Local immune response to gastric *Campylobacter* in non-ulcer dyspepsia

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**SUMMARY** Colonising *Campylobacter pyloridis* were identified histologically in gastric biopsy specimens from 89% of 83 patients with non-ulcer dyspepsia and chronic gastritis, but not in 58 dyspeptic patients with normal mucosa. The presence and population density of organisms was associated with the presence of intraepithelial neutrophils. In vivo coating of the organisms by host immunoglobulin was investigated by immunoperoxidase staining of IgA, IgG, and IgM in 54 biopsy specimens. IgA coated bacteria were seen in all cases of active gastritis, and in 60% of biopsy specimens without intraepithelial neutrophils. Coating with IgG or IgM, or both, was correlated with activity of gastritis and was rarely seen in the absence of a neutrophil infiltrate.

Since their recognition by Warren and Marshall in 1983¹ the presence of colonising gastric campylobacter like organisms often found in chronic gastritis has been confirmed in many countries.²⁻⁴ The prevalence of this bacteria, now officially named *Campylobacter pyloridis*,⁵ in antral gastritis (type B, non-autoimmune gastritis) is consistently reported to be around 90%. The bacteria can be grown in pure culture from biopsy specimens of the affected mucosa.³⁶ Marshall himself ingested a culture of *C pyloridis* and developed symptomatic gastritis associated with mucosal colonisation, thereby fulfilling Koch’s third and fourth postulates.⁷ *C pyloridis* are often present in patients with peptic ulcer, although this association may be due to concurrent gastritis, as most agents that heal ulcers, with the notable exception of bismuth compounds, do not eliminate either the *C pyloridis* or gastritis.⁶⁻⁸ Thus the association between *C pyloridis* and gastritis cannot be doubted⁹ and must rival that between many other infectious diseases and their causative organism.

The stomach is normally protected from bacterial infection by its high luminal acidity¹⁰ and a thick surface coating of gastric mucus¹¹ Immunoglobulin producing cells, which are sparse in the lamina propria of normal gastric mucosa, increase in number in chronic gastritis.¹² With the development of chronic gastritis, the gastric epithelium of the isthmic zone shows secretion of IgA, together with lysozyme,¹³ and lactoferrin.¹⁴ These defences, inappropriate against physical or chemical insults, would be well suited to combat a bacterial pathogen. Evidence of a local specific immune response to colonising *C pyloridis* would, therefore, favour a causative role for this organism in chronic gastritis.

We have previously shown anti-*C pyloridis* immunoglobulin in the serum and gastric juice of colonised patients.¹⁵ Our aim in the present study was to investigate the in vivo adsorption of host immunoglobulin onto mucosal *C pyloridis* in gastric biopsy specimens by the immunohistochemical demonstration of antibody coating the bacteria.

**Material and methods**

Paired full thickness endoscopic biopsy specimens of antral and body mucosa from 141 patients with non-ulcer dyspepsia were studied. The presence and severity of chronic gastritis was assessed by one pathologist (JW) on formalin fixed paraffin embedded 5 µm sections stained with haematoxylin and eosin, according to the criteria of Whitehead.¹² Colonising *C pyloridis* were identified by the Warthin-Starry stain.

The distribution of *C pyloridis* and the presence of IgG, IgA, and IgM coating the organisms were studied in detail in 30 cases. In these biopsy specimens the numbers of organisms present in three sites: the mucosal surface, the upper, and the deep portions of the gastric pits, which were separately estimated on a scale + to +++ (++, + = occasional *C pyloridis* found after staining, +++ = *C pyloridis* in most high

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power fields of surface and gastric pits; \(+ + + = C. pyloridis\) in all high power fields of surface and pits; \(+ + + + = C. pyloridis\) particularly numerous in all fields) (Fig. 1) and these values were added to quantify the density of bacterial colonisation, giving a possible range of 1 to 12+.

Paired biopsy specimens were stained by the indirect immunoperoxidase method, with rabbit polyclonal antiserum to human IgA, IgM, and IgG (Dako) at a dilution of 1 in 500, haematoxylin being used as a counterstain. The presence of labelled \(C. pyloridis\) on the surface and in the upper and deep pits of each specimen was recorded. Negative controls consisted of sections of normal non-colonised mucosa, colonised mucosa with omission of rabbit antiserum, and sections from an adhesion study, in which \(C. pyloridis\) from broth culture adhered in vitro to normal antral mucosa maintained in tissue culture for two hours. All immunoperoxidase sections were examined without knowledge of the histological diagnosis and graded as follows: no immunoperoxidase labelling of bacteria present (−); one or two weakly positive bacteria present (+); high incidence of weakly positive bacteria (±); high incidence of strongly positive bacteria (+ +).

Patterns of immunoperoxidase labelling of \(C. pyloridis\) on actively inflamed gastric mucosa were compared with those of normal body mucosa and mucosa from antrum and body showing inactive chronic gastritis (Figs. 2 and 3).

Statistical analysis was performed using the Mann-Whitney U test for comparing population densities of \(C. pyloridis\), and elsewhere by Fisher’s exact test.

Results

Table 1 shows the prevalence of chronic gastritis and colonising \(C. pyloridis\) in one or both biopsy specimens from 141 patients with non-ulcer dyspepsia. \(C. pyloridis\) were present in 74 of 83 (89%) patients with chronic gastritis and in none of the 58 patients in whom both antral and body mucosa were normal. \(C. pyloridis\) were detected on the morphologically normal body mucosa in 20 of 22 patients in whom gastritis affected only the antrum. There was no correlation between the severity of chronic gastritis (superficial or atrophic) and detection of \(C. pyloridis\), except that organisms were never present on areas of intestinal metaplasia. The association between the activity of gastritis and \(C. pyloridis\) on individual biopsies was, however, significant (Table 2). In active gastritis the neutrophils were predominantly between...
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Fig. 2  Mucosal biopsy from gastric body stained by indirect immunoperoxidase technique for IgA. (a) (b) C pyloridis of characteristic morphology are present on and above surface; these score ++ for IgA labelling. (c) A gastric pit in same case; IgA positive C pyloridis are present on surface and in upper pit; unlabelled organisms are present in deep pit (open arrow). Original magnification (a) × 80; (b) and (c) × 320.
Fig. 3  Antral mucosa stained for IgA and IgG. (a) *C* pyloridis strongly positive for IgA (+ +) are present on surface, while those in gastric pit are weakly positive (+) (arrow). (b) IgG ++ organisms are present on surface and IgG + in upper pit. Unlabelled *C* pyloridis are present in deeper part of pit (open arrow). Original magnification × 320.)
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Table 1  Prevalence of chronic gastritis and C. pyloridis in 141 patients with non-ulcer dyspepsia

<table>
<thead>
<tr>
<th>Age</th>
<th>Total</th>
<th>Normal gastric mucosa</th>
<th>Chronic gastritis</th>
<th>Chronic gastritis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C. pyloridis absent</td>
<td>C. pyloridis absent</td>
<td>C. pyloridis present</td>
</tr>
<tr>
<td>&lt;20</td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>21–30</td>
<td>29</td>
<td>18</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>31–40</td>
<td>22</td>
<td>13</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>41–50</td>
<td>24</td>
<td>4</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>51–60</td>
<td>25</td>
<td>12</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>61–70</td>
<td>20</td>
<td>5</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>71–80</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 2  Presence of C. pyloridis related to activity in 144 biopsy specimens showing chronic gastritis

<table>
<thead>
<tr>
<th>Gastritis</th>
<th>C. pyloridis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Active</td>
<td>95</td>
</tr>
<tr>
<td>Inactive</td>
<td>22</td>
</tr>
</tbody>
</table>

p < 0.001

epithelial cells rather than in the lumen of gastric glands (a pattern reminiscent of infective colitis16), and were most numerous in the deep portion of the pits.

Table 3 shows the histological diagnoses in the 30 pairs of biopsy specimens studied in detail. The specimens were of full thickness mucosa, with a mean number of 31-44 (SD 11-63) gastric pits per biopsy. The 30 cases included three with normal antral and body mucosae with no bacteria, included as negative controls. All of the remaining 54 biopsy specimens showed C. pyloridis colonisation; those of actively inflamed mucosa, however, carried a significantly greater population density of colonising bacteria than those showing inactive gastritis or normal mucosa (p = 0.0022) (Fig. 4).

C. pyloridis labelled by anti-IgA and anti-IgM were easily identified; background staining of gastric mucus in active gastritis occurred with anti-IgG, but labelled C. pyloridis could still be confidently detected. Unlabelled organisms were apparent due to their faint haematoxylinophilia in areas of mucosa in which immunoperoxidase positive C. pyloridis were sparse or absent. The anti-Ig labelled C. pyloridis were always less numerous than those apparent on the Warthin-Starry stain, suggesting that only a proportion of the organisms were coated by host immunoglobulin. In all sections examined immunoperoxidase negative C. pyloridis could be seen at the depths of the gastric pits.

The pattern of immunoperoxidase positivity of C. pyloridis for the three antibody classes in active gastritis was different to that in inactive gastritis and normal mucosa (Fig. 5). Numerous C. pyloridis, which were positive for IgG or IgM, or both, were present on the surface of 25 of 29 active gastritis biopsy specimens, but rarely seen on the others (6 of 25, p < 0.001). IgA positive C. pyloridis were always present on the mucosal surface in active gastritis and were usually seen within pits. In the inactive and normal groups IgA positive C. pyloridis were found on the surface in 60% of cases, but were less commonly found within the gastric pits.

No immunoperoxidase stained organisms were seen on the three pairs of biopsy specimens from normal patients, or in the negative control slides. Sections from the adhesion study provided a useful negative control; C. pyloridis were also unstained by immunoperoxidase for IgA, IgG, and IgM.

Discussion

We propose that the immunoperoxidase labelling of mucosal C. pyloridis by anti-IgA, IgG, and IgM is due to the in vivo adsorption of specific host antibodies on to the bacteria. Other workers have used similar techniques to detect in vivo adsorbed antibody on oral bacteria17 and on Legionella pneumophila.18 We have previously shown the presence of anti-C. pyloridis IgA and IgM in the gastric juice of colonised subjects. The characteristic morphology, position, and population density of C. pyloridis on the mucosal surface in tissue sections1 allowed the immunoperoxidase positive organisms to be identified with confidence. This posi-

Table 3  Histological diagnoses in 30 paired biopsy specimens for immunoperoxidase study

<table>
<thead>
<tr>
<th>Active chronic gastritis</th>
<th>Inactive chronic gastritis</th>
<th>Normal (includes three negative control cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antral mucosa</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>Body mucosa</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>
activity is unlikely to be due to a non-specific cross reaction as some unlabelled *C. pyloridis* were also present in each section.

Lymphocytes and plasma cells are sparse in the lamina propria of the normal stomach, and an increase in their number is the principal criterion for the histological diagnosis of chronic gastritis. Inter-"estingly, the intestinal mucosa of neonates and germ free animals is similarly devoid of mononuclear cells before contact with luminal antigens. The main class of antibody present in mucosal secretions is dimeric IgA, which is produced locally by plasma cells in the lamina propria and transported from the interstitium to the lumen by secretory component, a protein synthesised by the epithelial cells. This secretory mechanism, which is apparently inactive in the normal stomach, has been shown in inflamed gastric mucosa, together with the secretion of lysozyme and lactoferrin. The mucosal plasma cells throughout the gastrointestinal tract are predominantly of IgA class, although an increase in the IgG and IgM plasma cell population occurs during the active phase in various inflammatory conditions, including chronic gastritis. The IgG appears in secretions during active inflammation by means of a non-specific increase in transudation of serum proteins across the inflamed epithelium.

We detected coating of *C. pyloridis* by host IgA in all cases with active gastritis and in many with inactive gastritis, a finding in accordance with the predominance of IgA in mucosal secretions. IgA protects the mucosa by interfering with the adhesion of organisms to the epithelial surface. It has also recently been shown to be capable of inducing antibody dependent cell mediated activity against enteropathogenic bacteria. IgA does not, however, opsonise or fix complement and therefore does not enhance neutrophil phagocytosis of coated organisms. This is in keeping with our observation of IgA coated *C. pyloridis*, irrespective of the presence of a neutrophil infiltrate in the mucosa. IgG and IgM coated *C. pyloridis* were observed almost exclusively on actively inflamed mucosae. Both these antibodies fix complement, with IgG also opsonising bacteria; thus both would be expected to enhance neutrophil activity against *C. pyloridis*. Other authors have observed phagocytosed *C. pyloridis* in neutrophils on the mucosal surface. This association of active gastritis with the presence of IgM and IgG coated *C. pyloridis* is therefore corroborative evidence that the inflammatory response may, indeed, be elicited by this organism.

The consistent observation of unlabelled *C. pyloridis* deep in the gastric pits is of considerable interest. Although the self induced *Campylobacter* gastritis of Marshall resolved spontaneously by the fourteenth day, colonisation by organisms has been shown to persist for at least four months in asymptomatic subjects. Indeed, the prevalence of *C. pyloridis* in patients of all ages and with all grades of chronic gastritis suggests that colonisation, once established, may persist indefinitely. The host's immune response is therefore not effective in eradicating the organism. In their ecological niche deep in the gastric pits, *C. pyloridis* may evade contact with host immunoglobulin, at least in a concentration sufficient to be detected by our immunoperoxidase technique. The alternative explanation for their persistence—that *C. pyloridis* are merely commensals in the inflamed stomach—seems unlikely in view of the systemic antibody response in colonised subjects and their association with activity of gastritis, an observation recently confirmed by electron microscopy.

We do not yet know the clinical importance of *C. pyloridis* in the pathogenesis of chronic gastritis. The immune mechanisms of the stomach are equipped to combat a luminal antigen, and the histo-
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Fig. 5 Immunoperoxidase labelling of C pyloridis present on surface and in upper and deep gastric pits by anti-IgA, IgG, and IgM.

logical features of chronic gastritis are consistent with those of a response to chronic infection. We have presented evidence for the in vivo coating of C pyloridis by host antibody, the class of which varies with the activity of the gastritis. A chronic infection by C pyloridis, therefore, offers a plausible explanation for the histological and immunopathological features observed in type B chronic gastritis.

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