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Fractalkine (CX3CL1) and brain inflammation: Implications for HIV-1–associated dementia

R Cotter,[1,2] C Williams,[1,2] L Ryan,[1,2] David Erichsen,[1,2] A Lopez,[1,2] H Peng,[1,2] and J Zheng[1,2,3]

[1]The Laboratory of Neurotoxicology, the Center for Neurovirology and Neurodegenerative Disorders, and the Departments of [2]Pathology and Microbiology and [3]Pharmacology, University of Nebraska Medical Center, Omaha, Nebraska, USA

Leukocyte migration and activation play an important role in immune surveillance and the pathogenesis of a variety of neurodegenerative disorders, including human immunodeficiency virus (HIV)-1–associated dementia (HAD). A novel chemokine named fractalkine (FKN, CX3CL1), which exists in both membrane-anchored and soluble isoforms, has been proposed to participate in the generation and progression of inflammatory brain disorders. Upon binding to the CX3C receptor one (CX3CR1), FKN induces adhesion, chemoattraction, and activation of leukocytes, including brain macrophages and microglia (MP). Constitutively expressed in the central nervous system (CNS), mainly by neurons, FKN is up-regulated and released in response to proinflammatory stimuli. Importantly, FKN is up-regulated in the brain tissue and cerebrospinal fluid (CSF) of HAD patients. Together, these observations suggest that FKN and its receptor have a unique role in regulating the neuroinflammatory events underlying disease. This review will examine how FKN contributes to the recruitment and activation of CX3CR1-expressing MP, which are critical events in the neuropathogenesis of HAD. *Journal of NeuroVirology* (2002) **8,** 585–598.

Keywords: chemokine receptors; chemokines; fractalkine; HIV-1–associated dementia

Introduction

Human immunodeficiency virus (HIV)-1–associated dementia (HAD) is a late-stage complication of advanced HIV-1 disease (Carpenter *et al*, 2000; Krebs *et al*, 2000; McArthur *et al*, 1999). Clinically, HAD results in a spectrum of neurological and psychiatric symptoms, including cognitive impairment, hallucinations, delirium, coma, and ultimately death (Gelbard and Epstein, 1995; Janssen *et al*, 1991; Marder *et al*, 1996; Masliah, 1996; Navia *et al*, 1986). The histopathological correlate of HAD is HIV-1 encephalitis (HIVE), which occurs in most, but not all, cases of dementia related to HIV-1 infection (Glass *et al*, 1995; Masliah, 1996; Wiley, 1995). HIVE features blood-brain-barrier (BBB) damage, productive viral infection, immune activation of mononuclear phagocytes (MP; brain macrophage and microglia), astrogliosis, and neuronal injury, apoptosis, and loss (Asare *et al*, 1996; Dickson *et al*, 1994; Gabuzda and Wang, 1999; Gendelman *et al*, 1997; Glass *et al*, 1995; Masliah *et al*, 2000; McArthur *et al*, 1999; Nath and Geiger, 1998; Navia *et al*, 1986; Rappaport *et al*, 1999; Wiley and Achim, 1994). It is believed that MP, the predominate cell type infected in the brain, induce neuronal injury and death through the production of neurotoxins (Gendelman *et al*, 1997; Genis *et al*, 1992; Giulian *et al*, 1990; Koenig *et al*, 1986; Ma *et al*, 1994; Moses *et al*, 1993; Nath *et al*, 1995; Pulliam *et al*, 1991; Ranki *et al*, 1995; Tornatore *et al*, 1991; Wiley *et al*, 1991). Given what is known about the involvement of MP in HIVE, it is important to understand how MP become immune activated. Recently, it has been proposed that neurons may directly participate in the disease process by inducing MP

Address correspondence to Jialin Zheng, Laboratory of Neurotoxicology, Center for Neurovirology and Neurodegenerative Disorders, 985215 Nebraska Medical Center, Omaha, NE 68198-5215, USA. E-mail: jzheng@unmc.edu

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recruitment and activation through release of soluble chemotactic factors. This review will examine the role that neuronal chemokines play in MP recruitment and activation during HAD.

Chemokines and chemokine receptors in the CNS

Chemoattractant cytokines (chemokines) are soluble molecules that regulate the migration and activation of leukocytes into brain and other tissues (Kutsch *et al*, 2000; Wu *et al*, 2000). More than 46 chemokines have been identified (Baggiolini *et al*, 1997; Zlotnik and Yoshie, 2000) and are classified into the following four groups (Table 1): alpha (CXC), beta (CC), gamma (C-chemokines), and delta (CX3C), based on the arrangement of cysteine residues within the receptor-binding domain. For example, CXC chemokines have two cysteine residues separated by a single amino acid, whereas in CC chemokines the cysteines are adjacent. Chemokines exert their effects by binding to and activating a family of seven-transmembrane, G-protein–coupled receptors (GPCRs). These receptors are divided into four groups: α-chemokine receptors (CXCR1-6), β-chemokine receptors (CCR1-10), γ-chemokine receptors (XCR1), and δ-chemokine receptors (CX3CR1) (Hesselgesser and Horuk, 1999; Klein *et al*, 1999; van der Meer *et al*, 2000) (review in Gabuzda *et al*, 2002; Karpus, 2001; Miller and Meucci, 1999; Ransohoff, 1998). In addition to mediating leukocyte recruitment and activation, chemokine receptors, such as CCR5 and CXCR4, also serve as coreceptors for HIV-1 (Dragic *et al*, 1996; He *et al*, 1997). Importantly, the endogenous ligands (RANTES, macrophage inflammatory protein [MIP]-1α/β) for these receptors have been shown to block HIV-1 binding and entry, suggesting that the production of these factors may be an important defense mechanism against HIV-1 infection in the human host (Kornbluth *et al*, 1998).

A wide range of chemokines are expressed in the brain during diseases, including α-chemokines, such as interleukin-8 (IL-8, CXCL8) and stromal-derived factor-1 alpha (SDF-1α, CXCL12); β-chemokines, such as monocyte chemoattractant protein (MCP)-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4), and RANTES (CCL5); and the δ-chemokine, fractalkine (FKN, CX3CL1) (Conant *et al*, 1998; Cotter *et al*, 1999b; Coughlan *et al*, 2000; Desbaillets *et al*, 1994; Gabuzda and Wang, 2000; Kornbluth *et al*, 1998; Persidsky, 1999; Zheng *et al*, 1999). Chemokines, such as SDF-1α, IL-8, and fractalkine (FKN), are constitutively produced in the brain and play an important role in central nervous system (CNS) homeostasis and development (Coughlan *et al*, 2000; Gabuzda and Wang, 2000; Gleichmann *et al*, 2000; Harrison *et al*, 1998; Horuk *et al*, 1996; Meucci *et al*, 1998, 2000; Nagasawa *et al*, 1996). Upon binding to neuronal receptors (CXCR4, CXCR2, and CX3CR1), these chemokines activate signaling pathways that regulate neuronal survival, injury, and repair (Kaul and Lipton, 1999; Meucci *et al*, 1998, 2000; Peng *et al*, 2002; Tong *et al*, 2000; Zheng *et al*, 2001). For example, knockout mice lacking CXCR4 exhibit abnormal migration of cerebellar external granule layer cells and other nervous system defects (Zou *et al*, 1998). These findings underscore the importance of chemokines and their receptors in neuronal cell development and maintenance.

Table 1 Chemokine and chemokine receptor families*

Systematic name	*Human ligand*	*Chemokine receptors*
Alpha (CXC) chemokine-receptor family		
CXCL1	GROα/MGSAα	CXCR2, CXCR1
CXCL2	GROβ/MGSAβ	CXCR2
CXCL3	GROγ/MGSAγ	CXCR2
CXCL4	PF4	Unknown
CXCL5	ENA-78	CXCR2
CXCL6	GCP-2	CXCR1, CXCR2
CXCL7	NAP-2	CXCR2
CXCL8	IL-8	CXCR1, CXCR2
CXCL9	Mig	CXCR3
CXCL10	IP-10	CXCR3
CXCL11	I-TAC	CXCR3
CXCL12	SDF-1 (α/β)	CXCR4
CXCL13	BCA-1	CXCR5
CXCL14	BRAK/bolkine	Unknown
(CXCL15)	Unknown	Unknown
CXCL16		CXCR6
Beta (CC) chemokine-receptor family		
CCL1	I-309	CCR8
CCL2	MCP-1/MCAF/TDCF	CCR2
CCL3	MIP-1α/LD78α	CCR1, CCR5
CCL4	MIP-1β	CCR5
CCL5	RANTES	CCR1, CCR3, CCR5
(CCL6)	Unknown	Unknown
CCL7	MCP-3	CCR1, CCR2, CCR3
CCL8	MCP-2	CCR3, CCR5
(CCL9/10)	Unknown	CCR1
CCL11	Eotaxin	CCR3
(CCL12)	Unknown	CCR2
CCL13	MCP-4	CCR2, CCR3
CCL14	HCC-1	CCR1, CCR5
CCL15	HCC-2/Lkn-1/MIP-1δ	CCR1, CCR3
CCL16	HCC-4/LEC/LCC-1	CCR1, CCR2
CCL17	TARC	CCR4
CCL18	DC-CK1/PARC/AMAC-1	Unknown
CCL19	MIP-3β/ELC/exodus-3	CCR7
CCL20	MIP-3α/LARC/exodus-1	CCR6
CCL21	6Ckine/SLC/exodus-2	CCR7
CCL22	MDC/STCP-1	CCR4
CCL23	MPIF-1/CKβ8/CKβ8-1	CCR1
CCL24	Eotaxin-2/MPIF-2	CCR3
CCL25	TECK	CCR9
CCL26	Eotaxin-3	CCR3
CCL27	CTACK/ILC	CCR10
CCL28	MEC	CCR3/CCR10
Gamma (C) Chemokine/receptor family		
XCL1	Lymphotactin/SCM-1α	XCR1
XCL2	SCM-1β	XCR1
Delta (CX3C) chemokine/receptor family		
CX3CL1	Fractalkine/neurotactin	CX3CR1

*Modified from Zlotnik and Yoshie (2000).

Chemokines also play a critical role in the host response to CNS injury and infection. Indeed, the role of chemokines and their receptors in neurodegenerative disorders, such as multiple sclerosis, Alzheimer's disease, stroke, and HAD, has been extensively investigated and reviewed (Gabuzda *et al*, 1998, 2002; Karpus, 2001; Letendre *et al*, 1999; Minami and Satoh, 2000; Ransohoff, 1997; Sanders *et al*, 1998). Several reports have shown that SDF-1α, IL-8, MIP-1α, MIP-1β, RANTES, MCP-1, and FKN are up-regulated in brain tissue and cerebrospinal fluid (CSF) from HAD patients (Coughlan *et al*, 2000; Kelder *et al*, 1998; Persidsky *et al*, 1999; Tong *et al*, 2000; Zheng *et al*, 1999, 2000). It has been proposed that these chemokines contribute to HAD pathogenesis through recruitment of monocytes into the brain, through initiation of neuroinflammatory cascades that affect viral replication, through induction of neural signaling and apoptosis, or through initiation of neuronal protection and repair (Albright *et al*, 1999; Broder and Collman, 1997; Cotter *et al*, 2001; Endres *et al*, 1996; Ghorpade *et al*, 1998; He *et al*, 1997; Kitai *et al*, 2000; Lavi *et al*, 1997; Luster, 1998; Mackay, 1996; Shieh *et al*, 1998; Vallat *et al*, 1998; Vicenzi *et al*, 2000; Zheng *et al*, 1999) (review in Gabuzda *et al*, 2002; Miller and Meucci, 1999). Although it is evident that chemokines are an important component of the host immune response, the nature of their role in disease pathogenesis is only beginning to be understood.

Neuronal chemokines and MP activation, a "chicken or egg" question

Traditionally, it was believed that MP activation and chemokine production preceded neuronal injury in HIVE. However, new evidence suggests that neurons themselves may initiate MP recruitment and activation (Biber *et al*, 2001; Harrison *et al*, 1998). Indeed, it has been proposed that in response to injury, neurons produce chemokines, such as FKN (Harrison *et al*, 1998), that act as "distress signals." Upon release, these factors recruit MP to sites of injury and stimulate the production of inflammatory factors with the potential to repair or exacerbate neuronal damage (Tong *et al*, 2000; Zheng *et al*, 2000) (Figure 1).

There are several lines of evidence that provide support for this hypothesis. *First*, excitotoxin-mediated neuronal injury, as well as nerve axotomy, induces production of the neuronal chemokine FKN (Chapman *et al*, 2000a; Harrison *et al*, 1998; Zheng *et al*, 2000). *Second*, this neuronal chemokine is also up-regulated in HAD brain tissue (Pereira *et al*, 2001; Tong *et al*, 2000; Zheng *et al*, 2000) and released in response to neuronal apoptosis induced by HIV-1 progeny virions (IIIB and ADA) and gp120 (Zheng *et al*, 2000). *Third*, both the soluble and membrane-bound forms of FKN have been shown to attract and immobilize leukocytes, such as monocytes and lymphocytes (Boehme *et al*, 2000; Chapman *et al*, 2000a, 2000b; Combadiere *et al*, 1998; Dorf *et al*, 2000; Fong *et al*, 1998; Goda *et al*, 2000; Harrison *et al*, 1998; Imai *et al*, 1997; Tong *et al*, 2000). The monocytes, when recruited to the site of injury, could secrete other chemokines that recruit additional leukocytes to the site of tissue injury and induce inflammation. Indeed, the FKN-CX3CR1 pair may participate in the generation and progression of inflammatory disorders within the brain and periphery. FKN has already been shown to play a role in a variety of pathological conditions related to inflammation, including atherosclerosis (Alexander, 2001; Greaves and Gordon, 2001; Greaves *et al*, 2001; McDermott *et al*, 2001), renal inflammation (Cockwell *et al*, 2002; Feng *et al*, 1999; Furuichi *et al*, 2001), airway inflammation (Fujimoto *et al*, 2001), psoriasis (Raychaudhuri *et al*, 2001), arthritis (Ruth *et al*, 2001; Volin *et al*, 2001), cardiac allograft rejection (Haskell *et al*, 2001), progression of acquired immunodeficiency syndrome (AIDS) (Faure *et al*, 2000; Foussat *et al*, 2001), and CNS inflammation (Boehme *et al*, 2000; Harrison *et al*, 1998; Hughes *et al*, 2002; Maciejewski-Lenoir *et al*, 1999; Nishiyori *et al*, 1998; Schwaeble *et al*, 1998; Zujovic *et al*, 2000). These observations suggest that FKN and its receptor have a unique role in regulation of the host response to disease (Fong *et al*, 1998; Harrison *et al*, 1998).

The balance of data demonstrates overwhelming support for FKN mediated recruitment of leukocytes. However, studies with FKN and CX3CR1 knockout mice (Cook *et al*, 2001; Jung *et al*, 2000) have tempered the importance of FKN and CX3CR1 in leukocyte recruitment. In studies (Cook *et al*, 2001) with FKN-deficient mice, responses to a variety of inflammatory stimuli were indistinguishable from those of wild-type mice in an intestine inflammation model system (Cook *et al*, 2001). In other reports (Jung *et al*, 2000), the absence of CX3CR1 did not interfere with either monocyte extravasation or dendritic cell migration and differentiation in a peritonitis model. Further, CX3CR1-deficient microglia exhibited proficient responses to peripheral nerve injury, indicating unimpaired neuronal-glial cross-talk in the absence of CX3CR1 (Jung *et al*, 2000). These findings suggest that other means of neuronal-glial linkage exist. Nevertheless, to elucidate the exact role of FKN- and CX3CR1-expressing cells in disease pathogenesis, further studies are certainly required. Moreover, FKN-CX3CR1 interactions may mediate other inflammatory responses besides the recruitment of leukocytes. The following section will examine how FKN mediates communication between neurons and MP during homeostasis and disease.

FKN expression, structure, and regulation

FKN (neurotactin, CX3CL1) is a 373–amino acid, multidomain molecule found in a wide variety of tissues, including liver, intestine, kidney, and brain.

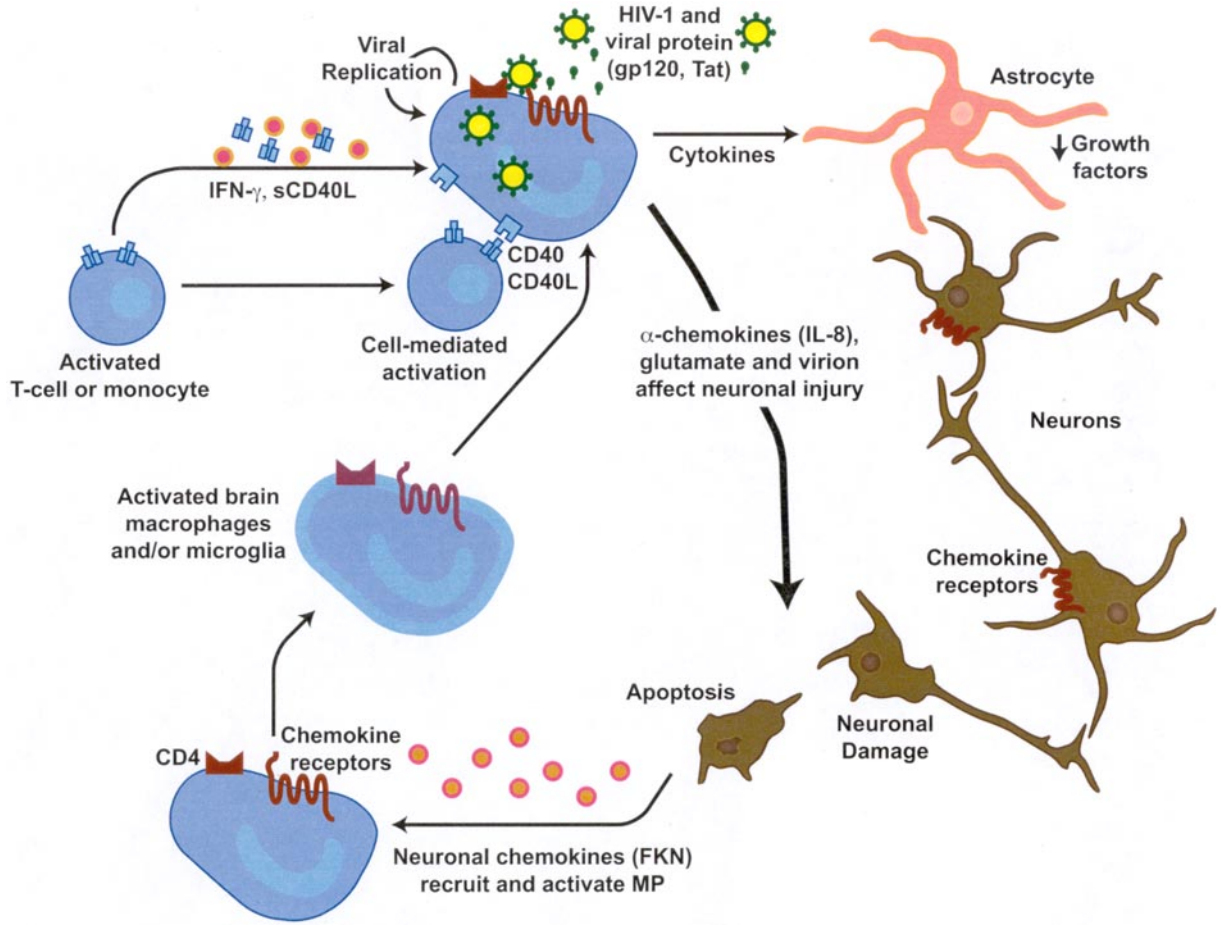

Figure 1 A proposed pathophysiological mechanism for how MP activation influences neuronal injury in HAD. During monocyte/macrophage maturation, macrophages acquire the ability to sustain productive HIV-1 infection. Release of progeny virion leads to infection of resident brain microglia. Uninfected or virus-infected MP can be immune-activated by a process that remains incompletely understood, but likely involves cytokines, chemokines, and cell-to-cell interactions. Released from injured neurons, the neuronal chemokine fractalkine (FKN) represents one pathway through which MP activation may occur. After recruiting MP to the site of injury, FKN may activate MP to produce neurotrophic/toxic factors that affect neuronal survival and induce CNS inflammation. In turn, recruited MP may also become infected and activation leading to the production of chemokines, cytokines, and glutamate. Chemokines, gp120, and whole virions may also interact with neuronal receptors to alter intracellular signal transduction pathways, leading to neuronal dysfunction and death.

Structural components of FKN include a 76–amino acid chemokine domain (CD) at the N-terminus, which is important in the binding, adhesion, and activation of its target cells (Harrison *et al*, 2001; Mizoue *et al*, 1999, 2001; Goda *et al*, 2000; Haskell *et al*, 2000). In addition, FKN has a 241–amino acid mucin-like stalk, which extends the chemokine domain away from the cell surface in order to aid in the adherence of CX3CR1-expressing cells (Fong *et al*, 2000). FKN also has an 18–amino acid stretch of hydrophobic residues that spans the cell membrane, and an extended C-terminus that anchors it to the cell surface (Cook *et al*, 2001; Hoover *et al*, 2000; Lucas *et al*, 2001). These unique structural features enable FKN to mediate chemotaxis, adherence, and activation of CX3CR1-expressing cells.

FKN is novel in that it is the only chemokine known to be expressed at higher levels within the CNS than in the periphery (Bazan *et al*, 1997). In the CNS, FKN is constitutively expressed by neurons (Harrison *et al*, 1998; Hughes *et al*, 2002) (Figure 2) and can be induced by astrocytes (Hughes *et al*, 2002; Pereira *et al*, 2001; Zheng *et al*, 2002). It is up-regulated and released in response to proinflammatory stimuli, such as lipopolysaccharide (LPS), IL-1β, tumor necrosis factor (TNF)-α, CD40L, and interferon (IFN)-γ (Fraticelli *et al*, 2001; Fujimoto *et al*, 2001; Garcia *et al*, 2000; Hughes *et al*, 2002; Imaizumi *et al*, 2000; Pereira *et al*, 2001; Yoshida *et al*, 2001; Zheng *et al*, 2002). This up-regulation is believed to occur through activation of nuclear factor (NF)-κB (Garcia *et al*, 2000).

FKN is also distinct from other chemokines, because it exists in both membrane-bound and soluble isoforms (Fong *et al*, 2000; Harrison *et al*, 2001; Mizoue *et al*, 2001). In response to excitotoxic stimuli, the membrane-spanning domain is rapidly cleaved and a soluble form of FKN is released from

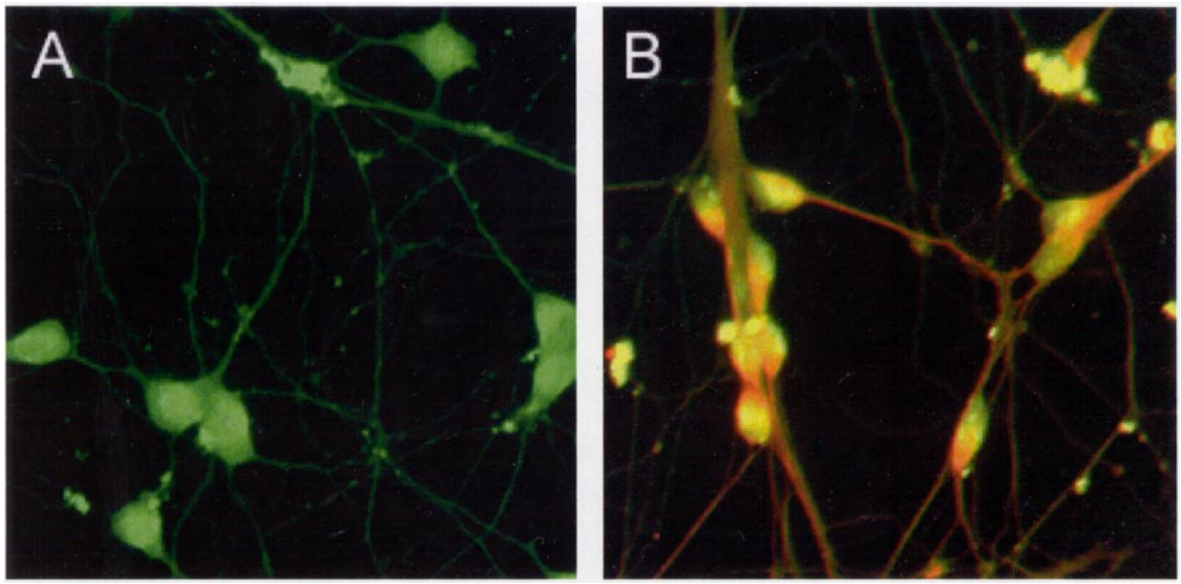

Figure 2 FKN expression in human neuronal cells. Panel (**A**) shows mixed human cortical cells in culture that were stained for FKN (green) and panel (**B**) shows neurons double-stained for FKN and MAP-2 (yellow, neuronal marker) (200×). Results are representative of three independent experiments.

the neuronal cell surface (Chapman *et al*, 2000a; Zheng *et al*, 2000). Proteolytic cleavage of FKN is proposed to occur at a di-arginine sequence located next to the transmembrane domain (Bazan *et al*, 1997; Cook *et al*, 2001; Fong *et al*, 2000; Harrison *et al*, 1998, 2001). However, the exact location of the cleavage site remains to be confirmed (Tsou *et al*, 2001). FKN cleavage can be mediated by two distinct metalloproteinase-dependent activities: a constitutive FKN sheddase, which is active under normal cell culture conditions, and an inducible FKN sheddase that can be rapidly activated by phorbol esters, such as phorbol 12-myristate 13-acetate (PMA) (Garton *et al*, 2001; Tsou *et al*, 2001). Recently, inducible cleavage has been shown to be mediated by the TNF-α–converting enzyme (TACE), which belongs to a family of proteins containing a metalloprotease domain (Garton *et al*, 2001; Tsou *et al*, 2001).

The receptor for FKN, CX3CR1 (Combadiere *et al*, 1998), is expressed on monocytes (Cambien *et al*, 2001; Chapman *et al*, 2000b), dendritic cells (Dichmann *et al*, 2001), T lymphocytes (Fong *et al*, 1998; Foussat *et al*, 2000; Fraticelli *et al*, 2001), natural killer cells (Fong *et al*, 1998; Imai *et al*, 1997; Inngjerdingen *et al*, 2001), astrocytes (Dorf *et al*, 2000), neurons (Hughes *et al*, 2002; Meucci *et al*, 2000; Tong *et al*, 2000), and brain microglia (Boehme *et al*, 2000; Chapman *et al*, 2000a; Harrison *et al*, 1998; Hughes *et al*, 2002). Like other chemokine receptors, CX3CR1 (previously called V28) belongs to a family of GPCRs, which feature a seven-transmembrane domain, an extracellular N-terminus, and a cytoplasmic C-terminus. GPCRs interact with and signal through heterotrimeric guanine nucleotide–binding regulatory proteins (G-proteins). Upon stimulation by a ligand, GPCRs undergo a conformational change that leads to activation of the G-protein by GDP-GTP exchange, followed by uncoupling of the G-protein from the receptor. Upon activation, G-proteins trigger a cascade of signaling events that regulate various cellular functions (Devi, 2000).

FKN functions: Cell adhesion and neuroprotection

In the brain, FKN is believed to regulate a complex network of paracrine and autocrine interactions between neurons and surrounding MP (Boehme *et al*, 2000; Harrison *et al*, 1998; Maciejewski-Lenoir *et al*, 1999), primarily through chemoattraction and adhesion. Both the soluble and membrane-bound forms of FKN are potent inducers of chemotaxis (Harrison *et al*, 2001). However, it is the membrane-bound form that enables FKN to immobilize CX3CR1-expressing cells, such as leukocytes (Boehme *et al*, 2000; Chapman *et al*, 2000a, 2000b; Combadiere *et al*, 1998; Dorf *et al*, 2000; Fong *et al*, 1998; Harrison *et al*, 1998; Imai *et al*, 1997; Tong *et al*, 2000). Mutation analyses and knockout mouse experiments have shown that specific residues within the FKN CD, such as Lys-7 and Arg-47, are important determinants in mediating binding, signaling, and adhesion of CX3CR1-expressing cells (Goda *et al*, 2000; Harrison *et al*, 2001; Haskell *et al*, 2000; Mizoue *et al*, 1999, 2001). Further, the adherence of FKN to CX3CR1-expressing leukocytes is believed to be integrin independent (Fong *et al*, 1998). Other studies suggest that adhesion of CX3CR1-expressing leukocytes is independent of G-protein activation (Haskell *et al*, 1999). It is

possible that the mucin-like domain of FKN may aid in adherence of CX3CR1-expression cells by extending the chemokine domain away from the cell surface in order to present it to trafficking leukocytes (Fong *et al*, 2000). Additionally, it is possible that the ability of FKN to mediate adhesion of trafficking cells may be a function of its slow receptor off-rate (Haskell *et al*, 2000). Nevertheless, it is clear that FKN and CX3CR1 fulfill important roles in leukocyte trafficking.

In addition to chemoattraction and adhesion, FKN may serve other functions, such as inhibition of HIV-1 infection and neuroprotection (Fong *et al*, 1998; Harrison *et al*, 1998; Haskell *et al*, 2000; Inngjerdingen *et al*, 2001; Tong *et al*, 2000). For example, FKN inhibits HIV-1 entry into CX3CR1-expressing cells (Faure *et al*, 2000) and inhibits neuronal injury induced by gp120 (Meucci *et al*, 2000), platelet-activating factor (PAF), and the regulatory HIV-1 gene product, Tat (Tong *et al*, 2000). FKN has also been shown to inhibit Fas-mediated death in microglia (Boehme *et al*, 2000). The protective functions of FKN are believed to be mediated through activation of signaling pathways involving the protein kinase, Akt (protein kinase B), and NF-κB, which are major components of prosurvival signaling pathways in neurons and microglia (Boehme *et al*, 2000; Meucci *et al*, 2000).

FKN and macrophage activation: Dysregulation of neurotrophic/toxic factors?

Because CX3CR1 is highly expressed on MP, it is possible that FKN-CX3CR1 interactions play an important role in mediating MP immune activation. Upon binding to CX3CR1, FKN has been shown to stimulate TNF-α and IL-8 production in MP (Figure 3) (Zheng *et al*, 2002; Zujovic *et al*, 2000). Although many of the individual factors secreted by FKN-activated MP remain to be determined, it is known that HIV-1–infected and immune-activated MP are capable of producing a wide variety of toxic factors. These factors include proinflammatory cytokines such as TNF-α, IL-1β (Sebire *et al*, 1993), glutamate (Jiang *et al*, 2001), arachidonic acid and its metabolites (Genis *et al*, 1992), PAF (Gelbard *et al*, 1994), quinolinic acid (Heyes *et al*, 1991; Kerr *et al*, 1998), NTox (Giulian *et al*, 1996), nitric oxide (NO) (Adamson *et al*, 1996), and reactive oxygen species (ROS) (Mollace *et al*, 2001). Alternatively, viral infection and FKN-mediated activation of MP may regulate production of trophic factors that mediate neuronal growth and repair (Lopez *et al*, 2001). A number of neurotrophic factors are secreted by MP (Barnea *et al*, 1996; Elkabes *et al*, 1996), including brain-derived neurotrophic factor (BDNF) (Kerschensteiner *et al*, 1999; Miwa *et al*, 1997), β-nerve growth factor (βNGF) (Caroleo *et al*, 2001; Garaci *et al*, 1999; Lopez *et al*, 2001), transforming growth factor-beta (TGF-β) (Chao *et al*, 1995), neurotrophin-3 (NT3) (Kullander *et al*, 1997; Loy *et al*, 1994; Mallat *et al*, 1989; Rocamora *et al*, 1996; Saad *et al*, 1991), and glial-derived neurotrophic factor (GDNF) (Batchelor *et al*, 1999). Withdrawal or dysregulation of these factors can result in neuronal injury and death (Deshmukh *et al*, 1996). Through enhanced neurotoxin secretion and dysregulated neurotrophin production, HIV-1–infected and FKN-activated MP may induce neuronal injury and death in HIVE (Aquaro *et al*, 2000; Conant *et al*, 1998; Cotter *et al*, 1999a; Fischer-Smith *et al*, 2001; Gabuzda *et al*, 1998; Gendelman, 1997; Glass *et al*, 1995; Koenig *et al*, 1986; Lopez *et al*, 2001; Nath and Geiger, 1998; Perno *et al*, 1997; Strizki *et al*, 1996; Wiley *et al*, 1986; Zheng and Gendelman, 1997).

FKN-induced secretory factor production is believed to occur through activation of intracellular signaling pathways (Cambien *et al*, 2001; Zheng *et al*, 2002). Therefore, the following section will discuss the relevant intracellular signaling pathways resulting from FKN-mediated activation of CX3CR1 expressing-MP.

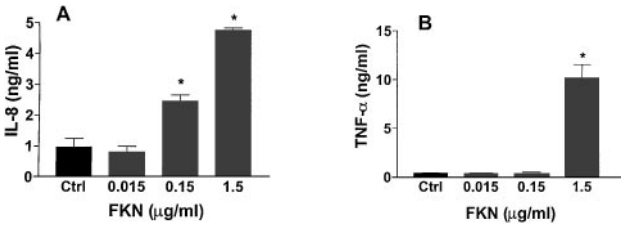

Figure 3 IL-8 (**A**) and TNF-α (**B**) production in cell supernatants from FKN-treated human monocyte-derived macrophages (MDM). After 14 days in culture, elutriated and recombinant human macrophage colony-stimulating factor (MCSF)-differentiated human MDM were treated with different concentrations of soluble FKN for 4 h. $^*P < .01$ as compared to control. Results are expressed as an average $\pm SD$ and are representative of three independent experiments.

FKN-mediated signal transduction pathways

Binding of FKN to CX3CR1 on MP initiates multiple signal transduction pathways and leads to the activation of a wide variety of protein kinases, including the tyrosine kinases (the Src tyrosine kinase family and Syk tyrosine kinase family), calcium calmodulin kinase (CaMK), protein kinase C (PKC), phosphatidylinositide 3-kinase (PI 3-kinase), protein kinase B, mitogen-activated protein kinases (MAP kinases), and NF-κB (Cambien *et al*, 2001; Garcia *et al*, 2000). Activation of these signal transduction pathways leads to elevation of cytosolic free calcium and modifications in enzymes, ion channels, transcriptional activators, and transcriptional regulators (Cambien *et al*, 2001; Iismaa *et al*, 1995).

Several studies have shown that binding of FKN to CX3CR1 induces the activation of MAP kinases (Figure 4) (Cambien *et al*, 2001; Zheng *et al*, 2002). Activation of MAP kinase pathways stimulate

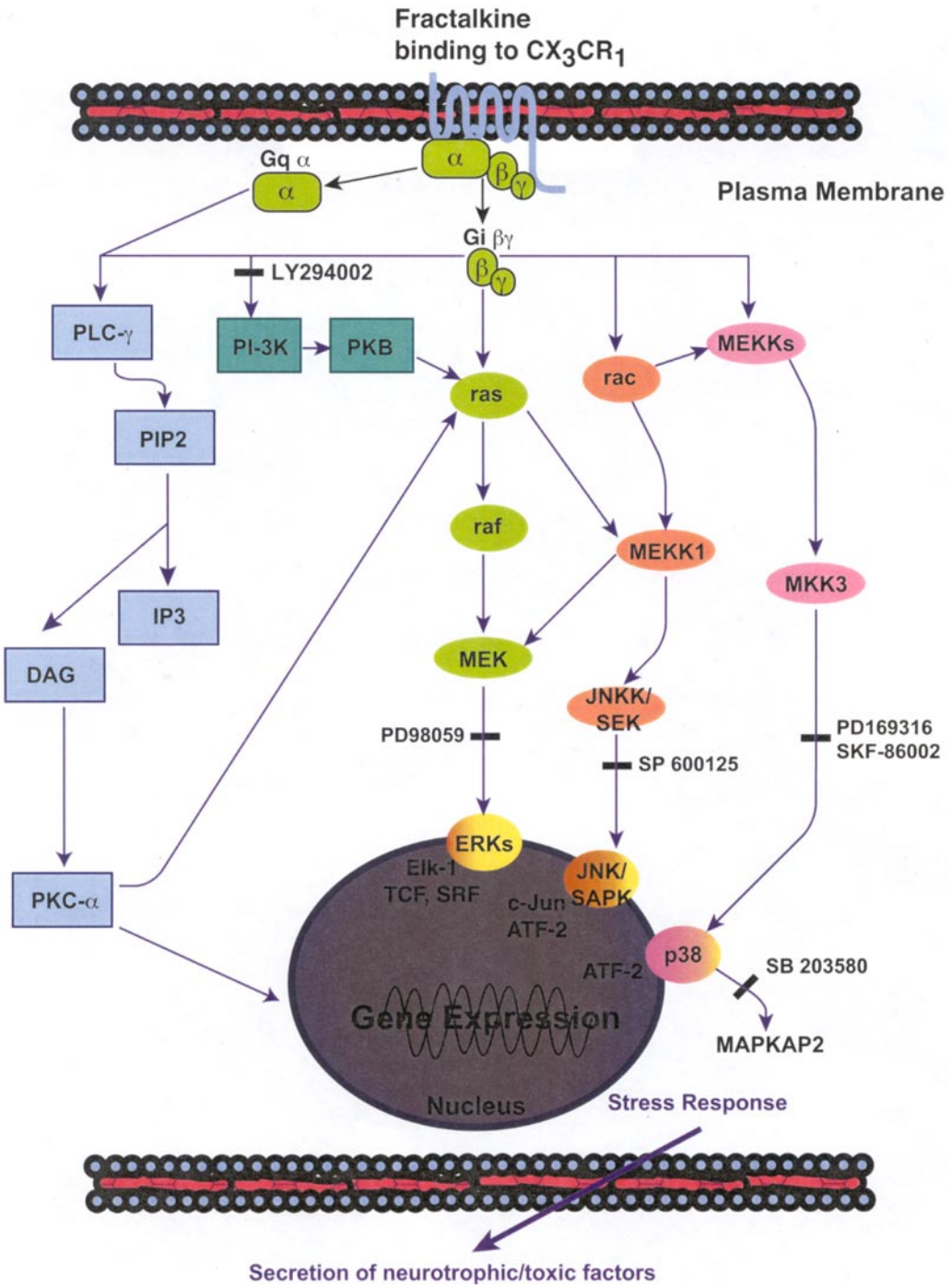

Figure 4 Overview of proposed MAP kinase and protein kinases B and C signal transduction events in mononuclear phagocyte (MP)-mediated production of proinflammatory factors or neurotrophic/toxic factors. FKN can bind the chemokine receptor CX3CR1 on MP and activate MAP kinase signaling through the α or $\beta\gamma$ subunit of G-protein, which can further activate one or more types of MAP kinases. These MAP kinases include extracellular signal-related kinases (ERK1 and ERK2) and stress-activated protein kinases (SAPK1/JNK1 and SAPK2/p38). In addition, FKN can also activate intracellular signaling pathways, such as increasing cytosolic free calcium, activation of phosphatidyl inositol 3-kinase (PI-3K) and protein kinase B (PKB), and alteration of protein kinase C, which further activate MP. This activation causes the production of proinflammatory factors or multiple neurotrophic/toxic factors. The inhibitors for different kinase pathways can be used as tools to elucidate the signaling pathways involved in MP activation events. Some of the stimulation pathways may increase the cellular activation state and cause overproduction of cytokines or neurotoxins, which mediate MP-induced neuronal injury in HAD.

cell growth and differentiation by regulating gene translation and expression (Lopez-Ilasaca, 1998; Lopez-Illasaca *et al*, 1997). There are three distinct MAP kinase cascades (Figure 4): c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), p38 (Lopez-Ilasaca, 1998), and extracellular signal–related kinases (ERK1/ERK2). The JNK/SAPK pathway is induced by exposure to ultraviolet radiation, heat shock, or inflammatory cytokines. The p38 pathway is activated in response to inflammatory cytokines, endotoxins, and osmotic stress. The ERK pathway is stimulated following binding of extracellular growth factors (for example, epidermal growth factor, EGF) to tyrosine kinase–linked receptors. It appears that each of the three MAP kinase pathways, ERK1/2, p38, and JNK, are activated during the binding of FKN to CX3CR1 on monocyte-derived macrophages (MDM) (Zheng *et al*, 2002). Interestingly, production of MIP-1β and IL-8 by FKN-activated MP can be blocked by MAP kinase inhibitors, such as PD98056 (ERK), SB203850 (p38), and SP600125 (JNK) (Zheng *et al*, 2002). Activation of MAP kinase signaling appears to be critical for FKN-induced capture, adhesion, and activation of MP (Cambien *et al*, 2001; Kansra *et al*, 2001; Zheng *et al*, 2002). Thus, multiple protein kinases appear to be involved in mediating the effects of FKN upon its target cells. The elucidation of specific pathways through which FKN induces MP activation and regulates production of neurotrophic/toxic factors will be critical to understanding disease pathogenesis in HAD and other neurodegenerative disorders.

Summary

In summary, the evidence presented within this review suggests that the expression and production of neuronal chemokines, such as FKN, may be a compensatory or reparative response to injury in the brain. As such, we propose that in HAD, viral proteins, such as gp120, induce neuronal injury, leading to the up-regulation or release of FKN. Acting as a "distress signal," FKN may then recruit and activate CX3CR1-expressing MP to the site of injury (Fong *et al*, 2000; Harrison *et al*, 2001). Dysregulation of this response may result in further neuronal injury as MP themselves become activated to produce neurotoxins. This, in turn, may induce a cycle of inflammation and injury. Identification of the pathways through which FKN induces MP activation could lead to the development of agents that impede or prevent further neuronal injury in HAD and other neurodegenerative disorders.

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