Proteinase-activated Receptor-1 Regulation of Macrophage Elastase (MMP-12) Secretion by Serine Proteinases*

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The serine proteinases plasmin and thrombin convert proenzyme matrix metalloproteinases (MMPs) into catalytically active forms. In addition, we demonstrate that plasmin(ogen) and thrombin induce a significant increase in secretion of activated murine macrophage elastase (MMP-12) protein. Active serine protease is responsible for induction, as demonstrated by the absence of MMP-12 induction in plasminogen(Plg)-treated urokinase-type plasminogen activator-deficient macrophages. Since increased MMP-12 protein secretion was not accompanied by an increase in MMP-12 mRNA, we examined post-translational mechanisms. Protein synthesis was not required for early release of MMP-12 but was required for later secretion of activated enzyme. Immunofluorescent microscopy demonstrated basal expression in macrophages that increased following serine proteinase exposure. Inhibition of MMP-12 secretion by hirudin and pertussis toxin demonstrated a role for the thrombin G protein-coupled receptor (protease-activated receptor 1 (PAR-1)). PAR-1-activating peptides were able to induce MMP-12 release. Investigation of signal transduction pathways involved in this response demonstrate the requirement for protein kinase C, but not tyrosine kinase, activity. These data demonstrate that plasmin and thrombin regulate MMP-12 activity through distinct mechanisms: post-translational secretion of preformed MMP-12 protein, induction of protein secretion that is protein kinase C-mediated, and extra-cellular enzyme activation. Most importantly, we show that serine proteinase MMP-12 regulation in macrophages occurs via the protein kinase C-activating G protein-coupled receptor PAR-1.

Serine and matrix metalloproteinases (MMPs)¹ have important roles in hemostasis and remodeling of extracellular matrices during fibrinolysis and tissue repair. Abnormal regulation of these proteinases may cause tissue destruction. MMPs are typically secreted in zymogen form and require extracellular activation. The serine proteinases plasminogen, plasmin, urokinase-type plasminogen activator (uPA), and tissue-type plasminogen activator (tPA) have classically been shown to function in fibrinolysis (tPA/uPA/plasmin), cell motility and invasion (uPA/plasmin), and MMP activation. The MMPs are a family of structurally related zinc-containing enzymes that are either secreted or membrane-associated (membrane-type MMPs; MT-1–5 MMPs). As a group, the MMPs are capable of degrading all extracellular matrix components.

In addition to fibrinolysis and extracellular matrix degrada- tion, MMPs and serine proteinases can cleave nonmatrix proteins with significant biological ramifications. For example, MMPs and related ADAMS (a disintegrin and metallopeptinase domain) can cleave and release a variety of active molecules from cell surfaces, such as tumor necrosis factor-α (1), and plasmin can activate transforming growth factor-β in plasma (2). In addition, both MMPs and serine proteinases generate angiotatin, an inhibitor of endothelial cell proliferation, from plasminogen (3–9).

Such redundant activity and interaction of the serine and metalloproteinase families have precedence in previous work. Plasmin cleaves many pro-MMPs, including the gelatinases MMP-2 and -9 (progelatinase A and B) (10) and MMP-12 (macrophage elastase) (11) within the N-terminal domain, altering conformation and exposing the active site zinc, which in turn releases the remainder of the proenzyme domain, resulting in a fully active MMP. At the cellular level, uPA-generated plasmin controls gelatinase activity in HT1080 cells (10). Carmeliet et al. (11) have recently shown that MMP activation in macrophages in culture is uPA/plasmin-dependent, and data suggests similar activation in a murine model of atherosclerotic microaneurysm formation. Another serine proteinase, thrombin, has also been reported to activate MMPs (progelatinase A, MMP-2) in microvascular endothelial cells (12) and to actually increase collagenase (MMP-1) protein and stromelysin (MMP-3) mRNA and protein in large vessel endothelial cells (human saphenous vein and mammary artery) (13).

Protease-activated receptor (PAR-1) is a unique cell surface-associated receptor activated by thrombin proteolysis that is characterizedly coupled to G protein heterodimers (14) and is also activated by plasmin (15). It is a member of the seven transmembrane domain family that is activated following proteolytic cleavage of its N terminus (16). This newly formed N terminus acts as a tethered ligand to activate the receptor. Peptides homologous to this tethered ligand can also activate the receptor but are not as effective as thrombin that cleaves between residues Arg⁴⁴ and Ser⁴⁵. Many cell types, including endothelial cells (17), macrophages (18), smooth muscle cells, and fibroblasts (19) express PAR-1.

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¹ The abbreviations used are: MMP, matrix metalloproteinase; MMP-12, murine macrophage elastase; Plg, plasminogen, Thr, thrombin; FTX, pertussis toxin; uPA, urokinase-type plasminogen activator; tPA, tissue-type plasminogen activator; PKC, protein kinase C; PBS, phosphate-buffered saline; DMEM, Dulbecco’s modified Eagle’s medium; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase.
While the activation of pro-MMPs to their active form by serine proteases has been previously described, the purpose of this study was to explore other mechanisms of MMP activation by the serine protease plasminogen and thrombin.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Recombinant mouse macrophage elastase (MMP-12, MMP-12) was expressed and purified from *Escherichia coli* to homogeneity as described (20). The *E. coli*-derived enzyme is spontaneously active, since only its catalytic domain is expressed upon purification.

Glu-human plasminogen (Plg), plasmin, cycloheximide, apotinin, proteinsin, taxol, hirudin, and gentamicin were purchased from Sigma. Glu-plasminogen was prepared from fresh frozen plasma by affinity chromatography on lysine-Sepharose, gel filtration, and ion exchange chromatography. Purity is >98% Glu-plasminogen and <2% Lys-plasminogen, as determined by acetic acid/urea polyacrylamide gel electrophoresis. Thrombin was obtained from American Diagnostics (Greenwich, CT). v-Phenylalanilyl-7-prolyl-l-arginine chloromethylketone was generated (TRAPs; TFLLR-NH2 and TFRIFD-NH2), as was an irrelevant peptide (FLFLR-NH2 and TFRFID-NH2), as was an irrelevant peptide. PAR-1-activating peptide sequences are identical in protein sequence to the receptor cleavage and duplication sites of thrombin (14).

A polyclonal monospecific antibody to mouse macrophage elastase was generated in rabbits as described (21) and used at 1:1000 for Western analysis unless otherwise stated. For immunohistochemistry, the antibody was used at 1:150. A Cy3-labeled goat anti-rabbit IgG secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA) was used for immunofluorescence at 1:500.

**Macrophage Culture Conditions**—Peritoneal macrophages were harvested from mice deficient in MMP-12 by targeted mutagenesis (MMP-12−/−) and wild-type littermates (MMP-12+/+). Peritoneal macrophages were also harvested from mice deficient in plasminogen, urokinase plasminogen activator, and tissue-type plasminogen activator (kindly provided by Dr. Peter Carmeliet, University of Leuven, Leuven, Brussels). Peritoneal macrophages from MMP-12−/+ and MMP-12−/− mice were obtained by peritoneal lavage. In some experiments, to increase macrophage yield, mice received a 1-ml intraperitoneal injection of 3% thioglycollate (22), peritoneal lavage. In some experiments, to increase macrophage yield, mice received a 1-ml intraperitoneal injection of 3% thioglycollate (22), peritoneal lavage. Peritoneal macrophages were also harvested from mice deficient in plasminogen, urokinase plasminogen activator, and tissue-type plasminogen activator (kindly provided by Dr. Peter Carmeliet, University of Leuven, Leuven, Brussels). Peritoneal macrophages from MMP-12−/+ and MMP-12−/− mice were obtained by peritoneal lavage. In some experiments, to increase macrophage yield, mice received a 1-ml intraperitoneal injection of 3% thioglycollate (22), and 5 days following injection, peritoneal macrophages were harvested and plated into 24-well tissue culture plates (Becton Dickinson, Franklin Lakes, NJ) at 1 × 10^6 cells/well overnight at 4 °C. The macrophages were then resuspended in ice-cold 4 °C DMEM (Life Technologies, Inc.). After 24 h of culture at 37 °C and 5% CO2, cells were plated in serum-free medium and treated with 20 μg/ml of plasminogen (10–100 units/ml of thrombin or buffer control in the presence or absence of the serum protease inhibitor apro- tinin for 48 h. Cell-conditioned media were harvested and subjected to Western blot analysis and casein zymography.

**Immunofluorescence and Confocal Microscopy**—Peritoneal murine macrophages (control and thyoglycollate-stimulated) from MMP-12−/− and wild-type littermates were cultured on Lab-Tech slides (Nalgen Nunc, Naperville, IL) at 5 × 10^4 cells/well in 10% fetal calf serum DMEM for 24 h. Monocytes were stimulated with 3 μg of MMP-12 cDNA, glyceraldehyde-3-phosphate dehydrogenase cDNA, and Bluescript (pBS) plasmid cDNA.

**RESULTS**

**Plasminogen Induces MMP-12 Secretion and Activation**—Murine peritoneal macrophages were harvested from MMP-12-deficient mice and incubated with Plg (20 μg/ml) (Sigma) in the presence or absence of apro tin (Sigma) for 48 h. Western analysis of the macrophage-conditioned media for MMP-12 demonstrated small amounts of pro-MMP-12 at 54 kDa in control macrophages and the generation of active MMP-12 (29 kDa) in the Plg-stimulated MMP-12−/+ macrophages (Fig. 1A, lane 2). The active MMP-12 was not, however, secreted by the Plg-stimulated MMP-12−/+ macrophages in the presence of apro tin, demonstrating dependence upon serine protease activity.

PERitoneal murine macrophages were stimulated with plasmin (Sigma) (20 μg/ml), 48-h conditioned medium was collected, and Western analysis for MMP-12 was performed. As
shown in Fig. 1B, treatment with either Plg or plasmin resulted in increased MMP-12 release and activation to a 29-kDa form. Densitometry of autoradiographs (n = 4) demonstrated a 2.5–3.6-fold increase in secreted protein over control levels (p < 0.05 for plasmin) (Fig. 1C). Casein zymography confirmed the 29-kDa band seen on Western in addition to the typical 22-kDa fully processed form (Fig. 1D). We presume that the 29-kDa form represents an active enzyme with an N-terminal AA sequence at the beginning of the catalytic domain extending through part of the C-terminal domain as described previously (23). Conversion on zymography probably is a function of continual processing of the C-terminal domain while running through the gel. Control macrophage-conditioned media produced a minimal lysis at 22 kDa, and Plg-treated MMP-12 −/− macrophages did not produce lytic bands at any molecular weight (data not shown).

To further investigate the role of plasmin(o)gen in MMP-12 production, thiglycollate-elicted peritoneal macrophages were harvested from serum proteinase uPA-deficient mice (uPA −/−) and mice deficient in Plg (Plg −/−) and tissue-type plasminogen activator (tPA −/−). The macrophages were plated on tissue culture plastic and incubated in the presence of Plg (20 μg/ml) or plasmin (20 μg/ml), and Western blot analysis for MMP-12 was performed (Fig. 2). Increased amounts of activated MMP-12 (29 kDa, arrow) were produced following both Plg and plasmin stimulation in wild-type, Plg −/−, and tPA −/− macrophages. Interestingly, increased MMP-12 secretion and activation was observed in the uPA −/− macrophages in response to plasmin but not Plg. This suggests that the active serine proteinase plasmin, which is generated from plasminogen via uPA, is required for the secretion and activation of MMP-12 in murine macrophages. Although equal numbers of macrophages were plated from wild-type and each serine proteinase-deficient animal, Plg −/− macrophages produced lesser amounts of constitutive pro-MMP-12 and less activated MMP-12 following Plg and plasmin stimulation. This was seen
mice were cultured in the absence or presence of plasmin (20 μg/ml). Conditioned medium was collected at varying times, and Western analysis was performed using an antibody specific for MMP-12.

in repeat experiments.

We then explored plasmin-mediated secretion of MMP-12 at varying time points (6, 12, 24, 48, and 72 h). As demonstrated in Fig. 3, MMP-12 protein secretion increases over time, without a coincident increase in pro-MMP-12. This further demonstrates a true increase in secretion and activation of MMP-12, as opposed to sole activation of proenzyme.

Plasminogen and Plasmin Do Not Induce Gene Transcription of MMP-12 mRNA in Murine Macrophages—Although the activation of MMP-12 by plasminogen has previously been described (11), we demonstrated an increase in total MMP-12 protein by Western analysis in the Plg- and plasmin-stimulated macrophages compared with control. Therefore, we investigated MMP-12 mRNA levels by Northern analysis following stimulation with Plg and plasmin. There was no change in MMP-12 mRNA levels at 12 h (data not shown) or at 24 h in the presence of Plg or plasmin (Fig. 4A). Similarly, nuclear run-on assays did not demonstrate Plg or plasmin induction of MMP-12 transcription (Fig. 4B).

Active Protein Synthesis Is Not Required for Early Plasminogen-induced Secretion of MMP-12 Protein—To determine if plasmin promotes an active secretion of basally expressed MMP-12 protein, macrophages were pretreated with either monensin (1 μM) or cycloheximide (10 μg/ml) 1 h prior to plasmin or plasminogen treatment. As demonstrated by Western analysis of conditioned media, monensin pretreatment blocked secretion of pro-MMP-12 (54 kDa) in control macrophages and both pro- and activated (29 kDa) MMP-12 in Plg- and plasmin-treated cells (Fig. 5A), demonstrating active secretion of protein. Pretreatment with cycloheximide limited the amount of activated MMP-12 secreted in Plg-treated macrophages, and this amount remained constant (Fig. 5B), indicating that active protein synthesis is required for the robust induction of secretion seen at later time points (24 and 48 h; Figs. 1 and 3). The slower initial induction of secretion in plasminogen-treated (12–18 h; Fig. 5B) compared with plasmin-treated (6–12 h; Fig. 3) macrophages may be reflective of the time required for the macrophage-mediated generation of effective amounts of plasmin from the parent protein plasminogen. Compared with control, monensin- and cycloheximide-treated cells showed no evidence of cell damage as examined by inverted microscopy (data not shown).

Macrophages Basally Express Cytoplasmic MMP-12 Protein That Increases following Plasmin and Thrombin Treatment—Control and plasmin-stimulated thioglycollate-elicited peritoneal macrophages and their cell-conditioned media that were examined at 6, 12, 24, 48, and 72 h for MMP-12 secretion by Western analysis (Fig. 3) were also examined by confocal immunofluorescent microscopy for MMP-12 protein expression. Non-thioglycollate-elicited peritoneal macrophages were examined at 24 h following plasmin stimulation. Western analysis of conditioned media from plasmin-treated thioglycollate-elicited peritoneal macrophages demonstrated a progressive increase in the active form of MMP-12 (29 kDa) as demonstrated in Fig. 3. Corresponding immunofluorescent confocal microscopy of control macrophages at 24 h (Fig. 6, upper left panel) demonstrated a positive signal for MMP-12 in a cytoplasmic pattern (red signal demonstrates positive staining for MMP-12 protein;
The Serine Proteinase Thrombin Activates and Induces Secretion of MMP-12 Protein and Involves the Thrombin Receptor, PAR-1—We therefore further explored the serine proteinase thrombin, due to its well-characterized signal transduction mechanism and its effect on of MMP-12 secretion. Western analysis of conditioned media from thrombin-stimulated murine peritoneal macrophages shows that thrombin also induces MMP-12 secretion and activation (Fig. 8A), consistent with our findings by confocal microscopy. Thrombin and plasmin are capable of activating the thrombin G protein-coupled receptor, protease activator receptor-1 (PAR-1). To determine if induction of MMP-12 was occurring through thrombin receptor activation, we attempted to block thrombin activity by preincubating thrombin with hirudin (Sigma) prior to exposure to macrophages. Hirudin, a leech-derived Thr-specific inhibitor, neutralizes the activity of thrombin by binding to its anion site, neutralizing the activity of thrombin by binding to its anion site, neutralizing the activity of thrombin by binding to its anion site, neutralizing the activity of thrombin by binding to its anion site, neutralizing the activity of thrombin by binding to its anion site, neutralizing the activity of thrombin by binding to its anion site.

Fig. 6. Macrophages basally express cytoplasmic MMP-12 protein that increases in the cytosol following plasmin treatment. Control and plasmin-stimulated macrophages were examined for MMP-12 at 6, 12, 24, 48, and 72 h by confocal immunofluorescent microscopy. Macrophages were plated on Tissue-Tek slides (Nalgen Nunc) fixed with 4% paraformaldehyde and stained with rabbit anti-mouse MMP-12 specific antibody. Secondary staining was performed with goat anti-rabbit Cy3 IgG. A red signal demonstrates positive staining for MMP-12 protein; a green signal indicates laser reflectance of macrophages. Left upper panel, control macrophages demonstrate positive signal for MMP-12 in a cytoplasmic pattern in certain cells. Magnification was ×600. Inset a demonstrates nonspecific, perinuclear staining found in MMP-12−/− macrophages. Right upper panel and lower panels, plasmin-treated macrophages demonstrate a cytoplasmic and cell surface staining pattern (arrow, inset b). At 24 h (left lower panel), there is positive staining for MMP-12 in the extracellular matrix (asterisk). By 48 h (right lower panel) in the presence of plasmin, MMP-12 protein expression continues in a cytosolic and cell surface-associated pattern, compared with control macrophages at 48 h having minimal MMP-12 expression (inset c).

Fig. 7. MMP-12 protein increases in the cytosol following thrombin treatment. Control and thrombin-stimulated (10 units/ml) peritoneal macrophages were examined for MMP-12 protein expression by confocal immunofluorescent microscopy as described in the legend to Fig. 7. A red signal (arrow) demonstrates positive staining for MMP-12 protein (shown without laser reflectance). Left panel, immunofluorescent confocal microscopy of control macrophages at 24 h demonstrated minimally detectable signal in the cytoplasm (arrow). Right panel, following thrombin treatment, MMP-12 expression was significantly increased within the cytoplasm (arrow), evident at 24 h. MMP-12 was also demonstrated extracellularly (asterisk).
To confirm that Thr was acting through activation of PAR-1 to induce MMP-12 protein, PAR-1 (thrombin receptor)-specific peptides were generated (TRAPs; TFLLR-NH₂ and TFRIFD-NH₂) as an irrelevant peptide (FLTRL-NH₂). PAR-MMP-12 protein (Fig. 8A) were capable of inducing active and intermediate forms of MMP-12 at 48 h. Supernatants were collected, and Western analysis was performed for MMP-12. B, thrombin-induced MMP-12 protein secretion is blocked by pertussis toxin. Murine macrophages were plated on tissue culture plastic and pretreated overnight with PTX (100 ng/ml) prior to exposure to increased doses of Thr (80 units/ml). Supernatants were harvested at 48 h, and Western analysis for MMP-12 was performed.

Serine Proteinase-stimulated MMP-12 Secretion Is Negatively Coupled to Adenylyl Cyclase and Is PKC- and MAPK-dependent—Plasminogen stimulation was performed in the presence of pharmacologic agents that alter signal transduction mediators, specifically tyrosine kinase (genistein, herbimycin, orthovanadate) and cyclooxygenase (indomethacin). Secretion of active MMP-12 was not inhibited in the presence of genistein, herbimycin, or orthovanadate, indicating no dependence of signal transduction upon tyrosine kinase(s) (Fig. 9A).

Thrombin-induced secretion of active and intermediate forms of MMP-12 was also demonstrated in the presence of genistein but was inhibited by forskolin, an inhibitor of adenylyl cyclase, the PKC inhibitor calphostin C, and the MAPK/ extracellular signal-regulated kinase 1 inhibitor PD 98059 (Fig. 9B). These findings demonstrate that the thrombin response is negatively coupled to cAMP as previously demonstrated in adrenal medullary microvascular endothelial cells (27). This also demonstrates PKC dependence of the serine proteinase-mediated MMP-12 effect. Failure of the PKC inhibitor H7 to block this response may reflect selective effects on different PKC isofoms by these inhibitors. The effect of inhibition of these signal transduction mediators on serine proteinase-mediated MMP-12 secretion is summarized in Table I.

DISCUSSION

Macrophage elastase is expressly produced by macrophages and is the primary metalloproteinase produced by murine macrophages (21). Macrophages also express the serine proteinases uPA and the urokinase receptor, uPAR, and are capable of generating plasmin from plasminogen. It has previously been demonstrated that plasmin is capable of extracellularly activating pro-MMP-12 (11) and generates angiostatin from plasminogen in tumor (7) and HT1080 cells (9). We have recently reported that macrophage-mediated generation of the
angiogenesis inhibitor angiotatin from plasminogen is dependent upon the metalloproteinase MMP-12 (3). In the present study, we demonstrate that the serine proteinases plasmin(o)gen and thrombin induce release of preformed MMP-12 stores and increase MMP-12 protein secretion and conversion of pro-MMP-12 to its active form. In our system, activation of pro-MMP-12 (54 kDa) results in a 29-kDa form, and detectable amounts of lower molecular weight forms by Western analysis, as previously reported (22 kDa) (23), are not seen. This may be reflective of the serine proteinase activity on secreted enzyme at the cell surface. Our investigations into the mechanism of this secretion demonstrate that it does not require tyrosine kinase activity and further show G protein-mediated protein kinase C/MAPK regulation via the thrombin receptor, PAR-1.

Interestingly, we found decreased amount(s) of control and active MMP-12 in the plasminogen-deficient (Plg<sup>-/-</sup>) macrophages following plasminogen and plasmin stimulation (Fig. 2). Although the mechanism of this is unclear, we postulate that in plasminogen-deficient animals, macrophages may be “primed” by the presence of plasmin(o)gen to respond to plasmin(o)gen stimulation. Supporting this hypothesis, we demonstrate that MMP-12 secretion involves PKC and MEK signaling mechanisms that may not be activated in the absence of plasmin(o)gen, and that inhibition of these signaling pathways inhibits, and in some cases totally blocks, not only activated but also constitutive MMP-12 secretion (Fig. 9).

In fact, the effect of decreased levels of plasin on MMP activity in vivo has significance for recent findings in certain animal disease models. We have demonstrated that macrophages from uPA-deficient animals cannot generate plasmin in vitro (Fig. 2). In vivo, however, these animals are capable of generating plasmin (although possibly in decreased amounts) via endothelial tPA. Consistent with this, previous studies have demonstrated that plasmin(o)gen-dependent activation of pro-MMPs in uPA-deficient animals does occur, but in reduced amounts (11). Interestingly, it has been shown that lower amounts of MMP-12 are expressed following vascular injury in these mice compared with wild type, and this finding is associated with lower macrophage numbers in the neointima (29). In that MMP-12 is required for macrophage matrix invasion in mice (21), our data suggest that decreased amounts, together with decreased activation, of secreted MMP-12 in uPA-deficient animals contribute to the impaired macrophage neointimal infiltration.

Increases in MMP-12 gene transcription or post-transcriptional mRNA stability did not account for the increase in MMP-12 protein. Examination of control and plasmin-treated murine macrophages by confocal immunofluorescent microscopy demonstrates a modest level of basal cytoplasmic expression in control macrophages that is not dependent upon thionglycollate elicitation. Within 24 h of treatment, plasmin-treated macrophages increase their expression of cytoplasmic MMP-12 that is then secreted, with some evidence of extracellular accumulation. Consistent with secretion of basal protein, initial increases in secreted MMP-12 remained stable in the presence of cycloheximide, as determined by Western blot analysis. Later (>24 h) increases in MMP-12, however, are dependent upon active protein synthesis, as demonstrated by the absence of increased MMP-12 over time in the presence of cycloheximide. These data also demonstrate that plasmin(o)gen, at concentrations that would be consistent with those at the macrophage cell surface (approximately 10-fold increase over plasma concentrations), is capable of regulating MMP-12 activity predominantly via post-translational secretion of preformed cytosolic MMP-12 protein and increase in protein secretion and extracellular enzyme activation.

To investigate the mechanism underlying this serine proteinase regulation of MMP-12, we then examined the effect of thrombin on MMP-12 secretion, due to its well characterized G protein-mediated receptor activation through PAR-1. In fact, thrombin increased the protein expression and secretion of MMP-12 from macrophages. Additionally, we recognized that plasmin has been reported to similarly cleave and consequently activate this receptor (15). Through the use of specific inhibitors of signal transduction molecules, we show that plasmin(o)- and thrombin-induced MMP-12 secretion of active enzyme in macrophages is not tyrosine kinase-dependent but is dependent upon other protein kinases (protein kinase C family members, MAPK). It is indeed interesting that LPS immunomodulation of macrophage function involves similar signal transduction pathways through its GPCR receptor CD14 (30). More specifically, LPS-induced tumor necrosis factor-α and IL-1 secretion in macrophages has been similarly reported to be PKC-dependent (31).

The control of MMP expression through several distinct regulatory mechanisms and signaling pathways has been demonstrated previously. In fact, LPS regulation of gene expression of another MMP, MMP-9, in a human monocyte-like cell line (U937) and in alveolar macrophages has been found to involve post-transcriptional mechanisms (32). Similarly, signal transduction pathways have been investigated in collagen-induced MMP-1 (collagenase) production in keratinocytes and have shown a PKC- and tyrosine kinase-dependent mechanism distinct from that of PKC-induced PMA up-regulation (33). Finally, during macrophage differentiation, PKC-β has been found to have a signaling role in the fibronectin matrix-mediated cell adhesion and gene expression of MMP-9 (34). Although these investigations primarily involve regulation of MMP expression via gene induction, our findings provide evidence that similar signaling mechanisms may ultimately be involved in serine proteinase-mediated mechanisms regulating MMP expression.

We propose that serine proteinase-dependent regulation of MMP-12 may have a role in biologic processes associated with inflammation, such as vascular injury, angiogenesis, tumor growth, and metastasis. Consistent with this hypothesis is the lowered concentration of MMP-12 seen in vascular injury in uPA<sup>−/−</sup> mice (29). In tumor biology, tumor cell proteinase expression has been characterizedly associated with increased tumor invasion and metastasis (35). Tumor stromal and inflammatory cell proteinase expression, however, does not always contribute to tumor progression but may function in host repair and defense. Macrophages are often recruited to tumor sites (tumor-associated macrophages) where plasmin(o)gen and thrombin are replete, due to both local hemostasis and tumor- and macrophage-derived factors (vascular endothel-
PAR-1-mediated Serine Proteinase-induced Secretion of MMP-12

41250

liial growth factor and tumor necrosis factor-α that contribute to vessel wall “leakiness” (36). In this context, the serine proteinase regulation of MMP-12 in macrophages may affect tumor growth and metastasis through the MMP-12-mediated generation of the angiogenesis inhibitor, angiotatin, from its parent serine proteinase protein plasminogen.

In sum, we show that the serine proteinases plasmin and thrombin directly the release of MMP-12 from the macrophage and activate the enzyme extracellularly. We hypothesize that control of MMP-12 expression through the G protein-coupled receptor PAR-1 provides a distinct and focused regulation of this MMP.

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