Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties in vivo

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Monocyte chemoattractant protein (MCP)–3 is inactivated upon cleavage by the matrix metalloproteinase (MMP) gelatinase A (MMP-2). We investigated the susceptibility to proteolytic processing of the 4 human MCPs by 8 recombinant MMPs to determine whether MCP-3 is an isolated example or represents a general susceptibility of chemokines to proteolytic inactivation by these important inflammatory proteases. In addition to MMP-2, MCP-3 is efficiently cleaved by membrane type 1 (MT1)–MMP, the cellular activator of MMP-2, and by collagenase-1 and collagenase-3 (MMP-1, MMP-13) and stromelysin-1 (MMP-3). Specificity was shown by absence of cleavage by matrilysin (MMP-7) and the leukocytic MMPs neutrophil collagenase (MMP-8) and gelatinase B (MMP-9). The closely related chemokines MCP-1, MCP-2, and MCP-4 were not cleaved by MMP-2 or MT1-MMP, but were cleaved by MMP-1 and MMP-3 with varying efficiency. MCPs were typically cleaved between residues 4 and 5, but MCP-4 was further processed at Val7-Pro8. Synthetic MCP analogs corresponding to the MMP-cleared forms bound CC chemokine receptor (CCR)–2 and CCR-3, but lacked chemoattractant activity in pre-B cells transfected with CCR-2 and CCR-3 or in THP-1 monocytic cells, a transformed leukemic cell line. Moreover, the truncated products of MCP-2 and MCP-4, like MCP-3, were potent antagonists of their cognate CC chemokine receptors in transwell cell migration assays in vitro. When they were injected 24 hours after the initiation of carrageenan-induced inflammation in rat paws, their in vivo antagonist activities were revealed by a greater than 66% reduction in inflammatory edema progression after 12 hours. We propose that MMPs have an important role in modulating inflammatory and immune responses by processing chemokines in wound healing and in disease.

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Introduction

Chemokines are potent chemoattractant cytokines that are produced locally in tissues and direct the migration and homing of leukocytes. Tissue gradients of inflammatory chemokines attract and maintain inflammatory cells at sites of host challenge in infection, inflammation, and cancer.1 Chemokines can be divided into families according to the position and spacing of N-terminal cysteine residues. Presently, the C, CC, CXC, and CX3C families are recognized,2 with more than 54 human chemokines currently identified. The monocyte chemoattractant proteins (MCPs) of the CC family consist of 4 proteins termed MCP-1, MCP-2, MCP-3, and MCP-4 (CCL-2, CCL-8, CCL-7, and CCL-13, respectively) that target multiple leukocyte subsets (monocytes, basophils, eosinophils, dendritic cells, and natural killer cells) whereas, in the initial phases of inflammation, CXC chemokines attract polymorphonuclear leukocytes. Therefore, chemokines are important mediators of many pathologies, including chronic inflammatory and autoimmune diseases where the coordinated expression of MCPs and resultant leukocyte infiltration correlate with disease progression.3,4 An important question in the pathogenesis of inflammation and disease is how chemoattractant signals are squelched to restrict new cell recruitment and to promote clearance of the inflammatory infiltrate as a prelude to tissue resolution. Proteolysis of chemokines may provide a mechanism for loss of chemoattractant signaling. Indeed, production of proteolytically processed chemokines in cell culture has been reported.5-8 We have demonstrated an N-terminal–truncated form of MCP-3 in human rheumatoid synovial fluid that had been cleaved at Gly4-Ile5, and the effects of engineered truncated chemokines on cell behavior have been characterized.9 Hence, the identification of proteinases that may process chemokines in vivo is important, but as yet poorly characterized. Very recently, the matrix metalloproteinase (MMP) gelatinase A (MMP-2) was shown to process the N-terminus of MCP-3 and stromal cell–derived factor-1α (SDF-1) and β.7,8 Prior to these studies, considerable emphasis was placed on the role of the serine protease dipeptidyl peptidase IV/CD 26,10 which can process many bioactive molecules with a penultimate N-terminal proline residue, including chemokines. The serine proteinase
cathepsin G was also recently reported to process SDF-1α.11 However, the activity of these serine proteases in chemokine cleavage and inactivation in vivo has not been demonstrated.

MMPs are either secreted or cell-membrane–bound proteinases with broad substrate specificity that have traditionally been proposed to degrade most components of the extracellular matrix12 although in vivo evidence for this is generally lacking. Matrix proteolysis is a hallmark of inflammation with MMPs considered to be important effectors of this process and also essential for leukocyte extravasation and migration. In particular, leukocyte-derived collagenase–2 (MMP-8) and gelatinase B (MMP-9) are the prominent early proteolytic mediators of matrix degradation and allow effector cell egress to the site of tissue damage.13,14 Following this initial leukocyte proteolytic phase, stromal cells, in response to proinflammatory cytokines secreted by the cellular infiltrate, produce MMPs that amplify the acute tissue-destructive phase. Notably, interstitial collagenase (MMP-1), stromelysin-1 (MMP-3), matrilysin (MMP-7), and collagenase-3 (MMP-13) have been suggested to play important roles in inflammatory tissue destruction.12 Hence, persistence of the inflammatory infiltrate results in continued tissue destruction that can progress to chronic inflammatory disease. Although MMPs may play a role in extracellular matrix degradation in inflammation, it is gradually becoming appreciated that these proteases have a wider substrate repertoire that includes many bioactive molecules. Indirectly, the MMP-mediated inactivation of serpins15,16 and chemokines7 can modulate healing with direct biological effects of MMP cleavage manifested by the activation and release of tumor necrosis factor–α (TNF–α),17 fibroblast growth factor receptor,18 Fox-ligand,19,20 and α-defensin.21 The MMP-mediated cleavage and inactivation of the complement protein mannone binding lectin22 was also very recently reported. MMP-9 is known to activate IL-8 and to cleave and inactivate SDF-1.8,23 Hence, MMP activity on nontraditional substrates may exert a biological impact on inflammatory processes as profound as those of MMP tissue-degradation effects.

We have recently reported the use of the yeast 2-hybrid system to screen for potential MMP substrates. Exosete scanning resulted in the identification of MCP-3 as a binding protein and substrate for MMP-2.7 This was a specific interaction, as MMP-2 did not cleave MCP-1, MCP-2, or MCP-4. We have also very recently identified SDF-1α and SDF-1β as MMP substrates that are cleaved by a number of MMPs, including MMP-2.8 Because MMP substrate specificity overlaps to form a robust extracellular proteolytic system, we investigated in the present report whether other MMPs also process MCP-3 and if the MCP family is generally susceptible to proteolysis by MMPs. Here we establish that MCP-1, MCP-2, MCP-3, and MCP-4 are all processed by multiple MMPs to typically remove an N-terminal tetrapeptide, resulting in reduced agonist activity and conversion to CC chemokine receptor (CCR) antagonists that are effective both in vitro and in vivo. Hence, the chemokine superfamily represents an emerging and important class of substrates for MMPs that, when cleaved by these inflammatory proteases, have novel modulatory effects in regulating inflammatory processes.

**Materials and methods**

**Production of MMPs and MCPs**

Recombinant human MMPs were expressed in Chinese hamster ovary (CHO) cells and purified from the conditioned CHO-SFM culture medium.8 Briefly, conditioned culture medium was collected and enzymatic activity was purified by gelatin-Sepharose chromatography for gelatinases. The other MMPs were purified by combinations of heparin-agarose and dye-ligand chromatography as appropriate.24 Membrane type–1 MMP (MT1-MMP [MMP-14]) was produced as a soluble, transmembrane segment–deleted form. Proteolytic activity was confirmed by cleavage of quenched fluorescent peptide substrates and inhibition by the tissue inhibitor of metalloproteinase–1 (TIMP-1).25 Human MCP-1, MCP-2, MCP-3, MCP-4, and truncated derivatives were chemically synthesized by means of solid-phase methods; the polypeptides were purified by reverse-phase high-performance liquid chromatography (HPLC) and folded by means of air oxidation as previously described.26 Homogeneity and fidelity of synthesis were confirmed by electrospray ionization mass spectrometry.

**Electrospray ionization mass spectrometry**

Chemokine samples were introduced into the mass spectrometer through a micro bore PLRP column (1 × 50 mm) on the Michrom HPLC system (Michrom BioResources, Auburn, CA) (solvent system: 20% to 100% solvent B [0.045% trifluoroacetic acid, 80% acetonitrile in water] for 10 minutes; 100% solvent B for 2 minutes). The quadrupole mass analyzer (in the single-quadrupole mode) was scanned over a mass-to-charge ratio range of 300 to 10 000 d with a step size of 0.5 d and a dwell time of 1 millisecond per step. The ion source voltage was set at 5 kV, and the orifice energy was 80 V. Protein molecular weights were determined from these data by means of the deconvolution software supplied by P E Sciex (Thornhill, ON, Canada).

**Proteinase assays**

P-aminophenylmercuric acetate (APMA)–activated MMP (1 ng) and 1 μg chemokine were mixed in buffer (100 mM NaCl, 5 mM CaCl₂, 20 mM Tris, pH 8.0) and incubated at 37°C. Aliquots were removed at 1-hour intervals, and product accumulation was monitored by densitometric analysis of Coomassie-stained Tris-tricine sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gels. The overall catalytic rate (Kcat/Km) specificity constant was calculated from graphical determination of the kcat/Km. Electrospray ionization mass spectrometry of the reaction products was used to confirm cleavage, and the data were deconvoluted to identify the scissile bond.

**Chemotaxis assays**

Cell migration of THP-1 monocytic leukemia cells (ATCC, Manassas, VA) or a pre-B cell line (murine B300 19; ATCC) stably transfected with either human CCR-2 or human CCR-3 (designated B300-CCR2 and B300-CCR3, respectively) was evaluated in disposable 12-well Transwell polystyrene trays (Corning Costar, Cambridge, MA) across a polycarbonate membrane with 5-μm size pores. Full-length and N-terminus–truncated synthetic chemokines corresponding to the MMP-cleaved form of the chemokines were diluted in Hepes-buffered RPMI 1640 supplemented with 10 mg/mL bovine serum albumin. Chemokine samples were added to the lower chamber, and THP-1 cells or B19 300 cell transfectants (1 × 10⁶ cells per milliliter), in the same media without the MCPs, were added to the upper chamber. After 3-hour incubation at 37°C in 5% CO₂ humidified atmosphere, migrated cells in the lower chamber were counted. Migrated cells in 5 separate fields per well from duplicate wells were enumerated on a hemacytometer by means of light microscopy.

125I-labeling of MCP-1 and measurement of chemokine receptor binding affinities

The binding affinity (dissociation constant [Kd]) of MCP-2, MCP-2(5-76), MCP-4, and MCP-4(8-75) was determined by competition for 125I-labeled MCP-1 on CCR-2 and Scatchard analysis. The 125I-labeling of MCP-2 and MCP-4 resulted in precipitation of the chemokines, which could not be used for binding assays. MCP-1 (10 μg) was labeled with 9.25 × 10⁶ Bq (250 μCi) 125I-Bolton Hunter reagent (NEN Life Science Products, Boston, MA) at 4°C for 30 minutes in 0.1 M borate buffer, and the reaction was terminated by incubation in 0.1 M glycine. The labeled MCP-1 was separated from free 125I-Bolton Hunter reagent by Sephadex G-25 size-
exclusion chromatography. Binding assays were performed by means of CCR-2-transfected pre-B cells (5 × 10^6) suspended in 200 μL HEPES-buffered RPMI 1640 media, 10 mg/mL bovine serum albumin, and 0.1% NaCl. The ^125^I-MCP-1 (4 nM) and increasing concentrations of unlabeled competitor were added and incubated for 30 minutes. Cell-bound ^125^I-MCP-1 was separated from the unbound ^125^I-MCP-1 by pelleting the cells through a 2.3 mixture of diacetylphosphate and dibutylphosphate. The specifically bound counts per minute in the cell pellet were calculated after subtracting the nonspecifically bound counts per minute (counts per minute bound in the presence of 100-fold molar excess of unlabeled competitor) and dividing by the total counts per minute bound in the absence of competitor. Assays were performed in duplicate, and the experiments repeated.

**Rat paw inflammation model**

Male Wistar rats (200 to 225 g) (Charles River Breeding Farms, Montreal, QC, Canada) were housed in a biocontainment facility according to the Canadian Animal Care Committee with the use of institutionally approved animal care protocols. Inflammation was induced in rat paws by intraplantar injection of 1% lambda carrageenan (Sigma, St Louis, MO) in sterile saline (total volume, 100 μL) under halothane anesthesia. Paw volumes were measured by means of a hydroplethysmometer (Ugo Basile, Milan, Italy) before and 24 hours after injection of carrageenan and the induction of inflammation.2 After 24 hours, the rats were anesthetized with halothane, and an intraplantar injection of chemokine analogs in sterile saline (50 μg in 50 μL) was administered according to a double-blind protocol. Five rats were used per treatment, and the experiment was repeated 2 or 3 times for each peptide. At 12 hours after peptide administration, paw volumes were measured in a randomized sequence by an observer unaware of the treatments. Changes in paw volume were then calculated by subtracting the final paw volume from the volume at the 24-hour time point.

**Statistical analysis**

Statistical analysis was performed by nonparametric (Kruskal-Wallis) analysis of variance followed by a Dunn multiple comparison test.

**Results**

**MCP-3 is cleaved by multiple MMPs**

The specificity of 8 MMPs that have been associated with wound healing22,28 in the proteolysis of MCP-3 was determined. Incubation of the chemokine with recombinant MMP-1, MMP-2, MMP-3, MMP-13, and MT1-MMP resulted in a small but distinct increase in electrophoretic mobility on Tris-tricine gels, whereas MMP-7, MMP-8, and MMP-9 did not, even with prolonged incubation (Figure 1). Cleavage of full-length chemokine was confirmed by electrospray ionization mass spectrometric identification of the new lower molecular mass product (Figure 1). Deconvolution of the mass spectrometry data revealed that the MCP-3 scissile bond for each proteolytically competent MMP was Gly4-Ile5 (Table 1). We designated this cleavage product MCP-3(5-76). MMP-mediated processing of MCP-3 was efficient, as shown by the high turnover rates (Table 1), with MMP-2 and MT1-MMP showing highest activity.

**Multiple MMPs cleave MCP-1, MCP-2, and MCP-4**

We tested the ability of the MMPs to cleave MCP-1, MCP-2, and MCP-4. As found before,7 MMP-2 was highly specific for MCP-3 with no activity against MCP-1, MCP-2, or MCP-4. Figure 2 shows that MCP-1 is cleaved at Ala4-Ile5, designated MCP-1(5-76); by MMP-1; to a lesser extent (20% of total) by densitometric analysis) by MMP-3; and to a very minor extent (5% of total) by MMP-8. In contrast, MCP-2 was susceptible to cleavage only by MMP-3 at Ser4-Val5, designated MCP-2(5-76). Interestingly, MCP-4 was processed at 2 sites, Asp3-Ala4 and Ala4-Leu5, by MMP-3. Both sites were also susceptible to MMP-1 activity, with proteolysis proceeding by a further cleavage at Val7-Pro8, designated MCP-4(8-75). Cleavage site susceptibility for MCP-1, MCP-2, and MCP-4 is summarized schematically in Figure 3.

**MCP-2(5-76) is a CCR-2 receptor antagonist**

To compare the biological activity of full-length MCP-2 and MCP-2(5-76), we performed chemotaxis assays using synthetically prepared MCP-2(5-76) and a pre-B cell line stably transfected with the predominant MCP-2 chemokine receptor CCR-2. Figure 4A shows that, unlike full-length MCP-2, MCP-2(5-76) was inactive as a CCR-2 agonist at all concentrations up to 10^-6 M in the chemotaxis assay. However, MCP-2(5-76) retained receptor-binding affinity (K_d, 4.5 nM) very similar to that for MCP-2 (K_d, 4.4 nM) (Figure 5) and was effective as a CCR-2 antagonist in cell migration assays (Figure 4B).

**MCP-4(8-75) is a CCR-2 and CCR-3 receptor antagonist**

The agonist and antagonist activity of MCP-4(8-75) was compared with that of MCP-4 with the use of 2 pre-B cell lines stably transfected with either CCR-2 or CCR-3, to which full-length MCP-4 binds with high affinity. Compared with the full-length chemokine, MCP-4(8-75) was unable to direct chemotaxis in either cell line (Figure 6A, B). Like the MMP-cleaved forms of MCP-2 and MCP-3, MCP-4(8-75) was a potent receptor antagonist of both
CCL-2 and CCL-3 (Figure 6C,D). MCP-4(8-75) was calculated to bind CCR-2 with a K_{d} of 14 nM, an affinity very similar to that of the full-length form (K_{d}, 16 nM) (Figure 5).

### THP-1 cell migration in response to MCP-2(5-76) and MCP-4(8-75)

As a model of inflammatory monocytes, we used THP-1 monocytic cells, a transformed leukemic cell line that expresses CCR-2, to further assess the effects of MMP cleavage of MCP-2 and MCP-4. Whereas the full-length MCPs directed chemotaxis across transwell filters, MCP-2(5-76) and MCP-4(8-75) showed no chemotactic activity (Figure 7A,B), but both were receptor antagonists as evident by the dose-dependent reduction in THP-1 cell chemotaxis (Figure 7C,D), confirming the previous results using the pre-B cells transfected with CCR-2.

### MMP-cleaved MCPs reduce inflammatory edema in vivo

To determine the biological effects of MMP cleavage of MCPs in vivo, the extent of inflammatory edema induced in a rat paw model following injection with carrageenan was measured after administration of synthetic N-terminal–truncated MCPs corresponding to the MMP-cleaved forms. Results are shown in Figure 8 as the change in paw volume from the 24-hour time point, when the chemokines were injected, to the 36-hour postcarrageenan time point. Without chemokine, the paw volume continued to increase following injection with carrageenan, to the 36-hour postcarrageenan time point. When the chemokines were added, the paw volume decreased significantly (1.80 ± 0.02 mL) as the inflammatory response progressed. MCP-1(5-76) and MCP-4(8-75) showed an approximately 66% reduction in the increase in inflammatory edema from 24 to 36 hours compared with the vehicle control (Figure 8). An engineered form of MCP-1(9-76), a potent CCR-2 antagonist, was found to reduce the paw volume to a similar extent. Most notable though was MCP-3(5-76), which reduced the paw volume 1.6-fold to below that at the time of injection. Hence, MMP inactivation of MCPs also generates CCR antagonists that retain cellular binding affinity and that modulate cell migration in vitro and dampen the inflammatory response to carrageenan in vivo.

### Discussion

MMPs are implicated in many physiological processes involving matrix turnover, but direct evidence for this is limited. In contrast, MMPs are clearly identified with pathologies, including arthritis and tumor metastasis where they have been assumed to primarily play a matrix degradative role. However, a broader MMP substrate degradome is now being revealed by recent studies that show proteolytic susceptibility of signaling proteins such as MCP-3, SDF-1α and SDF-1β, TNF-α, and Fas ligand. This indicates an equally important role for MMP activity in regulating inflammation and immune processes in which proteolytic targets include molecules involved in information networks. In view of the efficient cleavage of MCP-3, but not MCP-1, MCP-2, or MCP-4, by MMP-2, it was important to determine whether processing and inactivation of chemokines by MMPs are limited to a few isolated examples or are more general phenomena that may have broad biological implications for many pathological and physiological processes.

Because MMPs are differentially expressed in many tissues, in many cell types, and during inflammation and healing, we determined the specificity of 8 MMPs against the CC chemokines MCP-1, MCP-2, MCP-3, and MCP-4. In addition to MMP-2, MMP-1, MMP-3, MMP-8, MMP-13, and MT1-MMP cleave MCPs and all characteristically perform this at position 4-5, but the MMPs are not always functionally interchangeable and each MCP showed a different profile of proteolytic susceptibility. The pattern...
of MMP proteolysis of the MCP family is striking. MMP-3, a protease with broad extracellular matrix substrate specificity (see www.clip.ubc.ca/mmps.shtm for a comprehensive updated list of MMP substrates), can cleave all MCP chemokines, but MMP-2, MMP-13, and MT1-MMP are active only on MCP-3. The similar specificity of MMP-2 and MT1-MMP is interesting because MT1-MMP is the major cellular activator of proMMP-2, and both proteases assemble and function together in activation complexes.25,33,35 Most notable was the general inability of MMP-7 and of the primarily leukocytic enzymes MMP-8 and MMP-9 to process MCPs. Although MMP-8 could cleave MCP-1, catalytic efficiency was very low. MMP-9 can activate IL-8 by N-terminal processing, but does not cleave MCP-2,36 or any other MCP as shown here. It is important to distinguish between efficient and precise proteolytic processing, as shown for these chemokines, and the more general catabolic actions of proteases during protein degradation. MMP-9 has been reported to slowly degrade the chemokines PF-4, GRO-α/H9251, and CTAP-III, with the degraded products lacking activity. 23 Although in vitro biochemical assays do not replicate in vivo conditions and are often not performed in the presence of extracellular matrix components that may modulate protease activity, the slow turnover rates in vitro of PF-4, GRO-α/H9251, and CTAP-III, even at high enzyme-to-substrate ratios, 23 indicate that these may be poor MMP-9 substrates in vivo, reducing the physiological relevance of any such activity of MMP-9.

Figure 5. Cell binding of MCP-2(5-76) and MCP-4(8-75). CCR-2 binding of 125I–MCP-1 in the presence of increasing amounts of unlabeled MCP-1, MCP-2, MCP-4, or vehicle, and synthetic analogs corresponding to MCP-2(5-76), the MMP-cleaved form of MCP-2, and corresponding to MCP-4(8-75), the MMP-cleaved form of MCP-4. Mean values of duplicate assays are shown.

Figure 6. Receptor activity of full-length MCP-4 and MCP-4(8-75). (A) (B) Cell migration assays of B300-CCR2 (panel A) or B300-CCR3 (panel B) showing activity of MCP-4(8-75), MCP-4, or vehicle alone (0 M). (C) Cell migration assay of B300-CCR2 showing, as indicated, MCP-4 (300 nM) in the presence of increasing concentrations of MCP-4(8-75) or MCP-4 alone—ie, with 0 M MCP-4 (8-75). (D) Cell migration assay of B300-CCR3 in response, as indicated, to full-length MCP-4 (100 nM) and in the presence of increasing concentrations of MCP-4(8-75) or MCP-4 alone, ie, with 0 M MCP-4(8-75). Mean values of duplicate assays are shown.

Figure 7. The effects of MMP-cleavage of MCP-2 and MCP-4 on THP-1 monocytic cell chemotaxis. (A) (B) Cell migration assay of THP-1 cells in response to MCP-2 and MCP-2(5-76) (panel A) or MCP-4 and MCP-4(8-75) (panel B), as indicated. Cells migrated in the absence of chemokine are shown at 0 M chemokine. Mean values of duplicate assays are presented. (C) (D) Antagonist assays are shown for MCP-2(5-76) (panel C) and MCP-4(8-75) (panel D). Chemotaxis of THP-1 cells in response to 30 nM MCP-2 or 100 nM MCP-4 in the absence and presence of MMP-cleaved chemokine analog at the indicated concentrations is shown in the corresponding panels. Mean values of duplicate assays are presented.

Figure 8. Anti-inflammatory effects of MMP-cleaved MCP analogs in vivo. Rat paws were injected with carrageenan at time 0. After 24 hours, the volume of the paws was measured, and the paws were injected with either the indicated chemokine analog (50 μg, equivalent dose 250 μg/kg) or phosphate-buffered saline alone. Shown is the change in paw volume from 24 to 36 hours. The negative value for MCP-3(5-76) indicates a reduction in volume at 36 hours compared with 24 hours. *Group differed significantly from vehicle group (P < .05). **Group differed significantly from vehicle (P < .01). Data presented as the mean ± SD; n = 5 per group.
In addition to the amino acid sequence of the substrate, auxiliary elements of substrate specificity are pivotally important, including substrate binding by exosites on the MMP hemopexin C domain.7,31 The absence of a hemopexin C domain in MMP-7 may explain the lack of activity against these chemokines, and the hemopexin C domain of MMP-9 is suggested to be positioned away from the catalytic domain by an extended carbohydrate-rich linker peptide,34 possibly accounting for its lack of ability to process the N-terminus of the MCFs. Therefore, the same position of the MMP cleavage site in MCFs reported here and SDF-1α and SDF1-B suggests that chemokine processing is a more general property of MCFs and not an isolated example limited to MCF-3 by MCF-2, first reported by McQuiban et al.7

Neoeotipote antibodies have been used to show the generation of MCF-3(5-76) in human arthritis in vivo.7 Processing of SDF-1α has been observed by monocytic U937 cells in culture and MCF processing reported in cell culture following treatment with cytokines7,36 and concanavalin A,7 which activates the MMP-2 processing reported in cell culture following treatment with colchicine for receptor occupancy, with subsequent desensitization the receptor antagonists. The antagonistic effects are due to competitive inhibition of the MMP cleavage site in MCFs reported here and SDF-1α, and SDF-1α which cover a more restricted CCR spectrum, MCF-3(5-76) was previously suggested the potential importance of generating a broad-spectrum chemotactic antagonist for CCR-1, CCR-2, and CCR-3 in modulating inflammatory and immune processes in vivo. However, whether a connection exists between MCF activity and the modulation of CCR-1, CCR-2, and CCR-3–dependent cellular responses in disease remains to be elucidated and is currently under investigation in our laboratories.

The physiological likelihood of in vivo chemokine cleavage, specifically MCF-3, was revealed by the high kinetic turnover rates (Table 1). Notably, MCF-2 and MCF-14 were the most efficient at cleaving MCF-3 in vitro, but interestingly neither cleaved MCF-1, MCF-2, or MCF-4. This points to a unique role for MCF-3 cleavage by MT1-MMP and MCF-2. MT1-MMP is the physiological activator of MCF-2 and forms a cell-surface receptor for this enzyme.35 MT-MMPs are also critical for cell migration in collagen,37 an important feature of remodeling wounds. We have previously postulated that modulation of the MMP-2/MT1-MMP proteolytic axis at the cell surface changes the proteolytic profile of stromal cells from collagenolytic (predominantly manifested by MT1-MMP activity) to gelatinolytic (by MCF-2) during the conversion from a cytokine-stimulated resorptive cell to a matrix-depositing cell.31,33 Proteolysis of MCF-3 by MT1-MMP and MCF-2 recruited to the cell surface in both the collagenolytic and gelatinolytic phases of tissue remodeling is likely to also be augmented by the action of collagenase and stromelysin, which may process MCF-1, MCF-2, and MCF-4 during the tissue resorptive phase, to reduce chemokine activity directly and indirectly by creating CCR-1, CCR-2, and CCR-3 antagonist gradients. Although TIMPs present in the tissue reduce net proteolytic activity by MCFs, particularly at the interface between the inflammatory lesion and normal tissue, TIMP-2 binding to the hemopexin C domain of MCF-2 interferes with both MCF-3 binding and cleavage nor MCF-2 activation by MT1-MMP (G.A.M., C.M.O., unpublished data, May 2000).

Our data support the following model (Figure 9), which connects the activity of chemokines and MCFs in the stages that define the inflammatory reaction. Chemoattractant-directed leukocytes secrete MCFs, predominantly MCF-8 and MCF-9 but not MCF-2,38,40 that may degrade matrix and promote migration but do not cleave MCFs. As the inflammatory reaction progresses, cytokines such as interleukin-1 and TNF-α are also released by macrophages.38 The stromal cells respond by secreting MCFs and expressing cell surface MT1-MMP, which contributes toward the bulk removal of matrix. In addition, we propose that an important function of MCFs is to regulate the inflammatory response by processing MCFs to reduce agonist activity and to form CCR-1, CCR-2, and CCR-3 antagonist gradients. Ultimately this would deplete the cellular infiltrates of leukocytes expressing these receptors. In the tissue-resolution phase, transforming growth factor (TGF)-β1 elevates extracellular matrix deposition and stabilizes new matrix by repressing MCF and stimulating TIMP expression.41,42 TGF-β1 also stimulates MCF-2 levels,31 the most efficient MCF in the cleavage and inactivation of MCF-3. This may maintain cleaved MCF-3 levels despite the reduction in collagenase41 and stromelysin42 expression. Thus, MCFs not only are effectors of the inflammatory response but are important for its regulation.
The present and recent data from our group showing the efficient cleavage of MCPs 1, 2, 3, and 4 and SDF-1α and SDF-1β by MMPs indicate that the chemokine superfamily represents a large and biologically important new class of MMP substrate and that MCP-3 cleavage by MMP-2 is not a rare example. Notably, activity always includes cleavage at position 4-5, indicating that this site in other chemokines may also be susceptible to MMP processing. There are approximately 54 human chemokines presently identified, and we have initiated a comprehensive screen to determine chemokine susceptibility to MMP cleavage. It is difficult to predict from sequence analysis alone which chemokines will also be processed because we have found that both scissile bond sequence and precise domain structure contribute to the proteolytic susceptibility of the chemokine (G.A.M., C.M.O., unpublished data, May 2000). Overall, our data suggest that inflammatory-induced MMP proteolysis of chemokines may contribute to the normal dissipation of inflammatory cell infiltrates, which in turn results in reduced MMP expression in a homeostatic feedback loop—aberrations of which may contribute to chronic inflammatory pathologies.

Acknowledgment
We thank Shouming He for technical assistance in mass spectrometry analysis.

References

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