Isolation of a gastric campylobacter-like organism from the stomach of four Rhesus monkeys, and identification as *Campylobacter pylori*

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Summary. Campylobacter-like organisms, isolated from the gastric antrum of Rhesus monkeys, were compared with *Campylobacter jejuni* and *C. pylori*. They were similar to *C. pylori* by light microscopy, in ultrastructural morphology, in enzymic, fatty-acid-methyl-ester, and protein-profile analysis, and in antigenic reactivity with rabbit antisera to *C. jejuni* and *C. pylori* and with *C. pylori*-specific monoclonal antibody. Because this natural infection of the Rhesus monkey is associated with chronic gastritis, resembling the disease in humans colonised with *C. pylori*, we recommend the animal as a model for the investigation of human gastritis.

Introduction

It is widely recognised that histologically diagnosed chronic gastritis in man is frequently accompanied by colonisation of the antral mucosa with *Campylobacter pylori* (Blaser, 1987). Although limited human volunteer studies (Marshall *et al.*, 1985; Morris and Nicholson, 1987) support a pathogenic role for this organism in chronic gastritis, such uncontrolled studies are subject to criticism. The role of *C. pylori* in gastritis needs, therefore, to be investigated with suitable animal models, which may be useful also in establishing effective antimicrobial therapy. In the International Workshop on *C. pylori* Infections, at Keystone in 1987, the development of a good animal model of such gastritis was considered by the participants as the primary research priority (A. Lee, personal communication).

We have recently studied several primate species for susceptibility to gastritis (Baskerville and Newell, 1987), and have now isolated a gastric campylobacter-like organism (GCLO) from the chronically inflamed gastric mucosa of several Rhesus monkeys. To classify these isolates we have compared their morphological, structural, biochemical and antigenic properties with those of *C. jejuni* and *C. pylori*.

Materials and methods

Animals

Seven Rhesus monkeys (*Macaca mulatta*) aged 2–8

years, which came to autopsy for unrelated reasons, were examined microbiologically. The histopathological findings in the gastric mucosa of these animals have been described by Baskerville and Newell (1987).

Bacterial strains

C. jejuni strain 81116, isolated from human faeces, was kindly supplied by the Public Health Laboratory, Southampton. *C. pylori* strain NCTC 11638 was isolated from human gastric mucosa by Dr B. Marshall (Perth, Western Australia) and kindly supplied by Dr M. Skirrow (Royal Infirmary, Worcester). These isolates possessed the biochemical and antigenic properties characteristic of their respective species (Newell *et al.*, 1984; Newell, 1987).

GCLO strains 87008, 87010 and 87012 were isolated from the gastric mucosa of Rhesus monkeys (see below).

Culture and storage of campylobacter-like organisms

Tissue samples from the gastric antrum, fundus, pylorus and duodenum were processed within 1 h of death. Pieces of biopsy material c. 5 mm² were smeared directly on to agar plates containing defibrinated horse blood 5% and selective antibiotics (Skirrow, 1977). In some cases tissue was snap frozen and stored in liquid nitrogen before culture. Plates were cultured for up to 5 days at 37°C in micro-aerophilic conditions (micro-aerophilic gas generating kit, Oxoid). GCLOs, recognised by colonial morphology, were sub-cultured on blood agar alone, for purity, and identified by light microscopy with phase contrast and Gram's staining, by the catalase and oxidase reactions, and by the rapid hydrolysis of urea (Owen *et al.*, 1985). Isolates were suspended in proteose peptone 1% (Oxoid) with glycerol 10%, and were snap frozen and stored in liquid nitrogen.

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SDS-PAGE

The total protein profile of *C. jejuni* strain 81116, *C. pylori* NCTC 11638 and the monkey isolates was examined on a 10–25% gradient SDS-polyacrylamide gel as described by Newell (1987).

Western blotting

SDS-PAGE gels of total protein profiles were electro-blotted on to nitrocellulose paper with a semi-dry blotter (Dako Ltd)—see Kyhse-Andersen (1984). The blots were incubated with rabbit antisera to *C. jejuni* (Newell *et al.*, 1984) and *C. pylori* (Newell, 1987) and a mouse monoclonal antibody (CP1) specific for *C. pylori* (Newell, 1988). Bound antibodies were detected with goat anti-rabbit or goat anti-mouse IgG coupled to peroxidase (Bio-Rad Ltd) as described by Newell (1987).

Cell fatty acid (FA) analysis

Cell FAs were analysed by capillary gas-liquid chromatography (GLC) and GLC mass-spectrometry of their FA methyl esters (FAME) as described by Wait and Hudson (1985).

Results

GCLOs were isolated from four of the seven Rhesus monkeys examined, and could be cultured from snap-frozen as well as from fresh biopsy material. Morphologically, these organisms were motile Gram-negative rods, S-shaped or spiral, and frequently with characteristic ox-bow forms. By transmission electronmicroscopy the bacteria had the morphological features of *C. pylori* (fig. 1) including unipolar multiple sheathed flagella with

Fig. 1. Transmission electronmicrograph (with negative staining) of a gastric campylobacter-like organism from monkey stomach. Note the multiple unipolar sheathed flagella with terminal paddles. Bar = 1.0 μ m.

terminal paddles. Conversely *C. jejuni* had single bipolar unsheathed flagella.

All the isolates grew micro-aerophilically only, gave positive results in tests for oxidase and catalase, and produced large quantities of urease, similar to the *C. pylori* strain but unlike the *C. jejuni* strain which did not produce urease.

The FAME and lipid profiles of the monkey strains possessed major 14:0 and cyclopropane 19:0 acids, together with 18:0, 3-hydroxy 16:0 and 3-hydroxy 18:0 acids, characteristic of the *Campylobacter* genus. They lacked 16:1 acid; and the ratio of 18:0 to 16:0 acids was >1. This cell fatty acid profile was indistinguishable from that of *C. pylori*, but differed markedly from that of *C. jejuni* which possessed 16:1 acid and had an 18:0 to 16:0 ratio of <0.1.

The SDS-PAGE total protein profile (fig. 2) showed no obvious major outer membrane proteins, such as that of *C. jejuni* (43×10^3 mol. wt), but had three notable proteins with mol. wt (10^3) 62, 56 and 54, similar to the *C. pylori* strain.

Western blots of the total protein profiles of *C. jejuni*, *C. pylori* and a monkey GCLO, incubated with rabbit antisera to *C. jejuni* and *C. pylori*, are shown in fig. 3. Multiple prominent protein antigens were detected in the monkey GCLO with both antisera. None of these antigens corresponded with *C. jejuni* antigens, although rabbit antiserum to *C. jejuni* did cross-react with several antigens of the monkey strain. The antigenic profiles of *C. pylori* and the monkey GCLO were identical, with rabbit antiserum to *C. pylori*; and they were very similar

Fig. 2. SDS-PAGE of the total protein profile of *C. pylori* 11638 (track 1), Rhesus monkey GCLO strains 87008 (track 2), 87010 (track 3) and 87012 (track 4), and *C. jejuni* 81116 (track 5).

Fig. 3. Western blots of total protein profiles of *C. jejuni* 81116 (track 1), Rhesus monkey GCLO strain 87008 (track 2) and *C. pylori* NCTC 11638 (track 3), incubated with (A) rabbit antiserum to *C. jejuni*, (B) rabbit antiserum to *C. pylori*, (C) mouse monoclonal antibody to *C. pylori*.

with rabbit antiserum to *C. jejuni*. The *C. pylori*-specific monoclonal antibody reacted with a single protein (31×10^3 mol. wt) in the total protein profiles of both *C. pylori* and the monkey strain.

Discussion

The morphological, biochemical and antigenic properties of GCLOs isolated from the gastric antrum of Rhesus monkeys have been investigated. By Gram's stain and enzymic criteria, especially the rapid production of urease, these organisms were similar to *C. pylori*. This similarity was further supported by ultrastructural characteristics, in particular the possession of multiple unipolar sheathed flagella which distinguish *C. pylori* from other *Campylobacter* species (Jones *et al.*, 1985). The total protein profiles of the monkey strains were unlike that of *C. jejuni*, with its characteristic single major outer membrane protein of $(43-47) \times 10^3$ mol. wt (Newell *et al.*, 1984), but consistent with that of *C. pylori* (Megraud *et al.*, 1985; Newell, 1987). Total protein antigenic profiles obtained by immunolabelling with rabbit antisera to *C. jejuni*

and *C. pylori* demonstrated that a very close antigenic relationship existed with *C. pylori* but not with *C. jejuni*. However, some antigenic cross-reactivity was seen with anti-*C. jejuni* serum. This was consistent with the degree of inter-species cross-reactivity previously ascribed to campylobacter flagellins (Newell, 1986, 1987). Moreover, a monoclonal antibody, CP1, which is directed against a protein (31×10^3 mol. wt) of all *C. pylori* strains but does not cross-react with *C. jejuni* (Newell, 1988), was found to react in Western blots with a protein of the same mol. wt in the monkey strains.

The value of fatty-acid analysis in the chemotaxonomy of *Campylobacter* species has been demonstrated by Wait and Hudson (1985); and *C. pylori* was shown to have a unique profile within the genus *Campylobacter*, unlike that of other genera examined (Hudson *et al.*, 1988). The fatty-acid profiles of our monkey GCLOs were entirely consistent with those of the *C. pylori* strains previously studied.

All these investigations indicate that the monkey GCLO is indistinguishable from *C. pylori* by the techniques used.

Recently, campylobacter-like organisms associated with the gastric mucosa of several animals other than man have been reported. The GCLO isolated from the ferret was both morphologically (Fox *et al.*, 1986) and by fatty-acid profile (Hudson *et al.*, 1988) unlike *C. pylori*. Nevertheless, GCLOs apparently similar to *C. pylori* have been isolated from the monkey *Macaca nemestrina* (Bronston and Schoenknecht, 1988), a pig and a baboon (Jones and Eldridge, 1988). However, the relationship of the bacterial colonisation to gastritis in these animals is not known. In a recent survey of the stomachs from 10 baboons (*Papio* spp.), only *C. jejuni* was isolated and gastritis was never observed (Baskerville and Newell, 1987).

The Rhesus monkey GCLO described here appears to be identical to *C. pylori*, and the histopathological evidence (Baskerville and Newell, 1987) suggests that colonisation of the monkey with this organism results in lesions similar to *C. pylori*-associated gastritis in man. This naturally acquired monkey infection may therefore provide an appropriate model for the study of human gastritis. Moreover, the source of infection may be easier to investigate in a closed primate colony.

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