Enterohepatic *Helicobacter* species isolated from the ileum, liver and colon of a baboon with pancreatic islet amyloidosis

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Microaerobic bacteria were isolated from a baboon with pancreatic islet amyloidosis and hepatitis. Phenotypic and molecular analyses identified two distinct helicobacters. Analyses of 16S rRNA demonstrated ‘*Helicobacter macaceae*’ in the ileum and liver, and *Helicobacter cinaedi* in the colon. To the best of the authors’ knowledge, this is the first report describing the isolation of enterohepatic *Helicobacter* species from a baboon.

Introduction

Enterohepatic *Helicobacter* species (EHS) colonize the intestine and liver of humans and animals (Fox, 2002). *Helicobacter hepaticus*, the prototype EHS, induces chronic active hepatitis and hepatocellular carcinoma in genetically susceptible mouse strains (Fox et al., 1994; Ihrig et al., 1999). Selected EHS, but not *Helicobacter pylori*, play a role in the pathophysiology of cholesterol gallstone formation in C57L mice fed a lithogenic diet (Maurer et al., 2005, 2006). In addition, *Helicobacter* species DNA has been identified in bile and gall-bladder tissue of humans with chronic cholecystitis (Fox et al., 1998). In the present study, we describe the isolation of EHS from the ileum, liver and colon of a baboon with pancreatic islet amyloidosis and hepatitis.

Case report

An adult male baboon (*Papio anubis*) was euthanized due to a chronic wasting disease. Prior to euthanasia with an intravenous barbiturate, blood samples were collected for haematological analyses, and values were compared to clinical reference data for baboons (Hainsey et al., 1993). The complete blood count was within the normal range; however, the biochemical profile revealed hyperglycaemia (192 mg dl⁻¹; normal range 65–101 mg dl⁻¹), hypertriglyceridaemia (315 mg dl⁻¹; normal range 36–94 mg dl⁻¹), hyperlipasaemia (25 U l⁻¹; normal range 0–19 U l⁻¹) and hypoproteinaemia (5.7 mg dl⁻¹; normal range 6.3–7.9 mg dl⁻¹). On gross examination during necropsy, the liver appeared moderately enlarged. Representative samples were collected from various organs, fixed in 10 % neutral-buffered formalin, and processed for routine histopathological evaluation.

Microbiological and histological studies

Samples that had been collected from the ileum, caecum, colon, gall bladder, bile and liver, and placed in culture media containing 20 %, v/v, glycerol, with brucella broth, and frozen at −70 °C, were subsequently homogenized with PBS for microaerobic culture. Media used for culture included trypticase soy agar with 5 % sheep blood (BAP), medium impregnated with trimethoprim, vancomycin and polymyxin (TVP; Remel), and medium impregnated with ceftazidime, vancomycin and amphotericin B (CVA; Remel). In addition, selective antibiotic medium (ABM) contained the following components: blood agar base no. 2, 5 % horse blood (Remel), 50 μg amphotericin B ml⁻¹, 100 μg vancomycin ml⁻¹, 3-5 μg polymyxin B ml⁻¹, 200 μg bacitracin ml⁻¹ and 10-7 μg nalidixic acid ml⁻¹ (Sigma). A portion of each homogenized sample was applied directly to TVP, CVA, and ABM media, and filtered through a 0.45 μm pore-size filter and plated on BAP. Plates were incubated at 37 °C under microaerobic conditions for 2–4 weeks in vented jars containing N₂, H₂ and CO₂ (80:10:10). Bacterial isolates with characteristic growth on agar and microscopic morphology were subsequently used for biochemical analysis and to obtain genomic DNA for PCR, RFLP and 16S rRNA gene sequencing analyses.

Microaerobic bacteria were isolated from the ileum, liver and colon. The isolates grew at 37 °C under microaerobic conditions for 2–4 weeks in vented jars containing N₂, H₂ and CO₂ (80:10:10). Bacterial isolates with characteristic growth on agar and microscopic morphology were subsequently used for biochemical analysis and to obtain genomic DNA for PCR, RFLP and 16S rRNA gene sequencing analyses.

Microaerobic bacteria were isolated from the ileum, liver and colon. The isolates grew at 37 °C, at 42 °C, and in the presence of 1 % glycine. They were oxidase positive, weak catalase positive, urease negative, were unable to reduce nitrate, and were negative for alkaline phosphatase hydrolysis, indoxyl acetate hydrolysis and gamma glutamyl transpeptidase activity. The isolates from the ileum and liver were resistant to nalidixic acid (30 μg disk) but sensitive to cephalothin (30 μg), while the colon isolate was sensitive to nalidixic acid and resistant to cephalothin.

Abbreviation: EHS, enterohepatic *Helicobacter* species.
PCR was performed on individual bacterial isolates using *Helicobacter* genus-specific primers C97 and C05, as previously described (Fox et al., 1998). These primers were used to generate a 1200 bp PCR product of the 16S rRNA gene from *Helicobacter* species that was then subjected to RFLP (Fox et al., 1998). RFLP analysis revealed that ileal and hepatic isolates had identical banding patterns with *Alu* and *Hha*I restriction enzymes, but were distinct from the banding patterns of the colonic isolate. The RFLP patterns from the ileal and hepatic isolates were identical to the pattern of an isolate of 'Helicobacter macacae' (MIT 99-5504) obtained from the colon of a non-diarrheic rhesus monkey with chronic idiopathic colitis (Fox et al., 2001b). The RFLP pattern of the colonic isolate was identical to the pattern of an isolate of *Helicobacter cinaedi* (MIT 99-10781) obtained from the faeces of a rhesus monkey (J. G. Fox and others, unpublished results) (Fig. 1).

In order to determine the phylogenetic relatedness of the *Helicobacter* isolates, 16S rRNA sequencing and data analyses were performed as previously described (Fox et al., 2001b). The sequences of the 16S rRNA genes of the isolates from the ileum and liver were 99 % similar to that of 'H. macacae' (MIT 99-5501 and MIT 99-5504). The ileal isolate had an intervening sequence (IVS) that was not present in the liver isolate. The sequence of the 16S rRNA gene of the colonic isolate was 99 % similar to that of the Mainz strain (CCUG 33804) of *H. cinaedi* (Fig. 2).

Histological examination of the liver revealed that there was multifocal and mild portal infiltration by low to moderate numbers of lymphocytes and occasional macrophages (Fig. 3a). The hepatocytes were diffusely and moderately swollen with clear cytoplasm containing PAS-positive material (glycogen) (Fig. 3b). Pancreatic islets of Langerhans were diffusely effaced by deposits of pale eosinophilic hyaline extracellular material that demonstrated apple-green birefringence under polarized light after staining with Congo red (amyloid; Fig. 3c, d). In the caecum and colon (ascending, transverse and descending), there was mild to moderate separation of the crypts, and the lamina propria was mildly expanded by low numbers of lymphocytes, occasional plasma cells, macrophages, rare globular leukocytes and eosinophils (Fig. 3e, f). The inflammatory infiltrates in the lamina propria of the descending colon were slightly more prominent.

**Fig. 1.** PCR-RFLP patterns of the 16S rRNA gene of various *Helicobacter* isolates from monkeys. (a) DNA digested with *Alu*I; (b) DNA digested with *Hha*I. Lanes: M, 1 kb plus ladder; 1, MIT 99-5504 ('*H. macacae*') (Fox et al., 2001b); 2, MIT 03-7674, ileum (baboon); 3, MIT 03-7674, liver (baboon); 4, MIT 00-6197 (*H. cinaedi*), liver (rhesus monkey) (Fox et al., 2001a); 5, 03-7674, colon (baboon); 6, 99-10781, faeces (rhesus monkey).

**Fig. 2.** Phylogenetic tree constructed on the basis of 16S rRNA sequence similarity values. The scale bar is equal to a 3 % difference in nucleotide sequences as determined by measuring the lengths of the horizontal lines connecting two species. GenBank accession numbers are given in parentheses. Isolates from this study are shown in bold type.
Discussion

This report describes the isolation of EHS from the ileum, liver and colon of a baboon with pancreatic islet amyloidosis and hepatitis. Although a previous study has reported the histologic detection of Helicobacter-like organisms in the stomach of baboons with gastritis (Mackie & O’Rourke, 2003), to the best of our knowledge this is the first report of EHS being isolated from a baboon. ’H. macacae’ was isolated from the ileum and liver. ’H. macacae’ is a novel species that has been isolated from rhesus monkeys with and without chronic idiopathic colitis (Fox et al., 2001b; J. G. Fox and others, unpublished results). ’H. macacae’ (MIT 99-5504) has also been identified by PCR and sequence analysis in the stools of children with a history of gastroenteritis and with stool samples that were transiently positive for H. pylori antigen by ELISA (Haggerty et al., 2005).

H. cinaedi was isolated from the inflamed colon of the baboon. This strain was 99 % similar to the Mainz strain of H. cinaedi. The Mainz strain was originally isolated from the joint effusion of an AIDS patient with septic arthritis, and is 4 % distant from the H. cinaedi type strain (Dewhirst et al., 2005; Husmann et al., 1994). In humans, H. cinaedi is the most commonly reported EHS, and has been recovered from patients with diarrhoea, bacteraemia and other inflammatory conditions (Simons et al., 2004; Taylor et al., 2003). In a survey ascertaining the prevalence of enteric helicobacters using PCR-denaturing gradient gel electrophoresis (DGGE), in faecal samples from zoo animals, H. cinaedi was detected in a captive baboon (Al-Soud et al., 2003). H. cinaedi has also been isolated from the colon, liver and mesenteric lymph node of a rhesus monkey with chronic colitis and hepatitis (Fox et al., 2001a). H. cinaedi frequently colonizes captive rhesus monkeys without overt diarrhoea (Fernandez et al., 2002). Rhesus monkeys, in a similar manner to hamsters as well as other animals, could serve as potential reservoir hosts for human infection (Fernandez et al., 2002; Gebhart et al., 1989; Vandamme et al., 2000). Captive cotton-top tamarins (CTTs) with endemic colitis are also colonized with a novel intestinal Helicobacter species (Saunders et al., 1999). EHS have also been identified in human patients with inflammatory bowel disease (Bohr et al., 2004).

The accumulation of glycogen in the liver of the baboon, as well as the lymphohistiocytic portal lesions, suggested diabetes with hepatic involvement (Stone & Van Thiel, 1985). However, further clinical pathological evidence was not available to support a definitive diagnosis of chronic
hyperglycaemia and diabetes. The hepatitis and typhlocolitis were consistent with the lesions observed in rhesus monkeys infected with EHS (Fox et al., 2001a,b). The islet amyloid is a pathological feature in human type 2 diabetes that has also been identified in baboons with and without hyperglycaemia, as well as in other species of non-human primates (Hubbard et al., 2002; Kahn et al., 1999; Wagner et al., 2006). Interestingly, nine of 40 (22.5%) baboons with pancreatic islet amyloidosis had concurrent diarrhoea (Hubbard et al., 2002). In addition, the probable cause of death in 14 of 40 (35%) baboons with pancreatic islet amyloidosis was diarrhoea, colitis or typhlitis (Hubbard et al., 2002).

The occurrence of pancreatic islet amyloidosis in the baboon may be unrelated to the infection with EHS. However, it is interesting to note that in humans, the association between Helicobacter pylori infection and type 2 diabetes remains controversial (Dore et al., 2000; Gillum, 2004; Gulcelik et al., 2005; Ko et al., 2001; Quadri et al., 2000). There is additional evidence for the occurrence of pancreatic disease in the context of Helicobacter pylori infection (Manes et al., 2003). Furthermore, in a study of Syrian hamsters, isolation of Helicobacter cholecystus correlated strongly with the presence of cholangiobiosis and centrolobular pancreatitis (Franklin et al., 1996). The authors hypothesized that the pathological lesions in these hamsters could have been the result of an ascending infection of the common duct joining the bile and pancreatic ducts (Franklin et al., 1996).

Amyloid deposits occur in association with several distinct diseases and can be classified into two general forms, systemic and localized (Hull et al., 2004). Secondary amyloidosis is systemic and associated with chronic inflammatory disease. On the other hand, in type 2 diabetes, the amyloidosis is localized and islet amyloid polypeptide or amylin is deposited in the pancreatic islets (Hull et al., 2004). Amylin may be induced by oxidative stress, and once formed may be responsible for beta cell death through the process of oxidative stress (Hayden & Tyagi, 2002; Schubert et al., 1995). Although we only observed inflammation in the intestine and liver, and not in the pancreas of the baboon, it is conceivable that increased oxidative damage produced as a result of Helicobacter species infection may have directly or indirectly affected the pancreas (Sipowicz et al., 1997). Future experiments designed to investigate the pathogenic role of EHS in metabolic diseases are warranted.

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References


