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

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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, cytotoxin-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/cell ratio = 0.25). The cagA-positive, cytotoxin-producing strains (HP04, 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, HP04, and HP85) did not affect intracellular mucin synthesis. 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, 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.
RESULTS

Effect of \textit{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 [\textsuperscript{14}C]GlcNAc into acid-insoluble proteins. Basal rates of incorporation of [\textsuperscript{14}C]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 \((0.39 ± 0.02) \times 10^6\) and \((0.92 ± 0.08) \times 10^6\), respectively. [\textsuperscript{14}C]GlcNAc-labeled mucins in the culture medium represented 5.8 to 11% of the intracellular labeled mucins. Significant inhibition of mucin synthesis was noted after 12 h of cocultivation with a bacterial load of \(10^6\) bacteria (cytotoxin-negative strains, \(cagA^+\)-positive, cytotoxin-producing strains) and \(10^7\) bacteria (cytotoxin-negative strains, \(cagA^+\)-positive, cytotoxin-producing strains) caused stronger inhibition of intracellular mucin synthesis than the \(cagA^+\)-positive, non-cytotoxin-producing strains. Significant effects were noted at bacterial loads of \(10^2\) 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 [\textsuperscript{14}C]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 \textit{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 \(\approx 2 \times 10^6\) 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 \(10^5\), \(10^6\), and \(10^7\), PAS-positive material was significantly decreased to \(74.6\% ± 8\%, 73.6\% ± 7\%,\) and \(62.0\% ± 13\%\) of the level in controls. In contrast, protein content in the PAS-positive material was increased to \(110\% ± 11\%, 159\% ± 43\%,\) and \(167\% ± 28\%\) in controls (\(P < 0.05\) for \(10^7\) bacteria) (data not shown).

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 \textit{H. pylori} underminds 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 \textit{H. pylori} remains controversial (3, 26).

The results reported in this study indicated that \textit{cagA^+}-positive, cytotoxin-producing and non-cytotoxin-producing \textit{H. pylori} strains impaired mucin synthesis in gastric mucous cells. In contrast, \textit{cagA^+}-negative, non-cytotoxin-producing strains did not affect mucin synthesis. Significant alterations of intracellular mucins were found after incubation for 12 h with \(\approx 10^5\) bacteria (bacterium/cell ratio \(= 0.25\)). None of the \textit{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 Cl16E 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 fucose, and or threonine residues in the endoplasmic reticulum and/or Golgi complex, and formation of oligosaccharide cores in the Golgi system. The 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 increase 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). Iver 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 mucus 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.

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