Alimentary tract and pancreas

Systemic and local antibody responses to gastric *Campylobacter pyloridis* in non-ulcer dyspepsia

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SUMMARY Antibody titres to *Campylobacter pyloridis* in serum and gastric juice were estimated by an enzyme linked immunosorbent assay (ELISA) to whole organisms obtained from bacterial culture in 39 patients with non-ulcer dyspepsia. Whereas 20 of the 21 patients with chronic gastritis had gastric *C pyloridis*, 17 patients with no *C pyloridis* had normal histology in the gastric antrum and body. Significantly raised serum IgG and IgA antibody titres to *C pyloridis* were found in colonised patients with gastritis. Patients with raised IgG antibody to *C pyloridis* were also shown to have significantly raised titres to other *Campylobacter* species, suggesting antigenic cross reactivity. Gastric juice antibodies were also studied and IgA titres to *C pyloridis* were detected in a proportion of patients with gastritis, together with low levels of IgM, but no IgG.

The presence of spiral organisms in the stomach has been noted on a number of occasions dating back to 1938.¹⁻³ It was only recently, however, that the significance of gastric spiral organisms was recognised by Warren⁴ and Marshall⁵ who identified the previously detected curved bacilli on the gastric epithelium of the majority of patients with active chronic gastritis by the Warthin-Starry silver stain. Morphologically, and in respect to their atmospheric requirements and DNA base composition, these organisms are most closely related to the genus *Campylobacter*.⁶ Reports from a number of workers have all confirmed the presence of these organisms in the majority of patients with gastritis.⁷⁻¹¹ The organism was originally described as a 'Campylobacter-like organism' (CLO) but has now been formally named *Campylobacter pyloridis*.^{1,2} It remains to be seen whether *C pyloridis* is involved in the pathogenesis of chronic gastritis, or is merely a commensal organism.

Little is known of host defences to *C pyloridis*, either at the systemic or the local level. Jones *et al*¹¹ studied circulating antibody to *C pyloridis* by agglutination and complement fixation techniques. They showed raised titres in *C pyloridis* positive patients, but did not analyse the response by

antibody class, and hence shed little light on the host response.

To further characterise the serum and local antibody response, we have studied circulating and gastric juice antibodies to *C pyloridis*, with particular reference to antibody class. In addition, we compared serum antibody titres with other strains of gastric *C pyloridis* isolated from difference patients, and to other *Campylobacter* species.

Methods

PATIENTS

Thirty nine patients (21 men, mean age 41 years) without peptic ulceration were studied. Endoscopic biopsies were obtained for histology and bacterial isolation and culture. Serum and fasting gastric juice were collected for antibody assay.

ENDOSCOPY

The examination was carried out by one endoscopist using an Olympus GIF-T fiberoptic gastroduodenoscope, sterilised between patients with Dettol (Reckitt & Colman). Fasting gastric juice samples were collected and stored at -20°C. Biopsies were taken from the gastric body on the greater curve and the antral floor. The biopsies for histology were immediately fixed in 10% buffered formalin. An additional antral biopsy was also placed in Stuart's

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transport medium (Oxoid) before microaerophilic culture.

HISTOLOGY

Antral and body biopsies were routinely processed and stained with haematoxylin and eosin, to determine the presence of chronic gastritis, and with a Warthin-Starry silver stain technique to identify the *C. pyloridis*. One histopathologist (JIW), without knowledge of the clinical details, examined the sections using the classification of Whitehead *et al.*¹³ to determine the presence and degree of chronic gastritis.

Bacterial cultures

Gastric antral biopsies were suspended in 0.5 ml 1% tryptone diluent and homogenised using a glass Griffiths tube. Ten microlitre aliquots of homogenate were cultured on heated blood agar incubated at 37°C under microaerophilic conditions for at least four days. The organisms produced characteristic 0.5–2 mm light brown convex entire colonies, identified by their characteristic biotyping, being gram negative, oxidase positive, catalase positive, indole negative, hydrogen sulphide positive, urease positive and nalidixic acid resistant.¹⁴ The positively identified organisms were purified and stored in glycerol broth in liquid nitrogen.

C. pyloridis preparations for the ELISA (enzyme linked immunosorbent assay, see next section) were derived from stored strains, grown in freshly prepared brain heart infusion medium (Oxoid) supplemented with 10% calf serum and 5 µg/l haemin under microaerophilic conditions at 37°C for two days with continuous agitation. Cells were harvested by centrifugation at 1500 g for 10 minutes, washed twice in PBS (phosphate buffered saline) and fixed in 4% formaldehyde in PBS for 10 minutes. The *C. pyloridis* were then washed three times in PBS and stored in PBS containing 0.1% azide at 4°C until used.

Strains of *C. fetus* and *C. faecalis* were kindly provided by Dr M D Skirrow (Worcester). *C. sputorum* subspecies *sputorum* (NCTC 11528) was obtained from the National Collection of Type Cultures and *C. jejuni* from the departmental collection. For certain experiments a strain of *Escherichia coli* 0 type 01 was used in place of *C. pyloridis*.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

An indirect ELISA procedure¹⁵ was used wherein whole formalised bacteria from one patient were attached to 'Microtitre' plates (Dynatech Laboratories) at 5×10^7 organisms/well by means of methyl glyoxal.¹⁶ After washing in PBS containing 0.1% Tween 20 (PBS/Tween), active sites were quenched

with 0.5% bovine serum albumin (BSA) in PBS/Tween. Serum samples were diluted in PBS/Tween containing 1% BSA. Gastric juice samples were neutralised to pH 6.5–7 by 1/3 dilution in 0.67 M Tris-HCl pH 7.4 in 0.15 M saline. All samples were incubated in sextuplicate for 90 minutes at 37°C. Plates were washed four times in PBS/Tween, and re-incubated with alkaline phosphatase-conjugated anti-human IgG, IgA or IgM (Sigma) diluted 1/200 in PBS/Tween/BSA as above. After a further four washes, antibody binding was estimated by reactivity with p-nitrophenyl phosphate substrate at 25°C, and quantitated by optical density measurement at 405 nm.

Control sera and blanks were included in each assay. Values for test samples were normalised with respect to the control references to correct for minor day-to-day variations in the assay.¹⁵ The in-run coefficient of variation was <5% and the between-run coefficient <10%.

Total immunoglobulin contents were estimated by ELISA using polyvalent anti-Ig as the solid phase with class specific conjugates.

The ELISA data are presented as optical measurements at 405 nm and statistical analysis was by the two tailed Mann-Whitney U-test.

Results

Eighteen patients had entirely normal antral and body mucosa, 21 had chronic gastritis, which was limited to the gastric antrum in seven. There were no patients with an inflamed gastric body and a normal gastric antrum. The histology is summarised in Table 1. No *C. pyloridis* were detected on biopsies from patients with normal histology, while all but one of the patients with chronic gastritis had demonstrable *C. pyloridis* in one or both biopsies.

SERUM ANTIBODIES TO CLOS

C. pyloridis cultured from one patient were used to

Table 1 Chronic gastric and *Campylobacter pyloridis*

Patients	Degree of Gastritis*		
	Nil	Superficial or mild atrophic	Moderate or severe atrophic
<i>C. pyloridis</i> – positive	0	12	8
<i>C. pyloridis</i> – negative	18	0	1

The presence and degree of chronic gastritis in 39 patients with or without *Campylobacter pyloridis*.

*Assessed by the criteria of Whitehead *et al.*¹³

Fig. 1 Serum IgG, IgA and IgM titres to *C. pyloridis* in bacteria positive (●) and negative (○) patients. Statistical analysis by the two tailed Mann-Whitney U-test.

assay the circulating antibody titres (Fig. 1). While all sera examined contained antibody to *C. pyloridis*, the *C. pyloridis* positive patients had significantly higher IgG and IgA titres than the *C. pyloridis* negative patients; the difference being more marked for IgG. IgM titres were not statistically different between the two groups, each showing a wide range of values. Total serum IgG, IgA and IgM showed no significant difference in titres between the two groups, as did an assay of antibodies to *Escherichia coli* (Table 2).

CIRCULATING IgG TO DIFFERENT PATIENTS' *C. PYLORIDIS*

To investigate antigenic differences between strains of *C. pyloridis* from different patients, six samples of serum showing high IgG titres to our reference *C.*

pyloridis were compared with six low titre sera. Assays were carried out using five different *C. pyloridis* strains under identical conditions. The high titre sera to the reference *C. pyloridis* strain also showed consistently high titres to the other strains of *C. pyloridis*, implying antigenic cross reactivity (Fig. 2). There was marked variability in the ranking order of the high titre sera to different *C. pyloridis*, which suggests a degree of antigenic variation between the strains.

CIRCULATING IgG TO OTHER CAMPYLOBACTER SPECIES

Using the same six high and six low titre samples to the reference *C. pyloridis* strain, the assay was

Table 2 Immunoglobulin and antibody levels to *E. coli* in *Campylobacter pyloridis* positive and negative patients

Immunoglobulin class	Total serum immunoglobulins		Antibody* to <i>E. coli</i>	
	<i>C. pyloridis</i> Positive	Negative	<i>C. pyloridis</i> Positive	Negative
IgG	91 ± 16	95 ± 14	30 ± 12	35 ± 8
IgA	134 ± 10	128 ± 13	49 ± 17	52 ± 18
IgM	77 ± 32	72 ± 28	68 ± 23	72 ± 25

Mean values (± standard deviations) for total immunoglobulins and antibody titres of *E. coli* organisms for patients with and without *Campylobacter pyloridis*. Results are based on ELISA optical density measurement.

*Arbitrary values not directly comparable with serum immunoglobulin concentrations.

Fig. 2 IgG antibody titres to five different *C. pyloridis* strains (obtained from different patients) in six patients with high titres (H) and six patients with low titres (L) to the reference *C. pyloridis*. The differences between high and low titre groups were significant ($p < 0.05$) for all *C. pyloridis* strains.

but causing the active polymorphonuclear cell response which is usually seen in colonised patients.⁴

Whatever the role of the organism in gastritis and its mode of spread, it appears that *C pyloridis* is ubiquitous. It is not surprising to find that all patients tested had measurable serum antibodies to *C pyloridis*: what is of more interest is that although serum IgG and IgA titres in the *C pyloridis* positive group were significantly raised, the IgM titres in both groups were similar. This is most likely explained by the *C pyloridis* colonisation being relatively longstanding. One would only expect a raised IgM response to the initial infection, whereas with continued colonisation, the IgM response would decrease and, even with subsequent recolonisation or recrudescence of infection, no further IgM response would be expected.

The assay technique used was aimed at detecting surface coat antigens. Our results with other Campylobacter species suggest a degree of antigenic crossreactivity, although absorption experiments are necessary to confirm this and are the subject of further study. Using an acid extracted bacterial cell lysate, a specific assay for *C jejuni* antibodies has been developed¹⁷; by using a similar technique for antigen preparation, it may be possible to assay for antibodies specific for the various species of Campylobacter.

Circulating IgG antibodies are clearly of major importance in the immune defence against invasion by microorganisms, but they probably have little relevance to local mucosal surface immunity. The dominant antibody class of seromucous secretions is dimeric secretory IgA. Although the exact role of secretory IgA in local immunity is unclear, its ability to render bacteria mucophilic may be an important factor in the defence against microorganisms.¹⁸ In chronic gastritis, the ability to secrete IgA is enhanced in the mucous neck cells¹⁹; it has been proposed that this secretory mechanism develops as a result of increased epithelial immaturity,²⁰ which is secondary to the increased epithelial cell turnover associated with chronic gastritis.²¹ We have detected IgA antibodies to *C pyloridis* in the gastric juice of gastritic *C pyloridis* positive patients and have some evidence to suggest that *C pyloridis* on gastritic mucosae may be coated with IgA antibodies.²² Nevertheless, the presence of anti-*C pyloridis* IgA class antibody in the stomach clearly does not eliminate the infection in gastritic patients. The local gastric antibody response, possibly causing the organisms to be mucophilic, may itself partly explain the characteristic position of colonising *C pyloridis*, deep in the gastric mucus layer.

In conclusion, we suggest that *C pyloridis* are ubiquitous organisms capable of eliciting both a

systemic and local antibody response in patients with chronic gastritis. Our data suggest that the secretory antibody response does not eliminate colonisation. This antibody response, however, may be of some importance in inhibiting bacterial adherence and invasion.

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