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.^{3,6} 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 mucus.¹¹ 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 isthmus 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 on to 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

Fig. 1 Antral biopsy specimen stained by Warthin-Starry technique, showing colonising *C. pyloridis*. Organisms are present on surface (arrow) and in upper and deep sections of pits. Both pits contain organisms, although of varying population density. Dense colonies of bacteria (open arrow) present in upper pit (score 4+). This specimen scored 9+ for density of colonising *C. pyloridis* (surface 2+; upper pits 4+; deep pits 3+). Original magnification $\times 130$.

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 ($\pm$); 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

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) $\times 80$; (b) and (c) $\times 320$.

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 $\times 320$.

Table 1 Prevalence of chronic gastritis and *C. pylori* in 141 patients with non-ulcer dyspepsia

Age	Total	Normal gastric mucosa <i>C. pylori</i> absent	Chronic gastritis <i>C. pylori</i> absent	Chronic gastritis <i>C. pylori</i> present
≤20	11	6	0	5
21-30	29	18	1	10
31-40	22	13	2	7
41-50	24	4	1	19
51-60	25	12	1	12
61-70	20	5	2	13
71-80	10	0	2	8

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

Gastritis	<i>C. pylori</i>	
	+	-
Active	95	14
Inactive	22	13

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. pylori* 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. pylori 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. pylori* could still be confidently detected. Unlabelled organisms were apparent due to their faint haematoxyphilic in areas of mucosa in which immunoperoxidase positive *C. pylori* were sparse or absent. The anti-Ig labelled *C. pylori* 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. pylori* could be seen at the depths of the gastric pits.

The pattern of immunoperoxidase positivity of *C. pylori* for the three antibody classes in active gastritis was different to that in inactive gastritis and normal mucosa (Fig. 5). Numerous *C. pylori*, 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. pylori* 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. pylori* 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. pylori* were also unstained by immunoperoxidase for IgA, IgG, and IgM.

Discussion

We propose that the immunoperoxidase labelling of mucosal *C. pylori* 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. pylori* IgA and IgM in the gastric juice of colonised subjects. The characteristic morphology, position, and population density of *C. pylori* 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

	Active chronic gastritis	Inactive chronic gastritis	Normal (includes three negative control cases)
Antral mucosa	22	5	3
Body mucosa	7	6	17

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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References

- Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983;i:1273-5.
- Jones DM, Lessells AM, Eldridge J. Campylobacter-like organisms on the gastric mucosa: culture, histological and serological studies. *J Clin Pathol* 1984;37:1002-6.
- Langenberg ML, Tytgat GNJ, Schipper MEI, Reitra PJGM, Zanen HC. Campylobacter-like organisms in the stomach of patients and healthy individuals. *Lancet* 1984;i:1348.
- Lee WK, Gourley WK, Buck GE, Subramanyam K. A light and electron microscopic study of a campylobacter-like bacteria inhabiting the human stomach. *Gastroenterology* 1985;88:1470.
- Anonymous. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. *Int J Syst Bacteriol* 1985;35:223-5.
- Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984;i:1311-4.
- Marshall BJ, Armstrong JA, McGeachie DB, Glancy RJ. Attempt to fulfil Koch's postulates for pyloric campylobacter. *Med J Aust* 1985;142:436-9.
- Lambert JR, Hansky J, Davidson A, Pinkard K, Stockman K. Campylobacter-like organisms (CLO)—*in vivo* and *in vitro* susceptibility to antimicrobial and antiulcer therapy. *Gastroenterology* 1985;88:1462.
- Thomas JM. Campylobacter-like organisms in gastritis. *Lancet* 1984;ii:1217.
- Giannella RA, Broitman SA, Zamcheck N. Gastric acid barrier to ingested micro-organisms in man: studies *in vivo* and *in vitro*. *Gut* 1972;13:251-6.
- Gibbons RJ. Review and discussion of the role of mucus in mucosal defence. In: Strober W, Hanson LA, Sell KW, eds. *Recent advances in mucosal immunity*. New York: Raven Press, 1982:343-51.
- Whitehead R. *Mucosal biopsy of the gastrointestinal tract*. 3rd ed. Philadelphia: WB Saunders, 1984.
- Isaacson P. Immunoperoxidase study of the secretory immunoglobulin system and lysozyme in normal and diseased gastric mucosa. *Gut* 1982;23:578-88.
- Valnes K, Brandtzaeg P, Elgjo K, Stave R. Specific and non-specific humoral defence factors in the epithelium of normal and inflamed gastric mucosa. *Gastroenterology* 1984;86:402-12.
- Rathbone BJ, Wyatt JJ, Worsley BW, et al. Systemic and local antibody responses to gastric Campylobacter pyloridis in non-ulcer dyspepsia. *Gut* 1986;27:642-7.
- Price AB, Day DW. Pseudomembranous and infective colitis. In: *Recent advances in histopathology*. Vol 11. Edinburgh: Churchill Livingstone, 1981:99-117.
- Brandtzaeg P, Fjellanger I, Gjeruldsen ST. Adsorption of immunoglobulin A on to oral bacteria *in vivo*. *J Bacteriol* 1968;

- 96:242-9.
- ¹⁸ Boyd JF, McWilliams E. Immunoperoxidase staining of *Legionella pneumophila*. *Histopathology* 1982;6:191-6.
 - ¹⁹ Tomasi TB, Grey HM. Structure and function of immunoglobulin A. *Progress in Allergy* 1972;16:81-213.
 - ²⁰ Solari R, Kraehenbuhl JP. The biosynthesis of secretory component and its role in the transepithelial transport of IgA dimer. *Immunology Today* 1985;6:17-20.
 - ²¹ Rosekrans PCM, Meijer CJLM, Van Der Wal AM, Cornelisse CJ, Lindeman J. Immunoglobulin containing cells in inflammatory bowel disease of the colon: a morphometric and immunohistochemical study. *Gut* 1980;21:941-7.
 - ²² Brandtzaeg P, Baklien K, Fausa O, Hoel PS. Immunohistochemical characterisation of local immunoglobulin formation in ulcerative colitis. *Gastroenterology* 1974;66:1123-36.
 - ²³ Keren DF, Appelman HD, Dobbins WO, *et al*. Correlation of histopathologic evidence of disease activity with the presence of immunoglobulin-containing cells in the colons of patients with inflammatory bowel disease. *Hum Pathol* 1984;15:757-63.
 - ²⁴ Van Spreeuwel JP, Lindeman J, Van Maanen J, Meyer CJLM. Increased numbers of IgE containing cells in gastric and duodenal biopsies. An expression of food allergy secondary to chronic inflammation? *J Clin Pathol* 1984;37:601-6.
 - ²⁵ Jonard PP, Rambaud JC, Dive C, Vaerman JP, Galian A, Delacroix DL. Secretion of immunoglobulins and plasma proteins from the jejunal mucosa. *J Clin Invest* 1984;74:525-35.
 - ²⁶ Williams RC, Gibbons RJ. Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. *Science* 1972;177:697-9.
 - ²⁷ Tagliabue A, Boraschi D, Villa L, *et al*. IgA-dependent cell-mediated activity against enteropathogenic bacteria: distribution, specificity and characterisation of the effector cells. *J Immunol* 1984;133:988-92.
 - ²⁸ Tytgat GNJ, Langenberg ML, Rauws E, Rietra PJGM. Campylobacter-like organisms in the human stomach. *Gastroenterology* 1985;88:1620.
 - ²⁹ Steer HW. The gastro-duodenal epithelium in peptic ulceration. *J Pathol* 1985;146:355-62.

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