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Matrix Metalloproteinase Secretion by Gastric Epithelial Cells Is Regulated by E Prostaglandins and MAPKs*

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Because matrix metalloproteinases (MMPs) play roles in inflammatory tissue injury, we asked whether MMP secretion by gastric epithelial cells may contribute to gastric injury in response to signals involved in *Helicobacter pylori*-induced inflammation and/or cyclooxygenase inhibition. Tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and epidermal growth factor (EGF) stimulated gastric cell MMP-1 secretion, indicating that MMP-1 secretion occurs in inflammatory as well as non-inflammatory situations. MMP-1 secretion required activation of the MAPK Erk and subsequent protein synthesis but was down-regulated by the alternate MAPK, p38. In contrast, secretion of MMP-13 was stimulated by TNF- α /IL-1 β but not EGF and was Erk-independent and mediated by p38. MMP-13 secretion was more rapid (peak, 6 h) than MMP-1 (peak \geq 30 h) and only partly depended on protein synthesis, suggesting initial release of a pre-existing MMP-13 pool. Therefore, MMP-1 and MMP-13 secretion are differentially regulated by MAPKs. MMP-1 secretion was regulated by E prostaglandins (PGEs) in an Erk-dependent manner. PGEs enhanced Erk activation and MMP-1 secretion in response to EGF but inhibited Erk and MMP-1 when TNF- α and IL-1 β were the stimuli, indicating that the effects of PGEs on gastric cell responses are context-dependent. These data show that secretion of MMPs is differentially regulated by MAPKs and suggest mechanisms through which *H. pylori* infection and/or cyclooxygenase inhibition may induce epithelial cell signaling to contribute to gastric ulcerogenesis.

Whereas peptic ulcer disease (PUD)¹ affects millions of patients in the United States each year (1), gastric inflammation

is even more prevalent and predisposes to both PUD and adenocarcinoma of the stomach (2). Two major risk factors for gastritis and PUD are gastric colonization with *Helicobacter pylori* and nonsteroidal anti-inflammatory drug (NSAID) use (3–5). *H. pylori* colonizes more than half of the world's population. Eradication of *H. pylori* results in resolution of gastric inflammation, confirming its central role in pathogenesis (6). *H. pylori*-associated PUD is accompanied by tissue inflammation and secretion of cytokines, including interleukin (IL)-1 β and tumor necrosis factor (TNF)- α (7–12). Nevertheless, the mechanisms through which *H. pylori* induces gastric damage are not well established (5).

Matrix metalloproteinases (MMPs) are zinc-coordinating enzymes that remodel healthy connective tissue by specifically digesting collagen and other structural molecules (13, 14). In chronic inflammatory conditions, such as rheumatoid arthritis, the extracellular secretion of MMPs substantially contributes to stromal tissue destruction (15–21). Either directly or indirectly (via induction of cytokine synthesis), *H. pylori* stimulates gastric epithelial cells to produce MMPs *in vitro* (10, 22–27). Thus, *H. pylori* colonization may influence gastric cells to promote inflammation (5) and damage the gastric mucosa.

In contrast, NSAID-induced PUD is accompanied by relatively little inflammation (28). NSAIDs are presumed to damage the gastric mucosa by inhibiting cyclooxygenase and depleting E prostaglandins (PGEs). PGEs regulate gastric blood flow and production of bicarbonate and mucus; thus, down-regulated PGE synthesis may impair barrier function of the gastric mucosa (29). Conversely, replenishment of PGEs using the synthetic PGE analog misoprostol reduces the gastrotoxicity of NSAIDs (30). The possibility that prostaglandins directly regulate gastric cell function has not been rigorously examined, despite complex effects on signal transduction exerted by PGEs in many cell types (20, 31–35).

Mitogen-activated protein kinases (MAPKs) are serine/threonine protein kinases including the extracellular signal-regulated kinase (Erk), JNK, and p38 subfamilies (36). Whereas p38 and Jnk are implicated in inflammatory responses (37, 38), Erk has been regarded as a signal for cell growth and differentiation. However, Erk also may regulate inflammatory and tissue-destructive responses (20, 39). In fibroblast-like synoviocytes, which largely drive the destruction of cartilage in rheumatoid arthritis (16, 17, 21), Erk regulates MMP-1 secretion in response to inflammatory cytokines (20). PGEs inhibit fibroblast-like synoviocyte secretion of MMP-1 via inhibition of Erk (15, 20, 40). This model suggests that autodestruction of stromal tissue in response to inflammatory stimuli may be mediated by MMP production and regulated by MAPKs and prostaglandins.

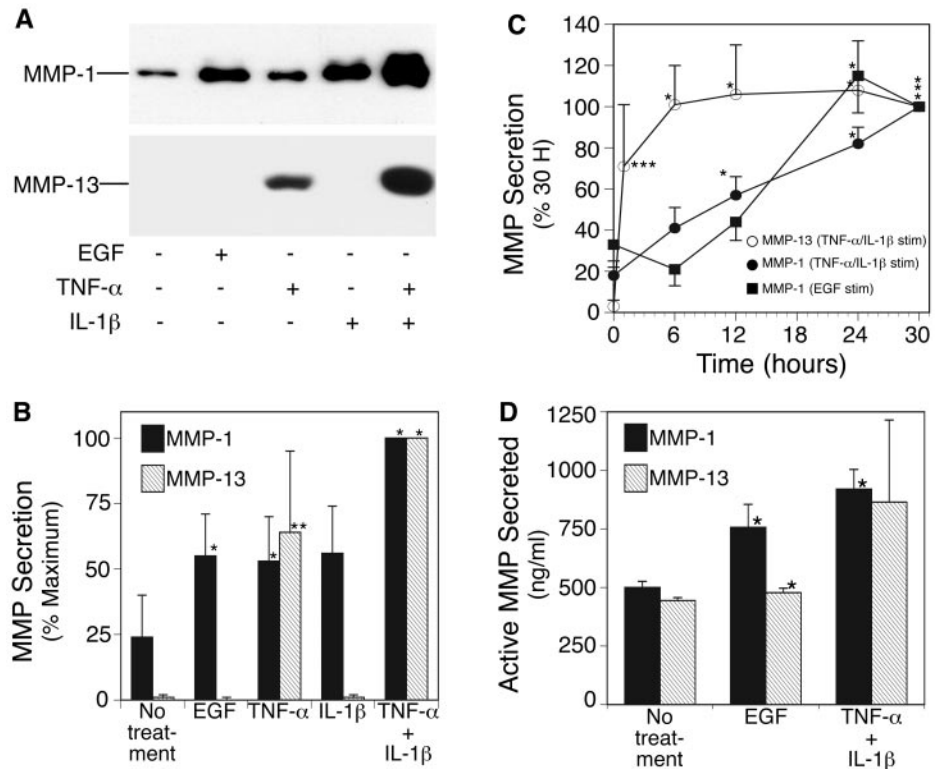
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¹ The abbreviations used are: PUD, peptic ulcer disease; NSAID, nonsteroidal anti-inflammatory drug; IL, interleukin; TNF, tumor necrosis factor; MMP, matrix metalloproteinase; PGE, E prostaglandin; MAPK, mitogen-activated protein kinase; EGF, epidermal growth factor; CHX, cycloheximide; Erk, extracellular signal-regulated kinase; JNK, c-Jun NH₂-terminal kinase; MEK, MAPK/Erk kinase.

FIG. 1. Effects of EGF, TNF- α , and IL-1 β on MMP-1 and MMP-13 secretion by AGS cells. A and B, AGS cells were serum-starved for 24 h; stimulated for 18 h \pm EGF, TNF- α , and/or IL-1 β (each at 20 ng/ml); and assayed for MMP-1 (A and B) or MMP-13 (A and B) secretion as described under "Experimental Procedures." C, time course. AGS cells were stimulated \pm EGF or TNF- α /IL-1 β and assayed for MMP-1 and MMP-13 secretion. D, activation state of secreted MMPs. AGS cells stimulated \pm EGF or TNF- α /IL-1 β were assayed for active MMP-1 and MMP-13 by an enzyme-linked immunosorbent assay-based method. Data shown are representative or the mean \pm S.E. of three experiments. *, $p < 0.05$; **, $p = 0.06$; ***, $p = 0.08$ (versus the appropriately matched condition, either no treatment (B and D) or $t = 0$ (C)).



In the present study, we examined gastric epithelial cell responses to signals relevant to exposure to *H. pylori* and to NSAIDs. We asked whether cytokine stimulation of gastric epithelial cells regulates MMP secretion and whether such regulation is MAPK- and/or prostaglandin-dependent. Our observations suggest a model in which gastric ulceration is propagated by the transduction of exogenous pro-inflammatory signals into the gastric cells themselves and by a gastric cell response that may result in autologous destruction of gastric tissue.

EXPERIMENTAL PROCEDURES

Materials—Unless otherwise stated, all materials were from Sigma. Anti-phospho-Erk, anti-Erk 1, anti-Erk 2, and anti-p38 antisera, as well as horseradish peroxidase-conjugated anti-rabbit antiserum, were from Santa Cruz Biotechnology. Anti-MMP-1 and anti-MMP-13 antisera were from Chemicon. 10% Tris-glycine/polyacrylamide gels were from Novex/Invitrogen. UO126, PD98059, and SB203580 were from Biomol. SB202190 was from Calbiochem. CentriconTM centrifugal filter devices were from Millipore. RPMI 1640 medium, fetal bovine serum, and 2.5% trypsin/EDTA were from BioWhittaker. Penicillin G sodium (10,000 units/ml)/streptomycin sulfate (10,000 μ g/ml) in 0.85% NaCl was from Invitrogen. ECL chemiluminescence kit was from Amersham Biosciences. AGS cells were from the American Type Culture Collection. MMP-1 and MMP-13 activity kits were from R and D Systems.

Cell Culture and Cell Treatment—AGS human cells derived from a gastric epithelial tumor (41) were cultured in 6-well plates in RPMI 1640 medium containing 10% fetal bovine serum and penicillin/streptomycin. Cells grown to near confluence were serum-starved for 24 h (0% fetal bovine serum) before stimulation and/or other treatment. Unless otherwise specified, the following concentrations of reagents were used: IL-1 β , TNF- α , and epidermal growth factor (EGF), 20 ng/ml each; PGE₁, PGE₂, UO126, SB203580, and SB202190, 10 μ M each; PD98059, 50 μ M; and cycloheximide, 10 μ g/ml.

Erk and p38 Phosphorylation and Activation—Erk activation was defined as the degree of phosphorylation of the activating tyrosine and threonines on Erk 1 and 2, as determined by immunoblotting with anti-phospho-Erk antiserum. Similarly, p38 activation was defined as p38 phosphorylation using anti-phospho-p38 antiserum. AGS cells were serum-starved for 24 h, followed by experimental treatment. Lysates were prepared as described, protein levels were measured, and equivalent amounts of protein were subjected to SDS-PAGE analysis and immunoblotting for the phosphorylated, active forms of Erk (20). Im-

munoblots imaged by chemiluminescence and autoradiography were quantitated by densitometry. Duplicate samples were immunoblotted for total Erk1/2 or p38, and Erk and p38 activation was reported as phosphorylated/total protein. Because the actual units of quantitation were arbitrary, results were normalized to either the unstimulated or maximally stimulated condition, as appropriate.

MMP Secretion—MMP secretion was determined as described previously (20). Briefly, after AGS cells were grown to near confluence and serum-starved for 24 h, supernatants were removed and replaced with fresh medium. After a 30–60-min period for equilibration, the cells were stimulated, and the supernatants were recovered and concentrated in CentriconTM centrifugal filter devices (M_r cutoff, 30,000) for 35 min at 5000 \times g at 4 $^{\circ}$ C. Aliquots of the concentrated supernatants were supplemented with Laemmli buffer and assayed for MMP-1 and MMP-13 by SDS-PAGE and immunoblotting with appropriate anti-MMP antisera (1:200 dilution) and horseradish peroxidase-conjugated anti-rabbit antibody. Visualization and quantitation were as described for Erk. Measurement of secretion of active MMP-1 and MMP-13 was performed using commercially available kits, according to the manufacturer's instructions.

RESULTS

MMP-1 and MMP-13 Secretion Stimulated by TNF- α , IL-1 β , and EGF—We first examined whether AGS cell stimulation could induce MMP-1 secretion. TNF- α , IL-1 β , or both (18 h) induced MMP-1 secretion (Fig. 1, A, top panel, and B). Because TNF- α /IL-1 β together induced more MMP-1 secretion than either alone (TNF- α /IL-1 β versus TNF- α , $p = 0.03$; TNF- α /IL-1 β versus IL-1 β , $p = 0.05$) and because both cytokines are implicated in gastrointestinal ulcerogenesis (7–12, 42, 43), most subsequent experiments were performed using TNF- α /IL-1 β together. Significant TNF- α /IL-1 β stimulation of MMP-1 secretion was first observed after 12 h, and MMP-1 continued to accumulate for at least 30 h (Fig. 1C). EGF also stimulated MMP-1 secretion (Fig. 1A, top panel, and B). MMP-1 secretion in response to EGF was also delayed (>12 h after stimulation), and MMP-1 continued to accumulate for 24 h (Fig. 1C). These data confirm that AGS cell MMP-1 secretion is responsive to both inflammatory cytokines and growth factors. Moreover, MMP-1 secreted in response to both EGF and TNF- α /IL-1 β was enzymatically active (Fig. 1D).

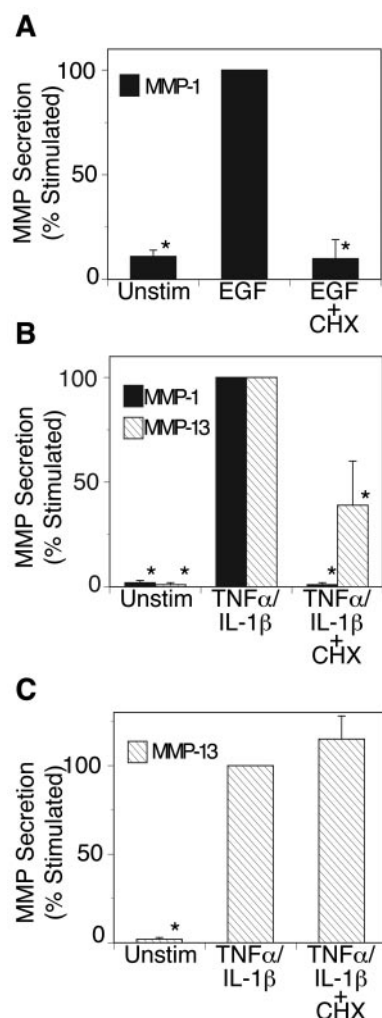


FIG. 2. CHX sensitivity of MMP-1 and MMP-13 secretion. A and B, AGS cells were incubated for 30 min \pm CHX (10 μ g/ml), stimulated \pm EGF (A) or TNF- α /IL-1 β (B) for 18 h, and assayed for MMP-1 (A and B) and MMP-13 (B) secretion. C, AGS cells were incubated for 30 min \pm CHX followed by TNF- α /IL-1 β stimulation for 1 h and assay of MMP-13 secretion. Data shown are the mean \pm S.E. of three (A) or four (B and C) experiments. *, $p < 0.05$ (versus the appropriate stimulated condition).

TNF- α /IL-1 β also significantly stimulated secretion of enzymatically active MMP-13 (Fig. 1A, bottom panel, B, and D). TNF- α , but not IL-1 β alone, also increased MMP-13 secretion. MMP-13 secretion occurred in response to TNF- α /IL-1 β as early as 1 h after stimulation and reached steady-state levels more rapidly (≤ 6 h) than MMP-1 (Fig. 1C). In contrast to MMP-1, EGF did not stimulate MMP-13 secretion (Fig. 1A, bottom panel, and B). Therefore, AGS cell MMP-1 and MMP-13 expression, secretion, and activity are differentially regulated in a stimulus-specific manner.

Role of Protein Synthesis in MMP-1 and MMP-13 Secretion—The differing kinetics of MMP-1 and MMP-13 secretion suggested that these MMPs might differ with regard to a requirement for protein synthesis. To evaluate this possibility, we tested the effects of cycloheximide (CHX), which inhibits protein synthesis by affecting binding and activity of transfer RNA (44). CHX almost completely inhibited MMP-1 secretion in response to both EGF (Fig. 2A) and TNF- α /IL-1 β (Fig. 2B), confirming the dependence of MMP-1 secretion on protein synthesis. In contrast, MMP-13 secretion after 1 h was almost entirely insensitive to CHX treatment (Fig. 2C), and MMP-13 secretion after 18 h was only partly inhibited (Fig. 2B), sug-

gesting initial release of pre-formed MMP-13, followed by later secretion of newly synthesized protein. These data provide further evidence that secretion of MMP-1 and MMP-13 is differentially regulated.

Erk Regulates MMP-1 but not MMP-13 Expression by Gastric Epithelial Cells—Because EGF, a canonical activator of Erk (45), stimulated MMP-1 secretion from AGS cells, we next asked whether Erk regulates MMP-1. EGF stimulation of AGS cells induced rapid but transient activation of Erk (peak at 15–30 min), with return to baseline by 4 h. TNF- α /IL-1 β also stimulated transient Erk activation, with similar kinetics (Fig. 3A–C). Thus, in both cytokine- and EGF-stimulated AGS cells, Erk activation and return to baseline activity precede MMP-1 secretion. Consistent with the effects of cytokines on MMP-1 secretion, TNF- α /IL-1 β stimulated greater Erk activation than either cytokine alone.

To determine whether Erk activation is required for MMP-1 expression, we tested the effects of UO126 (46), a specific inhibitor of MEK, the proximal activator of Erk (47). UO126 inhibited both EGF- and TNF- α /IL-1 β -stimulated Erk activation, as well as MMP-1 secretion (Fig. 4A and B). PD98059, another specific MEK inhibitor (48), also inhibited both Erk activation and MMP-1 secretion in response to EGF or TNF- α /IL-1 β (data not shown). Taken together, these studies confirm that Erk activation mediates MMP-1 secretion from AGS cells in response to either EGF or TNF- α /IL-1 β .

TNF- α /IL-1 β -stimulation of MMP-13 secretion after 18 h was not significantly inhibited by UO126 (Fig. 4B) or PD98059 (data not shown). MMP-13 secretion after 1 h of TNF- α /IL-1 β stimulation was also not inhibited by UO126 (data not shown). Thus, Erk activity appears not to regulate either the protein synthesis-dependent or synthesis-independent components of MMP-13 secretion. This observation is consistent with the failure of EGF to stimulate MMP-13 secretion.

Erk Activation Is an Early Event in MMP-1 Secretion—The observation that Erk activation returned to baseline hours before detectable MMP-1 secretion (Fig. 3C) suggested that Erk activation is an early step leading to MMP-1 secretion. To test this hypothesis, we exposed AGS cells to UO126 before or after EGF or TNF- α /IL-1 β stimulation. Incubation of AGS cells with UO126 prior to stimulation resulted in expected inhibition of MMP-1 secretion (Fig. 5), but adding UO126 90 min after EGF or TNF- α /IL-1 β stimulation resulted in little or no inhibition of MMP-1 secretion. Thus, Erk activation initiates a process leading to MMP-1 secretion, but continual Erk activity is not required.

p38 Activity Inhibits MMP-1 Secretion via Inhibition of Erk—Because p38 activity typically promotes inflammatory phenotypes (49, 50), we hypothesized that p38 would positively regulate MMP secretion and, conversely, that its inhibition would abrogate secretion. We first confirmed that TNF- α /IL-1 β transiently stimulated p38 phosphoactivation in AGS cells (Fig. 3D). In contrast, EGF only marginally stimulated p38 in these experiments. Unexpectedly, the specific p38 inhibitor SB203580 (51) enhanced MMP-1 secretion in AGS cells stimulated with either EGF or TNF- α /IL-1 β (Fig. 4, A and B). SB202190, another selective p38 inhibitor (52), had similar effects (data not shown). SB203580 (Fig. 4, A and B), as well as SB202190 (data not shown), also enhanced Erk activation in response to either EGF or TNF- α /IL-1 β . Thus, the effects of p38 inhibition on MMP-1 secretion and Erk activation were concordant. The observations that SB203580 alone stimulated Erk activation and MMP-1 secretion and that both of these processes were inhibited by UO126 (Fig. 4D) indicate that p38 tonically inhibits Erk activity and MMP-1 secretion, regardless

FIG. 3. Effects of EGF, TNF- α , and IL-1 β on Erk and p38 activation in AGS cells. A and B, AGS cells were incubated for 30 min \pm EGF, TNF- α , IL-1 β , or TNF- α /IL-1 β (each at 20 ng/ml), and lysates were assayed for Erk activation as described under "Experimental Procedures." A: top panel, immunoblot for phosphorylated Erk; bottom panel, immunoblot for total Erk. B, Erk phosphorylation normalized to total Erk levels. C and D, time courses of MAPK activation. AGS cells were stimulated with EGF or TNF- α /IL-1 β , followed by determination of Erk activation (C) or p38 activation (D), each normalized to total enzyme levels. Data shown are representative (A) or the mean \pm S.E. (B–D) of five experiments. *, $p < 0.05$; **, $p = 0.07$ (versus appropriate control, either no treatment (B) or $t = 0$ (C)).

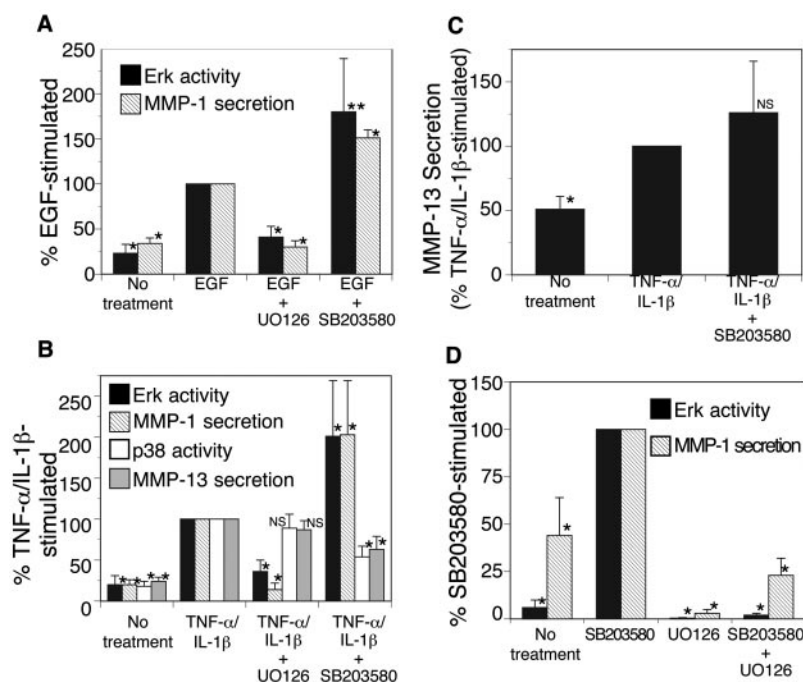
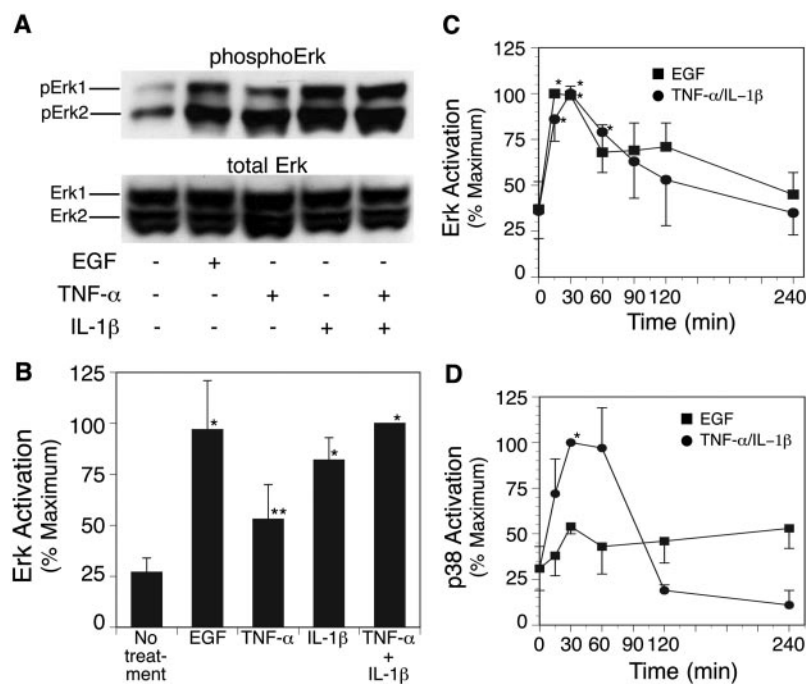


FIG. 4. Erk and p38 differentially regulate MMP-1 and MMP-13. AGS cells were incubated for 30 min with UO126 or SB203580 (10 μ M each) followed by stimulation with EGF (A) or TNF- α /IL-1 β (B) and assay for Erk or p38 activation (30-min stimulation), as well as MMP-1 or MMP-13 secretion (18-h stimulation). C, AGS cells were incubated \pm SB203580 (10 μ M, 30 min) followed by a 60-min stimulation with TNF- α /IL-1 β and determination of MMP-13 secretion. D, AGS cells were incubated \pm SB203580, UO126, or both and assayed for Erk activation (30-min incubation) or MMP-1 secretion (18-h incubation). Data shown are the mean \pm S.E. of three (A, MMP-1; B, Erk; and D), five (B, MMP-13; C), six (B, MMP-1), or seven (A, Erk) experiments for each condition. *, $p \leq 0.05$; **, $p = 0.06$; NS, not significant (all versus stimulated condition).

of cell stimulation status, and that its inhibitory action on MMP-1 is mediated, at least in part, via Erk inhibition.

In contrast to the enhancing effects of p38 inhibitors on Erk and MMP-1, both SB203580 (Fig. 4B) and SB202190 (data not shown) inhibited both p38 phosphoactivation and MMP-13 secretion in AGS cells stimulated with TNF- α /IL-1 β . These studies show that p38 provides a positive signal for MMP-13 secretion, further evidence that MMP-1 and MMP-13 expression is differentially regulated by MAPKs in AGS cells. The observation that SB203580 failed to inhibit the early, CHX-independent phase of MMP-13 secretion (Fig. 4C) indicates that p38 regulates the later, protein synthesis-dependent phase of MMP-13 secretion.

PGEs Regulate Erk Activation and MMP-1 but not MMP-13 Secretion in Gastric Cells—Because PGEs inhibit both Erk and

MMP-1 responses in other cell types (15, 20, 34, 35, 40, 53, 54), we asked whether they would affect AGS cells. Preincubation of AGS cells with PGE₁ or PGE₂ enhanced both Erk activation and MMP-1 secretion in response to EGF (Fig. 6A). In contrast, PGE₁ and PGE₂ inhibited Erk activation and MMP-1 secretion in cells stimulated with TNF- α /IL-1 β (Fig. 6B). In the absence of other stimuli, PGE₁ and PGE₂ independently stimulated both Erk phosphorylation (Fig. 6C) and MMP-1 secretion (Fig. 6D), and PGE-stimulated MMP-1 secretion was inhibited by UO126. In total, these studies demonstrate that PGEs exert stimulus-specific effects on Erk and MMP-1 in AGS cells and that the capacity of PGEs to independently induce MMP-1 secretion depends upon their ability to activate Erk. In contrast, neither PGE₁ nor PGE₂ significantly inhibited TNF- α /IL-1 β -stimulated MMP-13 secretion, consistent with the ob-

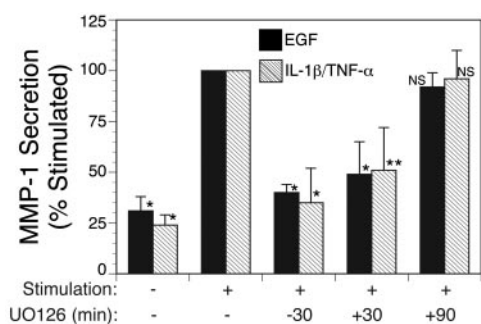


FIG. 5. Kinetics of Erk regulation of MMP-1 in AGS cells. AGS cells were incubated $\pm 10 \mu\text{M}$ UO126 30 min before, 30 min after, or 90 min after addition of EGF or TNF- α /IL-1 β . Stimulation was continued for a total of 18 h, and the supernatants were analyzed for MMP-1 secretion. Data shown are the mean \pm S.E. of three experiments for each condition. *, $p < 0.05$ versus the stimulated condition without inhibitors; **, $p = 0.07$; NS, not significant versus stimulated condition.

servation that MMP-13 secretion is Erk-independent (inhibition of TNF- α /IL-1 β -stimulated MMP-13 secretion: PGE₁, $27 \pm 24\%$, $p = 0.19$; PGE₂, $29 \pm 22\%$, $p = 0.15$).

DISCUSSION

The pathogenesis of peptic ulcer disease is not well understood. We considered whether MMP secretion from host cells might contribute to gastric tissue stromal destruction because MMPs have analogous roles in other inflammatory diseases such as rheumatoid arthritis (19, 20). Gastric epithelia secrete active MMPs including MMP-2, -3, -7, and -9 (10, 22–25, 27), but MMP regulation has not been well explored. We selected MMP-1 and MMP-13 to study because prior observations in other cell types have indicated that these MMPs are differentially regulated by MAPKs and therefore likely to exhibit contrasting properties (20, 43, 55, 56). Basement membrane is typically composed primarily of type IV collagen (57), which is not a major substrate for MMP-1. However, type I and type III collagens predominate in the gastric stroma (58) and are susceptible to both MMP-1 and MMP-13 (59). In a rat model of gastric ulceration, mRNAs for type I collagen also were up-regulated (60). The observation that arteries and veins employ types I, II (also susceptible to MMP-1) (59), and III collagen in their matrices (61) suggests that MMP-1 might also contribute to hemorrhage in ulcer disease. Gastric cells have previously been shown to secrete MMP-1 in a manner consistent with their ability to invade stroma. For example, Gööz *et al.* (24) reported that *H. pylori* infection results in MMP-1 secretion from AGS cells, and we recently observed that *H. pylori* stimulates AGS cell MMP-1 secretion in an Erk-dependent manner.² Similarly, MMP-1 production is increased in human gastric carcinomas (62). Membrane type-1 MMP shares with MMP-1 the capacity to degrade type I collagen (63) and has been implicated in invasiveness of gastric carcinoma cells (64). Thus, it is likely that MMP-1 plays a role in ulcerogenesis, perhaps in conjunction with other MMPs that are secreted from gastric cells in active forms and target other collagen types (65). To our knowledge, ours is the first demonstration that gastric cells also secrete MMP-13.

Secretion of both MMP-1 and MMP-13 by AGS cells was stimulated by inflammatory cytokines. Because *H. pylori* induces IL-1 β and TNF- α secretion in ulcers and gastritis (24, 66), these data implicate cytokine induction as a mechanism in infectious ulcer formation. The observation that MMP-13 se-

cretion was stimulated by TNF- α and IL-1 β , but not EGF, contrasts with MMP-1, which responded to all three, demonstrating that MMP-1 and MMP-13 are differentially regulated in AGS cells. The response of MMP-1 secretion to both a growth factor and cytokines suggests that MMP-1 may participate in homeostatic connective tissue remodeling, as well as in inflammation-induced injury. In contrast, MMP-13 is more likely to play a role solely in inflammatory responses.

Similarly, the kinetics of MMP-1 and MMP-13 secretion in response to TNF- α /IL-1 β differed markedly. Delayed (>12 h), CHX-inhibitable MMP-1 secretion indicates a requirement for protein synthesis, presumably of MMP-1 itself. MMP-13 secretion at 12 h also was partly CHX-inhibitable, but an early (1 h), CHX-insensitive component of MMP-13 secretion suggests a two-phase process: initial, rapid release of a pre-existing MMP-13 pool, followed by *de novo* MMP-13 synthesis.

MMP-1 and MMP-13 are differentially regulated by MAPKs in fibroblast-like synovial cells (20, 55), and we now show their differential regulation by MAPKs in AGS cells. Erk regulates MMP-1 secretion in response to either EGF or TNF- α /IL-1 β , but persistent Erk activation is not required because Erk inhibitors were not effective when added after peak Erk activity. The observation that Erk activation was not inhibited by CHX (data not shown) confirms that Erk activation precedes protein synthesis in the MMP response. The effects of Erk on MMP-1 synthesis likely are mediated, at least in part, through AP-1 transcriptional activation (67). In contrast, Erk did not regulate either the initial release of pre-formed MMP-13 from AGS cells or the subsequent synthesis of additional MMP-13. The observation that EGF (a canonical activator of Erk) did not stimulate MMP-13 secretion confirms that MMP-13 secretion is Erk-independent and differentially regulated compared with MMP-1 secretion.

In contrast to Erk, p38 activity inhibits AGS cell MMP-1 secretion, as deduced from the observation that p38 inhibitors enhanced release of MMP-1. These observations were unexpected because p38 is generally considered pro-inflammatory, and p38 inhibitors are under study as anti-inflammatory agents (68–70). Studies in keratinocytes and dermal fibroblasts have implicated p38 as positively regulating MMP-1 secretion (71–74); however, the effects of p38 on MMP-1 secretion are clearly cell type-specific because p38 inhibition has no effect on MMP-1 secretion from synovial fibroblasts (20, 75). In gastric cells, the observation that SB203580 enhanced both Erk and MMP-1 secretion in a UO126-inhibitable manner indicates that p38 acts by inhibiting Erk. This observation is not without precedent because p38 down-regulates and SB203580 stimulates Erk activity and Erk-mediated proliferation in another human gastrointestinal tumor epithelial cell line, pancreatic cells (76). The mechanism of p38-mediated Erk inhibition might relate to high level selectivity of distinct MAPK phosphatases for Erk and p38 (77). The ability of p38 inhibitors to stimulate and/or enhance gastric cell MMP-1 release suggests that they may prove ulcerogenic, consistent with a role for p38 in gastric and oral mucosal healing in humans and rats (78, 79). However, in contrast to MMP-1, the ability of p38 inhibitors to inhibit MMP-13 secretion suggests that MMP-13 secretion is positively regulated by p38. The net effect of p38 inhibition on gastric mucosal homeostasis may therefore depend on the balance between these two and probably other activities. The observation that p38 inhibition of MMP-1 secretion is Erk-dependent again points to Erk as a central regulator of MMP-1.

PGEs improve gastric barrier functions by increasing blood flow, mucus and bicarbonate secretion, and gastric epithelium proliferation while decreasing acid secretion (29). Our data

² M. H. Pillinger, N. Marjanovic, S.-Y. Kim, J. U. Scher, P. Izmirly, S. Tolani, V. Dinsell, Y.-C. Lee, M. J. Blaser, and S. B. Abramson, unpublished observations.

FIG. 6. Stimulus-specific effects of E prostaglandins on Erk activation and MMPs. AGS cells were incubated \pm PGE₁ or PGE₂ (each at 10 μ M) for 30 min, stimulated \pm EGF (A) or TNF- α /IL-1 β (B), and assayed for Erk activation (30-min stimulation) as well as MMP-1 secretion (18-h stimulation). C, AGS cells were incubated \pm PGE₁ or PGE₂ for 30 min and assayed for Erk activation (top panel) and total Erk (bottom panel). D, AGS cells were incubated \pm UO126 for 30 min, followed by addition of PGE₁ or PGE₂ for an additional 18 h, and assayed for MMP-1 secretion. Data shown are the mean \pm S.E. of six (A and B) or representative of three (C and D) experiments for each condition. *, $p \leq 0.05$; **, $p = 0.07$ (versus the appropriate stimulated condition).

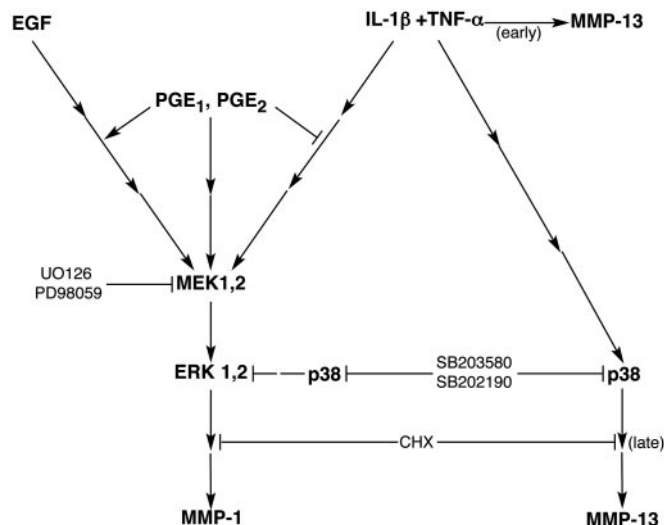
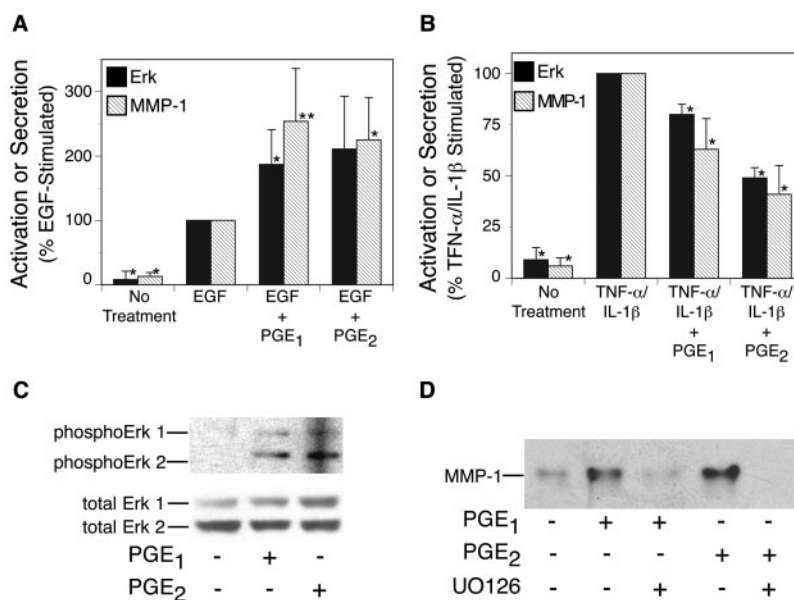


FIG. 7. Best-fit model of MMP regulation by MAPKs and prostaglandins in gastric epithelial cells. Our current studies indicate that both EGF and TNF- α /IL-1 β stimulate MMP-1 secretion from AGS cells. MMP-1 secretion is positively regulated by Erk, whereas p38 inhibits MMP-1 secretion via inhibition of Erk. Erk inhibitors (UO126 and PD98059) inhibit MMP-1 secretion, whereas p38 inhibitors (SB203580 and SB202190) enhance MMP-1 secretion. PGEs either inhibit or enhance MMP-1 secretion, depending upon the stimulatory context. In contrast, TNF- α /IL-1 β , but not EGF, stimulates MMP-13 secretion. MMP-13 secretion is both p38-dependent (late, CHX-sensitive phase) and independent (early, CHX-insensitive phase). Accordingly, p38 inhibitors down-regulate MMP-13 secretion in response to TNF- α /IL-1 β . Barbed arrows indicate stimulation, and blunt-ended lines indicate inhibition; arrows directed at other arrows indicate that the precise site of action has not been determined.

suggest an additional role: regulation of auto-catabolic processes such as MMP-1 secretion. However, MMP-1 secretion can be either enhanced or inhibited by PGEs, depending on the stimulus involved. Thus, the actual effects of PGEs on gastric barrier maintenance *in vivo* may vary with context, and the notion that PGEs are gastroprotective may be an oversimplification. Alternatively, it is possible that both stimulation and inhibition of MMP-1 secretion by PGEs are salutary with respect to gastric homeostasis. PGEs may serve to stimulate low levels of MMP-1 secretion in the absence of inflammation (or in the presence of growth factors) to facilitate its role in normal

tissue remodeling (80), but they may act to inhibit destructive MMP-1 secretion induced by inflammatory cytokines. Both the enhancing and inhibiting effects of PGEs on MMP-1 appear to be Erk-dependent. Although the mechanism(s) by which PGEs can both stimulate and inhibit Erk activation and MMP-1 expression is not clear, four PGE receptors (EP₁–EP₄) have been identified (81–83), and their differential engagement may promote divergent effects. Alternatively, PGEs might induce a single signal that can modulate distinct pathways, depending upon the specific activation state of the cell.

In summary, our data indicate that MMP secretion by gastric epithelial cells may be driven by both cytokines and EGF and implicate Erk and p38, as well as PGEs, as important regulators (Fig. 7). These observations suggest mechanisms by which gastric cells may participate in the propagation of gastric inflammation and ulcer disease in specific contexts, such as in *H. pylori*-induced inflammation and/or cyclooxygenase inhibition. This work opens possible pharmacologic avenues to minimize ulcer risk or to treat peptic ulceration.

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