et al*, 2001; [4]Boutet *et al*, 2001; [5]Coughlan *et al*, 2000; [6]Gillard *et al*, 2002; [7]Giovannelli *et al*, 1998; [8]Goldberg, 2001; [9]Jazin *et al*, 1997; [10]Klein *et al*, 1999; [11]Klein *et al*, 2001; [12]Horuk *et al*, 1997; [13]Lazarini *et al*, 2000; [14]Lacy *et al*, 1995; [15]Lavi *et al*, 1997; [16]Limatola *et al*, 1999; [17]Limatola *et al*, 2000a; [18]Luan *et al*, 2001; [19]McGrath *et al*, 1999; [20]Meucci *et al*, 1998; [21]Nguyen and Stangel, 2001; [22]Oh *et al*, 2001b; [23]Ohtani *et al*, 1998; [24]Puma *et al*, 2001; [25]Robinson *et al*, 2001; [26]Tanabe *et al*, 1997; [27]van der Meer *et al*, 2000; [28]van der Meer *et al*, 2001; [29]Xia *et al*, 1997; [30]Xia *et al*, 2000; [31]Zheng *et al*, 1999b; [32]Zou *et al*, 1998.

CXCR2 expression in the cerebellum

The first demonstration of CXCR2 expression in the cerebellum has been obtained by Horuk and colleagues (1997): Immunohistochemical analysis performed on human brain revealed that CXCR2 is widely expressed in many brain regions, and in several projecting neurons, including fibres in the cerebellum granular layer (Table 1). Positive labeling for CXCR2 is also demonstrated in human, in both fetal and adult neurons, hippocampal cultures, and hNT (human teratocarcinoma) differentiated cells (Hesselgesser *et al*, 1997; Xia *et al*, 1997; Meucci *et al*, 1998; Coughlan *et al*, 2000). Confocal microscopy analysis, performed on rodent cerebellar sections and cerebellar neuronal cultures, demonstrates the presence of CXCR2 both on Purkinje and granule neurons: sagittal cerebellar slices obtained from 8-week-old mice display intense reactivity for CXCR2 in the granular layer and Purkinje cell layer (Giovannelli *et al*, 1998). Positive immunostaining is also described for primary cultures of rat cerebellar granules (Limatola *et al*, 1999) and Purkinje neurons (Cristina Limatola, unpublished results). A positive staining for CXCR2 is shown in developing brain from mice at embryonic days 11.5 to 13.5 (Luan *et al*, 2001). Contrasting results on CXCR2 expression on astrocytes and microglial cells have been reported: (i) In human normal brain tissue, CXCR2 expression is detected at very low levels, but it increases noticeably in multiple sclerosis lesions (Muller-Ladner *et al*, 1996); (ii) human astrocytes express CXCR2 (Lacy *et al*, 1995), whereas (iii) mouse astrocytes are negative for CXCR2 mRNA (Heesen *et al*, 1996). In this respect, it is interesting that astrocyte cultures obtained from CXCR2-deficient mice still respond to KC/CXCL1 and macrophage inflam-

matory protein (MIP)-2/CXCL2 stimulation, inducing chemokine mRNA synthesis (Luo *et al*, 2000b), which suggests the presence of another CXC receptor. In addition, oligodendrocytes precursor cell lines, oligodendrogliomas, and oligodendrocyte progenitor cells express CXCR2, whose stimulation regulates their proliferation (Robinson *et al*, 1998, 2001; Wu *et al*, 2000; Nguyen and Stangel, 2001).

In conclusion, CXCR2 is constitutively expressed by cerebellar neurons, appearing in the cerebellum during embryogenesis. However, the importance of CXCR2 expression in CNS development is under question. The CXCR2 knockout mice does not show neurological defects, suggesting a redundant role of CXCR2 (Cacalano *et al*, 1994). The correlated expression of CXCR2 and its ligands in the developing mouse brain likely accounts for a developmental role, possibly exalted in stress conditions (Luan *et al*, 2001).

CXCR2 signaling pathways

Cerebellar neurons express functional CXCR2 and display activation of multiple signaling pathways upon CXCR2 stimulation (Table 2).

The monitoring of intracellular calcium transients is the most direct way to study the expression of functional chemokine receptors: With this experimental approach, CXCR2 expression is detected in Purkinje neurons (Giovannelli *et al*, 1998), cerebellar granules (Limatola *et al*, 1999) and hippocampal (Meucci *et al*, 1998), septal (Puma *et al*, 2001), and NT2.N (Coughlan *et al*, 2000) neurons. A direct evidence of the presence of functional CXCR2 receptors on cerebellar neurons was also obtained following the kinetics analysis of accumulation of second messengers upon chemokine treatment: IP_3 rapidly accumulates

Table 2 CXC receptors signaling in CNS cells

Receptor/agonist	$\uparrow[Ca^{2+}]i$	$\uparrow IP_3$	$\downarrow cAMP$	$\uparrow MAPK$	CREB	Neuromodulation
CXCR1/CXCR2						
CXCL1	PN,[8] hNT2[6]	PN[8]		*CN[24]*		LTD impairment in PNs[8]
CXCL2	GC[12]	GC[12]		**GC[12,14,20]**		
CXCL8	**PN,[7,8]** DRG,[17] HiN,[15] SeN[19]			GC[14]		$\downarrow$Ca^{2+} channels in SeN[19]
CXCL1/CXCL2/ CXCL8						SIC, $\uparrow$sPSC frequency, $\uparrow$eEPSC amplitude in PNs[8,20]
CXCR3						
CXCL10	GC, *PN*,[7] DRG,[17] MG[5]					
CXCL9/CXCL10				CN[23,24]		
CXCR4						
CXCL12	**hFN, hAs,**[9,25,26] **AG,[16] MG,[22]** *PN*,[7,13] HiN,[15] DRG,[17] CN,[2] hNT2,[6] hMG,[1] GC,[7,10,13]○ As,[2,3,22]○	*hFN*,[26] GC○	**hFN[25,26] hAs[25]**	CN,[11,24] NP,[11] **AG,[16]** CbS,[7] As,[3,11]○	CbS,[7] HiN[15]	Glutamate release,[4] $\downarrow$**Ca^{2+} channels in HEK,[18]** $\downarrow$spontaneous Ca^{2+} transients in HiN,[15] SIC, $\uparrow$sPSC frequency,[13,21] $\downarrow$eEPSC amplitude in PNs,[21] $\uparrow$PSP amplitude in HpS[26]

AG, CXCR4-expressing astroglioma cells; As, astrocytes; CbS, cerebellar slices; CN, cortical neurons; DRG, dorsal root ganglion neurons; eEPSC, evoked excitatory postsynaptic current; GC, granule cells; hAs, human astrocytes; HEK, Ca^{2+} channels–expressing HEK cells; hFN, human fetal neurons; HiN, hippocampal neurons; HiS, hippocampal slices; hMG, human microglia; hNT2, human teratocarcinoma cells; LTD, long term depression; MG, microglia; NP, neuronal progenitors; PN, Purkinje neurons, SeN, septal neurons; SIC, slow inward current; sPSC, spontaneous postsynaptic currents.
PTX-sensitive effects and references in **bold**; PTX-insensitive or partially sensitive effects and references in *italics*; ○contrasting results concerning PTX sensitivity; ○○Cristina Limatola, unpublished results.
References: [1]Albright *et al*, 2001; [2]Bajetto *et al*,1999; [3]Bajetto *et al*, 2001a; [4]Bezzi *et al*, 2001; [5]Biber *et al*, 2001; [6]Coughlan *et al*, 2000; [7]Gillard *et al*, 2002; [8]Giovannelli *et al*, 1998; [9]Klein *et al*, 1999; [10]Klein *et al*, 2001; [11]Lazarini *et al*, 2000; [12]Limatola *et al*, 1999; [13]Limatola *et al*, 2000a; [14]Limatola *et al*, 2002; [15]Meucci *et al*, 1998; [16]Oh *et al*, 2001a; [17]Oh *et al*, 2001b; [18]Oh *et al*, 2002; [19]Puma *et al*, 2001; [20]Ragozzino *et al*, 1998; [21]Ragozzino *et al*, 2002; [22]Tanabe *et al*, 1997; [23]Xia *et al*, 2000; [24]Xia and Hyman, 2002; [25]Zheng *et al*, 1999a; [26]Zheng *et al*, 1999b.

in the first seconds of CXCL1 and CXCL2 treatment, both in primary cultures of cerebellar granule and in Purkinje neurons (Giovannelli *et al*, 1998; Limatola *et al*, 1999).

In addition to Ca^{2+} signaling, other CXCR2-coupled signaling pathways, described both in neutrophils and human embryonic kidney (HEK) cells, comprising extracellular signal–regulated kinase (ERK), phosphatiolyl inositol 3 kinase (PI3-K), and Akt activation (Jones *et al*, 1995; Knall *et al*, 1996, 1997; Tilton *et al*, 1997), are activated in CXCL2-stimulated cerebellar neurons (Ragozzino *et al*, 1998; Limatola *et al*, 2002). However, when human neutrophils are stimulated with CXCL8, the block of the PI3-K/Akt pathway by wortmannin impairs chemokine-induced ERK phosphorylation (Knall *et al*, 1997). In contrast, in rat cerebellar neurons, the block of the PI3-K/Akt pathway with LY294002 does not prejudice CXCL2-mediated ERK activation, demonstrating that the two pathways are independent, as suggested by their different sensitivity to pertussis toxin (PTX) treatment (Limatola *et al*, 2002). Part of the reasons for these discrepancies could be the fact that in human neutrophils, CXCL8 induces the simultaneous activation of CXCR2 and CXCR1 (that is, the other known receptor for CXCL8), whereas, in general, central neurons do not express CXCR1 (Horuk *et al*, 1997, but see also Puma *et al*, 2001). However, different PI3-K

inhibitors were used in the two studies (Knall *et al*, 1997; Limatola *et al*, 2002). The direct activation of the ERK1/2 and PI3-K pathways is reported upon CXCR2 stimulation of other neurons: GROγ/KC stimulation of mouse cortical neurons induces a potent and sustained phosphorylation of both ERK1/2 and Akt, accompanied by a strong tau hyperphosphorylation (Xia and Hyman, 2002), which is associated with neurofibrillary tangle formation in Alzheimer's disease patients (Goedert, 1993). Interestingly, the activation of ERK1/2 mediated by fractalkine/CX3CL1 in hippocampal neurons is also insensitive to the inhibition of PI3-K/Akt pathway, suggesting a neuronal-specific pathway (Meucci *et al*, 2000).

A unique observation in the field of chemokine receptor signaling and, in general, of G-protein–coupled receptor signaling, is the description of CXCR2 being coupled to sphingomyelin hydrolysis and C-Jum N-terminal kinase (JNK) activation in cerebellar granule neurons (Limatola *et al*, 1999). The activation of this pathway by CXCL2 has been described only in neurons so far. It is worthwhile to note that the analysis of sphingomyelin hydrolysis and ceramide generation in other CXCR2-expressing cells, like neutrophils or transfected HEK cells, could give an insight on the specificity of this signaling route in neurons. The molecular mechanisms underlying CXCL2-induced sphingomyelin breakdown are not known yet,

although the activation of this signaling pathway has been widely studied using classical cytokine receptor activation, Fas and neurotrophin receptors, as well as cell treatment with damaging physical agents (Hannun and Luberto, 2000). The only additional example of G-protein–coupled receptor inducing sphingomyelin hydrolysis is the CB1 cannabinoid astrocyte receptor (Sánchez *et al*, 1998).

CXCR2 and neuromodulation
One of the most intriguing effects of chemokines in the CNS is certainly their ability to influence neurotransmission (Table 2). The neuromodulatory properties of chemokines have been described in several neuronal systems (Giovannelli *et al*, 1998; Ragozzino *et al*, 1998; Meucci *et al*, 1998; Limatola *et al*, 2000a; Puma *et al*, 2001). When cerebellar slices are exposed to the CXCR2 ligands CXCL1 or CXCL8, the frequency of spontaneous postsynaptic currents (PSCs) (both excitatory, EPSC, and inhibitory, IPSC) recorded from Purkinje neurons increases considerably, whereas PSC amplitude is maintained (Giovannelli *et al*, 1998). Similar effects are described for CXCL2 (Ragozzino *et al*, 1998), and, in both cases, these are not abolished when nerve cell excitability is blocked by tetroobtoxin (TTX) treatment, indicating that the target of chemokine action is mainly the neurotransmitter release. In cerebellar slices, CXCL1 and CXCL8 potentiate the evoked EPSCs on Purkinje neurons, likely acting presynaptically at the parallel fibres of granule cells. This hypothesis is supported by the mobilization of Ca^{2+} induced by both CXCL1 and CXCL2 in cultured cerebellar granules (Giovannelli *et al*, 1998; Limatola *et al*, 1999). The CXCL2-induced positive modulation of neurotransmitter release is impaired when the ERK kinase MEK is inhibited by the compound PD98059, indicating the involvement of the ERK kinase pathway in chemokine-mediated neurotransmitter release (Ragozzino *et al*, 1998). The involvement of the ERK kinase pathway in the modulation of neurotransmitter release is proved under different experimental conditions, using either the brain-derived neurotrophic factor (BDNF) (Jovanivic *et al*, 2000) or the cholinigic agomist nicotine (Cox and Parson, 1997). However, the MEK inhibitor PD98059 causes nonspecific effects on glutamate release from hippocampal synaptosomes (Pereira *et al*, 2002), making the role of MEK on neurotransmitter release still under question.

The full mechanism responsible for CXCR2 modulation of synaptic transmission observed in cerebellar slices is still unknown; however, a recent report describes a mechanism of modulation of neuronal properties potentially relevant to chemokine-induced synaptic modulation: The stimulation of CXCR1 and CXCR2 with CXCL8 in freshly isolated cholinergic septal neurons induces the fast and repetitive inhibition of the voltage-dependent Ca^{2+} currents (Puma *et al*, 2001). This study reports for the first time the expression of CXCR1 in central neurons, by single-cell reverse transcriptase–polymerase chain reaction (RT-PCR) analysis, in contrast to previous attempts to demonstrate CXCR1 in the CNS that gave negative results (Dunstan *et al*, 1996; Horuk *et al*, 1997). Most of these CXCL8-responsive cholinergic neurons express both CXCR1 and CXCR2, and the effects induced by CXCL8 likely derive from the simultaneous activation of the two classes of receptors. The inhibitory effects on the Ca^{2+} currents involves the G-protein–dependent inhibition of L- and N-type voltage-dependent Ca^{2+} channels, as demonstrated using selective Ca^{2+} channel antagonists, non-hydrolysable GTP analogues, and anti $G_{i\gamma}$-specific antibodies (Puma *et al*, 2001). In addition, Zamponi and Snutch (1998) report a partial voltage-dependence of CXCL8 inhibitory effect, suggestive of direct interaction between G-protein and Ca^{2+} channels. The observation that the partial blockage of Ca^{2+} currents does not desensitize upon repeated chemokine applications would indicate that CXCR1 and CXCR2 do not desensitize or internalize, the latter effect being measurable already after 1 min of CXCR2 stimulation in CXCR2-expressing HEK cells (Yang *et al*, 1999). The inhibition of Ca^{2+} currents by CXCL8 might represent a mechanism of chemokine-induced synaptic modulation, likely reducing evoked neurotransmitter release (Miller, 1998). Therefore CXCR2-induced potentiation of spontaneous and evoked neurotransmitter release in the cerebellum should be mediated through different mechanisms. Interestingly other chemokines modulate neurotransmitter release: MDC/CCL22 and fractalkine/CX3CL1 inhibit the excitatory transmission between hippocampal neurons, blocking the frequency but not the amplitude of spontaneous glutamatergic EPSCs. CCL22 and CX3CL1 stimulate different G-protein–coupled receptors and only CX3CL1 has an inhibitory activity on the voltage-dependent Ca^{2+} currents of hippocampal neurons (Meucci *et al*, 1998), suggesting that the inhibitory action of these two chemokines is due to different mechanisms (see also Oh *et al*, 2002). Several other chemokines, such as RANTES/CCL5, MIP-1γ/CCL3, and stromal cell–derived growth factor (SDF)-1γ/CXCL12, induce an inhibition of the spontaneous synaptic activity, reducing the frequency of the intracellular Ca^{2+} oscillations in synaptically coupled hippocampal neurons (Meucci *et al*, 1998).

In addition to these fast effects on synaptic transmission, the CXCR2 agonist CXCL1 is reported to modulate the induction of the long-term depression (LTD) at the synapse between the parallel fibres and the Purkinje neurons in the cerebellum; prolonged pretreatment of cerebellar slices with CXCL1 considerably reduces the percentage of successful inductions of LTD in adult mice (8-week-old), but is unable to reverse LTD once established (Giovannelli *et al*, 1998).

CXCR2 and neuroprotection
Ligands of the CXCR2 are involved in the modulation of proliferative, throphic, and antiapoptotic responses of CNS cells: These responses have been observed in cerebellar granules cells (Limatola *et al*, 2000b, 2002), hippocampal (Araujo and Cotman, 1993) and cortical neurons (Bruno *et al*, 2000), astrocytes (Saas *et al*, 1999), and oligodendrocytes precursors (Robinson *et al*, 1998; Wu *et al*, 2000). Neurotrophic activities of CXCR2-stimulating chemokines are reported for cerebellar neurons: CXCL2 and CXCL8 efficiently reduce the number of apoptotic neurons in granule cultures where the apoptotic cell death is induced by K^+ depletion from the culture medium (Limatola *et al*, 2000b, 2002). The molecular mechanisms involved in CXCR2-mediated cell survival requires the functional expression, on granule neurons, of the ionotropic glutamate receptors of the alpha-amino-3-hydroxy-s-methylisoxazole-4-propiomate (AMPA) type, as demonstrated with the use of specific AMPA receptor inhibitors and by cell treatment with antisense oligonucleotides specific for the AMPA receptor subunits (Limatola *et al*, 2000b). The neurotrophic effects induced by CXCR2 activation in cerebellar neurons are also dependent on the signaling coupling of CXCR2 with the PI3-K/Akt pathway, because the inhibition of PI3-K/Akt pathway by LY294002 efficiently abolishes chemokine-mediated neurotrophic effects (Limatola *et al*, 2002).

In addition, CXCL8 has a protective action against the toxic effects of *N*-methyl-D-aspartate (NMDA) in cortical mixed cultures: The cellular protection is specific for NMDA-mediated damages, because CXCL8 is apparently unable to protect cells from γ-amyloid peptide toxicity (Bruno *et al*, 2000). The effects exerted by IL-8/CXCL8 on hippocampal neurons are dependent on the time in culture (Araujo and Cotman, 1993) and specifically include an increased survival of cultured neurons treated with this chemokine.

CXCR2-dependent protective/proliferative effects have been reported also for glial cells: (i) Human cultured astrocytes, at early passages, are protected by CD95-mediated apoptosis through CXCL8 secretion, with an autocrine loop; when neutralizing γ-CXCL8–specific antibodies are present in the CD95-ligation medium, astrocytes become sensitive to CD95-mediated death (Saas *et al*, 1999). (ii) A proliferative response to CXCL1 was observed in oligodendrocytes precursors both under physiological (Robinson *et al*, 1998) and pathological conditions (Wu *et al*, 2000), suggesting a potential modulatory role for CXCR2-stimulating chemokines in the spinal cord both during development and following damaging events.

In conclusion, all these observations, together with the reported increased expression of CXCR2 and chemokine ligands for CXCR2 in several CNS pathologies (Van Meir *et al*, 1992; Liu, 1993; Muller-

Ladner *et al*, 1996; Xia *et al*, 1997; Desbaillets *et al*, 1997; Robinson *et al*, 2001; Xia and Hyman, 2002), indicate that, under physiological conditions and during development, CXCR2-stimulating chemokines exert trophic, neuroprotective, and neuromodulatory activities on CNS cells, and support the view that in neuroinflammatory diseases, chemokine production (mainly but not only) by glial cells are involved in the inflammatory processes and, in the meantime, act to defend neurons from the toxic effects of the inflammatory substance released.

CXCR3

CXCR3 is expressed on natural killer cells and on activated lymphocytes (Loetscher *et al*, 1996; Sallusto *et al*, 1998), playing the main role of inducing G-protein–dependent Ca^{2+} transients and cellular migration to the inflammatory areas. Three different agonists of CXCR3 have been identified up to now: γ-interferon–inducible protein (IP-10/CXCL10), monokine induced by γ-interferon (Mig/CXCL9), and interferon-inducible T cell γ-chemoattractant (I-TAC/CXCL11) (reviewed by Horuk, 2001). A potential fourth agonist is the CC chemokine secondary lymphoid tissue chemokine (SLC/CCL21) (Biber *et al*, 2001).

Functional expression of CXCR3 in the CNS
CXCR3 expression in the CNS increases following several types of inflammatory diseases or traumatic events and is likely to play an active role in recruiting activated lymphocytes to the injured regions (Westmoreland *et al*, 1998; Sorenson *et al*, 1999). However, CXCR3 is also constitutively expressed in the CNS (Table 1) (Coughlan *et al*, 2000; Xia *et al*, 2000; Goldberg *et al*, 2001; van der Meer *et al*, 2001). CXCR3 neuronal immunoreactivity is specific for cerebellar Purkinje neurons, while is diffusely present in astrocytes in many CNS areas, including cerebellum. *In situ* hybridization with CXCR3-specific probes confirms receptor localization on Purkinje neurons; microglia and oligodendrocytes do not express CXCR3 (Goldberg *et al*, 2001). CXCR3 expression in cerebellar Purkinje neurons also occurs in human fetal brain (van der Meer *et al*, 2001). The prominent expression of CXCR3 in astrocytes is associated with various pathological states. Astrocytic CXCR3 expression is confirmed in mixed glial primary cultures, where CXCR3 expression was larger than in native tissue, as all glial fibrillary acid protein (GFAP)-positive cells were also CXCR3-positive (Goldberg *et al*, 2001).

In contrast, Xia and colleagues (2000) report CXCR3 expression also in other CNS areas and cell types, describing CXCR3 immunoreactivity in cortical, striatal, spinal, hippocampal, and cerebellar neurons, including granule cells. Furthermore, the expression of CXCR3 is detected both on the cell

bodies and the neuronal processes of fetal neurons and hNT2 cells (Coughlan *et al*, 2000).

Expression of the CXCR3 ligands is also detected in human CNS (Xia *et al*, 2000). Such expression refers to subpopulations of both resting and active astrocytes, although the level is much higher in reactive astrocytes, and is more prominent in brains from Alzheimer's disease patients (Xia *et al*, 2000). Expression of CXCL10 is detected in neurons and astrocytes in ischemic rat brain (Wang *et al*, 1998) and in astrocytes following viral CNS infection in mice (Liu *et al*, 2000a). In addition, expression of CXCL9 and CXCL10 is reported in cultured human fetal astrocytes following stimulation with macrophage- or lymphocyte-activated medium (Poluektova *et al*, 2001).

There are a few reports on CXCR3 signaling in the nervous system (Table 2): CXCL10 stimulation of CXCR3 in mouse microglial cells elicits intracellular Ca^{2+} transients (Biber *et al*, 2001). The functional expression of CXCR3 in neurons is demonstrated in mouse primary cortical neurons, where both CXCL10 and CXCL9 activate the ERK1/2 pathway (Xia *et al*, 2000). In addition, CXCL10-induced calcium transients have recently been observed in cerebellar neurons (Gillard *et al*, 2002) and in cultured dorsal root ganglion neurons (Oh *et al*, 2001b).

In conclusion, CXCR3, although expressed in CNS cells, has not yet a well-defined physiological role besides in inflammatory processes. The neuronal expression of CXCR3, particularly diffused in the cerebellum, together with the expression of CXCR3 ligands on subpopulations of astrocytes, suggests that CXCR3 signaling may represent a pathway for glial-neuron interaction (Xia *et al*, 2000).

CXCR4

CXCR4 belongs to the seven-transmembrane family of receptors, and its responses are mediated by PTX-sensitive G-proteins (Hesselgesser *et al*, 1998a), leading to the induction of several intracellular pathways (Table 2). CXCR4 is peculiar among chemokine receptors, due to its monogamous relation with its natural agonist, SDF-1/CXCL12 (Bleul *et al*, 1996a; Oberlin *et al*, 1996; Ma *et al*, 1998; Wells *et al*, 1998). Both CXCL12 and CXCR4 are very well conserved between different species (Shirozu *et al*, 1995; Oberlin *et al*, 1996; Horuk, 2001), although splicing variants of CXCL12 (SDF-1γ, SDF-1γ, SDF-1γ; Tashiro *et al*, 1993; Shirozu *et al*, 1995; Gleichmann *et al*, 2000) and of CXCR4 (Heesen *et al*, 1997; Gupta and Pillarisetti, 1999) have been characterized.

CXCR4 expression in the cerebellum
CXCR4 and CXCL12 are constitutively and widely expressed through many CNS regions (Table 1), in many cell types including cortical, hippocampal, thalamic, olfactory bulbar, and cerebellar neurons (Jazin

et al, 1997; Lavi *et al*, 1997; Hesselgesser *et al*, 1997; Ohtani *et al*, 1998; McGrath *et al*, 1999; Klein *et al*, 1999; van der Meer *et al*, 2000; Banisadr *et al*, 2000; Tham *et al*, 2001; Boutet *et al*, 2001), microglial cells (He *et al*, 1997; Lavi *et al*, 1997; Tanabe *et al*, 1997), and astrocytes (Tanabe *et al*, 1997; Dorf *et al*, 2000; Bajetto *et al*, 1999; Klein *et al*, 1999). In the cerebellum, CXCR4 is expressed both in Purkinje neurons and granule cells (Bajetto *et al*, 1999; Limatola *et al*, 2000a; Klein *et al*, 2001) and in glial radial fibers (Limatola *et al*, 2000a). CXCL12 transcripts are present in subsets of neurons in several CNS regions, including cerebellum, and their expression is developmentally regulated (Tham *et al*, 2001). In addition, CXCL12 transcripts are detected in several types of cultured neurons, including cerebellar granule cells, and in astrocytes (Bajetto *et al*, 1999; Zheng *et al*, 1999b; Lazarini *et al*, 2000; Tham *et al*, 2001). In astrocytes, the expression of CXCL12 is up-regulated by lipopolysaccharide (LPS) treatment (Ohtani *et al*, 1998; Bajetto *et al*, 1999; Zheng *et al*, 1999), which also induces the down-regulation of CXCR4 expression and function (Bajetto *et al*, 1999). Cerebellar granule cells secrete CXCL12 under basal conditions (Bajetto *et al*, 1999). Furthermore, the regional expression of CXCL12 and CXCR4 is comparable (McGrath *et al*, 1999; Tham *et al*, 2001), suggesting that paracrine or autocrine mechanisms participate in the signaling involved in both physiological and inflammatory processes (Asensio and Campbell, 1999; Bajetto *et al*, 2001b).

The developmental pattern of CXCR4 expression is different between species. In rodents, CNS expression of CXCR4 begins during embryonic life, in the proliferative areas, and decreases after birth (Jazin *et al*, 1997). Difference from rodents, in human CNS, CXCR4 transcripts are not detected during embryogenesis or first childhood (van der Meer *et al*, 2001); and in macaque CNS, CXCR4 expression is detected in several fetal neurons and increases in the first 9 months after birth (Westmoreland *et al*, 2002). However, CXCR4 expression has been detected by flow cytometry in dissociated fetal neurons from human and macaque brains (Klein *et al*, 1999).

CXCR4 messengers are detected in the rodent cerebellum at late embryonic stages (Jazin *et al*, 1997; Zou *et al*, 1998; McGrath *et al*, 1999), increase until birth, and are reduced during the first 2 postnatal weeks (Klein *et al*, 2001). The decrease of CXCR4 expression in the cerebellum during postnatal development is confirmed by the low level of chemokine binding in this region in the adult rat (Banisadr *et al*, 2000). Similarly to CXCR4, the level of CXCL12 transcripts in rat cerebellum is high at birth and decreases progressively, although a delay between external granular layer (EGL) and the internal granular layer (IGL) is reported (Tham *et al*, 2001). Cerebellar neurons also express the recently cloned and characterized isoform SDF-1γ, whose transcripts are found both in granule cells and Purkinje cell layer (Gleichmann *et al*, 2000).

This isoform is expressed in the adult rat cerebellum, when the level of its transcripts in the whole brain is also maximal (Gleichmann *et al*, 2000).

CXCR4 signaling in cerebellar cells
CXCR4 expressed on central neurons and glial cells shows both PTX-sensitive (Bajetto *et al*, 1999; Klein *et al*, 1999; Zheng *et al*, 1999a, 1999b; Tanabe *et al*, 1997) and -insensitive signaling (Tanabe *et al*, 1997; Zheng *et al*, 1999b; Limatola *et al*, 2000a; Gillard *et al*, 2002), suggesting receptor coupling to a combination of $G_{\gamma}i$ and other G-proteins, likely cell type dependent (Fields and Casey, 1997). CXCR4 signaling cascade leads to the inhibition of adenylate cyclase: The inhibition of intracellular cAMP accumulation by forskolin treatment is reported for human cultured neurons (Zheng *et al*, 1999a, 1999b), although there are contrasting results in astrocytes (Zheng *et al*, 1999a; Bajetto *et al*, 1999).

A typical feature of chemokine receptor signaling is the induction of intracellular Ca^{2+} transients, which is confirmed for CXCR4 in hippocampal (Meucci *et al*, 1998), cortical (Bajetto *et al*, 1999), cerebellar (Limatola *et al*, 2000a; Klein *et al*, 2001; Gillard *et al*, 2002), human embryonic (Klein *et al*, 1999; Zheng *et al*, 1999a, 1999b), and dorsal root ganglion (Oh *et al*, 2001b) neurons, as well as in astrocytes (Tanabe *et al*, 1997; Bajetto *et al*, 1999, 2001a; Klein *et al*, 1999; Zheng *et al*, 1999a) and microglial cells (Tanabe *et al*, 1997; Albright *et al*, 2001).

In cerebellum, CXCR4 induces intracellular Ca^{2+} transients both in cultured granule and Purkinje neurons (Limatola *et al*, 2000a; Klein *et al*, 2001; Gillard *et al*, 2002) and in cultured astrocytes (our unpublished results). CXCR4-dependent Ca^{2+} transients are also reported in Purkinje neurons from rat cerebellar slices: These responses are not caused by the direct receptor activation on Purkinje neurons, but are mediated through a glutamatergic mechanism, being inhibited by an antagonist of metabotropic glutamate receptors (Limatola *et al*, 2000a). Cerebellar slice stimulation with CXCL12 likely causes the release of glutamate from either glial (Bezzi *et al*, 2001) and/or neuronal cells, stimulating metabotropic glutamate receptors on Purkinje neurons that mediate the Ca^{2+} transients (Limatola *et al*, 2000a). In cultured cerebellar granule cells, CXCR4-induced Ca^{2+} signaling seems to be subjected to some limitations, because contrasting results are reported on CXCL12 ability to induce increases of intracellular Ca^{2+} (Bajetto *et al*, 1999; Limatola *et al*, 2000a). However, the variability in neuronal response as a consequence of the culture conditions is a general feature of chemokines (Xia and Hyman, 2002) and may reflect the dependence on the activity of the neuronal network (Meucci *et al*, 1998) or different level of glial contamination. An alternative hypothesis is that the CXCR4 expressed on granule neurons may be inactivated due to its continuous stimulation by CXCL12, constitutively released from cultured granule cells (Bajetto *et al*, 1999). In

a recent study, cultured cerebellar granule neurons respond to CXCL12 treatment with an increase of intracellular Ca^{2+} concentration only following neuron treatment with 20 mM KCl or glutamate (Klein *et al*, 2001). The same result is obtained in fetal human and macaque neurons for other chemokines (Klein *et al*, 1999). The requirement of neuronal membrane depolarization for chemokine receptor–dependent Ca^{2+} increases may be suggestive of a glutamatergic mechanism, similar to that reported in cerebellar slices: Glutamate, released from granule cell terminals upon CXCR4 stimulation, acts on granule cells; the consequent depolarization of granule neurons, removing the Mg^{2+} blockage of NMDA receptors (Nowak *et al*, 1984), amplifies the Ca^{2+} transients. Accordingly, we observed that CXCL12-induced Ca^{2+} responses in cultured cerebellar granule cells are sensitive to glutamatergic antagonists (unpublished observations). Alternatively, it is possible that CXCR4-dependent Ca^{2+} signal is modulated by other intracellular signaling pathways (Scharenberg *et al*, 1998). In this respect, it is interesting to note that CXCR4 expressed on Jurkat T cells does not mobilize intracellular Ca^{2+} (Sostios *et al*, 1999). Depending on the cell type, CXCR4-induced Ca^{2+} transients rely on Ca^{2+} ions released from cellular stores, or fluxing from the extracellular space, or both (Hesselgesser *et al*, 1998a; Bajetto *et al*, 1999). CXCR4-mediated Ca^{2+} transients are insensitive to external Ca^{2+} removal in astrocytes but not in neurons (Bajetto *et al*, 1999; Coughlan *et al*, 2000). Ca^{2+} released from internal stores may be related to the CXCR4-induced stimulation of phospholipase C and to the corresponding accumulation of inositol phosphates, which is measured in embryonic neurons (Zheng *et al*, 1999a, 1999b), as well as in cerebellar granule neurons (Cristina Limatola, unpublished data). The CXCR4-induced intracellular Ca^{2+} increases may cause the regulation of membrane ionic channels, as it has been shown in primary cultures of macrophages. In these cells, CXCL12 elicits a transient Ca^{2+}-activated K^+ current and a sustained Ca^{2+}-independent $Cl^{\bigcirc}$ current (Liu *et al*, 2000b).

The stimulation of CXCR4 leads to the activation of the mitogen-activated protein kinases, ERK1/2, in cortical neurons, cultured astrocytes, cerebellar slices, and human astroglioma cells overexpressing CXCR4 (Lazarini *et al*, 2000; Bajetto *et al*, 2001a; Oh *et al*, 2001a; Gillard *et al*, 2002). At least in astrocytes, ERK1/2 activation is dependent on the upstream PI3-K pathway activity, because it is abolished by cell pretreatment with the inhibitor wortmannin (Bajetto *et al*, 2001a), and is mediated by PTX-sensitive G-protein in astrocytes, cortical cultures, and E15 neuronal progenitors (Lazarini *et al*, 2000; Bajetto *et al*, 2001a). Neither p38 nor JNK kinases are activated by CXCL12 on astrocytes (Bajetto *et al*, 2001a).

The possibility that different transduction pathways are involved in CXCR4 signaling in different cellular systems (Tanabe *et al*, 1997; Bajetto *et al*, 1999; Lazarini *et al*, 2000) may explain the

discrepancies found concerning the sensitivity to PTX (Zheng *et al*, 1999b; Limatola *et al*, 2000a; Gillard *et al*, 2002). In addition, differences arising from culture conditions and glial contamination might be considered.

Recently, it has been shown that CXCR4 stimulation triggers a signaling cascade leading to glutamate release from glial cells (Bezzi *et al*, 2001): By the use fluorimetric assay, it has been demonstrated that CXCR4 stimulation induces the Ca^{2+}-dependent release of glutamate from astrocytes by an exocytotic mechanism; in this model, glutamate release is the final step of a complex signaling cascade, involving tumor necrosis factor alpha (TNF-γ) release and its autocrine action. This pathway may be amplified by the activation of microglia; microglia-secreted TNF-γ would stimulate astrocytes in a paracrine fashion, further enhancing glutamate release (Bezzi *et al*, 2001). These mechanisms may be relevant upon neuronal injury in pathological states (Allen and Attwell, 2001): Due to its signaling pathway, leading to the modulation of membrane conductances (Liu *et al*, 2000b) and due to the possibility of inducing glutamate release (Bezzi *et al*, 2001), CXCR4 may have a key neuromodulatory role, altering neuronal properties and synaptic transmission.

Role of CXCR4 in cerebellar development
CXCR4 signaling has a key role in the development of cerebellar cytoarchitecture, as its expression is necessary for the correct organization of the EGL (Ma *et al*, 1998; Zou *et al*, 1998), where granule cell proliferation takes place (Goldowitz and Hamre, 1998). Deletion of CXCR4 gene induces errors in the embryonic development of the cerebellar cortex, including the formation of an abnormal EGL with a very small number of granule cells (Ma *et al*, 1998; Zou *et al*, 1998). CXCR4 ◯/◯ mice are also defective in the localization of Purkinje cells that do not form the typical layer and have ectopic localization (Ma *et al*, 1998), although the same defect is not observed by Zou *et al* (1998). The observation that CXCL12 knockout mice show similar phenotypes reinforces the specificity of CXCL12 interaction with CXCR4 (Ma *et al*, 1998; but see Zou *et al*, 1998). Besides cerebellar defects, deletion of CXCL12 or CXCR4 genes causes many other abnormalities, and both types of knockout mice die perinatally (Tachibana *et al*, 1998; Ma *et al*, 1998), rendering impossible to further characterize the role of CXCR4 in cerebellar development, which in mice is completed by the end of the 3rd postnanal week (Goldowitz and Hamre, 1998).

The abnormal cerebellar development in CXCR4 ◯/◯ mice is likely due to the premature migration of granule cells from the EGL towards the IGL (Ma *et al*, 1998; Zou *et al*, 1998). Therefore, the possible role of CXCR4 expressed on granule cells in the mouse EGL around birth (Zou *et al*, 1998; McGrath *et al*, 1999; Lu *et al*, 2001) is to prevent this premature migration. CXCL12 expressed on the surface of the pia mater (McGrath *et al*, 1999; Klein *et al*, 2001) likely acts as a signal to keep granule cells in the EGL at this stage (Zou *et al*, 1998; Klein *et al*, 2001), in analogy to lymphocyte localization in the secondary lymphoid organs (Bleul *et al*, 1998). The lack of CXCR4 expression in knockout mice might also cause the impairment of tangential migration of postmitotic granule cells, thus not allowing them to recognize their assigned migratory position (see Komuro *et al*, 2001).

The role of CXCL12 in cerebellar development and specifically in regulating the migration of granule cells has to be considered in light of its known chemotactic actions (Bleul *et al*, 1996b), which is also exerted on differentiated NT2N cells (Hesselgesser *et al*, 1997), neuronal progenitors (Lazarini *et al*, 2000), cerebellar granule cells (Klein *et al*, 2001; Lu *et al*, 2001), and microglial cells (Tanabe *et al*, 1997). CXCL12 does not stimulate astrocytes migration (Tanabe *et al*, 1997; Lazarini *et al*, 2000), but inhibits the chemotactic responses to other chemokines (Dorf *et al*, 2000). CXCL12 also causes repulsion from a chemokine gradient (Poznansky *et al*, 2000).

Despite its established roles in chemotaxis, CXCR4 is not a migratory signal for embryonic granule cells, rather exerting a static action (Ma *et al*, 1998; Zou *et al*, 1998). It is now emerging that granule cell migration, a key step of cerebellar development, requires multiple environmental cues and is regulated by complex signaling, including growth factors, voltage-activated Ca^{2+} channels, and NMDA receptors (reviewed in Komuro and Rakic, 1998).

In a recent paper, Lu *et al* (2001) describe a possible mechanism of action of CXCR4 signaling in the regulation of granule cells migration: In cultured granule cells, CXCL12-induced chemotaxis is selectively inhibited by the soluble Eph2B receptors, through the action of the regulatory cytoplasmic protein PDZ-RGS3. Because Eph2B and B ephrins are both expressed in the EGL of P3 mice, their interaction on granule cells at this stage could inhibit CXCR4 signaling, allowing cells to start inward migration. The authors propose a model of cerebellar development where pial CXCL12 acting on granule cells CXCR4 inhibits granule cells' inward migration (Lu *et al*, 2001). In addition, CXCL12 mRNA expression in cerebellar granules temporally correlates with granule cell migration to the IGL, as it terminates when the migration is completed (Tham *et al*, 2001). Thus, CXCL12 secreted from granule cells (Bajetto *et al*, 1999) might act as an autocrine signal coordinating cell migration (Lu *et al*, 2001).

CXCL12-CXCR4 interaction is potentially relevant in other processes, including granule cell proliferation in the EGL (Klein *et al*, 2001): (i) In cerebellar granule cells cultures, the stimulation of CXCR4 by CXCL12 increases the proliferative responses induced by the sonic hedgehog (Klein *et al*, 2001), which is physiologically released by Purkinje neurons in the cerebellum (see Dahmane and Ruitz-i-Altaba, 1999, for review). (ii) CXCR4 expression may

have an indirect effect on granule cell proliferation, prolonging their presence in the proliferative EGL (Klein *et al*, 2001). In addition CXCR4 are proposed to stimulate neurite outgrowth and the formation of synaptic contacts (Tham *et al*, 2001), also considering that CXCL12 expression in the IGL in the postnatal cerebellum coincides with an intense synaptogenesis (Altman, 1973).

In conclusion, CXCR4 signaling plays a crucial role in the orchestration of cerebellar development, being a determinant of granule cells number and positioning. It is not clear if such role may fully be extended to human brain where CXCR4 early expression has not been demonstrated.

CXCR4 and neuroprotection/neurodegeneration

The massive stimulation of chemokine receptors during inflammatory processes may lead to neuronal apoptosis and neurodegeneration (Glabinski and Ransohoff, 1999). Neurotoxic effects of the activation of CXCR4 have been extensively investigated in relation to the involvement of CXCR4 cascade in human immunodeficiency virus (HIV)-associated dementia (Miller and Meucci, 1999; Kaul *et al*, 2001). Chronic CXCL12 administration causes apoptosis in cultured human embryonic neurons and rat cerebellar granule cells (Zheng *et al*, 1999a, 1999b), antagonized by the anti-CXCR4 antibody 12G5. (Davis *et al*, 1997). Neurotoxic effects of CXCL12 are reported in cultured neurons (Hesselgesser *et al*, 1998b; Zheng *et al*, 1999b) and in mixed neuronal-glial cultures (Kaul and Lipton, 1999). NMDA receptors overstimulation is considered one of the key steps leading to neuronal death in neurological insults (Skaper *et al*, 2001). The role of glutamate in CXCR4-dependent neuroxicity is now confirmed by the report of Bezzi *et al* (2001), showing link between CXCR4 stimulation and glutamate release from astrocytes and reporting a protective effect of the NMDA receptor antagonist 2-amino-s-phosphonovaleric acid (APV) from CXCR4-induced apoptosis. On the other hand, Meucci and colleagues (1998) observed a protective effect of CXCL12 in cultured hippocampal neurons, when apoptosis was stimulated by glial deprivation or gp120 treatment. These phenomena represent general mechanisms of neuronal damages following CNS chronic inflammation. It is, however, intriguing to speculate if similar CXCR4-dependent apoptotic mechanism may be involved in the determination of neuronal number during cerebellar development (Goldowitz and Hamre, 1998).

CXCR4 and neuromodulation

Stimulation of CXCR4 modulates synaptic activity in both cultured neurons and brain slices (Meucci *et al*, 1998; Zheng *et al*, 1999b; Limatola *et al*, 2000a). Various chemokines alter neurotransmission in cultured hippocampal neurons. In synaptically connected neurons, the stimulation of CXCR4 by

CXCL12 induces a decrease in the frequency glutamatergic Ca^{2+} oscillations (Meucci *et al*, 1998). On the other hand, CXCR4 induces the increase of the evoked postsynaptic potentials recorded extracellularly in CA1 field of hippocampal slices (Zheng *et al*, 1999b).

In the cerebellum, CXCR4 stimulation modulates synaptic inputs: In cerebellar slices, CXCL12 applications induces a TTX-insensitive increase in the frequency of γ-aminobutyric acid (GABA)-ergic (Limatola *et al*, 2000a) and glutamatergic (Ragozzino *et al*, 2002) synaptic currents in Purkinje neurons. The modulation of synaptic transmission is of presynaptic origin, as shown by the absence of changes in the amplitude distribution of PSCs, and involves the activation of glutamate receptors, as it is absent in the presence of glutamatergic antagonists (Limatola *et al*, 2000a). In addition, CXCL12 elicits a slow inward current and the increase in intracellular Ca^{2+} in Purkinje neurons; membrane currents and Ca^{2+} transients are both of glutamatergic nature and are mediated, respectively, by AMPA-type ionotropic glutamate receptors and by metabotropic glutamate receptors (Limatola *et al*, 2000a), suggesting that CXCR4 stimulation induces glutamate release from astrocytes or synaptic terminals, as supported by the CXCR4-dependent glutamate release from glial cells (Bezzi *et al*, 2001). Consistently, it has been recently observed that CXCL12 reduces eEPSCs at parallel fibers–Purkinje neurons synapses in the cerebellum by a glutamatergic mechanism (Ragozzino *et al*, 2002). In addition, CXCL12 might interfere with neurotransmitter release through the regulation of Ca^{2+} channels, recently reported in transfected cells (Oh *et al*, 2002).

The physiological relevance of CXCR4-dependent neuromodulation in the cerebellum of adult rodents may be questioned in light of the decrease of CXCR4 and CXCL12 expression in rodent cerebellum during postnatal development (Banisadr *et al*, 2000; Tham *et al*, 2001). However, CXCR4 is detected in the adult rat by immunohistochemical techniques on Purkinje neurons, granule cells, and radial glial fibres (Limatola *et al*, 2000a); moreover, the recently characterized SDF-1γ/CXCL12 has been detected by *in situ* hybridization in Purkinje neurons and granule cells of the adult rat cerebellum (Gleichmann *et al*, 2000). These evidences confirm that CXCR4 participates in the modulation of neurotransmission under physiological conditions. CXCR4 neuromodulation may be more relevant in the adult human brain where CXCR4 is largely expressed (van der Meer *et al*, 2000).

In conclusion, in the mature cerebellum, the stimulation of CXCR4 may profoundly interfere with neurotransmission, inducing glutamate release from glial cells. Furthermore, glutamate receptors are known not only to mediate fast neurotransmission, but also to modulate neurotransmission both by pre- and postsynaptic mechanisms (Ziff, 1999; Shoepp, 2001). The

neuromodulatory effects would arise much faster than the neurotoxic effects, predictably playing a significant role during inflammatory processes.

Conclusions

It is now clear that chemokines and their receptors are constitutively present in the CNS and exert functions beyond their involvement in neuroinflammation. All three CXC chemokine receptors considered here are expressed in the cerebellum from embryonic stages and are candidate to a role in cell-cell communication within the CNS. Particular interest is now focused on CXCR2 and CXCR4, because they have been involved in several functional processes in the cerebellum and in other brain areas, including cell migration and proliferation during development, neuroprotection and neurodegeneration following CNS insults, and neuromodulation. On the other, hand little is known about the function of the *silent* Duffy antigen receptor (DARC), which is prominently expressed in cerebellar neurons (Horuk *et al*, 1997; Luo *et al*, 2000a; Luan *et al*, 2001). It is emerging that CXC chemokine receptors cascades modulate membrane ion channels and influence neurotransmission through short- and long-term mechanisms. Interestingly, they seem to interact preferentially with glutamatergic pathways, not only altering synaptic transmission, but also through more sophisticated mechanisms, including glia-neuron interactions and signaling cross-talk. In addition to CXC chemokines, other chemokines also likely exert relevant constitutive functions in the nervous system; among them, fractaline/CX3CL1 and its receptor CX3CR1 are widely expressed in the CNS (Harrison *et al*, 1998; Nishiyori *et al*, 1998) and have neuroprotective action, likely participating in neuron-glia communication (Meucci *et al*, 2000).

Much study is still necessary to shed full light on chemokine-dependent mechanisms in the CNS, with particular respect to the definition of the pathophysiological conditions in which these mechanisms may become relevant for brain function.

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