DETECTION OF LOCALLY PRODUCED IGA- AND IGG-ANTEBDIES AGAINST HELICOBACTER PYLORI IN HUMAN GASTRIC MUCOSA BY ELISPOT METHOD


The aim: Helicobacter pylori (H.p.)-infected individuals develop high IgG-antibody titers against H.p. in serum. IgA and IgG against H.p. have also been detected in gastric juice. The aim of the study was to define whether these specifically against H.p. and antigen directed immunoglobulins are locally produced by plasma cells within the gastric mucosa.

Methods: An enzyme-linked immunospot assay (ELISPOT) was developed, coded with water soluble proteins (WSP) from the H.p. reference strain NCTC 11637. Lymphocytes were separated by percoll gradient from single cell suspensions obtained from gastric biopsies of patients with gastritis, ulcer and of non-infected persons. H.p.-status was defined by serological (ELISA) and histopathological findings (Warthin-Starry-staining). The Sydney-system was used to classify chronicity and activity of gastritis.

Results: Immunoglobulin-producing cells were found in 63 % of H.p.-positive patients. All of them showed IgA-class immunospots and in 60 % in addition IgG-class immunospots. The detection of immunospots correlated significantly with the diagnosis gastritis or ulcer and with the extent of activity and chronicity. In some cases showing no gastritis or ulcer but high serum IgG-titers immunospots against H.p. were found. All H.p.-negative individuals were negative in the ELISPOT.

Conclusions: H.p. induces an antigen-specific local humoral immune response which correlates well with the grade of inflammation and the degree of H.p.-colonization. The specificity of the ELISPOT assay is high. It may be used as a method to detect antigen-specific local immunoglobulin production against selected H.p.-antigens.


The cause of H. pylori hypergastrinemia remains unknown. Pathogenic E.coli cause a rise in cytotoxic free calcium on binding. A similar process may occur if H. pylori bind to G cells. This rise in cytotoxic free calcium may then cause gastrin secretion. It is important to know if H. pylori binds specifically to G cells.

Methods

Human endoscopic antral biopsies were microdissected, digested with collagenase and enriched by centrifuge through a Histopaque density gradient. Using this method G cells made up 8% of the final cell population (range 6-10%). The G cells were mixed with a dense suspension of H. pylori at 37 degrees for one hour. The cells were heat fixed and stained using a monoclonal antibody to gastrin and a fluorescing monoclonal antibody to H. pylori.

Results

In biopsies from 5 different patients a total of 242 G cells were studied, of these 123 (50%) had H. pylori bound to them. Usually there were several H. pylori bound to each G cell.

Conclusion

H. pylori do bind to isolated G cells. Further work is needed to establish if this binding causes an increase in cytotoxic free calcium and subsequent secretion of gastrin.

Few and conflicting data are available about the possible correlation between H. pylori (HP) infection and disorders of gastrointestinal motility. The present study aimed at retrospectively evaluating 100 consecutive dyspeptic patients, who had undergone inter-digestive antralduodenal manometric recording, in order to verify whether the absence of phase 3 of the migrating motor complex (MMC) could be associated with a different prevalence of HP infection. All the patients underwent endoscopic examination of the upper gastrointestinal tract with at least two biopsies in both gastric antrum and corpus (for histological evaluation of Helicobacter-like organisms). Then, a 240-min interdigestive manometric recording, with evaluation of activity fronts (phase 3 of MMC) starting from the gastric antrum, was performed.

RESULTS: 

<table>
<thead>
<tr>
<th>N of antral phase</th>
<th>1/240 min</th>
<th>None</th>
<th>1 or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall patients</td>
<td>42</td>
<td>62</td>
<td>38</td>
</tr>
<tr>
<td>HP positive</td>
<td>44 (71.0%)</td>
<td>18 (47.3%)</td>
<td>26 (61.9%)</td>
</tr>
<tr>
<td>HP negative</td>
<td>18(29.0%)</td>
<td>20 (52.7%)</td>
<td></td>
</tr>
</tbody>
</table>

Patients with gastritis: 42
HP positive: 36 (75.0%) 13 (61.9%) HP negative: 6 (29.4%) 8 (18.2%)

Patients without gastritis 14
HP positive: 11 (78.6%) 5 (29.4%) HP negative: 4 (28.6%) 8 (17.8%)

Our data suggest that the prevalence of HP colonisation is significantly (p<0.018) higher in patients without evidence of antral phase 3 of MMC.

DIVERGENT EFFECTS OF H. PYLORI ON ACID SECRETION.

EL CHAMBLE, Wire A, McColl K E L. University Department of Medicine and Therapeutics, Western Infirmary, Glasgow, Scotland.

Background: H. pylori is associated with both DU disease - a disorder related to increased acid secretion, and gastric cancer a disease of acid hyposecretion. We have previously shown that in patients with DU disease, infection increases GRP stimulated acid secretion in DU patients and healthy volunteers by induction of acid secretion, but we have not yet evaluated whether the GRP effect can be maintained in DU patients who are positive for H. pylori. Therefore, for the first time, we have carried out a large number of GRP tests in a variety of H. pylori positive subjects. These included healthy volunteers, DU and non-ulcer dyspepsia patients. We have found 11 of the infected non-DU subjects to be achlorhydric to GRP. These subjects have been studied in further detail.

Subjects and Methods: The mean age of the 11 subjects was 61 (range: 43-79) 4 were males. All subjects had undergone endoscopic and biopsies from antrum and body of stomach for histological examination and CLO test. They also had 13C-urea breath test, measurement of parietal cell and intrinsic factor antibodies and a pentagastrin test (0.6ug/kg/h) to assess peak acid output. Six of the subjects were treated with eradication therapy and their tests repeated 3-6 months later.

Results: All subjects were positive for H. pylori by 13C-urea breath test, histology and CLO test. All had evidence of chronic active gastritis in the antrum and body. None showed evidence of malignancy and none had positive gastric autoantibodies. The median pentagastrin stimulated acid output in the 11 subjects was 0 (range: 0-6.0). Each of the 6 subjects who were eradicated of the infection showed a marked increase in acid secretion, including the 5 who were achlorhydric (p<0.01).

Discussion: These studies demonstrate that chronic H pylori infection can result in decreased as well as increased acid secretion. The reason for the different acid responses is unclear but may be related to different bacterial strains or genetic and environmental influences. This divergent effect on acid secretion explains how the bacterium can predispose to either DU disease or gastric cancer.
H PYLORI-INDUCED DISTURBANCE OF GASTRIC ACID SECRETION IS UNRELATED TO BACTERIAL CagA STATUS.

K.F. McColl, E El-Omar, A El-Nujumi, A Wirtz, A Covacci, T.E. Crabtree. University Department of Medicine & Therapeutics, Western Infirmary, Glasgow, Division of Medicine, University of Leeds, RD5, Huddersfield.

Subjects with H pylori who develop duodenal ulceration (DU) have a markedly increased acid secretory response to gastrin releasing peptide (GRP) which resolves following eradication of the infection. Subjects with H pylori who develop DU are also more likely to have bacterial strains which are CagA positive and consequently show a serological response to the bacterial protein. We have investigated whether the disturbance in gastric secretory function is related to the bacterial strain.

Patients and Methods Sixty seven subjects with non-ulcer dyspepsia (NUD) were examined. They had suffered from dyspepsia for >6 months and no microscopic lesion had been found despite at least two upper GI investigations. All were positive for H pylori by "C-urea breath test, and histology and CLO test of endoscopic antral biopsies. Any medication was discontinued at least 2 weeks prior to the study. Twenty five age and sex matched controls without H pylori infection were also examined. In all subjects acid output was measured in response to GRP (40 pmol/kg/min) administered IV for 60 mins. In those with H pylori, CagA status was determined indirectly by assaying serum CagA IgA antibodies by ELISA using a purified recombinant fragment of CagA.

Results: The median acid output to GRP in the 67 NUD subjects with H pylori was 18 mmol/kg (range 0-54) which was higher than that in the H pylori negative controls (8.1-22) (p<0.001) 37 of the 67 H pylori positive NUD subjects were CagA seropositive and their GRP stimulated acid output (median 19, 0-54) was similar to that in those who were CagA seronegative (17.5, 1-32).

Conclusions: These results indicate that the disturbance of gastric acid secretory function with H pylori is independent of bacterial CagA status. It must be related to other bacterial factors.

PROFOUND INCREASE OF HELICOBACTER PYLORI UREASE ACTIVITY IN GASTRIC ANTRAL MUCOSA AT LOW pH

S.E. Miedere, P. Gruber*. Medizinische Klinik des Evangelischen Johannes-Krankenhauses Bielefeld, Medizinische Poliklinik der Universität Bonn. *Division of Gastroenterology, St. Elizabeth's Medical Center, Boston, MA.

Purpose: The exact role that urease plays in the pathogenesis of H. pylori induced ulcer disease has not been fully elucidated. The aim of this study was to investigate the effect of pH on H. pylori urease activity in its ecological niche, the gastric antral mucosa.

Methods: Multiple gastric antral biopsy specimens were obtained from each of 20 patients with histologically documented H. pylori infection and 10 H. pylori negative patients and incubated in 10 mmol/L urea solutions at pH range 3.3 to 8.2. H. pylori positive antral biopsy specimens were also parallel incubated in a set of urea solutions at pH 7.0 and 8.0 until pH increased in the solution with initial pH 7.0 to 7.3. Specimens were removed, washed and reincubated in urea solution at pH 7.0. Activity of urease was studied by measuring the production of ammonia and change in pH of the solutions.

Results: Urease activity was reduced at pH 8.2 (1424 ±218μmol/L) but decreasing initial pH to neutral (7.0) and acidic values (5.0) resulted in significant maximal 6.5-fold increase in ammonia production (9491 ±1073μmol/L, p<0.0005) which considerably raised pH of the test solutions. Peak of urease activity was measured between pH 5.0 and 7.0. In contrast to specimens incubated initially at pH 8.0, reincubation of washed specimens from solutions with initial pH 7.0 showed 8-fold decreased urease activity.

Conclusions: Release of urease activity is markedly pH dependent with pH optima below the physiological mucosal surface pH. Furthermore, availability of urease is limited. We postulate that an impaired gastric mucosal integrity allowing back diffusion of hydrogen ions releases urease activity, which further weakens the mucous barrier and damages the gastric epithelium.

H. pylori and colonic adenomas. SK. Lam, S Pianko, JR Lambert, J Hansky, P Maddo, C Sovery. Department of Medicine, Mornington Peninsula Hospital and Monash Medical Centre, Melbourne, Australia

Previous studies have reported elevated plasma gastrin levels in subjects with colorectal adenocarcinoma and polyps. H. pylori infection also causes elevated fasting and meal stimulated gastrin release. The aim of this study was to assess the association between H. pylori infection and colonic adenomas. Methods: 100 Anglo-Celtic patients undergoing colonoscopy had fasting serum gastrin measured by specific RIA. 42 of 100 were diagnosed with colonic polyps, colonic cancer or previous colonic poly hist pathology. 269 representative Anglo-Celtic subjects, randomly sampled from the telephone directory, were used as controls. An ELISA technique (AMRAID) was used to detect H. pylori IgG antibodies. Logistic regression was performed with adjustments for the important confounding variable.

Results:

<table>
<thead>
<tr>
<th>Colonic adenoma</th>
<th>normal controls</th>
<th>All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odds ratio &amp; CI</td>
<td>1.7 (1.2-2.4) &amp; 0.001</td>
<td>1.7 (1.2-2.4) &amp; 0.001</td>
</tr>
</tbody>
</table>

Helicobacter pylori on expression of somatostatin mRNA in the gastric antrum and corpus.


Introduction: H. pylori (Hp) impairs reflex inhibition of acid secretion. This may be because Hp gastritis decreases mucosal expression of somatostatin (SST). A group from The Netherlands recently reported decreased SST peptide in the gastric corpus of Hp+ non-ulcer patients. This differs from our finding in duodenal ulcer (DU) disease, where eradication of Hp from DU patients significantly elevated antral SST mRNA but did not change SST mRNA in the gastric corpus. The discrepancy could be due to whether a difference between expression of mRNA and peptide, or a difference between DU and non-ulcer patients. Therefore we compared the expression of SST mRNA in the gastric corpus of non-ulcer patients with and without Hp infection.

Methods: Biopsies were taken from the gastric corpus and antrum of 9 Hp+ and 8 Hp– dyspeptic patients for total RNA extraction and Northern blotting. SST mRNA was detected using a 3' labelled cDNA probe, the signals being quantified by phosphorimager. Results: In the corpus, median SST mRNA was significantly lower in the Hp+ patients compared with the Hp–: 0.08 (0.02–0.5) vs 0.3 (0.2–0.5) (p<0.003). Median antral SST mRNA expression was also significantly less in the Hp+ patients: 0.10 (0.04–0.4) vs 0.39 (0.27–1.04). SST mRNA was significantly more abundant in the antrum than the corpus of Hp– patients (p<0.03).

Conclusions: Expression of SST mRNA is decreased in the corpus of Hp+ non-ulcer patients, consistent with the finding of decreased SST peptide in these people. This might be because corpus gastritis is typically present in Hp+ non-ulcer patients but characteristically absent in Hp– DU patients. Both groups have antral gastritis with consequent reduced SST mRNA expression.
EFFECT OF H. PYLORI CONSTITUENTS AND INFLAMMATORY CYTOKINES ON GASTRIN RELEASE FROM CULTURED CANINE G CELLS. I. Beales, L. Post, S. Sinivasan, J. Blaser*, J. Scheiman, J. Calam*, T. Yamada, J. Del Valle, Dept. of Internal Medicine, University of Michigan, Ann Arbor, USA, Vanderbilt University*, Nashville, TN, USA and Dept. of Medicine, Hammersmith Hospital, UK.

Introduction: We are asking how H. pylori(Hp) releases the acid-stimulating hormone gastrin. Release might be due to bacterial components, or cytokines released in Hp gastritis, which include tumour necrosis factor alpha (TNFα) and interleukin 8 (IL8).

Methods: Canine antral G cells were enriched by elution and cultured for 40 hours. G cells were incubated with test substances for 2 h. Gastrin release was measured by RIA. Results: Hp sonicates (0.5-50 μg/ml) and lipopolysaccharide (0.5-50 μg/ml) from different isolates had no significant effect on basal or stimulated gastrin release. IL8: 1 nM and 10 nM released gastrin (34 ± 16% and 43 ± 24% above control respectively, mean ± SE: both p<0.05). The effect of IL8 was inhibited by the somatostatin analogue octreotide (1μM), suggesting that gastrin release was a reversible regulatory effect. TNFα (0.1-100 ng/ml) did not affect basal gastrin release. Cell viability was not impaired by any of the test substances. When tested in the presence of IL8, Hp sonicates from 2 out of 3 strains enhanced the IL8-stimulated gastrin release in a dose dependent manner. In combination with IL8, 1 nM and 10 nM, maximal stimulation was 107-125% and IL8 285-362% above basal respectively.

Conclusions: The proinflammatory cytokine IL8 can stimulate gastrin release from antral G cells. The combination of Hp sonicate and IL8 was significantly more potent than either agent alone. An interaction between cytokines and Hp products may contribute to increased gastrin release in patients infected with Helicobacter pylori.

AN ACIDIC ENVIRONMENT INHIBITS H. PYLORI TOXIN-DEPENDENT CELL VACUOLATION IN VITRO. P. Sommer 1,2, V. Ricci 1, R. Fiocca 2, M. Romano 3, K. Ivey 4, E. Scolia 2, U. Ventura 1.

Instit. Human Physiology & Dept. Human Pathology, Univ.Pavia & IRCCS Policlinico San Matteo, Pavia, Dept. Medicine, Univ.Naples, ITALY; Dept. Medicine, VMHC, Long Beach, CA, USA.


AIM: To verify whether pH influences the vacuolating activity of H. pylori broh cell cultures (BCFs) on cultured gastric epithelial cells.

METHODS: We used BCFs from CCUG 17874 (tox+/urease+) and G21 (tox-/urease-) H. pylori strains. Culture was uninoculated broth filtrate. Cultures of H. pylori 28 cell line were incubated for 16h with control and BCFs at pH 6.2 and 7.4. After incubation, cell vacuolation was evaluated by neutral red dye uptake. Statistics: analysis of variance and Newman-Keuls’ Q test.

RESULTS: Neutral red dye uptake (ng/μg cell protein)

<table>
<thead>
<tr>
<th>pH</th>
<th>G21</th>
<th>CCUG 17874</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2</td>
<td>78 +/-2</td>
<td>110 +/-4</td>
</tr>
<tr>
<td>7.4</td>
<td>115 +/-7</td>
<td>533 +/-36</td>
</tr>
</tbody>
</table>

Means ± SEM * P<0.05 vs control at the same pH # P<0.05 vs G21 at the same pH ** P<0.05 vs itself at pH 6.2

CONCLUSIONS: an acidic environment: 1) inhibited the toxin-dependent cell vacuolation (CCUG 17874 vs G21 strain); 2) reduced the ammonia-dependent cell vacuolation (G21 strain at pH 6.2 vs pH 7.4).

A COMPARISON OF GASTRIC MUCOSAL LIPID COMPOSITION IN HELICOBACTER PYLORI INFECTED AND NON-INFECTED SUBJECTS. J.T. Anderson, C. Gallagher, D. Hopwood, P.E. Murray, P.E. Ross. Ninewells Hospital and Medical School, University of Dundee, Dundee, Scotland. DD1 9SY.

Introduction: Recent studies have suggested that H. pylori may ingest gastric lipid from the mucosal surface; and also that H. pylori infection may alter gastric mucosal lipid composition. The aim of this study was to compare phospholipids and their acyl groups in gastric mucosal biopsies from subjects with and without H. pylori infection.

Methods: We studied 15 patients with H. pylori infection and 22 age- and sex-matched controls with non-ulcer dyspepsia and normal endoscopic appearance. Gastric biopsies were taken for urease testing, histology, and lipid analysis. Phospholipid analysis was by TLC, and acyl group assay by capillary column gas liquid chromatography.

Results: Although gastric inflammation was consistently more severe in H. pylori infected subjects than controls, there was no significant difference in mucosal phospholipid composition (see table). Total phospholipid acyl groups were similar in both groups. Palmitate, stearate, oleate and linoleate comprised 18% and arachidonate 12% of the total. Neutral lipids were unchanged by H. pylori infection.