The gastric pathogen *Helicobacter pylori* is known to activate epithelial cell signaling pathways that regulate numerous inflammatory response genes. The aim of this study was to elucidate the pathway leading to extracellular signal-regulated kinase (ERK) 1/2 phosphorylation in *H. pylori*-infected AGS gastric epithelial cells. We find that *H. pylori*, via activation of the epidermal growth factor (EGF) receptor activates the small GTP-binding protein Ras, which in turn, mediates ERK1/2 phosphorylation. *cag*+ strains of *H. pylori* are able to induce greater EGF receptor activation than *cag*− strains, and studies with isogenic mutants indicate that an intact type IV bacterial secretion system is required for this effect. Blockade of EGF receptor activation using tyrophostin AG1478 prevents *H. pylori*-mediated Ras activation, inhibits ERK1/2 phosphorylation, and substantially decreases interleukin-8 gene expression and protein production. Investigations into the mechanism of EGF receptor activation, using heparin, a metalloproteinase inhibitor and neutralizing antibodies reveal that *H. pylori* transactivates the EGF receptor via activation of the endogenous ligand heparin-binding EGF-like growth factor. Transactivation of gastric epithelial cell EGF receptors may be instrumental in regulating both proliferative and inflammatory responses induced by *cag*+ *H. pylori* infection.

*Helicobacter pylori* is a pathogenic Gram-negative bacterium that colonizes human gastric mucosa. *H. pylori* infection has been connected etiologically to peptic ulcer disease (1), mucosa-associated lymphoid tissue lymphoma of the stomach (2), and gastric adenocarcinoma (3). *H. pylori* colonization invariably causes chronic active gastritis, a disease state characterized by neutrophil infiltration of the gastric mucosa and epithelial layer. Interaction of the bacterium with gastric epithelial cells leads to the production of chemokines, such as interleukin-8 (IL-8), which in turn causes activation and recruitment of neutrophils to the site of infection.

It is now known that attachment of *H. pylori* to gastric epithelial cells activates multiple signaling pathways that culminate in IL-8 gene transcription. Previous studies have shown that *H. pylori* is able to induce activation of the transcription factors NF-κB and AP-1, key regulators of many inflammatory genes including IL-8 (4–7). Moreover, we and others, have recently reported that infection of gastric epithelial cell lines with *H. pylori* results in the rapid activation of p38, JNK, and ERK1/2 mitogen-activated protein (MAP) kinases (6–8). However, the mechanisms whereby *H. pylori* are able to activate gastric epithelial cell MAP kinases are still unclear.

The ERK1/2 pathway has been linked to cellular proliferation and differentiation (9), however, our previous findings suggest these proteins also participate in proinflammatory cellular responses in gastric epithelial cells. By blocking upstream ERK1/2 phosphorylation using the MEK inhibitor PD98059 we were able to reduce the amount of IL-8 produced by AGS cells in response to *H. pylori* infection. Interestingly, this effect was not mediated via blockade of NF-κB activation (8). However, it has been demonstrated that *H. pylori*-mediated AP-1 activation is prevented by PD98059 (6).

The activity of ERK1/2 are generally regulated through the activation of cell surface receptors. One possible mechanism whereby *H. pylori* may modulate the ERK1/2 pathway is through the EGF receptor. The EGF receptor is a transmembrane receptor with intrinsic tyrosine kinase activity, known to regulate the ERK1/2 pathway, via activation of the small GTP-binding protein Ras. Numerous stimuli are known to cause transactivation of the EGF receptor, including Substance P (10), bradykinin (11), thrombin (12), insulin-like growth factor-1 (13), UV light (14), the dermonecrotic toxin produced by *Pasteurella multocida* (15) and the invasive bacterium *Salmonella typhimurium* (16). Here, we report that *H. pylori* is capable of inducing EGF receptor phosphorylation, which is, in part, responsible for ERK1/2 activation in *H. pylori*-infected AGS cells. In this study, we also examine the downstream effects of EGF receptor activation on IL-8 regulation by *H. pylori*, and explore the mechanism by which this bacterium induces EGF receptor phosphorylation.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Reagents**—AGS gastric epithelial cells (American Type Culture Collection, Rockville, MD) were grown in F-12 HAM medium (pH 7.4: Sigma-Aldrich) supplemented with 10% fetal bovine serum, 100 units/ml penicillin G sodium, and 100 μg/ml streptomycin sulfate. All cultures were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO₂. Cell culture experiments were carried...
H. pylori Induces EGF Receptor Phosphorylation

out using 100-mm dishes and 12- or 24-well polypropylene tissue culture plates (Corning Costar, Cambridge, MA). All experiments were carried out using confluent monolayers unless otherwise stated. In some experiments, cells were treated with AG1478 (Calbiochem, La Jolla, CA), batimastat (British Biochemical, Oxford, UK), U73122 (Bayer, Leverkusen, Germany), wortmannin or cyclohexamide (both from napoPharma, MN), heparin or cyclohexamide (both from Sigma-Aldrich).

H. pylori Clinical Isolates and Isogenic Mutants—H. pylori were plated onto Brucella agar supplemented with 5% horse blood (BBL, Becton Dickinson Microbiology, Cockeysville, MD) and incubated at 37 °C in a microaerophilic environment. After 3 days the bacteria were harvested into pyrogen-free Dulbecco’s PBS (Gibco, Mediatech, Herndon, VA). The bacteria were then pelleted by centrifugation at 4,000 × g for 10 min, and resuspended in antibiotic-free F-12 Ham’s medium. Unless otherwise stated experiments were performed using the cag−, vacuolating cytotoxin secreting H. pylori strain 43504 (American Type Culture Collection).

Isogenic H. pylori mutants lacking the pibC, cagA, or vacA genes were also studied together with their parental cag+ vacuolating cytotoxin secreting wild type strain (number 60190) (17). These strains plus the strain J44, were obtained from the culture collection of the Vanderbilt University Campylobacter and Helicobacter Laboratory (Nashville, TN) and have been described previously (17, 18). H. pylori-conditioned media were prepared by suspending bacteria in antibiotic-free medium for 3 h at 37 °C, pelleting at 4,000 × g for 10 min, and then filtering the medium through a 0.2-μm filter (Acrodisc; Gelman, Ann Arbor, MI).

Detection of EGF Receptor Phosphorylation—AGS gastric epithelial cells plated onto 100-mm dishes were serum-starved for 24 h prior to experiments. Cells were then incubated with H. pylori (1 × 106) bacteria for the indicated times. Monolayers were washed 5 times with ice-cold phosphate-buffered saline and lysed (45 min on ice) using 1 ml of lysis buffer. Lysis buffer contained 50 mM Tris-HCl, pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM NaF, 100 mM sodium orthovanadate, 100 μM phenylmethylsulfonyl fluoride, and a commercially available protease inhibitor mixture tablet (Complete; Roche Molecular Biochemical, Indianapolis, IN). Cells were then scraped, and transferred to Eppendorf tubes. Particulate material was removed by centrifugation, and the lysates collected. EGFR receptor immunoprecipitations were performed using 1 mg of lysate protein, which was incubated for 1 h at 4 °C with 2 μg of a monoclonal anti-EGF receptor antibody (Santa Cruz), followed by an overnight incubation at 4 °C with a 50-μl aliquot of Protein G Plus-agarose beads (Santa Cruz). The beads were washed three times with lysis buffer, and proteinase K was added by boiling for 5 min. SDS PAGE material was then digested with proteinase K and boiled for 5 min. The supernatant was then digested with 0.1% sodium dodecyl sulfate, and the supernatant was harvested and used for immunoblot analysis. EGFR immunoblot analysis was performed using the secondary antibody (Santa Cruz), and a chemiluminescence detection system.

EGF Receptor Immunoblot Analysis—AGS cells were grown on 12-well plates and maintained in serum-free medium for 24 h prior to the experiment. H. pylori were added in serum-free medium, and cultured for varying lengths of time. At the end of the experiment the monolayers were washed 3 times with phosphate-buffered saline and lysed with SDS sample buffer containing 62.5 mM Tris-HCl, pH 6.8, 2% w/v SDS, 10% glycerol, 50 mM dithiothreitol, 0.1% (w/v) bromphenol blue. Samples were then sonicated, heated to 100 °C for 5 min, and 20 μl of lysate loaded onto a 10% SDS-PAGE gel. After electrophoresis, the proteins were transferred onto nitrocellulose membranes and blocked for 1 h at room temperature with a 5% (w/v) solution of dried milk in Tris-buffered saline, pH 7.4, with 0.1% Tween 20 (TBS-T). This was followed by an overnight incubation at 4 °C with the phospho-specific MAP kinase antibodies diluted 1:1,000 in blocking buffer. The membranes were then washed 3 times with TBS-T, and incubated at room temperature for 1 h with peroxidase-conjugated goat anti-rabbit IgG (1:3000 dilution; Santa Cruz Biotechnology). A SuperSignal chemiluminescent substrate (Pierce, Rockford, IL) was used for detection.

A phospho-specific p44/p42 MAP kinase antibody was used to detect activated ERK1/2. This antibody detects p44 and p42 MAP kinase (ERK1 and ERK2) only when they are catalytically activated by phosphorylation at Thr180 and Tyr182. A phospho-specific p54/p46 MAP kinase antibody was used to detect JNK. This antibody detects p54 and p46 MAP kinase only when they are phosphorylated on Thr183 and Tyr185. All three antibodies were obtained from Cell Signaling Technology, Beverly, MA.

Preparation of an AGS Cell Line Stably Transfected with an IL-8 Reporter Gene—A 1,521-bp fragment containing nucleotides −1,481 to +40 of the promoter region of the IL-8 gene was cloned into the pGL2-basic luciferase expression vector between KpnI and HindIII restriction sites. The sequence was confirmed by DNA sequencing using primers specific for the pGL2-basic luciferase expression vector (GL primers 1 and 2; Promega Corp., Madison, WI). The IL-8 luciferase reporter gene and pcDNA3.1 vector (Invitrogen, Carlsbad, CA) expressing a genetin (G418) resistance gene were co-transfected into AGS gastric epithelial cells using LipofectAMINE according to the manufacturer’s instructions (Life Technologies, Gaithersburg, MD). AGS clones stably transfected with pcDNA3.1 were selected by adding G418 sulfate (400 μg/ml; Life Technologies) to the culture medium, and positive clones co-transfected with the IL-8 luciferase reporter were identified by their ability to respond to IL-1β (10 ng/ml; R&D Systems). The AGS clone identified as containing both the genetin resistance gene and the IL-8 luciferase reporter gene was subsequently maintained in G418 selection media.

IL-8 Enzyme-linked Immunosorbent Assay—IL-8 protein levels in AGS cell conditioned media were measured by enzyme-linked immunosorbent assay as previously described (14, 19, 20).

Statistical Analyses—Statistical analyses were performed using SigmaStat for windows version 2.0 (Jandel Scientific Software, San Rafael, CA). Unless stated otherwise, ANOVA followed by protected t tests were used for intergroup comparisons.

Ras Activation Assay—Ras activation was determined using a commercially available kit (Upstate Biotechnologies, Lake Placid, NY). Briefly, 1 mg of lysate protein from treated cells, preincubated with 20 μl of glutathione-agarose (Santa Cruz), for 30 min at 4 °C, was incubated for 30 min at 4 °C with 20 μl of Raf-1 RBD agarose. The beads were washed 3 times with lysis buffer and resuspended in 40 μl of PBS sample buffer, boiled for 5 min, and then loaded onto a 12% SDS-PAGE. Proteins were transferred to nitrocellulose and the membrane was stained for 1 h with 5% skim milk in phosphate-buffered saline (PBS) then visualized using an horseradish peroxidase-conjugated anti-mouse secondary antibody (Santa Cruz), and a chemiluminescence detection system.

Preparation of Ras-17N-expressing Retroviruses and Infection of AGS Cells—To construct a retroviral vector expressing the dominant negative Ras (Ras-17N), the human Ras-17N fragment was first excised from pZIP-Ras-17N by digestion with BspHI and BamHI. The resulting fragment was then ligated into the retroviral vector pMMP, digested with NcoI and BamHI. To generate Ras-17N-expressing retroviruses, 293T cells were seeded at 5 × 106 cells per 100-mm plate and incubated with 10 μl of 10% fetal bovine serum/Dulbecco’s modified Eagle’s medium for 24 h. The medium was replaced with fresh medium 4 h prior to transfection. The plasmids pMMP-gag-pol, pMDD-VSVG, and pMMP-Ras-17N were combined in a ratio of 3:1:4 and used to prepare transfection mixtures using Effectene Transfection Reagent (Qiagen Inc., Chatsworth, CA) according to the manufacturer’s instructions. Forty-eight h after transfection, the media were collected, filtered through 0.45-μm disc filters, and the supernatants were either used immediately or stored at −80 °C. A control retrovirus containing LacZ was generated in an identical manner. pMMP-LacZ, pMDD-gag-pol, and pMDD-VSVG were all kindly provided by Dr. Richard C. Mulligan (Children’s Hospital, Harvard Medical School, Boston, MA).

RESULTS

H. pylori Induces Phosphorylation of the EGF Receptor—To determine whether the EGF receptor is activated by H. pylori infection, lysates from AGS cells infected with H. pylori for 1 h were immunoprecipitated with a monoclonal anti-EGF receptor antibody. Western blot analysis of the immunoprecipitate using the phospho-specific EGF receptor antibody demonstrated the presence of a tyrosine-phosphorylated protein of approximately 170-kDa (Fig. 1A, upper panel). Pretreatment of the cells with the EGF receptor inhibitor tyrphostin AG1478 (1 μM) for 30 min was able to completely prevent H. pylori-mediated EGF receptor phosphorylation. To confirm that the immunoprecipitated protein was the EGF receptor the blot was rep-
**H. pylori Induces EGF Receptor Phosphorylation**

**Fig. 1. H. pylori promotes phosphorylation of the EGF receptor, which mediates ERK1/2 phosphorylation.** A, serum-starved confluent monolayers of AGS cells in 100-mm plates were infected with 1 × 10⁹ H. pylori cag+ strain 43504 for 1 h. Some AGS cells were pretreated with tyrphostin AG1478 (1 μM), a specific EGF receptor inhibitor, for 30 min prior to infection with H. pylori. The EGF receptor was immunoprecipitated (IP) with a monoclonal anti-EGF receptor antibody. The immunoprecipitated proteins were resolved on a 6% SDS-PAGE gel, and transferred to nitrocellulose. Phosphorylated EGF receptor (upper panel) was detected by Western blotting (WB) with an anti-phosphotyrosine monoclonal antibody (PY99). EGF receptor levels in the immunoprecipitates (lower panel) were assessed by reprobing with an anti-EGF receptor polyclonal antibody. B, serum-starved confluent monolayers of AGS cells in 12-well plates were pretreated with or without AG1478 at 1 μM for 30 min. The cells were then infected with 1 × 10⁹ H. pylori cag+ strain 43504 over a 30-min time course. Whole cell lysates were run on a 10% SDS-PAGE gel, and transferred to nitrocellulose. Levels of phosphorylated ERK1/2, p38, and JNK were assessed by Western blotting with phosphospecific antibodies. Blots were reprobed with control ERK1/2, p38, and JNK antibodies to demonstrate equal loading.

**H. pylori Activated IL-8 Gene Expression and Protein Production Is Down-regulated by Blockade of EGF Receptor Phosphorylation**—Previously, we demonstrated that activation of the ERK1/2 pathway by H. pylori is one of the mechanisms whereby the bacterium is able to regulate cytokine production (8). We hypothesized that blockade of the EGF receptor as an upstream activator of the ERK1/2 pathway may have functional effects on IL-8 gene regulation. To investigate this hypothesis, we used AGS cells stably transfected with an IL-8 luciferase reporter gene. As shown in Fig. 2A, treatment of these cells with H. pylori for 4 h caused a 30-fold increase in IL-8 luciferase reporter activity. However, reporter activity was reduced to ~22-fold when EGF receptor phosphorylation was prevented by treatment with AG1478 (1 μM). Consistent with our transcription data, there was a dramatic up-regulation of IL-8 protein production by AGS cells in response to infection with H. pylori for a 4-h period, which was reduced dose dependently when the cells were pretreated with the EGF receptor inhibitor AG1478 (Fig. 2B).

**H. pylori-mediated Activation of the EGF Receptor in AGS Gastric Epithelial Cells Is Dependent on cag Status**—We have previously observed that both cag+ and cag− strains of H. pylori were capable of activating the ERK1/2 pathway, but differ in the degree of activation (8). The ERK1/2 phosphorylation seen in AGS cells infected with cag− H. pylori was considerably reduced compared with those infected with cag+ strains. Therefore, we next examined whether activation of the EGF receptor was also dependent on the cag status of the bacteria. Fig. 3 shows the level of EGF receptor tyrosine phosphorylation when cells were infected with either a cag+ strain (43504) or cag− strain (J44) of H. pylori over a 4-h time course. We find that the cag− strain induces a relatively weak activation of the EGF receptor, as compared with the more substantial activation seen with cag+ strain. These data appear to correlate with the ability of the cag+ and cag− H. pylori to...
induce phosphorylation of ERK1/2, also shown in Fig. 3.

**EGF Receptor Activation by H. pylori Is Dependent Upon an Intact cag Secretion System, But Is Independent of the cagA and vacA Gene Products**—Having found a distinct difference between cag+ and cag− H. pylori in their ability to induce activation of the EGF receptor, we next examined whether isogenic mutants of H. pylori could induce the same response as a wild type cag+ strain (60190). As demonstrated in Fig. 4, phosphorylation of the EGF receptor by the picB− mutant was considerably less than for the isogenic wild type H. pylori strain. In contrast, the cagA− mutant induced a similar activation of the EGF receptor when compared with the wild type strain. These data suggest that full activation of the EGF receptor by H. pylori requires an intact type IV secretory apparatus, but not the translocation of the CagA protein into the host cell.

Since it has been reported that culture supernatant from vacA+ H. pylori was able to cause dephosphorylation of the EGF receptor (21), we also investigated the effect of a vacA− mutant of H. pylori on gastric epithelial cell EGF receptor phosphorylation. As shown in Fig. 4, the absence of the vacA gene had no evident effect on the ability of the bacteria to induce EGF receptor phosphorylation, as compared with the vacA+ wild type strain.

**H. pylori Causes Ras Activation That Is Prevented by the EGF Receptor Inhibitor AG1478**—Activation of the EGF receptor, as well as other cell surface receptors, stimulates the exchange of GDP for GTP on the small G protein Ras. Once in the active state, Ras can interact with several effector proteins such as Raf and phosphatidylinositol 3-kinase. Active Raf stimulates MEK, which in turn leads to the phosphorylation of ERK1/2.

To further delineate the mechanism leading to ERK1/2 phosphorylation, we investigated whether Ras becomes activated following H. pylori infection of AGS cells, and whether we could prevent this activation using AG1478. Our results (Fig. 5A, upper panel) show a marked activation of Ras, at 30 min, which is inhibited by pretreatment of the cells with AG1478 (1 μM). EGF (10 ng/ml), included as positive control, was found to stimulate Ras activation after 10 min. EGF activation of Ras was also prevented by pretreatment with AG1478. Whole cell lysates from these experiments were also probed for phospho-specific and control ERK1/2 to examine the correlation between Ras activation and ERK1/2 phosphorylation and to demonstrate equivalent levels of protein loading for the Ras assay (Fig. 5A, middle and bottom panels, respectively). Interestingly, EGF-mediated ERK1/2 phosphorylation was completely prevented with AG1478, whereas H. pylori activated phospho-ERK1/2 levels, although significantly reduced after AG1478 treatment, were still detectable. This finding suggests that there may be more than one mechanism involved in H. pylori activation of the ERK1/2 pathway.

**Dominant Negative Ras Inhibits H. pylori-mediated ERK1/2 Phosphorylation**—We next examined whether Ras activation by H. pylori was upstream of ERK1/2 phosphorylation. To do this we overexpressed dominant negative Ras in AGS cells using retroviral transfection, and examined ERK1/2 phosphorylation in response to H. pylori infection. As shown in Fig. 5B, ERK1/2 phosphorylation is absent in untreated control cells. However, H. pylori and EGF (10 ng/ml) each caused a strong induction of ERK1/2 phosphorylation in the LacZ (vector control)-transfected cells. EGF activation of ERK1/2 was almost completely abrogated in cells over expressing dominant negative Ras, whereas H. pylori-mediated ERK1/2 activation was only partially inhibited. These findings provide further evidence that ERK1/2 activation by H. pylori can be mediated via both Ras-dependent and Ras-independent pathways.

**H. pylori-conditioned Medium Is Unable to Cause EGF Receptor Phosphorylation**—To examine whether soluble factors secreted by H. pylori were responsible for phosphorylation of the EGF receptor, we incubated AGS cells with both whole bacteria or conditioned medium for 1 h. H. pylori-conditioned medium was unable to induce EGF receptor phosphorylation (Fig. 6), suggesting that contact between the bacterium and the host cell is necessary for induction of EGF receptor phosphorylation. These data agree with our previous finding, that H. pylori-conditioned medium is unable to induce ERK1/2 phosphorylation (8).

**H. pylori-mediated EGF Receptor Phosphorylation Is Prevented by Treatment with Heparin**—Previous studies have shown that, in a number of systems where the EGF receptor becomes transactivated, the underlying mechanism involves
cleavage of membrane-bound pro-HB-EGF (22). Cleaved HB-EGF then binds the EGF receptor leading to its phosphorylation. HB-EGF requires heparan sulfate proteoglycans as coreceptors of the EGF receptor and the addition of heparin has been shown to compete with heparan sulfate proteoglycans for HB-EGF binding (23). We therefore infected AGS cells with H. pylori in the presence of heparin (100 μg/ml). As shown in Fig. 6, heparin markedly inhibited H. pylori-mediated EGF receptor phosphorylation.

**HB-EGF Neutralizing Antibodies Prevent H. pylori-mediated EGF Receptor Phosphorylation, and Reduce ERK1/2 Phosphorylation**—The data resulting from the heparin co-culture experiments led us to examine whether HB-EGF, an endogenous ligand of the EGF receptor, was mediating EGF receptor activation in H. pylori-infected AGS cells. In support of this hypothesis we found that a HB-EGF neutralizing antibody (25 μg/ml) almost completely blocked H. pylori-mediated activation of the EGF receptor (Fig. 7A). The HB-EGF neutralizing antibody was also found to cause a substantial (48.5% by densitometry) reduction in H. pylori-activated ERK1/2 phosphorylation (Fig. 7B).

**Batimastat, a Metalloproteinase Inhibitor, Prevents EGF Receptor Activation and Reduces the ERK1/2 Phosphorylation Induced by H. pylori—Shedding of HB-EGF and other EGF receptor ligands has previously been reported to be dependent on the action of matrix metalloproteinases. We explored the possibility that H. pylori may be activating these proteases, and thus cause the shedding of the soluble form of HB-EGF. We investigated this by treating the cells with batimastat (5 μg/ml), a broad spectrum matrix metalloproteinase inhibitor. As shown in Fig. 8A, we found that batimastat could prevent EGF receptor phosphorylation by H. pylori. In addition, we found that this inhibitor could also markedly reduce ERK1/2 phosphorylation induced by H. pylori (Fig. 8B).**

**DISCUSSION**

We and others have previously reported that H. pylori activates a number of MAP kinases in gastric epithelial cell lines (6–8, 24). Activation of these pathways plays a key role in up-regulating the expression of the proinflammatory cytokine IL-8 (8). However, the exact mechanisms whereby H. pylori activates these signaling pathways are still unknown. We now...
report that *H. pylori* can induce phosphorylation of the EGF receptor in AGS gastric epithelial cells. *H. pylori*-induced EGF receptor phosphorylation leads to activation of Ras, which is able to mediate ERK1/2 phosphorylation, which in turn up-regulates IL-8 gene expression and protein production. We find that EGF receptor phosphorylation mediated by *H. pylori* is dependent upon an intact type IV bacterial secretory system.

Moreover, we find that the mechanism underlying the induction of EGF receptor phosphorylation by *H. pylori* involves activation of the EGF receptor ligand HB-EGF.

One important finding of our study is that there is a differential activation of the EGF receptor depending upon whether the *H. pylori* strain processes a 40-kb region of genes known as the *cag* pathogenicity island. The *cag* pathogenicity island encodes for ~30 proteins, that based on sequence homology appear to constitute a type IV bacterial secretion system (25). It has previously been reported that the absence of the whole
pathogenicity island or deletions of individual cag genes result in an impaired ability to activate NF-κB, AP-1, and MAP kinase pathways (6–8, 26). We have found that the picB–(cagE–) isogenic mutant of H. pylori is able to mediate a much weaker induction of EGF receptor phosphorylation compared with its parental wild type strain. These data suggest that the H. pylori type IV secretion system participates in host cell EGF receptor activation.

We also examined whether the CagA protein produced by H. pylori could be involved in EGF receptor activation. To date, CagA is the only H. pylori protein known to translocate from the bacterium into the cell via the type IV secretion system; HB-EGF release, which in turn activates EGF receptor and ERK1/2 phosphorylation, but via a Ras independent pathway (24). We have found that H. pylori induced phosphorylation of the EGF receptor is mediated through the release of EGF receptor ligand: HB-EGF. Previous studies have also examined the association between EGF receptor ligands and H. pylori. Romano et al. (30) reported that by incubating suspensions of H. pylori or conditioned broth with MKN-28 cells for several hours they were able to up-regulate both amphiregulin and HB-EGF mRNA levels. We find that rapid activation of the EGF receptor by H. pylori (within 15 min) is not mediated through the release of soluble bacterial factors, but instead appears to be contact dependent. Rapid release of HB-EGF from the cell is typically a result of post-translational modifications of membrane-bound HB-EGF, and does not appear to be mediated via production of newly synthesized proteins. Furthermore, replenishment of transmembrane HB-EGF requires 12–24 h (23). As our system examined the acute interaction between H. pylori and gastric epithelial cells we cannot rule out the possibility that soluble factors secreted by the bacteria may play a role in up-regulating EGF receptor ligand production during the later stages of infection.

Transactivation of the EGF receptor by numerous G-protein-coupled receptors has now been identified as a critical element in G-protein-coupled receptor-induced mitogenic signaling (13, 22, 31–33). The mechanism for activation of the EGF receptor under these circumstances appears to be through matrix metalloproteinases. Matrix metalloproteinases such as MMP-3, once activated, are able to cleave the membrane-bound precursor of HB-EGF causing shedding of its mature, soluble form, which in turn engages and activates the EGF receptor, and thus activates the MAP kinase cascade (22). We now report the novel finding that H. pylori-induced EGF receptor phosphorylation is mediated through HB-EGF, and is prevented by the metalloproteinase inhibitor batimastat. Engagement of the host gastric epithelial cell by H. pylori appears to induce HB-EGF release, which in turn activates EGF receptor and ERK1/2 signaling. We speculate that H. pylori may cause transactivation of the EGF receptor by interacting with a receptor already expressed on the surface of the gastric epithelial cell, or through insertion of bacterial proteins into the host cell. HB-EGF-mediated transactivation of the EGF receptor may be instrumental in inducing the augmented proliferative and inflammatory responses seen in infection of the gastric epithelium by cag+ H. pylori.

REFERENCES
1. Kuipers, E. J., Thijss, J. C., and Festen, H. P. (1995) Aliment. Pharmacol. Ther. 9, Suppl. 2, 59–69
2. Wodarsper, A. C., Ortiz-Hidalgo, C., Falzon, M. R., and Isaacsen, P. G. (1991) Lancet 338, 1175–1176
3. Huang, J. Q., Sridhar, S., Chen, Y., and Hunt, R. H. (1996) Gastroenterology 114, 1169–1179
