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Helicobacter Species in the Intestinal Mucosa of Patients with Ulcerative Colitis

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In a search for *Helicobacter* species in the intestinal mucosae of 42 patients with ulcerative colitis (UC) and 74 without UC, only *H. pylori* was found. Although the bacterium was detected in UC patients by culture (7.1%) and nested PCR (19.0%), its presence was not associated with the disease ($P = 0.13$).

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) of unknown etiology that is confined to the large bowel mucosa. It seems to be a multifactorial disorder involving both genetic and environmental components, particularly the bacterial gut microbiota (13). However, no specific pathogenic or commensal bacterium has been convincingly implicated as the etiologic agent (4). Numerous studies have demonstrated that indigenous *Helicobacter* species of the gut of mice—*Helicobacter hepaticus* and *Helicobacter bilis*—are able to induce a persistent inflammation of the colon and cecum in some immunocompromised mouse models (1, 5, 8, 15). Moreover, other *Helicobacter* species have been isolated from the gut of cottontop tamarins (14) and rhesus monkeys (7) with IBD-like disease. We hypothesize that species of the genus *Helicobacter* may also be associated with the pathogenesis of UC in human beings. Therefore, we investigated the presence of *Helicobacter* species in the intestinal mucosa of patients with UC and controls, by culture and PCR.

We studied prospectively 42 patients with UC and 74 without IBD (controls) who were undergoing colonoscopy. Prior consent of all patients and approval of the institutional ethics committee were obtained. The UC diagnosis was established by standard clinic, radiologic, endoscopic, and histologic criteria. The extent of ulcerative colitis was determined by histology. The gastric *H. pylori* status was assessed by serology (Cobas-Core EIA; Roche Diagnostic Systems, Basel, Switzerland) (12) and ^{13}C urea breath test (^{13}C-UBT; NDIRIS; Wagner Analysen Technik, Bremen, Germany) (13). Patients' characteristics, including gastric *H. pylori* status, are shown in Table 1.

Colonoscopic biopsy fragments were taken from the rectum, the sigmoid, descending, transverse, and ascending colon, the cecum, and the terminal ileum of each patient for culture and PCR. For culture, one fragment from each region was ground separately in a glass tissue grinder and plated onto Belo Horizonte medium (11) supplemented with polymyxin B plus bacitracin (Sigma Chemical Co., St. Louis, Mo.). The culture conditions and detailed characterization of the isolates were as previously described (10).

DNA from bacteria and mucosal fragments was extracted with a QIAamp DNA minikit (Qiagen GmbH, Hilden, Germany).

Primers C70 and B37 were used to amplify a product of ~1.5 kb from the 16S rRNA gene of the isolated strains (10). The amplicons were then purified and directly sequenced as described by Queiroz et al. (10). The sequences were aligned and compared with those in the GenBank database.

For the detection of *Helicobacter* DNA in fragments of the intestinal mucosa, the 16S rRNA gene was amplified by nested PCR using an outer primer pair (C70 and C37) and two inner primer pairs for the genus *Helicobacter* (C97 and C98) and the species *H. pylori* (HP1 and HP2) (3, 6, 10). Another nested PCR for *ureA* specific to *H. pylori* was also used (16) (Table 2).

We used the two-tailed chi-square or Fisher exact test for nonparametric values and Student's *t* test for comparison of mean age and disease duration. The significance level was set at a *P* value of ≤ 0.05.

Gastric *H. pylori* infection was detected in 53.7% of the UC patients and in 52.1% of the controls, with no significant difference between the groups ($P = 1.0$). This result agrees with those obtained by Duggan et al. (2) and Piodi et al. (9).

Helicobacter strains were isolated from the intestinal mucosa throughout the colon in three (7.1%) patients with and one (1.4%) without UC, with no significant difference ($P = 0.13$). The isolates were gram negative and motile and had a slightly spiral appearance. All strains showed the same biochemical characteristics and were urease, catalase, oxidase, alkaline phosphatase, and γ-glutamyl transpeptidase positive but did not reduce nitrate or hydrolyze hippurate. They did not produce hydrogen sulfide and were resistant to nalidixic acid and sensitive to cephalothin. More than 96% of the complete 16S rRNA gene sequence was determined for all isolates. Comparison of consensus sequences showed >99% similarity to *H. pylori*.

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TABLE 1. Characteristics of patients with UC and controls (without IBD)

Characteristic	Ulcerative colitis patients	Non-IBD controls
No. of patients	42	74
Mean age (yr) ± SD (range)	38.9 ± 14.7 (18–76)	49.4 ± 4.1 (18–79)
No. of males/no. of females	6/36	20/54
No. of *H. pylori*-positive patients[a]	22	38
No. with diarrhea	30	22
Mean duration (yr)	2.7	
No. with activity	30	
No. with distal disease/no. with extensive disease	31/11	
No. using sulfasalazine/no. using corticosteroid	10/15	

[a] One patient with UC and one non-IBD control were excluded from *H. pylori* status analysis because the values of enzyme-linked immunosorbent assay and ^{13}C-UBT were questionable.

Helicobacter DNA was detected by PCR in the intestinal mucosa of eight (19.0%) UC patients and seven (9.5%) controls. Although the sensitivity of the PCR assay was higher than that of the culture, no association was detected between the presence of *Helicobacter* in the intestine of controls and UC patients ($P = 0.23$). All DNA detected by specific 16S rRNA/*ureA* nested PCR belonged to *H. pylori* species.

All patients from whom *Helicobacter* strains were isolated or in whom *Helicobacter* DNA was detected in the intestinal mucosa were *H. pylori* positive, as assessed by ^{13}C-UBT and serology.

Occurrence of diarrhea was not associated with *Helicobacter* isolation in UC patients ($P = 1.0$) or in controls ($P = 1.0$). Also, no association was found between *H. pylori* detection and activity ($P = 0.19$), extent ($P = 0.32$), or duration ($P = 0.86$) of UC, and treatment with sulfasalazine ($P = 0.56$) or corticosteroids ($P = 0.29$).

TABLE 3. GenBank accession numbers of the *H. pylori* strains isolated from the intestinal mucosa of patients with ulcerative colitis and controls (without IBD)

Strain	Disease	No. of bp sequenced	Accession no.
LPB 582-99	UC	1,482	AY364437
LPB 473-00	UC	1,472	AY364438
LPB 427-01	Non-IBD control	1,478	AY364439
LPB 10-02	UC	1,471	AY364440

All *Helicobacter* strains isolated belonged to *H. pylori*, although the culture conditions adopted allow the isolation of most *Helicobacter* species, including those that have been recovered from the intestines of rodents and nonhuman primates with IBD. This result indicates that other cultivable *Helicobacter* did not colonize the lower gastrointestinal tracts of the patients and controls. Even considering that other *Helicobacter* species could be missed by culture, because they are fastidious, our hypothesis is reinforced by the concordant data obtained by a more sensitive method, the nested PCR assay, that is able to detect one copy of *Helicobacter* DNA fragment, equivalent to 10^{-3} fg (16). Again, the only *Helicobacter* species detected by this method was *H. pylori*. However, neither the presence of viable *H. pylori* in the intestine, detected by culture, nor the presence of *H. pylori* DNA, detected by nested PCR, showed significant association with UC.

In conclusion, although we cultured *H. pylori* from the intestinal mucosae of a small number of UC patients, its presence was not associated with the disease. Since no other *Helicobacter* species was detected in the intestinal mucosa of the patients, we may speculate that the *Helicobacter* genus is not involved in the genesis of UC.

Nucleotide sequence accession number. The sequences obtained in this study were deposited in GenBank (Table 3).

TABLE 2. Oligonucleotide primers used for PCR amplification of 16S rRNA and urease A genes

Primer and sequence (5′-3′)[a]	Amplicon size (bp)	PCR conditions
16S rRNA		
Outer primers		
C70: AGAGTTTGATYMTGGC B37: TACGGYTACCTTGTTACGA	1,500	94°C (45 s), 50°C (45 s), 72°C (45 s), with 5 s per cycle; 24 cycles
Inner primers		
C97: GCTATGACGGGTATCC C98: GATTTTACCCCTACACCA	400	94°C (1 min), 55°C (2 min), 72°C (3 min); 34 cycles
HP1: TGGCAATCAGCGTCAGGTAATG HP2: GCTAAGAGATCAGCCTATGTCC	500	94°C (1 min), 55°C (1 min), 72°C (3 min); 39 cycles
Urease A		
Outer primers		
HPU1: GCCAATGGTAAATTAGTT HPU2: CTCCTTAATTGTTTTTAC	411	94°C (1 min), 45°C (1 min), 72°C (1 min); 35 cycles
Inner primers		
AGTTCCTGGTGAGTTGTTCT AGCGCCATGAAAACCACGCT	361	96°C (30 s), 56°C (15 s), 74°C (30 s); 40 cycles

[a] Base designations are standard International Union of Biochemistry designations for bases and ambiguity.

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REFERENCES

1. **Cahill, R. J., C. J. Foltz, J. G. Fox, C. A. Dangler, F. Powrie, and D. B. Schauer.** 1997. Inflammatory bowel disease: an immune mediated condition triggered by bacterial infection with *Helicobacter hepaticus*. Infect. Immun. **65:**3126–3133.
2. **Duggan, A. E., I. Usmani, K. R. Neal, and R. F. A. Logan.** 1998. Appendicectomy, childhood hygiene, Helicobacter pylori status, and risk of inflammatory bowel disease: a case control study. Gut **43:**494–498.
3. **Engstrand, L., A. M. H. Nguyen, D. Y. Graham, and F. A. K. El-Zaatari.** 1992. Reverse transcription and polymerase chain reaction amplification of rRNA for detection of *Helicobacter* species. J. Clin. Microbiol. **30:**2295–2301.
4. **Farrel, R. J., and J. T. La Mont.** 2002. Microbial factors in inflammatory bowel disease. Gastroenterol. Clin. N. Am. **31:**41–62.
5. **Foltz, C. J., J. G. Fox, R. J. Cahill, R. C. Murphy, L. Yan, B. Shames, and D. B. Shauer.** 1998. Spontaneous inflammatory bowel disease in multiple mutant mouse lines: association with colonization by *Helicobacter hepaticus*. Helicobacter **3:**69–78.
6. **Fox, J. G., F. E. Dewhirst, Z. Shen, Y. Feng, N. S. Taylor, B. J. Paster, R. L. Ericson, C. N. Lau, P. Correa, J. C. Araya, and I. Roa.** 1998. Hepatic *Helicobacter* species identified in bile and gallbladder tissue from Chileans with chronic cholecystitis. Gastroenterology **114:**755–763.
7. **Fox, J. G., L. Handt, S. Xu, Z. Shen, F. E. Dewhirst, B. J. Paster, C. A. Dangler, K. Lodge, S. Motzel, and H. Klein.** 2001. Novel *Helicobacter* species isolated from rhesus monkeys with chronic idiopathic colitis. J. Med. Microbiol. **50:**421–429.
8. **Kullberg, M. C., J. M. Ward, P. L. Gorelick, P. Caspar, S. Hieny, A. Cheever, D. Jankovic, and A. Sher.** 1998. *Helicobacter hepaticus* triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12- and gamma interferon-dependent mechanism. Infect. Immun. **66:**5157–5166.
9. **Piodi, L. P., M. T. Bardella, C. Rocchia, B. M. Cesana, A. Baldassarri, and M. Quatrini.** 2003. Possible protective effect of 5-aminosalicylic acid on *Helicobacter pylori* infection in patients with inflammatory bowel disease. J. Clin. Gastroenterol. **36:**22–25.
10. **Queiroz, D. M. M., A. Santos, A. G. Oliveira, G. A. Rocha, S. B. Moura, E. R. S. Camargo, P. R. Valle, L. A. F. Bicalho, and R. Dani.** 2002. 2001. Isolation of a *Helicobacter* strain from the human liver. Gastroenterology **121:**1023–1028. (Erratum, **122:**250.)
11. **Queiroz, D. M. M., E. M. Mendes, and G. A. Rocha.** 1987. Indicator medium for isolation of *Campylobacter pylori*. J. Clin. Microbiol. **25:**378–2379.
12. **Rocha, G. A., A. M. R. Oliveira, D. M. M. Queiroz, E. N. Mendes, S. B. Moura, C. A. Oliveira, and T. C. A. Ferrari.** 1998. Serodiagnosis of *H. pylori* infection by Cobas Core ELISA in adults from Minas Gerais, Brazil. Braz. J. Med. Biol. Res. **31:**1263–1268.
13. **Sartor, R. B.** 1995. Current concepts of the etiology and pathogenesis of ulcerative colitis and Crohn's disease. Gastroenterol. Clin. N. Am. **24:**475–507.
14. **Saunders, K. E., Z. Shen, F. E. Dewhirst, B. J. Paster, C. A. Dangler, and J. G. Fox.** 1999. Novel intestinal *Helicobacter* species isolated from cotton-top tamarins (*Saguinus oedipus*) with chronic colitis. J. Clin. Microbiol. **37:**146–151.
15. **Shomer, N. H., C. A. Dangler, M. D. Schrenzel, and J. G. Fox.** 1997. *Helicobacter bilis* induced inflammatory bowel disease in *scid* mice with defined flora. Infect. Immun. **65:**4858–4864.
16. **Wang, J. T., J. T. Lin, J. C. Sheu, J. C. Yang, D. S. Chen, and T. H. Wang.** 1993. Detection of *Helicobacter pylori* in gastric biopsy tissue by polymerase chain reaction. Eur. J. Clin. Microbiol. Infect. Dis. **1:**367–371.