Clinical and histological associations of cagA and vacA genotypes in *Helicobacter pylori* gastritis

V J Warburton, S Everett, N P Mapstone, A T R Axon, P Hawkey, M F Dixon

Abstract

Aims—To determine the relation among the cytotoxin associated gene (cagA) and vacuolating cytotoxin gene (vacA) status of *Helicobacter pylori* isolates, the associated clinical diseases, and the severity and pattern of chronic gastritis.

Methods—*Helicobacter pylori* was cultured from gastric biopsies obtained from dyspeptic patients. DNA was extracted from the isolates and the cagA and vacA status determined by the polymerase chain reaction (PCR). The prevalence of the different cagA and vacA genotypes in three clinical groups, duodenal ulcer, gastric ulcer, and non-ulcer dyspepsia was compared. The histological features in sections from two antral and two corpus biopsies were graded by one blinded observer. The grades were compared with age and sex matched groups with different cagA and vacA genotypes, and with duodenal ulcers, or non-ulcer dyspepsia.

Results—Isolates from 161 patients were included. One hundred and nine (68%) harboured a cagA+ strain and 143 (89%) harboured a vacA s1 strain. The prevalence of cagA+ strains in duodenal ulcer patients (94%) was highly significantly greater than in those with non-ulcer dyspepsia (56%). However, of the patients infected with a cagA+ strain, almost equal numbers had non-ulcer dyspepsia or peptic ulceration. Chronic inflammation, polymorph activity, surface epithelial degeneration, atrophy, and intestinal metaplasia were all significantly more severe in the cagA+ than in the cagA− group, whereas only corpus epithelial degeneration was significantly more severe in the vacA s1 group compared with the vacA s2 group. Patients infected with cagA+ strains were almost four times more likely to have antral intestinal metaplasia than cagA− patients. An antral predominant gastritis was present in duodenal ulcer patients compared with matched non-ulcer dyspepsia patients, but this was not attributable to cagA or vacA status.

Conclusions—*Helicobacter pylori* strains showing cagA positivity and the vacA s1 genotype are associated with more severe gastritis but these virulence factors do not appear to determine the overall pattern. The pattern is closely linked to clinical disease. Therefore, it is likely that the nature of the disease complicating chronic infection is determined by host and environmental factors, while bacterial factors determine the magnitude of the risk of developing such disease.

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Helicobacter pylori is a major aetiological agent in a range of gastroduodenal diseases including chronic gastritis, peptic ulceration, gastric cancer, and lymphoma. Although *H pylori* infection always results in histological gastritis, only a minority of infected subjects develop an associated clinical disease. The fact that individuals with pangastritis and multifocal atrophy are more likely to develop gastric ulcer disease and cancer, and those with antral predominant gastritis have a propensity to develop duodenal ulcers indicates an association between the pattern of *H pylori* induced inflammation and disease outcome. This pattern could be determined by the virulence of the infecting strain or host and environmental factors.

An important virulence determinant of *H pylori* is the vacuolating cytotoxin (VacA). This unique type of extracellular toxin is found more often in strains from patients with duodenal ulcer disease.[1] Anti-VacA antibodies from such patients are found to neutralise the toxin itself, which suggests that it has an important role in vivo.[2] Carriage of VacA+ strains has also been shown to increase polymorph infiltration in the antrum.[2 3] All strains carry a single copy of the vacA gene, but a proportion do not produce the cytotoxin.[3] The vacA gene is known to differ between cytotoxin producing and non-cytotoxic strains,[4] with three different signal sequences having been identified: s1a, s1b, and s2. Strains with the s1 genotype (both a and b) are able to produce cytotoxin, whereas those with the s2 genotype are not.[5]

A second gene that was thought initially to be implicated in cytotoxin production is the cytotoxin associated gene (cagA).[6] Previous work indicates that about 60% of strains possess the cagA gene, of which nearly all express the immunodominant gene product, the CagA protein.[7] It is now appreciated that the cagA gene product is not itself a virulence factor, but that the gene is a member of a 40 kb cluster of genes (a "pathogenicity island"), some of which do contribute to bacterial pathogenicity.[8] Thus, cagA+ strains induce inflammatory cytokines (especially interleukin 8 (IL-8)) in vivo more readily than do cagA− strains.[9] Interestingly, anti-CagA antibodies are significantly more prevalent in patients with duodenal ulcers[10 11] and gastric cancer[12 13] than in patients with non-ulcer dyspepsia. Given

that these two diseases are associated with different patterns of gastritis, it is likely that cagA is not a determinant of the pattern itself, but this has not been investigated. To examine the relation between putative virulence factors and the severity and distribution of gastritis, we endeavoured to type *H pylori* strains with respect to their cagA and vacA gene status, and to correlate these findings with the pattern of gastritis and the disease associations present in the patient.

Methods

SOURCE AND ISOLATION OF *H PYLORI*
Patients attending an open access endoscopy service for the investigation of dyspepsia had mucosal biopsies taken from the antrum (four) and corpus (three). One of the antrum biopsies was used for an immediate rapid urease test and, if positive, initially one antral, and later one antral and one corpus biopsy specimen were sent in transport medium for culture. The other biopsy specimens were immersed in 10% formalin and submitted for histology. The patients were asked specifically about recent use of non-steroidal anti-inflammatory drugs (NSAIDs) and antisecretory drugs (H_2 blockers and proton pump inhibitors). Patients were not included if they had been on such treatment in the preceding four weeks.

ISOLATES
Stored isolates of *H pylori* (kept at −70°C) from antral biopsies alone were available from 39 patients while fresh isolates from both antrum and body biopsies were obtained from 122 patients. For all experiments, the *H pylori* type strain NCTC 11637 was used as a control, to compare with other published studies. Isolates were grown up on selective media (GC agar base; Oxoid, Basingstoke, UK), supplemented with lysed blood, L-glutamine, cocarboxylase, ferric nitrate, L-cystine, and dextrose (GC Supplement; Oxoid). The medium also contained the following antibiotics: vancomycin (3 μg/ml), polymyxin B (6 μg/ml), amphotericin (1 mg/ml), and trimethoprim (6.5 μg/ml). Plates were incubated at 37°C in a gas mixture of 10% CO_2, 5% oxygen balanced with nitrogen for 3–7 days. Isolates were identified as *H pylori* if they were catalase, oxidase, and urease positive, with the appearance of Gram negative curved bacilli. Isolates were harvested for storage in Brain Heart Infusion (Oxoid) containing 20% glycerol and stored at −70°C.

HISTOLOGY
Sections were stained by haematoxylin and eosin, alcian blue-periodic acid Schiff, and modified Giemsa. Only cases with two adequately sized biopsies of both the antral and oxyntic (corpus) mucosa were accepted for histological assessment. Cases were assessed by a single blinded observer, scoring the following features from 0–3 according to the updated Sydney System[14] for both antral and corpus biopsies: chronic inflammation, polymorphonuclear activity (neutrophil infiltration), surface epithelial degeneration, atrophy, intestinal metaplasia, and *H pylori* colonisation density.

The degree of antral predominance for these features was assessed by subtracting corpus scores from antral scores for each patient.

DNA EXTRACTION
DNA was extracted from the fresh isolates before storage at −70°C and after two passages for stored isolates. Three colonies were picked from a plate of *H pylori* with a sterile toothpick and suspended in 50 μl of sterile, double distilled water. Tubes were then boiled at 95°C for five minutes and 2 μl of a 1/5 dilution of this extract (containing ~ 100 ng of DNA) was used as template to determine cagA prevalence and the signal sequence of the vacA gene by the polymerase chain reaction (PCR).

POLYMERASE CHAIN REACTION
Detection of cagA
A cagA fragment of 349 base pairs was amplified from a 50 μl reaction mixture containing the following: 3 mM $MgCl_2$, 1× reaction buffer (Advanced-Biotechnologies, Surrey, UK), 200 μM of each dNTP (Boehringer Mannheim, Mannheim, Germany), and 0.5 U of Thermoprime (Advanced Biotechnologies). Fifty picomoles of primers F1 and B1[7] were used and the whole mixture was overlaid with ~ 30 μl of sterile mineral oil.

Determination of vacA signal sequence
The reaction mixture contained the following: 2 mM $MgCl_2$, 1× reaction buffer (Advanced Biotechnologies), 150 μM of each dNTP (Boehringer Mannheim), 0.5 U of Thermoprime (Advanced Biotechnologies), 50 pmols of primers VAR-1 and VAR-2,[5] made up to 50 μl with double distilled water, and overlaid with ~ 30 μl of sterile mineral oil. Standard specimen negative controls (water) were included.

Reaction conditions
For both PCRs: 94°C for five minutes, 60°C for one minute, then 35 cycles of 94°C for one minute and 60°C for 30 seconds, followed by a final extension step of 72°C for five minutes.

Viewing
A 12 μl aliquot of the PCR product was combined with 3 μl of loading dye and run on a 2% agarose gel, stained with 10 mg ethidium bromide, at 120–150 volts for 25–45 minutes (depending on the volume of the gel) in 1× Tris acetate EDTA buffer. Bands were viewed under ultraviolet excitation. For the vacA signal sequence PCR, a band 259 base pairs in size, corresponded to the s1 phenotype and a band size of 286 base pairs, indicated an s2 phenotype.[5]

The presence of *H pylori* DNA in cagA− strains was confirmed using a 16S rRNA PCR technique validated previously in this department[15] (fig 1).

STATISTICS
The significance of differences in histological features between the cagA and vacA groups and the duodenal ulcer and non-ulcer dyspepsia groups was determined by comparing
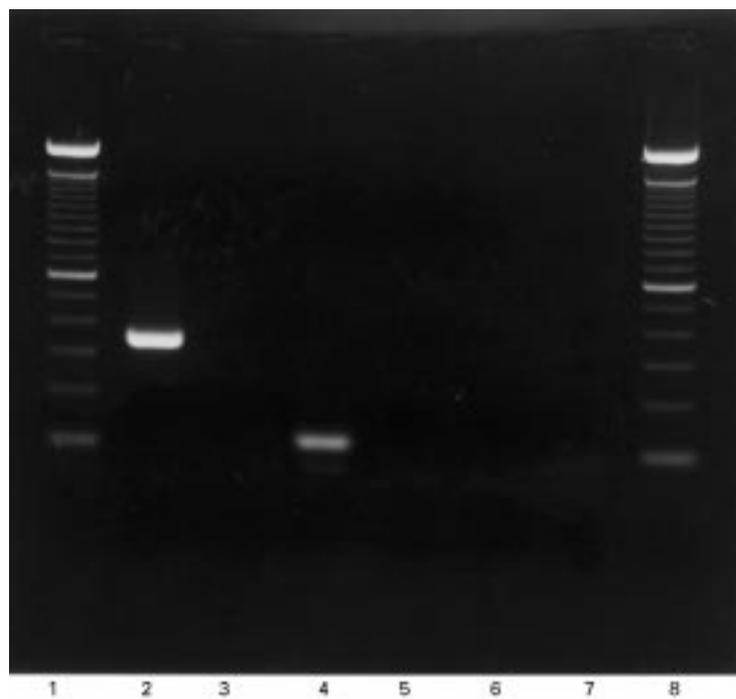
Figure 1 16S rRNA PCR technique. Lane 1, 100 bp DNA ladder; lane 2, 349 base pair cagA product from cagA+ Helicobacter pylori; lane 3, cagA− H pylori (no product); lane 4, 109 base pair 16S rRNA H pylori product from cagA− H pylori sample; lane 5, cagA PCR negative control; lane 6, 16S rRNA PCR negative control; lane 7, DNA extraction negative control; lane 8, 100 base pair DNA ladder.

individual grades using the Mann-Whitney U test. The χ^2 test was used to compare the prevalences of clinical diseases between groups.

Results

A total of 161 (74 female) H pylori infected patients were entered into the study. The distribution of clinical disease was as follows: 50 patients (age range, 17–77 years; median, 52 years) had current or past duodenal ulcer disease; 12 patients (age range, 33–77 years; median, 56 years) had gastric ulcer disease; and 99 patients (age range, 17–88 years; median, 54 years) had non-ulcer dyspepsia; in the latter group eight had oesophagitis, eight had duodenitis, and four had gastric erosions. The remainder had no endoscopic lesions.

The cagA and vacA status of isolates was determined on cultures from antral biopsies alone in 39 patients and from antral and corpus biopsies in 122 patients. Four of this latter group had cagA+ H pylori from one biopsy and cagA− from the other.

One hundred and nine patients (68%) were infected with cagA+ strains (including four patients with both cagA+ and cagA− strains),

and in 143 patients (89%) the infecting strain was of the vacA s1 type (table 1). One hundred and four (73%) vacA s1 types were also cagA+ compared with 36 that were cagA−. Likewise, only one vacA s2 type was cagA+ compared with 17 that were cagA−. Thus, cagA was highly significantly associated with vacA s1 (p < 0.0001). Of the four patients with both positive and negative cagA strains, three were of the vacA s1 type and one was of the s2 type.

cagA, vacA, AND CLINICAL DISEASE
The prevalence of cagA+ strains in duodenal ulcer patients (94%) was highly significantly greater than in those with non-ulcer dyspepsia (56%; p < 0.0001). Likewise, the distribution of vacA s1 was also significantly different in patients with duodenal ulcer disease compared with non-ulcer dyspepsia (p = 0.049). However, of the 109 patients infected with cagA+ strains, an almost equal number have non-ulcer dyspepsia as have peptic ulcer disease (55 versus 54). In contrast, only eight of 52 patients (15%) with cagA− strains have peptic ulceration. Similarly, 59 of 143 (41%) of vacA s1 strains were associated with peptic ulceration, while only three of 18 (17%) patients with s2 strains had ulcers.

cagA MATCHED SUBGROUP
Fifty two patients were infected with cagA− strains and, of these, 41 patients had biopsies suitable for assessment. These patients (age range, 26–76 years; median, 54 years) were age (± 5 years) and sex matched to 41 patients with cagA+ H pylori without regard to their clinical group (age range, 25–77 years; median, 55 years; p = 0.99). The following histological features of antral mucosa were significantly (p < 0.05) more severe in the cagA+ group compared with the cagA− group: chronic inflammation (p = 0.039), polymorph activity (p = 0.011), surface epithelial degeneration (p = 0.006), atrophy (p = 0.012), and intestinal metaplasia (p = 0.005) (table 2). Corpus features of chronic inflammation (p = 0.003), polymorph activity (p = 0.007), and surface epithelial degeneration (p = 0.004) were also significantly more severe in the cagA+ group. Of particular interest, 15 patients in the cagA+ subgroup had antral intestinal metaplasia compared with four in the cagA− group (odds ratio = 3.75; 95% confidence limits = 2.22–6.34).

The prevalence of duodenal ulcers was significantly greater in the cagA+ (34%) compared with the cagA− subgroup (7%; p = 0.032) but the prevalence of gastric ulcers was not.

vacA MATCHED SUBGROUP
Of 18 patients with vacA s2 H pylori, 16 were suitable for histological assessment. These patients (age range, 26–76 years; median, 56 years) were age and sex matched to 16 patients with vacA s1 H pylori (age range, 25–77 years; median, 55.5 years; p = 0.98). Corpus surface epithelial degeneration was found to be significantly (p = 0.004) more severe in the s1 group compared with the s2 group. Other features

Table 1 Prevalence of cagA and vacA s1 or s2 Helicobacter pylori

		cagA status		vacA status	
	Number	Positive (%)	Negative (%)	vacA s1 (%)	vacA s2 (%)
Duodenal ulcer	50	47 (94)	3 (6)	49 (98)	1 (2)
Gastric ulcer	12	7 (58)	5 (42)	10 (83)	2 (17)
Non-ulcer dyspepsia	99	55 (56)	44 (44)	84 (85)	15 (15)
Total	161	109 (68)	52 (32)	143 (89)	18 (11)

Both cagA+ and vacA s1 have a significantly greater association with duodenal ulcer than non-ulcer dyspepsia.

Table 2 Histological scores for the cagA matched groups (n = 41 in each group)

	Antrum						Corpus					
	Ci	Ac	SED	At	IM	Hp	Ci	Ac	SED	At	IM	Hp
cagA+												
Mean	1.951	1.366	1.268	0.780	0.537	1.610	1.561	0.878	0.732	0.537	0.146	1.244
Range	1–3	0–3	0–3	0–1	0–3	0–3	1–3	0–2	0–2	0–2	0–3	0–3
Median	2	1	1	1	0	2	1	1	1	0	0	1
cagA−												
Mean	1.659	0.951	0.805	0.415	0.146	1.488	1.195	0.463	0.341	0.293	0.049	1.146
Range	1–3	0–2	0–2	0–2	0–2	0–3	1–3	0–2	0–3	0–3	0–1	0–3
Median	2	1	1	0	0	1	1	0	0	0	0	1
z	2.065	2.557	2.754	2.521	2.811	0.741	3.011	2.723	2.870	1.736	0.863	1.177
p	0.039	0.011	0.006	0.012	0.005	0.459	0.003	0.007	0.004	0.083	0.388	0.239

Mean value is given for illustration. The individual grades for each histological feature were used in a Mann-Whitney test to explore differences.
Ci, Ac, and SED in both antrum and corpus as well as At and IM in the antrum are all significantly more severe in the cagA+ group.
Ci, chronic inflammation; Ac, polymorph activity, SED surface epithelial degeneration; At, atrophy; IM, intestinal metaplasia; Hp, *Helicobacter pylori*; z, standard normal deviate; p, significance.
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Table 3 Histological scores for the vacA s1 and s2 matched groups (n = 16 in each group)

	Antrum						Corpus					
	Ci	Ac	SED	At	IM	Hp	Ci	Ac	SED	At	IM	Hp
vacA s1												
Mean	1.875	0.875	1.125	0.375	0.375	1.500	1.375	0.750	0.688	0.313	0.125	1.313
Range	1–3	0–2	0–2	0–2	0–2	0–3	1–2	0–2	0–2	0–1	0–1	1–3
Median	2	1	1	0	0	1	1	1	1	0	0	1
vacA s2												
Mean	1.438	0.750	0.625	0.375	0.063	11.563	1.250	0.313	0.125	0.375	0.063	1.063
Range	1–3	0–2	0–1	0–1	0–1	1–3	1–3	0–1	0–1	0–2	0–1	0–2
Median	1	1	0	0	0	1	1	0	0	0	0	1
z	1.663	0.285	1.893	-0.227	1.492	-0.225	1.010	1.916	2.895	-0.117	0.597	1.202
p	0.096	0.776	0.058	0.820	0.136	0.822	0.312	0.055	0.004	0.907	0.551	0.229

Mean value is given for illustration. The individual grades for each histological feature were used in a Mann-Whitney test to explore differences.
Corpus SED was significantly more severe in s1 compared with s2 group.
Ci, chronic inflammation; Ac, polymorph activity, SED surface epithelial degeneration; At, atrophy; IM, intestinal metaplasia; Hp, *Helicobacter pylori*; z, standard normal deviate; p, significance.

that showed greater (but not statistically significant) severity in the s1 group were antral surface epithelial degeneration and corpus polymorph activity (table 3).

Duodenal ulcer, gastric ulcer, and non-ulcer dyspepsia prevalence did not differ significantly between the vacA s1 and s2 subgroups.

HISTOLOGICAL FEATURES AND CLINICAL DISEASE
Twenty five patients with duodenal ulcers (age range, 26–72 years; median, 54 years) were selected randomly from the overall series and matched to 25 patients with non-ulcer dyspepsia, not including those patients with identifiable endoscopic disease such as duodenitis, gastric erosions, and oesophagitis (age range, 25–77 years; median, 56 years; p = 1.0),

regardless of strain genotype. The prevalence of cagA or vacA type did not differ significantly between the duodenal ulcer and the non-ulcer dyspepsia subgroups. The features of corpus polymorph activity (p = 0.002) and surface epithelial degeneration (p = 0.015) were significantly more severe in patients with non-ulcer dyspepsia than in those with duodenal ulceration. However, patients with duodenal ulceration demonstrated a significantly higher grade of antral chronic inflammation (p = 0.008) and *H pylori* density (p = 0.005) (table 4). When the 12 patients with gastric ulceration were age and sex matched with non-ulcer dyspepsia patients (regardless of strain type), the histological scores showed no significant differences (data not shown).

Table 4 Comparison of histological scores for age matched patients with duodenal ulcer or non-ulcer dyspepsia irrespective of cagA or vacA status (n = 25 in each group)

	Antrum						Corpus					
	Ci	Ac	SED	At	IM	Hp	Ci	Ac	SED	At	IM	Hp
Non-ulcer dyspepsia												
Mean	1.640	1.240	0.960	0.480	0.360	1.320	1.480	0.960	0.720	0.520	0.160	1.120
Range	1–3	0–3	0–2	0–2	0–2	0–3	1–3	0–2	0–2	0–2	0–1	0–3
Median	2	1	1	1	0	1	1	1	1	0	0	1
Duodenal ulcer												
Mean	2.120	1.400	1.080	0.840	0.320	1.920	1.240	0.360	0.280	0.280	0.120	1.040
Range	1–3	0–3	0–3	0–2	0–3	1–3	1–2	0–2	0–2	0–2	0–3	0–2
Median	2	1	1	1	0	2	1	0	0	0	0	1
z	−2.670	−0.729	−0.478	−1.931	−0.134	−2.785	1.334	3.159	2.443	1.634	1.324	0.349
p	0.008	0.466	0.633	0.054	0.893	0.005	0.182	0.002	0.015	0.102	0.186	0.727

Mean value is given for illustration. The individual grades for each histological feature were used in a Mann-Whitney test to explore differences.
Antral *H pylori* density and Ci are significantly more severe in duodenal ulcer patients while corpus Ac and SED are more severe in non-ulcer dyspepsia patients.
Ci, chronic inflammation; Ac, polymorph activity, SED surface epithelial degeneration; At, atrophy; IM, intestinal metaplasia; Hp, *Helicobacter pylori*; z, standard normal deviate; p, significance.

Table 5 Antral predominance (antral minus corpus grades) in patients with duodenal ulcer compared to matched non-ulcer dyspepsia patients (n = 25 in each group)

	Ci	Ac	SED	At	IM	Hp
Duodenal ulcer	0.880	1.040	0.800	0.560	0.200	0.880
Non-ulcer dyspepsia	0.160	0.280	0.240	-0.040	0.200	0.200
z	3.204	2.888	2.844	2.387	0.670	2.725
p	0.001	0.004	0.005	0.017	0.503	0.006

The mean net value is given for illustration. The individual net grades (antrum minus corpus) were used in a Mann-Whitney test to explore differences.
The net grades for all features (except IM) are significantly greater in patients with duodenal ulcer than in non-ulcer dyspepsia patients.
Ci, chronic inflammation; Ac, polymorph activity, SED surface epithelial degeneration; At, atrophy; IM, intestinal metaplasia; Hp, *Helicobacter pylori*; z, standard normal deviate; p, significance.

Table 6 Antral predominance (antral minus corpus scores) and cagA status (n = 41 in each group)

	Ci	Ac	SED	At	IM	Hp
cagA+	0.390	0.488	0.537	0.244	0.390	0.366
cagA−	0.463	0.488	0.463	0.122	0.098	0.341
z	−0.404	0.140	0.261	0.669	2.592	0.524
p	0.686	0.889	0.794	0.504	0.010	0.600

The mean net value is given for illustration. The individual net grades (antrum minus corpus) were used in a Mann-Whitney test to explore differences.
No antral predominance demonstrated apart from IM.
Ci, chronic inflammation; Ac, polymorph activity, SED surface epithelial degeneration; At, atrophy; IM, intestinal metaplasia; Hp, *Helicobacter pylori*; z, standard normal deviate; p, significance.

INVESTIGATION OF ANTRAL PREDOMINANT GASTRITIS

Clinical disease

When corpus scores were subtracted from antral scores to determine antral predominance in the matched duodenal ulcer and non-ulcer dyspepsia patients, it was found that duodenal ulcer patients had a significant increase in net grades for all features of antral gastritis except intestinal metaplasia when compared with the net grades in non-ulcer dyspepsia patients (table 5). Of interest, non-ulcer dyspepsia patients exhibited a greater degree of atrophy in the corpus than in the antrum (mean net grade = −0.04), although this did not achieve statistical significance.

cagA

When antral predominance was sought in relation to cagA status (for the 82 matched subjects), intestinal metaplasia was significantly more severe (p = 0.01) in the antrum relative to the corpus in the cagA+ group compared with the cagA− group. No other net score differed significantly between the two groups (table 6). In keeping with this finding, when the 82 subjects were divided (on the basis of the net grade for chronic inflammation) into antral predominant (net score > 0) and diffuse or pangastritis (net score ≤ 0) categories, the prevalence of cagA+ strains was almost identical (20 of 39 and 21 of 43, respectively). In order to investigate the effect of cagA status on the pattern of gastritis separately from the influence of ulcer phenotype, 22 patients with non-ulcer dyspepsia infected with cagA− *H pylori* (age range, 29–74 years; median, 59.5 years) were age matched with non-ulcer dyspepsia subjects infected with cagA+ strains (age range, 26–76 years; median, 59 years; p = 0.9344) and antral predominance sought as above. The outcome was essentially similar to that found with the subjects from mixed clinical groups (data not shown); however, for intestinal metaplasia there was a significantly greater degree of antral predominance in the cagA+ group (p = 0.027).

vacA

Unlike the cagA matched group, no antral predominance was demonstrated for any feature of the vacA s1 or s2 subgroups.

Discussion

This study is the first to investigate *H pylori* cagA and vacA status in relation to the distribution of gastritis, a major correlate of clinical disease. While other studies have examined cagA and vacA status in relation to clinical associations and histological severity, our investigation addresses some important shortcomings of previous studies. We have investigated a sufficiently large series to yield a substantial number of cagA− subjects, thereby enabling statistically significant results to be obtained. Our patients represent a typical and well characterised population of dyspeptic subjects attending a single endoscopy unit and a wide age range is included. The majority of *H pylori* infections commence in childhood so that, to a large extent, age reflects the duration of gastritis. Indeed, we have shown previously, in a multivariate analysis, that age is related significantly to the degree of polymorph activity, atrophy, and intestinal metaplasia, irrespective of clinical disease.[16] With this in mind, we selected age matched subjects to make detailed comparisons of the severity and pattern of gastritis in antral and corpus biopsies according to cagA and vacA status. Finally, the pattern of gastritis is modified readily by acid suppressant drugs,[17] and certain histological features, in particular surface epithelial degeneration, will be affected by concurrent NSAID use. Therefore, we eliminated subjects who had taken such drugs in the four weeks before endoscopy.

The prevalence of cagA and vacA s1 strains was significantly different in patients with duodenal ulcers compared with those with non-ulcer dyspepsia (p < 0.0001, p = 0.049, respectively) (table 1). As in other reports,[5 18] we also found that cagA and vacA s1 were highly significantly associated with each other (p < 0.0001). However, when considering cagA+ *H pylori* isolates, a higher percentage was found in non-ulcer dyspepsia patients (51%) than in patients with duodenal ulcers (43%). Therefore, our data indicates, as others have found,[19] that a clinical diagnosis of duodenal ulcer is very likely to be associated with the cagA+ genotype of *H pylori* but the presence of a cagA+/vacA s1 strain cannot be taken to predict current duodenal ulcer disease. Nevertheless, there must be a risk that some non-ulcer dyspepsia patients with cagA+ strains will develop duodenal ulceration in the future. The magnitude of this risk can only be determined by longitudinal studies. Interestingly, we found a similar prevalence of cagA+ strains in gastric ulcer and non-ulcer dyspepsia patients (58% and 56%, respectively), although the number of ulcer patients was small. Turning to the strains lacking these virulence markers, 15% of cagA− and 17% of vacA s2 strains were

associated with peptic ulcer disease. Thus, these genotypes alone cannot be used to select out patients with "good" or "neutral" *H pylori* strains who need not receive eradication treatment.[20]

The presence of cagA was shown to have a significant association with increased severity of chronic inflammation, polymorph activity, and surface epithelial degeneration in both the antrum and corpus. Atrophy and intestinal metaplasia were only significantly greater in the antrum (table 2). The greater degree of polymorph activity and epithelial degeneration found with cagA+ strains was not associated with increased *H pylori* colonisation as judged by histology, despite the finding of a fourfold increase in antral colonisation density for cagA+ versus cagA− strains using quantitative culture.[21] As would be expected, the prevalence of duodenal ulcers was significantly different in the cagA+ and cagA− matched subgroups. However, when matched patients were grouped according to clinical disease, regardless of strain type, other histological features emerged as significantly different. Duodenal ulcer patients exhibited a greater density of *H pylori* colonisation in the antrum compared to age matched subjects with non-ulcer dyspepsia (who had normal findings on endoscopy), while the latter showed more severe polymorph activity and surface epithelial degeneration in the corpus than duodenal ulcer patients (table 4).

In contrast to cagA, vacA s1 was not associated with significantly higher scores for chronic inflammation, activity, atrophy, or intestinal metaplasia. However, in the light of a recently published report, one could argue that other regions of the vacA gene (subgroups of s1 and the middle region) may be linked more closely to histological changes and duodenal ulcer disease.[18] Atherton *et al* found that the signal sequence s1a was associated closely with peptic ulcer disease; however, the effect of the middle region subtypes (m1 and m2) on gastritis scores was not substantial.[18] When we originally set out on our investigation, the s2 genotype had been shown to be non-cytotoxic and the s1 genotype was found to produce cytotoxin, with the middle region subtypes associated with the amount of cytotoxin produced.[5] Because we were concerned with the potential for a strain to produce cytotoxin, we investigated the relation between the s1 and s2 types only. The fact that ours is a large study should have increased the chances of finding a significant influence of the vacA signal type on the severity and pattern of gastritis. When 16 subjects with the vacA s2 genotype were age matched with s1 cases, the only statistically significant difference in histology was the greater degree of surface epithelial degeneration in the corpus in patients with the s1 genotype, a finding in keeping with cytotoxin production by this strain. Surface epithelial degeneration in the antrum and polymorph activity in the corpus were also substantially more severe in the s1 group, but the differences did not reach statistical significance (table 3).

It is well recognised that duodenal ulcer disease is associated with an antral predominant gastritis and this was confirmed in the present study. In order to assess and compare antral predominance we subtracted the individual grades given to the histological features in corpus mucosa from those given to the corresponding antral biopsies. Duodenal ulcer subjects had a significantly greater degree of antral predominance over non-ulcer dyspepsia patients for all the features assessed except intestinal metaplasia (table 5). However, when the same approach was applied to cagA positive and negative subjects, the only feature with significant antral predominance was intestinal metaplasia (table 6). When antral predominance was sought for vacA s1 strains, none of the features achieved significance. It was thought that the influence of virulence factors on the pattern of gastritis might be seen to better effect in patients who do not have peptic ulcers. However, when antral predominance was sought in cagA+ versus cagA− non-ulcer dyspepsia patients, a significant difference was confined to intestinal metaplasia, as with subjects from mixed clinical groups. Thus, it was concluded that in contrast to the severity of histological change, the overall pattern of gastritis is not dictated by the infecting strain, at least as indicated by cagA and vacA status. Inevitably, this conclusion points to a fundamental role for host factors in determining the outcome in terms of the gastritis phenotype and associated disease. How can we reconcile this finding with the known association between cagA positivity and both duodenal ulcer disease and gastric cancer? As we have speculated previously,[17 22] the answer probably lies in differing levels of acid production.

In patients with an enlarged parietal cell mass and high acid output, the intolerance of *H pylori* to high local acid levels renders the corpus hostile to colonisation so that inflammation becomes concentrated in the antrum. Infection with "pro-inflammatory" cagA+/vacA s1 strains could increase cytokine effects on antral somatostatin producing D cells and result in hypergastrinaemia. In keeping with this suggestion, serum gastrin concentrations in patients infected with cagA+ strains are higher than their cagA− counterparts.[23] An increased acid load reaching the first part of the duodenum leads to gastric metaplasia which will become colonised by *H pylori*. It is reasonable to assume that by inducing more epithelial damage and inflammation, cagA+/vacA s1 strains infecting this metaplastic epithelium will result in more active chronic duodenitis ("bulbitis") than cagA− strains and, thereby, increase the risk of duodenal ulceration. This hypothesis remains to be tested, but it offers a plausible explanation for the closer association between cagA positivity and ulceration.

In subjects with normal, or subnormal levels of acid secretion, colonisation will be more evenly spread throughout the stomach. Thus, non-ulcer dyspepsia subjects show more corpus inflammation and atrophy than their age matched counterparts with duodenal ulceration. Corpus inflammation exerts cytokine effects on parietal cells, decreasing their sensitivity to gastrin so that even in the face of

hypergastrinaemia caused by antral inflammation there is no corresponding rise in acid output.[23] The appearance of multifocal atrophy principally affecting corpus mucosa and the ensuing hypochlorhydria are major risk factors for the development of gastric cancer.[24] Antral atrophy does not have a direct effect on acid production and in isolation is of lesser importance as a risk factor. We have shown that cagA+ strains are associated with more atrophy and intestinal metaplasia compared with cagA− strains, and a similar association has been demonstrated between the presence of atrophy and anti-CagA antibodies.[25] However, the extent of atrophy will be dependent upon the topography of the *H pylori* gastritis. Thus, duodenal ulcer patients with antral predominant gastritis will develop antral atrophy with little or no involvement of the corpus, and be at much lower risk of gastric cancer than non-ulcer dyspepsia or asymptomatic individuals with a corpus predominant gastritis.[26]

In our study, cagA+ patients were almost four times more likely to have antral intestinal metaplasia than their age matched cagA− counterparts. Although we did not demonstrate significant links between vacA status and atrophy and intestinal metaplasia, an association between in vitro cytotoxic activity of *H pylori* isolates and the presence of atrophic gastritis has been demonstrated in a population at high risk of gastric cancer in Japan.[27] It is known that cytotoxic strains induce more polymorph infiltration in vivo,[28] and a greater oxidative burst in polymorphs in vitro, than non-cytotoxin producers.[29] Furthermore, reactive oxygen metabolites have been implicated in the pathogenesis of atrophy and DNA mutations.[30] Thus, if host factors, and principally reduced acid output, permit colonisation throughout the stomach then cagA+/vacA s1 *H pylori* strains will have a greater propensity to produce multifocal atrophy, intestinal metaplasia and, possibly, DNA damage than cagA−/vacA s2 strains. Multifocal atrophy and intestinal metaplasia are characteristic of the gastric mucosa in which the intestinal type of gastric cancer develops. It remains to be established whether cagA+/vacA s1 strains increase free radical damage to DNA in gastric epithelial cells or increase epithelial cell proliferation relative to cagA−/vacA s2 strains and, thereby, exert a more direct role in gastric carcinogenesis.

In conclusion, this study has shown that while the possession of the cagA and vacA s1 genotype is linked to the severity of chronic *H pylori* gastritis these putative virulence markers do not appear to determine its overall pattern. In so far as the pattern of gastritis is strongly linked to the nature of the associated disease, our findings indicate that host or environmental factors largely determine the type of disease that will complicate long standing *H pylori* infection, while the magnitude of the risk of developing the disease is related to bacterial virulence factors.

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