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Urokinase-Type Plasminogen Activator Stimulation of Monocyte Matrix Metalloproteinase-1 Production Is Mediated by Plasmin-Dependent Signaling through Annexin A2 and Inhibited by Inactive Plasmin

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Chronic inflammatory diseases are associated with connective tissue turnover that involves a series of proteases, which include the plasminogen activation system and the family of matrix metalloproteinases (MMPs). Urokinase-type plasminogen activator (uPA) and plasmin, in addition to their role in fibrinolysis and activation of pro-MMPs, have been shown to transduce intracellular signals through specific receptors. The potential for uPA and plasmin to also contribute to connective tissue turnover by directly regulating MMP production was examined in human monocytes. Both catalytically active high m.w. uPA, which binds to the uPAR, and low m.w. uPA, which does not, significantly enhanced MMP-1 synthesis by activated human monocytes. In contrast, the N-terminal fragment of uPA, which binds to uPAR, but lacks the catalytic site, failed to induce MMP-1 production, indicating that uPA-stimulated MMP-1 synthesis was plasmin dependent. Endogenous plasmin generated by the action of uPA or exogenous plasmin increased MMP-1 synthesis by signaling through annexin A2, as demonstrated by inhibition of MMP-1 production with Abs against annexin A2 and S100A10, a dimeric protein associated with annexin A2. Interaction of plasmin with annexin A2 resulted in the stimulation of ERK1/2 and p38 MAPK, cyclooxygenase-2, and PGE2, leading to increased MMP-1 production. Furthermore, binding of inactive plasmin to annexin A2 inhibited plasmin induction of MMP-1, suggesting that inactive plasmin may be useful in suppressing inflammation.

Monocytes and macrophages are prominent at chronic inflammatory lesion sites in which there is extensive degradation and remodeling of connective tissue. Connective tissue turnover is believed to be mediated through the action of a series of proteases. These proteases include the plasminogen activation system, the matrix metalloproteinase (MMP) family of enzymes, and lysosomal cathepsins (1–4). In this study, we examined whether the urokinase plasminogen system, in addition to the activation of MMPs, also regulated the production of MMPs by human primary monocytes. The urokinase plasminogen activation system has been shown to be an important regulator of monocyte migration (5, 6). Urokinase-type plasminogen activator (uPA) when bound to its receptor, uPAR, cleaves cell surface plasminogen, resulting in the generation of plasmin. uPA is synthesized as pro-uPA, a single chain protein, with low catalytic activity, and is activated by a proteolytic cleavage of the Lys158 lle159 peptide bond, resulting in high m.w. two-chain form, high m.w. uPA (HMW-uPA) (7, 8). uPA is comprised of the following three domains: an N-terminal growth factor domain, a kringle domain, and a C-terminal catalytic domain. The first two domains comprise the N-terminal fragment (ATF), which can bind to uPAR, but lacks the catalytic domain and therefore cannot cleave plasminogen to generate plasmin. A low m.w. form of uPA (LMW-uPA) is produced as a result of the cleavage of HMW-uPA by plasmin and other proteases (9, 10). LMW-uPA lacks the growth factor domain, and therefore does not bind to uPAR, but does cleave plasminogen. The activity of uPA is regulated by soluble inhibitors, such as plasminogen activator inhibitor 1 (PAI-1). The plasmin generated by the action of uPA, in addition to cleaving fibrin, also activates many of the MMPs, thus playing a major role in the facilitation of connective tissue degradation (11).

The family of MMP enzymes is comprised of over 20 members (12, 13). These enzymes, grouped according to their domain structures and substrate preferences, include collagenases, gelatinases, stromelysins, membrane-type MMPs, and others. Collectively, MMPs can degrade all extracellular matrix components. MMPs have been linked to most diseases that involve tissue destruction, including cancer, rheumatoid arthritis, periodontal disease, and atherosclerosis (2, 13). The presence of monocytes/macrophages and their production of MMPs at these disease sites may contribute to the pathology associated with the degradation of extracellular matrix. A major MMP produced by monocytes is MMP-1 (interstitial collagenase), which degrades fibrillar collagens such as types I, II, and III. Activation of monocytes/macrophages by various agonists results in the production of MMP-1 that occurs, in part, through a PGE2-dependent pathway (14–19) and MAPKs (20).

Although plasmin has been reported to be involved in the activation of pro-MMP-1, -3, -7, -9, -10, and -13 (11), we explored the...
of this is shown in the comparison of MMP-1 production by monocytes that had been cultured for 8 h after the addition of PAI-1 or HMW-uPA to control cultures or exposure of LPS-treated cultures to HMW-uPA or HMW-uPA (30 nM) that had been preincubated with PAI-1 (10 μg/ml). Data are representative of at least three independent experiments.

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Materials and Methods

Reagents

HMW-uPA, LMW-uPA, ATF-uPA, and α2-antiplasmin were obtained from American Diagnostica. Plasmin (≥3 U/mg protein) was purchased from Sigma-Aldrich, and inactive plasmin and a stable mutant form of human PAI-1 were from Molecular Innovations. Annexin A2 polyclonal Ab was obtained from Santa Cruz Biotechnology, and mAbs against annexin A2 and F(ab)2, Abs against annexin A2 (p36) and S100A10 (p11) were from BD Biosciences. LPS (Escherichia coli 05:B5) was purchased from Sigma-Aldrich, ERK1/2/phospho-ERK1/2 and p38/phospho-p38 Abs were from Cell Signaling Technology. MMP-1 Abs used were rabbit polyclonal Abs against MMP-1 (provided by H. Birkedal-Hansen, National Institute of Dental and Craniofacial Research/National Institutes of Health, Bethesda, MD) and a mouse anti-human MMP-1 mAb (Chemicon International). Rabbit anti-cyclooxygenase (COX)-2 Abs were obtained from Cayman Chemical, and mouse anti-β-actin Abs were from Chemicon International.

Purification and culture of human monocytes

Human peripheral blood cells were obtained by leukapheresis of normal volunteers at Department of Transfusion Medicine at the National Institutes of Health. The monocyte fraction was purified by counterflow centrifugal elutriation, as previously described (21, 22), and contained >90% monocytes, as determined by morphology and flow cytometry. Monocytes were cultured in serum-free DMEM (Cambrex) supplemented with 2 mM L-glutamine (Mediatech) and 10 μg/ml gentamicin (Cambrex).

Western blot analysis of MMP-1, MAPKs, and COX-2

For MMP-1 determination, purified monocytes were cultured at a density of 5 × 10⁶/ml DMEM in 12-well polystyrene plates (Corning Life Sciences). After 36–48 h of treatment with reagents, the conditioned medium was centrifuged and collected. BSA (40 μg/ml) was added to the culture supernatants before the precipitation of the proteins with cold ethanol (final concentration, 60%) for at least 15 min at −70°C. The proteins were pelleted by microcentrifuging at 20,800 × g for 12 min, washed with ethanol, and lyophilized by rotary evaporation. The lyophilized proteins were resuspended in SDS-Laemmli buffer (500 mM Tris-HCl (pH 6.8)/10% SDS/0.01% bromphenol blue/20% glycerol), reduced with 5% 2-ME, heated for 4 min at 100°C, and electrophoresed on a 10 or 12% Tris-glycine gel in SDS running buffer (25 mM Tris-HCl (pH 8.3)/192 mM glycine/10% SDS). The proteins were transferred onto 0.45 μm nitrocellulose in a buffer containing 25 mM Tris-HCl (pH 8.3)/192 mM glycine/20% methanol and blocked with 50 mM Tris-HCl (pH 7.5)/150 mM NaCl (TBS) containing 5% nonfat dry milk for at least 1 h. The blots were then incubated overnight with Abs against MMP-1.

For detection of the levels of activated ERK1/2 and p38 MAPKs, monocytes were cultured in DMEM at 5 × 10⁶/ml in suspension. Ten to 60 min after the addition of reagents, the cells were pelleted and lysed with SDS loading buffer (prepared as described above), and the supernatants were loaded onto 12% Tris-glycine gels. The gels were then incubated overnight with mouse anti-human Abs against the phosphorylated forms of ERK1/2 and p38 and with rabbit anti-human Abs against total ERK1/2 and p38 as a measure of equal loading of the gels.

Cell protein isolation for the determination of COX-2 protein levels was prepared, as previously described (23). Briefly, 20 × 10⁶ monocytes in 4 ml of DMEM were cultured in suspension in 17-ml polypropylene tubes overnight in the presence or absence of reagents. The cells were then washed in PBS with protease inhibitors. The cell pellets were resuspended
in 250 mM sucrose-containing protease inhibitors and sonicated (Ultra-sonic Cell Disruptor; Kontes). Nuclear debris and unbroken cells were pelleted at 100,000 × g, and the supernatant was microfuged at 1500 × g to pellet cell membrane proteins. Equal amounts of protein were loaded onto 12% Tris-glycine gels. The blots were incubated overnight with rabbit anti-COX-2 Abs. Equal loading was measured with mouse anti-β-actin Abs.

Western blots for MMP-1, MAPKs, and COX-2 were incubated overnight with primary Abs, washed, and analyzed by the addition of Alexa Fluor 680 goat anti-rabbit or Alexa Fluor 750 goat anti-mouse Abs (Molecular Probes) and the infrared fluorescence detected with the Odyssey infrared imaging system (LI-COR). Densitometry analysis of the bands on the Western blots was determined with the LI-COR software analysis program or the ImageQuant software analysis program (Amersham Biosciences).

Plasmin activity assay

Plasmin activity was determined with the SPECTROZYME PL kit (American Diagnostica), which uses the chromogenic substrate H-D-norleucyl-hexahydrotryosyl-lysine-para-nitroanilide diacetate. For detection of cell-associated plasmin activity, monocytes were cultured at 1 × 10⁶/well in HBSS containing calcium and magnesium (HyClone) with the plasmin substrate. Kinetic absorbance readings were measured at 405 nm.

RT-PCR

Total cellular RNA was extracted with the RNasey Mini Kit (Qiagen) 8 h after stimulation of monocytes with LPS. Transcript levels of MMP-1 were determined using semiquantitative RT-PCR with GADPH as an internal control. The primer sets for MMP-1 were 5'-TGTGGTGTCACAGCTTCC-3' and 5'-CACATCAGGCACTCCACATC-3', and for GADPH 5'-TCGGAATCAACGATTTGCGTA-3' and 5'-ATGAGTCC-3'. OneStep RT-PCR kit (Qiagen) was used with the following reaction components: 5 µl of 5× OneStep RT-PCR buffer, 10 mM dNTP, 10 µM MMP-1 primer mix, 6 µM GADPH primer mix, 0.5 µg of RNA template, 1 µl of OneStep RT-PCR enzyme mix, and RNase-free water were added for a total of 25 µl. PCR times were as follows: 30 min at 50°C for reverse transcription, 15 min at 95°C for initial PCR, 36 cycles of 40 s at 94°C, 45 s at 57°C, and 1 min at 72°C for the final extension. The amplified DNA was separated by 1.7% agarose gel electrophoresis and stained with SYTO 60 red fluorescent nucleic acid stain (Molecular Probes), and the intensity of the stained bands was analyzed with the Odyssey infrared imaging system.

Cell staining and flow cytometry analysis

Plasmin and BSA were FITC conjugated, as previously described (24), using a FluoroTag FITC conjugation kit (Sigma-Aldrich). Purified human monocytes were fixed and permeabilized (buffers from eBioscience). A total of 3–10 × 10⁵ cells was preincubated with 5 µg of human whole IgG (Jackson ImmunoResearch Laboratories) in 90 µl of PBS containing 0.5% BSA and 2 mM EDTA at 4°C for 30 min to minimize the effect of non-specific FcR binding sites. FITC-conjugated BSA, plasmin (2.5 µg in 10 µl), or anti-human Abs (1–2.5 µg/10 µl) then were added, respectively, incubated for 30 min, washed, and analyzed by the FACSCalibur system (BD Biosciences). For annexin A2 binding site blocking experiments, polyclonal goat anti-human annexin A2 IgG (Santa Cruz Biotechnology), mouse anti-human annexin A2 (p36) IgG (F(ab)2), mouse anti-human S100A10 (p11) IgG (F(ab)2) (BD Biosciences), and inactive plasmin (10 µg/ml), respectively, were preincubated with the cells at 4°C for 30 min before the addition of the 2.5 µg/ml plasmin-FITC. For plasmin-FITC binding to monocytes, an equal amount of BSA-FITC served as negative control. Plasmin and inactive plasmin were also preincubated with monocytes to block the binding sites of annexin A2 recognized by anti-human annexin A2 Abs. For indirect immunostaining, polyclonal goat anti-human annexin A2 IgG (Jackson ImmunoResearch Laboratories) or goat IgG, as negative control, was added to the cells, followed by the addition of FITC-labeled rabbit anti-goat IgG F(ab)2 as secondary Ab. FACS results were analyzed by CellQuest software (BD Biosciences).

Statistical analysis

Comparison between group means was analyzed using ANOVA. The statistic data represent the mean ± SEM. A value of p < 0.01 is regarded as significant.

Results

Catalytically active uPA enhances MMP-1 production by activated monocytes

To determine the effect of uPA on human monocyte MMP-1 production, the catalytically active two-chain form of uPA (HMW-uPA) was added to control monocytes or monocytes activated by LPS. HMW-uPA caused a dose-dependent increase in the protein levels of MMP-1 by LPS-stimulated monocytes, whereas it did not induce MMP-1 in unstimulated monocytes (Fig. 1A). The majority of MMP-1 produced by monocytes is detected in the active 45- and 43-kDa form (active collagenase). In some experiments, depending on the time of incubation and the donor, bands corresponding to procollagenase were detected. We next examined whether the catalytic activity and/or binding to uPAR were required to mediate an increase in MMP-1 production by comparing HMW-uPA, LMW-uPA, and ATF-uPA. All experiments are representative of at least three independent experiments; an example of this is shown in the
comparison of MMP-1 production by monocytes from three donors that were cultured in the presence or absence of LPS and the indicated concentrations of LMW-uPA, HMW-uPA, and ATF-uPA (Fig. 1B). Catalytically active HMW-uPA, which binds to uPAR, and LMW-uPA, which does not bind to uPAR, increased LPS-induced production of MMP-1 protein. In contrast, ATF-uPA, which lacks the catalytic domain, but binds to uPAR, did not stimulate MMP-1 production. The requirement of catalytically active uPA in the induction of MMP-1 expression by monocytes was also verified at the mRNA level (Fig. 1C) as well as in experiments with a PAI-1 and HMW-uPA complex (Fig. 1D), which binds uPAR, but is inactive. These findings demonstrate that the increased production of monocyte MMP-1, mediated by uPA, is dependent on the catalytic activity of this enzyme and does not require signaling through uPAR.

**uPA stimulation of MMP-1 production is mediated by plasmin**

Stimulation of MMP-1 by catalytically active HMW-uPA and LMW-uPA, but not ATF-uPA, indicated that uPA was mediating its effect through the generation of plasmin from cell-bound plasminogen. This possibility was examined with a cell-based assay to determine plasmin levels in monocyte cultures. Addition of HMW-uPA or LMW-uPA to either control or LPS-stimulated monocytes induced a significant increase in plasmin activity (Fig. 2A). In contrast, ATF-uPA caused little, if any, increase in plasmin activity in unstimulated or LPS-stimulated monocytes.

We next added plasmin to monocyte cultures to determine its direct effect on MMP-1 production. Plasmin caused a dose-dependent increase in MMP-1 in LPS-stimulated monocytes, as shown at the protein and mRNA level, but did not induce MMP-1 in control (unstimulated) monocytes (Fig. 2, B and C). These findings demonstrate that plasmin is generated from cell-associated plasminogen by uPA and that plasmin requires a costimulant, such as LPS, to enhance MMP-1 production by monocytes.

**Plasmin induces MMP-1 production in monocytes through annexin A2**

Previous studies have shown that the heterotetramer of annexin A2 can serve as a receptor for plasmin on monocytes (25). To examine whether plasmin stimulated MMP-1 production through annexin A2, a polyclonal Ab against annexin A2 (Gt anti-Ann A2), goat IgG (GtIgG), or the F(ab')2 portion of mAbs against p36 (annexin A2) or p11 (S100A10) (F). Data are representative of at least three individual experiments.
annexin A2 (p36) and S100A10 (p11), components of the annexin A2 heterotetramer, was used to determine whether both components were involved in the induction of MMP-1. Both Abs inhibited MMP-1 production induced by LPS, and the enhancement by HMW-uPA and plasmin (Fig. 3, D and E). These findings demonstrate that uPA generation of endogenous plasmin or exogenous plasmin induction of MMP-1 production occurs through components that form the annexin A2 heterotetramer.

Further evidence of the interaction of plasmin with annexin A2 was determined by FACS analysis. Preincubation of monocytes with the polyclonal Ab against annexin A2 (Fig. 3C) or the mAbs against p36 (annexin A2) or p11 (S100A10) (Fig. 3F) blocked the binding of FITC-labeled plasmin to monocytes.

HMW-uPA and plasmin signaling leading to MMP-1 production occurs through PGE2 and MAPKs

We have previously shown that the production of MMP-1 by activated monocytes is regulated, in part, by PGE2, which is synthesized as a result of the induction of COX-2 (17). Western blot analysis of the protein levels of COX-2 in the membranes of the monocytes revealed that the LPS-induced COX-2 was increased by HMW-uPA or plasmin (Fig. 4A), which corresponded to increased PGE2 levels in the monocyte culture supernatants (Fig. 4B). PGE2 was also detected in control monocytes treated with HMW-uPA, most likely derived from COX-1 because COX-2 was not induced. The LPS-stimulated increase in MMP-1 and the further enhancement of MMP-1 by HMW-uPA or plasmin were inhibited by indomethacin, which was restored, in part, by the addition of PGE2 (Fig. 4C). These results demonstrate that HMW-uPA or plasmin induction of PGE2 is involved in the increase of MMP-1.

We have previously shown that LPS stimulation of monocyte MMP-1 production is also regulated by the p38 MAPK and ERK1/2 pathways (20). To determine whether HMW-uPA-generated plasmin or the direct addition of plasmin also induced MMP-1 through MAPKs, inhibitors of ERK1/2 (PD98059) (PD) or p38 (SB203580) (SB) were added to monocyte cultures. Both MAPK inhibitors suppressed the enhancement of MMP-1 by HMW-uPA or plasmin (Fig. 5A), indicating that p38 and ERK1/2 were involved in mediating the induction of MMP-1 by plasmin. This conclusion was further supported by HMW-uPA or plasmin-induced increases in the phosphorylation of p38 and ERK1/2 in LPS-stimulated monocytes (Fig. 5B). HMW-uPA also caused a slight increase in the phosphorylation of both MAPKs in unstimulated monocytes.
Inactive plasmin blocks plasmin-induced synthesis of MMP-1 by monocytes

We next examined whether catalytically active plasmin was required for the induction of MMP-1 synthesis. Inactive plasmin, in which the catalytic site had been irreversibly blocked with a peptide inhibitor, was added to monocytes before the addition of LPS or LPS plus plasmin. Inactive plasmin inhibited the production of MMP-1 by LPS and LPS plus plasmin. Inactive plasmin inhibited the production of MMP-1 by LPS and LPS plus plasmin. This inhibition was not related to direct blocking of plasmin activity, as shown in a cell-free plasmin activity assay in which the combination of inactive plasmin and plasmin exhibited the same activity as plasmin alone (Fig. 6B). FACS analysis revealed that inactive plasmin blocked the binding of FITC-labeled plasmin (Fig. 6C) and the binding of Abs against annexin A2 to monocytes (Fig. 6D). These findings demonstrate that inactive plasmin can function as an effective inhibitor of plasmin-mediated signaling in monocytes.

Discussion

Studies on uPA and plasmin have, in large part, focused on the role of these enzymes in pericellular matrix degradation. However, there is now increasing evidence that these enzymes may also function to induce cellular activation through various signaling pathways. Binding of uPA to cell surface uPAR has been shown to activate several signal transduction pathways, including intracellular tyrosine kinases and serine/threonine kinases such as ERK/MAPK (26). Evidence that uPA can signal through uPAR in macrophages, resulting in activation of ERK1/2, has been reported in RAW264.7 cells, a murine macrophage cell line, transfected with human uPAR (27). Support for uPA signaling through uPAR has been demonstrated, in part, by the effect of ATF-uPA, which binds to uPAR, but lacks the catalytic domain. Functions affected by ATF-uPA include proliferation, stimulation of early response genes, HIV-1 replication, migration, and apoptosis (28–34). However, our findings show that ATF-uPA did not induce signaling that led to increased MMP-1 production, nor did it inhibit MMP-1 production by binding to uPAR due to the rapid turnover of uPAR when bound to uPA (35, 36). Rather, only the catalytically active HMW-uPA or LMW-uPA, which does not bind to uPAR, increased MMP-1 production by activated monocytes. HMW-uPA tended to induce more MMP-1 than LMW-uPA, possibly due to more efficient activation of cell surface plasminogen when uPA is bound to uPAR. Collectively, these findings indicated that plasmin generated from the cleavage of cell surface-bound plasminogen by uPA was responsible for mediating the increase in MMP-1 production.

Plasmin has been demonstrated to induce changes in cellular functions in a variety of cells. Responses and signal pathways affected in monocytes by plasmin include the following: stimulation of the 5-lipoxygenase pathway, chemotaxis through stimulation of cyclic guanosine monophosphate, production of cytokines and tissue factor mediated by AP-1, and NF-kB activation and expression of MCP-1 and CD40 via activation of p38 MAPK and JAK/STAT pathways (25, 37–39). In our study, we show that plasmin causes a dose-dependent increase in MMP-1 production by LPS-activated monocytes, but has no effect on unstimulated or control cells, demonstrating that plasmin requires a costimulator, such as LPS, to
exert its effect on MMP-1 production. Moreover, we demonstrate that plasmin activity is generated from endogenous cell surface plasminogen most likely synthesized by monocytes, as a result of low levels of plasminogen mRNA that have been previously shown in human monocytes (40). Alternatively, plasminogen acquired in vivo may remain bound to the monocytes even though these cells have undergone extensive washing in the purification by elutriation and subsequently cultured in the absence of serum. Low levels of plasmin activity were detected in control monocytes, which would be in agreement with the constitutive production of uPA by monocytes (41, 42). These findings indicate that the uPA/plasmin system plays a significant role in the regulation of MMP-1 production by activated monocytes.

The ability of plasmin to increase MMP-1 inferred that it was acting through a specific cell surface receptor. Putative receptors for plasmin on monocyte cell lines and primary monocytes include β2- and β1-integrins, annexin A2 (25, 43–46). Recently, the annexin A2 heterotetramer has been identified as a receptor for plasmin-induced signaling in human monocytes (25). Moreover, annexin A2 has been linked to plasminogen-dependent matrix invasion by monocytes (46, 47). Annexin A2, a member of a family of proteins that bind anionic phospholipids in a Ca2+-dependent manner, exists in three forms, a monomer (p36), a heterodimer, or a heterotetramer. The heterotetramer is comprised of two annexin A2 monomers and two S100A10 (p11) monomers. Our data show that addition of Abs against annexin A2 and S100A10 to activated monocytes in the absence or presence of plasmin inhibited MMP-1 production. The lack of complete inhibition of MMP-1 production by Abs against annexin A2 is most likely due to uPA signaling through additional receptors and/or the binding of plasmin to other receptors that affect MMP-1 synthesis. These findings demonstrate that the components of the annexin A2 heterotetramer are involved in signaling by endogenous and exogenous plasmin that leads to MMP-1 production by LPS-stimulated monocytes.

Generation of plasmin by uPA from cell-associated plasminogen or the direct addition plasmin in the absence of a costimulator such as LPS failed to induce MMP-1 production. This is in contrast to MMP-9, which has been reported to be up-regulated in the THP-1 and U937 monocyctic cell lines by uPA alone (48, 49). We also observed that uPA or plasmin induces low levels of MMP-9 in primary monocytes in the absence of LPS (data not shown).

We have previously shown that LPS stimulation of MMP-1 production by primary human monocytes is dependent on signaling pathways that include COX-2, PGE2, p38 MAPK, and ERK1/2 (17, 20). These studies demonstrated that stimulation of monocytes results in the activation of MAPKs within 30 min, followed by detection of COX-2 expression as early as 3 h and PGE2 by 4 h. In this study, we show that uPA or plasmin increased COX-2 and PGE2 in activated monocytes. Moreover, the PG inhibitor indomethacin decreased the levels of MMP-1 induced by uPA or plasmin, which could be restored, in part, by exogenous PGE2. Signaling leading to PG and MMP production is regulated by upstream pathways that include the MAPKs. Analysis of the MAPK pathways revealed that uPA or plasmin increased the activation of ERK1/2 and p38 MAPK in LPS-stimulated monocytes. A slight increase in phosphorylation of p38 and ERK1/2 was detected in unstimulated cells treated with HMW-uPA. uPA has been shown to increase phosphorylation of ERK1/2 in RAW264.7 cells (27). We did not see an increase in activation of p38 or ERK1/2 by plasmin in control monocytes, although plasmin has been shown to stimulate p38 MAPK in monocytes, but not ERK1/2 (38). This discrepancy may be due to the concentration of plasmin used in these experiments. Specific inhibitors of p38 MAPK or ERK1/2 suppressed HMW-uPA or plasmin-mediated enhancement of MMP-1 production by LPS-stimulated monocytes, demonstrating that both of these MAPK pathways are involved in uPA and plasmin-mediated increase in MMP-1. We reported previously that p38 MAPK regulates MMP-1 primarily through transcription factors that are affected by PGE2 because inhibition of MMP-1 by p38 MAPK inhibitors can be reversed by PGE2 or dibutyryl cAMP (20). In contrast, suppression of MMP-1 by ERK1/2 inhibitors cannot be reversed by PGE2 due to activation of additional transcription factors whose regulation is PGE2 independent.

Our data demonstrate that the urokinase plasminogen activation system through generation of plasmin has a major role in the regulation of monocyte MMP-1 production, which is thought to be involved in the pathology associated with chronic inflammatory diseases. Plasmin has also been shown to induce MCP-1 and TNF-α, cytokines associated with inflammatory responses (25, 38). Our results also show that inactive plasmin blocked the binding of active plasmin to annexin A2 and the subsequent enhancement of MMP-1 production by activated monocytes. Thus, inactive plasmin may be useful in suppressing plasmin-mediated inflammatory responses without affecting tissue-type PA-mediated plasmin activity and its beneficial effects on clot lysis.

In summary, uPA increases MMP-1 production by LPS-activated monocytes through a series of events that include the generation of plasmin, which in turn signals through the components of the annexin A2 heterotetramer, resulting in the stimulation of the COX-2 and MAPK pathways. These findings demonstrate that the urokinase plasminogen activation system plays a major role in the regulation of the pathway leading to MMP-1 production by monocytes. Moreover, inactive plasmin is shown as an inhibitor of plasmin induction of MMP-1, which may have implications for decreasing inflammatory responses.

Disclosures

The authors have no financial conflict of interest.

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