Relationship between mucosal levels of *Helicobacter pylori*-specific IgA, interleukin-8 and gastric inflammation

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ABSTRACT

Mucosal IgA is important in local immune defence. *Helicobacter pylori* induces a specific IgA response in antral mucosa, but its immunopathology is unknown. Interleukin-8 (IL-8) has been suggested to be important in *H. pylori*-induced inflammation. Current information on the relationship between *H. pylori*-induced IgA and mucosal inflammation is limited. To investigate possible associations between mucosal-specific IgA, the toxinogenicity of *H. pylori*, mucosal levels of IL-8 and gastric inflammation, 52 endoscoped patients were studied. These comprised 28 patients with peptic ulcer and 24 with non-ulcer dyspepsia. Of these patients, 38 had *H. pylori* infection: 28 with peptic ulcer and 10 with non-ulcer dyspepsia. Antral biopsies were taken for histology, *H. pylori* culture and measurement of mucosal levels of IL-8 (pg/mg) and specific IgA (*A*450<1000) by ELISA. Mucosal *H. pylori* IgA was detectable in 35 out of 38 patients with *H. pylori* infection, with a median (interquartile) level of 220 (147, 531) units. There was no significant difference in mucosal levels of the IgA antibodies between patients infected with cytotoxin-positive or *cagA*-positive strains of *H. pylori* and those with toxin-negative or *cagA*-negative strains. The IgA levels in those patients with severe neutrophil infiltration were lower than in those with mild or moderate infiltration (*P*<0.05). There was a weak inverse correlation between antral mucosal IgA and IL-8 in infected patients (*r*=-0.36; *P*=0.04). *H. pylori* infection induced a significant local mucosal IgA response in most infected patients. The level of IgA antibodies does not appear to be correlated with the toxinogenicity of *H. pylori*. However, patients with severe active inflammation appear to have decreased levels of IgA. An inverse correlation between mucosal IL-8 and IgA may suggest that IL-8-induced inflammation compromises the mucosal IgA defence and renders the mucosa susceptible to further damage.

INTRODUCTION

Generation of mucosal IgA is an important local immune defence mechanism. *Helicobacter pylori* induces a significant IgA response in antral mucosa in infected patients [1,2]. Despite the persistence of a local and systemic immune response, *H. pylori* is not eliminated. However, animal studies have shown that repetitive oral immunization with *H. pylori* antigens and choler toxin induces a strong local IgA anti-*H. pylori* response in mice and ferrets [3–5]. Mice can be protected from infection by *H. felis* by immunization with sonicated bacterial extracts or recombinant urease [3–5]. Studies have also shown that oral administration of purified urease in combination with choler toxin to mice or ferrets resulted in eradication of the bacteria in significant numbers of animals [6,7]. Passive immunization with monoclonal IgA was shown to prevent *H. felis* infection in mice [3,8]. It was

Key words: *Helicobacter pylori*, IgA, interleukin-8, mucosal inflammation.
Abbreviations: IL-8, interleukin-8; PCR, polymerase chain reaction.
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also shown that the development of *H. pylori* infection was delayed among infants who were breast-fed by *H. pylori*-positive mothers whose milk had higher levels of *H. pylori*-specific IgA [9]. These results suggest that the mucosal-specific IgA antibodies may have a protective role against gastric *Helicobacter* infection. Although the mucosal IgA antibodies failed to eliminate the organism in humans, it may play a role in limiting *H. pylori*-induced mucosal damage.

Interleukin-8 (IL-8), a potent inflammatory mediator, has been suggested as being important in *H. pylori*-induced mucosal inflammation, and higher levels of IL-8 have been associated with more severe mucosal inflammation [10,11]. Studies have also suggested that cagA-positive strains of *H. pylori* are associated with a higher IL-8 production in epithelial cell lines and with more serious pathology, such as peptic ulcer disease and more active mucosal inflammation [12–17]. There is little information available on the relationship between active mucosal inflammation [12–17]. There is little information available on the relationship between mucosal-specific IgA antibodies and disease activity.

The aims of the present study were to investigate whether any association exists between mucosal levels of *H. pylori* IgA, the toxinogenicity of *H. pylori*, mucosal IL-8 and gastric inflammation.

**METHODS**

**Patients**

A total of 52 dyspeptic patients undergoing endoscopy were recruited. They included 28 patients with peptic ulcer (25 duodenal ulcer; three gastric ulcer) and 24 with non-ulcer dyspepsia. Among these patients, 12 were from a previous study [11]; extra samples were available from these subjects for the measurement of IgA at the later stage of that study. Patients who had had eradication therapy or had taken non-steroidal anti-inflammatory drugs were excluded from the study. Patients were also advised to stop any anti-acid treatment 2 weeks before endoscopy. During endoscopy, biopsies were taken for histology, CLO-test (Delta West), *H. pylori* culture and measurement of mucosal levels of IgA anti-*H. pylori* antibodies and IL-8. Informed consent was obtained from each patient. The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and was approved by Glasgow Royal Infirmary Ethics Committee.

**H. pylori culture and histology**

For *H. pylori* culture, gastric biopsies were transported in saline (0.9% NaCl) and homogenized in 0.5 ml of saline using a tissue grinder (BDH). Samples of 50 µl of homogenates were inoculated on to Columbia blood agar plates and incubated microaerobically at 37 °C for 3–7 days.

Histological identification of *H. pylori* in histological sections stained with Haematoxylin–eosin and Cresyl Violet was assessed by an independent pathologist, and mucosal inflammation was also scored according to the degree of infiltration of neutrophils and mononuclear cells, as described previously [11].

**Measurement of mucosal levels of IL-8 and anti-*H. pylori* IgA**

Each biopsy was weighed and placed in an Eppendorf tube containing PBS (pH 7.3). The volume of PBS in each tube was adjusted to 1 mg of biopsy in 100 µl of PBS. Each biopsy was then homogenized separately using a tissue grinder (BDH) at 4 °C. The homogenate was centrifuged at 2000 g for 10 min. The supernatant was removed and stored at −70 °C, until the measurements of IL-8 and IgA. IL-8 was measured by ELISA (Bio-Whittaker) as described elsewhere [11]. The lower detection limit of the assay is 3 pg/ml of homogenate supernatant. The levels of IL-8 were calculated and converted into units of pg/mg of biopsy.

Mucosal anti-*H. pylori* IgA antibodies were measured by ELISA (Sigma) in duplicate according to the manufacturer’s instructions. The antigen used in the assay contains partially purified proteins of high molecular mass (400–700 kDa), including urease from *H. pylori* strains. It has been found that the assay using this antigen has a very high specificity and sensitivity (100% and 98.7% respectively) in the serodiagnosis of *H. pylori* infection [18]. The procedure was as follows. The samples were diluted 1:50 in PBS/Tween 20 (0.25%, v/v), and 100 µl was added to each well. The plates were incubated at room temperature for 40 min and then washed three times in PBS/Tween 20. Diluted (1:5000 in PBS/Tween 20) peroxidase-conjugated goat anti-(human IgA) was added to each well (Sigma). The plates were then incubated at room temperature for 30 min. After washing three times, 100 µl of substrate solution (containing tetramethylbenzidine and H₂O₂) was added and incubated at room temperature for 20 min. The reaction was stopped by adding 100 µl of 1.25 M sulphuric acid. The *A₄₅₀* was measured using a Titertek Multiscan plate reader (Flow Laboratories, Irvine, Scotland, U.K.). Two reference samples (one positive and one negative) were also included in each experiment. Antibody positivity was defined as an absorbance value greater than 2 × S.D. of the mean absorbance of *H. pylori*-negative samples. The levels of IgA antibody were expressed as follows:

\[
\text{Antibody level} = \frac{\text{sample } A_{450} - (\text{mean} + 2 \times \text{S.D. of negative } A_{450})}{\text{S.D.}} \times 1000.
\]

**Detection of cytotoxin production by strains of *H. pylori***

To detect cytotoxin production, strains of *H. pylori* were cultured in Brucella broth containing 5% (v/v) foetal calf
serum at 37 °C microaerobically. After 48 h, the culture was centrifuged (2000 g; 15 min) and each culture supernatant of H. pylori was concentrated (20-fold). Concentrated culture supernatants were used to test cultured Vero cells for intracellular vacuolation, which was measured by phase-contrast microscopy and a Neutral Red uptake assay according to the methods described previously [19,20].

Detection of cagA gene expression by polymerase chain reaction (PCR)

To detect cagA expression by strains of H. pylori, chromosomal DNA was extracted from each strain. Approx. 20 ng of the DNA preparation was used in a PCR mixture of 25 µl (Ready To Go PCR beads; Pharmacia) containing 1.5 units of Taq polymerase, 10 mM Tris/HCl, 50 mM KCl, 3 mM MgCl₂, 200 µM of each dNTP and 0.2 µM of each primer. A pair of primers was selected (primer 1, 5’-AGGAATCTCGCAATTAAGGG-3’; primer 2, 5’-TTCTATGCCATTATGACCTCCCC-3’) from published sequences [21,22]. Chromosomal DNA extracted from reference strain NCTC11637, which is known to be cagA-positive, was used as a positive control. The reaction conditions were as follows: 5 min at 95 °C; 30 cycles of 1 min at 95 °C, 1 min at 60 °C and 1.5 min at 72 °C; followed by 4 min at 72 °C. The PCR products were analysed by agarose (1.6%, w/v) gel electrophoresis. The estimated size of the cagA PCR product was 750 bp (Figure 1).

Statistical analysis

Non-parametric statistical analysis was used. The Mann–Whitney U test was used for two-group comparisons, and the Kruskal–Wallis test was used for multiple-group comparisons. P < 0.05 was considered to be statistically significant.

RESULTS

H. pylori infection and mucosal IgA anti-H. pylori antibodies

All 28 peptic ulcer patients and 10 of 24 patients with non-ulcer dyspepsia were infected with H. pylori. Antral mucosal IgA anti-H. pylori antibodies were detectable in 35 of 38 patients with H. pylori infection, with a median (interquartile) level of 220.0 (147.5, 531.0) units (calculated as described in the Methods section), while in the 14 patients without H. pylori infection, the IgA antibody was detectable (at a low level) in only one.

No correlation was seen between mucosal IgA anti-H. pylori antibodies and patients’ sex, smoking habits or alcohol consumption. There was no significant correlation between patients’ age and antibody level, although those subjects below the age of 30 years had slightly lower levels of IgA than those aged 30–40 years (Table 1).

No significant difference was found in the mucosal IgA levels of patients with peptic ulcer compared with those with non-ulcer dyspepsia infected with H. pylori (median (interquartile) levels of 235 (165, 620) units and 153 (34, 345) units respectively).

Mucosal IgA antibodies and toxigenicity of H. pylori

H. pylori isolates from the 38 infected patients were analysed for expression of the VacA cytotoxin and

<table>
<thead>
<tr>
<th>No.</th>
<th>H. pylori IgA (units)</th>
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</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18 255 (168, 643)</td>
</tr>
<tr>
<td>Female</td>
<td>20 190 (45, 316)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>20–30</td>
<td>7 69.0 (0.0, 260.0)*</td>
</tr>
<tr>
<td>31–40</td>
<td>12 313.0 (154.5, 735.0)</td>
</tr>
<tr>
<td>41–50</td>
<td>7 235.0 (185.0, 659.0)</td>
</tr>
<tr>
<td>51–60</td>
<td>6 219.0 (128.5, 395.0)</td>
</tr>
<tr>
<td>≥ 61</td>
<td>6 260.0 (129.0, 539.0)</td>
</tr>
<tr>
<td>Smoking habit</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>16 240 (160, 713)</td>
</tr>
<tr>
<td>Moderate</td>
<td>16 234 (92, 420)</td>
</tr>
<tr>
<td>Heavy</td>
<td>6 230 (130, 946)</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10 197 (115, 574)</td>
</tr>
<tr>
<td>Moderate</td>
<td>25 260 (115, 574)</td>
</tr>
<tr>
<td>Heavy</td>
<td>3 144 (128, 135)</td>
</tr>
</tbody>
</table>

* P < 0.05 compared with age group 31–40 years.
Table 2  Antral mucosal levels of IgA anti-\textit{H. pylori} antibodies in patients with \textit{H. pylori} infection with reference to toxinogenicity of strains

IgA levels were calculated as (A$_{450}$ × 1000), and are given as median (interquartile range). $P$ values were 0.34 for toxin-positive compared with toxin-negative, and 0.51 for \textit{cagA}-positive compared with \textit{cagA}-negative.

<table>
<thead>
<tr>
<th>Strains associated with infection</th>
<th>No. of patients</th>
<th>Mucosal IgA antibodies (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxin-positive</td>
<td>24</td>
<td>219.0 (71.7, 348.0)</td>
</tr>
<tr>
<td>Toxin-negative</td>
<td>14</td>
<td>260.0 (148.0, 565.0)</td>
</tr>
<tr>
<td>\textit{cagA}-positive</td>
<td>8</td>
<td>224.5 (107.0, 510.5)</td>
</tr>
<tr>
<td>\textit{cagA}-negative</td>
<td>30</td>
<td>220.0 (90.0, 505.0)</td>
</tr>
</tbody>
</table>

possession of the \textit{cagA} gene. Table 2 summarizes the mucosal levels of IgA anti-\textit{H. pylori} antibodies in patients colonized with \textit{H. pylori} strains with or without VacA or \textit{cagA}. There was no significant difference in mucosal levels of the IgA antibodies between patients infected with cytotoxin-positive or \textit{cagA}-positive strains on the one hand and those with toxin-negative or \textit{cagA}-negative strains of \textit{H. pylori} on the other.

**Mucosal IgA anti-\textit{H. pylori} antibodies and mucosal inflammation**

When antral mucosal inflammation was assessed according to the degree of infiltration of neutrophils and mononuclear cells, there was no significant correlation between the mucosal levels [median (interquartile)] of IgA antibodies and the mononuclear cell infiltration score: mild infiltration, 210 (128, 562) units; moderate, 216 (63, 586) units; severe, 214 (126, 418) units. However, the IgA levels in those patients with severe neutrophil infiltration [200 (80, 250) units] were lower than in those with mild [316 (201, 603) units] or moderate [355 (171, 960) units] infiltration (Figure 2; $P < 0.05$).

**DISCUSSION**

It was found in this study that most infected patients had raised mucosal levels of anti-\textit{H. pylori} IgA. The results confirm the presence of mucosal-specific IgA in \textit{H. pylori} infection. In a previous report, it was found that about 98% of infected patients had mucosal-specific IgA [23]. While there were large variations in the mucosal levels of IgA among patients, there was no significant difference between patients infected with toxinogenic strains (containing either VacA or \textit{cagA}) and those infected with strains not expressing either VacA or \textit{cagA}. There was no significant correlation between patients’ age and the mucosal levels of IgA, although a slightly lower level of...
the IgA antibodies in patients below the age of 30 years was apparent. The lack of correlation between the toxigenicity of *H. pylori* and mucosal IgA suggests that the variation in host’s immune response may be more important, and the time for which patient has been infected may also be relevant.

Although animal studies showed that oral immunization with *H. pylori* antigens induced a significant secretory IgA response and protected mice from infection by *H. felis*, and even eradicated the bacteria from infected mice [3–7], the role of mucosal IgA antibodies in the immunopathology of *H. pylori*-related gastroduodenal disease in humans is unclear. Mucosal secretory IgA has been suggested to be important in inhibiting antigen uptake, in blocking bacterial adherence and the motility of the organism, and also in toxin neutralization [24]. It has been shown that the IgA inhibits the cell vacuolation induced by *H. pylori* cytotoxin in vitro [25]. Previous studies also showed that *H. pylori* in gastric biopsy was coated with IgA, but that only a proportion of the bacteria were coated [26]. Thus it is possible that some bacteria evaded the IgA defence mechanism for some reason.

Current information on the association between the mucosal antibodies and the disease activity is limited. Serum IgG titres were shown to be correlated with the severity of inflammation both in the antrum and in the body of the stomach [27]. Serum IgG responses to the 54 kDa heat-shock protein and to the vacuolating cytotoxin were correlated with acute mucosal inflammation [28]. Interestingly, the serum IgA responses to a whole-cell sonicate of *H. pylori* and to the cytotoxin were inversely related to chronic inflammatory scores [28]. In the present study, it was found that patients whose mucosa was severely infiltrated with neutrophils displayed lower levels of IgA than those patients whose mucosa had mild to moderate neutrophil infiltration. Previous studies have shown that higher levels of IL-8 are associated with a stronger mucosal inflammatory response, especially neutrophils. In the present study, a weak inverse relationship was found between levels of specific IgA and IL-8 in antral mucosa. These results may suggest that, in some patients, *H. pylori*-induced high levels of the pro-inflammatory cytokine IL-8 may cause more severe inflammation with intense neutrophil infiltration, which could damage the integrity of the mucosa [29,30] and compromise the mucosal IgA defence. Insufficient mucosal IgA secretion may allow more bacterial colonization and more severe mucosal damage.

In conclusion, *H. pylori* infection induced a significant local mucosal IgA response in most infected patients. The level of the IgA antibodies does not appear to be correlated with the toxigenicity of *H. pylori*. However, the mucosal production of IL-8 induced by *H. pylori* infection may mediate severe inflammation, which may compromise the mucosal IgA defence, thus rendering the mucosa susceptible to further damage.

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Received 30 November 1998/6 January 1999; accepted 6 January 1999