ELSEVIER

FEMS Immunology and Medical Microbiology 36 (2003) 181–185

FEMS Immunology and Medical Microbiology

www.fems-microbiology.org

Clinical relevance of the *cag*A, *vac*A and *ice*A genotypes of *Helicobacter pylori* in Brazilian clinical isolates

Marcelo Lima Ribeiro, Anita Paula Ortiz Godoy, Yune Helena Borges Benvengo, Sergio Mendonça, José Pedrazzoli Jr. *

Clinical Pharmacology and Gastroenterology Unit, São Francisco University Medical School, Av. São Francisco de Assis, 218, 12916-900 Bragança Paulista, SP, Brazil

Received 4 July 2002; received in revised form 16 October 2002; accepted 1 December 2002

First published online 22 February 2003

Abstract

Infection with *Helicobacter pylori* strains harboring determinants of pathogenicity may lead to a strong inflammatory response in gastric mucosa. In this work, we examined the frequency of the *cag*A, *vac*A and *ice*A genotypes in *H. pylori* strains isolated from Brazilian patients and correlated these with the clinical manifestations. *H. pylori* was isolated from 165 patients [30 with non-ulcer dyspepsia cases (NUD); 93 peptic ulcer disease (PUD): 31 gastric ulcers (GU) and 62 duodenal ulcer disease (DU); 18 with erosive gastritis (EG); and 24 gastroesophageal reflux disease (GERD)]. Allelic variants of *cag*A, *vac*A and *ice*A were identified using the polymerase chain reaction. More than one *H. pylori* strain was detected in 28 cases (17%), and these were excluded from the statistical analysis. We were unable to confirm an association between *ice*A status and clinical outcome. There was a strong association between the genotype *cag*A-positive *vac*A s1 and PUD. However, logistic regression analysis showed that *vac*A s1 was the only predictive factor for PUD (OR = 4.19; 95% CI 1.95–8.98). The presence of the less virulent strain *vac*A s2 was related to GERD (OR = 8.59; 95% CI 2.85–25.91). Our results support the hypothesis that virulent strains may protect against the development of GERD.

Keywords: *cag*A; *vac*A; *ice*A; *H. pylori*

1. Introduction

Helicobacter pylori is a Gram-negative, microaerophilic, curved bacterium that persistently colonizes the human stomach, where it establishes a long-term infection of gastric and duodenal mucosa [1,2]. *H. pylori* has worldwide distribution, and its prevalence ranges from 25% in developed countries to more than 90% in developing areas, although not all infected individuals develop clinically relevant disease [2]. Colonization with *H. pylori* results in chronic superficial gastritis, which increases the risk for the development of duodenal and gastric ulcers, gastric adenocarcinoma, or non-Hodgkin's gastric lymphoma [3].

The first strain-specific gene identified in *H. pylori* was *cag*A (cytotoxin-associated gene), considered as a marker for the presence of a pathogenicity island about 40 kb. The presence of an intact *cag* pathogenicity island is associated with higher interleukin-8 levels and mucosal inflammation [4]. As a result, individuals infected with *cag*A-positive *H. pylori* strains have a higher risk for peptic ulcers and gastric cancer [5,6].

The *vac*A gene encodes a vacuolating toxin excreted by *H. pylori* that can damage epithelial cells [7]. This gene is present in all strains and comprises two variable parts [8]. The s-region encoding the signal peptide is located at the 5′ end of the gene, and exists as an s1 or s2 allele [5]. The m-region (middle) occurs as an m1 or m2 allele. The production of vacuolating cytotoxin is related to the mosaic combination of s and m allelic types. Strains that possess a *vac*A s1 signal allele are associated with peptic ulcer disease [5,6,8,9]. The mosaic combination of the *vac*A gene has been associated to specific genotypes with different outcomes, especially duodenal ulcer disease [5,8,10].

Recently, a novel gene designated *ice*A (induced by contact with epithelium) has been described. There are two main allelic variants of this gene, *ice*A1 and *ice*A2, but only *ice*A1 RNA is induced following adherence in vitro

* Corresponding author. Tel.: +55 (11) 4034 8134; Fax: +55 (11) 4034 1825.
E-mail address: pedrazzoli@saofrancisco.edu.br (J. Pedrazzoli Jr.).

0928-8244/03/$22.00
doi:10.1016/S0928-8244(03)00029-4

[11]. Although *ice*A1 may be associated with peptic ulcer disease [5,11], other studies have failed to confirm this correlation, and some groups have suggested a reverse relationship [9]. Peek et al. [12] demonstrated that *ice*A1 expression was significantly related to the host mucosal response, and this led to the hypothesis that the levels of transcription within the host environment may contribute to disease development. In contrast, *ice*A2 expression may be more influenced by gene structure.

Gastroesophageal reflux disease (GERD) results from exposure of the esophagus to acid [13]. There is no apparent causal relationship between *H. pylori* colonization and the presence of GERD and its complications [14], although GERD patients are infected with less virulent *H. pylori* strains, most of which do not have *cag*A, *vac*A s1 or *ice*A1. This finding supports the hypothesis that virulent strains may protect against the development of GERD [6,15].

Since infection with *H. pylori* harboring of determinants pathogenicity may lead to a strong inflammatory response in the gastric mucosa [4], we have examined the *cag*A, *vac*A and *ice*A genotypes of *H. pylori* strains isolated from Brazilian patients and correlated these with the clinical manifestations.

2. Materials and methods

2.1. Patients and definition of lesions

H. pylori isolates were obtained from 165 patients (95 males and 70 females; age 45.9 ± 15.8 years) living in southeastern Brazil. There was no significant difference in the age distribution of the different groups. Thirty patients with non-ulcer dyspepsia (NUD), 93 with peptic ulcer disease [PUD; which included gastric ulcers (GU, 31 patients) and duodenal ulcers disease (DU, 62 patients)], 18 with erosive gastritis (EG) and 24 with gastroesophageal reflux disease (GERD) were included in the study. The study was approved by the Ethics Committee of São Francisco University Medical School, and was done in accordance with the Declaration of Helsinki. Each patient signed a written informed consent prior to entering the study.

2.2. H. pylori culture and DNA extraction

H. pylori isolates were obtained by inoculating the specimens into selective and non-selective media followed by incubation for 3–5 days at 37°C under microaerophilic conditions, as previously described [16]. The colonies were identified by Gram staining and by oxidase, catalase and urease production. Genomic DNA was extracted with DNAzol™ reagent (Gibco BRL, Cincinnati, OH, USA), and the integrity of the DNA was assessed by electrophoresis in 0.8% agarose gels stained with ethidium bromide.

2.3. PCR amplification

The presence of *H. pylori* was confirmed by polymerase chain reaction (PCR) of the 16S rRNA [17] and glmM [18] genes. The *cag*A gene was analyzed using the primers D008 and R008, as described by Covacci et al. [19]. For analysis of the *vac*A m region, primers VA3-F and VA3-R were used, whereas primers VA4-F and VA4-R were used to amplify the m1 and m2 subtypes, respectively. The *vac*A s region was analyzed using the primers VA1-F and VA1-R, as previously described [8]. For *ice*A genotype analysis, primers iceA1-F, iceA1-R, iceA2-F and iceA2-R were used. The primers iceA2-F and iceA2-R yielded a fragment of 124, 229 or 334 bp, depending on the existence of repeated sequences of 105 nucleotides [5].

2.4. Statistical analysis

The correlation between *H. pylori* genotypes and clinical disease was evaluated using either the χ^2 test with Yates continuity correction or Fisher exact test. Only cases containing single genotypes were included. Student's *t*-test was used to analyze the age distribution among different groups of patient. Logistic regression analysis was used to examine the relationship between the *cag*A, *vac*A and *ice*A genotypes of *H. pylori* and the clinical data. The results are presented as odds ratios (OR) with 95% confidence intervals (CI). A P value of < 0.05 was considered statistically significant.

3. Results

More than one *H. pylori* strain was detected in 28 (17%) of the 165 patients studied, 9 of whom had NUD, 4 had EG, 5 had GERD, 4 had GU and 6 had DU. None of these patients was included in the analysis of the relationship between clinical disease and the putative virulence factors (*vac*A, *cag*A, *ice*A).

Based on the *vac*A and *ice*A genotypes, 137 (83%) specimens were colonized by a single *H. pylori* strain. In seven (4.2%) cases, multiple *vac*A genotypes were detected. Sixteen isolates (9.7%) were positive for both *ice*A1 and *ice*A2, and 4 (2.4%) of these contained multiple *vac*A and *ice*A genotypes. One isolate did not yield a result for the *vac*A s region and could not be classified.

The *vac*A genotype was determined in patients with single strain. Most of the isolates had the s1 signal sequence allele (68.8%). Considering all of patients with non-heterogeneous infections, there was a strong association between the *vac*A s1 strains and the presence of PUD ($\chi^2 = 12.95$; $P = 0.0003$) and DU ($\chi^2 = 11.55$; $P = 0.0007$), and between *vac*A s2 and GERD [$\chi^2 = 16.11$; $P = 0.0001$, (Table 1)].

The m1 allele was found in 48 (35%) and the m2 in 89 (65%) patients. There was a significant association only

Table 1
The *vac*A, *ice*A and *cag*A status of *H. pylori* strains obtained from 137 patients with different clinical outcomes

Genotype status	Clinical outcome					Total *n* (%)
	Non-ulcer or dyspepsia *n* (%)	Peptic ulcer*		Erosive gastritis *n* (%)	Gastroesophageal reflux*** *n* (%)	
		Gastric *n* (%)	Duodenal** *n* (%)			
*vac*A						
s1	13 (61.9)	19 (70.4)	48 (85.7)	9 (64.3)	5 (26.3)	94 (68.6)
s2	8 (38.1)	8 (29.6)	8 (14.3)	5 (35.7)	14 (73.7)	43 (31.4)
m1	7 (33.3)	12 (44.4)	22 (39.3)	4 (28.6)	3 (15.8)	48 (35)
m2	14 (66.7)	15 (55.6)	34 (60.7)	10 (71.4)	16 (84.2)	89 (65)
s1m1	7 (33.3)	12 (44.4)	22 (39.3)	4 (28.6)	3 (15.8)	48 (35)
s1m2	6 (28.6)	7 (25.9)	26 (46.4)	5 (35.7)	2 (10.5)	46 (33.6)
s2m2	8 (38.1)	8 (29.7)	8 (14.3)	5 (35.7)	14 (73.7)	43 (31.4)
*ice*A						
*ice*A1	2 (9.5)	3 (11.1)	11 (19.6)	2 (14.3)	4 (21)	22 (16)
*ice*A2	15 (71.4)	22 (81.5)	40 (71.4)	10 (71.4)	14 (73.7)	101 (73.7)
*ice*A$^-$	4 (19.1)	2 (7.4)	5 (9)	2 (14.3)	1 (5.3)	14 (10.3)
*cag*A						
*cag*A-positive	10 (47.6)	22 (81.5)	41 (73.2)	9 (64.3)	10 (52.6)	92 (67.1)
*cag*A-negative	11 (52.4)	5 (18.5)	15 (26.8)	5 (35.7)	9 (47.4)	45 (32.9)

*$P < 0.001$; *cag*A-positive $P = 0.001$; *cag*A-positive *vac*A s1 $P < 0.01$; **$P < 0.001$; *vac*A s1m2 $P = 0.01$; *cag*A-positive *vac*A s1 $P < 0.01$; ***$P = 0.0001$; *vac*A s2m2 $P = 0.03$.

between NUD and the presence of the *vac*A m2 allele ($\chi^2 = 6.10$; $P = 0.01$).

Although statistical analysis showed no association between the s1m2 genotype and PUD ($\chi^2 = 2.94$; $P = 0.08$), a significant association was observed between this genotype and DU ($\chi^2 = 6.07$; $P = 0.01$). There was an association between *vac*A s2m2 genotype and patients with GERD ($\chi^2 = 4.56$; $P = 0.03$).

*Ice*A1 was detected in 22 (16%) of all the 137 isolates analyzed, and *ice*A2 was found in 101 isolates (73.7%). There was no relationship between the size of the iceA2 amplicon and the patient's disease. Fourteen isolates (10.2%) yielded no PCR product for *ice*A (Table 1). The *ice*A type was not associated with the *vac*A genotypes, or with the clinical outcome.

Of the 137 *H. pylori* isolates, 92 (67.1%) were *cag*A-positive, and 45 (32.9%) were *cag*A-negative (Table 1). There was an association between *cag*A-positive strains and PUD ($\chi^2 = 6.33$; $P = 0.001$).

The genotype *cag*A-positive *vac*A s1 was associated with PUD ($\chi^2 = 7.99$; $P = 0.004$) and DU ($P = 0.004$), and *cag*A-positive *vac*A s2 with GERD ($P = 0.007$). On the other hand, *cag*A-negative strains was associated with the *vac*A s1 genotype in PUD ($P = 0.02$) and with *vac*A s2 in GERD ($P = 0.01$). Statistical analysis also showed an association between the *cag*A-positive s1m2 genotype and PUD and DU ($P = 0.05$ and $p = 0.02$, respectively). Among patients with GERD, there was a relationship with *cag*A-positive *vac*A s2m2 genotype ($P = 0.006$) and *cag*A-negative *vac*A s2m2 combination ($P = 0.01$).

Logistic regression showed that *vac*A s1 was the only predictive factor for PUD (OR = 4.19; 95% CI 1.95–8.98) and DU (OR = 4.57; 95% CI 1.92–10.87). And the less virulent strain *vac*A s2 was related to GERD (OR = 8.59; 95% CI 2.85–25.91).

4. Discussion

The clinical relevance of putative virulence-associated genes of *H. pylori* is still a matter of controversy. The present study investigated the relationship between some virulence factors (*vac*A, *cag*A, and *ice*A) of *H. pylori* and clinical disease.

Evidence for the presence of multiple strains, based on *vac*A and *ice*A genotyping was found in 17% of the cases. The prevalence of multiple *H. pylori* strains may be still underestimated, especially in areas with a high prevalence of *H. pylori* infection [5,6,20].

Particular *vac*A genotypes have been considered markers for the pathogenesis of individual *H. pylori* strains since production of cytotoxin in vitro, epithelial damage in vivo, and the development of peptic ulcer disease are related to specific *vac*A genotypes [5,10]. Our results suggest a higher prevalence of the *vac*A s1 allele in patients with PUD and DU, in agreement with other reports [5,6,8,9]. The elevated toxicities of s1 strains may contribute to the development of ulcerations. Indeed, *vac*A s1 strains secrete larger amounts of cytotoxin than *vac*A s2 strains in vitro, the latter supposedly being less virulent [8,10]. In relation to the nucleotide sequence of the *vac*A middle region, our data did not indicate any *vac*A m genotype as a significant virulence factor, as previously described [6].

The relationship between *vac*A s1m1 and peptic ulcer remains controversial even though this genotype has

been correlated with clinical outcome [5,6,8,9]. Although our data indicated that the *vac*A s1m2 genotype was associated with DU, logistic regression analysis suggested that this genotype could be due to a higher prevalence of the *vac*A s1 allele among patients with DU, as noted before [5].

The *ice*A contains two main allelic variants, *ice*A1 and *ice*A2, of which *ice*A1 is associated with PUD [5,11]. Recently, *ice*A1 expression was found to be significantly related to the host mucosal response [12]. However, the *ice*A1 genotype was not associated with peptic ulceration [9,20]. In contrast to results obtained from Dutch, Japanese and Korean patients [5,9,20], *ice*A2 was the most frequent genotype detected in our population, as previously described [20]. These results may reflect important geographic differences between *H. pylori* strains and patients. The present study in Brazilian patients did not identify any *ice*A genotype as a significant virulence factor. This finding agrees with previous reports and suggests that this gene should not be used as a marker for predicting the clinical outcome of *H. pylori* infection [20].

With regard to *cag*A, this gene was detected in 67% of our patients. Although PCR-based methods for *cag*A detection may result in false negative results, in silico analysis from sequences available in GenBank indicated that the sequences for the primers set used in this study were highly conserved, as noted before [21].

Our data are supported by previous reports and suggest that persons colonized with *cag*A-positive *H. pylori* strains are at increased risk of developing peptic ulceration [5,6]. Our results also indicate that both *cag*A-positive and *vac*A s1 are associated with PUD and DU. This agrees with other reports, and suggests a possible role for these factors in the pathogenesis of *H. pylori*-related peptic ulceration [5,6,10,19]. The present study also demonstrated an association between the *cag*A-positive *vac*A s1m2 genotype and the presence of PUD and DU. However, a logistic regression analysis pointed to *vac*A s1 as a predictor factor for underlying disease. This finding indicates that the presence of *cag*A is not independently associated with PUD or DU, as shown by similar data from patients with a peptic ulcer in the Netherlands [6]. Thus, the *vac*A m allele, *cag*A, and *ice*A are not useful for discriminating or predicting a specific disease risk in our patient population.

Patients with *cag*A-positive strains have a threefold lower risk of complications in GERD when compared with patients carrying *cag*A-negative strains [22]. Additionally, infection by more virulent strains may protect against GERD, so these patients may have a lower frequency of the *vac*A s1 genotype and a higher frequency of the *vac*A s2 and m2 genotypes [6,15]. Our data support this hypothesis. When the putative virulence factors were combined, a higher relationship was detected only between *cag*A-positive *vac*A s2m2 and *cag*A-negative *vac*A s2m2 genotypes and GERD. However, a logistic regression analysis showed a relationship between the presence of a less virulent strain (*vac*A s2) and this particular disease.

Despite the limitations of the present study, the analysis of virulence genes revealed a specific association between *H. pylori* strains and clinical outcome. We were unable to confirm an association between *ice*A genotypes and clinical status, suggesting that this gene is not useful for the universal discrimination or prediction of specific disease risk. Our data did reveal an association between *vac*A s1 and peptic ulceration in Brazilian patients. This study also supports the hypothesis that virulent strains may protect against the development of GERD. The identification of bacterial virulence factors predicting a pathological outcome would be beneficial not only for the understanding the pathogenesis of peptic disease but also for targeting treatment to those in greatest need of therapy.

Acknowledgements

A.P.O.G is supported by a fellowship from Fundação Clara de Assis, São Francisco University.

References

[1] Marshall, B.J. and Warren, J.R. (1984) Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1, 1311–1315.
[2] Blaser, M.J. (1997) Ecology of *Helicobacter pylori* in the human stomach. J. Clin. Invest. 100, 759–762.
[3] Parsonnet, J., Hansen, S., Rodriguez, L., Gelb, A.B., Warnke, R.A., Jellum, E., Orentreich, N., Vogelman, J.H. and Friedman, G.D. (1994) *Helicobacter pylori* infection and gastric lymphoma. New Engl. J. Med. 330, 1267–1271.
[4] Censini, S., Lange, C., Xiang, Z., Crabtree, J.E., Ghiara, P., Borodovsky, M., Rappuoli, R. and Covacci, A. (1996) *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. USA 93, 14648–14653.
[5] van Doorn, L.J., Figueiredo, C., Sanna, R., Plaisier, A., Schneeberger, P., de Boer, W. and Quint, W. (1998) Clinical relevance of the *cag*A, *vac*A, and *ice*A status of *Helicobacter pylori*. Gastroenterology 115, 58–66.
[6] Arents, N.L., van Zwet, A.A., Thijs, J.C., Kooistra-Smid, A.M., van Slochteren, K.R., Degener, J.E., Kleibeuker, J.H. and van Doorn, L.J. (2001) The importance of *vac*A, *cag*A, and *ice*A genotypes of *Helicobacter pylori* infection in peptic ulcer disease and gastroesophageal reflux disease. Am. J. Gastroenterol. 96, 2603–2608.
[7] Telford, J.L., Ghiara, P., Dell'Orco, M., Comanducci, M., Burroni, D., Bugnoli, M., Tecce, M.F., Censini, S., Covacci, A. and Xiang, Z. (1994) Gene structure of the *Helicobacter pylori* cytotoxin and evidence of its key role in gastric disease. J. Exp. Med. 179, 1653–1658.
[8] Atherton, J.C., Cao, P., Peek, R.M., Tummuru, M.K., Blaser, M.J. and Cover, T.L. (1995) Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vac*A types with cytotoxin production and peptic ulceration. J. Biol. Chem. 270, 17771–17777.
[9] Yamaoka, Y., Kodama, T., Gutierrez, O., Kim, J.G., Kashima, K. and Graham, D.Y. (1999) Relationship between *Helicobacter pylori* *ice*A, *cag*A, and *vac*A status and clinical outcome: studies in four different countries. J. Clin. Microbiol. 37, 2274–2279.
[10] Atherton, J.C., Peek, R.M., Tham, K.T., Cover, T.L. and Blaser,

M.J. (1997) Clinical and pathological importance of heterogeneity in *vac*A, the vacuolating cytotoxin gene of *Helicobacter pylori*. Gastroenterology 112, 92–99.

[11] Peek, R.M., Thompson, S.A., Donahue, J.P., Tham, K.T., Atherton, J.C., Blaser, M.J. and Miller, G.G. (1998) Adherence to gastric epithelial cells induces expression of a *Helicobacter pylori* gene, *ice*A, that is associated with clinical outcome. Proc. Assoc. Am. Phys. 110, 531–544.

[12] Peek, R.M., van Doorn, L.J., Donahue, J.P., Tham, K.T., Figueiredo, C., Blaser, M.J. and Miller, G.G. (2000) Quantitative detection of *Helicobacter pylori* gene expression in vivo and relationship to gastric pathology. Infect. Immun. 68, 5488–5495.

[13] Castell, D.O. (1975) Physiology and pathophysiology of the lower esophageal sphincter. Ann. Otol. Rhinol. Laryngol. 84, 569–575.

[14] Werdmuller, B.F. and Loffeld, R.J. (1997) *Helicobacter pylori* infection has no role in the pathogenesis of reflux esophagitis. Dig. Dis. Sci. 42, 103–105.

[15] Loffeld, R.J., Werdmuller, B.F., Kusters, J.G., Perez-Perez, G.I., Blaser, M.J. and Kuipers, E.J. (2000) Colonization with *cag*A-positive *Helicobacter pylori* strains inversely associated with reflux esophagitis and Barrett's esophagus. Digestion 62, 95–99.

[16] Mendonca, S., Ecclissato, C., Sartori, M.S., Godoy, A.P., Guerzoni, R.A., Degger, M. and Pedrazzoli Jr., J. (2000) Prevalence of *Helicobacter pylori* resistance to metronidazole, clarithromycin, amoxicillin, tetracycline, and furazolidone in Brazil. Helicobacter 5, 79–83.

[17] Ho, S.A., Hoyle, J.A., Lewis, F.A., Secker, A.D., Cross, D., Mapstone, N.P., Dixon, M.F., Wyatt, J.I., Tompkins, D.S. and Taylor, G.R. (1991) Direct polymerase chain reaction test for detection of *Helicobacter pylori* in humans and animals. J. Clin. Microbiol. 29, 2543–2549.

[18] Bickley, J., Owen, R.J., Fraser, A.G. and Pounder, R.E. (1993) Evaluation of the polymerase chain reaction for detecting the urease C gene of *Helicobacter pylori* in gastric biopsy samples and dental plaque. J. Med. Microbiol. 39, 338–344.

[19] Covacci, A., Censini, S., Bugnoli, M., Petracca, R., Burroni, D., Macchia, G., Massone, A., Papini, E., Xiang, Z. and Figura, N. (1993) Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc. Natl. Acad. Sci. USA 90, 5791–5795.

[20] Ashour, A.A., Collares, G.B., Mendes, E.N., de Gusmao, V.R., Queiroz, D.M., Magalhaes, P.P., de Carvalho, A.S., de Oliveira, C.A., Nogueira, A.M., Rocha, G.A. and Rocha, A.M. (2001) *ice*A genotypes of *Helicobacter pylori* strains isolated from Brazilian children and adults. J. Clin. Microbiol. 39, 1746–1750.

[21] Owen, R.J., Peters, T.M., Varea, R., Teare, E.L. and Saverymuttu, S. (2001) Molecular epidemiology of *Helicobacter pylori* in England: prevalence of cag pathogenicity island markers and IS605 presence in relation to patient age and severity of gastric disease. FEMS Immunol. Med. Microbiol. 30, 65–71.

[22] Vicari, J.J., Peek, R.M., Falk, G.W., Goldblum, J.R., Easley, K.A., Schnell, J., Perez-Perez, G.I., Halter, S.A., Rice, T.W., Blaser, M.J. and Richter, J.E. (1998) The seroprevalence of cagA-positive *Helicobacter pylori* strains in the spectrum of gastroesophageal reflux disease. Gastroenterology 115, 50–57.