Isolation of a Campylobacter-Like Organism from Healthy Syrian Hamsters (Mesocricetus auratus)

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Received 17 April 1989/Accepted 19 July 1989

A Campylobacter-like organism was isolated from the ilea of normal hamsters. The organism was isolated from an ileal homogenate which was passed through a filter (0.65-μm pore size) and cultured on blood-agar plates in a microaerophilic atmosphere at 37°C. Pinpoint translucent colonies were first observed after 120 h of incubation. The isolated organism measured 2.0 to 3.5 μm in length (excluding flagella) by 0.17 to 0.25 μm in width and typically had a single terminal sheathed flagellum. The organism was oxidase, catalase, and urease positive, reduced nitrates, and was susceptible to nalidixic acid (30-μg disk) and resistant to cephlothin (30-μg disk). Unlike Campylobacter pylori subsp. mustelae, this organism did not hydrolyze indoxylacetate. Immunofluorescence studies with a Campylobacter species-specific monoclonal antibody (8322-2E6) revealed the presence of numerous positively stained organisms within the crypt epithelial cells of the hamsters from which this organism was isolated. The role of this organism in the pathogenesis of proliferative ileitis in hamsters is uncertain, as is the taxonomic relationship of this organism to other members of the genus Campylobacter.

Proliferative ileitis is a serious disease of Syrian hamsters (Mesocricetus auratus) which is characterized by mucosal hyperplasia and pyogranulomatous inflammation of the ileum. Members of the genus Campylobacter, especially Campylobacter jejuni, have been implicated as the etiologic agents of proliferative ileitis. C. jejuni has been repeatedly isolated from hamsters with both naturally occurring and experimentally induced proliferative ileitis (3, 8, 9). Light and electron microscopic studies of ileal sections from hamsters with proliferative ileitis (4, 7, 17) have also implicated members of the genus Campylobacter. Recent studies, however, have suggested that C. jejuni is only minimally involved in the pathogenesis of proliferative ileitis and that other members of the genus Campylobacter may be involved (15, 16).

The inability to derive colonies of Syrian hamsters by cesarian section and produce nongenetic animals has hampered investigations of proliferative ileitis. Microbiologic culture techniques have so far been unable to differentiate the suspected Campylobacter-like etiologic agent of proliferative ileitis from normal flora. Only Campylobacter hyointestinalis (5) and C. jejuni (3, 8, 9) have been isolated from the intestines of hamsters. Oral inoculation of young hamsters with C. hyointestinalis, C. jejuni, C. coli, and C. mucosalis has been uniformly unsuccessful in reproducing proliferative ileitis (12).

We have previously reported the presence of intracellular Campylobacter-like organisms in normal hamsters (15, 16). These organisms were demonstrated during immunofluorescence studies with a monoclonal antibody that has consistently recognized all Campylobacter species tested. Microbiologic culture techniques failed to isolate any Campylobacter-like organism in either study.

In this paper we report a partial morphologic and biochemical characterization of a Campylobacter-like organism isolated from the ilea of normal hamsters.

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MATERIALS AND METHODS

Animals. Fifteen 28- to 30-day-old Syrian hamsters (CR: RGH) were obtained from a large commercial supplier's barrier colony. This large barrier colony had been repeatedly surveyed and found to be free of both C. jejuni and proliferative ileitis by repeated ileal cultures and necropsies.

The hamsters were housed individually in polycarbonate cages with autoclaved bedding for 20 days prior to sacrifice. During this period, the animals were provided with a commercial laboratory rodent chow (RMH 3000; Agway, Inc., Syracuse, N.Y.) and autoclaved water ad libitum. No experimental manipulations were performed during this period.

Media. Campylobacter blood-agar plates (CBAP; Blaser's formula), rapid urease test reagent, and the rapid hippurate test were purchased from Remel, Lenexa, Kans. Brain-heart infusion broth with 0.16% agar (BHI broth) and BHI broth with 1.5% NaCl, 3.5% NaCl, 1% glycine, 1% KNO3, 0.02% cysteine hydrochloride, and 1% glucose with 0.002% phenol red were prepared from commercially obtained reagents (BBL Microbiology Systems, Cockeysville, Md.). Indoxyl-acetate disks were commercially purchased (American Type Culture Collection, Rockville, Md.) and used following the manufacturer's directions.

Monoclonal antibody production. The production and characterization of the anti-CAMPYLOBACTER genus-specific monoclonal antibody 8322-2E6 and anti-C. jejuni monoclonal antibody 841-2A11 have been reported previously (16). Monoclonal antibodies for immunohistochemistry were purified from hybridoma culture supernatant by ammonium sulfate precipitation followed by protein A-Sepharose affinity chromatography as described previously (15, 16).

Necropsy and tissue processing. Hamsters were killed in a CO2-flooded chamber at 48 to 50 days of age. The terminal 3 cm of the ileum was aseptically removed and divided into two equal portions. One portion was used in the microbiologic isolation procedures, while the other section was further divided into two sections for histopathology. Tissue for light microscopy was fixed in McDowell-Trump fixative.
(11) and routinely processed, embedded in paraffin, cut at 4 μm, and stained with hematoxylin and eosin and with Warthin-Starry silver stains (10) prior to microscopic evaluation. The other section of tissue, for immunofluorescence staining, was fixed in 95% cold ethanol and processed by the cold-ethanol method (13).

**Immunofluorescence staining.** Sections for immunofluorescence evaluation were stained by the streptavidin-biotin method as described previously (15). Hydrated sections (4 μm) were placed in 0.1 M glycine hydrochloride buffer, pH 2.5, for 5 min, washed in three changes of phosphate-buffered saline (PBS) (10 min each), and incubated for 20 min in PBS containing 0.02% (vol/vol) Tween 20 (PBS-Tween 20) and 0.5% (wt/vol) nonfat powdered milk. The sections were covered with purified monoclonal antibody appropriately diluted in PBS-Tween 20 and 0.5% nonfat dry milk and incubated in a humidified chamber overnight at 4°C. After incubation, the sections were washed in three changes (10 min each) of PBS-Tween 20, covered with affinity-purified streptavidin–caproyl–anti-mouse immunoglobulin G (Biomedical Corp., Foster City, Calif.) appropriately diluted in PBS-Tween 20, and incubated in a humidified chamber overnight at 4°C. Following incubation, the sections were again washed in changes of PBS-Tween 20, covered with fluorescein isothiocyanate-labeled streptavidin (Biomedical Corp.) appropriately diluted in PBS, and incubated at room temperature in a humidified chamber for 3 h. After three washes in PBS, the sections were mounted in an aqueous mounting medium (Biomedical Corp.) and examined with an Olympus BHS epifluorescence microscope equipped with a 100-W high-pressure mercury light source and the appropriate fluorescein isothiocyanate filters. Bacterial smears and similarly processed sections from hamsters with documented proliferative ileitis were simultaneously stained and examined as controls.

**Microbiologic procedures.** The ileum of each hamster was individually cultured for the presence of *Campylobacter*-like species within 1 h of removal. The section of each ileum for microbiologic culture was homogenized in a 1:5 dilution (wt/vol) of BHI broth in a Potter-Elvehjem tissue grinder. Two CBAP were inoculated with 250 μl of homogenate from each animal and incubated at 37 and 43°C in an atmosphere consisting of approximately 6% O₂, 7% CO₂, 7% H₂, and 80% N₂, as described previously (6). The remaining homogenate was filtered through a nitrocellulose filter (0.65-μm pore size; Whatman Co., Clifton, N.J.), and 250 μl of the resulting filtrate from each animal was inoculated onto two CBAP and two BAP which were incubated at 37 and 43°C in the atmosphere listed above. Additional CBAP and BAP were inoculated with *C. jejuni* ATCC 29428 and incubated with all test cultures as positive controls. Plates were examined daily for 10 days for bacterial colonies of gram-negative organisms with *Campylobacter*-like morphology.

Isolates with *Campylobacter*-like morphology were further identified by standard *Campylobacter* microbiologic techniques (14, 16), including oxidation reaction, catalase reaction, malidic acid (30 μg) and cephalothin (30 μg) susceptibility, and growth in BHI broth and BHI broth with 1.5% NaCl, 3.5% NaCl, and 1% glycine. Additional tests included glucose utilization, urease production, hippurate hydrolysis, indoxylacetate hydrolysis, and hydrogen sulfide production in 0.02% cysteine hydrochloride-supplemented BHI broth with a lead acetate-impregnated strip. Control organisms included *C. jejuni* ATCC 29428, *C. pylori* ATCC 43504, and *C. pylori* subsp. *mustelae* ATCC 43772.

**ELISA technique.** Isolates compatible with members of the genus *Campylobacter* were tested for reactivity with the anti-*Campylobacter* genus-specific monoclonal antibody 8322-2E6 and the anti-*C. jejuni* monoclonal antibody 841-2A11. Bacterial isolates were suspended in 2% Formalin in PBS for 4 h before application to 96-well assay plates (Falcon 3912; Becton Dickinson Labware, Oxnard, Calif.) at 50 μl per well. Plates were air-dried at 37°C and incubated for 1 h at room temperature with 100 μl of PBS containing 0.10% (vol/vol) Tween 20 and 2.5% (wt/vol) nonfat powdered milk. The wells were washed once with enzyme-linked immunosorbent assay (ELISA) wash fluid (PBS containing 0.05% [vol/vol] Tween 20) and incubated with 50 μl of the appropriate hybridoma culture supernatant for 1 h at room temperature. After being washed, the wells were incubated with peroxidase-labeled goat anti-mouse immunoglobulin G (Coo- per Biomedical, Inc., West Chester, Pa.) for 1 h at room temperature. After the wells were washed, bound antibody was detected by the addition of 50 μl of 2,2’-azino-bis(3-ethylbenzthiazoline sulfonic acid) substrate (Sigma Chemical Co., St. Louis, Mo.) for 15 min, and the A405 was determined with an automated ELISA reader (Bio-Tek Instruments, Winooski, Vt.).

**Transmission electron microscopy.** Isolates compatible with members of the genus *Campylobacter* were further characterized by electron microscopy. Bacterial cultures were scraped from BAP, suspended in PBS (0.1 M, pH 7.4), and centrifuged at 11,000 × g. The bacterial pellet was resuspended in PBS, and 10% phosphate-buffered Formalin was added to a final concentration of 1% Formalin. After 24 h at 4°C, the bacterial suspension was centrifuged and suspended in sterile distilled water four times. The organism suspension was placed on 300-mesh carbon-coated grids, negatively stained with 2% phosphotungstic acid, and examined with a Hitachi H-600 transmission electron microscope.

**RESULTS**

**Necropsy results.** All hamsters appeared grossly normal at necropsy. No proliferative or inflammatory ileal lesions were noted in any hamster during either gross or microscopic examination. No intracellular organisms were noted on examination of the Warthin-Starry silver-stained sections from any hamster.

**Microbiological results.** No known *Campylobacter* spp. or *Campylobacter*-like organisms were isolated from either the CBAP or BAP at 43°C or from the CBAP incubated at 37°C. A *Campylobacter*-like organism was isolated from 4 of the 15 hamsters on the BAP incubated at 37°C. The pinpoint translucent colonies of this organism were first observed on initial isolation following 5 days of incubation. On reisolation and later passages, colonies were visible by 72 h of incubation. The results of standard biochemical tests are listed in Table 1.

**Transmission electron microscopy results.** Transmission electron microscopic examination of the organism revealed a typical *Campylobacter*-like morphology (Fig. 1). The organism measured 2.0 to 3.5 μm (length excluding flagellum) by 0.17 to 0.25 μm. Typically, a single terminal sheathed flagellum, as long as or longer than the body, was present. Organisms with two or three terminal flagella were occasionally observed.

**ELISA results.** The anti-*Campylobacter* genus-specific monoclonal antibody 8322-2E6 reacted positively (>0.35 optical density [OD] unit) with *C. jejuni*, *C. coli*, *C. pylori*, *C. pylori* subsp. *mustelae*, and the *Campylobacter*-like organisms isolated from all four hamsters. The anti- *C. jejuni*
TABLE 1. Phenotypic properties of the Campylobacter-like organisms isolated from hamsters compared with those of selected other Campylobacter spp.\(^a\)

<table>
<thead>
<tr>
<th>Property</th>
<th>C. jejuni</th>
<th>C. coli</th>
<th>C. pylori</th>
<th>C. pylori subsp. mustelae</th>
<th>Hamster Campylobacter-like organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>42°C</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>V</td>
</tr>
<tr>
<td></td>
<td>37°C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Susceptibility</td>
<td>Nalidixic acid (30-μg disk)</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Cephalothin (30-μg disk)</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Glucose fermentation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Growth in BHI with:</td>
<td>1% Glycine</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.5% NaCl</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3.5% NaCl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>H₂S production</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hippurate hydrolysis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Indoxylacetate hydrolysis</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>Urease production</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>No. of flagella</td>
<td>1</td>
<td>1</td>
<td>≤4</td>
<td>≤4</td>
</tr>
<tr>
<td></td>
<td>Sheathed flagella</td>
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<td>-</td>
<td>+</td>
<td>+</td>
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</table>

\(a\) +, Positive; -, negative; V, variable; S, susceptible; R, resistant.

monoclonal antibody 841-2A11 reacted positively (>0.35 OD unit) with C. jejuni and C. coli but did not react (<0.02 OD unit) with C. pylori, C. pylori subsp. mustelae, or any of the Campylobacter-like hamster isolates.

**Immunohistochemistry results.** Six of the 15 hamsters, including the four from which the Campylobacter-like organism was cultured, were positive for intracellular organisms when stained with the anti-Campylobacter genus-specific monoclonal antibody 8322-2E6. The positively stained organisms were primarily confined to the intestinal crypt epithelial cells, with organisms occasionally present within the villus epithelial cells adjacent to the intestinal crypts (Fig. 2 and 3). Rarely were more than five epithelial cells in a single crypt positive by immunofluorescence, with the heavier areas of infection usually overlying the lymphoid tissue of the ileum. No specific immunofluorescence was

![FIG. 1. Electron micrograph of the isolated Campylobacter-like organism. A single polar sheathed flagellum is present (inset). Phosphotungstic acid negative stain. Bar, 1 μm.](http://jcm.asm.org/)

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FIG. 2. Ileal section from a normal hamster. *Campylobacter* species-specific monoclonal antibody 8322-2E6 was used as the primary antibody, followed by affinity-purified biotin-caproyl-anti-mouse immunoglobulin G and fluorescein isothiocyanate-streptavidin. Fluorescing bacteria are limited to the crypt epithelial cells (arrows). Bar, 50 μm.

FIG. 3. Ileal section from a normal hamster stained as in Fig. 2. Fluorescing bacteria are present within the crypt epithelial cells (small arrow) and the villus epithelial cells adjacent to the crypt (large arrows). Bar, 20 μm.
detected in any of the 15 hamsters when the anti-C. jejuni monoclonal antibody 841-2A11 was used.

DISCUSSION

The Campylobacter-like organism isolated in this study differs from previously reported members of the genus Campylobacter. The organism isolated hydrolyzed urea, a characteristic of only C. pylori and C. pylori subsp. mustelae (1, 2) in the genus Campylobacter. The isolated hamster organism reduced nitrates and had a pattern of susceptibility to nalidixic acid (30 μg) and cephalothin (30 μg) similar to that of C. pylori subsp. mustelae (1, 2) but different from that of C. pylori. C. pylori subsp. mustelae differed from both C. pylori and the isolated Campylobacter-like organism in its ability to hydrolyze indoxylacetate.

The growth characteristics and morphology of the organism isolated were different from those of C. pylori subsp. mustelae. The initial isolation of the hamster Campylobacter-like organism required 120 h of incubation versus the 72 h reported for initial isolation of C. pylori subsp. mustelae (1). Colonies were considerably smaller, with culture-adapted organisms producing only pinpoint colonies following 72 h of incubation. The hamster organism was substantially smaller than C. pylori subsp. mustelae, with a single sheathed terminal flagellum instead of the multiple terminal sheathed flagella of C. pylori subsp. mustelae. Further investigations are needed to clarify the taxonomy and relationships of this hamster Campylobacter-like organism to C. pylori subsp. mustelae, C. pylori, and other members of the genus Campylobacter. In two previous studies (15, 16), we reported the presence of specific immunofluorescence staining with the anti-Campylobacter genus-specific monoclonal antibody 8322-2E6 in noninfected control hamsters. The Campylobacter-like organism isolated and partially characterized in this report may be that organism. The reactivity of this Campylobacter-like organism with the anti-Campylobacter genus-specific monoclonal antibody 8322-2E6 and the positive immunofluorescence of the ileal sections from the four hamsters from which this organism was isolated suggest that this organism may be responsible for the previous findings. The positive immunofluorescence of the ileal sections of two hamsters from which this organism was not isolated may be due to a failure of the microbiologic methods to isolate this organism from these hamsters or to the presence of an additional different Campylobacter-like organism(s) not isolated in this study.

The isolation of this Campylobacter-like organism from normal healthy hamsters obtained from a colony with no reported history of proliferative ileitis suggests that this organism is not the etiologic agent of proliferative ileitis. Development of additional monoclonal antibodies and improved culture techniques will be necessary to resolve the involvement of this and other members of the genus Campylobacter in the ilea of normal hamsters and hamsters with proliferative ileitis.

ACKNOWLEDGMENT

This work was supported in part by a grant from The Ohio State University, Office of Research and Graduate Studies (221193).

LITERATURE CITED