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

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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.⁵⁻⁸ Two types of *H pylori* have been described: type I produces a vacuolating cytotoxin which plays a fundamental role in *H pylori* pathogenicity.⁵⁻¹¹ 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* aggres-

siveness and is located inside a pathogenicity island of *H pylori*, which encodes specific virulence factors.¹³

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.¹⁴⁻¹⁷ 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.^{4, 20-24}

In the inflammatory process, mast cells play a pivotal role as initiators and regulators of inflammation.²⁵ 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 mucosae of the respiratory, genitourinary, and gastrointestinal tracts.²⁵ As they play a key role in infection and immunity and are present in large numbers (about 20 000/mm³) 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 peritoneum²⁶; water extracts of *H pylori* cause rat mesentery mast cells to degranulate²⁷; and in patients with *H pylori* infection, the density of gastric mucosal mast cells is increased.²⁸

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

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Table 1 Comparison of histological scores for patients with duodenal ulcer or non-ulcer dyspepsia

	Antrum			Body		
	CI	PMN	Hp	CI	PMN	Hp
H pylori positive patients						
Non-ulcer dyspepsia						
Range	0–3	1–3	1–3	0–3	0–3	1–3
Median	2.0	1.5	2.0	2.0	1.0	2.0
Duodenal ulcer						
Range	1–3	1–3	1–3	0–2	0–3	1–3
Median	2.0	2.0	2.0	2.0	1.0	2.0
H pylori negative patients						
Non-ulcer dyspepsia						
Range	0–2	0	0	0–1	0	0
Median	1.0	0	0	1.0	0	0
Duodenal ulcer						
Range	1–3	0	0	0–2	0	0
Median	1.0	0	0	2.0	0	0

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

H pylori infection (Giemsa and/or Wartin–Starry staining); the gastric juice sample was used for pH measurement (Crison 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 CO_2 acetone bath and then stored at -20°C until homogenised using a technique described elsewhere.²⁹ For both antral and body biopsies the following features were evaluated and graded according to the updated Sydney system³⁰: atrophy, chronic inflammation, polymorphonuclear cell activity (neutrophil infiltration), and *H pylori* colonisation density. DNA was extracted from gastric juice.³¹ We then amplified the following *H pylori* genes by PCR: urease A (ureA), cagA, and vacA polymorphism under previously described conditions.³² In mucosal homogenates we measured the following: (1) total protein content by Bradford's method,³³ 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 ($\chi^2 = 4.55$, 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

Diagnosis	Tryptase (U/g)		Gastrin ($\mu\text{g/g}$)		Histamine ($\mu\text{g/g}$)		SMS ($\mu\text{g/g}$)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
DU Hp pos (n=30)	104	14	12	1.5	21	7.3	925	113
DU Hp neg (n=19)	121	20	12	1.9	24	7.5	914	89
NUD Hp pos (n=27)	155	16	25*	3.1	28	7.1	934	64
NUD Hp neg (n=21)	125	15	14	3.1	51	12.3	1044	110
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.

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.

with duodenal ulcer and 15 of the 27 with non-ulcer dyspepsia. Alleles *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.

H. PYLORI, TRYPTASE, GASTRIN, SOMATOSTATIN, HISTAMINE, AND pH

Table 2 shows mean values, standard errors of the mean, and a statistical analysis of tryptase, gastrin, histamine, and somatostatin mucosal concentrations in the patients studied.

Gastric juice pH (mean (SEM)) was significantly higher in *H. pylori* infected than in *H. pylori* non infected patients, at 3.04 (0.33) *v* 1.91 (0.22) (Student's *t* test: $t = 2.77$, $p < 0.01$). Gastric juice pH correlated with the grade of polymorphonuclear cell infiltration in the body (Spearman's $r = 0.248$, $p < 0.05$) but not in the antrum (Spearman's $r = 0.211$, NS).

There was a positive correlation between mucosal tryptase and gastrin values ($r = 0.290$, $p < 0.01$); mucosal gastrin was also correlated with gastric juice pH values ($r = 0.456$, $p < 0.001$). None of the mucosal indices evaluated was correlated with the grade of gastritis or with the grade of polymorphonuclear cell infiltrate of the antrum or the body.

cagA, *vacA*, TRYPTASE, GASTRIN, SOMATOSTATIN, AND HISTAMINE

The individual data for tryptase and gastrin in patients with non-ulcer dyspepsia subdivided on the basis of the presence or absence of *cagA* are shown in fig 1, together with data on *vacA*

polymorphism for each subject. The mean levels of tryptase were significantly higher in patients with the *cagA* negative genotype (Student's *t* test: $t = 2.30$, $p < 0.05$), or who were positive for the *S2* ($t = 2.05$, $p < 0.05$) or *M2* alleles ($t = 2.90$, $p < 0.01$) of *vacA*. A similar pattern was observed for mucosal gastrin, although significant differences were found only in relation to the *M1* and *M2* alleles of *vacA* ($t = 3.62$, $p < 0.01$).

Mucosal somatostatin and histamine values were not influenced by *cagA* or by the *vacA* genotype.

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,^{6-8, 32} 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.*,¹⁹ 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.²⁵ 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.³⁴⁻³⁶ 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 previously¹⁵⁻¹⁷ may depend upon the different approaches used to measure somatostatin: Moss *et al.*¹⁵ and Graham *et al.*¹⁶ measured somatostatin mRNA and the number of antral D cells, respectively, while we evaluated somatostatin concentration. Haruma *et al.*,¹⁷ 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 hypothesised 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.²⁵⁻³⁸ *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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- 1 Bayerdorffer E, Neubauer A, Rudolph B, *et al.* Regression of primary gastric lymphoma of mucosa-associated lymphoid tissue type after cure of *Helicobacter pylori* infection. *Lancet* 1995;345:1591-4.
- 2 Calvert R, Randerson J, Evans P, *et al.* Genetic abnormalities during transition from *Helicobacter pylori*-associated gastritis to low-grade MALToma. *Lancet* 1995;345:26-7.
- 3 McFarlane GA, Munro A. *Helicobacter pylori* and gastric cancer. *Br J Surg* 1997;84:1190-9.
- 4 Peek RM, Blaser MJ. Pathophysiology of *Helicobacter pylori*-induced gastritis and peptic ulcer disease. *Am J Med* 1997;102:200-7.
- 5 Blaser MJ. *Helicobacter pylori* phenotypes associated with peptic ulceration. *Scand J Gastroenterol* 1994;29(suppl 205):1-5.
- 6 Atherton JC, Cao P, Peek RM, *et al.* Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995;270:17771-7.
- 7 Cover TL. The vacuolating cytotoxin of *Helicobacter pylori*. *Mol Microbiol* 1996;20:241-6.
- 8 Atherton JC, Peek RM, Tham KT, *et al.* Clinical and pathological importance of heterogeneity in vacA, the vacuolating cytotoxin gene of *Helicobacter pylori*. *Gastroenterology* 1997;112:92-9.
- 9 Atherton JC. The clinical relevance of strain types of *Helicobacter pylori*. *Gut* 1997;40:701-3.
- 10 Phadnis SH, Ilver D, Janzon L, *et al.* Pathological significance and molecular characterization of the vacuolating toxin gene of *Helicobacter pylori*. *Infect Immun* 1994;62:1157-65.
- 11 Xiang Z, Censini S, Bayeli PF, *et al.* Analysis of expression of CagA and VacA virulence factors in 43 strains of *Helicobacter pylori* reveals that clinical isolates can be divided into two major types and that CagA is not necessary for expression of the vacuolating cytotoxin. *Infect Immun* 1995;63:94-8.
- 12 Covacci A, Censini S, Bugnoli M, *et al.* Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* 1993;90:5791-5.
- 13 Censini S, Lange C, Xiang Z, *et al.* cagA, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci USA* 1996;93:14648-53.
- 14 Graham DY, Opekun A, Lew GM, *et al.* *Helicobacter pylori*-associated exaggerated gastrin release in duodenal ulcer patients. *Gastroenterology* 1991;100:1571-5.
- 15 Moss SF, Legon S, Bishop AE, *et al.* Effect of *Helicobacter pylori* on gastric on gastrin somatostatin in duodenal ulcer disease. *Lancet* 1992;340:930-2.
- 16 Graham DY, Lew GM, Lechago J. Antral G-cell and D-cell numbers in *Helicobacter pylori* infection: effect of H. pylori eradication. *Gastroenterology* 1993;104:1655-60.
- 17 Haruma K, Sumii K, Okamoto S, *et al.* *Helicobacter pylori* infection is associated with low antral somatostatin content in young adults. *Scand J Gastroenterol* 1995;30:550-3.
- 18 Plebani M, Basso D, Cassaro M, *et al.* *Helicobacter pylori* serology in patients with chronic gastritis. *Am J Gastroenterol* 1996;91:954-8.
- 19 Warburton VJ, Everett S, Mapstone NP, *et al.* Clinical and histological associations of cagA and vacA genotypes in *Helicobacter pylori* gastritis. *J Clin Pathol* 1998;51:55-61.
- 20 Crabtree JE, Farmery SM, Lindley IJD, *et al.* CagA/cytotoxic strains of *Helicobacter pylori* and interleukin-8 in gastric epithelial cell lines. *J Clin Pathol* 1994;47:945-50.
- 21 Gionchetti P, Vaira D, Campieri M, *et al.* Enhanced mucosal interleukin-6 and -8 in *Helicobacter pylori*-positive dyspeptic patients. *Am J Gastroenterol* 1994;89:883-7.
- 22 Moss SF, Legon S, Davies J, *et al.* Cytokine gene expression in *Helicobacter pylori* associated antral gastritis. *Gut* 1994;35:1567-70.
- 23 Crowe SE, Alvarez L, Dytoc M, *et al.* Expression of interleukin 8 and CD54 by human gastric epithelium after *Helicobacter pylori* infection in vitro. *Gastroenterology* 1995;108:65-74.
- 24 Basso D, Scrigner M, Toma A, *et al.* *Helicobacter pylori* infection enhances mucosal interleukin-1 beta, interleukin-6, and the soluble receptor of interleukin-2. *Int J Clin Lab Res* 1996;26:207-10.
- 25 Abraham SN, Malaviya R. Mast cells in infection and immunity. *Infect Immun* 1997;65:3501-8.
- 26 Bechi P, Dei R, Di Bello MG, *et al.* *Helicobacter pylori* potentiates histamine release from serosal rat mast cells in vitro. *Dig Dis Sci* 1993;38:944-9.
- 27 Kurose I, Granger DN, Evans DJ, *et al.* *Helicobacter pylori*-induced microvascular protein leakage in rats: role of neutrophils, mast cells, and platelets. *Gastroenterology* 1994;107:70-9.
- 28 Nakajima S, Krishnan B, Ota H, *et al.* Mast cell involvement in gastritis with or without *Helicobacter pylori* infection. *Gastroenterology* 1997;113:746-54.

- 29 Plebani M, Di Mario F, Dal Santo PG, et al. Measurement of pepsinogen group I in endoscopic gastroduodenal biopsies. *Clin Chem* 1990;36:682-4.
- 30 Dixon MF, Genta RM, Yardley JH, et al. Classification and grading of gastritis. The updated Sydney system. *Am J Surg Pathol* 1996;20:1161-81.
- 31 Basso D, Navaglia F, Cassaro M, et al. Gastric juice polymerase chain reaction: an alternative to histology in the diagnosis of *Helicobacter pylori* infection. *Helicobacter* 1996;1:159-64.
- 32 Navaglia F, Basso D, Piva MG, et al. *Helicobacter pylori* cytotoxic genotype is associated with peptic ulcer and influences serology. *Am J Gastroenterol* 1998;93:227-30.
- 33 Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
- 34 Plebani M, Basso D, Busatto G, et al. Are tryptase and cathepsin D related to *Helicobacter pylori* infection and mucosal gastrin in peptic ulcer? *Res Exp Med* 1994;194:1-8.
- 35 Bechi P, Romagnoli P, Panula P, et al. Gastric mucosal histamine storing cells. Evidence for different roles of mast cells and enterochromaffin-like cells in humans. *Dig Dis Sci* 1995;40:2207-13.
- 36 Plebani M, Basso D, Busatto G, et al. Gastrin stimulates gastric mast cells in rabbits. *Res Exp Med* 1995;195:365-71.
- 37 Gillen D, El-Omar EM, Wirtz AA, et al. The acid response to gastrin distinguishes duodenal ulcer patients from *Helicobacter pylori*-infected healthy subjects. *Gastroenterology* 1998;114:50-7.
- 38 Cairns JA, Walls AF. Mast cell tryptase is a mitogen for epithelial cells. Stimulation of IL-8 production and intercellular adhesion molecule-1 expression. *J Immunol* 1996;156:275-83.