Fractalkine Cleavage from Neuronal Membranes Represents an Acute Event in the Inflammatory Response to Excitotoxic Brain Damage

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Fractalkine is a recently identified chemokine that exhibits cell adhesion and chemoattractive properties. It represents a unique member of the chemokine superfamily because it is located predominantly in the brain in which it is expressed constitutively on specific subsets of neurons. To elucidate the possible role of neuronally expressed fractalkine in the inflammatory response to neuronal injury, we have analyzed the regulation of fractalkine mRNA expression and protein cleavage under conditions of neurotoxicity. We observed that mRNA encoding fractalkine is unaffected by experimental ischemic stroke (permanent middle cerebral artery occlusion) in the rat. Similarly, *in vitro*, levels of fractalkine mRNA were unaffected by ensuing excitotoxicity. However, when analyzed at the protein level, we found that fractalkine is rapidly cleaved from cultured neurons in response to an excitotoxic stimulus. More specifically, fractalkine cleavage preceded actual neuronal death by 2–3 hr, and, when evaluated functionally, fractalkine represented the principal chemokine released from the neurons into the culture medium upon an excitotoxic stimulus to promote chemotaxis of primary microglial and monocytic cells. We further demonstrate that cleavage of neuron-derived, chemoattractive fractalkine can be prevented by inhibition of matrix metalloproteases. These data strongly suggest that dynamic proteolytic cleavage of fractalkine from neuronal membranes in response to a neurotoxic insult, and subsequent chemoattraction of reactive immune cells, may represent an early event in the inflammatory response to neuronal injury.

Key words: chemokines; fractalkine; chemotaxis; neuroinflammation; proteolytic cleavage; excitotoxicity; stroke

It has been reported recently that a number of chemotactic cytokines are expressed in the CNS (Mennicken et al., 1999). These proinflammatory proteins and their respective receptors have been implicated in a number of diseases of the CNS, including stroke, AIDS dementia, and Alzheimer's disease (Luster, 1998; Asensio and Campbell, 1999; Miller and Meucci, 1999; Xia and Hyman, 1999). Although chemokines are generally thought to play a beneficial role in host defense by recruiting and activating leukocytes, inappropriate activation of these cells may culminate in autoimmune diseases, such as multiple sclerosis (Hesselgesser and Horuk, 1999).

Fractalkine is the most recently discovered chemokine in the CNS. It represents a novel CX3C chemokine and is unusual in that it exists as two distinct forms. The protein is encoded as a transmembrane molecule that displays adhesion properties. However, it can also be cleaved from the cell membrane to produce a soluble form that creates a chemotaxic gradient for receptive inflammatory cells. Thus, two distinct forms of fractalkine can exist with intrinsically different spatial properties and functions (Bazan et al., 1997; Pan et al., 1997). Both the adhesion and chemotactic properties of fractalkine are mediated via a specific G-protein-coupled, seven transmembrane domain receptor (CX3CR1) (Imai et al., 1997; Combadiere et al., 1998). Unlike fractalkine, which is expressed by central neurons, CX3CR1 is found predominantly on resident microglial cells. This complimentary distribution pattern suggests that fractalkine may play a role in signaling between neurons and microglia (Harrison et al., 1998; Nishiyori et al., 1998; Maciejewski-Lenoir et al., 1999). Should fractalkine play a role in microglial recruitment during CNS inflammation, a change in the absolute levels of fractalkine may be anticipated under conditions of neuronal injury. However, initial studies have shown that the levels of mRNA encoding fractalkine are unaffected by experimental allergic encephalomyelitis *in vivo* or excitotoxicity *in vitro* (Schwaeble et al., 1998; Maciejewski-Lenoir et al., 1999). It can be argued that analysis of fractalkine mRNA rather than protein may be misleading because this fails to distinguish between membrane-bound, and soluble cleaved forms of fractalkine. This is important because a translocation of the fractalkine pool from the membrane to the extracellular compartment, without a change in the absolute amount of either fractalkine mRNA or protein, would create the necessary gradient for chemotaxis of inflammatory cells in response to neuronal damage.

To address this issue, we have used cultured primary cortical neurons subjected to glutamate-induced excitotoxicity and analyzed the dynamics of fractalkine cleavage during the time course

Received Feb. 28, 2000; revised April 28, 2000; accepted May 17, 2000.

We thank Dr. Laura Facci for technical assistance, and Drs. David L. Simmons, Stephen E. Moore, and Stephen D. Skaper for continuing support.

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This article is published in *The Journal of Neuroscience*, Rapid Communications Section, which publishes brief, peer-reviewed papers online, not in print. Rapid Communications are posted online approximately one month earlier than they would appear if printed. They are listed in the Table of Contents of the next open issue of JNeurosci. Cite this article as: JNeurosci, 2000, 20:RC87 (1–5). The publication date is the date of posting online at www.jneurosci.org.

http://www.jneurosci.org/cgi/content/full/4390

of neurotoxicity. We report that fractalkine is readily cleaved off neuronal membranes after an excitotoxic stimulus, and importantly, that cleavage occurs several hours before neuronal death. Furthermore, excitotoxicity-triggered fractalkine cleavage is critically dependent on NMDA receptors and activation of matrix metalloproteases. Collectively, our data suggest that dynamic proteolytic cleavage of fractalkine from neuronal membranes in response to a neurotoxic insult, and subsequent chemoattraction of reactive immune cells, may represent an early event in the inflammatory response to neuronal injury.

MATERIALS AND METHODS

Primary cell culture

Primary microglial cells were prepared from neonatal rat cortices as described previously (Skaper et al., 1990). Primary cortical neuronal cell cultures were prepared from fetal rat brain (embryonic day 18) as described previously (Strijbos and Rothwell, 1995), with the following modifications. Cortices were trypsinized (0.08%, 30 min) and mechanically dissociated. Cells were seeded at a density of $1.1 \times 10^5/cm^2$ in Neurobasal medium, supplemented with N2, 25 μM glutamate, 1 mM sodium pyruvate, 1 mM glutamine, 100U/ml penicillin, and 100 μg/ml streptomycin onto poly-D-lysine-coated tissue culture plates (10 μg/ml). After 5 d in culture, half the medium volume was replaced with fresh medium lacking glutamate. Experiments were performed after 9–11 d in culture. During this period, neurons developed extensive neuritic networks and formed functional synapses.

Induction of excitotoxicity

Mature cortical cultures were washed twice with prewarmed Locke's solution, pH 7.4, and exposed for 10 min at room temperature to magnesium-free Locke's solution, pH 7.0, supplemented with 100 μM D-serine and 100 μM glutamate. Thereafter, cells were again washed twice with prewarmed Locke's solution, pH 7.4, and placed in their original culture medium for 24 hr, before the extent of neurotoxicity was assessed. Control samples were prepared by harvesting cell lysates–medium immediately after exposure to glutamate–Locke's solution ($t = 0$). In separate experiments, culture medium and cells were collected at various time points after glutamate treatment and analyzed for fractalkine content.

Assessment of excitotoxicity

Neuronal death was quantified by measuring dehydrogenase activity retained in the cultured cells (MTT assay) or released into the culture medium [lactate dehydrogenase (LDH) assay] at various time points after glutamate treatment (Strijbos and Rothwell, 1995). To check the reliability of these assays and to determine the time course of cell death accurately, phase-contrast photomicrographs were taken at various time points after application of glutamate, and the extent of visible neuronal death was compared with MTT–LDH results. Loss of MTT signal, and, conversely, the increase in medium LDH content, correlated well with microscopic evidence of cell loss.

Stable expression of human fractalkine

Human umbilical vein endothelial cells (HUVEC) (BioWhittaker, Workingham, UK) were treated with tumor necrosis factor-α (TNF-α) (25 ng/ml, 12 hr) (Chapman et al., 2000) before isolation of total RNA (Tri-reagent; Sigma, Poole, UK). First-strand cDNA was synthesized using oligo-dT_{12-18} (Life Technologies, Paisley, UK), and human fractalkine was amplified using specific primers (GenBank accession number U84487): (5′-GGACTCTTGCCCACCCTCAGC, 5′-GGCACGAGGGCACTGAGC). The amplified product (1475 bp) was cloned into pCDNA3 (Invitrogen, Groningen, The Netherlands) and transfected into the human endothelial cell line ECV304 (European Collection of Cell Cultures), using Lipofectamine plus (Life technologies). Geneticin was used to select stable clones (600 μg/ml; Sigma).

Cytokine stimulation of HUVEC

HUVECs (BioWhittaker) were stimulated with TNF-α as described previously (Chapman et al., 2000). Conditioned media was harvested and analyzed by Western blotting for content of soluble fractalkine.

Inhibition of fractalkine cleavage by batimastat

ECV304 stably expressing human fractalkine (ECV304-hfrac) and HUVEC (exposed to TNF-α, 6 hr) were treated with single application of batimastat (1–20 μM; SmithKline Beecham Pharmaceuticals, Harlow, UK) (Wojtowicz-Praga et al., 1997) and incubated for 24 hr, after which medium content of cleaved fractalkine was determined by Western blotting and chemotaxis assay.

Detection of fractalkine

Human. Fractalkine content of cultured HUVEC/ECV304-hfrac cells and conditioned culture medium was analyzed by SDS-PAGE and Western blotting (Chapman et al., 2000).

Rat. Fractalkine content of cultured neurons and conditioned culture medium, obtained at various time points after glutamate treatment, was analyzed as described above with the following modifications. Separation was performed on a 6% Tris-glycine gel. Membranes were blocked using 4% casein–PBS and incubated overnight with a specific anti-fractalkine polyclonal antiserum (1:500; Torrey Pines Biolabs, San Diego, CA). Preincubating the primary antiserum with recombinant rat fractalkine (10 μM; SmithKline Beecham Pharmaceuticals) abolished detection of fractalkine.

Chemotaxis assays

Chemotaxis of primary microglial cells and monocytic WEHI cells (European Collection of Cell Cultures) in response to culture medium obtained from control or glutamate-treated rat primary cortical neurons, or recombinant rat fractalkine chemokine domain (1 nM; SmithKline Beecham Pharmaceuticals) was performed using a standard chemotaxis assay (Turner et al., 1998). The contribution of fractalkine to chemotaxis was investigated by immunoneutralization of the CX3CR1 receptor expressed on the various cells by a specific antiserum (1:200; Torrey Pines Biolabs). Chemotaxis of these cells to monocyte chemoattractant protein-1 (MCP-1) (1 nM) was unaffected by the CX3CR1 antiserum, demonstrating the specificity of the antiserum for the fractalkine receptor (data not shown).

Permanent middle cerebral artery occlusion

Permanent middle cerebral artery occlusion (pMCAO) or a parallel sham surgery was performed as described previously (Zea-Longa et al., 1989). Animals were allowed to recover for 6 hr before being killed. All animal experimentation was approved by and complied with Home Office regulations (UK).

Real-time Taqman quantitative reverse transcription-PCR

mRNA isolation, cDNA production, and Taqman analysis from cerebral cortices subjected to surgery was performed as described previously (Harrison et al., 2000). Primer and probe sets for fractalkine, interleukin-1β (IL-1β), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed against known rat sequences: fractalkine (GenBank accession number AF030358), forward, 5′-GCTCCTAAGGTAGAGGAACCCATT; reverse, 5′-CAGGGACAGGAGTGATAAACACACT; probe, 5′-TGCCACTGCAGATCCCCAGAAACT; IL-1β (GenBank accession number M98820), forward, 5′-GATGGCTGCACTATTCCTAATGC; reverse, 5′-AGACTGCCCATTCTCGACAAG; probe, 5′-CCCCAGGACATGCTAGGGAGCCC; GAPDH (GenBank accession number AF106860), forward, 5′-GAACATCATCCCTGCATCCA; reverse, 5′-CCAGTGAGCTTCCCGTTCA; probe, 5′-CTTGCCCACAGCCTTGGCAGC.

Because GAPDH mRNA expression does not change in this model of stroke (Harrison et al., 2000), all data were normalized to GAPDH, and statistical significance was determined by ANOVA at the 95% confidence interval.

RESULTS

Effects of pMCAO on mRNA expression of fractalkine and IL-1β

A 10-fold increase in IL-1β mRNA was observed in the ipsilateral cortex in pMCAO-treated rats compared with contralateral cortex and sham-operated controls (Fig. 1). In contrast, there were no significant changes in fractalkine mRNA between any of the tissues or treatment groups (Fig. 1).

Time courses of glutamate-triggered neurotoxicity and fractalkine cleavage *in vitro*

Primary cortical neurons were subjected to a transient, 10 min exposure to a neurotoxic concentration of glutamate (100 μM), after which the neurons were allowed to recover in glutamate-free medium for various periods of time. In analogy with previous studies, these experimental conditions triggered a delayed, submaximum degree of excitotoxicity sensitive to NMDA receptor blockade by (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine maleate (MK801) (10 μM) (Fig. 2*A*) (Strijbos and Rothwell, 1995). To ensure accurate determination of the time course of neurotoxicity, we used MTT and LDH assays combined with phase-contrast microscopy. Using these assays, we

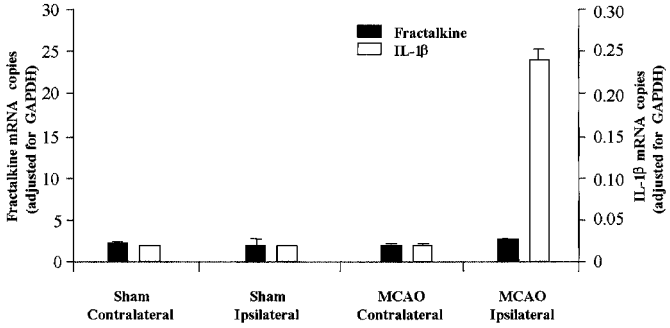

Figure 1. Cortical mRNA expression 6 hr after MCAO as assessed by real-time Taqman RT-PCR. Copies of mRNA encoding fractalkine or IL-1β per nanogram of total RNA–copies of mRNA encoding GAPDH (mean ± SD; $n = 4$). Changes in gene expression between treatment groups was analyzed by using ANOVA on log-transformed data accommodating for levels GAPDH by incorporating them as covariates. Pairwise comparisons of the ipsilateral MCAO group relative to the other groups were made, and the 95% confidence interval for this difference was calculated using pooled estimates of variability from the ANOVA.

observed that neuronal viability was unaffected by glutamate treatment for up to 3 hr, after which a gradual decline in neuronal viability was observed, approximating 50% cell loss at 24 hr after the insult (Fig. 2).

We failed to detect a change in mRNA encoding fractalkine in response to an excitotoxic stimulus in cultured neurons. We therefore analyzed fractalkine protein on these cultured cells. Under control conditions, cell-associated fractalkine was readily detected (Fig. 2*B*). Upon glutamate treatment, however, a rapid time-dependent decline in membrane-associated fractalkine was observed. This effect was evident within 1 hr of transient glutamate treatment, and membrane-tethered fractalkine was reduced by 50% within 3 hr of treatment (Fig. 2*B*). At 24 hr after glutamate treatment, a time point at which ~50% of all neurons in the culture are degenerated (Fig. 2*A*), virtually all fractalkine had been removed from the neuronal membranes. Interestingly, the reduction in membrane-bound fractalkine occurred several hours before onset of neuronal death, suggesting that fractalkine cleavage is a dynamic process in the events leading up to toxicity rather than a nonspecific consequence of neuronal death (Fig. 2).

Chemotaxis induced by fractalkine

Because these results imply that fractalkine is released into the culture medium upon excitoxicity, we next analyzed the culture medium, sampled at various time points after the glutamate insult, for its ability to stimulate chemotaxis of receptor (CX3CR1)-expressing primary rat microglia and WEHI cells (a murine monocytic cell line). We tested the medium sampled at 3 hr post-glutamate treatment because this represents a time point at which significant loss of membrane-associated fractalkine occurs, in the absence of neuronal death (Fig. 2).

We observed a significant degree of chemotaxis of both primary rat microglia and WEHI cells in response to neuronal culture medium taken at 3 hr after glutamate treatment (Fig. 3). Although monocytic cells will undergo chemotaxis to a variety of chemokines, we observed that fractalkine accounted for the majority of chemoattractive activity present in the culture medium because a neutralizing antiserum against the fractalkine receptor inhibited >85% of the chemotaxis (Fig. 3).

Mechanism of proteolytic cleavage of fractalkine

To investigate the proteases involved in (glutamate-triggered) fractalkine cleavage, we examined a number of putative protease inhibitors for their efficacy to modulate this process. Cleavage, and inhibition thereof, was assessed by measurement of the fractalkine content of conditioned medium–cell lysates from

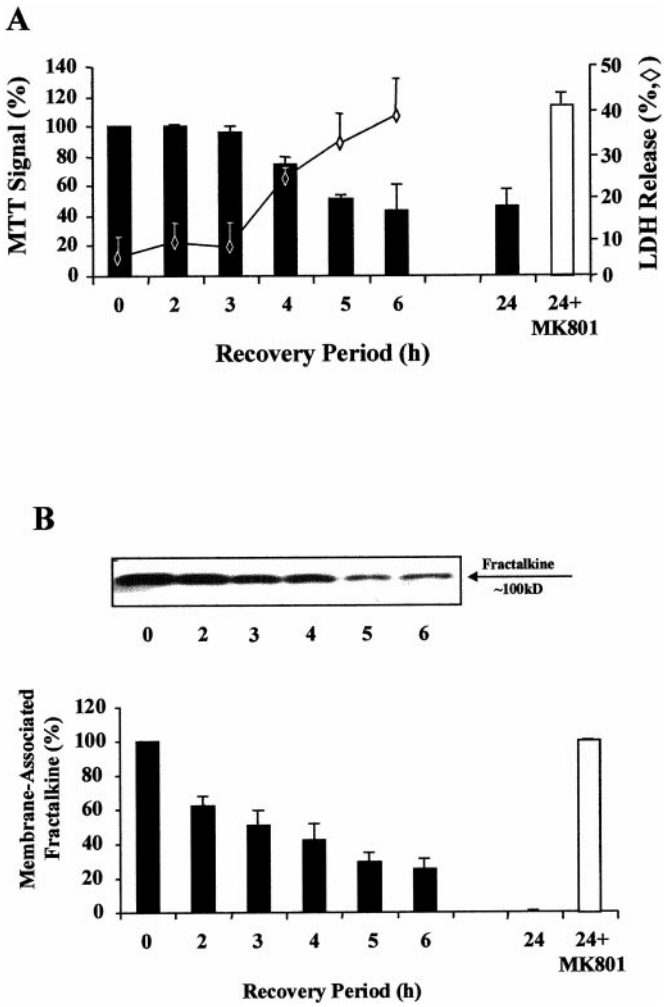

Figure 2. Time course of neuronal viability (*A*) and neuronal membrane-associated fractalkine levels (*B*) of rat primary cortical neurons in response to a transient, 10 min exposure to glutamate (100 μM), followed by various recovery periods in glutamate-free medium. Loss of membrane-associated fractalkine occurs several hours before neuronal death. Neuronal viability was assessed by MTT and LDH assays (mean ± SD; $n = 6$–9). Levels of membrane-associated fractalkine were determined by Western blotting, followed by densitometric analysis. Levels of fractalkine detected at all time points after exposure are significantly different from those at time 0 ($p < 0.01$). Data are expressed as a percentage of the fractalkine content of sham-exposed cultures at time 0 ($n = 3$; the *top panel* in *B* represents a typical Western blot).

ECV304-hfrac (a stable cell line expressing human fractalkine), TNF-α-stimulated HUVEC cells, and glutamate-treated primary cortical neurons. Accumulation of fractalkine in the culture medium of these cells was not affected by pepstatin A (an aspartic protease inhibitor), E64 (a cysteine protease inhibitor), or aprotinin (serine protease inhibitor; data not shown). However, application of the matrix metalloprotease inhibitor batimastat dose dependently inhibited cleavage of fractalkine from the membranes of HUVEC and ECV304-hfrac, with an IC_{50} of ~4 μM (Fig. 4*B*,*D*). In analogy, when applied to glutamate-treated primary cortical neurons, batimastat also inhibited the cleavage of fractalkine from these cells, as judged by the loss of chemotactic activity of the culture medium (Fig. 3).

DISCUSSION

The data presented here demonstrate that fractalkine can act as a neuron-derived intercellular signaling molecule to attract proinflammatory cells after excitotoxic injury and suggest that fractal-

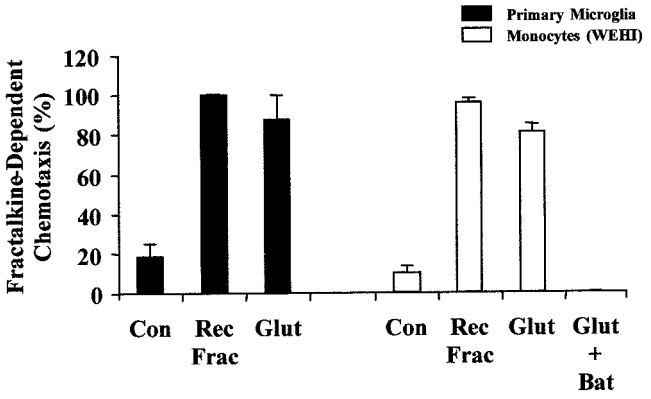

Figure 3. Chemotaxis of primary microglial cells and monocytic WEHI cells in response to culture medium obtained from sham-treated (*Con*), glutamate-treated (*Glut*) primary cortical neurons, or rat recombinant fractalkine (1 nM; *Rec Frac*). The effect of the matrix metalloprotease inhibitor batimastat on chemotaxis was also determined (*Glut+Bat*). Neuronal culture medium used for chemotaxis was obtained at 3 hr after sham or glutamate treatment. Data are expressed as the percentage of chemotaxis that is sensitive to inhibition by an CX3CR1 neutralizing antiserum (mean ± SD; $n = 4$). Fractalkine-dependent chemotaxis of cells toward media conditioned with glutamate-treated neurons was significantly different from control conditioned media ($p < 0.01$). Batimastat abolished the fractalkine-dependent chemotaxis of WEHI to media conditioned with glutamate-treated neurons ($p < 0.001$).

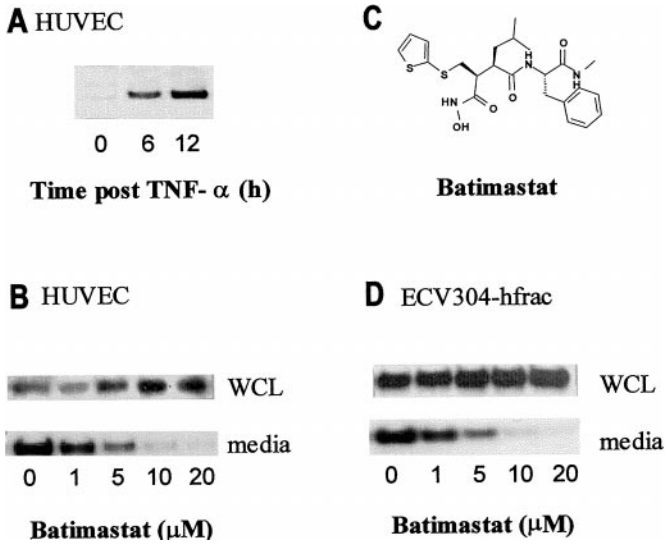

Figure 4. Expression and cleavage of fractalkine from HUVEC and ECV304-hfrac cells and inhibition thereof by the matrix metalloprotease inhibitor batimastat. HUVEC cells were stimulated with TNF-α (25 μM), and fractalkine content of the culture medium–cell lysate was analyzed at various time points after stimulation (*A*). ECV304-hfrac and HUVEC, previously exposed to TNF-α (6 hr), were treated with batimastat (20 μM; *C*), after which accumulation of cleaved fractalkine in the culture medium was determined (*B*, *D*).

kine cleavage under these conditions may precipitate the neuroimmune response that frequently accompanies brain trauma. We further reveal that neuronal fractalkine cleavage is an inducible, dynamic process in the events leading up to neuronal death, regulated by post-translational modification rather than transcriptional control.

Previous studies have demonstrated that the expression of fractalkine mRNA in the CNS is unaffected by neuroinflammatory processes, such as that produced by experimental allergic encephalomyelitis *in vivo* or in cultured primary neurons subjected to excitotoxicity or stimulated with inflammatory cytokines (Schwaeble et al., 1998, Maciejewski-Lenoir et al., 1999). Using real-time quantitative reverse transcription (RT)-PCR technology, we extend these findings by demonstrating that fractalkine mRNA is equally unaffected by experimental cerebral ischemia in the rat, despite coincident brain tissue inflammation as judged by significant increases in the expression of the proinflammatory cytokine IL-1β (Rothwell and Strijbos, 1995; Harrison et al., 2000). Conversely, mRNA encoding the fractalkine receptor CX3CR1 increases in several animal models of neuroinflammation, although this can probably be explained by microglial proliferation and infiltration–migration of CX3CR1-bearing immune cells to the site of injury (Harrison et al., 1998; Jiang et al., 1998). Collectively, this suggests that fractalkine activity during neuroinflammation is unlikely to be regulated by transcriptional control but instead by post-translational modification.

To address this issue, we have used cultured primary cortical neurons subjected to glutamate-induced excitotoxicity and analyzed the dynamics of fractalkine cleavage during the time course of neurotoxicity. We found that fractalkine protein is constitutively expressed on the membrane of these cells, and, in accordance with previous studies, its mRNA levels remained constant after excitotoxicity (data not shown) (Maciejewski-Lenoir et al., 1999). However, when examined at the protein level, we observed that fractalkine is readily cleaved off neuronal membranes after an excitotoxic stimulus. Importantly, glutamate-triggered fractalkine cleavage occurred several hours before actual neuronal death, as assessed by several viability criteria, suggesting that this represents an active event in the excitotoxic pathway rather than a nonspecific consequence of cell death. The NMDA receptor antagonist MK801 prevented both neuronal death and fractalkine cleavage, indicating that in the CNS, fractalkine cleavage, and thus activation of the responsible protease, can be regulated via the NMDA subtype of excitatory glutamate receptors. It is important to note that, although MK801 is neuroprotective, it will not effect glutamate activation of non-NMDA receptors, suggesting that the NMDA receptor pathway constitutes an inducible primary signal for fractalkine cleavage under neurotoxic conditions. This contrasts with endothelial cells that require TNF-α for fractalkine expression and in which cleavage is regulated by a constitutively active protease, allowing a constant turnover of the fractalkine pool. This strongly suggests that regulation of protease activity, and indeed its resting activation state, may vary between cell types. The importance of the differential regulation of fractalkine-cleaving proteases for chemotaxis during inflammation remains unknown.

As predicted, culture medium sampled at 3 hr after the excitotoxic insult, a time point at which significant loss of membrane-tethered fractalkine was observed without actual neuronal death, strongly stimulated chemotaxis of rat primary microglia and monocytic WEHI cells. Interestingly, chemotaxis was reduced by 85% after immunoneutralization of CX3CR1, suggesting that, because fractalkine appears the sole ligand for this receptor, fractalkine represents the principal chemokine released from neurons upon excitoxicity capable of attracting cells of a monocytic lineage. It must be noted that here we analyze the ability of conditioned media to chemoattract monocytic cells, and it is quite possible that other chemokines (e.g., CXC-type) are also released after this insult. The relative contribution of fractalkine to chemotaxis is critically dependent on cell type. For example, TNF-α-stimulated HUVEC cells release large amounts of chemoattractants, although the net contribution of fractalkine to the chemoattraction of monocytes to these cells is negligible relative to that of MCP-1 (Chapman et al., 2000). Soluble fractalkine may therefore represent a major signal for chemoattraction in the brain but not peripheral organs and tissues in which its adhesion properties may be more important.

Mechanistically, it is unclear how the excitotoxic signaling cascade couples to the protease responsible for fractalkine cleav-

age. Comparison of the primary amino acid sequence of fractalkine with that of consensus cleavage sites, combined with size analysis of cleaved- and full-length fractalkine fragments, suggests that cleavage may occur between a pair of alanine residues at the extracellular C terminus of the protein. This cleavage site is situated N terminal to the predicted transmembrane domain of the protein and appears to resemble that of a syndecan-like cleavage motif. In addition, a role for a calcium-dependent serine protease, such as the mammalian proteases furin and PC3, in fractalkine cleavage has also been suggested. In a first attempt to identify the proteases involved in fractalkine cleavage, we examined a number of putative protease inhibitors for their efficacy to modulate this process.

Of all compounds tested, only batimastat, a broad spectrum inhibitor of matrix metalloproteases, prevented fractalkine cleavage from HUVEC and ECV304-hfrac cells. Batimastat also inhibited excitotoxicity-triggered fractalkine cleavage in primary neurons, as judged by the loss of chemotaxic activity, without affecting ensuing neuronal death. These data strongly support the premise that excitotoxicity-induced cleavage of fractalkine from primary cortical neurons is mediated by a matrix metalloprotease(s), although the exact mechanisms linking stimulation of excitatory glutamate receptors to activation of the protease(s) remain unknown. Although it is widely accepted that batimastat is a general matrix metalloprotease inhibitor, it is plausible that it may affect other metalloproteases.

The data presented here advance our understanding of the role of fractalkine in the inflammatory response to central neurodegeneration in several ways. First, we demonstrate in a well characterized *in vitro* model of excitotoxicity that fractalkine cleavage occurs very early on in processes leading to neuronal death. Although the cleavage event itself does not precipitate neurotoxicity in that inhibition of cleavage by batimastat does not confer neuroprotection, it is dynamically regulated on overstimulation of the NMDA subtype of excitatory glutamate receptors because it occurs several hours before actual cell death and is prevented by MK801. Second, we report that inhibition of matrix metalloproteases by batimastat inhibits fractalkine cleavage from a variety of cell types. These observations convincingly demonstrate that this subclass of proteases mediates fractalkine cleavage, although it appears that these enzymes may be subjected to differential regulation depending on cell type (constitutively active enzyme in endothelial cells vs glutamate-stimulated enzyme in neurons). More specifically, because batimastat inhibits excitotoxicity-associated fractalkine cleavage, activation of matrix metalloprotease appears to be a component of the events leading to neuronal death, although it does not trigger neurotoxicity per se. Third, and finally, it appears that the release of fractalkine from neurons after excitotoxicity plays a pivotal role in attracting microglia and other cells derived from the monocytic lineage. Should neurodegeneration *in vivo* trigger similar events, it is anticipated that interference with fractalkine cleavage may reduce the ensuing neuroinflammatory response and possibly limit the extent of secondary neuronal damage.

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