Inverse relation of serum *Helicobacter pylori* antibody titres and extent of intestinal metaplasia

H Osawa, F Inoue, Y Yoshida

Abstract

**Aims**—To clarify the relation between the serum titre of anti-*Helicobacter pylori* (*H pylori*) antibody and the extent of intestinal metaplasia of the gastric mucosa.  

**Methods**—The serum anti-*H pylori* IgG titres of 95 asymptomatic individuals (mean age 65 years) undergoing an annual health examination were measured and compared with the extent of intestinal metaplasia (absent, moderate, or extensive), determined by examination of multiple endoscopic mucosal biopsy specimens. Serum pepsinogen I (PGI) levels, as a marker for gastric atrophy, were also measured.  

**Results**—The prevalence of seropositivity for *H pylori* antibody was high (>80%), regardless of the extent of metaplasia. However, there was a negative association between the extent of metaplasia and the anti-*H pylori* titre: 75% of the subjects in the group without metaplasia had high (3+) antibody levels, as did 43% with moderate, and 37% with extensive metaplasia (absent v extensive). The inverse relation between the titre and the extent of metaplasia was evident when examined in those with normal PGI (>30 ng/ml), whereas no such relation was apparent in subjects with low PGI (≤30 ng/ml).  

**Conclusions**—The anti-*H pylori* titre correlates inversely with the extent of intestinal metaplasia, particularly in subjects with less marked gastric atrophy.


Keywords: extent of intestinal metaplasia, anti-*Helicobacter pylori* IgG titre, gastric atrophy

*Helicobacter pylori*, first isolated by Warren and Marshall in 1983, has been implicated in the pathogenesis of peptic ulcer. Its relation to gastric cancer has been also noted recently, particularly in patients with intestinal-type carcinoma with underlying intestinal metaplasia. Conversely, *H pylori* has been reported to occur infrequently in areas of intestinal metaplasia. Until recently, the techniques available for the detection of *H pylori* (isolation-culture, assessment of urease activity, and microscopic examination after Gram staining) all required biopsy specimens of the gastric mucosa. The problem with all these techniques is that they yield no information about infection in any part of the gastric mucosa other than the site of biopsy. The difficulty in distinguishing endoscopically mucosa with mild to moderate intestinal metaplasia from normal mucosa has complicated these direct sampling methods further, when the relevance of *H pylori* infection to intestinal metaplasia is discussed.

Recently, enzyme linked immunosorbent assays (ELISA) for anti-*H pylori* antibodies in serum have been developed. Studies on the effect of bacterial eradication on the antibody titre suggest strongly that the antibody titre reflects the current state of *H pylori* infection in the entire gastric mucosa. We have used one of these ELISAs to evaluate the status of *H pylori* infection in a cohort study for the screening of gastrointestinal disorders. We estimated the anti-*H pylori* antibody titres and compared them with the degree of intestinal metaplastic extension evaluated histologically by examination of multiple site biopsy specimens. The relation between *H pylori* infection and gastric atrophy, which frequently leads to intestinal metaplasia, was also examined by comparing the antibody titres with the serum levels of pepsinogen I (PGI), a marker of atrophic gastritis.

**Methods**

**Subjects**  
Subjects were 95 asymptomatic individuals in their sixties (30 men and 65 women, mean age 64.8 years) from 104 who requested gastroscopy as a part of a health examination in a town in Kyushu island in 1992. None of the 95 individuals recruited was taking any medication at the time of the examination. Nine individuals who had a history of peptic ulcer or gastric surgery, abdominal pain and dyspepsia, or were currently taking antacids, H2 receptor antagonists, proton pump inhibitors, antibiotics, steroids, or non-steroidal anti-inflammatory drugs, were excluded from the study. All gave informed written consent.

**Serum and biopsy specimens**

After endoscopic examination for gastric mucosal abnormality including cancer and polyp, two mucosal biopsy specimens were taken from each of the following five sites on the lesser curvature: (1) antrum, (2) angulus, (3) lower body, (4) middle body, and (5) upper body. The specimens were fixed immediately in 4%...
neutral buffered formalin and processed for histological examination. Sera were separated within one hour of blood sampling and stored at −20°C until assayed.

ELISA FOR H PYLORI IgG
Serum anti-H pylori antibody titres were measured using a semiquantitative IgG ELISA kit (GAP Test IgG, Biomerica) in accordance with the manufacturer’s instructions. This ELISA uses a DEAE ion exchange, chromatography purified H pylori antigen. Serial two- or threefold dilutions (1:2–1:16) of positive and negative control sera were assayed in duplicate on each ELISA plate to determine the cut off index for semiquantitative analysis based on the positive/negative ratio. Semiquantitative titres of 3+, 2+, 1+, and 0 were assigned to the positive/negative ratio obtained with 1:2, 1:4, 1:8, and 1:16 dilutions respectively of the positive control. Subjects with semiquantitative titres of 3+, 2+, and 1+ were defined as seropositive. Subjects with a titre of 0 or positive/negative ratio lower than the 1:16 dilution of the positive control serum were considered to be seronegative.

SERUM CONCENTRATIONS OF PEPSONIEN I
Serum pepsinogen I concentrations were determined using a competitive binding double antibody radioimmunoassay kit (Dainabot) following the manufacturer’s instructions.

PATHOLOGICAL ASSESSMENT AND CLASSIFICATION
The biopsy specimens were routinely processed and stained with haematoxylin and eosin. The presence or absence of intestinal metaplasia was determined by a single pathologist throughout the study.

The extent of histological changes was assessed from the biopsies taken from the five different sites previously described, and was assigned to groups showing absent, moderate, or extensive intestinal metaplasia.

As metaplastic changes appear first at the antrum or angulus of the stomach and extend towards the upper body, subjects who showed metaplasia in the upper gastric body were defined as the group with extensive intestinal metaplasia. Subjects without metaplasia showed no metaplastic changes in any biopsy specimen. The group with moderate intestinal metaplasia included subjects in whom changes were detected in one or more specimens from the antrum, angulus, lower body, or middle body, but not in the upper body.

We also allocated subjects to two subgroups according to their serum PGI concentrations: a low PGI group (PGI ≤ 30 ng/ml) with severe atrophic gastritis, and a normal PGI group (PGI > 30 ng/ml) with absent or lesser degrees of atrophic gastritis, irrespective of the extent of intestinal metaplasia.

Table 1 The prevalence of subjects with H pylori infection in three groups classified according to the extent of intestinal metaplasia

<table>
<thead>
<tr>
<th>Intestinal metaplasia</th>
<th>None</th>
<th>Moderate</th>
<th>Extensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal PGI</td>
<td>19/19</td>
<td>11/12</td>
<td>18/19</td>
</tr>
<tr>
<td>Low PGI</td>
<td>5/7</td>
<td>10/11</td>
<td>20/27</td>
</tr>
<tr>
<td>Total</td>
<td>24/26(92%)</td>
<td>21/23(91%)</td>
<td>38/46(83%)</td>
</tr>
</tbody>
</table>

PGI = pepsinogen I.

STATISTICAL METHODS
The Wilcoxon rank sum test was used to compare the titre of anti-H pylori antibody between the groups with intestinal metaplasia (extensive or moderate) and the group without changes. The Bonferroni correction with rest was used to compare the PGI levels among the three groups. Probability (p) values less than 0.05 were considered to indicate a significant difference.

Results

ANTI-H PYLORI IgG TITRE AND INTESTINAL METAPLASIA
Eighty of the 95 subjects (87%) examined had positive antibodies to H pylori. The prevalence of H pylori positivity was uniformly high in all groups irrespective of the extent of intestinal metaplasia (table 1). Subjects who were anti-H pylori IgG negative were excluded from further analysis since they were considered not to have any history of H pylori infection.

Among the 83 seropositive subjects, 24 had no intestinal metaplasia according to our classification, 21 had moderate intestinal metaplasia, and 38 had extensive intestinal metaplasia. The percentage of seropositive patients in these groups was similar (92%, 91%, and 83% respectively). However, there was a negative association between the extent of metaplasia and the anti-H pylori titre. The prevalence of a high (3+) titre of anti-H pylori antibody was 75% in the group without metaplasia, 43% in the group with moderate metaplasia, and 37% in the group with extensive metaplasia. The difference was statistically significant between the group without metaplasia and the group with extensive metaplasia (p = 0.007, table 2).

ANTI-H PYLORI IgG TITRE AND GASTRIC ATROPHY
Table 3 shows the proportions of subjects with low (1+), medium (2+), and high (3+) titres

Table 2 The prevalence of subjects with high titre (3+), medium titre (2+), and low titre (1+) of anti-H pylori IgG in three groups classified by the extent of intestinal metaplasia

<table>
<thead>
<tr>
<th>Anti-H pylori IgG titre, n(%)</th>
<th>I+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without metaplasia (n = 24)</td>
<td>1 (4)</td>
<td>5 (21)</td>
<td>18 (75)</td>
</tr>
<tr>
<td>Moderate intestinal metaplasia (n = 21)</td>
<td>1 (5)</td>
<td>11 (52)</td>
<td>9 (43)</td>
</tr>
<tr>
<td>Extensive intestinal metaplasia* (n = 38)</td>
<td>5 (13)</td>
<td>19 (50)</td>
<td>14 (37)</td>
</tr>
</tbody>
</table>

* p = 0.007 v without metaplasia.
metaplasia

in the group with normal PGI (>30 ng/ml) and in that with low PGI (≤30 ng/ml). The proportion of subjects with high titre is significantly higher in the normal PGI group compared with that in the low PGI group (p = 0.040). This inverse relation between the anti-H pylori IgG titre and the extent of intestinal metaplasia was more evident among patients with normal PGI levels (>30 ng/ml). The prevalence of a high (3+) titre of anti-H pylori antibody was 84% in the group without metaplasia, 45% in the group with moderate metaplasia, and 39% in the group with extensive metaplasia. The difference in prevalence was statistically significant between the group without metaplasia and the group with moderate metaplasia (p = 0.025), as well as between the group without metaplasia and the group with extensive metaplasia (p = 0.004) (table 4). In the low PGI group, only five of 35 subjects (14%) had biopsies showing no intestinal metaplasia, while 20 (57%) had extensive intestinal metaplasia. The anti-H pylori IgG titres in the low PGI group were not significantly different between these groups, and the prevalence of high titres in each group tended to be lower than in the normal PGI group (table 5).

Table 4 The prevalence of subjects with high titre (3+), medium titre (2+), and low titre (1+) of anti-H pylori IgG in three groups classified by the extent of intestinal metaplasia, in the subgroup with normal pepsinogen I levels

<table>
<thead>
<tr>
<th>Anti-H pylori IgG titre, n(%)</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without metaplasia (n=19)</td>
<td>0</td>
<td>3</td>
<td>16 (84)</td>
</tr>
<tr>
<td>Moderate intestinal metaplasia (n=11)</td>
<td>0</td>
<td>0</td>
<td>6 (55)</td>
</tr>
<tr>
<td>Extensive intestinal metaplasia (n=18)</td>
<td>2 (11)</td>
<td>9 (50)</td>
<td>7 (39)</td>
</tr>
</tbody>
</table>

*p = 0.025 v without metaplasia; †p = 0.004 v without metaplasia.

PGI CONCENTRATIONS AND INTESTINAL METAPLASIA

Serum PGI was 38.5 (SD 18.2) ng/ml in the group without intestinal metaplasia, 32.5 (15.7) ng/ml in the group with moderate intestinal metaplasia, and 30.1 (22.7) ng/ml in the group with extensive intestinal metaplasia. Thus PGI concentrations tended to decrease as intestinal metaplasia extended, although the differences were not statistically significant among these groups.

Discussion

Although it is thought that H pylori may play a role in the malignant transformation of intestinal metaplasia, the bacteria have not often been detected in the gastric mucosae of patients with intestinal metaplasia. The altered milieu in the areas of intestinal metaplasia, including an increase of secretory IgA and less acidity compared with the normal gastric mucosa, may explain the decreased colonisation of H pylori in the metaplastic mucosa. IgA in the gastric juice may be important in inhibiting bacterial adhesion to the epithelial surface, and the decreased acidity may inhibit the proliferation of H pylori. Consequently, it is essential to take biopsy specimens of mucosa not involved in the metaplasia and examine them for H pylori in such patients. However, the endoscopic diagnosis of mild or moderate metaplasia is quite difficult without histological examination. Therefore H pylori detection by biopsy specimen examination may not be very reliable, particularly in elderly Japanese patients in whom intestinal metaplasia of the stomach is common.

Recently, serological techniques for detecting H pylori have been developed and several studies have been conducted to compare the results of serological tests and direct detection in biopsy specimens. However, no studies referring to the serological titre and extent of intestinal metaplasia have, to our knowledge, been reported. In the present study, we compared the anti-H pylori IgG titre, evaluated by a sensitive and highly specific ELISA and the extent of intestinal metaplasia, evaluated by histological examination of biopsy specimens taken from multiple sites.

Anti-H pylori antibodies were detected in over 80% of the subjects examined, regardless of the extent of intestinal metaplasia. This result agrees with a previous report that the prevalence of H pylori infection is high (70–80%) and relatively constant in Japanese persons born before 1950. The anti-H pylori IgG titre was related inversely to the extent of intestinal metaplasia. As the serum antibody titre is considered to reflect the current state of H pylori infection, our results suggest that the number of bacteria present in gastric mucosa decreased in proportion to the extent of intestinal metaplasia. Consequently, the prevalence of serum positivity in patients in whom H pylori is not detected in biopsy specimens would increase as intestinal metaplasia extends. The discrepancies between serological evaluation and direct detection in previous studies can thus be explained.

Next, we examined the relation between the anti-H pylori titre and the severity of gastric
The severity of mucosal atrophy, because the bacterium has also been reported to be absent in severe atrophic mucosa. 8 The severity of mucosal atrophy was evaluated by measuring the serum PGI levels. Samloff et al 10 reported that in mild or moderate atrophic gastritis, PGI concentrations did not differ significantly from normal, whereas they were reduced in severe atrophic gastritis. We found that the prevalence of high anti- H pylori antibody titres was greater in the group with normal PGI concentrations than in the group with low PGI. The inverse relation between the anti- H pylori IgG titre and the extent of intestinal metaplasia was statistically significant among patients with normal PGI concentrations, but not among those with a low PGI. This serological result suggests that severe gastric mucosal atrophy itself suppresses the number of bacteria, regardless of the presence of intestinal metaplasia, although the small number of patients without intestinal metaplasia in this group made precise evaluation difficult. The interpretation of the relation between H pylori infection and intestinal metaplasia in the low PGI group is further complicated by the high frequency of intestinal metaplasia in patients with severe atrophic gastritis. These results show that the anti- H pylori IgG titre has a strong inverse correlation with the extent of intestinal metaplasia, at least in subjects with less marked atrophic changes.

Our study showed that H pylori seropositivity is quite common in elderly patients, even in those with intestinal metaplasia, despite reports that the organism cannot be detected histologically in the gastric mucosa of such patients. This indicates that H pylori infection could occur concurrently or at least may have occurred in the recent past. Another interesting finding of this serological study was that the antibody titre showed an inverse correlation with the extent of metaplasia, implying that the bacterial load may decrease quantitatively as the metaplasia extends. Considering the high risk of gastric cancer in elderly patients with intestinal metaplasia, 4-23 in whom endoscopic diagnosis is not necessarily easy, those with low anti- H pylori antibody titres should be observed closely, even if marked gastric atrophy is not present.

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