Helicobacter pylori Extract Induces Nuclear Factor-kappa B, Activator Protein-1, and Cyclooxygenase-2 in Esophageal Epithelial Cells

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Helicobacter pylori infection is recognized as the major cause of gastritis and gastric cancer; however, its role in the development of gastroesophageal reflux disease and Barrett's adenocarcinoma is unclear. The expression of NF-κB, AP-1, and COX-2 may be important in inflammation and tumorigenesis in the esophagus. The aim of this study was to examine the effect of live *H pylori* or *H pylori* extract (HPE) on these factors in the esophageal epithelial cell lines SKGT-4 and OE33. NF-κB and AP-1 activity were assessed by gel shift assay and COX-2 by Western blotting. Coculture of SKGT-4 and OE33 with live *H pylori* and HPE induced NF-κB and AP-1 DNA-binding activity, and also decreased IκB-α levels. Treatment with the specific MEK1/2 MAPK inhibitor PD98059, but not the p38 MAPK inhibitor SB203580, inhibited NF-κB and AP-1 activity. The antioxidant vitamin C inhibited *H pylori*-induced NF-κB activation, but increased AP-1 expression. Moreover, HPE induced COX-2 expression and IL-8 production, and PD98059 inhibited COX-2 expression, ERK1/2 phosphorylation, and IL-8 production. These data demonstrate that both live *H pylori* and HPE induce NF-κB and AP-1 expression in esophageal epithelial cells. The induction of such transcription factors may play a role in the specific immune response within Barrett's mucosa and may indirectly cause inflammation of the gastric cardia and the distal esophagus. (J GASTROINTEST SURG 2006;10:551–562)

KEY WORDS: AP-1, esophageal cancer, *H pylori*, HPE, NF-κB

Helicobacter pylori infects over half the world's population. The infection is associated with chronic gastritis, peptic ulceration, gastric lymphoma, and gastric adenocarcinoma.[1,2] *H pylori* eradication is effective in the treatment of gastritis, peptic ulcers, and early lymphoma of mucosal-associated lymphoid tissue.[3] The relationship, however, between *H pylori* infection and gastroesophageal reflux disease (GERD), the onset of Barrett's esophagus, and the development of adenocarcinoma of the esophagus and the esophagogastric junction is controversial. A significantly higher prevalence of esophageal *H pylori* infection has been reported in patients with Barrett's and esophageal adenocarcinoma compared with Barrett's patients without cancer (75% vs. 32.6%).[4] Nondysplastic Barrett's epithelium is also frequently colonized with *H pylori*.[5] Moreover, the prevalence of inflammation at the esophagogastric junction, so-called carditis, is similar in patients with and without GERD and is associated with *H pylori* infection.[6–9]

In GERD, *H pylori* gastritis may protect against the development of esophageal erosions.[10] The infection exerts a protective effect on the esophagus, probably mediated by the ammonia buffer produced by *H pylori* and refluxing in the esophagus.[11] Patients carrying cagA+ strains of *H pylori* have greater protection against the complications of GERD, especially Barrett's metaplasia, dysplasia, and adenocarcinoma.[12,13] The studies suggest that the distribution and severity of *H pylori*- related gastritis and atrophy, rather than the mere presence of *H pylori* infection, play a role in the pathophysiology of GERD, and that *H pylori* colonization of the esophagus may occur by a mechanism that is different from that observed in the gastric mucosa.

The specific immune environment within Barrett's metaplasia is an important driver in the development of GERD and cancer of the esophagus. In inflammatory disease states, proinflammatory mediators known to be regulated by transcription factors

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1091-255X/06/$—see front matter
doi:10.1016/j.gassur.2005.09.003

such as nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1) are thought to play an important role in the regulation of various cellular functions, including cellular activation, proliferation, and apoptosis. NF-κB resides in the cytoplasm, in an inactive form, as a heterodimer consisting of p50 and p65 (RelA) subunits complexed to the inhibitory molecule IκB, which prevents the migration of the heterodimer to the nucleus. After a range of stimuli in many cell types, NF-κB translocates to the nucleus and binds to its specific DNA site and subsequently upregulates the gene expression.[14,15] We have previously reported that NF-κB is increasingly expressed from inflammation through Barrett's metaplasia to dysplasia and adenocarcinoma.[16]

The relationship between *H pylori* infection and the transcription factors NF-κB and AP-1 and related cytokines has not previously been addressed. Jones et al.[17] demonstrated that wild-type *H pylori* strains increased Fas protein expression in OE33 cells, and that *H pylori* induced apoptosis at a higher rate in the Barrett's-derived human esophageal adenocarcinoma cells than in normal esophageal cells. This *H pylori*-induced apoptosis was primarily dependent on intact bacteria and the presence of the cagA and picB/cagE gene products. It is known that transcription factors such as NF-κB and AP-1 regulate a wide variety of genes involved in epithelial inflammation, growth, and apoptosis, including Fas.[14,15]

In this study we have sought to determine whether *H pylori* infection has any effect on the expression of NF-κB and AP-1 in esophageal epithelial cells and to further explore the molecular mechanisms involved. We report herein that both live *H pylori* and *H pylori* extract induce NF-κB, AP-1, and COX-2 expression in the esophageal epithelial cells SKGT-4 and OE33 cells, and that *H pylori* also activates ERK1/2 phosphorylation. Moreover, incubation of esophageal epithelial cells with HPE increased IL-8 production, and HPE-induced IL-8 was inhibited by the MEK1/2 MAPK inhibitor PD98059 and vitamin C.

MATERIAL AND METHODS

Material

NF-κB and AP-1 consensus oligonucleotides were obtained from Promega (Promega Corp., Madison, WI). Antibody to IκB-α, anti-phospho-ERK1/2, and non-phospho-ERK1/2, anti-p50 (sc-114X), anti-p65 (sc-109X), anti-c-Rel (sc-70X), anti-Fra-1, anti-c-Fos, anti-c-Jun, and anti-Jun-D for gel super-shift assays were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal COX-2 antibody was purchased from Cayman Chemical Company (Ann Arbor, MI). [γ^{32}P]ATP (35 pmol, 3000 Ci/mmol) was from Amersham International (Aylesbury, UK). Poly(dI-dC) was obtained from Pharmacia (Biosystems, Milton Keynes, UK). Vitamin C was obtained from Sigma (Poole, Dorset, UK). PD98059 and SB203580 were purchased from Calbiochem, Novabiochem Corp., La Jolla, CA.

Cell Culture

The esophageal epithelial cell line OE33 (derived from the adenocarcinoma of the lower esophagus; Barrett's metaplasia) was obtained from the European Collection of Animal Cell Cultures (Porton Down, Salisbury, UK). SKGT-4 cells (established from a well-differentiated adenocarcinoma arising in Barrett's epithelium of the distal esophagus) were a gift from Dr. David S. Schrump (Thoracic Oncology Section, Surgery Branch, National Cancer Institute, NIH, Bethesda, MD). OE33 and SKGT-4 cells were grown in RPMI 1640 medium supplemented with 10% filtered fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mmol/L L-glutamine.

H pylori Culture

H pylori reference strains NCTC 11637 and NCTC 11638 obtained from the National Collection of Type Cultures (Colindale, UK) were used in this study. The strains were grown in a microaerobic-humidified atmosphere on 7% lysed horse blood Columbia agar at 37° C. After 48–72 hours, bacteria were harvested in RPMI 1640 medium without antibiotics and resuspended to a concentration of 2×10^8 colony-forming units (cfu)/ml using the McFarland standard kit. Live *H pylori* were cocultured with SKGT-4 or OE33 for different periods of time (30 min – 240 min). The ratio of *H pylori* to esophageal epithelial cells is 100:1. For preparation of *H pylori* extract (HPE), suspended bacteria were placed on ice for 20 minutes and centrifuged at 15000*g* for 10 minutes at 4° C. Serial dilutions of HPE were used to infect the cells and 1 ml of HPE was found to induce a pronounced NF-κB and AP-1 activation, therefore 1 ml HPE was used in all experiments. The total protein content of this HPE was 300 μg/ml as measured by the method of Bradford.[18]

Cell Culture Treatments

Confluent SKGT-4 and OE33 cells (1×10^6 cells/ml) grown in 6-well plates were cocultured with 1 ml of freshly harvested *H pylori* suspension (2×10^8 cfu/ml) or HPE. In the case of all inhibitors, appropriate dilutions were made in cell culture medium just