Campylobacter like organisms in duodenal and antral endoscopic biopsies: relationship to inflammation

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SUMMARY In 66 endoscopic duodenal biopsies studied retrospectively and in 84 paired duodenal and antral biopsies assessed prospectively campylobacter like organisms were seen in 22 (33%) of the retrospectively examined duodenal biopsies and in 61 (73%) examined prospectively, and in 70 (83%) of antral biopsies. In the duodenum the organisms were mostly confined to the surface epithelium in areas showing gastric metaplasia, while in the antrum they were also found within superficial glands. A strong association was shown between the presence of campylobacter like organisms and histological active duodenal inflammation.

There has been much recent interest in the presence of campylobacter like organisms in gastric biopsies and discussion as to their possible role in the aetiology of various disorders. As early as 1906 Krienitz¹ isolated spirochaetes from the stomach of a patient with gastric cancer and this was confirmed later by others including Freedberg and Barron² who found spirochaetes in the gastric mucosa of 13 of 35 patients undergoing gastric resection for carcinoma. Since the report by Marshall and Warren,³ others have reported a positive association between the presence of campylobacter like organisms and histological gastritis⁴⁻⁵ and a negative one when chronic atrophic gastritis is associated with intestinal metaplasia as in pernicious anaemia.⁶ The reports of a possible association with peptic ulceration are more varied ranging from no association⁴ or no definite association,⁷ to finding an association with peptic ulceration.⁸⁻¹⁴ There is little information available, however, about duodenal campylobacter like organisms in peptic ulceration, therefore we examined duodenal endoscopic biopsies both retrospectively and prospectively for campylobacter like organisms presence to study further their relationship with duodenal inflammation. Antral biopsies were also taken to allow comparison with other published results.

Methods

PATIENTS

Two groups of patients were studied. Group 1 were all those in whom duodenal biopsies were obtained at routine endoscopy between January 1980 and August 1984; 70 biopsies were taken from 69 patients. Four biopsies were inadequate, two being very superficial and two contained material from the base of an ulcer only, leaving 66 biopsies from 65 patients. Group 2 consisted of 87 patients studied between September 1984 and March 1985 and included those with duodenal ulcers or duodenal inflammation at endoscopy, some with a macroscopically normal duodenal and gastric mucosa and 12 patients comprising two consecutive endoscopy lists. Paired duodenal and antral biopsies were taken from all group 2 patients. If either the duodenal or antral mucosa appeared inflamed at endoscopy, biopsies were taken from the inflamed area, and when duodenal ulcer was present then two biopsies were taken, one from the base and the other from the ulcer margin. Paired biopsies were suitable for study in 84 patients.

LIGHT MICROSCOPY

The biopsies were fixed in neutral buffered formol saline and routinely processed, sections were cut at 4 µm and stained with haematoxylin and eosin. Campylobacter like organisms were recognised on H & E sections as haematoxyphilic curved structures approximately the size of red blood cells, usually lying along the mucosal surface (Fig. 1). The

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Fig. 1 Large numbers of campylobacter like organisms (arrowed) within the superficial layers of the duodenal surface epithelium. H & E.

sections were viewed using a $\times 40$ objective. In order to aid histological identification of the organisms some additional sections were stained using PAS, Giemsa and Warthin-Starry techniques (Fig. 2). Duodenitis was diagnosed by the presence of increased plasma cells and lymphocytes in the lamina propria, and activity by the presence of polymorph infiltration, oedema of the lamina propria and degeneration and or regeneration of the surface epithelium. Duodenal ulceration was assessed by the presence of inflammatory exudate. The presence or absence of gastritis in the antral sections was judged using standard criteria¹⁵ and the cases were subdivided into superficial or atrophic gastritis and into active or inactive phases. Where biopsies were deemed unsuitable for grading of inflammation or where no recognisable tissue pattern could be found, the cases were excluded from further investigation. All results are based on the histological findings.

Results

Campylobacter like organisms were identified in duodenal biopsies in areas showing gastric metaplasia and were found on the surface epithelium and to a lesser extent in superficial glands, but sparing the

adjacent uninvolved small intestinal mucosa. The density of campylobacter like organisms tended to increase towards histological features indicative of ulceration. They were found to be associated with signs of activity and in particular were often seen close to polymorphs in the surface epithelium (Fig. 3). In the antral biopsies the campylobacter like organisms were seen on the surface epithelium and within superficial glands. A striking feature in the antral biopsies was the absence of the organisms in areas with intestinal metaplasia, even when there was colonisation of the adjacent surface epithelium. The organisms were sometimes seen to form a dense purple belt along the antral surface associated with mucus. The campylobacter like organisms only stained with PAS when surrounded by mucus but were easily identifiable using Giemsa and Warthin-Starry. The use of these stains did not identify organisms in any sections found to be negative on H & E staining.

Twenty two (33%) of the 66 duodenal biopsies in group 1 patients were found to contain campylobacter like organisms. Such organisms also were identified in 61 (73%) of the duodenal biopsies and in 70 (83%) of the antral biopsies from group 2 patients. The discrepancy in the incidence of campylobacter like organisms in duodenal biopsies examined

Fig. 2 Numerous campylobacter like organisms are seen both in isolation and in groups on the surface epithelium. Warthin-Starry.

retrospectively and prospectively can be accounted for by a higher proportion of normal duodenal biopsies in the retrospective group.

If group 1 and 2 duodenal biopsies are considered together (Fig. 4) it can be seen that no campylobacter like organisms were found in an histologically normal duodenal mucosa ($n=38$) or in biopsies showing features of duodenitis without activity ($n=20$). In biopsies showing active duodenal inflammation ($n=39$) campylobacter like organisms were seen in three (92%). Of the 36 patients negative for campylobacter like organisms one was taking non-steroidal anti-inflammatory drugs for rheumatoid arthritis, while in the others the inflammation could not be attributed to any obvious cause. Fifty three patients had histological evidence of active duodenal ulceration and of these 45 (85%) had demonstrable campylobacter like organisms. One patient with a recurrent phaeochromocytoma and a campylobacter like organisms negative duodenal ulcer is represented twice as he had an ulcer biopsied on two occasions.

Campylobacter like organisms were found in only

one of 11 histologically normal antral biopsies. This patient had active duodenal inflammation and duodenal campylobacter like organisms. A good correlation was found between the presence of campylobacter like organisms in antral biopsies and gastritis (Fig. 5), but the grade of gastritis (superficial or atrophic) and the presence or absence of activity did not correlate with presence of campylobacter like organisms.

Discussion

Burnett *et al*⁷ were unable to show campylobacter like organisms in biopsies taken from the duodenal cap of patients with duodenal ulcer, although four of the seven patients studied did have campylobacter like organisms in the antral tissue. Steer⁸ used scanning electron microscopy to view the surface of duodenal biopsies taken from 11 patients with duodenal ulcers and identified campylobacter like organisms on the surface of biopsies from eight patients, but was unable to detect campylobacter like organisms in 14 patients with histologically

Fig. 3 Numerous *Campylobacter* like organisms (arrowed) are shown opposing polymorphs within the superficial epithelium in active duodenitis. H & E $\times 600$.

Fig. 4 Proportion of duodenal biopsies (Groups 1 & 2) found to contain campylobacter like organisms subdivided by histological diagnosis.

normal duodenum. Phillips *et al*¹⁶ were only able to find campylobacter like organisms in duodenal biopsies showing gastric metaplasia. The frequency with which campylobacter like organisms have been shown in antral biopsies from patients with active duodenal ulceration ranged between 57 and 100%^{3 7 9 10 11 17} – our result of 80% is compar-

Fig. 5 Proportion of antral biopsies (Group 2) found to contain campylobacter like organisms subdivided by histological diagnosis.

able. We were unable to see campylobacter like organisms in histologically normal duodenum confirming the findings of others.^{7 8} It was noteworthy that there was total absence of campylobacter like organisms around the goblet cells in the duodenal biopsies, even if campylobacter like organisms were present in adjacent goblet cell free mucosa.

In gastritis all reports to date agree that there is a definite association between campylobacter like

organisms presence and gastritis, but there is disagreement as to whether the activity and grade of gastritis is of importance in determining campylobacter like organisms presence. Warren and Marshall³ found a positive correlation with gastritis, but found that the association with inactive gastritis was less pronounced. Phillips *et al*¹⁶ also reported an increase in campylobacter like organisms with activity and in particular association with polymorphonuclear cells. McNulty *et al*¹⁰ found a strong correlation of campylobacter like organisms presence with the severity of the gastritis. In contrast Jones *et al*⁵ found that the organisms appeared to be similarly correlated with both inactive and active gastritis, and also that there was no correlation between the degree of gastritis and the level of the antibody response in the blood. These data are supported by those of Rollason *et al*⁴ who found that the type of gastritis made no difference to the percentage of biopsies found to contain campylobacter like organisms. Our results are in agreement with those of the latter two groups. No campylobacter like organisms were found at sites of intestinal metaplasia even when they colonised the adjacent mucosa, this is a similar finding to that reported by Meyrick-Thomas *et al*,¹² Price *et al*⁹ and Marshall *et al*.¹⁷ In this study campylobacter like organisms were only found in one of 11 histologically normal antral biopsies. Other groups have reported a similar result – Marshall and Warren³ found campylobacter like organisms in two of 31 normal biopsies, Rollason *et al*⁴ in one of 11 and Burnett *et al*⁷ in six of 25. Three groups, however, were unable to show campylobacter like organisms in histologically normal antrum.^{5 8 10} The significance of the presence of campylobacter like organisms in histologically normal antrum is unknown. In the patient in our study there was accompanying duodenal ulceration and campylobacter like organisms colonisation. The other groups did not comment as to whether their patients had other relevant pathology.

It has been suggested that the presence of campylobacter like organisms in biopsies is merely a consequence of contamination,⁴ but the inclusion in group 2 of the patients from two complete endoscopy lists (Table), and the lack of campylobacter like organisms presence in normal tissue taken from patients endoscoped immediately after patients in whom campylobacter like organisms were demonstrated, excluded their introduction by the endoscope. Antral campylobacter like organisms presence can not be explained by contamination from the duodenum as seven of the group 2 patients were found to have antral, but no duodenal campylobacter like organisms identified. If con-

Table Details of consecutively endoscoped patients.

Age	Sex	Endoscopic diagnosis	CLO presence	
			Duodenum	Antrum
74	M	Duodenitis	–	–
59	M	Duodenal Ulcer	+	+
71	F	Hiatus Hernia & Oesophagitis	–	–
43	M	Duodenitis	+	+
71	F	Oesophagitis	–	+
24	M	Oesophagitis	–	–
53	M	Duodenal Ulcer	+	+
39	M	Duodenitis	+	+
51	M	Duodenal Ulcer	+	+
64	M	Duodenal Ulcer	+	+
46	M	Duodenal Ulcer	+	+
60	F	Oesophagitis	–	–

CLO=campylobacter like organism.

tamination was the major cause for campylobacter like organisms presence then one could expect a high incidence in histologically normal tissue. A uniform distribution of the organisms was not observed in the material studied. In particular campylobacter like organisms were never observed adjoining goblet cells in either the duodenal or antral mucosa. This could be explained by a possible production by goblet cells of a substance which makes for an unfavourable environment for colonisation. The presence of antibodies to campylobacter like organisms in the blood has been reported and it is probable that tissue penetration by the organisms is necessary for antibody formation,⁹ again ruling out superficial contamination as a cause for campylobacter like organisms presence. Kaldor *et al*¹⁸ found that patients with peptic ulcers had significantly higher IgG antibody titres to campylobacter pyloridis than normal controls.

Campylobacter like organisms are related to active duodenal inflammation and ulceration and are associated with gastric metaplasia. They were not seen in histologically normal duodenal biopsies. Marshall *et al*¹⁹ have recently shown, by administering campylobacter like organisms orally to a previously healthy volunteer, the organisms are able to colonise histologically normal mucosa and induce gastritis. They have theorised that infection with campylobacter like organisms leads to a syndrome of acute campylobacter like organisms gastritis which may progress to a chronic infection and thereby predispose to peptic ulceration. A high proportion of our duodenal ulcer patients were found to have campylobacter like organisms in both the duodenum and antrum, and all but one of the duodenal ulcer patients had histological evidence of gastritis data that would support Marshall's hypoth-

esis, which was further reinforced by data presented at the Third International Workshop on Campylobacter Infections.²⁰

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