Helicobacter pylori induces apoptosis of rat gastric parietal cells

BRUNO NEU,1 PAMELA RANDLKOFER,1 MATHILDE NEUHOFTER,1 PETRA VOLAND,1 ARTUR MAYERHOFER,2 MARKUS GERHARD,1 WOLFGANG SCHEPP,3 AND CHRISTIAN PRINZ1
1Department of Medicine II and 3Krankenhaus München Bogenhausen, Technical University of Munich, Munich; and 2Anatomisches Institut der Universität München, München, Germany

Received 27 December 2001; accepted in final form 19 March 2002

THE GASTRIC MUCOSA IS FREQUENTLY infected with the gram-negative bacterium Helicobacter pylori, colonizing the antral part of the gastric epithelium in which the pH is elevated to 3–4, which allows optimal growth (7). Previous studies have revealed that urease activity of H. pylori depends on urea uptake through a specific channel, which is open at pH values between 3 and 6 but closes at levels >7 (6, 19, 28). During long-term infection as well as long-term therapy with proton pump inhibitors, the infection may also proceed to the gastric corpus, resulting in atrophy of gastric glands, hypochlorhydria, and possibly the development of gastric adenocarcinoma (14, 32, 33).

One mechanism by which H. pylori generates mucosal atrophy may be the induction of apoptosis of the epithelial mucosal barrier followed by destruction of the remaining mucosa by exposure to luminal acid. Indeed, in vivo and in vitro studies demonstrate that infection with H. pylori is associated with apoptosis of gastric epithelial cells (20, 31); coculture of human gastric cancer cell line (AGS) cells with H. pylori in vitro resulted in growth inhibition predominantly at the G(0)–G(1) checkpoint, possibly mediated by changes of the regulatory proteins p53, p21, and cyclin E (1). Another mechanism may be a more indirect effect. These studies have suggested that adherence of H. pylori to the gastric mucosa triggers the production of proinflammatory cytokines such as interleukin (IL)-8, tumor necrosis factor (TNF)-α, and IL-1β (12). The release of these cytokines in turn induces the accumulation of inflammatory cells and also leads to a sustained functional impairment of mucosal cells. For example, incubation of parietal cells (PC) and enterochromaffin-like cells with IL-1β induced apoptosis of these cells that are crucial for acid secretion (9, 12, 18, 25, 26).

It may also be of special interest to investigate direct effects of the bacteria on gastric PC. As mentioned above, long-term infection as well as long-term therapy with proton pump inhibitors may lead to the colonization of the gastric corpus, and the only spaces in which...
the bacterium may survive appear to be the acidic spaces inside of the gastric gland, where the bacteria are in close contact with the acidic lumen of parietal cells. Indeed, pathologists have detected bacteria inside the gastric gland; it may therefore be possible that the bacteria affect PC directly by interfering with the life cycle of this important cell type.

Some *H. pylori* strains appear to be more virulent than others and may, therefore, also affect PC differentially. Several virulence factors of *H. pylori*, especially the cag pathogenicity island (cagPAI), encode type IV secretion machinery. Products encoded by the cagPAI, such as CagA or CagE, have been associated with increased mucosal inflammation (9, 12, 34). Also, *H. pylori* strains expressing vacuolating toxin (VacA) have been considered to be associated with increased pathogenic potential of the bacterium (4, 5).

Our current work investigated direct effects of *H. pylori* on programmed cell death in gastric PC. Our results show a significant stimulation of apoptosis, which appears to depend on the presence of the cagE (also called picB) gene (34). It appears that the effect of *H. pylori* involves the generation of nuclear factor-κB (NF-κB) but not the generation of the rat IL-8 homolog cytokine-induced neutrophil chemoattractant (CINC)-1. In this sense, *H. pylori* has a direct effect on parietal cells that is distinct in its signal transduction in epithelial cells, in which IL-8 is released in large amounts. Our data thus contribute to the understanding of gastric atrophy and carcinogenesis and may present a model for the events during late stages of mucosal destruction.

**MATERIALS AND METHODS**

**Cell isolation and primary cell culture.** Highly enriched rat gastric PC were prepared as previously described (27). A total of 80 preparations was used (5 rats/preparation). All killing experiments were performed in accordance with the ethical guidelines of the Technical University of Munich. The experiments comply with all relevant local and institutional regulations. Briefly, the stomachs were treated with pronase E (1.3 mg/ml; Roche, Mannheim, Germany) for enzymatic digestion, and the dispersed cells were then subjected to counterflow elutriation (JE-6 elutriation rotor, Beckman Instruments, Palo Alto, CA) and density gradient centrifugation. Enriched PC were placed on six-well plates precoated with Matrigel diluted 1:500 with sterile dH2O (Becton Dickinson, Heidelberg, Germany). For immunocytochemistry and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay, 4–5 × 10⁶ cells were grown on sterile glass coverslips coated with Cell-Tak (Becton Dickinson; dillusion 1:1 with 0.5 M NaHCO₃). Cells were cultured in DMEM (GIBCO BRL, Eggenstein, Germany) supplemented with 5 μg/ml insulin, 5 μg/ml transferrin, and 5 μg/ml sodium selenite, 100 μg/ml gentamicin, 10 μg/ml hydrocortisone, and 1 g/500 ml BSA. For stimulation experiments, PC were incubated in serum-free medium. Cell purity in this cell isolation and primary cell culture system was ≥97%, as measured by Giemsa staining. Cell viability was determined by trypan blue staining.

**Bacterial strains and infection.** *H. pylori* isolates used in this work were as follows: G27 and HP2808, both wild-type laboratory strains and both expressing the cagPAI and the vacA genes, and G27 knockout mutants were used. These strains were deficient for the cagE, cagF, cagH, cagN, or the vacA gene. The wild-type strain and the isogenic mutants were kindly provided by A. Covacci, Immunobiological Research Institute, Chiron, Siena, Italy. Tx50 is a laboratory strain lacking the cagPAI and vacA. The isolates were kept at −80°C in Brucella broth with 10% fetal bovine serum containing 10% (vol/vol) glycerol. *H. pylori* strains were cultured on WC-agar plates containing 10% horse serum in a microaerophilic atmosphere at 37°C over 48–72 h. Bacteria were harvested in PBS (pH 7.4) and diluted corresponding to the multiplicity of infection (MOI) desired, using a McFarland densitometer. The scale is graduated in both McFarland standard units and the average bacterial concentrations. The viability of bacteria was monitored by light microscopy. The parietal cells cultured in DMEM at 37°C were then incubated with the bacteria. In some experiments, *H. pylori* strain G27 was heat inactivated (30 min, at 60°C), sonicated, or treated with chloramphenicol (25 μg/ml for 30 min). *S. aureus* and *C. jejuni* were used as controls. They were grown in their corresponding environment and were as equally diluted as the *H. pylori* strains.

**Electrophoretic mobility shift assay.** For electrophoretic mobility shift assay (EMSA), nuclear extracts (NE) of enriched PC were used. Per sample, 1 × 10⁷ cells were incubated with *H. pylori* over 8 h. Nuclear extracts were prepared as follows. Briefly, parietal cells were detached from the tissue culture plate with 1 × trypsin-EDTA and washed once in ice-cold PBS. After centrifugation (1,000 rpm, 5 min), they were resuspended in *buffer 1* (15 mM NaCl, 15 mM β-mercaptoethanol, 15 mM Tris·HCl, 2% protein inhibitor cocktail, and 0.5% phenylmethylsulfonyl fluoride, all from Sigma, Munich, Germany). After centrifugation, cells were resuspended in *buffer 1* and kept on ice for the next 30 min. They were centrifuged again and resuspended in *buffer 2* (*buffer 1* supplemented with 2 mM EDTA, 0.5 mM EGTA, and 0.34 M sucrose). The lysates were transferred into special tubes and homogenized over 11 min to obtain the nuclei. After saccharose density gradient centrifugation with *buffer 3* (*buffer 1* supplemented with 1 mM EDTA, 0.25 mM EGTA, and 1.37 M sucrose), the pellets were resuspended in binding buffer (250 mM HEPES, 5 mM EDTA, 20 mM 1,4-dithiothreitol, 40% glycerol, 1 M NaCl) and sonicated (Branson Sonifier, Heidelberg, Germany). NE were immediately placed on dry ice and stored at −80°C. For EMSA, 10 μg nuclear protein in 20 μl H₂O and binding buffer were incubated with 4 μg of poly-(dI: dC) for 5 min at room temperature. Then, 25 ng of nonlabeled (cold) oligonucleotides (ODN) containing the HIV-NF-κB consensus sequence or the SP-1 sequence (Gelshift assay kit; Stratagene, Cambridge, UK) were incubated over 20 min with the lysates of unstimulated cells for competition assay. Finally, 2 μl of [α³²P]-labeled oligos (~100,000 counts/min, labeled with T4 polynucleotide kinase, Roche) containing the NF-κB consensus site or the SP-1 site were added to each probe for another 20 min. The probes were kept on ice, mixed with 2.2 μl of sample buffer [20% Ficoll 400 and 0.25% bromophenol blue (in Tris-borate EDTA (TBE)) and loaded on a 7% acrylamide gel. Electrophoresis was carried out at room temperature for 7–10 h with TBE. After completion of the electrophoresis, the gel was washed in H₂O, dried, and exposed to either an X-ray film at ~70°C for 2–5 days or to a phosphor imaging screen (Molecular Dynamics, Sunnyvale, CA) for ~2 days.

Supershift analysis was performed using the following antibodies: anti-NF-κB p50 and anti-NF-κB p65 (Santa Cruz; Heidelberg, Germany). The antibodies were added before addition of radiolabeled ODNs for 20 min at room temperature.

AJP-Gastrointest Liver Physiol • VOL 283 • AUGUST 2002 • www.ajpgi.org
RESULTS

Induction of apoptosis in highly enriched PC by incubation with H. pylori. After 12 h of culture, purified rat gastric PC were incubated with the H. pylori strain G27 over 8 h in regular media. Bacterial densities were examined by McFarland assay. At the indicated MOI of 2, a concentration of two bacteria per cell was used. As visualized in the Fig. 1A, a significant stimulation of apoptosis was already observed at an MOI of 10 corresponding to 10 bacteria per cell. At higher concentrations, the stimulation was even more prominent (P < 0.01). Maximal effects were observed at an MOI of 20 (n = 5 independent experiments).

Figure 1B shows a time course of PC in culture under basal conditions and after addition of H. pylori strain G27. Bacteria were added over 4, 8, and 12 h of incubation. With the use of TUNEL assay and staining with peroxidase, 15% (+/- 2%) of the PC showed apoptosis under basal conditions and 35% (+/- 3%) of PC were apoptotic after 12-h coculture with H. pylori strain G27. Apoptosis was stimulated significantly after 4 h of incubation, and further prolongation of the incubation time enhanced the stimulatory effect (total of n = 5 experiments, P < 0.05). Similar results were observed for trypan blue staining during 4–8 h of incubation, indicating that cell death was due to apoptosis but not necrosis. At a longer incubation interval (>24 h), a higher percentage of cells was found to stain positive for trypan blue, which was larger than the number of apoptotic cells (~2-fold). Therefore, subsequent experiments were performed over an 8-h incubation period, because this period was found to produce a significant and reproducible stimulation, and a longer period was considered to produce nonspecific effects on cell death by induction of necrosis. Moreover, three independent experiments were performed using filtered supernatants of the H. pylori strain G27, and apoptosis of parietal cells was measured by TUNEL reaction. However, no significant differences were observed.

Electron microscopy (EM) was performed to validate these results, and a representative figure is shown in Fig. 1, C and D. Figure 1C shows a PC incubated for 8 h with vehicle only. The normal cell is characterized by a large nucleus with heterochromatin, many mitochondria, and microvilli, which are found on the surface of the cell and inside the canaliculi. Figure 1D shows a representative PC after coculture with H. pylori G27 for 8 h. More than 40% of PC showed this typical apoptotic appearance with pyknotic cell nuclei and apoptotic bodies on the surface. The EM thereby confirmed the TUNEL results.

Specificity of H. pylori effects. To demonstrate the specificity of the H. pylori effects, several other bacteria were added, and their effect on PC was investigated. As depicted in Fig. 2A, TNF-α and the H. pylori strain G27 induced a twofold increase of apoptosis of PC after 8 h of incubation. In contrast, S. aureus and C. jejuni did not significantly induce programmed cell death at an MOI of 10 (n = 4 experiments). Next, different H. pylori strains characterized by the presence or absence of the vacA and cagA gene were incubated simultaneously with enriched PC. As shown in Fig. 2B, TNF-α at 20 ng/ml, the laboratory strains H. pylori strain G27 as well as the H. pylori laboratory strain 2808 at an MOI of 10 induced apoptosis two- to threefold compared with the basal rate. In contrast, the H. pylori strain Tx30 lacking the vacAs1 and cagA genes did not exert any significant effect on PC apoptosis. To determine the effect of heat inactivation, the H. pylori G27 strain was sonicated or heat inactivated over 30 min. The procedures were used to discriminate between effects of lipopolysaccharides (LPS) present in the bacteria or potential proteins. As shown in Fig. 2C, sonication and heat inactivation at 60° over 30 min
completely abolished the effects, indicating that LPS are not mediating these effects. Similarly, addition of chloramphenicol at 25 μg/ml over 30 min completely prevented the effect of G27 on PC apoptosis.

Costimulatory effects of H. pylori and TNF-α. Highly enriched PC were cultured and incubated with the H. pylori strain G27 over 8 h. Apoptosis was measured by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) reaction. The percentage of apoptosis was set to 100% in the cells incubated under basal conditions. Data are shown as mean ± SE. *P < 0.01 vs. basal in n = 5 independent experiments (1-way ANOVA followed by Newman-Keuls test). B: time dependence of apoptotic PCs. Cells were incubated with H. pylori strain G27 for 4–12 h. Apoptosis was measured by TUNEL reaction. Data are shown as means ± SE. *P < 0.01 in n = 5 independent experiments. C, D: electron microscopy (EM) of an isolated rat PC incubated over 8 h with vehicle (C) and the H. pylori strain G27 (D). In untreated cells, PC can be recognized by numerous mitochondria and microvilli on the surface. The EM in D shows the characteristic pyknotic nuclei in PCs after incubation with H. pylori. Apoptotic PCs are recognized by the condensed, coarse aggregates of chromatin, by the shrunken nucleus, and, in earlier stages, by the intact cytoplasm and mitochondria compacted together (35). MOI, multiplicity of infection.

Effects of knockout mutants deficient in genes of the cagPAI. Different mutants of the cagPAI were used to examine the importance of the proteins encoded by the genes. These mutants were provided by A. Covacci (Siena, Italy). As shown in Fig. 4, the cagA, cagH, cagF, and cagN mutants stimulated apoptosis of PC after 8 h of incubation. In contrast, addition of the cagE mutant did not induce apoptosis of rat gastric PC, indicating that this effect depends on the cagE protein.

Signal transduction in H. pylori-induced apoptosis of PC. The G27 strain was used to stimulate apoptosis in PC after 8 h of coculture. p65 AS ODN (100 μM) were used to block the effect of NF-κB. As shown in Fig. 5, the AS ODN completely inhibited the effect, whereas the MS ODN did not block G27-induced programmed cell death. In the experiments shown in Fig. 5, the was reproducible in three independent experiments (not shown as a figure). IL-8 was also not detected in rat peripheral mononuclear blood cells (PMBC; 10⁶ cells/ml). The rat IL-8 homolog CINC-1 (36) was also measured in identical supernatants of isolated rat PCs using specific ELISA (Amersham, Heidelberg, Germany). The rat IL-8 homolog CINC-1 was not detected in rat PCs but in rat PMBC (10⁶ cells/ml) at a concentration of 20 pg/ml, which was stimulated 10-fold by the addition of LPS (10 ng/ml).

Effects of knockout mutants deficient in genes of the cagPAI. Different mutants of the cagPAI were used to examine the importance of the proteins encoded by the genes. These mutants were provided by A. Covacci (Siena, Italy). As shown in Fig. 4, the cagA, cagH, cagF, and cagN mutants stimulated apoptosis of PC after 8 h of incubation. In contrast, addition of the cagE mutant did not induce apoptosis of rat gastric PC, indicating that this effect depends on the cagE protein.

Signal transduction in H. pylori-induced apoptosis of PC. The G27 strain was used to stimulate apoptosis in PC after 8 h of coculture. p65 AS ODN (100 μM) were used to block the effect of NF-κB. As shown in Fig. 5, the AS ODN completely inhibited the effect, whereas the MS ODN did not block G27-induced programmed cell death. In the experiments shown in Fig. 5, the was reproducible in three independent experiments (not shown as a figure). IL-8 was also not detected in rat peripheral mononuclear blood cells (PMBC; 10⁶ cells/ml). The rat IL-8 homolog CINC-1 (36) was also measured in identical supernatants of isolated rat PCs using specific ELISA (Amersham, Heidelberg, Germany). The rat IL-8 homolog CINC-1 was not detected in rat PCs but in rat PMBC (10⁶ cells/ml) at a concentration of 20 pg/ml, which was stimulated 10-fold by the addition of LPS (10 ng/ml).
transfection rates were 84 (±6) % in three different experiments. The apoptotic rate was 15% (±3), and the rate in the H. pylori incubated fraction was 35% (±2), indicating that the majority of cells that die was also transfected with ODN.

Gel shift assays of H. pylori-induced activation of NF-κB. PCs were incubated with vehicle, the H. pylori strain G27, or the G27 knockout mutants cagH, cagA, cagE, cagF, and cagN. Nuclear extracts were prepared, and EMSA was performed as described above. The extracts were incubated with radiolabeled, double-stranded ODNs containing the NF-κB consensus sequence. A total of three independent experiments was performed using different strains mutated in the cagPAI. As indicated in Fig. 6A, the G27 strain strongly activated the binding of NF-κB to the consensus sequence (Fig. 6A, lane 2). Only the cagE mutant failed to induce NF-κB binding to the consensus sequence (lane 5). Densitometric analysis of three independent experiments was used to quantitate the intensity of both bands relative to the intensity under basal conditions (Fig. 6B), indicating a strong stimulation with most strains but a lack of stimulation using the cagE mutant (lane 5, Fig. 6B).

Fig. 2. Apoptosis of highly enriched PCs after the addition of different bacteria. A: specific induction of apoptosis by H. pylori strain G27 compared with other bacteria. PCs were incubated with H. pylori strain G27, S. aureus, and C. jejuni, each with an MOI of 10 for 8 h. Tumor necrosis factor (TNF)-α (20 ng/ml) was used as a positive control. The percentage of apoptosis was set to 100% in the cells incubated under basal conditions. Data are shown as means ± SE. *P < 0.01 vs. basal in n = 5 independent experiments. B: specific induction of apoptosis by H. pylori strain G27 compared with other H. pylori strains. PCs were incubated for 8 h with different H. pylori strains at an MOI of 10: G27 (cagA+ and vacA+), 2808 (cagA+ and vacA+), and Tx30 (cagA+ and vacA-). C: induction of apoptosis by H. pylori strain G27 before and after different treatments of the bacteria. PCs were incubated with H. pylori G27 over 8 h at an MOI of 10. Alternatively, H. pylori G27 was heated for 30 min at 60°C, sonicated or treated with chloramphenicol (25 μg/ml) for 30 min, and apoptosis was determined after incubation of 8 h.

Effects of proteasome inhibitors and L-NMMA on apoptosis. Rat gastric PCs were incubated with vehicle or the H. pylori strain G27 over 8 h, and apoptosis was
measured using TUNEL assay. Simultaneously, the proteasome inhibitor PSI (1 mM) or the NOS inhibitor L-NMMA (10^{-4} M) was added. As indicated in Fig. 8, both inhibitors prevented the induction of apoptosis in rat PCs induced by the addition of the H. pylori strain G27.

**DISCUSSION**

*H. pylori* colonizes the gastric corpus mucosa, and this bacterial expansion often results in multifocal atrophic gastritis. During this long-term infection, *H. pylori* may also colonize the crypts of the gastric glands, and a direct contact of the bacterium may be given, enabling direct interaction of *H. pylori* also with PCs. In the current study, we were able to show a direct effect of the bacteria on gastric PC. Culturing bacteria together with the acid-producing cells resulted in programmed cell death at MOI rates of 1–20, as shown by TUNEL and specific features determined by EM. This effect can be considered specific, because it was observed with defined bacterial subtypes, it was not affected by antibodies against cytokines known to affect PC function (such as TNF-α), and the effect was abolished by short-term heat inactivation, indicating that LPS or related structures do not contribute to the...
**H. pylori**-induced apoptosis of PC. Addition of chloramphenicol completely prevented the effects of live **H. pylori** on apoptosis, indicating that de novo bacterial protein synthesis, for example, active production of proteins potentially involved in this effect, is required during this process.

Incubation with **H. pylori**-induced apoptosis in a terminally differentiated cell. PCs originate from stem cells, and their approximate lifespan is <1 wk. Incubation may effectively alter the equilibrium between differentiation and apoptosis at a specific cellular signal-transduction step. Previous studies have revealed that **H. pylori** leads to a G1 arrest of a gastric epithelial cell line (1). **H. pylori** initiates epithelial cell signaling events that resulted in activation of the transcription factor NF-κB (21). Direct inhibition of NF-κB abolished **H. pylori**-stimulated apoptosis in epithelial cells; **H. pylori** activated NF-κB, which was blocked by cotreatment with peroxisome proliferator-activated receptor (PPAR)-γ agonist (16). These results suggested that activation of a PPAR-γ pathway attenuates the ability of **H. pylori** to induce NF-κB-mediated apoptosis in...
gastric epithelial cells. In the current study, we also observed apoptosis in PCs dependent on the activation of NF-κB. Specific AS ODNs and a proteasome inhibitor PSI completely prevented the \textit{H. pylori} effects, indicating that NF-κB has a proapoptotic function similar to observations made in other cell types (10, 15).

In human epithelial cells, activated NF-κB translocates to the nucleus, where it upregulates IL-8 gene transcription (30). Previous studies have shown that \textit{H. pylori} infection activates IL-8 gene expression in gastric epithelial cells in vitro and in vivo (2). IL-8 mRNA and protein levels are increased in the gastric mucosa of patients with \textit{H. pylori} gastritis, and immunohistochemical studies demonstrate increased IL-8 protein in gastric epithelial cells from infected individuals (3, 12). \textit{H. pylori} also increases IL-8 mRNA levels and protein production in cultured monolayers of AGS cells and other gastric epithelial cell lines (2). Gene expression of IL-8, in turn, was considered to be of crucial importance for the attraction of granulocytes and monocytes, leading to the release of products that affect epithelial cell death.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure7}
\caption{Stimulation of NF-κB activation in PCs by \textit{H. pylori} (HP) and inhibition of NF-κB activation by incubation with p65 AS ODN. A: cells were preincubated with p65 AS and MS ODN for 8 h. After culture medium change, they were further incubated with HP G27 at an MOI of 10 for again 8 h. Then, NE were prepared as previously described. Lane 1 contains NE of unstimulated cells, lane 2 contains NE of G27, lane 3 contains NE of p65 AS ODN incubated cells, and lane 4 contains NE of p65 MS ODN incubated cells. B: densitometric analysis using One D scan software. C: stimulation of SP-1 activation in PCs by HP and cells incubated with p65 AS and MS ODN. The SP-1 band contains the same NE as in Fig. 6C. The SP-1 EMSA serves as control, showing equal protein loading. Lane 1 contains NE of unstimulated cells, lane 2 contains NE of G27, lane 3 contains NE of p65 AS ODN incubated cells, and lane 4 contains NE of p65 MS ODN incubated cells. Lane 5: competition assay with cold NF-κB ODNs (25 ng/ml). D: densitometric analysis of the SP-1 labeling.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure8}
\caption{N-CBZ-isoleucin-glutamin-(o-t-butyl)-alanin-leucin (PSI) and N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) effects on G27-induced PC apoptosis (TUNEL). Cells were incubated with vehicle, \textit{H. pylori} G27 (8 h), or G27 together with PSI (1 mM) or L-NMMA (10\textsuperscript{-4} M). *\textsuperscript{1}Significant differences relative to the apoptotic rate under basal conditions; *\textsuperscript{2}, *\textsuperscript{3} significant differences relative to G27-caused apoptosis.}
\end{figure}
In the current study, we observed that *H. pylori* did not induce IL-8 production in rat gastric PCs. This may not be surprising, because in rats, no gene sequence has been detected thus far encoding for IL-8. Thus this finding may only represent a species difference between humans and rats; however, we also measured concentrations of the rat IL-8 homolog CINC-1 (36) in the supernatants of rat gastric PCs in the presence and absence of *H. pylori*. Because we did not detect CINC-1 in rat PCs but in rat mononuclear blood cells (positive control), it may be concluded that the signaling cascade activated by *H. pylori* does not involve a signal pathway associated with the transcription of the rat IL-8 homolog CINC-1.

It appears that the differential activation process for NF-κB and the rat IL-8 homolog CINC-1 in PCs may reflect different cellular transduction mechanisms. Indeed, recent studies reported that *H. pylori*, when cocultured with AGS epithelial cells, rapidly activated MAP kinases (17). With the use of specific inhibitors, the authors found that p38 and MEK-1 activity was required for *H. pylori*-induced IL-8 production, but activation of these proteins was not essential for *H. pylori*-induced NF-κB activation in epithelial cells (17). Thus it may be possible that the missing effect on IL-8/CINC-1 secretion in PCs may be the missing activation of p38 and MEK-1 in PCs too, and activation of NF-κB may require other kinases involved in the induction of cell death.

Our data further support the view that the cagE (picB) protein encoded by the cagPAI is responsible for the induction of this programmed cell death. The cagPAI of *H. pylori* is a 40-kb region immediately upstream from the cagA gene that encodes >40 putative bacterial proteins. On the basis of sequence homology, the cagPAI encodes a secretion system that is involved in the export or surface expression of bacterial virulence factors (11). Gene products of the cagPAI are also known to participate in epithelial cell activation by *H. pylori*. *H. pylori* cagA+ strains are more potent in activating epithelial cell IL-8 production than cagA− bacteria (9, 23, 24, 24). *H. pylori* picB, a homolog of the Bordetella pertussis toxin secretion protein, is required for induction of IL-8 in gastric epithelial cells (34). Furthermore, disruption of specific cag region genes markedly reduces *H. pylori*-mediated tyrosine phosphorylation of gastric epithelial cell proteins, NF-κB activation, and IL-8 gene transcription (21, 22, 29).

*H. pylori* strains deficient in cagE, but not mutated in the cagA sequence, did not induce apoptosis of PC in the current work. Similar observations regarding a differential effect of cagE-deficient mutants on cellular signal transduction were also observed by Keates at al. (17). The authors reported differential activation of mitogen-activated kinases and c-jun kinases by cagA+ and cagE-deficient *H. pylori*, similar mechanisms may also be present in gastric PC. Thus c-Jun kinases may also play an important role in the activation of NF-κB in rat gastric PC too.

A specific inhibitor of a possible NF-κB downstream target was further investigated. As indicated, the NOS inhibitor L-NMMA completely inhibited the effect of *H. pylori* on apoptosis determined by TUNEL reaction in PCs. These results indicate that a possible downstream target of NF-κB is gene expression of NOS and NO production, thereby leading to DNA breakdown. Similar observations were made in pancreatic β-cells following IL-1β stimulation. In β-cells, NF-κB activation is linked to apoptosis, and this effect appeared to be mediated by induction of inducible NOS (iNOS) and generation of NO (13). The promoter region of the rat iNOS gene contains the NF-κB consensus sequence at several positions (bp 71–79, 134–142, 888–896, and 930–938), underlining the functional interaction between NF-κB activation and iNOS transcription. Our data therefore support the idea that iNOS may be a key enzyme mediating *H. pylori*-induced cell death, leading to the generation of NO and subsequent apoptosis in PCs.

In summary, our data suggest that gastric PCs may be directly affected by *H. pylori*, that this effect is mediated by the cagE product, and that it involves activation of the transcription factor NF-κB and the generation of NO.

The wild-type strain and the isogenic mutants were kindly provided by A. Covacci, Immunobiological Research Institute, Chiron, Siena, Italy.

This work was supported by the Else Kröner Fresenius Stiftung (to B. Neu), Kuratorium für Klinische Forschung der TU München (KKF-8733156 to C. Prinz), Deutsche Forschungsgemeinschaft (DFG-411/7–1 to C. Prinz), and Gastrofoundation, Munich, Germany. C. Prinz is a recipient of the Heisenberg Award from the Deutsche Forschungsgemeinschaft.

REFERENCES


