Association of peptic ulcer with increased expression of Lewis antigens but not cagA, iceA, and vacA in Helicobacter pylori isolates in an Asian population

P Y Zheng, J Hua, K G Yeoh, B Ho

Abstract

Background—Studies in Western populations suggest that cagA, iceA, and vacA gene status in Helicobacter pylori isolates is associated with increased virulence and peptic ulcer disease.

Aim—To investigate the relationship between peptic ulcer and expression of Lewis (Le) antigens as well as cagA, iceA, and vacA in H. pylori isolates in Singapore.

Methods—Expression of Le antigens in H pylori isolates obtained from patients with dyspepsia was measured by enzyme linked immunosorbent assay. The cagA, iceA, and vacA status was determined by polymerase chain reaction.

Results—Of 108 H pylori isolates, 103 (95.4%) expressed Le' and/or Le', while Le' and Le' were expressed in 23 (21.3%) and 47 (43.5%) isolates, respectively. Expression of two or more Le antigens (Le', Le', Le', or Le') was significantly higher in H pylori isolated from ulcer patients than in non-ulcer patients (89.6% vs 73.2%, p=0.035). There were no significant differences in the prevalence of cagA or iceA1 in H pylori isolates from peptic ulcer and non-ulcer patients (86.6% vs 90.2% for cagA; 70.1% vs 68.3% for iceA), and no association of peptic ulcer with any specific vacA genotype.

Conclusions—The present study indicates that peptic ulcer disease is associated with increased expression of Lewis antigens but not cagA, iceA, or vacA genotype in H pylori isolates in our population. This suggests that cagA, iceA, and vacA are not universal virulence markers, and that host-pathogen interactions are important in determining clinical outcome.

(Gut 2000;47:18–22)

Keywords: Lewis blood group antigens; cagA; iceA; vacA; Helicobacter pylori; peptic ulcer

Helicobacter pylori is the major aetiologial agent of chronic active gastritis and is generally accepted as having a causative role in the pathogenesis of peptic ulcer (PU) disease. H pylori infection has also been aetiological linked to the development of gastric carcinoma.1,2 It is estimated that more than 50% of the world’s population are infected with H pylori. However, only a minority of H pylori infected subjects develop PU or gastric cancer. The reasons for this are not well understood.

Vacuolating cytotoxin gene (vacA) s1a genotype and the cytotoxin associated gene (cagA) have been demonstrated to be related to the virulence of H pylori infection and the development of peptic ulcer.3,4 However, there are also reports to the contrary.5,6 A novel gene iceA (induced by contact with epithelium gene) has been reported and two allelic variants of the gene (iceA1 and iceA2) described.7 Studies based on Western populations suggested that iceA1 is associated with PU.8,9 Recent studies showed that the lipopolysaccharides (LPS) of most H pylori isolates express Lewis’ (Le’) and/or Le’ blood group antigens,10 and these antigens are also expressed on human gastric mucosa.11 It is postulated that this molecular mimicry may play a role in the pathogenesis of H pylori infections.12 Peptic ulcer disease has been suggested to be associated with H pylori expression of Le'/Le’, and an association between cagA gene and expression of Le'/Le’ has also been reported.13 Expression of Le’ antigens and the prevalence of iceA have not been fully investigated in Asian countries where the prevalence of the cagA gene is high regardless of the presence of the disease.14 H pylori strains may differ in various geographical regions7 and studies in different populations may clarify the importance and universality of putative virulence factors. In the present study expression of Le’ antigens and the prevalence of cagA as well as iceA and vacA were investigated in 108 H pylori isolates in Singapore.

Materials and methods

PATIENTS AND H PYLORI ISOLATES

H pylori strains were isolated from the gastric biopsies of 108 patients undergoing upper gastrointestinal endoscopy for dyspepsia at the National University Hospital, Singapore. Informed consent was obtained from all patients for gastroscopy and biopsies. All patients included in the study were H pylori positive as assessed using the rapid urease test and culture. The patient population comprised 88 Chinese, 13 Indians, and seven Malays. Of these, 67 were males and 41 females. Mean age

Abbreviations used in this paper: cagA, cytotoxin associated gene; iceA, induced by contact with epithelium gene; vacA, vacuolating cytotoxin gene; Le, Lewis blood group antigen; PCR, polymerase chain reaction; PU, peptic ulcer; LPS, lipopolysaccharides; NUD, non-ulcer dyspepsia; OD, optical density.
was 46 years (16–78). Based on history and endoscopic examination, patients were classified into the following groups: duodenal ulcer (36), gastric ulcer (31), and non-ulcer dyspepsia (41). Non-ulcer dyspepsia (NUD) was defined as patients with neither a history of ulcer disease nor endoscopic evidence of ulcer disease. Upper gastrointestinal endoscopy was performed, and after completion of mucosal examination two biopsies were obtained from the gastric antrum within 2 cm of the pylorus. Our previous study on multiple antrum biopsies showed a predominance of a single strain of *H pylori*. Bacteria isolated from gastric biopsies were grown on blood agar plates (blood agar base 2 supplemented with 5% horse blood) in a humidified incubator supplied with 5% CO2 (Forma Scientific, USA) at 37°C for three days. Isolates were identified as *H pylori* based on Gram stain morphology and positive tests for urease, oxidase, and catalase.

### Antigen expression in LPS

Expression of various LPS epitopes in the *H pylori* isolates was measured using an enzyme linked immunosorbent assay (ELISA) as described by Simoons-Smit and colleagues. The following murine monoclonal antibodies (Mabs) were used: Mab 54-1F6A, specific for Lea; Mab 1E52, specific for Leb; Mab 3-3A, specific for Lea (Bioprobe, Netherlands); Mab 225Le, specific for Lea (Bioprobe); Mab 3-3A, specific for blood group A antigen; and Mab 3E7, specific for blood group B antigen (Dako, USA). Optical density (OD) was read at 490 nm. An OD value of 0.2 was chosen as the cut off value because the sum of non-specific background binding values for Mabs and for the conjugate protein linked Le antigens (that is, Lea, Leb, Le and Leb (IsoSep, Sweden)) were used as positive controls for the Mabs.

### PCR analysis

#### Detection of *cagA* and *iceA*

DNA of each *H pylori* strain was isolated by chloroform-phenol extraction. The polymerase chain reaction (PCR) was carried out in an amplification thermal cycler (Perkin-Elmer, USA) programmed according to the protocol of Zheng and colleagues. The primers used for *cagA*, *iceA1*, and *iceA2* and the expected PCR fragment lengths are listed in table 1.

#### Genotyping of *vacA* alleles

The combined expressions described by Atherton and colleagues and Wang and colleagues were used to amplify the *vacA* gene fragments of all *H pylori* isolates. Typing of *vacA* for signal sequence region alleles and middle region alleles was carried out using the primers and methods as described by Atherton and colleagues for *s1a, s1b, s2, m1*, or *m2* and Wang and colleagues for *m1T* or *m1Tm2*. These seven pairs of primers and the expected PCR fragment lengths are listed in table 1.

## Results

### Expression of Lewis Antigens in Clinical Outcome

Of the 108 *H pylori* isolates, 86 were found to express both *Le* and *Le*, while 11 and six isolates expressed only *Le* and *Le*, respectively. Three isolates expressed only *Le* while two other isolates were not-typeable. The study revealed that 23 (21.8%) isolates expressed *Le* while 47 (43.5%) isolates expressed *Le* (table 2). Only one isolate expressed blood group A antigen while none expressed blood group B antigen.

### Relationship between Le Expression and Clinical Outcome

Table 2 shows that of 67 isolates from patients with PU, 64 (95.5%) were positive for *Le* and/or *Le* antigens compared with 39 (95.1%) of 41 isolates from patients with NUD (p=1.000). Furthermore, the mean OD level of *Le* or *Le* expression was not significantly different between isolates from patients with or without ulcer disease (1.0150 (0.6515) vs 1.0105 (0.6515)) v

### Table 1: Polymerase chain reaction (PCR) for amplification of *H pylori* cagA, iceA, and vacA genes

<table>
<thead>
<tr>
<th>Region</th>
<th>Primer</th>
<th>Nucleotide sequence (5'→3')</th>
<th>Size of PCR product (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>cagA</em></td>
<td><em>cagA</em>-F</td>
<td>AATACACAAACCGCTCCAAAG</td>
<td>400</td>
<td>22</td>
</tr>
<tr>
<td><em>cagA</em>-R</td>
<td>TGGTCTGCTGCTGCTGCTG</td>
<td>352</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td><em>iceA1</em></td>
<td><em>iceA1</em>-F</td>
<td>GTGTTGTGCTGCTGCTGCTG</td>
<td>352</td>
<td>23</td>
</tr>
<tr>
<td><em>iceA1</em>-R</td>
<td>TGGTCTGCTGCTGCTGCTG</td>
<td>352</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td><em>iceA2</em></td>
<td><em>iceA2</em>-F</td>
<td>GGTCTGCTGCTGCTGCTG</td>
<td>352</td>
<td>23</td>
</tr>
<tr>
<td><em>iceA2</em>-R</td>
<td>TGGTCTGCTGCTGCTGCTG</td>
<td>352</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td><em>m1</em></td>
<td><em>m1</em>-F</td>
<td>GGTCTGCTGCTGCTGCTG</td>
<td>352</td>
<td>23</td>
</tr>
<tr>
<td><em>m1</em>-R</td>
<td>TGGTCTGCTGCTGCTGCTG</td>
<td>352</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td><em>s1a</em></td>
<td><em>s1a</em>-F</td>
<td>CACACCTACCTACCTACCTA</td>
<td>190</td>
<td>23</td>
</tr>
<tr>
<td><em>s1a</em>-R</td>
<td>TGGTCTGCTGCTGCTGCTG</td>
<td>352</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td><em>s1b</em></td>
<td><em>s1b</em>-F</td>
<td>AGCCTGCTGCTGCTGCTG</td>
<td>190</td>
<td>23</td>
</tr>
<tr>
<td><em>s1b</em>-R</td>
<td>TGGTCTGCTGCTGCTGCTG</td>
<td>352</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td><em>s2</em></td>
<td><em>s2</em>-F</td>
<td>CACACCTACCTACCTACCTA</td>
<td>190</td>
<td>23</td>
</tr>
<tr>
<td><em>s2</em>-R</td>
<td>TGGTCTGCTGCTGCTGCTG</td>
<td>352</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Relationship between *H pylori* expression of Le antigens, cagA, iceA1, vacA, and cagA

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Isolate</th>
<th>Lea</th>
<th>Leb</th>
<th>Lea and/or Leb</th>
<th>Lea and/or Leb, and/or Leb</th>
<th>≥ 2 Le antigens</th>
<th>cagA</th>
<th>iceA1</th>
<th>iceA2</th>
<th>vacA s1a/m1T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic ulcer</td>
<td>67</td>
<td>61 (91.0)</td>
<td>58 (86.6)</td>
<td>64 (95.5)</td>
<td>18 (26.9)</td>
<td>34 (50.7)</td>
<td>60 (89.6)*</td>
<td>58 (86.6)</td>
<td>47 (70.1)</td>
<td>16 (23.9)</td>
</tr>
<tr>
<td>Non-ulcer</td>
<td>41</td>
<td>36 (87.8)</td>
<td>34 (82.9)</td>
<td>39 (95.1)</td>
<td>5 (12.2)</td>
<td>13 (31.7)</td>
<td>30 (73.2)</td>
<td>37 (90.2)</td>
<td>28 (68.3)</td>
<td>10 (24.4)</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
<td>97</td>
<td>92</td>
<td>103</td>
<td>23</td>
<td>47</td>
<td>90</td>
<td>95</td>
<td>76</td>
<td>26</td>
</tr>
</tbody>
</table>

Values are number (percentage).

*Le* antigens (Lea, Leb, Le, or Leb).

†p<0.05 compared with non-ulcer group.
1.0813 (0.6496) for Le⁺ (p=0.608); 1.3549 (1.1200) vs 1.3774 (1.1917) for Le⁻ (p=0.890). Sixty (89.6%) of 67 isolates from PU patients expressed two or more Le antigens (Le⁺, Le⁻, Le⁺ or Le⁻) compared to 30 (73.2%) of 41 isolates from NUD patients (p=0.035). Furthermore, 32/67 (47.8%) isolates from PU patients expressed three or more Le antigens compared with 11/41 (26.8%) isolates from NUD patients (p=0.043).

Levels of expression of Le⁺ and Le⁻ were not significantly different in the H pylori isolates from patients with peptic ulcer compared with those without ulcers (26.9% vs 12.2% (p=0.091) for Le⁺ and 50.7% vs 31.7% (p=0.072) for Le⁻, respectively). The results also showed that Le⁺ coexpressed with Le⁻.

RELATIONSHIP BETWEEN cagA STATUS AND CLINICAL OUTCOME
The cagA gene was positive in 95 (88%) of 108 H pylori isolates. As shown in table 2, the cagA gene was found in 58 (86.6%) of 67 PU isolates compared with 37 (90.2%) of 41 non-ulcer isolates (p=0.763). This shows that there was no significant difference for the cagA gene in H pylori isolates from PU and NUD patients.

Of the 95 cagA positive isolates, 91 (95.8%) expressed Le⁺ and/or Le⁻ antigens compared with 12 (92.3%) of 13 cagA negative isolates (p=0.480). Furthermore, the mean OD level of Le⁺ or Le⁻ expression was not significantly different between cagA positive and cagA negative isolates (1.0490 (0.6125) vs 0.9862 (0.8928) for Le⁺ (p=0.745); 1.2860 (1.0970) vs 1.8615 (1.3687) for Le⁻ (p=0.088).

RELATIONSHIP BETWEEN iceA STATUS AND CLINICAL OUTCOME
Of 108 isolates, iceA1 was positive in 75 isolates and iceA2 was detected in 26 isolates. Four isolates were positive for both iceA1 and iceA2, while three isolates did not yield either iceA1 or iceA2 fragments. There was no significant difference in the presence of iceA1 in H pylori isolates from PU and NUD patients (70.1% vs 68.3%; p=0.833). iceA1 was not associated with cagA status (p=0.531). Similarly, there was no significant difference in the presence of iceA2 in H pylori isolates from PU and NUD (23.9% vs 24.4%; p=1.000).

Of 75 iceA1 positive isolates, 72 (96.0%) expressed Le⁺ and/or Le⁻ antigens compared with 31 (93.9%) of 33 iceA1 negative isolates (p=0.640). The mean OD level of Le⁺ or Le⁻ expression was not significantly different between iceA1 positive and iceA1 negative isolates (1.0106 (0.6242) vs 1.0699 (0.7026) for Le⁺ (p=0.663); 1.2570 (1.0831) vs 1.4669 (1.2140) for Le⁻ (p=0.373)).

RELATIONSHIP BETWEEN vacA GENOTYPE AND CLINICAL OUTCOME
Of 108 isolates, 107 were typed as s1a of the vacA genotype and the other isolate was typed as s2. Four vacA genotypes (s1a/m1T, s1a/m1Tm2, s1a/m2, and s2/m2) of H pylori isolates were identified (table 3), with the distribution as follows: 39 s1a/m1T, 4 s1a/m1Tm2, 64 s1a/m2, and 1 s2/m2. There was no significant difference between PU and NUD patients for infection by s1a/m1T genotype H pylori isolates (37.3% vs 34.1%; p=0.837).

Discussion
In this study we observed the occurrence of Le⁺, Le⁻, Le⁺, Le⁻, and blood group A antigen in H pylori isolates. Of 108 isolates, 106 (98.1%) were typeable with Mabs specific for Lewis and other blood group antigens. The two isolates which were non-typeable showed O side chain (data not shown), indicating the existence of other serotypes that were not reactive with the Mabs used as described by Simoons-Smit and colleagues.15 One strain of H pylori expressed blood group A antigen which has so far been reported in one other Helicobacter species, H mustelae.24 However, the low prevalence of this antigen in H pylori isolates suggests that it does not have an important role in the pathogenesis of gastric diseases. The chemical structures of Le⁺, Le⁻, and Le⁺ or H pylori were elucidated earlier,25 while the chemical structure of Le⁻ has recently been determined.26

Le⁺ and Le⁻ were frequently encountered in our local H pylori isolates. Expression of Le⁺ and Le⁻ was similar to the finding in Canada,27 but was higher than the findings in the USA,14 Europe.15 Expression of Le⁺ and Le⁻ in H pylori isolates in our study was much higher than that found in the USA,14 Netherlands,15 and Canada.27 Broadhurst and Lin-Chu reported that the Le(+a - b+) phenotype is frequent in Chinese patients but rare or absent in Caucasians.28 The relationship between Le antigen expression by H pylori and host phenotype is not clear.27 29 Our observation of higher expression of Le⁺ and Le⁻ in our population of predominantly Chinese patients lends support to the suggestion by Wirth and colleagues29 that H pylori Le antigen expression is related to the host phenotype. However, Taylor and colleagues did not find such a correlation in their study.27

Wirth and colleagues29 suggested that the risk of peptic ulcer increases with expression of Le antibodies in H pylori isolates. They found that expression of Le⁺ or Le⁻ in H pylori isolates was significantly higher in PU than in NUD patients. It is important to note that only three of 96 subjects in their multicentre study were of Chinese origin. In contrast, in our predominantly Chinese patients (88/108 (81.5%)), we found equally high expression of Le⁺ and Le⁻ in H pylori isolates from both PU and NUD patients. Furthermore, the mean OD level of Le⁺ or Le⁻ expression was not significantly different between the two groups.

In this study we showed that the presence of two or more Le antigens (Le⁺, Le⁻, Le⁺, or Le⁻)
Lewis antigens, H pylori and peptic ulcer

was significantly higher in H pylori isolates from PU than from NUD patients. Expression of Le" and Le£ antigen in H pylori isolates from patients with PU was not significantly different from NUD patients.

As this was an exploratory study on the expression of Le antigens in H pylori strains and indeed the first such study in our population, statistical analyses were performed on multiple parameters. We cannot exclude the possibility that some findings may be due to chance. However, the finding of higher expression of Le antigens in peptic ulcer associated H pylori strains is novel in our population because it holds true on testing for ≥ 2 Le antigens and ≥ 3 Le antigens.

Several studies in Caucasian populations suggest an association between infection by cagA positive H pylori and PU disease. Our study indicates that cagA status is not predictive of gastroduodenal disease in the Singapore population, and adds to the growing evidence that the cagA gene should not be regarded as a universal virulence marker of peptic ulcer disease.

An association of peptic ulcer and infection by iceA1 positive H pylori isolates was described in two previous studies of Western patients. In accordance with van Doorn and colleagues, we found that the presence of iceA1 was independent of the cagA gene. Similar to cagA status, iceA1 of H pylori was not associated with PU risk in our population. This constitutes further evidence that distinct H pylori genotypes circulate in Western and Asian countries.

Four vacA genotypes (s1a/m1T, s1a/m1Tm2, s1a/m2, and s2/m2) of H pylori isolates were found in the Singapore population. In the present study almost all of the H pylori isolates were typed as s1a, which is similar to reports from China and Taiwan. The majority (95.4%) of H pylori isolates in Singapore were typed as s1a/m1T or s1a/m2, suggesting that there is less mosaicism in vacA alleles of H pylori in Singapore where the population is of mainly Chinese origin. The s1a/m1 genotype H pylori that was reported to be associated with PU was not detected in our population. In contrast with the finding by Wang and colleagues, there was no association between infection of s1a/m1T H pylori isolates and peptic ulcer in the present study.

The present study showed that peptic ulcer disease was not associated with cagA status or iceA or vacA genotypes but there was an association with increased expression of a combination of Le antigens. This suggests that the pathogenesis of H pylori induced gastric diseases may be due to host-pathogen interactions rather than H pylori itself. Expression of Le antigens may favour development of disease in the host by two mechanisms. Firstly, H pylori strains expressing Le antigens may adapt more readily to a host possessing similar antigens. Expression of host related antigens on the surface of bacteria could allow bacteria to elude elimination by the host immune response and thus facilitate persistence of infection in the host. Chronicity of infection may lead to the development of disease by increasing inflammation and promoting mucosa atrophy. Secondly, expression of host related antigens on H pylori may increase the pathogenic potential of the bacterium via induction of autoantibodies that cross react with gastric mucosa. However, it is noted that expression of Le antigens in H pylori was also high in NUD patients, which indicates that additional unidentified factors also contribute to the pathogenesis of H pylori associated peptic ulcer in our population.

The project was supported by the National University of Singapore (NUS) and the Ministry of Education (MOE) of Singapore (grant RSR-2001-0047). The authors thank Drs. Hiroyuki Horiuchi (University of Tsukuba, Japan) and Dina Applemeld (University of Melbourne, Australia) for helpful discussion. ZPY is a Research Scholar of NUS.

22 Lago AP, Goddard E, Faulconner A, et al. Diagnosis of Helicobacter pylori infection by PCR: comparison with


