Helicobacter pylori Encodes the Pathogenicity Island
Activates Matrix Metalloproteinase 1 in Gastric Epithelial Cells via JNK and ERK*

Received for publication, October 11, 2005, and in revised form, November 23, 2005  Published, JBC Papers in Press, December 1, 2005, DOI 10.1074/jbc.MS11053200

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**Helicobacter pylori** colonizes the human gastric epithelium and induces an inflammatory response that is a trigger for gastric carcinogenesis. Matrix metalloproteinases (MMPs) have recently been shown to be up-regulated in gastric epithelial cells infected with *H. pylori* and might contribute to the pathogenesis of peptic ulcer. The aim of this study was to extend the knowledge about the effect of *H. pylori* infection on MMP-1 expression by gastric epithelial cells, the kinetics of induction, the pathogenetic properties of the bacterium, and the intracellular signaling pathways required for MMP-1 up-regulation. Expression of MMP-1 was induced more than 10-fold by co-culture of AGS cells with *H. pylori* strains carrying the pathogenicity island (PAI1). *H. pylori* strains with mutations in the PAI and a defective type IV secretion system had no effect on MMP-1. Double immunofluorescence revealed strong MMP-1 staining in epithelial cells of gastric biopsies at sites of bacterial attachment. In vitro, MMP-1 is up-regulated by interleukin-1β and tumor necrosis factor-α, but these regulatory mechanisms are not operating in *H. pylori* infection as shown by inhibitory antibodies. Specific inhibitors of JNK kinase and ERK1/2 kinase were found to suppress the *H. pylori*-induced MMP-1 expression and activity. AGS cells treated with antisense MMP-1 showed a significantly reduced potential to degrade reconstituted basement membrane. Our results suggest that in gastric epithelial cells, *H. pylori* up-regulates MMP-1 in a type IV secretion system-dependent manner via JNK and ERK1/2. Induction of MMP-1 is further implicated in complex processes induced by *H. pylori*, resulting in tissue degradation and remodeling of the gastric mucosa.

Infection with *Helicobacter pylori* leads to chronic inflammation in the stomach, which may evolve to complications such as peptic ulcerations and gastric neoplasia (1–4). In gastric cancer development a complexity of factors involving genetic diversity of the organism, host susceptibility, environmental factors, and bacterial factors of *H. pylori* including a vacuolating cytotoxin (VacA), adhesins (BabA), a cytotoxin-associated antigen A (CagA), and the cag pathogenicity island (PAI2), encoding a specialized type IV secretion system (T4SS) (5, 6). In contrast to PAI- strains, PAI+ strains induce more notable phenotypic changes in vivo and in vitro, such as higher expression of cytokines like IL-8, IL-1β, or TNF-α (7), activation of transcription factors NF-κB (8–11) and AP-1 (11), stress response kinases JNK, p38, and ERK (11–14), Rho GTPases Rac1 and Cdc42 (15), and reorganization of the host actin cytoskeleton (15) and induction of cellular motility (16, 17).

In response to *H. pylori* occurs an enhanced release of cytokines and chemokines, which, in turn, stimulates gastric epithelial cells to produce matrix-degrading enzymes implicated in connective tissue remodeling. Matrix metalloproteinases (MMPs), embedded in a complex network of proteases, are involved in turnover and remodeling of extracellular matrix components, which in turn activate other assorted proteins, including growth factors and protease inhibitors (18–20). Activity of MMPs is regulated both at the level of gene expression and in proteolytic activity control. MMP mRNA expression can be induced by growth factors, cytokines, tumor promoters, and oncogene products. Once translated, MMPs were rapidly secreted in a proenzyme form, which could be activated by proteolytic cleavage and inhibited by their tissue inhibitors (TIMPs) (21).

Different MMPs and TIMPs have been reported to be overexpressed in gastric cancer, and a role in gastric cancer invasion and peritoneal metastasis was suggested. Recent studies have addressed the potential role of gastric MMPs and their inhibitors in early stages of gastric carcinogenesis. Besides the overexpression of MT1-MMP (22), MMP-1 (23), MMP-2 (24), MMP-3 (25), MMP-7 (26–28), and MMP-9 (24, 29), some cell culture experiments suggest that different regulatory factors may be involved in the activation process in the stomach. Interleukin 1β was found to mediate increased secretion of MMP-3 (25) and MMP-1 (30) in AGS gastric epithelial cells. Up-regulation of MMP-7 required adhesion of PAI+ bacteria to epithelia cells, and is mediated by activation of ERK1/2 (26), NF-κB, and AP-1 transcription factors (27). NF-κB is also essential for *H. pylori*-induced activation of MMP-9 gene expression (29).

In the present study, we analyzed how *H. pylori* elicits the up-regulation of MMP-1 in gastric epithelial cells. We tried to ascertain the pathogenetic factors of *H. pylori* indispensable for the MMP-1 induction and the signals and regulatory pathways stimulated by exposure to *H. pylori*, which are mostly relevant for the overexpression of MMP-1 and the increase of basement membrane destruction in response to the pathogen.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and *H. pylori* Infection**—The human gastric epithelial cells (AGS) were maintained in RPMI 1640 (PAA, Linz, Austria) medium containing 10% fetal calf serum (Biochrom, Berlin, Germany)
TABLE 1
Sequences of PCR primers, length of PCR products, and optimal annealing temperature

| Primers (5’–3’) | Sense | Antisense | Template (bp) | Annealing (°C) |
|----------------|-------|-----------|---------------|---------------|
| MMP-1          | CTGAAGGTGATGAAAGCAGCC | AGTCCCAAGAGAATGCGGAG | 428 | 57 |
| MMP-3          | CTCACAGACGCTGACCCTTGT | GACTGATGGGCCCTGAGG | 294 | 57 |
| uPA            | CTTCGCTGAAGATGGGCTTTCA | TCTCTCTTGCTGAGACCTGGC | 447 | 60 |
| TIMP-1         | CTTGTTGTTCGGGCTGATAT | CGTTCCCAAGAACATGAGT | 481 | 57 |
| 18 S RNA       | CCGTACCACATCCAGG | GCTGGAATTACCGCGGCT | 186 | 50 |

and antibiotics/antimycotics (PAA). Cells were grown at 37 °C in a humidified atmosphere containing 5% CO₂. The adherent cells were detached using trypsin/EDTA (Biochrom, Berlin, Germany). Prior to functional assays, 0.02% EDTA (Sigma) was alternatively used to avoid the destruction of cell surface antigens. Cells were counted in the Coulter Counter ZII (Coulter Immunotech, Marseille, France).

The cells were infected with H. pylori according to the protocol of Churin et al. (17). H. pylori strains were cultured on agar plates containing 10% horse serum under microaerophilic conditions at 37 °C for 48 h. For the infection, bacteria were harvested in phosphate-buffered saline, pH 7.4, and added to the serum-starved host cells at a multiplicity of infection (m.o.i.) of 50. We used wild-type H. pylori strain P1; isogenic mutant strains cagA and virB7; and wild-type strain Hp26695; and isogenic mutant strains HpPAI, Hp0539, and Hp0543.

**cDNA Array Analysis**—We used the GEArray Q series Human Tumor Metastasis Array designed to establish the expression profile of 96 genes important in tumor metastasis (Superarray Bioscience Corp., Bethesda, MD). The complete list of genes can be found at the Superarray web site. Total RNA was prepared by TRIzol reagent (Invitrogen, Groningen, The Netherlands). Two micrograms of RNA was reverse transcribed, and the probe was simultaneously labeled using the Ampo-Labeling-LRP Kit (Superarray Bioscience Corp.) according to the manufacturer’s instructions. Prehybridization, washing steps, hybridization, and chemiluminescence detection were carried out following the protocol of the manufacturer. Array images were detected using the Syngene BIO Imaging system. The raw signal intensities were corrected for background by subtracting the signal intensities of the average of the three negative controls and the four blanks, and were expressed as fold changes given as the relative expression ratio: gene/housekeeping gene.

**Quantification of mRNA by RT-PCR**—Using TRIzol reagent (Invitrogen), we extracted total cellular RNA according to the manufacturer’s instructions. RNA was reverse-transcribed using oligo(dT)₁₂₋₁₈ primers by Moloney murine leukemia virus reverse transcriptase (Promega). Amplification of selected cDNA was done with the primer pairs listed in Table 1. Integrity of RNA and adequate cDNA synthesis were confirmed by using 18 S RNA specific primers. The lengths of the resulting amplification products in base pairs are given in Table 1. Complete PCR were initially heated to 94 °C for 5 min for denaturation, and specific fragments were amplified in 30 cycles (0.5 min at 94 °C, 1 min at 50–60 °C, and 1 min at 72 °C). The PCR products were analyzed on a 0.8% agarose gel stained with ethidium bromide and scanned. All oligonucleotide primers were custom synthesized by Eurogentec (Seraing, Belgium).

**Patient Populations**—Biopsy specimens were obtained from 15 patients who had undergone upper esophagogastroduodenoscopy at the University Hospital Magdeburg. The patients included in the study had to be older than 18 years. All patients gave written informed consent to participate in the study. H. pylori infection was detected by the urease breath test, rapid urease test, and histological examination. Six patients were H. pylori positive (3 cag+ patients) and 9 patients had no H. pylori infection. The patients without H. pylori infection had clinical indications, such as dyspeptic symptoms or gastroesophageal reflux disease, making upper gastrointestinal endoscopy necessary. The investigation conforms with the principles outlined in the Declaration of Helsinki, and was approved by the local Ethics Committee.

**Immunofluorescence Analysis**—Tissue sections were deparaffinized in xylene and dehydrated in ethanol. The tissue was blocked in 10% horse serum/phosphate-buffered saline and incubated with rabbit polyclonal anti-Helicobacter antibody (Dako, Glostrup, Denmark) diluted 1:50 and monoclonal anti-MMP-1 antibody (Calbiochem, distributed by Merck) diluted 1:100, each for 16 h at 4 °C. After washing with phosphate-buffered saline/Triton, primary antibodies were detected with fluorescein isothiocyanate-conjugated anti-rabbit antibody and Texas red-conjugated anti-mouse antibody (Vector Laboratories, Burlingame, CA) diluted 1:100 for 2 h at 37 °C. The tissue sections were thoroughly washed in phosphate-buffered saline/Triton and mounted in VECTASHIELD® mounting medium with 4’,6’-diamidino-2-phenylindole (Vector Laboratories). The specimens were analyzed using a fluorescence microscope (Leica DMRE7, Leica Wetzlar, Germany) equipped with a CCD camera (Spot RT, Diagnostic Instruments, Burlough, MI) and a 20/1.25 objective. Separate images were taken in the corresponding channels, and later merged using the Photoshop® software. Image acquisition of the controls and data processing were carried out under the same conditions.

**Protein Extraction and Quantification**—Tissue samples were homogenized in phosphate buffer at pH 6.0 (50 mM sodium phosphate, 0.2 M NaCl, 5 mM EDTA, 100 μM E-64, 1 mM phenylmethylsulfonyl fluoride) using sonication. Homogenates were centrifuged for 10 min at 4 °C at 10,000 × g. Protein contents were measured in all samples using the Bio-Rad DC Protein Assay (Bio-Rad).

**MMP-1 Protein Secretion and Activity**—Culture supernatants were 10-fold concentrated in Centricron® centrifugal filter devices (molecular mass cutoff, 30 kDa) (VIVAScience, Hannover, Germany). The supernatants and tissue homogenates were reduced by adding 5× Laemmli buffer containing 20% dithiothreitol and were boiled. 25–μl Supernatants or 50 μg of protein were separated by SDS-PAGE and blotted onto nitrocellulose membranes. The membranes were blocked with 3% dry milk in TBS/Tween and incubated for 2 h at room temperature with monoclonal anti-MMP-1 antibody (1:100) (Calbiochem). This was followed by incubation with the secondary, peroxidase-conjugated rabbit anti-mouse antibody (1:25,000) for 30 min. West Pico Supernatant substrate (Perbio, Bonn, Germany) was left on the membrane until distinct bands had developed. A MagicMark standard (Invitrogen) was used to identify the molecular weights. The ECL membrane images were quantified using the Genegnome and GeneTools image scanning and analysis package (Syngene BioImaging Systems, Synoptics Ltd.).

Measurement of secreted active MMP-1 was performed using Fluorokine® E Human active MMP-1 Fluorescent Assay (R&D Systems) according to the manufacturer’s instructions. 150-μl culture supernatants (unconcentrated) were applied from each sample.
activation at 95 °C for 0 s, annealing at 50 °C or 57 °C (primer dependent) for 5 s, and an extension at 72 °C for 11 s. The PCR products were checked on a 0.8% agarose gel stained with ethidium bromide. All oligonucleotide primers were custom synthesized by Eurogentec (Seraing, Belgium).

Antisense Oligonucleotides—The antisense oligonucleotide (asMMP-1) used in this study was complementary to the AUG codon of the MMP-1 mRNA: 5′-GGAAAGCTGTGCATACTGGC-3′. A random oligonucleotide was used as a control (nSO DN): 5′- TGTAGTGTTAGTAT-3′. Both oligonucleotides were protected against exo- and endonucleases by phosphorothioate backbones. The uptake of oligonucleotides in cultured cells was monitored by 3′-fluorescein isothiocyanate labeling.

Transfection was performed as described previously (31). 10 μM asMMP-1 or the nSO DN oligonucleotides was added to the culture medium without a transfection reagent. The ability of the antisense oligonucleotide to decrease the MMP-1 mRNA and protein level (concentrated culture supernatants) was controlled by quantitative RT-PCR and in Western blots 24 h after transfection.

In Vitro Invasion Assay—Cellular invasion of AGS cells infected with H. pylori was evaluated in 24-well Transwell chambers (Costar, Bodenheim, Germany) as described previously (32). The upper and lower culture compartments were separated by polycarbonate filters with 8-μm pore size. Prior to the invasion assays, the polycarbonate filters were coated with 100 μg of Matrigel matrix per cm². AGS cells (1.5 × 10⁶ cells/cm²) or high and low m.o.i. were preincubated on the reconstituted basement membrane for 3 h in serum-free RPMI 1640 culture medium. Following infection with H. pylori strains, cells were incubated for 24 h in the invasion chambers. Cells passing the filters and attaching to the lower sites of Matrigel-coated membranes (invasive cells) were harvested using trypsin/EDTA; the cell number was quantified in a Coulter Counter ZII. The percentage of invasive cells was calculated after DNA quantification.

Using TRIzol reagent (Invitrogen), we extracted total cellular RNA according to the manufacturer’s instructions. RNA was reverse-transcribed using oligo(dT)₁₂₋₁₈ primers by Moloney murine leukemia virus reverse transcriptase (Promega). PCR was done with the quantitative PCR Mastermix kit (Qiogene, Heidelberg, Germany) according to the manufacturer’s instructions. The standard temperature profile included initial denaturation for 30 s at 95 °C, followed by 40 cycles of denatur
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RESULTS

Analysis of mRNA Expression for Genes Known to Be Involved in Extracellular Matrix Remodeling by Gastic Epithelial Cells after Coculture with H. pylori—To characterize changes in the gene expression profile of gastric epithelial cells in response to H. pylori (wild-type strain P1), we performed cDNA microarray analysis using the GEArray Q Series Human Tumor Metastasis Gene Array (Biomol), which contains 96 genes including growth factors and their receptors, extracellular matrix components, proteases and their inhibitors, oncogenes and suppressors, and other related genes. We used mRNA isolated from AGS cells compared with mRNA of AGS cells co-cultivated for 24 h with H. pylori strain P1 (m.o.i. 50). The majority of genes examined showed only small differences in expression. However, there were 15 different genes (Table 2), the expression of which was more than 2-fold up-regulated by infection with H. pylori. Interestingly, 10 of these 15 genes were proteases or protease inhibitors, whereas only one cell-matrix molecule or oncogene was found to be up-regulated. The most abun-

dant gene found to be up-regulated 25-fold was MMP-1, followed by MMP-3 (12.5-fold) and MMP-8 (5.3-fold). Down-regulated genes were not involved in this study. To confirm the results of cDNA microarray analysis, we performed semiquantitative RT-PCR (Fig. 1). Control RT-PCRs on primary cultures of gastric epithelial cells revealed similar results, allowing for the reproduction of the results obtained from carcinoma cells as shown in Table 2 (data not shown).

MMP-1 Overexpression in Gastric Mucosa Is Associated with H. pylori Infection—To analyze the effects of H. pylori-induced inflammation on MMP-1 production in vivo, we localized MMP-1 expression in gastric biopsy specimens. Double immunofluorescence staining revealed strong expression of MMP-1 (red fluorescence) in epithelial cells of H. pylori-infilitrated foveolae (Fig. 2m), whereas none of the H. pylori-negative foveolae showed this strong positivity for MMP-1 (Fig. 2, A, B, and E). Staining of MMP-1 was localized mainly to sites of bacterial infection and was also identified in fibroblasts.

H. pylori-Induced MMP-1 Overexpression in Gastric Epithelial Cells in Vivo and in Vitro—The analysis of the MMP-1 protein expression pattern in gastric biopsies indicates that MMP-1 is not or only weakly expressed in normal gastric mucosa, but significantly up-regulated in biopsies of H. pylori-infected gastric mucosa (Fig. 3). The two patients with very high levels of MMP-1 were infected with cag+ strains. These data were consistent with the immunohistochemical results and correspond to the array data.

The time course of MMP-1 gene expression and secretion in response to H. pylori infection was investigated in AGS cells by Western blot analysis and in an activity assay. Significant H. pylori stimulation (wild-type strain P1, m.o.i. 50) of MMP-1 protein secretion was observed after 4 h. Active MMP-1 increased significantly within 20 h of stimulation (Fig. 4). We next determined whether induction of MMP-1 overexpression depended on pathogenetic factors that vary among H. pylori strains. AGS cells were cocultured for 24 h with the wild-type H. pylori strains P1 and Hp26695 or the corresponding mutant strains cagA, virB7, HpaPl, Hp0543, and Hp0539. Like the wild-type strains, the isogenic cagA and Hp0543 mutant strains induced high MMP-1 expression. The cagA mutant does not show CagA expression and the Hp0543 mutant is defective in CagA translocation, but both mutants still induce activation of JNK and NF-κB (16). Thus, H. pylori-induced MMP-1 expression is CagA-independent. The mutant strains HpPAI,
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Hp0539, and virB7, which are either missing or defective in the T4SS, completely failed to increase MMP-1 expression and activity (Fig. 5).

**Role of Proinflammatory Cytokines and Growth Factors in Regulation of MMP-1 Secretion**—H. pylori stimulates the production of proinflammatory cytokines and chemokines in host cells. Therefore, we tested whether any of these cytokines stimulated by H. pylori in vivo increases MMP-1 transcription in vitro. AGS cells were treated for 24 h with 50 ng/ml IL-1β, IL-6, TNF-α, or TGF-β. MMP-1 activity was measured by the MMP-1 activity assay. The addition of IL-1β and TNF-α resulted in significantly increased levels of active MMP-1, whereas TGF-β addition induced a weaker stimulation. IL-6 seems to have no effect on MMP-1 induction (Fig. 6A).

We next studied if the cytokines found to be inducers of MMP-1 were also implicated in H. pylori-induced MMP-1 up-regulation. AGS cells were infected with H. pylori strain P1 and simultaneously treated with neutralizing antibodies for IL-1β, IL-6, TNF-α, or TGF-β. As shown in Fig. 6, B and C, neither P1-dependent induction of MMP-1 protein expression nor MMP-1 activity will be significantly reduced by adding cytokine-blocking antibodies. The concentrations of neutralizing antibodies were tested to block direct stimulations with the corresponding cytokine as described above (data not shown).

**JNK and ERK1/2 Activation Is Required for MMP-1 Regulation in Gastric Epithelial Cells Colonized by H. pylori**—The contact between H. pylori and gastric epithelial cells leads to the activation of a variety of signal transduction pathways, such as ERK1/2, JNK, and p38, and to the induction of early response transcription factors, such as NF-κB and AP-1 (33). Therefore, we investigated these H. pylori-activated molecular signaling pathways for their role as regulators of MMP-1 expression in gastric epithelium. AGS cells were infected with wild-type H. pylori strain P1 at a m.o.i. 50 in the presence of vehicle (dimethyl sulfoxide) alone or with the NF-κB inhibitors IKK-NBD and APDT, the p38 inhibitor SB203580, the JNK inhibitor SP600125, and the MEK1/2 inhibitor U0126. MMP-1 levels were measured by quantitative RT-PCR (Fig. 7A) and in concentrated supernatants using Western blot and activity assay (Fig. 7B) after 24 h of infection. ERK and JNK inhibition by U0126 and SP600125 completely abolished stimulated MMP-1 mRNA expression and protein secretion in response to H. pylori infection, whereas inhibition of NF-κB and p38 MAPK had no effect on the MMP-1 mRNA, protein, and activity levels stimulated by H. pylori. Because of the long time point used in the assay, we expect that in addition to the indicated signaling pathways transcriptional regulation of unknown factors contributes to MMP-1 regulation.
MMP-1 Inhibition Decreases H. pylori-stimulated Basement Membrane Degradation and Migration of AGS Gastric Epithelial Cells—Cellular proteases are known to enhance the motility and invasive behavior of tumor cells. We tested the influence of H. pylori and MMP-1 expression on the migration of AGS cells through basement membranes using Transwell chambers. Infection with wild-type H. pylori strains P1 and Hp26695 significantly enhanced the cellular migration of AGS cells (Fig. 8A). To determine the participation of MMP-1 overexpression on cellular migration, we applied 10 μM MMP-1-antisense oligonucleotides. These oligonucleotides were tested in advanced experiments to suppress MMP-1 mRNA (quantitative RT-PCR, Fig. 8B) and protein expression (Western blot, Fig. 8C) without any changes of cellular proliferation (not shown). The reduced expression of MMP-1 protein by antisense oligonucleotides resulted in much lower cellular migration, indicating the functional significance of MMP-1 in H. pylori-induced cellular migration. A random oligonucleotide (nsODN) had no effect.

DISCUSSION

In the present study, we demonstrated that infection of gastric epithelial cells with H. pylori stimulates the expression of MMP-1 in vitro as well as in human gastric biopsies. Up-regulation of MMP-1 expression in gastric epithelial cells was found to be dependent on infections with PAI-1 H. pylori strains, and involves activation of JNK and ERK1/2 signaling pathways. Moreover, we found that induction of MMP-1 by H. pylori plays a major role in the stimulation of gastric epithelial migration.

Among the 15 up-regulated genes detected in the cDNA array in this study, we identified 5 proteases, 3 protease inhibitors, and 1 protease receptor, indicating that the induction of the proteolytic network is one of the most adverse consequences of H. pylori infection. For MMP-2 and MMP-9, no alteration was found in our culture system, indicating a sophisticated stimulation mechanism for the individual MMPs caused by H. pylori. MMP-2 has already been reported to be increased in H. pylori-infected individuals (24), but no correlation was detected in vitro (29, 34). MMP-9 was found to be overexpressed in response to H. pylori in vitro and in vivo. However, they used MKN45 cells for this study, and not the AGS cells as in our present investigation (29). This means that the artificial in vitro systems are limited, and interpretations should be made carefully. In control experiments with primary cultures of epithelial cells from gastric biopsies, we were able to corroborate our findings in AGS cells for 10 selected genes that were down- or up-regulated in response to H. pylori (results not shown).

We selected MMP-1 because it was the most abundant gene found to be up-regulated in AGS cells by H. pylori and has been reported to be overexpressed in gastric ulcers (35, 36) and advanced gastric cancers (37–39). We found MMP-1 to be highly up-regulated in gastric biopsies of H. pylori-infected patients compared with non-infected patients, with the highest expression spots localized at sites of bacterial attachment. Gooz et al. (23) corroborated this finding in vitro studies. They noted enhanced expression and activation of MMP-1 in AGS cells after infection with H. pylori (23). The kinetics of MMP-1 secretion in response to H. pylori shown in our study are consistent with the results of Pillinger et al. (30) who used TNF-α/IL-1β as stimulants. Because of delayed secretion, they suggested the requirement of new MMP-1 protein synthesis. MMP-1 activation was found to be once more delayed, as shown in our study. This makes sense, because unstimulated AGS cells express only small amounts of MMP-1 near the detection limit.

Many H. pylori-induced diseases are strongly associated with the presence of a functional T4SS and CagA (40, 41). H. pylori strains lacking the functional T4SS failed to induce MMP-1 expression in our study, whereas cagA-defective strains obviously up-regulate MMP-1. These findings perfectly fit in results from our previous study. Here, H. pylori PAI1 mutants lacking the whole PAI did not induce cell scattering and migration, whereas mutants that did not translocate CagA, but induced strong IL-8 secretion surprisingly still stimulated obvious cell motility via a so far unknown mechanism (16). MMP-1 seems to be one downstream factor implicated in the complex regulation process of H. pylori-induced destruction of tissue architecture and tissue infiltration in vivo.

The epithelial cytokine/chemokine response to H. pylori infections may be particularly important for the regulation of MMP expression. Epidermal growth factor, TNF-α, and IL-1β were reported to induce MMP-1 secretion in AGS cells (20, 30). This is in line with our observations in activity assays analyzing cytokine stimulations; however, using neutralizing antibodies, the blocking of IL-1β, IL-6, TNF-α, and TGF-β in parallel with H. pylori infection did not result in a significantly weaker MMP-1 stimulation. Our results indicate that although cytokines can stimulate MMP-1 expression in general, other factors than cytokines must be responsible for MMP-1 induction in H. pylori-infected AGS cells.
Several reports indicate that the MMPs are divergently regulated by the ERK1/2, JNK, and p38 pathways (42–44). H. pylori selectively activate ERK1/2, JNK, p38, and NF-κB in AGS cells (33). Our results show that H. pylori caused MMP-1 up-regulation depends on activation of ERK1/2 and JNK. Inhibition of NF-κB and p38 signaling showed no or only a slight opposite effect on the MMP-1 expression, indicating their minor role in the regulation of MMP-1 in gastric epithelial cells. ERK1/2- and/or JNK-dependent activation of MMP-1 has already been reported for human skin and gingival fibroblasts (45, 46), monocytes (44), invasive melanomas (47), chondrocytes (48), and TNF-α/IL-1β-stimulated gastric epithelial cells (30). In contrast to ERK and JNK, p38 inhibitor studies provided divergent results, ranging from no effects at all to significant stimulations of MMP-1 expression. This is possibly because of different concentrations used for the inhibitor SB203580 or even a cell type-specific effect. Otherwise, using the same AGS cell line and equal amounts of inhibitor, Pillinger et al. (30) detected a significantly enhancing effect of p38 inhibitor SB203580 on MMP-1 mediated by simultaneous ERK activation (30). Possibly, the TNF-α/IL-1β-mediated stimulation of MMP-1 they used may not reflect in total the complex activation mechanisms initiated by H. pylori infection in gastric epithelial cells. In addition, other MMPs, such as MT1-MMP (47), MMP-9 (29), or MMP-13 (30), were described to be regulated by different signaling pathways and factors, whereas MMP-7 is also regulated through ERK1/2 in H. pylori infections. As some known stimulators of MMP-1, such as HGF and u-PAR/u-PA, were also activated via ERK signaling, it seems likely that overexpression of MMP-1 in response to H. pylori depends on different interacting factors (49, 50). Despite the similarities within the MMP family, their regulation and activation turned out to be different and needs to be investigated further.

Only four members of the MMPs, including MMP-1, MMP-8, MMP-13, and MT1-MMP, can degrade fibrillar collagens, as collagen I and collagen III are the predominant structure proteins in the gastric stroma. MMP-1 was found to be up-regulated in H. pylori-induced gastric ulcers, but a causal role in the ulcerogenesis was doubted as nonsteroidal anti-inflammatory drug (NSAID)-induced ulcers lack MMP-1 overexpression (36). Otherwise, these findings underline the specific interplay of H. pylori and MMP-1 overexpression as previously found and pointed out in this study (23). Induction of cell scattering (motility), migration, and cellular elongation of gastric epithelial cells by H. pylori was described as cag PAI- and CagA-dependent mechanisms (16, 17). The increase of the migration potential of AGS cells in response to PAI+ H. pylori could be strongly inhibited by addition of MMP-1 ODNs, indicating an important role of MMP-1 in tissue remodeling processes caused by mucosal inflammation. Whereas collagen I, which is the major substrate of MMP-1, is not included in the reconstituted basement membrane, it seems likely that not only the collagenolytic activity of MMP-1 but also the interaction with diverse bioactive molecules is implicated in the degradation process. As Rho-GTPases also represent important key regulators of both migration and increased cell motility driven by remodeling of the cytoskeleton (50), it is noteworthy that Rac1 and Cdc42 have antagonistic effects on MMP-1 expression in human skin fibroblasts (45). As Rac1 activity is not affected by silencing Cdc42, the authors hypothesized that WASP/WAVE proteins might effect MMP-1 expression. Recently, Sossey-Alaoui et al. (51) clearly identified WAVE3 as an activator of MMP-1 in neuroblastoma cells (51). This pathway could display a possible third mechanism besides ERK1/2 and JNK regulation, stimulating MMP-1 expression in response to H. pylori in the gastric mucosa.

In summary, we suggest that H. pylori-induced overexpression of MMP-1, dependent on the PAI status, possibly increases gastric tumor incidence. Obviously, MMP-1 expression in gastric epithelial cells is regulated by ERK1/2 and JNK signaling pathways including the activity of other factors transcriptionally induced by H. pylori, resulting in tissue architec-
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A representative Western blot (A) of AGS cells infected with wild-type H. pylori and the related regulation cascades of MMP-1 should provide a better understanding of the functional relationship between inflammation of the gastric mucosa and increased stomach cancer incidence.

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