Detection and Isolation of *Campylobacter* Species Other than *C. jejuni* from Children with Crohn's Disease[▽]

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The presence of *Campylobacter* species other than *Campylobacter jejuni* and antibodies to *Campylobacter concisus* in children were investigated. A significantly greater presence of *C. concisus* and higher levels of antibodies to *C. concisus* were detected in children with Crohn's disease (CD) than in controls. *Campylobacter* species other than *C. jejuni* were isolated from intestinal biopsy specimens of children with CD.

Inflammatory bowel diseases (IBD), which include Crohn's disease (CD) and ulcerative colitis (UC), are chronic relapsing idiopathic inflammatory diseases of the gastrointestinal tract (12). Strong evidence from both animal and human studies indicates that a bacterial component is involved in the pathogenesis of IBD (2, 4, 13–16, 18). Despite a number of putative causative agents being proposed, the exact organism(s) that causes human IBD remains unknown (9, 21). *Campylobacter* species other than *Campylobacter jejuni* have recently gained considerable attention as emerging human intestinal pathogens; however, little is known regarding their role in human IBD.

In this study, we investigated, using molecular and cultural methods, the presence of *Campylobacter* species other than *C. jejuni* in intestinal biopsy specimens of children with CD and controls. Antibodies specific to *Campylobacter concisus* were also examined.

Intestinal biopsy specimens were collected from 85 children (51 males; ages, 2 to 16 years) undergoing diagnostic colonoscopy. In children with endoscopically normal mucosae, three biopsy specimens were collected from the cecum, and in those with endoscopic abnormalities, three biopsy specimens were collected from the inflamed region. To assess the potential effect of inflammation upon the detection of *Campylobacter* species, in 13 children, three additional biopsy specimens from endoscopically normal areas near the inflamed region were collected. Following collection, DNA was extracted from one biopsy specimen (Gentra Systems, Minneapolis, MN) and the further two biopsy specimens were used for bacterial cultivation and histological examination. Diagnosis of CD was based upon standard endoscopic, histologic, and radiologic investigations (5). On the basis of their diagnosis, patients were grouped into a CD group ($n = 33$) and a control group ($n = 52$).

The 16S rRNA gene of *Campylobacter* species was amplified from DNA using a previously described *Campylobacter* PCR (7, 8a) with the following modifications: 400 ng of DNA was used in a 50- μ l PCR mixture, and the number of thermal cycles was 40. PCR products (15 μ l) were examined on agarose gels. All PCR products were sequenced, and the sequences obtained were compared to gene sequences of known identities using the BLAST search program (<http://www.ncbi.nlm.nih.gov>). Five samples whose sequencing results revealed mixed sequences were subjected to a *C. concisus*-specific PCR (1). This *C. concisus*-specific PCR was initially designed to group *Campylobacter* species into two genotypes; however, in this study, a sample positive for either of the genotypes was considered positive for *C. concisus*.

Biopsy specimens were cultured on agar plates prepared using blood agar base no. 2 supplemented with 6% sterile defibrinated horse blood, trimethoprim (10 μ g/ml), and vancomycin (10 μ g/ml) (Oxoid Limited, Hampshire, United Kingdom) and incubated under microaerophilic conditions generated by a *Campylobacter* gas generating system (Fisher Scientific catalog no. BR0056A; Oxoid). Colonies were identified using the Oxoid biochemical identification system and sequencing of the nearly complete 16S rRNA gene (7, 8a).

Antibodies specific to *C. concisus* were determined in sera available from 8 CD and 12 control children using a previously described enzyme-linked immunosorbent assay (22). A whole-cell lysate of the *C. concisus* isolated in this study was used as the antigen.

The *Campylobacter* PCR positivity rate for children with CD (82%) was significantly higher than that for controls (23%) ($P < 0.001$, Fisher's exact test) (17). Sequencing of PCR products revealed these to be similar to those of various *Campylobacter* species (Table 1). *C. concisus* detection was significantly higher in children with CD (51%) than in controls (2%) ($P < 0.0001$). The prevalence of other *Campylobacter* species in CD children was not significantly different from that in controls ($P > 0.05$).

In the 13 CD children from whom two sets of biopsy spec-

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TABLE 1. Detection by PCR sequencing of *Campylobacter* species in intestinal biopsy samples of children with CD and controls^a

Subject group	No. of PCR products	Sequence length (bp)	Organism (% similarity to organism) ^b
CD patients	17	610–805	<i>C. concisus</i> (99–100)
	3	770–794	<i>C. showae</i> (98–99)
	2	767–785	<i>C. hominis</i> (99–100)
	2	703–709	<i>Campylobacter gracilis</i> (99)
	1	751	<i>Campylobacter rectus</i> (99)
	1	786	<i>C. jejuni</i> (100)
	1	764	<i>B. ureolyticus</i> (100)
Controls	1	802	<i>C. concisus</i> (99)
	2	744–776	<i>C. hominis</i> (99–100)
	2	733–751	<i>C. rectus</i> (99)
	3	758–791	<i>C. jejuni</i> (99–100)
	2	734–786	<i>B. ureolyticus</i> (100)
	2	— ^c	NA

^a Twenty-seven of 33 patients with CD and 12 of 52 controls were positive for *Campylobacter* by PCR. In one CD patient, *C. hominis* and *C. showae* were detected from the two biopsy specimens collected from macroscopically inflamed and noninflamed areas, respectively.

^b The taxonomic position of *B. ureolyticus* is uncertain, and this species is being considered for inclusion in the *Campylobacter* genus (19). NA, not available.

^c —, mixed sequences.

imens were collected, 76% of the noninflamed tissues were *Campylobacter* PCR positive, compared with 46% of the inflamed tissues from the same patients. This difference was not significant ($P > 0.05$).

Four different *Campylobacter* species were isolated from each of four CD patients. Based on sequencing of the nearly complete 16S rRNA gene, these isolates were shown to be similar to *C. concisus* (99% similarity), *Campylobacter hominis* (100% similarity), *Campylobacter showae* (99% similarity), and *Bacteroides ureolyticus* (99% similarity). For each patient, the *Campylobacter* species isolated was the same as that detected by *Campylobacter* PCR. Biochemical testing showed all four isolates to be negative for L-alanyl aminopeptidase (Oxoid biochemical identification system) and catalase activity, one of four (*B. ureolyticus*) to be urease positive, and two of four (*C. hominis* and *B. ureolyticus*) to be oxidase positive.

The level of immunoglobulin G (IgG) antibodies (optical density at 405 nm) specific to *C. concisus* was 0.991 ± 0.447 (mean $\pm$ standard deviation) in children with CD, which was significantly higher than that in the controls (0.329 ± 0.303 , $P < 0.001$, unpaired t test) (17).

In this study, a significantly higher prevalence of *C. concisus* DNA and a higher level of *C. concisus*-specific IgG antibodies were detected in children with CD than in controls. Although the exact clinical relevance of *C. concisus* remains undetermined, a recent review by Newell reported this bacterium to be an emerging human pathogen of intestinal infectious diseases (10). The facts that *C. concisus* has been shown to have the potential to damage epithelial integrity (3, 6) and that the immune response detected in this study to *C. concisus* in CD children was greater than in controls suggest that *C. concisus* may contribute to CD pathogenesis.

The finding that the *Campylobacter* PCR positivity rate for endoscopically normal biopsy specimens was higher (76%), although not significantly higher, than for biopsy specimens

from inflamed areas (46%) may relate to the fact the mucous layer may be depleted in inflamed areas, thus, potentially reducing the bacterial load in these areas (20). In future studies to detect mucus-associated bacteria, such as *Campylobacter* species, collection of biopsy samples from the edges of the inflamed areas where the mucus is more likely to be intact may prove more useful.

In addition to detecting *Campylobacter* species other than *C. jejuni* using PCR sequencing, we also isolated four *Campylobacter* species other than *C. jejuni*, including *C. concisus*, *C. showae*, *B. ureolyticus*, and *C. hominis*, from four different children with CD. To our knowledge, this represents the first report of successful isolation of these organisms from CD patients.

The low isolation rate of *Campylobacter* species other than *C. jejuni* in this study may relate to the fact that prior to colonoscopy, bowel preparation using osmotic laxatives is required. Indeed, a study of rodents has shown that induced diarrhea removes the majority of spiral bacteria (*Campylobacter* species are spiral or curved) from the intestinal mucous layer and crypts (11). Furthermore, osmotic changes due to bowel preparation may affect the viability of *Campylobacter* species. Given this, in future studies, we plan to also include fecal samples collected prior to bowel preparation to culture *Campylobacter* species (8).

In conclusion, in a newly diagnosed pediatric population, we have demonstrated for the first time a significantly higher presence of *C. concisus* and significantly higher levels of IgG antibodies specific to *C. concisus* in children with CD than in controls. The isolation of a range of *Campylobacter* species other than *C. jejuni*, including *C. concisus*, from children with CD not only represents the first reported isolation of these bacteria from intestinal tissue of humans with CD but also provides important evidence that in the early stages of disease *Campylobacter* species other than *C. jejuni* are viable in the intestinal tracts of children with CD. Further studies of the possible role of *Campylobacter* species other than *C. jejuni*, particularly *C. concisus*, in the pathogenesis of human IBD are clearly warranted.

Nucleotide sequence accession numbers. All sequences of PCR products and the nearly complete 16S rRNA gene sequences of the four *Campylobacter* isolates have been submitted to GenBank under accession no. EU781595 to EU781631.

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