Role of vacA and cagA in Helicobacter pylori Inhibition of Mucin Synthesis in Gastric Mucous Cells

WINFRIED BEIL,1,* MARIE LUISE ENNS,2 SIMONE MÜLLER,3 BARBARA OBST,1 KARL-FRIEDRICH SEWING,1 AND SIEGFRIED WAGNER3

Department of General Pharmacology,1 Institute for Laboratory Animal Science,2 and Department of Gastroenterology and Hepatology,3 Medizinische Hochschule Hannover, D-30625 Hannover, Germany

Received 23 August 1999/Returned for modification 15 December 1999/Accepted 27 March 2000

The aim of this study was to investigate the effect of Helicobacter pylori on the function of gastric mucous cells. H. pylori (10^4 to 10^7 CFU/well) was incubated with the mucin-producing gastric cell line HM02 for 12 and 24 h. Mucin synthesis and secretion were determined by the incorporation of d-[N-acetyl-14C]glucosamine into intracellular and released high-molecular-weight glycoproteins. cagA-positive, cytoxin-producing and non-cytotoxin-producing H. pylori strains impaired the incorporation of d-[N-acetyl-14C]glucosamine into intracellular glycoproteins. Significant inhibition of mucin synthesis was noted after 12 and 24 h of cocultivation with a bacterial load of ≥10^8 bacteria (bacterium/10^6 cells) ratio = 0.25. The cagA-positive, cytoxin-producing strains (HP64, HP57, and HP87) caused significantly stronger inhibition of intracellular mucin synthesis than the cagA-positive, non-cytotoxin-producing strains (HP05, HP83, and HP84). The cagA-negative, non-cytotoxin-producing strains (HP01, HP64, and HP85) did not affect intracellular or released mucins. The results indicate that H. pylori directly impairs mucin synthesis in gastric mucous cells and that cytotoxic cagA-positive strains cause more profound inhibition of mucin synthesis. We suggest that the increased inhibitory effect of cagA-positive, cytoxin-producing strains on mucin synthesis can be considered one possible factor responsible for the increased risk of developing peptic ulceration with these H. pylori strains.

The human pathogen Helicobacter pylori is the cause of antral gastritis and peptic ulceration (11). In the human stomach, H. pylori is found within the gastric mucus layer and is primarily associated with gastric mucus cells (8, 31). The gastric mucus layer acts as a protective barrier for the gastric epithelium against luminal attack by acid and pepsin. The polymeric structure of mucin leads to the formation of a water-insoluble gel. Bicarbonate trapped within the mucus helps the mucus layer to maintain a pH gradient between the acidic gastric lumen and the neutral epithelial surface (27). H. pylori infection is associated with a decrease in mucus thickness (24). The bacteria are able to elaborate protease and phospholipase (28, 34), enzymes capable of degrading the major components of gastric mucus. This degradation may compromise the mucus layer and facilitate reversed diffusion of hydrogen ions.

It is not clear what distinguishes patients with H. pylori infection who develop peptic ulceration from those who develop gastritis only. H. pylori is characterized by a high genomic variability which forms the basis for interstrain differences in pathogenic potential (4). For instance, the vacuolating cytoxin vacA is encoded by a gene occurring in multiple alleles that may or may not affect the secretion of a more or less active gene product (1). A second marker for pathogenicity is the product of cagA, which is usually coexpressed with vacA (32). Cytotoxin-producing strains seem to cause ulcers more than non-cytotoxin-producing strains (9, 14).

The aim of this work was to investigate whether H. pylori directly affects mucus synthesis and secretion in a cellular in vitro system and, if so, whether there are differences between different H. pylori strains.

(Part of this work has been presented at the 100th meeting of the American Gastroenterological Association, Orlando, Fla., and has been published in abstract form [Gastroenterology, 116:A354, 1999].)

MATERIALS AND METHODS

H. pylori culture. Nine clinical isolates of H. pylori were used: the cagA-negative, non-cytotoxin-producing strains HP01, HP04, and HP85; the cagA-positive, non-cytotoxin-producing strains HP05, HP83, and HP84; and the cagA-positive, cytotoxin-producing strains HP64, HP57, and HP87. The presence of cagA mRNA in the H. pylori strains was analyzed by reverse transcription-PCR using the primers (5' GAT AAC AGG CAA GCT TTT GAG 3' and 5' CTT CCA AAG ATT GTT TGG CAG 3') and incubation conditions described by Tummuru et al. (32). The presence of the vacuolating toxin was determined by incubation of HM02 cells with concentrated supernatants from H. pylori broth medium as described by Cover et al. (10). Bacteria were grown on blood agar plates under microaerophilic conditions for 2 days at 37°C. Bacteria were harvested in phosphate-buffered saline solutions. For estimation of the desired final bacterial numbers, optical densities at 600 nm were measured and were correlated to viable CFU. Comparative experiments were conducted with Campylobacter jejuni (ATCC 35560; American Type Culture Collection, Manassas, Va.).

Cell culture and cocultivation experiments. HM02 cells, which were derived from a human well-differentiated mucus-producing gastric carcinoma, were used in all experimental protocols. Previous studies have shown that H. pylori specifically adheres to HM02 cells and that this cell line provides a suitable in vitro model for the study of the interaction of H. pylori and the gastric epithelium (33).

Cells were seeded at a density of 4 × 10^5 cells/well in six-well plastic plates (Greiner, Nürtlingen, Germany), incubated overnight, and washed in RPMI 1640 (Greiner, Nürtlingen, Germany). Incubation conditions were as described above. Bacteria were grown on blood agar plates under microaerophilic conditions for 2 days at 37°C. Bacteria were harvested in phosphate-buffered saline solutions. For estimation of the desired final bacterial numbers, optical densities at 600 nm were measured and were correlated to viable CFU. Comparative experiments were conducted with Campylobacter jejuni (ATCC 35560; American Type Culture Collection, Manassas, Va.).

The human pathogen Helicobacter pylori is the cause of antral gastritis and peptic ulceration (11). In the human stomach, H. pylori is found within the gastric mucus layer and is primarily associated with gastric mucus cells (8, 31). The gastric mucus layer acts as a protective barrier for the gastric epithelium against luminal attack by acid and pepsin. The polymeric structure of mucin leads to the formation of a water-insoluble gel. Bicarbonate trapped within the mucus helps the mucus layer to maintain a pH gradient between the acidic gastric lumen and the neutral epithelial surface (27). H. pylori infection is associated with a decrease in mucus thickness (24). The bacterium is able to elaborate protease and phospholipase (28, 34), enzymes capable of degrading the major components of gastric mucus. This degradation may compromise the mucus layer and facilitate reversed diffusion of hydrogen ions.

It is not clear what distinguishes patients with H. pylori infection who develop peptic ulceration from those who develop gastritis only. H. pylori is characterized by a high genomic variability which forms the basis for interstrain differences in pathogenic potential (4). For instance, the vacuolating cytoxin vacA is encoded by a gene occurring in multiple alleles that may or may not affect the secretion of a more or less active gene product (1). A second marker for pathogenicity is the product of cagA, which is usually coexpressed with vacA (32). Cytotoxin-producing strains seem to cause ulcers more than non-cytotoxin-producing strains (9, 14).

The aim of this work was to investigate whether H. pylori directly affects mucus synthesis and secretion in a cellular in vitro system and, if so, whether there are differences between different H. pylori strains.

(Part of this work has been presented at the 100th meeting
RESULTS

Effect of *H. pylori* on mucin synthesis and secretion. The ability of HM02 cells to synthesize and to secrete mucin HMG was studied by determination of the rates of incorporation of [14C]GlcNAc into acid-insoluble proteins. Basal rates of incorporation of [14C]GlcNAc into cellular mucin were 4,793 ± 1,066 and 7,725 ± 1,231 cpm/well after 12 and 24 h of incubation, respectively. The cell numbers per well were (0.39 ± 0.02) × 10^6 and (0.92 ± 0.08) × 10^6, respectively. [14C]GlcNAC-labeled mucins in the culture medium represented 5.8% to 10.6% of the intracellular labeled mucins.

Cocultivation of HM02 cells with HP05, HP83, and HP84 (cagA-positive, non-cytotoxin-producing strains) and HP64, HP57, and HP87 (cagA-positive, cytotoxin-producing strains) impaired the incorporation of [14C]GlcNAc into intracellular mucins. Significant inhibition of mucin synthesis was noted after 12 h of cocultivation with a bacterial load of ≥10^6 bacteria (bacterium/cell ratio = 0.25). A smaller bacterial load (10^5 bacteria) did not significantly alter mucin synthesis. The amount of labeled mucins recovered from the culture medium of HM02 cells remained unchanged. The cagA-positive, cytotoxin-producing strains caused stronger inhibition of intracellular mucin synthesis than the cagA-positive, non-cytotoxin-producing strains. Significantly different effects were noted at bacterial loads of 10^6 bacteria (cytotoxin-negative strains, 33.5% ± 4.6%; cytotoxin-positive strains, −56.3% ± 4.4%) and 10^7 bacteria (cytotoxin-negative strains, −33.5% ± 4.7%; cytotoxin-positive strains, −50.0% ± 7.9%) (Fig. 1). Similar effects were observed at the end of the 24-h incubation period. The cytotoxin-negative strains at 10^5, 10^6, and 10^7 bacteria/ml reduced [14C]GlcNAc incorporation by 22.5% ± 7.7%, 31.2% ± 7.6%, and 26.7% ± 11%, respectively; the cytotoxin-positive strains reduced intracellular incorporation rates by 55.3% ± 11%, 56.7% ± 8.4%, and 56.3% ± 11%, respectively (P < 0.05 relative to values for cytotoxin-negative strains at 10^5, 10^6, and 10^7 bacteria). In contrast, the cagA-negative, non-cytotoxin-producing strains (HP01, HP04, and HP85) did not affect intracellular mucin synthesis (data not shown).

Isolation of intracellular HMG from *H. pylori*-treated HM02 cells. The vast majority of PAS-positive material in HM02 cells eluted in the voided volume (fractions 8 to 14) after gel chromatography, indicating the presence of glycoproteins with a molecular mass of ≥2 × 10^6 Da (HMG) in the cells. In agreement with the tracer studies, HP87, HP57, and HP64 impaired the carbohydrate content of HMG. At bacterial loads of 10^5, 10^5, and 10^7, PAS-positive material was significantly decreased to 74.6% ± 8%, 73.6% ± 7%, and 62.0% ± 13% the level in controls. In contrast, protein content in the PAS-positive material was increased to 110% ± 11%, 159% ± 43%, and 167% ± 28% the level in controls (P < 0.05 for 10^7 bacteria) (data not shown).

Effect of *H. pylori* on cell viability. Cocultivation of HM02 cells with *H. pylori* (cagA-positive, non-cytotoxin-producing and cytotoxin-producing strains) for 24 h was not associated with a reduction in gastric cell count. At the highest bacterial load (10^7/ml), cell counts were (0.92 ± 0.03) × 10^6 (cytotoxin-negative strains) and (0.82 ± 0.05) × 10^6 (cytotoxin-positive strains) cells/well, respectively. Likewise, LDH release did not increase, indicating that *H. pylori* did not exert a major cytotoxic effect.

Effect of *C. jejuni* on mucin synthesis and secretion. In contrast to *H. pylori*, *C. jejuni* significantly stimulated the incorporation of [14C]GlcNAc into intracellular mucins and the release of labeled mucins into the culture medium during the 12-h (Fig. 2) and 24-h (data not shown) incubation periods.

DISCUSSION

Under normal conditions, the gastric mucus layer acts as a protective barrier for the gastric epithelium against luminal attack by acid, pepsin, and exogenous damaging agents. Available data indicate that *H. pylori* undermines the integrity of the mucus layer, i.e., accelerates mucus degradation, perhaps through lipolytic and proteolytic activities (20, 22, 28, 34). However, the importance of proteolytic enzymes in the pathophysiology of *H. pylori* remains controversial (3, 26).

The results reported in this study indicated that cagA-positive, cytotoxin-producing and non-cytotoxin-producing *H. pylori* strains impaired mucin synthesis in gastric mucous cells. In contrast, cagA-negative, non-cytotoxin-producing strains did not affect mucin synthesis. Significant alterations of intracellular mucins were found after incubation for 12 h with ≥10^5 bacteria (bacterium/cell ratio = 0.25). None of the *H. pylori* strains affected the baseline mucin secretion of HM02 cells. In contrast to these results, Micots et al. (21) reported a modest...
inhibition of mucin secretion from colonic Cl.16E cells incubated with H. pylori. The reasons for this discrepancy may be attributed to the different cellular models used for testing (gastric cells versus colonic cells). However, the baseline mucin secretion of HM02 cells is rather low. Therefore, changes in the amount of secreted radioactive mucins may be detected only after long-term incubation.

The intracellular synthesis of mucin proceeds through the steps of ribosomal synthesis of core peptides, formation of sugar nucleotides, addition of steps of ribosomal synthesis of core peptides, formation of oligosaccharide cores are then elongated, producing chains of from 2 to 20 sugars in length that have a variety of linear or branched patterns. Finally, other posttranslational modifications, such as sulfation, complete the molecule. The mucins are packed in secretory vesicles and are ready for migration to the cell apex (15).

vacA induces acidic vacuoles in the cytoplasm of eukaryotic cells. The membranes of cytotoxic-induced vacuoles react with anti-rab 7 antibodies, suggesting that these vacuoles are derived, at least in part, from late endosomes (23). Such compartments are crucial crossroads in the complex network of intracellular membrane traffic in eukaryotic cells. Garner and Cover (16) noted that vacA interacts with endocytic vesicles, and Satin et al. (25) have recently shown that vacA modifies the intracellular sorting of endogenous proteins. Based on these observations, we suggest that the increased activity of cytotoxic H. pylori strains on mucin synthesis may be caused by the interaction of vacA with the intracellular processing or sorting of glycoproteins. This hypothesis is supported by the finding that exposure of HM02 cells to cytotoxic H. pylori strains induces the formation of “immature” glycoproteins; i.e., the bacteria decrease carbohydrate content and increases protein content in glycoproteins.

The fucosylated blood group antigens Lewis b and H-1 mediate the adherence of H. pylori to gastric mucus cells (5, 13). cagA-positive strains show a fivefold higher density of bacterial cells in the gastric antrum than cagA-negative strains (2). Ilver et al. (19) have provided evidence that the presence of cagA is associated with bacterial binding to the Lewis b antigen. The cagA gene encodes a protein involved in the export of bacterial virulence factors (7). Based on these observations, we propose that cagA-mediated adherence of H. pylori to mucous cells may play a critical role in the delivery to HM02 cells of bacterial factors that alter mucin synthesis. However, the exact biochemical mechanism of the effect of cagA on mucin synthesis remains unclear.

Much evidence indicates that H. pylori vacA- and cagA-positive strains are endowed with an increased pathogenic ulcerative potential. Purified vacA induces ulceration in the stomach of mice (30), and vacuolating cytotoxic bacteria are more often isolated from patients with peptic ulcer disease (9, 14, 29). Our data demonstrate that cytotoxic cagA-positive H. pylori strains induce amore profound inhibition of mucin synthesis than cagA-positive, non-cytotoxin-producing strains, whereas cagA-negative, non-cytotoxin-producing strains do not affect mucin synthesis. A decrease in the synthesis of glycoproteins and changes in gastric mucin structure accompany the development of gastritis and are prominent features in the etiology of peptic ulcers (17). Recognizing that the results of our in vitro study cannot be directly extrapolated to the interaction of H. pylori with mucin synthesis in vivo, the findings suggest that the inhibitory effect of cagA-positive, cytotoxin-producing strains on mucin synthesis can be considered one possible factor responsible for the increased risk of developing peptic ulceration with these H. pylori strains.

ACKNOWLEDGMENTS

This work was supported by a grant from Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 280, TP A7). We thank U. Stiass for skilful technical assistance.

REFERENCES


