An Ultrastructural Study of the Gastric Campylobacter-like Organism
‘Campylobacter pyloridis’

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Microaerophilic spiral organisms may be isolated frequently from samples of gastric mucus taken from patients undergoing gastroscopy. The ultrastructure of these gastric campylobacter-like organisms (‘Campylobacter pyloridis’) shows that they have greater affinities with Spirillum than with Campylobacter.

INTRODUCTION

In the last century ‘spirochetes’ were observed in the gastric mucosa of the dog (Bizzozero, 1893) and early this century similar organisms were observed by Krienitz (1906) in the stomach of humans with gastric cancers. Doenges (1939) observed ‘spirochetes’ in 43% of human stomachs examined in routine autopsies and somewhat similar results were reported by Freedberg & Barron (1940) from the examination of resected surgical specimens of the stomach. Recently there has been a resurgence of interest in the spiral bacteria of the stomach. In addition to confirming these early histological studies, it has been possible, using modern culture methods, to grow a campylobacter-like organism from the gastric mucus (Warren & Marshall, 1984). These findings have been amply confirmed by other workers (McNulty & Watson, 1984; Langenberg et al., 1984; Pearson et al., 1984; Jones et al., 1984). The possible role of these bacteria in the pathogenesis of gastritis and of peptic ulceration is now being widely investigated. We report here our observations on the structure of these spiral bacteria known as ‘Campylobacter pyloridis’ and recently designated type I gastric campylobacter-like organism (GCLO-1; Owen et al., 1985), that frequently can be seen in and isolated from endoscopic biopsies taken from patients who undergo gastroscopy.

METHODS

Pieces of tissue from endoscopic biopsy of gastric antral mucosa were fixed in Carson’s buffered formalin and processed for electron microscopy (Curry et al., 1984). Other non-fixed pieces of tissue were rubbed on to the surface of blood agar plates [Blood Agar Base no. 2 (Oxoid CM271) containing 7% defibrinated horse blood]. These plates were incubated for 4 d at 37°C in an atmosphere of 10% CO₂, 5% O₂ and 85% H₂. The method for negative staining, the preparation of sarcosinate-treated extracts and their digestion with trypsin have been described previously (Curry et al., 1984).

RESULTS

Isolates of spiral bacteria from different patients were morphologically and culturally similar. The bacteria were gently sinusoidal in shape and approximately 3.5 μm long by 1 μm wide. The terminal region had a domed appearance with no obvious depressions and was not tapered (see below). Although some bacteria from culture did not possess flagellar filaments, most did, and these were at one end only. The number of flagella varied between 1 and 5 per cell and they were sheathed (Fig. 1). Occasionally the internal filament was seen protruding through the end of the membrane-like sheath and conversely some flagella had distal regions lacking the internal
filament. Generally these filaments were up to 2.5 μm long. The flagellar sheath was approximately 30 nm in diameter, enclosing a filament 15 nm wide.

In sarcosinate-treated cell suspensions (Fig. 2a) discs were seen that were generally similar to those observed previously in *Campylobacter fetus*, *Campylobacter jejuni* and *Aquaspirillum serpens* (Curry et al., 1984) and those reported in *Vibrio cholerae* (Ferris et al., 1984). The discs from the spiral organism were approximately 90 nm in diameter and had a central hole which was surrounded by a more densely staining inner annular zone 40 nm in diameter. Some negatively stained preparations showed the location of these discs at the flagellar insertions through the apical wall (Fig. 2b). In sarcosinate preparations that had been treated with trypsin, the outer annulus of the discs appeared to be undergoing degeneration (Fig. 2c). Also visible in the sarcosinate preparations were structures that were roughly 12 nm in diameter with a central darkly staining hole 4 nm in diameter. These ‘doughnut’ structures were present in very large numbers and appeared to be associated with the cell surface (Fig. 2b). When the sarcosinate preparations were treated with trypsin the ‘doughnut’ structures disappeared (Fig. 2c). The ‘doughnut’ structures also disappeared when the sarcosinate extracts were heated to 100 °C for 5 min.

The overall appearance of *A. serpens* was similar to this campylobacter-like organism (Fig. 3a). The most obvious difference was the absence of the flagellar sheath. Sarcosinate extracts of *A. serpens* contained large featureless discs (Curry et al., 1984) that were associated with the terminal flagellar region of the organism (Fig. 3b). More remarkable are the large number of ‘doughnut’ structures that were present in these preparations (Fig. 3c). These were 10 m in diameter and otherwise morphologically indistinguishable from those seen in sarcosinate preparations of the campylobacter-like organism. Thin sections of the cultured campylobacter-like organism showed that the cell wall had a double membrane similar to that of other Gram-negative bacteria, but with terminal specializations. These consisted of an electron-lucent region immediately below the terminal region of the plasma membrane (Fig. 4). There were additional structures on the inner surface of this membrane for a distance of about 400 nm from the tip of the organism; these consisted of an additional electron-dense layer 6 nm thick immediately underlying the terminal plasma membrane, with another electron-dense layer 20 nm thick internally (Fig. 4a). In favourable sections links could be found between these two dense layers. For comparison the appearances of the structures at the terminal region of *C. jejuni* are shown in Fig. 4(b) and those of *A. serpens* in Fig. 4(c).
Fig. 2. Negatively stained preparations of sarcosinate-treated cultures of spiral organisms from the stomach. (a) In addition to short lengths of flagellar filaments and fragments of membrane, discs perforated by a central hole and 'doughnut'-shaped surface proteins are present; (b) the location of the discs at the insertions of the flagellar filaments can be seen; (c) in this trypsin-treated sarcosinate extract of spiral organisms note the lack of 'doughnut' structures and the degradation of flagellar discs. Bars, 100 nm.
Fig. 3. Negatively stained preparations of *Aquaspirillum serpens* (a) showing the overall shape of the organism and the unsheathed flagellar filaments which insert into one end of the organism only; bar, 1 μm. (b) Sarcosinate preparation of *A. serpens* showing the discs at the flagellar insertion points; bar, 100 nm. (c) Sarcosinate preparation of *A. serpens* showing flagellar filaments, membrane fragments and the ‘doughnut’ structures normally located on the outer membrane; bar, 100 nm.
Fig. 4. Thin sections of the terminal regions of (a) 'C. pyloridis', (b) C. jejuni and (c) A. serpens. In (a) note the electron-lucent zone just beneath the terminal cell wall. The inner surface of the true cytoplasmic membrane has a closely adherent electron-dense layer (arrowed). An additional electron-dense layer is found a short distance internally from this membrane-associated dense layer. Note the membrane-like nature of the flagellar sheath. In (b) note the conical shape of the terminal region and in (c) the dome-shaped terminal region. Both these organisms show arrangements of electron-dense layers similar to that in (a). Bars, 100 nm.
In sections of the biopsy material the spiral bacteria were sparsely distributed or localized into small groups on the gastric mucosa. These stained bacteria are morphologically identical to those described above from cultured material. When mucus was present, the bacteria were either attached to the microvilli of the gastric mucosa or a short distance (up to 10 μm) above this epithelial surface. In the absence of visible mucus above the gastric surface, bacteria were attached by threads 5 nm wide to the microvilli.

**DISCUSSION**

The spiral organisms isolated from human gastric biopsy material were all morphologically similar and appeared to be an antigenically homogeneous group (D. M. Jones & J. Eldridge, unpublished observations). A different type of campylobacter-like organism has been isolated from the stomach that more closely resembles the true campylobacters (Kasper & Dickgiesser, 1985). For this reason, organisms of the type described here have been temporarily designated GCLO-1 (Owen et al., 1985), being synonymous with ‘C. pyloridis’. Although they have been described provisionally as campylobacter-like organisms, from the results of this study it can be seen that they have morphological differences from the campylobacters. Unlike campylobacters, the ends of these bacteria do not taper, nor do they have a terminal concavity at the location of the single unsheathed flagella that is found in campylobacters. The large flagellar discs did not have the radial structures characteristic of those seen in C. jejuni and C. fetus (Curry et al., 1984). The terminal structures internal to the plasma membrane are present in these organisms, as they are in many spiral bacteria.

The large number of 12 nm 'doughnut'-like structures seen in sarcosinate extracts have not been seen in similar extracts of campylobacters although they are present in extracts of A. serpens. The cell surface of A. serpens is composed of a layer of regularly arranged protein sub-units very similar to those of this spiral organism and many other bacterial species have similar structures (Kist & Murray, 1984). The results of trypsin and heat treatment suggest that the 'doughnut' structures are protein in nature and that they are seen in the extracts as a result of the breakdown of the cell surface by detergent.

The overall morphology, possession of flagellar discs without radial structures and the protein sub-unit arrangement of the cell wall all suggest that these campylobacter-like organisms may be more closely related to the genus *Aquaspirillum* than to the genus *Campylobacter*. However, the possession of sheathed flagella clearly distinguishes this organism from A. serpens.

In vivo the campylobacter-like organism appears to have a preferred habitat within the mucus that normally lines the stomach. We have been unable to isolate the organism from samples of mucus taken from the small and large bowel at colonoscopy (D. M. Jones & J. Eldridge, unpublished observations). It is probably a commensal although clearly it has some association with gastritis. It has been more frequently reported in association with histologically defined gastritis than with entirely normal gastric mucosa (Warren & Marshall, 1984; Jones et al., 1984). The spiral body form and the sheathed flagella filaments may be specializations for existence in the peri-epithelial mucus.

As yet there is not sufficient data to place these spiral bacteria in their exact taxonomic position, but they do not seem to be campylobacters. If the organisms noted earlier this century in the stomachs of monkeys and dogs are related to these strains from the human stomach, they may all represent a previously unrecognized taxonomic group of organisms.

**REFERENCES**


