Relation between Seroreactivity to Low-Molecular-Weight Helicobacter pylori-Specific Antigens and Disease Presentation

Ratha-Korn Vilaichone,1,2 Varocha Mahachai,3 Chomsri Kositchaiwat,4 David Y. Graham,2 and Yoshio Yamaoka2*

Gastroenterology Unit, Department of Medicine, Thammasat University Hospital, Pathumthani,1 and Gastroenterology Unit, Department of Medicine, Ramathibodi Hospital, Mahidol University,4 Bangkok, Thailand, and Department of Medicine, Veterans Affairs Medical Center, and Baylor College of Medicine, Houston, Texas2

Received 9 June 2003/Returned for modification 6 August 2003/Accepted 26 August 2003

The identification of Helicobacter pylori-strain specific factors that correlate with clinical outcome has remained elusive. We investigated possible relationships between a group of H. pylori antigens and clinical outcome and compared an immunoblot assay kit (HelicoBlot, version 2.1 [HB 2.1]; Genelabs Diagnostics) with an established serological test, the high-molecular-weight cell-associated protein test (HM-CAP). We used sera from 156 Thai patients with different disease presentations, including 43 patients with gastric cancer, 64 patients with gastric ulcer, and 49 patients with nonulcer dyspepsia (NUD). HB 2.1 was compared to HM-CAP as a diagnostic test for H. pylori infection. The seroprevalence of H. pylori was significantly higher among gastric cancer patients than among patients with NUD (93 and 67%, respectively; P < 0.01). Among the H. pylori-seropositive patients, the presence of the antibody to the 37,000-molecular-weight antigen (37K antigen) was inversely related to the presence of gastric cancer (e.g., for gastric cancer patients compared with NUD patients, odds ratio [OR] = 0.28 and 95% confidence interval [CI] = 0.1 to 0.8). The presence of antibody to the 35K antigen was higher in gastric ulcer patients than in NUD patients (OR = 11.5; 95% CI = 2.4 to 54.3). The disease associations of antibodies to the 35K and 37K antigens are consistent with the possibility that these antigens are either indirect markers for H. pylori-related diseases or have specific active or protective roles in H. pylori-related diseases.

Although histological gastritis is essentially universal among Helicobacter pylori-infected individuals, only a fraction of those infected develop a clinically important outcome, such as peptic ulcer and gastric carcinoma (4). Experience with other bacterial pathogens suggests that H. pylori strain-specific factors may influence the pathogenicity as well as the risk of developing different H. pylori-related outcomes. There has been considerable interest in putative bacterial virulence factors. The most studied of these factors are the cag pathogenicity island (for which the cagA gene is the marker) and the vacuolating cytotoxin (for which the vacA gene is the marker) (2, 7, 8, 10, 12, 13). However cagA gene-positive, vacuolating cytotoxin-producing strains are predominant, irrespective of the clinical outcome, especially in Asia (e.g., Japan and Korea) (19). Thus, at least in Asia, the presence of these antigens has little or no value in predicting the clinical outcome.

In a previous study, immunoblot assays were used to examine the prevalence of antibodies to the various H. pylori antigens in a Japanese population (18). We found that antibody to a low-molecular-weight antigen, the 33,000- to 35,000-molecular weight antigen (33K-35K antigen), was linked to or was a marker for peptic ulcer diseases (18). This hypothesis has not been tested previously. In Thailand gastric cancer is one of the most common causes of cancer death (the sixth leading cause of cancer death in men) (16). Like the strains from other Asian countries, Thai strains are predominantly cag pathogenicity island positive and have vacA s1 (vacuolating cytotoxin-producing) genotypes, irrespective of the clinical outcome (R.-K. Vilaichone et al., unpublished data). This study investigated the anti-H. pylori antibody profiles in a Thai population by using immunoblot analyses to correlate the H. pylori antigens used in the immunoblot assay and the outcomes of gastric cancer or gastric ulcer.

MATERIALS AND METHODS

Patients. Thai patients with gastric cancer, gastric ulcer, or nonulcer dyspepsia (NUD) were entered into the study. Gastric ulcers were identified endoscopically. NUD was defined as dyspeptic symptoms without peptic ulcer, gastric cancer, or erosive esophageal disease. Gastric cancers were confirmed histologically. All gastric cancers were of an advanced stage and of the distal type. Patients were excluded if they had previously received anti-H. pylori therapy, had had gastric surgery, or had used antibiotics or proton pump inhibitors within the last 4 weeks. Informed consent was obtained from all patients, and the protocol was approved by the hospital ethics committee. Serum samples were obtained

TABLE 1. Demographic data for patients with gastric cancers, gastric ulcers, and NUD

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Gastric cancer</th>
<th>Gastric ulcer</th>
<th>NUD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>43</td>
<td>64</td>
<td>49</td>
</tr>
<tr>
<td>No. of male/no. of females</td>
<td>23/20</td>
<td>42/22w</td>
<td>17/32</td>
</tr>
<tr>
<td>Age range (yr)</td>
<td>33–85</td>
<td>29–85</td>
<td>19–83</td>
</tr>
<tr>
<td>Mean ± SD age (yr)</td>
<td>60.4 ± 13.6w</td>
<td>62.1 ± 13.1w</td>
<td>45.8 ± 17</td>
</tr>
</tbody>
</table>

w P < 0.01 compared to NUD patients.
TABLE 2. Accuracy HB 2.1 compared to that of HM-CAP

<table>
<thead>
<tr>
<th>Disease (no. of patients)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Accuracy (%)</th>
<th>NPV (%)</th>
<th>PPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric cancers (43)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Gastric ulcers (64)</td>
<td>100</td>
<td>85</td>
<td>97</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>NUD (49)</td>
<td>97</td>
<td>89</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>Total patients (156)</td>
<td>99</td>
<td>88</td>
<td>97</td>
<td>97</td>
<td>97</td>
</tr>
</tbody>
</table>

from each patient on the day of the endoscopic procedure and were stored at −80°C until serology tests were performed.

Endoscopy was performed after an overnight fast. Gastric biopsy specimens, one from the antrum and one from the corpus, were obtained for histopathological examination and to identify H. pylori infection. Biopsy specimens for histology were fixed in 10% buffered formalin, embedded in paraffin, cut in sequential 4-μm sections, and stained with Giemsa stains. The presence of H. pylori in the gastric biopsy specimens was recognized by detection of the characteristic organism by microscopic examination.

Serological analyses. Serum samples were analyzed with an immunoblot assay kit (Helicoblot, version 2.1 [HB 2.1]; Genelabs Diagnostics, Singapore, Republic of Singapore) in accordance with the instructions of the manufacturer. HB 2.1 contains a special antigen line, designated the current infection marker (CIM). CIM was originally identified by screening immunogenic proteins of H. pylori and was synthesized by using recombinant technology. CIM is located at the bottom of the strip as an independent band, such that the location does not correspond to the apparent molecular weight. The HB 2.1 criteria for H. pylori seropositivity were (i) a positive result for the 116K band (CagA) and one or more of the 89K (VacA), 37K, 35K, 30K (UreA), and 19.5K bands, together with (ii) the presence of the 89K, 37K, or 35K band and (iii) the presence of both the 30K and 19.5K bands. CIM was noted but was not used to define the presence or absence of infection. All strips were interpreted blindly by one of the authors (Y.Y.) according to the instruction manual. The results were compared with those of another established serologic test (high-molecular-weight cell-associated protein [HM-CAP]; Enteric Products, Inc., Westbury, N.Y.).

Statistical analysis. The sensitivity, specificity, and accuracy were calculated against the diagnosis of H. pylori infection determined by using HM-CAP as the “gold standard.” Data were analyzed by using the Statistical Package for the Social Sciences. If normality assumptions were satisfied, the parametric two-sample t test was used; otherwise, the Mann-Whitney U test was applied. Associations between categorical variables were determined by using the \( \chi^2 \) test, with odds ratios (ORs) presented where applicable. Logistic regression analysis was used to relate the risk of gastric cancer or gastric ulcer adjusted by age and sex. A P value less than 0.05 was considered significant.

RESULTS

HB 2.1 results and clinical outcome. We examined 156 Thai patients (43 patients with gastric cancer, 64 patients with gastric ulcer, and 49 patients with NUD). The ages of the patients with gastric cancer (mean ± standard deviation [SD], 60.4 ± 13.6 years; range, 33 to 85 years) and gastric ulcer (mean ± SD, 62.1 ± 13.1; range, 29 to 85 years) were significantly higher than those of the NUD patients (mean ± SD, 45.8 ± 17; range, 19 to 83 years) (Table 1). The male/female ratio for gastric ulcer patients was also significantly higher than that for NUD patients (42 males and 22 females versus 17 males and 32 females, respectively). Therefore, logistic regression analysis was used to adjust age and sex before comparison of these groups of patients in the analyses for the relationship between the presence of H. pylori-specific antigens and clinical outcome.

By using HM-CAP as the gold standard, the overall sensitivity, specificity, accuracy, negative predictive value (NPV), and positive predictive value (PPV) of HB 2.1 for H. pylori infection status were 99, 88, 97, 97, and 97%, respectively (Table 2). The prevalence of H. pylori infection was significantly higher in gastric cancer patients than in NUD patients (Table 3).

In contrast, the prevalence of H. pylori infection determined by histology or CIM was significantly lower than that determined by either HB 2.1 or HM-CAP (Table 3). If histology was considered the standard, the overall sensitivity, specificity, accuracy, NPV, and PPV of CIM for H. pylori status were 87, 84, 86, 82, and 89%, respectively. These data are consistent with the suggestion that CIM antigen identifies active infection and that CIM-negative and HB 2.1- or HM-CAP-positive patients represent those with prior H. pylori infection.

H. pylori-specific antigens in HB 2.1 and clinical outcome. As expected, almost all infected Thai patients possessed the CagA and VacA antigens, and there was no relationship between the presence of these antigens and the clinical outcomes (Table 4). Logistic regression analysis showed that the presence of antibody to the 37K antigen was uncommon in patients with gastric cancer, and this difference was statistically significant compared with the findings for NUD patients (OR = 0.28; 95% confidence interval [CI] = 0.1 to 0.8) (Table 4). The presence of antibody to the 35K antigen was associated with gastric ulcer (OR = 11.5; 95% CI = 2.4 to 54.3 for gastric ulcer patients compared with NUD patients) (Table 4). Both gastric cancer and gastric ulcer are associated with corpus gastritis. The patients with gastric cancer and gastric ulcer together had a prevalence of antibody to the 35K antigen that was greater than that among those with NUD (OR = 3.7; 95% CI = 1.2 to 11.7). Interestingly, 17 patients had antibody to the 35K antigen, but antibody to the 37K antigen was absent from the patients. This pattern was completely absent among the NUD patients.

DISCUSSION

Serologic tests are widely used for the diagnosis of H. pylori infection because they are both simple and convenient. The immunoblot assay has the advantage that one can also investigate possible relationships between disease presentation and the presence of specific H. pylori antigens. A number of studies have reported on the use of the immunoblot assay, including those that have used commercial assay kits (9, 15, 18). Probably the most widely used commercial immunoblot assay is the one from Genelabs Diagnostics, which produces HB 2.0 and the revised version, HB 2.1. We recently evaluated the accuracy of HB 2.1 with a Japanese population by using a combination of histology, culture, and serology (HM-CAP) as the gold standard and found HB 2.1 to be accurate for the diagnosis of H.
studies conducted in Thailand (14, 17), which used only the gastric cancer patients was higher than that found in previous infection (9). In this study, we showed that HB 2.1 and other countries (3, 20). The prevalence of infection in gastric cancer patients was higher than that found in previous studies conducted in Thailand (14, 17), which used only the rapid urease test and histology to detect H. pylori infection. In fact, if we used only histology as a marker of H. pylori infection, the prevalence of H. pylori infection in gastric cancer patients would be only 53% (Table 3). It is well known that very advanced atrophic gastritis is an unhealthy environment for H. pylori and that the infection is frequently lost in these patients (5, 6, 11). The results of histology and tests for the presence of antibody to CIM were similar, suggesting that this explanation is likely correct.

The disease associations shown in these studies suggest that several H. pylori antigens may be protective (e.g., the 37K antigen) or have a role in disease pathogenesis (e.g., the 35K antigen), or, alternately, they may simply be markers for the presence of as yet unidentified factors or antigens that have disease associations. In this study, gastric cancer was inversely associated with the presence of antibody to the 37K antigen (OR = 0.28; 95% CI = 0.1 to 0.8). Studies are needed to investigate whether this antigen is not expressed in patients who subsequently develop gastric cancer or whether expression is switched off in response to the gastric environment in gastric cancer patients. The original version of HelicoBlot (HB 2.0) did not include the 37K antigen, which prevents comparison of these results to those obtained with the Japanese population (18). Despite the relatively low prevalence of antibody to the 35K antigen, this study confirmed prior observations regarding a relationship between the 35K antigen and peptic ulcer (1, 18). Recently, studies have shown that the 35K antigen does not represent OipA (9). The 37K and the 35K antigens remain unidentified, and their identification is required in order to ascertain whether the proposed associations are biologically plausible.

**ACKNOWLEDGMENTS**

The material presented here is based on work supported in part by the Office of Research and Development, Medical Research Service, U.S. Department of Veterans Affairs, and by Public Health Service grant DK56338, which funds the Texas Gulf Coast Digestive Diseases Center. R.-K.V. received an American College of Gastroenterology International GI Training Grant Award.

We thank Genelabs Diagnostics, which kindly provided HB 2.1.

**REFERENCES**


**TABLE 4. Results of multivariable model of each immunoreactive band for H. pylori infection**

<table>
<thead>
<tr>
<th>Immunoreactive band</th>
<th>% (no.) of the following patients:</th>
<th>OR (95% CI) for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gastric cancer (40)b</td>
<td>Gastric cancer vs NUD</td>
</tr>
<tr>
<td>CIM</td>
<td>57 (23)</td>
<td>0.8 (0.2–2.3)</td>
</tr>
<tr>
<td>19.5K</td>
<td>70 (28)</td>
<td>0.9 (0.2–2.3)</td>
</tr>
<tr>
<td>30K (arc4)</td>
<td>88 (35)</td>
<td>0.4 (0.1–1.3)</td>
</tr>
<tr>
<td>35K</td>
<td>25 (10)</td>
<td>2.0 (0.5–7.4)</td>
</tr>
<tr>
<td>37K</td>
<td>23 (9)</td>
<td>0.28 (0.1–0.8)</td>
</tr>
<tr>
<td>89K (vac4)</td>
<td>95 (38)</td>
<td>2.8 (0.2–4.2)</td>
</tr>
<tr>
<td>116K (cag4)</td>
<td>95 (38)</td>
<td>100 (53)</td>
</tr>
</tbody>
</table>

a The total number of patients in each group.
b The OR was statistically significant.

The prevalence of H. pylori infection was significantly higher among gastric cancer patients than among NUD patients, in agreement with the results of previous studies conducted in other countries (3, 20). The prevalence of H. pylori infection in gastric cancer patients was higher than that found in previous studies conducted in Thailand (14, 17), which used only the rapid urease test and histology to detect H. pylori infection. In fact, if we used only histology as a marker of H. pylori infection, the prevalence of H. pylori infection in gastric cancer patients would be only 53% (Table 3). It is well known that very advanced atrophic gastritis is an unhealthy environment for H. pylori and that the infection is frequently lost in these patients (5, 6, 11). The results of histology and tests for the presence of antibody to CIM were similar, suggesting that this explanation is likely correct.

The disease associations shown in these studies suggest that several H. pylori antigens may be protective (e.g., the 37K antigen) or have a role in disease pathogenesis (e.g., the 35K antigen), or, alternately, they may simply be markers for the presence of as yet unidentified factors or antigens that have disease associations. In this study, gastric cancer was inversely associated with the presence of antibody to the 37K antigen (OR = 0.28; 95% CI = 0.1 to 0.8). Studies are needed to investigate whether this antigen is not expressed in patients who subsequently develop gastric cancer or whether expression is switched off in response to the gastric environment in gastric cancer patients. The original version of HelicoBlot (HB 2.0) did not include the 37K antigen, which prevents comparison of these results to those obtained with the Japanese population (18). Despite the relatively low prevalence of antibody to the 35K antigen, this study confirmed prior observations regarding a relationship between the 35K antigen and peptic ulcer (1, 18). Recently, studies have shown that the 35K antigen does not represent OipA (9). The 37K and the 35K antigens remain unidentified, and their identification is required in order to ascertain whether the proposed associations are biologically plausible.