IgG2 response and low IgG titre specific to Helicobacter pylori CagA as serological markers for gastric cancer

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Infection with Helicobacter pylori cytotoxin-associated gene A (CagA)-positive strains is associated with the development of gastric cancer (GC). However, some reports have failed to demonstrate an increased frequency of CagA antibodies in GC patients. This study evaluated the response of IgG antibody and subclasses IgG1 and IgG2 against both CagA and H. pylori membrane antigens in patients with pre-cancerous lesions and cases with GC. A total of 137 patients with a positive serum IgG response to H. pylori were selected: 46 with intestinal metaplasia, 41 with gastric adenocarcinoma and 50 with non-atrophic gastritis (NAG) considered as controls. The response of total IgG, IgG1 and IgG2 was investigated by immunoblot and ELISA using an in-house recombinant CagA and membrane antigens from a local strain, and possible associations were estimated using a logistic regression model. Compared with NAG patients, GC patients showed a higher frequency of IgG2 CagA antibodies (55.2 vs 15.4 %, \( P < 0.001 \)), but a lower frequency (80.5 vs 96.0 %, \( P = 0.021 \)) and diminished levels of IgG2 H. pylori antibodies [12.5 vs 21.9 ELISA units (EU), \( P = 0.007 \)]. GC patients also presented lower levels of CagA (32.6 vs 42.4 EU, \( P = 0.004 \)) and H. pylori total IgG (33.7 vs 38.7 EU, \( P = 0.029 \)). GC was associated with a positive IgG2 CagA response [odds ratio (OR) = 3.74, 95 % confidence interval (CI) 1.81–5.37; \( P = 0.002 \)] and with a low titre of total IgG CagA antibodies (OR = 2.18, 95 % CI 1.35–2.69; \( P = 0.006 \)). These results suggest that the IgG2 response to CagA could be used as a novel serological marker to identify patients with H. pylori-associated GC.

INTRODUCTION

According to estimations based on seroprevalence studies, half of the world population is infected with Helicobacter pylori (Brown, 2000). In 1994, the International Agency for Research on Cancer classified H. pylori as a class 1 human carcinogen (IARC Working Group, 1994). As reported by meta-analyses, a two- to threefold increased risk of stomach cancer is associated with this infection (Huang et al., 1998; Eslick et al., 1999). Gastric malignancy is the fourth most common type of cancer and the second most common cause of cancer-related deaths worldwide (Parkin et al., 2005). H. pylori infection induces a chronic inflammation of the gastric mucosa that is intensified by high levels of pro-inflammatory cytokines during the immune response, especially in the case of strains possessing the cagA gene (Peek et al., 1995; Yamaoka et al., 1997). In a small proportion of infected individuals and over a decades-long process, this chronic inflammation leads to the development of gastric cancer (GC) through a series of progressive stages that include non-atrophic gastritis (NAG), atrophic gastritis, metaplasia and dysplasia (Correa, 1996).

The cagA gene of H. pylori is located in the cag pathogenicity island (cag-PAI). The CagA toxin is injected into the gastric

Abbreviations: cagA, cytotoxin-associated gene A; cag-PAI, cag pathogenicity island; CI, confidence interval; DC, dendritic cell; EU, ELISA units; GC, gastric cancer; IFN-\( \gamma \), gamma interferon; IL-4, interleukin-4; IM, intestinal metaplasia; NAG, non-atrophic gastritis; OR, odds ratio; Th, T helper.
epithelial cells through a type IV secretion system also encoded in the cag-paI; once translocated into the cytoplasm, CagA can act as a master key that is involved in several cell-signalling cascades, which include the induction of membrane dynamics, actin cytoskeletal rearrangements and the disruption of cell–cell junctions, as well as proliferative, pro-inflammatory and anti-apoptotic responses that in turn favour neoplastic transformation (Backert et al., 2010). A meta-analysis showed that infection with CagA-positive strains further increases the risk for GC in comparison with \textit{H. pylori} infection alone (Huang et al., 2003). However, some reports have failed to demonstrate a high prevalence of anti-CagA antibodies among GC patients (Yamaoka et al., 1999; Schumann et al., 2006; Chomvarin et al., 2009). Moreover, there also exists evidence that both age and the severity of the mucosal lesions negatively influence the performance of CagA as a serological marker for GC (Camorlinga-Ponce et al., 2008). An association between the presence of GC and a low titre of anti-CagA antibodies has also been reported (Nomura et al., 2002; Suzuki et al., 2007). These heterogeneous results make it difficult to use simple CagA-positive serology as a marker for malignancy.

The response of the IgG subclass to \textit{H. pylori} comprises the four IgG subclasses and it varies according to the associated disease, age and ethnic groups analysed (Mitchell et al., 2001, 2002; Dzierzanowska–Fangrat et al., 2003). In particular, a selective reduction in IgG2 levels in subjects with GC has been observed in comparison with non-malignant diseases (Ren et al., 2005). To date, there are only two reports on the IgG subclass response to CagA in children and young adults with benign diseases; CagA is predominantly recognized by IgG1, followed by IgG3 and then IgG2, and it fails to stimulate an IgG4 response (Dzierzanowska-Fangrat et al., 2003; Du et al., 2006). As in many infections, the IgG subclass response to infecting pathogens has been associated with the severity of clinical symptoms, and because patients with malignant diseases exhibit a significant alteration in the composition of serum IgG subclasses (Hussain et al., 1995; Lagacé et al., 1999; Schauenstein et al., 1997; Anderhuber et al., 1999), the present work aimed to evaluate the total IgG antibody response and that of the subclasses IgG1 and IgG2 against \textit{H. pylori} antigens in patients with GC, intestinal metaplasia (IM) or NAG.

**METHODS**

**Patients.** Serum samples were obtained from a previous study (Camorlinga-Ponce et al., 2008) carried out between October 1999 and July 2002 from patients attending the Gastroenterology Unit at the Hospital General, Secretaria de Salud, and the Centro Medico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, both in Mexico City. Informed consent was obtained from each subject, and they were interviewed using a previously validated questionnaire on clinical and demographic characteristics (Camorlinga-Ponce et al., 2008). For the study, 137 patients who were \textit{H. pylori} positive by ELISA and immunoblot were included: 46 with IM, 41 with GC and 50 with NAG. The protocol was approved by the Research and Ethics Committees of both participating hospitals.

**Clinical diagnosis and serum samples.** Diagnosis was based on endoscopic and histopathological findings. Six biopsies (four from the antrum and two from the corpus) were taken from each patient to perform histopathological studies, as described previously (Camorlinga-Ponce et al., 2008). The diagnosis was determined as described previously (Camorlinga-Ponce et al., 2008) and corresponded to the most severe histological lesion in any of the six biopsies analysed. A 5 ml sample of blood was drawn from each subject, centrifuged to separate the serum and stored at $-70^\circ$C until tested.

**Antigens.** \textit{H. pylori} membrane antigens were obtained from a strain isolated from a Mexican patient with active chronic gastritis according to a previously reported procedure (Mattsson, et al., 1998), modified as follows. \textit{H. pylori} cells from a broth culture were pelleted at 15 000 g for 20 min at 4°C and resuspended in PBS. The suspension was sonicated and centrifuged at 15 000 g for 20 min at 4°C. The supernatant was ultracentrifuged at 100 000 g for 1 h at 4°C. After removal of the supernatant, the pellet of membrane antigens was resuspended in PBS and the protein concentration was determined using the Lowry method (Lowry et al., 1951). SDS-PAGE analysis (10 % acrylamide) showed that this fraction was composed of different molecular mass proteins. Recombinant CagA was produced in our laboratory from the same local strain. In brief, the cagA gene was cloned in frame with a tag in the Xhol site (5’–3’) of pIVEX2.4Nde (Roche). The His-tagged CagA (100 kDa) was purified with Ni-nitrotriacetic acid resin (Qiagen) according to the denaturing protocol in the manufacturer’s instructions. The recombinant protein was characterized by immunoblotting with antibodies specific to CagA (Santa Cruz Biotechnology) and His (Roche). To confirm the identity of the recombinant CagA, the protein was sequenced using mass spectrometry.

**Immunoblotting for IgG.** Immunoblotting to test for \textit{H. pylori}-specific IgG was performed as described by Ayala et al. (2008). The immunoblot for anti-CagA was performed as follows. CagA (15 ng) was dotted onto strips of nitrocellulose (Protran; Perkin Elmer). The strips were blocked for 3 h with 5 % skimmed milk in PBS and further incubated overnight with serum samples at a 1 : 2000 dilution in 1 % skimmed milk in PBS. The strips were washed with 0.05 %Tween 20 in PBS and incubated for 1 h with horseradish peroxidase-conjugated goat anti-human IgG (Zymed) at a 1 : 40 000 dilution in 1 % skimmed milk in PBS. After washing the strips, chemiluminescent horseradish peroxidase substrate (Perkin Elmer) was added, and the strips were exposed to radiographic film (Kodak) in the dark.

**ELISA for IgG.** After chequerboard titration of reagents, the following conditions were used. All incubations were performed at room temperature, and flat-bottomed 96-well Polysorb plates (Nunc) were coated with 5 μg \textit{H. pylori} membrane antigens or 100 ng CagA in 50 μl 50 mM NaHCO3 (pH 9.6) for 2 h. The wells were washed with 0.05 % Tween 20 in PBS and blocked with 1 % BSA in PBS for 2 h. After washing the wells, serum samples at a 1 : 200 dilution in 1 % BSA were added to individual wells in duplicate and incubated for 1 h. After washing the wells, alkaline phosphatase-conjugated goat anti-human IgG (Zymed) at a 1 : 40 000 dilution in 1 % skimmed milk in PBS. After washing the strips, chemoluminescent horseradish peroxidase substrate (Perkin Elmer) was added, and the strips were exposed to radiographic film (Kodak) in the dark.

**ELISA for IgG subclasses.** Before conducting the IgG subclass assays, chequerboard titration of reagents was carried out to determine the
optimal concentrations of patients’ serum and IgG subclass conjugate that would provide maximal discrimination between positive and negative subjects. Flat-bottomed 96-well Polysorb plates were coated with 5 μg H. pylori membrane antigens or 125 ng CagA. Serum samples were added in duplicate at 1:200 dilutions for membrane antigens or 1:10 dilutions for CagA. After washing the wells, biotinylated anti-human IgG1 or IgG2 mAb (Invitrogen) was added at 1:300 dilution in PBS. The wells were washed and alkaline phosphatase-conjugated streptavidin (Invitrogen) was added at 1:600 for CagA, respectively. After 1.5 h incubation, the wells were washed and colour reactions were developed as described above. The A405 was read and the cut-off for positivity and final value were determined as described above.

Statistical analyses. For the analyses, patients with NAG were considered as controls. Bivariate analyses were compared using Student’s t-test (normal variables), Mann–Whitney test (no normal variables), Fisher’s exact test and χ² statistics. Trends were determined using the Cuzick test for tendency. An adjusted odds ratio (OR) with 95% confidence intervals (CI) was estimated using the logistic regression model. The OR and CI were corrected according to the method of Zhang & Yu (1998). Data were analysed using the statistical software program Stata 10.0.

RESULTS

General characteristics of the study subjects and total IgG response

As shown in Table 1, IM and GC patients were significantly older than the controls, and being male and smoking were more frequent among GC patients. Table 2 shows the frequency and titre of total IgG antibodies against CagA and H. pylori membrane antigens. When compared with controls, no differences in the frequency of response to CagA in disease groups were found. In contrast, when the magnitude of the response was analysed, a significantly reduced IgG titre to CagA (P=0.004) and to H. pylori (P=0.029) was observed in the GC group when compared with the controls.

IgG1 and IgG2 subclass response

Table 3 describes the frequency of IgG1 and IgG2 subclasses specific for CagA and H. pylori membrane antigens. We observed no significant difference in the frequency of IgG1 to either of the two antigens tested, although the IM patients showed lower levels of CagA IgG1 compared with NAG controls (10.4 vs 17.9 EU, P=0.004). In contrast, the frequency of IgG2 did change and increased from NAG (15.4%) to IM (30.6%) to GC (55.2%), and this increment was significant for GC patients compared with the NAG group (P=0.001). With regard to the IgG2 response to H. pylori, we observed a lower frequency and a reduced magnitude in the cancer group compared with NAG patients (80.5 vs 96.0%, P=0.021, and 12.5 vs 21.9 EU, P=0.007). When the IgG1:IgG2 ratio was analysed, we observed that, for H. pylori membrane antigens, the ratio was higher in GC patients than in the controls (P=0.038). In contrast, for CagA, the IgG1:IgG2 ratio was significantly lower in both patients with IM and patients with GC compared with the controls (P=0.004 and P=0.003, respectively) (Table 4).

Regression analysis of the association of IgG and IgG2 responses with GC and IM

Possible associations of the total IgG and IgG2 subclass responses to CagA and H. pylori membrane antigens were analysed further using a logistic regression model, adjusting for the confounding factors of sex, age and smoking.

Table 1. General characteristics of the study subjects

Statistically significant results are shown in bold.

<table>
<thead>
<tr>
<th>Status</th>
<th>NAG (%) (n=50)</th>
<th>IM (%) (n=46)</th>
<th>GC (%) (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years (std)</td>
<td>50.2 (11.8)</td>
<td>56.8 (13.0)†</td>
<td>59.1 (14.3)†</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18 (36.0)</td>
<td>17 (37.0)</td>
<td>26 (63.4)§</td>
</tr>
<tr>
<td>Female</td>
<td>32 (64.0)</td>
<td>29 (63.0)</td>
<td>15 (36.6)</td>
</tr>
<tr>
<td>Years of education, mean (sd)</td>
<td>5.9 (4.1)</td>
<td>4.6 (3.4)</td>
<td>5.9 (4.6)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>19 (38.0)</td>
<td>14 (30.4)</td>
<td>25 (61.0)§</td>
</tr>
<tr>
<td>No</td>
<td>31 (62.0)</td>
<td>32 (69.6)</td>
<td>16 (39.0)</td>
</tr>
<tr>
<td>Ever drinking alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24 (48.0)</td>
<td>19 (41.3)</td>
<td>20 (48.8)</td>
</tr>
<tr>
<td>No</td>
<td>26 (52.0)</td>
<td>27 (58.7)</td>
<td>21 (51.2)</td>
</tr>
</tbody>
</table>

*P=0.01 (Student’s t-test), compared with controls.
†P=0.002 (Student’s t-test), compared with controls.
§P=0.009 (χ² test), compared with controls.
$P=0.029 (χ² test), compared with controls.
During the progression to cancer, changes in the gastric mucosa have been suggested that affect the ability of H. pylori to colonize, causing fading of the IgG response (Nomura et al., 2002; Suzuki et al., 2007). However, little is known about changes in the response of IgG subclasses to H. pylori antigens during disease progression. In this study, we aimed to characterize the IgG1 and IgG2 responses to CagA, the best characterized virulence protein of H. pylori, in patients with cancer.

**DISCUSSION**

In this study, we found that the magnitude of the total IgG response to H. pylori membrane and CagA antigens was lower in patients with cancer compared with NAG patients. This reduced response has been observed previously and has been suggested that changes in the gastric mucosa during the progression to cancer affect the ability of H. pylori to colonize, causing fading of the IgG response (Nomura et al., 2002; Suzuki et al., 2007). However, little is known about changes in the response of IgG subclasses to H. pylori antigens during disease progression. In this study, we aimed to characterize the IgG1 and IgG2 responses to CagA, the best characterized virulence protein of H. pylori, because of its strong association with disease, and we compared the results with the response to other H. pylori antigens.

The frequency of IgG1 against H. pylori was similarly high in all disease groups, but the IgG2 frequency was lower in GC compared with NAG patients ($P=0.021$). In contrast, the response to CagA differed and, whereas the frequency of the IgG1 response was similar in all groups, the IgG2 response showed an almost twofold increase from NAG (15.4%) to IM (30.6%) and again to GC (55.2%), and this increment was statistically significant (OR 3.74, 95% CI 1.81–5.37; $P=0.002$). These results suggested a disease-associated shift in the IgG2 response to CagA.

Consistent with our results, previous studies have reported that CagA is recognized predominantly by IgG1 and to a lesser extent by IgG2 (Dzierzanowska-Fangrat et al., 2003; Du et al., 2006). However, we analysed an older population and a wider spectrum of diseases, and observed that the frequency of IgG2 increased from NAG (15.4%) to IM (30.6%) and again to GC (55.2%), and this increase was statistically significant (OR 3.74, 95% CI 1.81–5.37; $P=0.002$). These results suggested a disease-associated shift in the IgG2 response to CagA.

<table>
<thead>
<tr>
<th>Status</th>
<th>NAG (n=50)</th>
<th>IM (n=46)</th>
<th>GC (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CagA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency [%]</td>
<td>39 (78.0)</td>
<td>36 (78.3)</td>
<td>29 (70.7)</td>
</tr>
<tr>
<td>Titre [median, (range)]</td>
<td>42.4 (4.7–91.8)</td>
<td>38.8 (11.1–72.9)</td>
<td>32.6 (1.2–100)$^*$</td>
</tr>
<tr>
<td>H. pylori</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency [%]</td>
<td>38.7 (17.4–61.6)</td>
<td>36.6 (15.1–48.8)</td>
<td>33.7 (22.7–100)$^+$</td>
</tr>
</tbody>
</table>

*P=0.004 (Mann–Whitney test), compared with controls.
†P=0.029 (Mann–Whitney test), compared with controls.

**Table 3. Frequency of IgG1 and IgG2 antibodies specific for CagA and H. pylori membrane antigens**

Statistically significant results are shown in bold.

<table>
<thead>
<tr>
<th>Status</th>
<th>NAG</th>
<th>IM</th>
<th>GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CagA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total n</td>
<td>39</td>
<td>36</td>
<td>29</td>
</tr>
<tr>
<td>IgG1 $^+$ [%]</td>
<td>31 (79.5)</td>
<td>31 (86.1)</td>
<td>25 (86.2)</td>
</tr>
<tr>
<td>IgG2 $^+$ [%]</td>
<td>6 (15.4)</td>
<td>11 (30.6)</td>
<td>16 (55.2)$^*$</td>
</tr>
<tr>
<td>H. pylori</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total n</td>
<td>50</td>
<td>46</td>
<td>41</td>
</tr>
<tr>
<td>IgG1 $^+$ [%]</td>
<td>44 (88.0)</td>
<td>43 (93.5)</td>
<td>40 (97.6)</td>
</tr>
<tr>
<td>IgG2 $^+$ [%]</td>
<td>48 (96.0)</td>
<td>42 (91.3)</td>
<td>33 (80.5)$^+$</td>
</tr>
</tbody>
</table>

*P=0.001 (J$^2$ test), compared with controls.
†P=0.021 (Fisher’s exact test), compared with controls.
itself, as patients with cancer usually show a characteristic and significant alteration in the pattern of serum IgG subclasses, with an increase in IgG2 and a reduction in IgG1 (Schauenstein et al., 1997; Anderhuber et al., 1999). Analogous results have been observed in rodent models (Haddada et al., 1984; Weblacher et al., 1993). In addition, CagA might also be involved in this tumour-associated phenomenon. It was found that CagA can be directly translocated into human B lymphoid cells by H. pylori in a type IV secretion system-dependent manner, triggering its activation by tyrosine phosphorylation. Activation of translocated CagA resulted in the upregulation of anti-apoptotic molecules, thereby enhancing the survival of human B lymphoid cells by preventing apoptosis (Lin et al., 2010).

As reported previously, the magnitude of the anti-CagA IgG response was lower in GC patients (Camorlinga-Ponce et al., 2008). However, in this study, we measured the anti-CagA response only in patients who were H. pylori positive by serology and extended the observation to IgG1 and IgG2 subclasses. Our results suggested that a low anti-CagA IgG response is a risk factor for GC (OR 2.18, CI 95% 1.35–2.69, for the lowest tertile of IgG magnitude), and documented a shift in the response to CagA from IgG1 to IgG2.

Table 4. Ratio of IgG1 : IgG2 anti-CagA and anti-H. pylori responses in patients with IM and GC

<table>
<thead>
<tr>
<th>IgG1 : IgG2 ratio</th>
<th>NAG</th>
<th>IM</th>
<th>GC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Median (range)</td>
<td>n</td>
</tr>
<tr>
<td>CagA</td>
<td>31</td>
<td>1.14 (0.94–1.98)</td>
<td>31</td>
</tr>
<tr>
<td>H. pylori</td>
<td>50</td>
<td>0.98 (0.70–1.25)</td>
<td>46</td>
</tr>
</tbody>
</table>

*P=0.004 (Mann–Whitney test), compared with controls.
†P=0.003 (Mann–Whitney test), compared with controls.
‡P=0.038 (Mann–Whitney test), compared with controls.

As reported previously, the magnitude of the anti-CagA IgG response was lower in GC patients (Camorlinga-Ponce et al., 2008). However, in this study, we measured the anti-CagA response only in patients who were H. pylori positive by serology and extended the observation to IgG1 and IgG2 subclasses. Our results suggested that a low anti-CagA IgG response is a risk factor for GC (OR 2.18, CI 95% 1.35–2.69, for the lowest tertile of IgG magnitude), and documented a shift in the response to CagA from IgG1 to IgG2.

It is well known that, in response to chronic H. pylori infection, the development of adaptive immunity leads to the differentiation of naive Th cells into Th1 or Th2 effector cells (Monack et al., 2004). Th1 cells produce gamma interferon (IFN-γ) and are associated with inflammation.

Table 5. Association between the magnitude of total IgG and the frequency of IgG2 against CagA and H. pylori in patients with IM and GC

All patients were positive for anti-H. pylori IgG. Patients with NAG were considered the control group. The OR was adjusted for sex, age and smoking and corrected according to the method of Zhang & Yu (1998). Statistically significant results are shown in bold.

<table>
<thead>
<tr>
<th>Status</th>
<th>NAG</th>
<th>IM</th>
<th>GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAG</td>
<td>n</td>
<td>n</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Total anti-CagA IgG Magnitude (EU) Medium–high (39.7–100)</td>
<td>26</td>
<td>17</td>
<td>1.0</td>
</tr>
<tr>
<td>Low (1.0–39.6)</td>
<td>13</td>
<td>19</td>
<td>1.52 (0.83–2.20)</td>
</tr>
<tr>
<td>Anti-CagA IgG Negative</td>
<td>33</td>
<td>25</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive</td>
<td>6</td>
<td>11</td>
<td>2.50 (0.97–4.49)</td>
</tr>
<tr>
<td>Total anti-H. pylori IgG Magnitude (EU) Medium–high (33.8–100)</td>
<td>34</td>
<td>36</td>
<td>1.0</td>
</tr>
<tr>
<td>Low (1.0–33.7)</td>
<td>16</td>
<td>10</td>
<td>0.77 (0.35–1.44)</td>
</tr>
<tr>
<td>Anti-H. pylori IgG Positive</td>
<td>48</td>
<td>42</td>
<td>1.0</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>4</td>
<td>1.64 (0.27–7.74)</td>
</tr>
</tbody>
</table>

*P=0.006 (logistic regression), compared with controls.
†P=0.002 (logistic regression), compared with controls.
IFN-γ promotes the production of IgG2 by B-cells. Th2 cells produce interleukin-4 (IL-4) and are involved in the humoral response. IL-4 induces IgG1 and IgE class switching and downregulates IFN-γ (Wan & Flavell, 2009). Our results confirmed the findings of Ren et al. (2005) that GC patients show a selective reduction in their H. pylori-specific IgG2 response compared with those with uncomplicated gastritis. We showed that H. pylori-specific IgG2 predominated in NAG (IgG1 : IgG2 ratio < 1), whilst H. pylori-specific IgG1 predominated in GC (IgG1 : IgG2 ratio > 1) (Table 4). This finding is in agreement with the report by Ren et al. (2001) that IFN-γ, which induces IgG2 production, is the dominant cytokine secreted in gastric mucosal cultures from H. pylori-infected patients with uncomplicated gastritis. In contrast, IL-4, which induces IgG1 production and downregulates IgG2, prevailed in cultures from GC patients. Thus, the shift in the IgG1 : IgG2 ratio might be a consequence of the shift in the Th1/Th2 response; this seems to be related to the different antigenic capabilities of the H. pylori LPS. The LPS from H. pylori contains Lewis blood-group antigens whose expression varies within a single strain of H. pylori as a result of phase variation (on and off) (Bergman et al., 2006). H. pylori variants that express Lewis antigens are able to bind the dendritic cell (DC)-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) receptor present on DCs blocking Th1-cell development; in contrast, those variants that do not express Lewis antigens escape binding to DC-SIGN and induce a strong Th1-cell response (Bergman et al., 2004). There is evidence that LPS from H. pylori strains isolated from non-tumoral gastric mucosa does not express Lewis antigens and displays a high immunogenic capability, whereas that isolated from tumoral gastric mucosa expresses Lewis antigens and displays a reduced immunogenic capability (Yokota et al., 1997). Furthermore, IgG2 is the subclass that reacts with the highly immunogenic LPS (Yokota et al., 1998). Regulation of the Th1 and Th2 response by H. pylori seems to be related to the chronicity of infection, as chronic exposure to H. pylori impairs DC function and inhibits Th1-cell development (Mitchell et al., 2007). Thus, the shift in the IgG1 : IgG2 ratio might be related to modulation of the Th1/Th2 balance through phase variation between H. pylori LPS and DCs from the early stages of infection (NAG) with a predominantly Th1 response and upregulation of IgG2 to the most advanced stages (GC) with a predominantly Th2 response.

Notably, we observed a gradual reduction in the titre of the H. pylori-specific IgG and IgG2 antibodies relative to the severity of the mucosal lesion. Neither the absence of an IgG2 response nor the magnitude of the IgG response reached a significant OR value (OR 4.19, 95% CI 0.84–13.47) for the IgG2 response, and the OR was 1.64 (95% CI 0.93–2.33) for the lowest tertile of IgG magnitude. This lack of significance of our results could be a consequence of the sample size (n=41) and the age (mean 59.1 years) of the GC patients studied; as Yamaji et al. (2002) analysed >10 000 patients and found that those over the age of 60 years with a weakly positive antibody response showed the highest risk for GC.

This study is the first to report the anti-CagA IgG2 response as a marker for H. pylori-associated GC. We have confirmed the results of previous reports indicating that GC patients show lower levels of anti-CagA IgG as well as H. pylori-specific IgG and IgG2. We also found a shift in the anti-H. pylori IgG1 : IgG2 ratio, with a predominance of IgG2 among patients with NAG and of IgG1 among those with gastric malignancy. As these findings are supported by evidence from reports on other chronic infections and other types of cancer, we believe they are suitable for evaluating the risk of developing GC in H. pylori-infected subjects.

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REFERENCES


