

Activation of IL-8 Gene Expression by *Helicobacter pylori* Is Regulated by Transcription Factor Nuclear Factor- κ B in Gastric Epithelial Cells¹

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In vivo, gastric infection with *Helicobacter pylori* leads to substantial production of the inflammatory cytokines IL-1, IL-6, TNF- α , and IL-8. *H. pylori* strains that contain the *cag* pathogenicity island (*cag*⁺) and are associated with ulceration and gastric carcinoma induce greater cytokine production than *cag*⁻ strains. Expression of these cytokines is often regulated by the transcription factor complex, nuclear factor- κ B (NF- κ B) through κ B-binding elements in the enhancer/promoter regions of their genes. We report that more virulent *cag*⁺ *H. pylori* strains induce increased NF- κ B-DNA binding activity, which elevates IL-8 expression in AGS gastric epithelial cells. The *cag*⁺ *H. pylori* strains induce significant stimulation of IL-8 promoter-driven reporter activity, while *cag*⁻ strains do not. Furthermore, mutation of specific genes within the *cag* island (*picA1* and *picB*) ablates enhanced NF- κ B activation and IL-8 transcription. Increased IL-8 expression is inhibited by mutation in either the NF- κ B or NF-IL-6 binding element. The *cag*⁺ strains, compared with the *cag*⁻ strains, induce enhanced nuclear localization of a RelA-containing NF- κ B binding complex, but no increase in NF-IL-6 binding activity. These studies demonstrate that the ability of different types of *H. pylori* strains to activate NF- κ B correlates with their ability to induce IL-8 transcription and indicate a mechanism for the heightened inflammatory response seen in subjects infected with *cag*⁺ *H. pylori* strains. *The Journal of Immunology*, 1998, 160: 2401–2407.

Infection with the Gram-negative bacterium *Helicobacter pylori* leads to diverse clinical and pathologic outcomes in humans, including chronic superficial gastritis, duodenal or gastric ulceration, and adenocarcinoma of the stomach (1–6). One hypothesis is that infection with specific *H. pylori* strains that possess the *cag* pathogenicity island (*cag*⁺) causes peptic ulceration and gastric carcinoma (7–9). Recent studies reveal that the *cag* region is comprised of approximately 40 kb of DNA that is absent in *cag*⁻ strains (10). An 87-kDa secreted cytotoxin (10, 11) and the *picB* gene product, both of which are usually expressed by *cag*⁺ strains, have been implicated in *H. pylori* pathogenesis (10–16). The cytotoxin produces vacuolation of cells (11, 17), whereas the *picB* product is linked to inflammatory processes (10, 16). The *picB* product is homologous to proteins known to be involved in secretion from bacterial cells (10, 16), and other homologues of this pathway have been identified in the *cag* island (10).

Studies of gastric biopsies from patients infected with *H. pylori* indicate that *cag*⁺ strains induce significantly more IL-1 α , IL-1 β , and IL-8 than do *cag*⁻ strains (18). IL-8, a potent T cell and neutrophil chemoattractant and activating agent, is considered an important factor in the pathogenesis of inflammatory diseases (19).

Consistent with the in vivo observations, *cag*⁺ *H. pylori* strains induce significantly greater IL-8 mRNA and protein in gastric epithelial cells in vitro than do *cag*⁻ strains (20, 21). The *H. pylori* *picB* gene (also called *cagE*) product is required for IL-8 expression, since its mutation abrogates this response (10, 16). Recent studies indicate that mutation of homologues of other genes in the secretory pathway ablates IL-8 secretion (10).

However, little is known about the early events following host-bacterium interactions that influence the course of *H. pylori* colonization. Signals generated in response to infection, stress, and injury activate nuclear factor- κ B (NF- κ B),³ a ubiquitous transcription factor complex belonging to the Rel family of proteins (22–25). In most cells, except mature B cells, macrophages, and some neurons, NF- κ B complexes are sequestered in the cytoplasm by inhibitory molecules, termed I κ Bs (24, 27). Upon stimulation of cells, specific I κ Bs are degraded, and certain NF- κ B factors are stimulated to enter the nucleus, bind its specific cognate DNA site, and subsequently regulate gene transcription (22, 27). In most cells, activation of NF- κ B is critical for the inducible expression of the proinflammatory response genes, including cytokines IL-1 β , IL-6, IL-8, and TNF- α (24, 27). IL-1 and TNF- α also activate NF- κ B to initiate an autoregulatory pathway that is thought to be responsible for increasing the magnitude of the inflammatory response (23). The translocation of Rel proteins is induced by bacteria and viral pathogens, immune and inflammatory cytokines, and cytotoxic agents (23, 24, 27). NF- κ B activity is induced in HeLa cells by *Shigella flexneri* (26) and in macrophages by *Staphylococcus aureus* and several of its exotoxins (27). There also is enhanced nuclear transport of p50/RelA NF- κ B components in macrophages before *Listeria monocytogenes* invasion (28).

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³ Abbreviations used in this paper: NF- κ B, nuclear factor- κ B; AP-1, activating protein-1; wt, wild type; CAT, chloramphenicol acetyltransferase; EMSA, electrophoretic mobility shift assay.

Activation of NF- κ B is the most crucial step for IL-8 gene transcription in most cells, but C/EBP-like (NF-IL-6), and AP-1 binding sites also are required for IL-8 transcriptional activation by IL-1 or TNF- α (29). Synergistic interactions between NF- κ B and NF-IL-6 may play an important role in the expression of the acute phase response genes, especially in the transcription of IL-6 and IL-8 genes (30, 31). Depending on the cell line, co-operation between NF- κ B and either NF-IL-6 or AP-1 is sufficient for IL-8 gene activation (29). NF-IL-6 may be the primary co-operator, and in its absence or when the NF-IL-6 site is mutated, an interaction between NF- κ B and AP-1 occurs (29).

In this study we investigated the molecular mechanisms by which *H. pylori* activates IL-8 gene expression in gastric AGS epithelial cells. We show that both NF- κ B and NF-IL-6 elements are essential for activation of IL-8 gene expression by *H. pylori*. On exposure to *cag*⁺ *H. pylori* strains, gastric epithelial cells exhibit increased RelA-containing NF- κ B activity, which elevates IL-8 expression and thus contributes to the heightened inflammatory response seen in patients infected with these strains (18).

Materials and Methods

Bacterial strains and growth conditions

The *H. pylori* clinical isolates used in this study were from the culture collection of the Vanderbilt University *Campylobacter/Helicobacter* Lab (Nashville, TN). Wild-type strains 88-23 (60190) and N6 express the vacuolating cytotoxin (*tox*⁺) in vitro and possess the *cag* region, including *cagA* and upstream genes *picA1*, *picA2*, and *picB* (16, 32); we recently found that the *picA* reported previously (32) actually consists of two overlapping open reading frames, now termed *picA1* and *picA2*. Strains 88-22 (Tx-30a) and 87-203 are wild-type isolates that do not possess the *cag* region and do not express detectable cytotoxin activity (*tox*⁻) in vitro (32). Isogenic *cagA*, *picA1* or *picB* mutants had been prepared by insertion of a kanamycin resistance gene within the *cagA*, *picA1* or *picB* loci, respectively, in *H. pylori* strains 88-23, as previously described (16, 32). All *H. pylori* isolates were cultured on 5% sheep blood agar plates in a microaerobic atmosphere (generated by Campy Pak-Plus, BBL Microbiology Systems, Cockeysville, MD) at 37°C for 24 to 48 h. For all incubations with cell cultures, the bacterial cultures were harvested in PBS (pH 7.4) and resuspended to yield a concentration of 5×10^{10} CFU/ml. Stock cultures were maintained at -70°C in brucella broth (BBL Microbiology Systems) supplemented with 15% glycerol.

DNA constructs

The pIL-8(wt)/CAT, pIL-8(- κ B)/CAT, and pIL-8(-5'NF)/CAT were provided by Dr. Charles Kunsch and have been described previously (30). Briefly, in these reporter constructs, chloramphenicol acetyltransferase (CAT) expression is under control of the human IL-8 genomic sequence from -420 to +101 bp. Specific substitution mutations were introduced into the IL-8 promoter region to disrupt the NF- κ B binding site (designated pIL-8(- κ B)/CAT) and a 5' NF-IL-6 binding site (designated pIL-8(-5'NF)/CAT) located between -92 and -71 bp (30).

Cell culture and transient transfection

Human gastric cancer cell line AGS (CRL 1739) obtained from American Type Culture Collection (Rockville, MD) was grown in DMEM containing 5% FCS (HyClone Laboratory, Logan, UT), gentamicin (20 mg/l), and L-glutamine (2 mM) in a humidified incubator containing 5% CO₂, as previously described (21). For transient transfection assays, AGS epithelial cells were grown in individual 100-mm³ culture plates to ~60% confluence, at which time 10 μ g of the CAT reporter constructs under control of the IL-8 promoter were transfected by the calcium phosphate/DNA coprecipitation procedure (33, 34). These constructs were cotransfected with 1 μ g of Rous sarcoma virus- β -galactosidase construct for determination of transfection efficiency. The transfected AGS cells then were incubated in 0.5% FCS medium without antibiotics for 24 h in the absence or the presence of TNF- α (10³ U/ml) or *H. pylori* cells at a concentration of 10⁹/ml (cell to bacteria ratio of 1:1000), as described previously (21). AGS cells were collected by scraping, suspended in 100 μ l of 0.25 M Tris-HCl (pH 8.0), and lysed by four repeated freeze-thaw cycles. Equal amounts of protein from different cell extracts were assayed for CAT activity, as previously described (35). Each experiment was performed four times with at least two different plasmid preparations.

Nuclear extract preparation

Nuclear extracts from AGS cells were prepared as previously described (36). After the treatment indicated, cells were scraped, centrifuged, and washed in cold Tris-buffered saline (pH 7.9), and then resuspended in 400 μ l of cold buffer A (10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 1 mM aprotinin, 14 mM leupeptin, 1 mM pepstatin, and 80 μ g of benzamide/ml). After the cells were allowed to swell on ice for 15 min, they were lysed by vigorous vortexing for 10 s in the presence of Nonidet P-40 (final concentration, 1.0%). The homogenate was centrifuged for 30 s at 13,000 \times g, and the nuclear pellet was resuspended in 50 μ l of cold buffer C (20 mM HEPES (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 1 mM aprotinin, 14 mM leupeptin, 1 mM pepstatin, and 80 μ g of benzamide/ml). This suspension was agitated at 4°C for 15 min with a Vortex-Genie 2 (Scientific Industries, Inc., Bohemia, NY) with a multiple sample head at setting 5, followed by microcentrifugation for 5 min at 4°C. The resulting supernatant was stored in small aliquots at -80°C. Protein concentrations were determined as previously described (37).

Preparation of radiolabeled DNA probes and electrophoretic mobility shift assay (EMSA)

EMSAs were performed as previously described (38). All oligonucleotides were synthesized by the Vanderbilt University DNA Core. Double-stranded oligonucleotide- κ B (5'-CGTGGAAATTCCTCTG-3', -83 to -68 bp) and wild-type CEBP/NF-IL-6 (5'-CATCAGTTGCAAATCGTGG-3', -97 to -79 bp) binding sites from the IL-8 promoter were end labeled with [α -³²P]dATP (6000 Ci/mmol) using the Klenow fragment of *Escherichia coli* DNA polymerase I (Pharmacia, Piscataway, NJ), were used as probes. The binding reactions (38) were performed on ice in a volume of 25 μ l and contained 5 ml of 5 \times binding buffer (50 mM Tris-HCl (pH 7.9), 250 mM NaCl, 2.5 mM EDTA, 50% glycerol, and 5 mM DTT), 5 to 10 μ g of nuclear proteins, and 2.0 μ g of poly(dI-dC) (Boehringer Mannheim, Indianapolis, IN). After 30 min on ice, 30,000 cpm of ³²P-labeled DNA probe was added to the reaction mixture for 30 min at ambient temperature. The DNA-protein complexes were separated on native 4% polyacrylamide gels (prerun at 150 V for 1.5 h) in Tris glycine buffer at 150 V for 2 to 4 h at ambient temperature. After electrophoresis was performed, the gels were dried and exposed for autoradiography at -70°C for 24 to 36 h. In competition studies, a 25-fold molar excess of unlabeled wild-type oligonucleotides or oligonucleotides containing mutant NF- κ B (5'-CGTtaAcTTTCCTCTG) or CEBP/NF-IL-6 (5'-CATagcT TGCAAATCGTGG-3') binding sites was included in the reaction mixture along with the radiolabeled probe. For supershift experiments, affinity-purified rabbit Abs (2 μ g/reaction) to p50, RelA, c-Rel, or p52 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) were included in the standard reaction mixture and incubated on ice for 30 min before the labeled oligonucleotides were added.

Results

Activation of IL-8 gene transcription by *H. pylori* is mediated by NF- κ B and NF-IL-6 in AGS epithelial cells

Molecular analysis of the 5' flanking region of the IL-8 gene has revealed that putative C/EBP, NF- κ B, and AP-1 binding sites are important in regulating IL-8 gene transcription (29, 30, 39). Sequences from -94 and -71 bp of the IL-8 promoter consisting of NF- κ B and NF-IL-6 binding sites are essential for responsiveness to IL-1 or TNF- α in human fibrosarcoma 8387 cells and glioblastoma T98G cells (29), whereas in Jurkat T cells, both are required for maximal IL-8 transcriptional activation by phorbol ester and TNF- α (30). Since *H. pylori* induces IL-8 mRNA and protein levels in gastric epithelial cells (20, 21, 40), we examined whether this induction is mediated at the level of transcription. To this end, human gastric AGS epithelial cells were transiently transfected with a wild-type IL-8 promoter/CAT reporter gene construct (pIL-8(wt)/CAT, which spans from -420 to +101 bp of the IL-8 gene) and then stimulated with live cells of *cag*⁺ *H. pylori* strain 88-23 or with TNF- α as a positive control. Assay of CAT activity revealed 10.8- and 4.5-fold increases in response to TNF- α and *H. pylori* stimulation, respectively (Fig. 1). These results indicated that *H. pylori* induces IL-8 expression in AGS cells at the level of transcription.

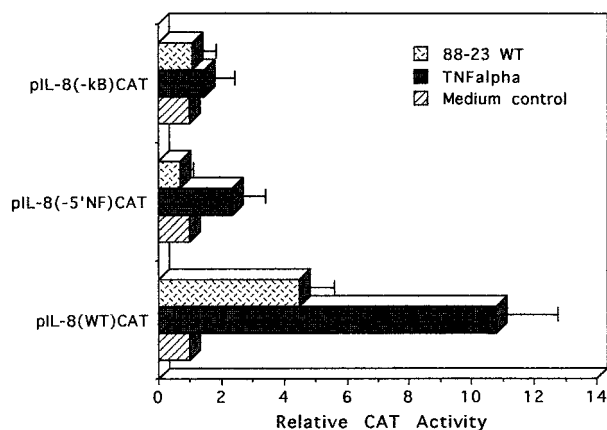


FIGURE 1. NF- κ B and NF-IL-6 binding sites are required for *H. pylori*- and TNF- α -mediated induction of IL-8 expression in AGS epithelial cells. AGS cells were transfected with 10 μ g of the indicated reporter plasmids for 24 h and were treated in the absence of antibiotics with 0.5% FCS medium alone or 0.5% FCS medium containing either live *H. pylori* cells or 10^3 U of TNF- α for an additional 24 h before samples for CAT assays were harvested. The CAT activity from the reporter plasmid was normalized to the β -galactosidase activity from the cotransfected Rous sarcoma virus- β -galactosidase expression plasmid. The results are presented relative to the use of FCS medium control alone, as the mean \pm SE of four experiments.

To assess whether the NF- κ B and/or the NF-IL-6 binding sequences in the IL-8 promoter may contribute to the stimulatory activities by *H. pylori*, transient transfections with mutant IL-8 promoter/CAT reporter gene constructs with specific mutations in either the NF- κ B (designated pIL-8(- κ B)CAT) or the NF-IL-6 (designated pIL-8(-5' NF)CAT) binding site (30) were performed in parallel studies (Fig. 1). Mutation in either the 5' NF-IL-6 site or the NF- κ B site in the IL-8 promoter significantly reduced TNF- α -mediated CAT activity in gastric cells and essentially abolished activation by *H. pylori*. These experiments indicated that activation of the IL-8 promoter in gastric AGS epithelial cells in response to *H. pylori* stimulation requires intact binding sites for both the NF- κ B and the NF-IL-6 elements.

H. pylori pic gene products are required for induction of IL-8 promoter activity in AGS epithelial cells

Since recent studies indicated that in AGS epithelial cells, the *pic* gene products were essential for the enhanced IL-8 mRNA and protein levels induced by *cag*⁺ strains (16), we sought to determine whether these products also are essential for *H. pylori*-induced IL-8 promoter activity in AGS cells. Inducible activation of IL-8 promoter activity was assayed in AGS cells by transient transfection analysis, as described above. After stimulation with the *cag*⁺ *H. pylori* strain 88-23, there was a 5.2-fold increase in CAT activity (Fig. 2). Isogenic *picA1* and *picB* mutants of *cag*⁺ strain 88-23 and the wild-type *cag*⁻ strain 88-22 did not induce IL-8 promoter activity. An isogenic *cagA*⁻ mutant of strain 88-23 induced IL-8 promoter activity, but at a reduced level. These results indicate that the *H. pylori* *pic* gene products are essential for induction of IL-8 promoter activity in AGS cells, which parallels the previously reported IL-8 mRNA and protein responses (16).

Stimulation with *H. pylori* induces a single predominant κ B binding complex, while NF-IL-6 is constitutively expressed in AGS cells

The transient transfection analysis experiments with the wild-type and mutant IL-8 promoter/CAT reporter gene constructs indicate

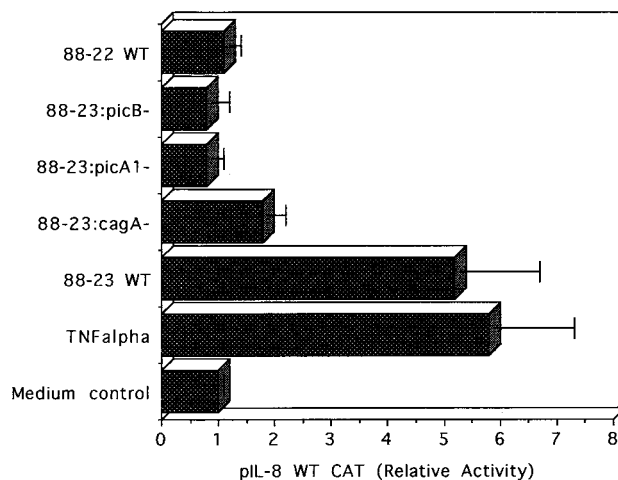


FIGURE 2. Products of the *cag* pathogenicity island are required for induction of IL-8 expression in AGS epithelial cells by *cag*⁺ *H. pylori* cells. AGS cells were transfected with 10 μ g of the pIL-8 WT CAT plasmid for 24 h. Samples were treated with 0.5% FCS medium alone (medium control) or with 0.5% FCS medium containing either *H. pylori* cells or 10^3 U of TNF- α . AGS cell lysates were prepared 24 h after stimulation and analyzed for β -galactosidase and CAT activities. The CAT activity results are presented as described in Figure 1, representing a mean of four transfections.

that both NF-IL-6 and NF- κ B sites are essential for *H. pylori* and also for TNF- α -mediated activation of the IL-8 promoter in gastric AGS epithelial cells. To further determine whether the increase in IL-8 expression seen in *H. pylori*-stimulated gastric epithelial cells was related to alteration in NF- κ B or NF-IL-6 binding activity in the nuclei, nuclear extracts from AGS cell monolayers were prepared, and EMSAs were performed using as probes radiolabeled oligonucleotides representing wild-type IL-8 promoter NF- κ B and NF-IL-6 element sequences. A single predominant κ B binding complex (absent in unstimulated cultures; Fig. 3, lane 1) was induced in AGS cells after stimulation with live *H. pylori* wild-type strain 88-23 (lane 2). The specificity of this inducible κ B-binding factor was confirmed by competition analysis with unlabeled wild-type and mutant oligonucleotides. Rel/NF- κ B proteins appeared to mediate this *H. pylori*-induced binding, since unlabeled wild-type (lane 3), but not mutant κ B oligonucleotides (lane 4) effectively competed with the induced NF- κ B complex. NF-IL-6 binding was constitutively present in nuclei from unstimulated cultures, appearing as two bands on EMSA (lane 5). Stimulation with *H. pylori* did not alter this binding (lane 6).

To understand further the mechanism of NF- κ B activation, we studied the kinetics of *H. pylori*-induced NF- κ B binding in AGS epithelial cells by EMSA. A single DNA-protein complex was induced in nuclear extracts prepared from stimulated AGS cells 1 h after coculture with *H. pylori* strain 88-23, and similar κ B-DNA binding activity levels were maintained through 4 h (Fig. 4). To identify the subunit composition of the induced κ B-DNA binding complex, Abs to Rel family members, Rel A, c-Rel, p50, and p52, were preincubated with the nuclear extracts from AGS cells and used in supershift analysis (Fig. 5). As expected, nuclear extracts from AGS cells cocultured with strain 88-23 showed significant stimulation of κ B binding activity (Fig. 5, lane 2) compared with unstimulated cells (lane 1). Anti-RelA Ab specifically recognized this stimulated κ B-DNA binding complex, resulting in supershifted complexes (lane 3). Preincubation with anti-c-Rel or anti-p52 Ab did not recognize any of the κ B binding complexes (lanes

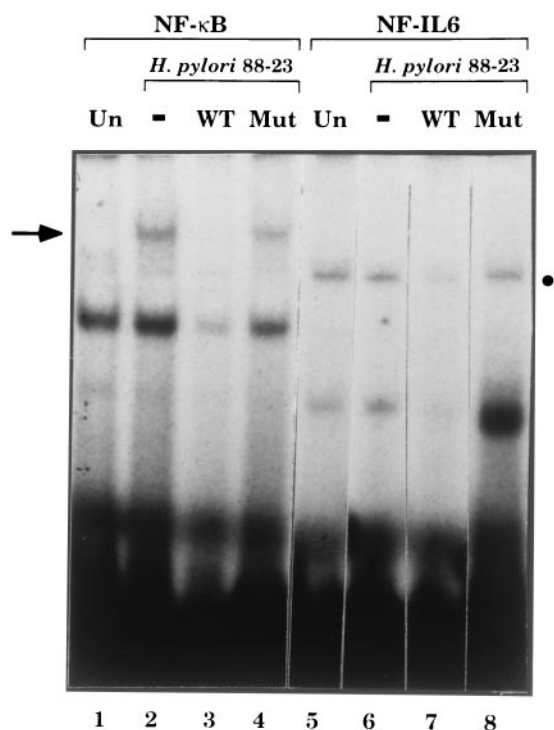


FIGURE 3. Live cells of *H. pylori* wild-type strain 88-23 induce a prominent NF- κ B binding complex, whereas the NF-IL-6 binding complex is constitutively expressed in AGS cells. Nuclear extracts containing equal protein concentrations from AGS cells unstimulated or stimulated with *H. pylori* strain 88-23 were analyzed for NF- κ B or NF-IL-6 binding activity by gel-shift assays. The NF- κ B probe (lanes 1–4) or the NF-IL-6 probe (lanes 5–8) was incubated with nuclear extracts from unstimulated (lanes 1 and 5) or *H. pylori*-stimulated AGS cells alone (lanes 2–4 and 6–8), without (lanes 2 and 6) or with a 25-fold excess of cold wild-type probe (lanes 3 and 7) or a 25-fold excess of mutant probe (lanes 4 and 8). The arrow indicates the location of induced κ B binding complex, and the dot indicates the specific NF-IL-6 binding complex.

4 and 5), but preincubation with anti-p50 Ab resulted in the formation of a very faint supershifted complex (lane 6). These results indicate that in AGS cells, a RelA NF- κ B binding complex is rapidly induced in response to *H. pylori* infection.

The pic gene products expressed by cag⁺ H. pylori strains are essential for induction of NF- κ B DNA binding activity in epithelial cells

Since we (21) and others (20) have previously shown that *cag*⁺ strains induce significantly more IL-8 mRNA and protein than do *cag*[−] wild-type *H. pylori* strains, we sought to further ascertain whether that difference was related to NF- κ B activation (Fig. 6). Markedly increased RelA-NF- κ B-DNA binding activity was induced by wild-type *cag*⁺ *H. pylori* strains (lanes 2–5) compared with the wild-type *cag*[−] strains (lanes 6–9). To determine whether the observed difference in κ B-DNA binding activity was specific to the *cag* region, we next examined a wild-type *cag*⁺ *H. pylori* strain (88-23) and isogenic *cagA*, *picA1* or *picB* mutants. From previous studies, we know that the *picA1* mutation has a polar effect on *picB*. As expected, stimulation with the wild-type strain 88-23 induced a predominant NF- κ B binding complex in AGS cells that was not seen in unstimulated cells (Fig. 7, lanes 1 and 2). The isogenic mutants that lacked expression of either *picB* or *picA1* did not induce κ B-DNA binding activity (lanes 4 and 5). The isogenic *cagA*[−] mutant induced κ B-DNA binding activity (lane 3), and the dichotomy between the results for the *cagA*[−]

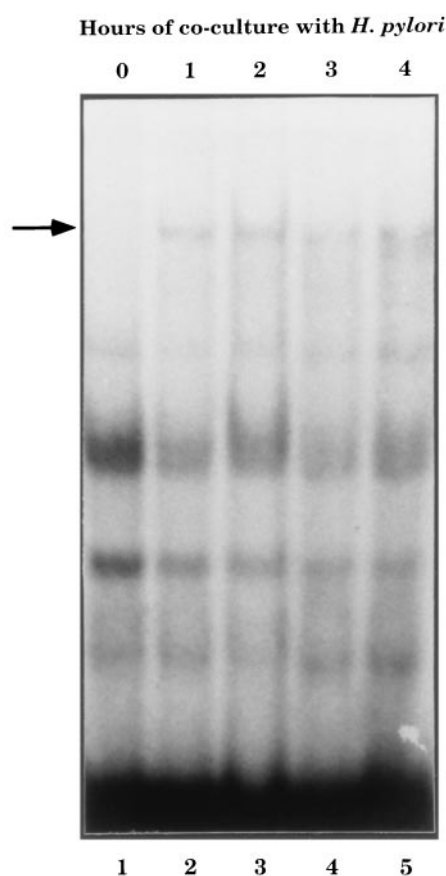


FIGURE 4. A κ B binding complex is induced in AGS cells within 1 h of coculture with *H. pylori*. Nuclear extracts containing equal protein concentrations from AGS cells that were unstimulated or cocultured with *H. pylori* strain 88-23 were analyzed for κ B binding activity by gel-shift assays using an IL-8 κ B oligonucleotide as a probe. Lane 1, Nuclear extract from unstimulated AGS cells. Lanes 2 to 5, Nuclear extract from AGS cells cocultured with *H. pylori* for 1 to 4 h. The arrow indicates the position of the induced κ B binding complex.

mutant and the *picA1*[−] and *picB*[−] mutants, exactly parallel earlier results on induction of IL-8 mRNA and protein (16, 21). While the laboratory *cagA*[−] mutant still induces detectable κ B binding activity in EMSA, the reproducible observation that *H. pylori* 88-23:*cagA*[−] induces significantly less transcriptional activity remains paradoxical. These data might suggest that the NF- κ B complexes released by 88-23:*cagA*[−] are able to associate with DNA but are not in a properly modified, hyperphosphorylated form to activate transcription. Alternatively, given the diminished κ B binding activities of wild-type *cagA*[−] *H. pylori* strains (Fig. 6, lanes 6–9), the laboratory 88-23:*cagA*[−] strain might use other gene products to facilitate the full transcriptional activation of κ B-containing genes (e.g., *picA1* or *picB*). As such, these results indicate that the increase in NF- κ B binding activity may play an essential role in the observed activation of the IL-8 promoter induced by *cag*⁺ *H. pylori* strains, and this effect is dependent on the *picA1* and/or *picB* gene products.

Discussion

IL-8 is an important cytokine in the host inflammatory response to *H. pylori* (18, 41, 42), which correlates with its induction in gastric epithelial cells cocultured with *H. pylori* in vitro (20, 21, 40). Up-regulation of IL-8 by *H. pylori* may lead to free radical generation and the release of proteolytic enzymes from activated neutrophils,

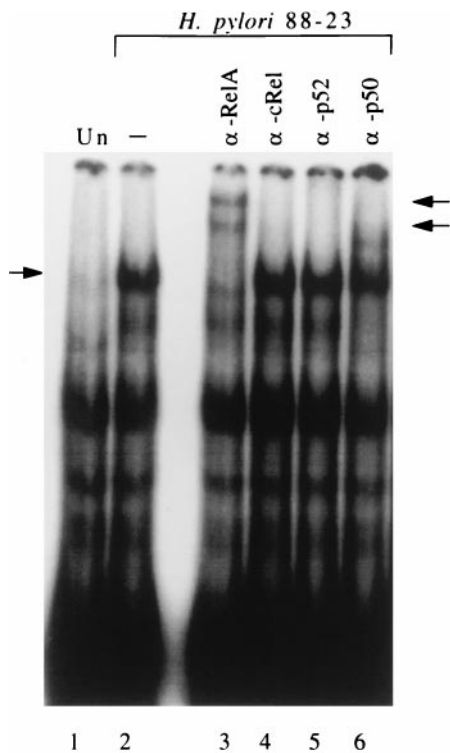


FIGURE 5. Anti-RelA sera recognizes *H. pylori*-stimulated κ B binding complex in nuclear extracts from AGS cells. Nuclear extracts from AGS cells that were unstimulated or cocultured with *H. pylori* strain 88-23 were analyzed for κ B binding by gel-shift assays. The κ B probe was incubated with nuclear extracts from AGS cells that were unstimulated (lane 1) or cocultured with *H. pylori* strain 88-23 (lanes 2–6). The nuclear extracts were preincubated for 30 min with Abs against RelA (lane 3), c-Rel (lane 4), p52 (lane 5), or p50 (lane 6). Arrows indicate the locations of supershifted complexes in lanes 3 and 6.

affecting mucosal integrity (43). That eradication of *H. pylori* in ulcer patients results in a reduction in antral IL-8 mRNA expression, in neutrophil infiltration, and in surface epithelial lesions (41), suggests that inflammatory cytokines may play an important role in the mucosal damage seen in *H. pylori* infection.

In this report we investigated the molecular mechanisms by which IL-8 gene expression is regulated upon exposure of gastric AGS cells to *H. pylori* variants. Our studies demonstrate that both NF- κ B and NF-IL-6 elements are required for induction of IL-8 promoter activity in gastric AGS epithelial cells by both *H. pylori* and TNF- α , findings consistent with observations that intact binding sites of both κ B and NF-IL-6 elements are required for maximal IL-8 expression in Jurkat T cells, by stimuli such as IL-1, TNF- α , and phorbol esters (30).

Our study further shows that a RelA-containing NF- κ B binding complex is rapidly induced in response to *H. pylori* stimulation of AGS cells, whereas there is no increase in NF-IL-6 binding activity. Although, the NF- κ B site is required for inducibility of the IL-8 enhancer in all cell types examined (29), the NF-IL-6 element also is necessary as a co-operator for IL-8 gene transcription (29, 30). In vitro DNA binding studies and transient transfection assays in Jurkat T cells and fibrosarcoma cells implicate RelA homodimer as the transcriptional activator binding to the κ B-like site in the IL-8 enhancer (39), providing direct evidence for the role of RelA in the regulation of IL-8 gene expression. The classical NF- κ B p50/RelA heterodimer does not appear to bind the IL-8 site in vitro and does not *trans*-activate transcription from the IL-8 promoter

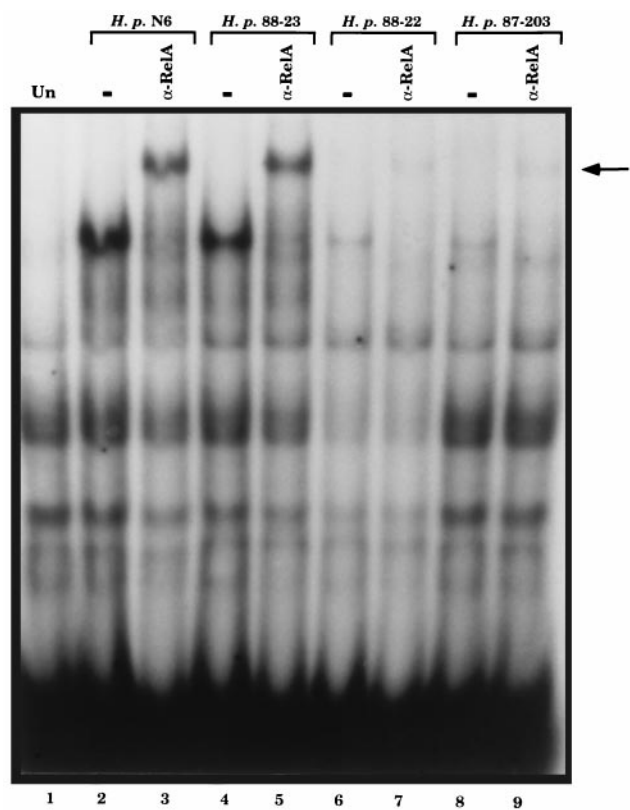


FIGURE 6. Wild-type *cag*⁺ *H. pylori* strains show enhanced stimulation of κ B binding activity in AGS cells compared with wild-type *cag*⁻ *H. pylori* strains. Equal protein concentrations of nuclear extracts from AGS cells that were unstimulated or cocultured with *H. pylori* were analyzed for κ B binding by gel-shift assays. The κ B probe was incubated with nuclear extracts from unstimulated AGS cells (lane 1) or cells cocultured with *cag*⁺ *H. pylori* strains N6 (lanes 2 and 3) or 88-23 (lanes 4 and 5) or *cag*⁻ strains 88-22 (lanes 6 and 7) or 87-203 (lanes 8 and 9). For lanes 3, 5, 7, and 9, the nuclear extracts were preincubated with RelA Ab for 30 min. The arrow indicates the location of the supershifted complex.

despite its ability to do so in cells transfected with Ig κ or HIV κ B site reporters (39).

Compared with *cag*⁻ *H. pylori* strains, the more virulent *cag*⁺ strains showed an enhanced ability to induce RelA-NF- κ B binding activity and IL-8 promoter activity, paralleling earlier observations that increased IL-8 induction in AGS cells was *cag*⁺ strain specific (20, 21). The current data indicate that increased IL-8 expression in epithelial cells in response to *cag*⁺ *H. pylori* strains may result from an increase in RelA binding to the cognate enhancer element in the IL-8 promoter. That the *pic*-encoded determinants expressed by *cag*⁺ strains required for IL-8 expression (10, 16) also are essential for inducing NF- κ B binding and IL-8 promoter activity in AGS gastric cells confirms that the ability of wild-type and mutant *H. pylori* strains to activate NF- κ B correlates with their ability to induce IL-8 expression.

Recent studies suggest that the *cag* pathogenicity island may encode a secretion system in *H. pylori* analogous to the hybrid-type secretion systems present in *Yersinia*, *Bordetella*, and *Agrobacterium* species that secrete factors involved in bacteria-host cell interactions (10). Whether inactivation of *cag* genes other than *picA1* and *picB* affects NF- κ B activation and IL-8 promoter activity in gastric epithelial cells, as they affect IL-8 secretion (10), remains to be determined.

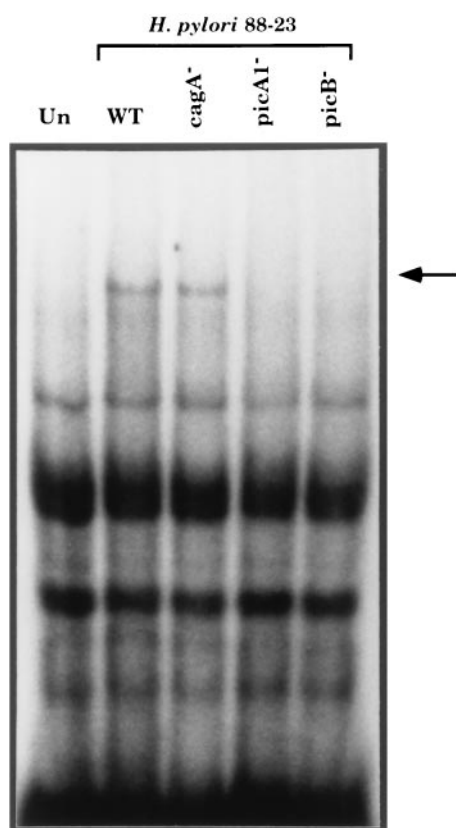


FIGURE 7. The *pic* gene products of *H. pylori* are required for induction of NF- κ B binding activity in gastric epithelial cells. Equal protein concentrations of nuclear extracts from unstimulated AGS cells (lane 1) and cells cocultured with wt (lane 2), *cagA*⁻ (lane 3), *picA1*⁻ (lane 4), or *picB*⁻ (lane 5) *H. pylori* strains were analyzed for κ B.

The finding that *H. pylori* is a potent activator of NF- κ B has important implications, since other NF- κ B-responsive genes, including TNF- α , IL-1, and IL-6, which have been found to be elevated in the gastric mucosa of persons with *H. pylori* (44, 45), also may participate in the pathophysiology of *H. pylori* colonization. Co-operation between the NF-IL-6 and the NF- κ B/Rel families of proteins in regulating immune, inflammatory response and acute phase response genes allows the perpetuation and amplification of inflammatory signals (24, 30, 31).

Colonization by *H. pylori* may lead to altered homeostasis in the gastric mucosa (3), causing an imbalance between proinflammatory and anti-inflammatory cytokines. Lack of type 2 cytokine responses in subjects colonized with *H. pylori* (46) helps explain the elevated levels of proinflammatory cytokines in the gastric mucosa (18, 41, 44, 45), since by inhibiting NF- κ B activation, IL-10 suppresses the synthesis of proinflammatory cytokines, whereas IL-4 blocks cytokine mRNA accumulation (47).

In summary, the present studies demonstrate that IL-8 induction in AGS gastric cells by *H. pylori* is regulated via an NF- κ B-dependent transcriptional process. The *pic* determinants expressed by *cag*⁺ strains play a critical role in NF- κ B-mediated up-regulation of IL-8 expression, and the presence of these secretory pathway homologues may explain the heightened inflammatory response observed in subjects colonized with *cag*⁺ strains.

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