MICROBIAL CHARACTERISATION

Novel *Helicobacter* species isolated from rhesus monkeys with chronic idiopathic colitis

JAMES G. FOX, LAWRENCE HANDT*, SHI LU, ZELI SHEN, FLOYD E. DEWHIRST†, BRUCE J. PASTER*, CHARLES A. DANGLER, KEN LODGE*, SHERRI MOTZEL* and HILTON KLEIN*

Massachusetts Institute of Technology, Cambridge, MA, *Merck Research Laboratories, West Point, PA and 1Forsyth Institute, Boston, MA, USA

Chronic, idiopathic diffuse colitis is a well recognised clinical and pathological entity in captive rhesus monkeys. Six rhesus monkeys were diagnosed with clinically debilitating chronic diarrhoea. Histologically, colonic tissues were characterised as chronic, moderate to severe colitis and typhilitis, with diffuse mononuclear inflammation of lamina propria, reactive lymphoid hyperplasia and multifocal micro-abscesses. Colonic tissues were cultured for *Salmonella* spp. and *Shigella* spp.; all results were negative. Samples were negative for *Clostridium difficile* A and B toxins, and special stains of colonic tissue for acid-fast bacteria were also negative. The six diarrhoeic monkeys tested gave negative results for serum IgG antibodies to herpes B virus, STLV, SRV and SIV. Colonic tissue from the six diarrhoeic and two clinically normal monkeys with histologically confirmed colitis from the same colony were also subjected to micro-aerobic culture. Micro-aerobic cultures from all eight monkeys incubated at 37°C and 42°C revealed pinpoint or spreading colonies on antibiotic-containing media. Bacteria were identified as gram-negative, oxidase positive and urease negative. Of the nine strains characterised biochemically, two separate biotypes (corresponding to different species by 16S rRNA analysis) were identified. One biotype (type 1), from non-diarrhoeic monkeys and the second biotype (type 2) from diarrhoeic animals with subclinical chronic colonic inflammation, differed by catalase activity, ability to reduce nitrate to nitrite and sensitivity to cephalothin. Complete 16S rRNA analysis of five of the nine strains characterised biochemically indicated that the organisms isolated were two novel *Helicobacter* spp. By electron microscopy, these novel helicobacters had spiral morphology with bipolar sheathed flagella. This is the first report describing the isolation of novel *Helicobacter* spp. from inflamed colons of rhesus monkeys. Studies are needed to determine whether these novel *Helicobacter* spp. play a causal role in the initiation and progression of chronic colitis in macaques. Further microbiological and histological analysis of this chronic idiopathic colitis syndrome in macaques may prove useful in understanding the aetiology and pathogenesis of inflammatory bowel disease in man.

Introduction

Chronic idiopathic colitis in young rhesus monkeys (*Macaca mulatta*) is a well recognised clinical syndrome in animals maintained in captivity [1]. Monkeys with this disease have a debilitating persistent diarrhoea accompanied by progressive weight loss and dehydration. Histologically, the inflammatory lesions in the colon are characterised by lymphoplasmacytic infiltration, crypt epithelial hyperplasia, goblet cell depletion, presence of multifocal crypt abscesses and mucosal erosion and ulceration. The pathology of this disease mimics many characteristics of ulcerative colitis in man and cotton top tamarins (CTT) [2]. Approximately 50% of colony-maintained CTT develop active colitis, with the disease progressing to adenocarcinoma in 25–40% of those primates with clinically recognised colitis [3–5]. In CTT, there are supportive data which suggest that both environmental and infectious agents may be responsible for the disease [4, 6].

Received 23 Aug. 2000; revised version received 22 Oct. 2000; accepted 2 Nov. 2000.
Corresponding author: Dr J. G. Fox (e-mail: jgfox@mit.edu).
A novel Helicobacter sp. from inflamed colons of CTT was recently isolated and characterised and it was proposed that this microbe could be linked to the development of inflammatory bowel disease (IBD) in this species [2]. A specific serological response to this novel Helicobacter sp. has also been reported in CTTs with chronic colitis [7]. Helicobacter spp. are capable of inducing typhlocolitis in both immunocompetent and immune dysregulated mice and rats [8–11]. Furthermore, H. cinaedi and H. fennellae have been isolated from inflamed colonic tissue of diarrhoeic, immunocompromised human patients [12]. When inoculated into pig-tailed macaques (M. nemestrina), these organisms produce diarrhoea and bacteraemia [13].

Although the gastric helicobacters, H. pylori and H. heilmannii, are increasingly recognised in the gastric mucosa of macaques and organisms with spiral morphology have been noted by light and electron microscopy on surfaces of colonic epithelia in normal rhesus monkeys, enteric Helicobacter spp. have not been isolated previously from macaque monkeys [14]. Given the increasingly recognised important role of gastric and enterohepatic helicobacters in causing gastrointestinal disease, it was hypothesised that novel Helicobacter spp. may also be present in inflamed colons of rhesus monkeys with chronic idiopathic colitis. This study examined a group of rhesus monkeys with protracted diarrhoea and chronic colitis for the presence of Helicobacter spp.

Materials and methods

Clinical history

The rhesus monkeys were received at the research facility weighing between 2 and 4 kg. Before their arrival they had been treated with enrofloxacin 5 mg/kg i.m. once daily for 10 days for prophylactic treatment of subclinical shigella infections. All rhesus monkeys were housed individually after arrival and underwent an initial standardised conditioning regimen. All were treated with praziquantel (5.7 mg/kg i.m. once) and ivermectin (100 μg/kg i.m. once) during this period. The diarrhoea in the six clinically affected animals was characterised by frequent, low-volume liquid faeces occasionally containing blood. Eventually, the monkeys lost weight and terminally became anorexic and dehydrated. The clinical signs of three treated animals improved after antimicrobial treatment, but they continued to have intermittent diarrhoea until their death and necropsy. Two other monkeys without diarrhoea were also assessed for presence of Helicobacter spp. in colonic tissue.

Gross necropsy and microbial analysis

A gross necropsy was conducted on the six diarrhoeic monkeys, with collection of gastrointestinal tissues for histopathological examination. Swabs were collected from various levels of the intestinal tract for aerobic, micro-aerobic and anaerobic culture and scrapings were collected for endoparasite evaluation by direct smear (performed by the Diagnostic Laboratory, Merck Research Laboratory). Samples were also submitted to MIT for micro-aerobic bacterial culture. Stools from five animals were tested for Giardia by ELISA ProSpecT Giardia Rapid Assay (Alexon-Trend, Ramsey, MN, USA), for Clostridium difficile toxins A and B at Cornell University Veterinary Diagnostic Laboratory with the Tox A/B kit (Techlab, Blacksburg, VA, USA) and for Shigella spp. by PCR (performed by Dr Hou-Shu Houng of the Walter Reed Army Institute of Research). Serum was taken from the six animals for haematology, clinical chemistry (Merck Diagnostic Laboratory) and viral serology, including herpes B virus, STLV, SRV and SIV (Bioreliance Laboratories). Evaluation of the two clinically normal monkeys consisted of collection of gastrointestinal tissue for culture and histopathological evaluation.

Micro-aerobic culture

Full-thickness colonic biopsy samples were collected at necropsy from anterior, transverse and descending portions of the large intestine. The samples were placed in brucella broth with glycerol 20% and frozen at −70°C before micro-aerobic culture. After transport to MIT in dry ice, the samples were homogenised with phosphate-buffered saline (PBS). A portion of the tissue homogenate was passed through a 0.45-μm filter and plated on to CVA medium (Remel Labs, Lenexa, KS, USA) containing cefoperazone, vancomycin and amphotericin B, and helicobacter selective medium containing nalidixic acid, polymyxin B, amphotericin B, bacitracin and vancomycin. The remaining unfilttered homogenate was streaked on CVA or helicobacter selective medium. The cultures were incubated for 14 days in vented jars in an atmosphere of 80%, N₂, H₂ 10% and CO₂ 10%. To analyse the bacteria biochemically, ultrastructurally, by 16S rRNA sequencing and RFLP, cultures of bacteria compatible with Helicobacter spp. were subsequently passaged on sheep blood agar plates.

Electron microscopy

Three Helicobacter spp. isolates were examined after negative staining with a JEOL model JM 100EX transmission electron microscope operating at 100 kV. MIT 99-5503 and MIT 99-5504 (representing novel Helicobacter sp. rhesus type 1), and MIT 99-5512 (representing novel Helicobacter sp. rhesus type 2) were obtained from three different monkeys; the first two from control monkeys and the third from an animal with protracted diarrhoea.
Biochemical and physical characterisation of Helicobacter spp.

Nine isolates from monkeys were subjected to a detailed biochemical characterisation as described previously [15]. The isolates were examined for catalase, oxidase and urease activities. The RapID NH System (Innovative Diagnostic Systems, Norcross, GA, USA) was used to examine the isolates for alkaline phosphatase hydrolysis, indoxyl acetate hydrolysis, gamma-glutamyl transpeptidase and hydrolysis of urea. The isolates were also tested for their ability to reduce nitrate with nitrate broth (Gibco Laboratories, Grand Island, NY, USA) and diagnostic reagents as described previously [2]. Growth was examined at 25°, 37° and 42°C under aerobic, micro-aerobic and anaerobic conditions at 3–4 day intervals for up to 2 weeks. The organisms were also grown in the presence of glucose 1%. Susceptibility to cephalothin (30-μg disk) and nalidixic acid (30-μg disk) was determined (Difco Laboratories, Detroit, MI, USA). The bacteria were also gram-stained and examined for motility in sterile PBS by phase contrast microscopy.

DNA extraction

DNA was extracted from growth on primary plates by InstaGene Matrix (BioRad Laboratories, Hercules, CA, USA). Bacteria were selected from CVA plates and resuspended in 1 ml of double-distilled H2O in a microfuge tube. After centrifugation and removal of the supernate, 200 μl of Instagene matrix was added to the pellet and incubated at 56°C for 30 min. The samples were then boiled for 10 min, centrifuged for 5 min at high speed and 10 μl of the supernate were used for the PCR reaction.

PCR amplification of bacterial isolates

Helicobacter genus-specific primers C97 (5ʹ-GCT ATG ACG GGT ATC C–3ʹ) and C05 (5ʹ-ACT TCA CCC CAG TCG CTG–3ʹ) were used to amplify a 1200-bp PCR product from Helicobacter spp. Campylobacter genus-specific primers C98 (5ʹ-GAT TTT ACC CC TAC ACC A-3ʹ) and C99 (5ʹ-GCG TGG AGG ATG ACA CTT-3ʹ) were used to amplify a 298-bp PCR product from Campylobacter spp. PCR amplification reactions were performed with a thermal cycler and Taq polymerase (Roche Molecular Biochemical, Indianapolis, IN, USA). The reaction mixture (100 μl) contained 1 × polymerase buffer (supplied by the manufacturer but supplemented with 1 M MgCl₂ to a final concentration of 2.25 mM), 0.5 μM each of the two primers, 200 μM of each deoxyribonucleotide and bovine serum albumin 200 μg/ml. Samples were heated at 94°C for 4 min, briefly centrifuged and cooled to 58°C. Taq polymerase (2.5 U) was then added. Amplification was achieved by denaturation at 94°C for 1 min, annealing at 58°C for 2 min and elongation at 72°C for 2 min. A 15-μl portion of the sample was then electrophoresed through a Visigel 6% separation matrix (Stratagene, LaJolla, CA, USA).

Amplification of 16S rRNA from Helicobacter spp. by PCR and purification of PCR products

Sequences of the 16S rRNA genes of five bacterial isolates (MIT 99-5501, 99-5504, 99-5507, 99-5512, 99-5513) were determined. The 16S rRNA cistrons were amplified with eight sequencing primers used for Helicobacter spp. 16S rRNA and for intervening sequences present at Escherichia coli position 210 by methods described previously [16]. PCR was performed in thin-walled tubes with a Perkin-Elmer 9700 Thermocycler. One μl of the DNA template was added to a reaction mixture (50 μl final volume) containing 20 pmole of each primer, 40 nmole of dNTPs, 1 unit of Taq 2000 polymerase (Stratagene) in buffer containing Taqstart Antibody (Sigma Chemical Co.). In a hot start protocol, samples were preheated at 95°C for 8 min followed by amplification under the following conditions: denaturation at 95°C for 45 s, annealing at 60°C for 45 s and elongation for 1.5 min with an additional 5 s for each cycle. A total of 30 cycles were performed and then followed by a final elongation step at 72°C for 10 min. The results of PCR amplification were examined by electrophoresis in agarose 1% gel. DNA was stained with ethidium bromide and visualised under short wavelength UV light.

16S rRNA sequencing

Purified DNA from PCR was sequenced with an ABI Prism cycle-sequencing kit (BigDye® Terminator Cycle Sequencing kit with AmpliTaQ DNA polymerase, FS, Perkin-Elmer). The primers used for sequencing were as described previously [17]. Quarter dye chemistry was used with 80-μmole of primers and 1.5 μl of PCR product was used in a final volume of 20 μl. Cycle sequencing was performed with an ABI 9700 with 25 cycles of denaturation at 96°C for 10 s, and annealing and extension at 60°C for 4 min. Sequencing reactions were run on an ABI 377 DNA sequencer.

16S rRNA data analysis

Sequence data were entered into RNA, a programme set for data entry, editing, sequence alignment, secondary structure comparison, similarity matrix generation and dendrogram construction for 16S RNA in Microsoft QuickBasic for use with PC computers, and were aligned as described previously [18]. The database contains >1000 sequences obtained in this laboratory and >500 obtained from GenBank. Den- drograms were constructed by the neighbour-joining method [19].
Restriction fragment-length polymorphism of Helicobacter 16S rRNA gene

A 1.2-kb PCR fragment obtained from nine bacterial isolates from eight monkeys with Helicobacter genus-specific primers was subjected to restriction fragment-length polymorphism (RFLP) analysis [20]. Amplified DNA (20 μl) was digested with 10 U of AluI and HhaI in restriction buffer (New England Biolabs, Beverly, MA, USA) at 37°C for 3 h. Restriction patterns were compared after the digested PCR products were separated in a Visigel 6% separation matrix.

Histopathology

Colonial necropsy samples were fixed in neutral buffered formalin 10%, processed by standard methods and embedded in paraffin. Then 5-μm sections were stained with haematoxylin and cosin or Warthin-Starry silver stains. These sections were examined by light microscopy for evidence of lesions and for the presence of a bacterium with a morphology consistent with members of the genus Helicobacter.

Nucleotide sequence accession numbers

The 16S rRNA sequences for strains MIT 99-5501 and 99-5513 have been deposited in GenBank as AF333338 and AF333339. Sequences for strains MIT 99-5507, 99-5512 and 99-5513 were identical and have accession number AF333340.

Results

Microbial analysis

No single bacterial species was found in a majority of the animals, with the exception of Helicobacter spp. and E. coli. A Campylobacter sp. was isolated from two diarrhoeic monkeys. Tests for endoparasites, Giardia, C. difficile toxins and Shigella spp. by PCR were all negative (data not shown). Haematology and clinical chemistry parameters were within the normal range. All animals were serologically negative for the viruses tested.

Isolation, growth, biochemical and physical characteristics of Helicobacter spp

After incubation for 3–5 days, small pinpoint colonies developed on the agar surfaces. Helicobacter spp. were isolated from all three colonic (anterior, transverse, descending) biopsy samples from five monkeys (three with diarrhoea, two without) and from one of three colonic tissues from three other diarrhoeic monkeys. Bacteria were gram-negative and appeared spiral and motile under phase-contrast microscopy. The bacteria grew under micro-aerobic conditions at 37°C and 42°C, but not 25°C. Six isolates classified as type 1 were oxidase and catalase positive; all were urease negative. The six isolates did not reduce nitrate nor hydrolyse alkaline phosphatase or indoxyl acetate, and they did not have gamma-glutamyl transpeptidase activity. They were also resistant to nalidixic acid and cephalothin. In contrast, three isolates classified as type 2 were oxidase positive, urease negative and weakly catalase positive. These bacteria were also distinguished from the type 1 strains by their ability to reduce nitrate and their sensitivity to cephalothin.

Ultrastructure

Cells had a spiral appearance and measured approximately 0.2 μm × 2–3 μm (Fig. 1). They possessed bipolar, sheathed flagella.

PCR identification of strains

DNA from pure bacterial isolates was amplified with a Helicobacter genus-specific primer set. A 1.2-kb fragment was amplified from 17 of the 24 bacterial isolates tested (Fig. 2).

Phylogenetic analysis

Full 16S rRNA sequences were obtained for five isolates from rhesus monkeys. Sequence lengths were 1490 bp for four isolates and 1654 bp for MIT 99-5501, which contained an intervening sequence (IVS) of 164 bp. The IVS sequence was different from that of other helicobacter IVS, but typical in structure and location. With the exception of the IVS, sequences for MIT 99-5501 and MIT 99-5504 differed by only one bp and were 92–96% similar to most other helicobacter sequences. The closest sequence similarity, 97%, is to a sequence from a Helicobacter isolate from a woodchuck, MIT 98-6070. This cluster was designated as Helicobacter sp. rhesus type 1. Each of these isolates was from a non-diarrhoeic monkey with moderate colitis noted on histological examination of the tissues.

The sequences for MIT 99-5507, MIT 99-5512 and MIT 99-5513 were identical. This cluster was designated as Helicobacter sp. rhesus type 2. All these isolates were from monkeys with diarrhoea. They are also 92–96% similar to most other helicobacter sequences. Sequences for rhesus type 2 isolates are 96% similar to several helicobacter sequences in our database, but they clustered loosely with H. fennelliae. Thus, based on a 16S rRNA sequence difference of 3–4% from known Helicobacter species, the rhesus isolates represent two novel Helicobacter species (Fig. 3).

RFLPs

The isolates subjected to RFLP analysis gave two banding patterns with AluI which differentiated the two novel Helicobacter spp. (Fig. 4). Helicobacter strains MIT 99-5501 and 99-5504 had different RFLP patterns.
Histopathology

Chronic colitis was observed in tissues from the non-diarrhoic and diarrhoic animals; however, the lesions were notably more intense in the diarrhoic animals. In the diarrhoic animals, colonic mucosal alterations were diffuse and characterised by moderate to marked degrees of mucosal hyperplasia and inflammation (Fig. 5). There was vascular congestion in segments of the colonic mucosa and the surface epithelium was irregular with swelling, flattening and loss of epithelial cells. The hyperplastic colonic crypts were elongated and lined by a hyperchromic epithelium with diminished goblet cell differentiation. Crypts were occasionally microcystic and contained necrotic epithelial and inflammatory cells. The inflammatory cell infiltrates were chiefly lymphoplasmacytic and histiocytic. Granulocytes were observed less frequently, usually in sites of epithelial cell disruption. In contrast, colonic tissues from the non-diarrhoic animals were characterised by mild to moderate inflammation, typically mild mucosal hyperplasia, limited surface epithelial disruption and well-maintained goblet cell differentiation. Foci of necrotic inflammatory cells (including macrophages) were present in the superficial lamina propria, immediately beneath the surface epithelium of both diarrhoic and non-diarrhoic animals. Argyrophilic, spiral bacteria were observed in two primary patterns. Dense colonisation was observed along the surface and superficial crypt epithelium in samples from non-diarrhoic animals. In these areas long, slender, spiral bacteria were distinguished at the edges of the dense colonies (Fig. 6). This pattern of colonisation was observed with segmental distribution in only two of the diarrhoic animals. In addition to the absence of the dense surface colonisation, a different pattern of colonisation was observed in diarrhoic animals, occupying the middle to deep zone of the colonic crypts. In this region, short spiral bacteria (Fig. 6) and longer, slender spiral bacteria were observed, which were not tightly associated with the crypt epithelium. Variably sized bacilli were also observed.

Discussion

This report describes, for the first time, the isolation of enteric helicobacters from macaques. The isolation of novel Helicobacter spp. from inflamed colons of diarrhoic and non-diarrhoic rhesus monkeys increases the number of Helicobacter spp. isolated from the intestines of animals and man to at least 15. Six of these enterohpatic helicobacters (H. fennelliae, H. cinaedi, H. pullorum, H. canis, 'H. rappini' and H. canadensis) have been isolated from the faeces of diarrhoic humans [17, 21]. Phylogenetically, the two novel Helicobacters spp. isolated from rhesus monkeys with and without diarrhoea are clustered in a group of Helicobacter spp. with H. fennelliae which, like the two novel rhesus Helicobacter spp., is urease negative.

Fig. 1. Transmission electron micrograph of two novel Helicobacter spp. The cells had a spiral appearance and measured c. 0.2 μm × 2–3 μm; they possessed bipolar, sheathed flagella. (a) strain MIT 99-5503 (rhesus type 1); (b) strain MIT 99-5504 (rhesus type 1); (c) strain MIT 99-5512 (rhesus type 2). Bar = 0.5 μm.

because the IVS was present in strain 99-5501 and not in strain 99-5504. A fourth RFLP pattern was noted with one helicobacter 1.2-kb PCR product. The identity of this organism is unknown at present. There were no discriminating differences in RFLP with HhaI.
However, both urease-negative and urease-positive helicobacters have been isolated from intestinal tissue of animals; notably *H. hepaticus* and *H. bilis*, both urease-positive organisms, can induce typhlocolitis in rodents, whereas another two urease-negative novel *Helicobacter* spp. have similar pro-inflammatory ability in rodents [8, 10, 22, 23]. The novel *Helicobacter* sp. isolated from cotton top tamarins with ulcerative colitis is also urease negative, as is *H. canis* which has been isolated from diarrhoeic and non-diarrhoeic dogs and...
cats [2, 24, 25]. These clinical and experimental data suggest that urease is not essential for eliciting inflammation in the large intestine [2, 8, 24].

It is not known whether the novel Helicobacter spp. isolated in these clinically affected rhesus monkeys are the same spiral organisms previously identified historically and by electron microscopy in clinically normal rhesus monkeys [26]. These authors described a natural infection in rhesus monkeys as intestinal spirochaetosis. Organisms observed by electron microscopy were embedded side by side in the brush border, with occasional penetration into the apical cytoplasm [26]. There was also loss of the glycocalyx, microvilli and terminal web of the colonic epithelia. These Warthin-Starry-positive organisms appeared by electron microscopy as two structurally different bacteria, one as a spirochaete, the other a spiral, flagellate bacterium [26]. The authors acknowledged that these organisms could somehow be involved in colitis and dysentery of undetermined aetiology. Clearly, the two novel Helicobacter spp. we examined by electron microscopy in the present study had spiral morphology and bipolar sheathed flagella. The diffuse pattern of brush border microbial colonisation along the surface epithelium and into the superficial crypts, as observed in the non-diarrhoeic animals, was disrupted or absent in the colonic sections from the diarrhoeic animals. The loss of surface colonisation was associated with the degeneration and necrosis of the surface epithelium in the diarrhoeic animals. Deeper colonisation within the

---

**Fig. 4.** PCR-RFLP patterns of 16S rRNA gene from the monkey isolates. (a) DNA from monkey isolates digested by *Alu*I; (b) DNA from monkey isolates digested by *Hha*I. The RFLP patterns of *Helicobacter* sp. rhesus type 1 and *Helicobacter* sp. rhesus type 2 can be distinguished by *Alu*I digestion. Lanes 2–5, *Helicobacter* sp. rhesus type 2; 6–9, *Helicobacter* sp. rhesus type 1; lane 9 had an intervening sequence confirmed by 16S rRNA sequence. Lane 1 had a different RFLP distinct from *Helicobacter* sp. Rhesus types 1 and 2. *Hha*I digestion produced the same pattern for all the monkey isolates.

---

**Fig. 5.** Widespread multifocal superficial necrosis distorts the surface epithelium in diarrhoeic animals. There is a marked, diffuse, lymphoplasmacytic and histiocytic infiltrate in the lamina propria. The colonic crypts are elongated and hyperplastic and goblet cell density is diminished. Aggregates of necrotic cells are present in some crypts. Haematoxylin and eosin, bar = 200 µm.
illness is classified as IBD or chronic colitis, and is
differentiated from other causes of acute bacterial
colitis where specific aetiopathological agents such as
_Campylobacter_ and _Shigella_ spp. have been identified.
In these types of acute colitis there is a scarcity of
mononuclear inflammatory cells in the lamina propria
and lack of mucosal hyperplasia and dysplastic glands
[1]. The affected rhesus monkeys described in the
present report did not have _Shigella_ spp. identified, and
_Campylobacter_ spp. were present in only two of six
diarrhoeic monkeys. In the majority of the idiopathic,
chronic colitis cases seen in captive rhesus monkey
colonies, the aetiology of the colitis is never deter-
mined. These animals suffer from a protracted episodic
diarrhoea and weight loss, and eventually develop
chronic colitis. Although the animals may initially
respond to various antimicrobial agents, the diarrhoea
recurs and becomes chronic. In this regard the disease
is analogous to IBD in humans.

Although poorly defined, IBD is probably the result of
a combination of genetic and environmental factors.
Microbial flora are considered an important component
of the IBD process in man, in which microbial antigens
initiate and promote inflammation – particularly in the
presence of immune dysregulation or an impaired
mucosal barrier in the susceptible host [27–29].

With regard to a specific role for _Helicobacter_ spp. in
IBD, _H. hepaticus_, which induces a strong Th1
immune response, can cause IBD and hepatitis in
A/Jcr and recombinant AXB mice as well as IBD in
immune dysregulated rodents [10, 22, 30]. Although
the causative role of a novel _Helicobacter_ sp. isolated
from CTT with ulcerative colitis has not been estab-
lished, the systemic immune response to this helico-
bacter in infected monkeys is robust and appears to be
specific [2, 7]. Others investigating CTT ulcerative
colitis by electron microscopy have noted a bacterium
in affected colonic epithelia with the same morphology
as that of the novel _Helicobacter_ sp. isolated by
Saunders et al. [2, 31]; _H. cinaedi_ and _H. fennelliae_
have been associated with proctitis, colitis and diar-
rhoea in immunocompromised patients [12] and
experimental studies demonstrated that these organisms
can cause diarrhoea and bacteraemia in pig-tailed
macaques [13]. Co-infection of _Helicobacter_ sp. and
_Campylobacter_ sp. has also been recognised recently
in both man and animals [32, 33]. It is now known that
_C. jejuni_ and enterohepatic _Helicobacter_ spp. express a
cytolethal distending toxin [34, 35]. Whether this toxin
is in part responsible for tissue inflammation or
otherwise affects the host immune response requires
further investigation. Given these data, it is reasonable
to hypothesise that the novel _Helicobacter_ sp. isolated
from rhesus monkeys in this report may play a role in
the development and persistence of chronic colitis in
these animals. However, further studies are warranted
to elucidate the role of _Helicobacter_ sp. in the
development of idiopathic colitis in rhesus monkeys.

The clinical signs and pathologic descriptions of the
rhesus monkeys described in this report fulfill the
criteria for a well known idiopathic syndrome in
captive juvenile rhesus monkeys [1]. This chronic

crypts by other bacteria, or a subset derived from the
brush border flora, was also observed in the diarrhoeic
animals. It should be noted that the surface colonisa-
tion in the non-diarrhoeic animals was also associated
with a lower grade of colitis. In these animals, there
were small foci of necrosis in the superficial lamina
propria; however, there were limited epithelial altera-
tions, as manifested in the diarrhoeic animals. Thus,
both diarrhoeic and non-diarrhoeic monkeys had
chronic colitis as well as colonisation with _Helicobac-
ter_ sp. It is tempting to speculate that – like _H.
pylori_-induced gastritis where the majority of indivi-
duals with this infection are asymptomatic despite the
presence of chronic inflammation – lower bowel
helicobacter infection causes a chronic inflammation
but only occasionally does the infection elicit clinical
signs.

The clinical signs and pathological descriptions of the
rhesus monkeys described in this report fulfill the
criteria for a well known idiopathic syndrome in
captive juvenile rhesus monkeys [1]. This chronic

Fig. 6. (a) The non-diarrhoeic animals have prominent
lamina of colonising bacteria on the surface epithelium,
which extends into the superficial crypts. Warthin-Starry
staining, bar = 50 µm. Inset: long, slender, spiral bacteria
are visible at the deep margin of the colonies. Warthin-
Starry staining, bar = 3 µm. (b) Aggregates of short,
curved or spiral bacteria are also present in scattered
crypts of diarrhoeic animals. Warthin-Starry staining,
bar = 10 µm.
This work was supported in part by NIH grants R01 DK 52413, CA67529 in LCD and DE10374 to LC in captive bamboo dwarf giant and Walter Pouch for microbiology and parasitology support, and Edgar Brown and Marc Washington for haematological and serological analyses.

References