Helicobacter pylori Activates the Histidine Decarboxylase Promoter through a Mitogen-activated Protein Kinase Pathway Independent of Pathogenicity Island-encoded Virulence Factors*

Silja Wessler‡§, Michael Höcker§, Wolfgang Fischer, Timothy C. Wang‡‡, Stefan Rosewicz‡, Rainer Haas, Bertram Wiedenmann‡, Thomas F. Meyer‡, and Michael Naumann‡ ‡‡

From the ‡Max-Planck-Institut für Infektionsbiologie, Abteilung Molekulare Biologie, Berlin, the §Medizinische Klinik mit Schwerpunkt Gastroenterologie und Hepatologie, Universitätsklinikum Charité, Campus Virchow-Klinikum, Humboldt Universität Berlin, the ¶Max von Pettenkofer Institut für Medizinische Mikrobiologie und Hygiene, Abteilung Bakteriologie, 80336 München, Germany, and the **Gastrointestinal Unit and Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, 02114

Helicobacter pylori infection of the gastric mucosa is accompanied by an activated histamine metabolism. Histamine plays a central role in the regulation of gastric acid secretion and is involved in the pathogenesis of gastroduodenal ulcerations. Histidine decarboxylase (HDC) is the rate-limiting enzyme for histamine production in gastric epithelial cell line (AGS) and analyzed the underlying molecular mechanisms. The present study investigated the effect of H. pylori infection on the transcriptional activity of the human HDC promoter (HDC) in a gastric epithelial cell line (AGS) and analyzed the underlying molecular mechanisms. Our studies demonstrate that H. pylori infection potently transactivated the HDC promoter. The H. pylori-responsive element of the hHDC gene was mapped to the sequence +1 to +27 base pairs, which shows no homology to known cis-acting elements and also functions as a gastrin-responsive element. H. pylori regulates the activity of this element via a Raf-1/MEK/ERK pathway, which was activated in a Ras-independent manner. Furthermore, we found that H. pylori-induced transactivation of the hHDC promoter was independent of the cag pathogenicity island and the vacuolating cytotoxin A gene and therefore may be exerted through (a) new virulence factor(s). A better understanding of H. pylori-directed hHDC transcription can provide novel insights into the molecular mechanisms of H. pylori-dependent gene regulation in gastric epithelial cells and may lead to new therapeutic approaches.

Helicobacter pylori has been identified as a major pathogen associated with the development of chronic gastritis and gastroduodenal ulcer disease as well as gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma (1–5). H. pylori strains expressing the vacuolating toxin A and genes encoded by the cytotoxin-associated gene A (cagA)

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§ The first two authors contributed equally to this work.

¶ To whom correspondence should be addressed: Max-Planck-Institut für Infektionsbiologie, Abteilung Molekulare Biologie, Monbijoustrasse 2, 10117 Berlin, Germany. Tel.: 49-30-28460-410; Fax: 49-30-28460-401; E-mail: naumann@mpiib-berlin.mpg.de.

† The abbreviations used are: cagA, cytotoxin-associated gene A; PAI, pathogenicity island; NF-xB, nuclear factor xB; AP-1, activator protein 1; IL, interleukin; ECL, enterochromaffin-like; HDC, histidine decarboxylase; hHDC, human HDC; JNK, c-Jun NH2-terminal kinase; MAP kinase, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PMA, phorbol 12-myristate 13-acetate; EGF, epidermal growth factor; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; PM, phorbol 12-myristate 13-acetate; EGF, epidermal growth factor; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; kf, kilobase(s); TK, thymidine kinase; m.i., multiplicity of infection; IxB, inhibitory protein kB; luc, luciferase; EMSA, electrophoretic mobility shift assay; GAS-RE, gastrin-responsive element; bp, base pair(s); GAS-RE-BP, GAS-RE-binding protein; HIV, human immunodeficiency virus.
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development of gastroduodenal ulceration (31–35). Furthermore, we found that oxidative stress, which is commonly increased in ulcerative and inflammatory diseases affecting the gastric mucosa, is capable of transactivating the hHDC promoter in vitro (36). Aside from its ulcerogenic potential, histamine has also been shown to possess immunomodulatory properties in the context of mucosal inflammations, contributes to the healing of ulcerative lesions of the gastric and intestinal mucosa, and has also been shown to stimulate the growth of gastric epithelial cells (37–41). A current in vivo study demonstrated that H. pylori infection is associated with increased mucosal histamine levels as well as an expansion of the gastric ECL cell lineage (42). The hypothesis that these changes could be at least in part be attributed to a direct effect of H. pylori on ECL cells has been substantiated by the finding that H. pylori can stimulate histamine secretion from isolated rat ECL cells as well as ECL cell proliferation in vitro (43). Although these findings strongly suggested that H. pylori can directly influence the histamine metabolism of gastric ECL cells, potential molecular mechanisms that could underlie this effect are unclear.

It has been demonstrated that in gastric epithelial cells H. pylori infection elevates the abundance of "classical" second messenger molecules such as Ca²⁺, cyclic adenosine monophosphate (cAMP), and inositol trisphosphate (44). Although H. pylori has been shown to stimulate phosphorylation of several cellular proteins in gastric cancer cells in vitro (44, 45), intracellular signaling cascades activated by the bacterium are largely unknown. In a current study, we investigated the effect of H. pylori on the AP-1 transcription factor complex in gastric cancer cells in vitro and found that AP-1 activity is regulated by H. pylori through activation of c-Jun NH₂-terminal kinase (JNK) (13), which belongs to the superfamily of "mitogen-activated protein kinases" (MAP kinases) (46, 47). In addition to the JNK cascade, the MAP kinase superfamily comprises the extracellular-regulated kinase (ERK) pathway (46). Although some overlap between both pathways has been described (46), the JNK cascade activates primarily transcription factors involved in the "stress response" of eukaryotic cells, whereas the ERK pathway has been linked to genes involved in cellular proliferation and differentiation (46–48). Recently we demonstrated that ERK-related signaling cascades also play a central role in the transmission of the effects of gastrin and oxidative stress on the hHDC promoter in gastric cancer cells, whereas the JNK pathway is not involved in hHDC gene regulation (36, 49). Therefore, it is highly likely that the ERK cascade represents a potential target signaling route through which activators of hHDC gene transcription exert their transactivating effect on the hHDC promoter.

To investigate whether H. pylori can directly influence the transcriptional activity of the hHDC promoter, we performed in vitro studies employing hHDC-luciferase reporter gene constructs as well as various hHDC promoter mutants. Furthermore, we aimed to analyze the signal transduction pathways and nuclear factors responsible for transmission of this effect on the hHDC promoter. Finally, we analyzed the virulence factors involved in regulation of the hHDC promoter by H. pylori.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—The human gastric adenocarcinoma cell line (AGS) was grown in RPMI 1640 (Life Technologies, Inc., Heidelberg, Germany) supplemented with 4 mM glutamine, 100 units ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin, and 10% fetal calf serum (Life Technologies, Inc.) in a humidified 5% CO₂ atmosphere. AGS-B cells express the cholecystokinin-B/gastrin receptor through stable transfection and were described before (30). Where indicated, cells were treated with 50 µM phorbol 12-myristate 13-acetate (PMA, Sigma, St. Louis, MO) for 4 h or treated with 200 ng/ml EGF (Promega, Heidelberg, Germany) for 6 h. AGS-B cells were stimulated with 10⁻⁷ M gastrin (Calbiochem, San Diego, CA) for 12 h. To block MEK activation, cells were preincubated with 50 µM PD98059 (Calbiochem) for 30 min before infection.

**Bacteria and Infection**—The following H. pylori strains were used for infection experiments: P12 strain (wild type) and the isogenic cagA⁻ (cagA with a probable polar effect), nvac (50), and PAI (missing the cag pathogenicity island [PAI]; and G27 (wild type) and the isogenic cagI strain (58). For the construction of the PAI strain two approximately 2-kb DNA fragments upstream of the cag-PAI (region 545254–547164) and downstream of the PAI (584570–586563) (51) were amplified by polymerase chain reaction and cloned into pBluescript separated by a kanamycin resistance gene. The plasmid was transformed into H. pylori (P12), and one transformant was analyzed by polymerase chain reaction for correct allelic exchange of the PAI with the resistance gene. H. pylori strains were grown on agar plates containing 10% horse serum in a microaerophilic atmosphere (generated by Campy-Gen, Oxoid, Basingstoke, U. K.) at 37°C for 48–72 h. 24 h after infection H. pylori was harvested in phosphate-buffered saline (pH 7.4), diluted corresponding to the multiplicity of infection (m.o.i.), and incubated with the epithelial monolayer. Infection with H. pylori was monitored routinely by light microscopy.

**Transient Transfections and Luciferase Reporter Assays**—24 h prior to transfection, cells were seeded in tissue culture plates and grown to 60–70% confluence. Transfections of 1–2 µg of reporter constructs were carried out using cationic liposomes (Dac-30, Eugenotergent, Sart Tilman, Belgium) according to a protocol reported previously (13, 52). Transfection of hHDC promoter-luciferase reporter gene constructs was measured after transfection of 5'-deletion constructs (hHDC1000, hHDC480, hHDC400, hHDC125, +1 to +27 TK luc) as described previously (49, 53, 54). Transactivation activity of NF-κB and AP-1 was measured as described previously (13, 52). Cotransfection of dominant negative kinase cDNA mutants (DNERK1(k71R), DNERK2(k52R), DNRaf-1, DNRAf1, DNRAfs15(G15A), DNRAfs17(S17N), and DNNMEKK1(K432M)) with appropriate hHDC constructs has been described previously (49, 52). After transfection the cells were deprived of serum and maintained in RPMI 1640 supplemented with 4 mM glutamine and 0.1% fetal calf serum for 20–24 h. The expression of the transfection dominant negative kinase constructs was controlled by immunoblotting. For measurement of transactivation activity transfected cells were harvested, and luciferase activity was assayed as recommended by the manufacturer's instructions (Promega). The results were recorded on a Wallac 400 β-counter (Berthold-Wallac, Bad Wildbach, Germany). The data represent the mean ± S.D. calculated from three independent experiments as fold activation compared with the control. Activities varied <15% among transfection experiments.

**Immunoblotting**—To detect activated MEK1/2 and ERK1/2 total and phosphorylated forms of MEK1/2 and ERK1/2, total and phosphorylated MEK1/2 and ERK1/2 were separated in SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Western blot analysis was performed using phospho-specific antibodies (New England Biolabs, Beverly, MA) to detect pMEK1/2 and pERK1/2. Each sample was probed with anti-MEK1 and anti-ERK2 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) to indicate equivalent protein amounts in all lanes. To detect IκBα, samples were probed with an anti-IκBα antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

**Electrophoretic Mobility Shift Assay (EMSA)**—Nuclear extracts were prepared by using a non-ionic detergent method as described previously (55). For the detection of gastrin-responsive element (GAS-RE) DNA binding activity, equal amounts of nuclear protein extracts were incubated with labeled oligonucleotides containing the +1 +27 GAS-RE binding site sequence of the human HDC promoter: 5’-ACCTTTAATAAAGGCCCCACACTG-3’.

The oligonucleotide containing the GAS-RE recognition site was labeled using T4 kinase (Roche Molecular Biochemicals GmbH, Mannheim, Germany) in the presence of [γ-³²P]ATP. The DNA binding reactions were performed by binding a containing 10 µM Tris (pH 7.5), 5mM KCl, 0.2 mM EDTA, 10 µM K₃[HPO₄], 1 mM Na₂[V₃O₄], 10 mM NaF, 1.25% Nonidet P-40, and 10% glycerol. Equal amounts of protein extracts were separated in SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose membranes. Western blot analysis was performed using phospho-specific antibodies (New England Biolabs, Beverly, MA) to detect pMEK1/2 and pERK1/2. Each sample was probed with anti-MEK1 and anti-ERK2 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) to indicate equivalent protein amounts in all lanes. To detect IκBα, samples were probed with an anti-IκBα antibody (Santa Cruz Biotechnology, Santa Cruz, CA).
To identify the *H. pylori*-responsive element of the hHDC promoter sufficient for the transcriptional response, we analyzed a series of hHDC 5′-deletion constructs (Fig. 1B). Removal of nucleotides from the 5′-end down to 125 bp upstream of the Cap site (+1) had no significant influence on the *H. pylori*-induced luciferase activity. Similar results were obtained in cells treated with PMA (data not shown). Because this region comprises the +1 to +27 bp GAS-RE of the hHDC proximal promoter, we used a luciferase construct in which the GAS-RE sequence hHDC +1 to +27 was ligated upstream of the enhancerless herpes simplex virus 1 thymidine kinase (TK) promoter. We found that this element was capable of conferring *H. pylori* responsiveness to the same extent as the longest hHDC 5′-flanking fragments.

To underline that the GAS-RE is activated by *H. pylori* through enhanced binding of nuclear factors, we analyzed the DNA binding activity of nuclear proteins to the +1 to +27 sequence in EMSAs. Enhanced binding of AGS nuclear proteins was observed within 30 min postinfection (Fig. 1C, lanes 1–4). Treatment of AGS cells with PMA, a strong inducer of hHDC promoter activity, resulted in a strong increase of DNA binding activity of transcription factors within 10 min (Fig. 1C, lanes 5–7). Because *H. pylori* and PMA-induced complexes produced identical bandshifts it can be concluded that the *H. pylori*-stimulated complex consists of GAS-RE-binding proteins (GAS-RE-BPs). This was further confirmed by the finding that the complex stimulated by *H. pylori* could be competed away by an excess of unlabeled hHDC +1/+27 bp oligonucleotide (Fig. 1C, lanes 8–10). Based on these data, *H. pylori* was identified to induce very specific (m.o.i. of 5–50) enhanced hHDC promoter activity through activation of the GAS-RE-BP1/2 transcription factors that bind to the +1 to +27 bp minimal element.

**Activation of the hHDC Promoter Is Independent of cag-PAI-encoded Gene Expression**—To investigate the role of cag genes for activation of the hHDC promoter by *H. pylori*, AGS cells were colonized with different isogenic mutants lacking certain cag genes. AGS cells transfected with the 1.8-kb hHDC-luc construct exerted after infection with *H. pylori* wild type strains (P12 and G27) a strong increase in luciferase activity (Fig. 2A, left panel). Similar activation was obtained in AGS cells, infected with isogenic mutants, lacking the *vacA*, *cagA*<sup>+</sup>, and *cagI* genes. Additionally, the isogenic *H. pylori* strain PAI, which lacks the entire PAI, potently induced the hHDC promoter activity, supporting the notion of a *cag*-independent activation of the hHDC promoter by *H. pylori*. In contrast, in AGS cells transfected with a reporter gene construct in which luciferase expression was under control of the NF-κB consensus element of the HIV promoter, *H. pylori* mutants lacking *cagA*<sup>−</sup>-*cagI* genes or the entire PAI did not stimulate reporter gene expression (Fig. 2A, right panel). The *cag*-independent activation of the HDC promoter activity was also investigated by EMSA. Enhanced DNA binding of the GAS-RE-BP1/2 to the HDC enhancer element was observed in AGS cells infected with any *H. pylori* (Fig. 2B, lane 1–8, left panel). Using the Igκ-NF-κB oligonucleotide to detect NF-κB DNA binding activity, colonization with the *H. pylori* strains P12, *vacA*, and G27 induced strong activation of NF-κB (Fig. 2B, lanes 1–3 and 6, right panel), whereas the knockout mutants *cagA*<sup>+</sup>, *cagI*, and PAI showed a strongly reduced NF-κB DNA binding activity (Fig. 2B, lanes 4, 7, and 8, right panel).

**H. pylori-stimulated hHDC Promoter Activity Involves Activation of the ERK / MEK Kinases**—To study the signaling that is induced by *H. pylori* in a *cag*-independent manner, we investigated the capability of *H. pylori* to induce activation of certain MAP kinase pathways. Subconfluent monolayers of...
AGS cells were infected with \textit{H. pylori} (P12), and cell lysates were prepared after different time points postinfection and analyzed for activated and phosphorylated ERK1/2 and MEK1/2 by Western blot analysis using phospho-specific antibodies. \textit{H. pylori} activated both ERK1 and ERK2 in AGS cells within 30 min after infection (Fig. 3A, lanes 1–5), comparable to the activation induced by stimulation with PMA (Fig. 3A, lanes 6–9). Similar results were obtained using phospho-specific antibodies to detect activated MEK1/2. MEK1 and MEK2 were also phosphorylated within 30 min after \textit{H. pylori} infection or PMA treatment (Fig. 3B, lanes 1–9). To investigate whether the \textit{H. pylori}-induced activation of the ERK/MEK kinases is \textit{cagA}-independent, we studied the effects of the \textit{H. pylori} mutants on the activation of ERK1/2 and MEK1/2. In contrast to the AP-1-activating kinase pathway (13) the infection of AGS cells with \textit{H. pylori} knockout mutants (\textit{vacA}, \textit{cagA}, \textit{PAI}, and \textit{cagI}) in all cases stimulated activation of ERK1/2 and MEK1/2 (Fig. 3A and B, right panel). As a control, we used the same extracts to analyze the \textit{H. pylori}-infected cells for degradation of the NF-\textit{kB} inhibitor IxB. Colonization of AGS cells with \textit{H. pylori} (P12) for different periods of time resulted in degradation of IxB by the NF-\textit{kB} activity (Fig. 2B, lower panel). IxB is degraded in response to infection with \textit{H. pylori} strains P12, \textit{vacA}, and G27 (Fig. 3C, lanes 10, 11, and 14) but remains unaffected after infection with \textit{H. pylori} strains \textit{cagA*}, \textit{PAI}, and \textit{cagI} (Fig. 3C, lanes 12, 13, and 15). These data indicate that \textit{H. pylori} infection has the capacity to induce ERK/MEK activation independent of \textit{H. pylori} \textit{cag} gene expression.

To examine whether these \textit{H. pylori}-activated MAP kinases are involved in the upstream signaling regulating the hHDC promoter activity, we cotransfected AGS cells with the 1.8-kb hHDC-luciferase construct and infected with \textit{H. pylori} strains P12 and G27, their isogenic mutants \textit{vacA}, \textit{cagA*}, \textit{PAI}, and \textit{cagI} at a m.o.i. of 50 for 4 h, or left untreated (–) (left panel). As a control, the effect of \textit{H. pylori} on NF-\textit{kB}-dependent transactivation was analyzed in AGS cells transfected with 0.5 \mu g of a HIV-NF-\textit{kB} luciferase reporter construct (right panel). Results of three independent experiments are expressed as fold induction compared with the control. B, the influence of various isogenic \textit{H. pylori} mutants on the binding of nuclear factors to the hHDC +1 to +27 bp element was analyzed after infection of AGS cells with \textit{H. pylori} strains at a m.o.i. of 50. For EMSAs, nuclear extracts were prepared after a 60-min infection with \textit{H. pylori} strains and analyzed for DNA binding activity to the hHDC promoter using a 32P-labeled probe representing a consensus Igf NF-\textit{kB} binding site. For these experiments, nuclear extracts were prepared after a 90-min infection with \textit{H. pylori} strains (right panel). The sections of protein-DNA complexes of the autoradiograms are shown. The positions of protein-DNA complexes are indicated with arrows.

\textbf{Fig. 2.} \textit{H. pylori}-induced activation of the hHDC promoter is independent of the expression of \textit{vacA} and \textit{cag} genes. A, AGS cells were transfected with 1 \mu g of the 1.8-kb hHDC promoter luciferase construct and infected with \textit{H. pylori} strains P12 and G27, their isogenic mutants \textit{vacA}, \textit{cagA*}, \textit{PAI}, and \textit{cagI} at a m.o.i. of 50 for 4 h, or left untreated (–) (left panel). As a control, the effect of \textit{H. pylori} on NF-\textit{kB}-dependent transactivation was analyzed in AGS cells transfected with 0.5 \mu g of a HIV-NF-\textit{kB} luciferase reporter construct (right panel). Results of three independent experiments are expressed as fold induction compared with the control. B, the influence of various isogenic \textit{H. pylori} mutants on the binding of nuclear factors to the hHDC +1 to +27 bp element was analyzed after infection of AGS cells with \textit{H. pylori} strains at a m.o.i. of 50. For EMSAs, nuclear extracts were prepared after a 60-min infection with \textit{H. pylori} strains and analyzed for DNA binding activity to the hHDC promoter using a 32P-labeled probe representing a consensus Igf NF-\textit{kB} binding site. For these experiments, nuclear extracts were prepared after a 90-min infection with \textit{H. pylori} strains (right panel). Only the sections of protein-DNA complexes of the autoradiograms are shown. The positions of protein-DNA complexes are indicated with arrows.

\textbf{Fig. 3.} Infection with \textit{H. pylori} stimulates phosphorylation of ERK1/2 and MEK1/2 kinases. AGS cells were infected with \textit{H. pylori} (P12) at a m.o.i. of 100 or stimulated with 50 nM PMA for the indicated time points (left panels). The \textit{H. pylori} mutants, AGS cells were infected for 30 min (right panels). Cells were lysed, and 30 \mu g of lysates were separated by SDS-polyacrylamide gel electrophoresis and blotted onto membranes. Phosphorylation of ERK1/2 (A) and MEK1/2 (B) was detected using phospho-specific antibodies in Western blot analysis (upper panels). As a loading control, the same amounts of the cell lysates were blotted and probed with non-phospho-specific anti-ERK2 and anti-MEK1 antibodies (lower panels). Additional bands, besides ERK2 and MEK1, observed in some lanes represent a cross-reactivity with other antigens recognized by the antibodies. C, as an additional control we determined the IxB abundance in response to \textit{H. pylori} colonization using an IxB antibody for immunodetection. The positions of the recognized proteins are indicated.
cells were transfected with 1 μg of the 1.8-kb hHDC promoter luciferase construct and either 0.5 μg of a dominant-negative ERK1 mutant (DNERK1), 0.5 μg of an ERK2 mutant (DNERK2) (or in combination 0.25 μg for each construct), or empty vector. The total amount of plasmid DNA was kept constant. Transfected cells were either infected with H. pylori (P12) at a m.o.i. of 50, treated with 50 nM PMA for 4 h, treated with 10−7 M gastrin (AGS-B cells) for 12 h, or left untreated (left panel). Further, as a control AGS cells were transfected with a NF-κB-dependent luciferase reporter construct (HIV/NF-κB Luc) and DNERK constructs (right panel). B, to determine the role of the upstream ERK-activating kinase MEK, AGS cells were transfected with 1 μg of the 1.8-kb hHDC reporter construct and treated with 50 μM PD98059 (PD) for 30 min followed by infection with H. pylori (P12) at a m.o.i. of 50 or treatment with 50 nM PMA for 4 h (left panel). Further, as a control, AGS cells were transfected with a NF-κB-dependent luciferase reporter construct and treated as described above (right panel). The data represent the means ± S.D. calculated from three independent experiments as fold induction compared with the control.

Based on a Ras-independent Signaling—The small G-protein Ras is one known upstream regulator of Raf-1. In the following experiments we studied whether Ras activation contributes to the stimulation of the hHDC promoter activity. To show the functional dominant negative effect of the MEKK1 construct, we cotransfected the DNMEKK1 cDNA with the AP-1 luciferase construct. Expression of DNMEKK1 inhibited the EGF-induced AP-1 transactivation activity that was not affected by overexpression of dominant negative MEKK1 (Fig. 5B, right panel). To show the functional dominant negative effect of the MEKK1 construct, we cotransfected the DNMEKK1 cDNA with the AP-1 luciferase construct. Expression of DNMEKK1 inhibited the EGF-induced AP-1 activation (Fig. 5B, right panel).

Activation of the H. pylori-induced hHDC Promoter Activity Based on a Ras-independent Signaling—Possible upstream activators of MEK1/2 are represented by molecules like Raf-1 or MEKK1 (MEK kinase 1) (46). Cells expressing a DNRAf-1 construct and infected with H. pylori or treated with PMA or gastrin were analyzed for transactivation activity of the hHDC promoter. Compared with mock-transfected cells, the hHDC promoter transactivation activity was strongly reduced (Fig. 5A, left panel), whereas expression of DNRAf-1 had no influence on the activation of NF-κB induced by H. pylori (Fig. 5A, right panel). These data lead to the suggestion that the Raf-1 kinase lies upstream in the specific signal pathway leading to MEK/ERK-directed activation of the hHDC promoter. The possible role of MEKK1 in the H. pylori-induced activation of the hHDC promoter was investigated using constructs expressing DNMEKK1. Colonization of transiently transfected AGS cells with H. pylori or treatment with PMA induced hHDC promoter activity that was not affected by overexpression of dominant negative MEKK1 (Fig. 5B, left panel). To show the functional dominant negative effect of the MEKK1 construct, we cotransfected the DNMEKK1 cDNA with the AP-1 luciferase construct. Expression of DNMEKK1 inhibited the EGF-induced AP-1 activation (Fig. 5B, right panel).

Activation of the H. pylori-induced hHDC Promoter Activity Based on a Ras-independent Signaling—The small G-protein Ras is one known upstream regulator of Raf-1. In the following experiments we studied whether Ras activation contributes to the stimulation of the hHDC promoter activity. To explore the capacity of Ras to induce H. pylori-mediated hHDC promoter activity, we...
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In the present study we demonstrated that colonization of a permanent gastric epithelial cell line with H. pylori stimulates the transcriptional activity of the hHDC promoter in vitro. Furthermore, we found that the transactivating effect of H. pylori is independent of the vacA gene and genes encoded by the cag-associated pathogenicity island, which have been implemented in more severe clinical outcomes of chronic gastric H. pylori infections (1, 7, 8). The analysis of cis- and trans-activating factors involved in H. pylori-dependent hHDC regulation revealed that a proximal element at +1 to +27 bp, which has been identified previously to be responsible for gastric-independent regulation of the hHDC gene, is also mediating the effect of H. pylori on the hHDC promoter (53, 54).

H. pylori has been shown to stimulate the expression of proinflammatory cytokines such as IL-1β, IL-6, tumor necrosis factor-α, and IL-8 (18, 19). Studies about H. pylori-induced IL-8 secretion (11, 14, 15) demonstrated that activation of NF-κB is a central mechanism through which H. pylori transactivates the IL-8 gene promoter, whereas the H. pylori-dependent transactivation of the hHDC promoter does not involve NF-κB. The proximal hHDC +1 to +27 element does not display any homology to known transcription factor binding sites, and competition studies with oligonucleotides representing consensus binding elements of various known transcription factors demonstrated that this element is not bound by NF-κB or other well characterized nuclear factors such as AP-1, Sp1, or CREB (53). The view that H. pylori-dependent transactivation of the hHDC promoter does not require NF-κB is substantiated further by the finding that isogenic H. pylori mutants (cagA, PAI, cagI), which were not able to transactivate a NF-κB-luciferase reporter gene construct, stimulated the binding of nuclear proteins to the hHDC +1 to +27 element and transactivated a hHDC 1.8-kb luciferase reporter construct in AGS cells. Therefore, it can be concluded that the hHDC +1 to +27 bp element represents a new nuclear target sequence of H. pylori through which the bacterium can influence gene expression independent of vacA- or PAI-encoded genes in gastric epithelial cells (Fig. 7).

Previous analysis of the nuclear proteins regulating the hHDC +1 to +27 bp element demonstrated that it is bound by two so far unknown transcription factors (54). These proteins with a molecular size of 35 and 52 kDa, respectively, were termed GAS-RE-BPs because their binding to the +1 to +27 element is indispensable for full gastrin responsiveness of the hHDC promoter (54). Similar to gastrin and PMA, H. pylori infection of AGS cells stimulated binding of nuclear proteins to this element. EMSA analysis of H. pylori- and PMA-stimulated AGS cells demonstrated that the nuclear proteins stimulated by both factors displayed identical bandshifts. Additionally, the H. pylori-induced complex binding to the hHDC +1 to +27 sequence could be competed away with an excess of cold +1 to +27 bp oligonucleotide. Therefore, it appears very likely that the transcription factors binding to the +1 to +27 bp element in response to H. pylori represent the previously described GAS-RE-BPs. Because the GAS-RE-BPs represent two novel transcription factors, further analysis of these factors may lead to the identification of genes that have so far not been linked to the epithelial response to H. pylori. Further, our findings indicate that in addition to the well characterized vacA and cag genes, H. pylori expresses (a) virulence factor(s), which enable(s) the bacterium to activate alternative target genes. The fact that this/these factor(s) appear to be independent of genetic regions that have been associated with enhanced pathogenic potential of H. pylori strains indicates that additional gene loci outside the so far characterized virulence factors may contribute to the overall pathogenesis of H. pylori on the gastric mucosa.

To understand the molecular pathways by which the effect of H. pylori colonization of AGS cells is transmitted into the nucleus, we analyzed signal transduction cascades involved in H. pylori-dependent hHDC transactivation. We found that the transactivating effect of H. pylori is transmitted via a signaling cascade comprising ERKs and their upstream activating kinases MEK and Raf-1, respectively. ERKs and MEKs are activated by H. pylori colonization with a similar time course showing peak phosphorylation after 30 min of infection. In contrast to the effect of H. pylori on the hHDC promoter, activation of NF-κB and AP-1 by the bacterium strictly required cag gene expression (13). Therefore, the virulence factor(s) underlying H. pylori-dependent activation of Raf-1/MEK/ERK-dependent signaling cascades resulting in enhanced HDC transcription appears to act independently of the bacterial factors controlling the acute cytokine response featuring activation of stress response signaling pathways and NF-κB activation (Fig. 7). Our results indicate that different virulence factors of H. pylori are capable of activating distinct branches of the MAP kinases signaling system, which appear to result in transactivation of different epithelial target genes. This observation further suggests that dependent on the virulence factors expressed, H. pylori strains may be able to elicit a differential epithelial signaling response, which may lead to transactivation of a specific set of genes.

A recent study described that incubation of KATO III gastric carcinoma cells with H. pylori supernatants resulted in inhibition of ERK-signaling stimulated by EGF and that this effect was vacuolating toxin A-dependent (56). In contrast to this study, we found that exposure of AGS cells to two different strains of intact H. pylori (P12 and G27) resulted in enhanced MEK/ERK phosphorylation. Furthermore, in contrast to the findings by Pai et al. (56), the effect of H. pylori on the MEK/ERK cascade was vacuolating toxin A-independent. Although we did not investigate the effect of H. pylori on EGF-stimulated ERK signaling in the AGS model, the robust ERK/MEK phos-
phylation in response to intact H. pylori demonstrates that the bacterium is capable of stimulating this mitogenic pathway. Activation of the MEK-1/ERK cascade by classical mitogens such as EGF is typically induced through activation of Ras and Raf-1 (46). Because application of two different dominant negative Ras mutants did not influence the transactivating effect of H. pylori on the HDC promoter, the functional involvement of Ras in this context is unlikely. Previous studies from our laboratory demonstrated that alternatively to the Ras/Raf-1 sequence, the MEK/ERK cascade in AGS cells can be also activated through a Ras-independent, protein kinase C/Raf-1-dependent pathway (49). Whether protein kinase C-dependent activation of Ras in this context is unlikely. Previous studies from our laboratory demonstrated that alternatively to the Ras/Raf-1 sequence, the MEK/ERK cascade in AGS cells can be also activated through a Ras-independent, protein kinase C/Raf-1-dependent pathway (49). Whether protein kinase C-dependent signaling events are involved in the coupling of the Raf/MEK/ERK cascade to upstream signaling events triggered by H. pylori is currently under investigation in our laboratory.

Based on the clinical data currently available, the exact impact of H. pylori-stimulated HDC transcription on the overall pathophysiology of H. pylori in the stomach is currently unclear and has to be further clarified in in vivo studies. It can be speculated that dependent on the onset, duration, and/or intensity of activation, enhanced H. pylori-stimulated hHDC transcription could contribute to either tissue damage or processes of mucosal restitution and repair in the stomach (32, 33, 37, 38). Furthermore, a direct effect of H. pylori on HDC gene expression resulting in enhanced histidine production and secretion could help to maintain an acidic gastric environment, favoring the survival of H. pylori over non-acid-resistant bacteria. Overall, H. pylori-dependent transactivation of the hHDC gene represents a new molecular model for the interaction of the bacterium with gastric epithelial cells. Our data for the first time demonstrate that H. pylori is capable of activating a gastric target gene through an ERK-dependent signaling pathway, independent of vacuolating toxin A- and PAI-related virulence factors. Therefore, a detailed analysis of the molecular mechanisms underlying the effect of H. pylori on the hHDC gene could contribute to a better understanding of the molecular pathogenesis of H. pylori infections and could probably lead to new approaches for the treatment of H. pylori-associated gastric diseases.

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