**Helicobacter pylori** non-cytotoxic genotype enhances mucosal gastrin and mast cell tryptase

D Basso, F Navaglia, L Brigato, F Di Mario, M Rugge, M Plebani

**Abstract**

**Aims**—To determine the association, if any, between *H pylori* genotype and the gastric mucosal variations in the levels of gastrin, somatostatin, tryptase, and histamine.

**Methods**—49 patients affected by duodenal ulcer and 48 by non-ulcer dyspepsia were studied. To identify the *H pylori* genotype, the presence of the cagA gene and vacA alleles m1, m2, s1, and s2 were analysed by polymerase chain reaction. Gastrin, somatostatin, tryptase, and histamine were measured in antral mucosal biopsies.

**Results**—57 patients were infected with *H pylori* (30 with duodenal ulcer and 27 with non-ulcer dyspepsia). Gastrin and tryptase were increased in patients with *H pylori* infection, although the variations were statistically significant only for gastrin; somatostatin and histamine were not influenced by *H pylori* infection. In patients with non-ulcer dyspepsia the absence of the cagA gene and the presence of vacA alleles s2 and m2 were associated with higher values of tryptase and to a lesser extent of gastrin. These associations were not found in patients with duodenal ulcer.

**Conclusions**—The cagA negative s2m2 strain of *H pylori* may be less dangerous for the gastric mucosa than other *H pylori* strains since it enhances tryptase production by gastric mucosal mast cells; this enzyme is thought to stimulate tissue turnover and favour wound healing.

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**Keywords:** Helicobacter pylori; mast cells; tryptase; gastrin

The Gram negative bacterium *Helicobacter pylori* colonises the gastric mucosa, at the antrum in particular. *H pylori* infection has several sequelae, including antral gastritis, chronic atrophic gastritis, gastric and duodenal ulcer, gastric adenocarcinoma, and MALToma.1–4 This wide spectrum of diseases from the same microorganism may depend on differences in the host response, but also on differences between the strains of *H pylori* causing infection.5–7 Two types of *H pylori* have been described: type I produces a vacuolating cytotoxin which plays a fundamental role in *H pylori* pathogenicity.8–11 This cytotoxin is not produced by type II strains. The cagA gene is also often found in type I strains.12 Encoding a highly immunogenic protein, this gene, almost completely absent in type II strains, is considered to be a marker of *H pylori* aggressiveness and is located inside a pathogenicity island of *H pylori*, which encodes specific virulence factors.13–15

*H pylori* infection results in a cascade of biochemical events in the gastric mucosa, including enhanced gastrin release after a meal caused by reduced somatostatin production.16–17 In addition, microscopic mucosal inflammation characterised by infiltration with mononuclear and polymorphonuclear leucocytes is found in most *H pylori* infected patients.18–19 Gastric mucosal inflammation associated with *H pylori* infection is mediated by release of cytokines, which is greater when the infecting strain is cagA positive.18

In the inflammatory process, mast cells play a pivotal role as initiators and regulators of inflammation.20–24 Found in relatively large numbers next to blood and lymphatic vessels, mast cells are most prominent immediately beneath the epithelial surfaces of the skin and the mucosas of the respiratory, genitourinary, and gastrointestinal tracts.25–27 As they play a key role in infection and immunity and are present in large numbers (about 20 000/mm²) in the mucosa of the gastrointestinal tract, it is likely that mast cells participate in the pathophysiology of *H pylori* associated gastroduodenal diseases. In particular, *H pylori* downregulates histamine release from mast cells isolated from rat peritoneum29; water extracts of *H pylori* cause rat mesentery mast cells to degranulate30; and in patients with *H pylori* infection, the density of gastric mucosal mast cells is increased.31

As no data are available on the different effects of *H pylori* strains on gastric mast cells, our aims in this study were to determine whether there is an association between the *H pylori* genotype and the function of gastric mucosal D, G, and mast cells, and to verify whether *H pylori* induced gastritis may play any role in this context.

**Methods**

We studied 97 patients (52 males, 45 females, age range 17 to 79 years). The diagnoses, made on the basis of endoscopic and microscopic findings and taking into account the clinical history of each patient, were duodenal ulcer (n = 49) and non-ulcer dyspepsia (n = 48). All the patients underwent upper gastrointestinal endoscopy, during which samples of gastric juice and six antral and two body biopsies were obtained. Two of the antral and one body biopsy were used for the histological evaluation (haematoxylin and eosin staining); two further antral biopsies and the remaining body biopsy were used for the histological assessment of
Table 1 Comparison of histological scores for patients with duodenal ulcer or non-ulcer dyspepsia

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Antrum</th>
<th>Body</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CI</td>
<td>PMN</td>
</tr>
<tr>
<td>H pylori positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-ulcer dyspepsia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0–3</td>
<td>1–3</td>
</tr>
<tr>
<td>Median</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>1–3</td>
<td>1–3</td>
</tr>
<tr>
<td>Median</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>H pylori negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-ulcer dyspepsia</td>
<td></td>
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</tr>
<tr>
<td>Range</td>
<td>0–2</td>
<td>0</td>
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<tr>
<td>Median</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td>1–3</td>
<td>0</td>
</tr>
<tr>
<td>Median</td>
<td>1.0</td>
<td>0</td>
</tr>
</tbody>
</table>

CI, chronic inflammation; Hp, H pylori colonisation density; PMN, polymorphonuclear cell infiltrate.

**H pylori** infection (Giems and/or Warthin–Starry staining); the gastric juice sample was used for pH measurement (Crisom pHmeter) and for the polymerase chain reaction (PCR); the remaining two antral biopsies were used for histamine, somatostatin, gastrin, and tryptase determinations.

Immediately after sampling, the latter two specimens, were transferred to a test tube containing hexane and cooled to −70°C in a solid CO₂ acetone bath and then stored at −20°C until homogenised using a technique described elsewhere. For both antral and body biopsies the following features were evaluated and graded according to the updated Sydney system: atrophy, chronic inflammation, polymorphonuclear cell activity (neutrophil infiltration), and H pylori colonisation density. DNA was extracted from gastric juice. We then amplified the following H pylori genes by PCR: urease A (ureA), cagA, and vacA polymorphism under previously described conditions. In mucosal homogenates we measured the following: (1) total protein content by Bradford’s method, using reagents from Bio-Rad Laboratories; (2) tryptase, using a solid phase immunoradiometric assay (Kabi Pharmacia Tryptase Riact); (3) gastrin, histamine, and somatostatin by radioimmunoassay (Sorin Biomedica; Pharmacia; and Euro-Diagnostica, respectively). The results for tryptase, gastrin, histamine, and somatostatin were referred to the protein concentration.

**STATISTICS**

Statistical analysis of the data was made using analysis of variance (ANOVA one way), Bonferroni’s test for pairwise comparisons, the Kruskal–Wallis test, the Mann–Whitney U test, linear regression analysis, and Spearman’s regression analysis.

**Results**

**H pylori infection and gastritis**

The histological assessment of H pylori and ureA positivity in gastric juice by PCR gave overlapping results. With both procedures, H pylori infection was found in 57 cases (30 duodenal ulcer and 27 non-ulcer dyspepsia), the remaining 40 patients being H pylori negative. Of these, 32 (16 of 19 duodenal ulcer and 16 of 21 non-ulcer dyspepsia) had been treated for H pylori infection at least three months before the study.

The frequency of antral mucosal atrophy did not differ between H pylori positive and H pylori negative patients with duodenal ulcer or non-ulcer dyspepsia (χ² = 4.55, NS).

Table 1 shows the histological scores found in the antrum and the body for H pylori positive or negative patients with duodenal ulcer or non-ulcer dyspepsia. Chronic inflammation in the antrum was more severe in H pylori positive than in H pylori negative patients; when H pylori positive and H pylori negative patients were considered separately, higher grades of antral inflammation were found in duodenal ulcer patients than in patients with non-ulcer dyspepsia (Kruskal–Wallis H = 4.52, p < 0.05 for H pylori positive and H = 6.51, p < 0.05 for H pylori negative).

Chronic inflammation in the body of the stomach more severe in H pylori positive patients than in H pylori negative patients (H = 51.38, p < 0.001), but no differences between duodenal ulcer and non-ulcer dyspepsia were found (H = 1.88, NS for H pylori positive and H = 0.11, NS for H pylori negative).

In H pylori positive patients, polymorphonuclear infiltration in the antrum was more pronounced in duodenal ulcer than in non-ulcer dyspepsia (H = 5.09, p < 0.05). This was not the case for the body of the stomach (H = 0.44, NS).

No differences between duodenal ulcer and non-ulcer dyspepsia patients were found in relation to H pylori colonisation density, either in the antrum or in the body (H = 2.13, NS, and H = 0.47, NS). The grade of both the antral and the body polymorphonuclear infiltrate was correlated with the corresponding H pylori density (Spearman’s r = 0.397, p < 0.001, and r = 0.750, p < 0.001).

The cagA gene was positive in 39 cases, which included 24 of the 30 H pylori positive patients.

Table 2 Antral tryptase, gastrin, histamine, and somatostatin (SMS) in the patients studied

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Tryptase (µg/g)</th>
<th>Gastrin (µg/g)</th>
<th>Histamine (µg/g)</th>
<th>SMS (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>DU Hp pos (n=30)</td>
<td>104</td>
<td>14</td>
<td>12</td>
<td>1.5</td>
</tr>
<tr>
<td>DU Hp neg (n=19)</td>
<td>121</td>
<td>20</td>
<td>12</td>
<td>1.5</td>
</tr>
<tr>
<td>NUD Hp pos (n=27)</td>
<td>155</td>
<td>16</td>
<td>25*</td>
<td>3.1</td>
</tr>
<tr>
<td>NUD Hp neg (n=21)</td>
<td>125</td>
<td>15</td>
<td>14</td>
<td>3.1</td>
</tr>
</tbody>
</table>

ANOVA, one way F=2.07 NS, F=6.38 p<0.001, F=2.24 NS, F=0.33 NS.

DU, duodenal ulcer; Hp, H pylori; neg, negative; pos, positive; NUD, non-ulcer dyspepsia.
with duodenal ulcer and 15 of the 27 with non-ulcer dyspepsia. Alles s1 and m1 were found in 37 of 39 and in 28 of 39 cagA positive patients, and in seven of 18 and two of 18 cagA negative patients. The cagA gene and the m1 allele of vacA were significantly associated with a higher grade of polymorphonuclear cell infiltration of both the antrum (Mann-Whitney U test: z = 1.82, p < 0.05 and z = 2.47, p < 0.01) and the body (z = 3.28, p < 0.001 and z = 2.16, p < 0.05). The s1 allele of vacA was associated with a higher degree of polymorphonuclear cell infiltration (z = 2.39, p < 0.01) and inflammation (z = 1.82, p < 0.05) in the antrum but not in the body.

**Figure 1** Individual values of mucosal tryptase and gastrin in *H pylori* positive patients with non-ulcer dyspepsia subdivided on the basis of cagA positive (cagA+) or negative (cagA−) findings. For each patient the results for vacA alleles are also reported.

**Discussion**

We have confirmed here that gastric histology and the polymerase chain reaction give overlapping results in diagnosing *H pylori* infection.31 Also, and in agreement with previous findings by Warburton et al,19 we found that the infection was localised to the antrum and the body of the stomach, and *H pylori* colonisation density was directly correlated with the degree of polymorphonuclear cell infiltration. In *H pylori* positive patients, the degree of chronic inflammation and polymorphonuclear infiltration involving the gastric body was slightly more severe in patients with duodenal ulcer than in those with non-ulcer dyspepsia, in contrast to the findings of Warburton et al.19

It is now clear that the *H pylori* species is heterogeneous, comprising different strains. We typed the genome of the infecting strain of *H pylori* in our patients by defining cagA and vacA status. As in previous studies,31−33 patients with duodenal ulcer were infected mainly by cagA positive *H pylori* strains possessing the s1 and m1 alleles of vacA (type I), while the cagA gene was present in about half of the patients with non-ulcer dyspepsia. In agreement with Warburton et al,33 we observed that infections caused by cagA positive, vacA s1m1 strains tended to be associated with a major degree of polymorphonuclear cell infiltration of the gastric mucosa.

*H pylori* associated chronic mucosal inflammation might alter normal gastric physiology, and in particular peptic acid secretion, either directly or through alterations to cells involved in the regulation of the gastric secretory process, such as antral G or D cells, enterochromaffin-like (ECL) cells, and mast cells. Mast cells play a crucial role in the pathophysiology of several chronic gastrointestinal inflammatory conditions.33 To investigate the influence of *H pylori* on mast cells, we measured the mucosal levels of tryptase and histamine. In human gastric mucosa the former is present in mast cells only,34 while the latter originates both from mast cells and ECL cells.35 We found no significant differences in mucosal tryptase and histamine levels between the groups studied. These preliminary findings suggest that *H pylori* infection does not affect the function of mast cells. However, in patients with non-ulcer dyspepsia, tryptase levels tended to be somewhat higher than in patients with other causes of gastric inflammation. To clarify this finding we focused our attention on gastrin, since in our previous papers we showed the presence of a gastrin–
tryptase link. This was confirmed in the present study. Furthermore, mucosal gastrin levels were significantly higher in *H pylori* positive patients with non-ulcer dyspepsia than in all other patient groups. These findings confirm recent data from Gillen et al showing that *H pylori* seems to influence gastrin levels mainly in non-ulcer dyspepsia patients since it reduces their sensitivity to gastrin more than in patients with duodenal ulcer.\(^37\)

The mechanism whereby *H pylori* influences gastrin is not clear, although it has been suggested that it promotes enhanced gastrin release by reducing somatostatin production.\(^15\)\(^-\)\(^17\) To verify this, we measured mucosal levels of somatostatin in our material, but we found no correlation with *H pylori* infection, nor was somatostatin correlated with gastrin. The discrepancy between these results and those published previously\(^16\)\(^-\)\(^17\) may depend upon the different approaches used to measure somatostatin: Moss et al\(^3\) and Graham et al\(^4\) measured somatostatin mRNA and the number of antral D cells, respectively, while we evaluated somatostatin concentration. Haruma et al\(^5\), who also evaluated the mucosal concentration of somatostatin, found low levels of this hormone in *H pylori* positive patients, but the numbers were very small (six cases). We postulated that *H pylori* may affect the function of antral G cells directly or indirectly, through mucosal inflammation or by enhancing gastric juice pH, or both. Chronic mucosal inflammation, either of the antrum or of the body, was not related to mucosal gastrin or somatostatin levels, but gastric body polymorphonuclear infiltration was correlated with gastric juice pH. This was higher in *H pylori* positive patients with non-ulcer dyspepsia and was correlated with mucosal gastrin. This suggests that the involvement of the gastric body by *H pylori* infection in patients with non-ulcer dyspepsia may impair gastric acid secretion, leading to hypergastrinaemia.

We then ascertained whether the *H pylori* genotype may influence mucosal levels of tryptase, gastrin, histamine, or somatostatin. In patients with duodenal ulcer no significant variations in these were found, and cagA positive s1m1 *H pylori* strains played a major role in causing the infection. Therefore, as expected, no significant association between these variables was found. On the other hand, in patients with non-ulcer dyspepsia, the higher levels of mucosal tryptase were associated with the s2 or m2 alleles of vacA and with cagA negative strains of *H pylori*. In these patients, higher levels of mucosal gastrin were associated with the m2 allele of vacA, while histamine and somatostatin were not influenced by *H pylori* strains. These findings are surprising—in non-ulcer dyspepsia the infection sustained by less virulent *H pylori* strains causes increased production of tryptase by mast cells, possibly through gastrin stimulation. These data might be interpreted in the light of the role of tryptase in wound healing: this mast cell enzyme has been found to be a potent mitogen for epithelial cell proliferation, which is one of the final steps in the healing process.\(^38\)\(^-\)\(^39\) *H pylori* infection sustained by type II strains may cause less gastric damage than type I strains, since the former may not only cause less extensive epithelial cell damage, but may also favour the defence mechanisms of the stomach against mucosal injury.

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