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Experimental *Campylobacter jejuni* Infection in *Macaca nemestrina*

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Experimental infection of four specific-pathogen-free *Macaca nemestrina* monkeys (aged 3.5 and 4.5 months) with *Campylobacter jejuni* 81-176 caused acute diarrheal illness, characterized by fluid diarrhea, bloody stools, and fecal leukocytes, which lasted for approximately 7 to 11 days. Histologic examination of intestinal biopsies showed acute colitis characterized by infiltration of the mucosa with neutrophils and lymphocytes, and cryptitis. There were no histologic changes in the small intestine. Excretion of *C. jejuni* was demonstrated for 2 to 4 weeks postchallenge. Plasma antibodies to *C. jejuni* group antigen were elevated after challenge. Only mild diarrhea occurred after rechallenge with the same strain or with a heterologous *C. jejuni* strain (79-168) followed by further elevation in specific immunoglobulins A, M, and G. Four 1-year-old juvenile *M. nemestrina* monkeys which had experienced multiple infections with *Campylobacter* spp. did not exhibit illness when challenged with *C. jejuni* 81-176. All had elevated immunoglobulin A, M, and G plasma antibodies prior to challenge, and these humoral antibody levels were indicative of the immunity to challenge. The results demonstrate that *C. jejuni* infection in *M. nemestrina* caused colitis with clinical and pathologic results similar to those found in humans and indicate that prior infection protects against subsequent challenge.

Campylobacter jejuni is an important enteric bacterial pathogen of humans in both developed and developing countries (8, 10). In developed countries, infection usually manifests itself as an acute illness with fever and with blood and leukocytes in stools (6, 10, 12, 18; T. J. Cover and M. J. Blaser, Annu. Rev. Med., in press). In developing countries, infection often results in mild illness or no symptoms at all (10, 15, 21). The reasons for this dichotomy of clinical findings has not been fully resolved, but differences in the acquisition of immunity have been reported and proposed as a possible explanation (3, 9, 25). The mechanisms of the diarrheal disease are not yet adequately elucidated, and studies of the pathophysiology of *C. jejuni* have been hampered by the lack of a good animal model. Experimental infection of rodents, chickens, dogs, rabbits, and other species has been limited by either the failure to induce clinical signs and intestinal pathology reflective of human disease or the necessity for surgical manipulation that diminishes the value of the model (27). These problems prompted recent experimental inoculation of humans (2), which duplicated the findings of natural infection; however, wide-scale experimentation with humans is not practical.

Campylobacter spp. are commonly isolated from laboratory-housed monkeys with diarrhea, but no clear etiologic significance has been established (11, 22, 23, 26). A previously described experimental model of *Campylobacter* infection in *Macaca mulatta* was characterized by very mild diarrhea of short duration and absence of detectable intestinal pathology (13). This leaves the questions of the enteropathogenic role of *Campylobacter* spp. in monkeys and the validity of a monkey model for *Campylobacter* infection in humans unresolved.

Because of the possibility that prior *Campylobacter* infection protects against experimental inoculation, we studied infant *Macaca nemestrina* monkeys that had been reared free of *Campylobacter* exposure. This model mimics *Campylobacter* infection in developed countries. In comparison, we also inoculated juvenile *M. nemestrina* monkeys which differed only in that they had experienced repeated exposure to *Campylobacter* spp., a situation which parallels populations in developing countries (15, 25). We report here the clinical, pathologic, and serologic aspects of experimental inoculation or subsequent challenge (or both) of these animals with *C. jejuni*.

MATERIALS AND METHODS

Animals. Four *M. nemestrina* monkeys (infants 1 to 4) delivered by caesarean section were reared in isolation in a room with restricted entry and housed individually in Perspex-partitioned cages. Protective clothing, including coveralls, masks, gloves, and boots, were worn by all personnel entering the room. The infants were fed an infant milk formula (SMA-R; Wyeth Laboratories, Philadelphia, Pa.) throughout the experiment. Monkey biscuits (Purina Monkey Chow 5405, 25% protein diet; Ralston Purina Co., Richmond, Ind.) were available ad libitum.

Rectal swabs were cultured weekly from birth to monitor for *Campylobacter* and *Shigella* infection. The infants were specific pathogen free (SPF) for these organisms when experimentally challenged.

Four *M. nemestrina* monkeys (juveniles 5 to 8) that had been housed in an infant primate nursery from birth were moved to the isolation facility when they were 12 months old. They had recently completed a prospective study in which rectal swabs were obtained weekly and cultured for *Campylobacter* spp. (R. G. Russell et al., unpublished observations). During the 12-month observation period, each animal had been infected with both *Campylobacter coli* and *C. jejuni*, and juveniles 6, 7, and 8 were known to have been infected with multiple serotypes (see Results). The juveniles were challenged with *C. jejuni* 81-176 to determine whether prior exposure to *C. jejuni* and *C. coli* imparted protective immunity.

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Bacteria. *C. jejuni* 81-176 (Penner serotype 23/36) was isolated during an outbreak of campylobacteriosis in Minnesota caused by consumption of contaminated raw milk (16). The isolate of 81-176 received on a brucella agar slant contained two colony types, consisting of a large spreading colony and a smaller, discrete, nonspreading colony. Subcultures of the former colonies were used for all experimental inoculations. *C. jejuni* 79-168 (Penner serotype 3) was isolated from a household outbreak of diarrhea (5). The person from whom this isolate was obtained had bloody diarrhea with fecal leukocytes and colitis confirmed by proctoscopy.

Stock cultures were stored at −70°C in tryptic soy broth (Difco Laboratories, Detroit, Mich.) containing 15% (vol/vol) glycerol. Subcultures on brucella anaerobic agar plates were incubated at 42°C in an atmosphere of 6% oxygen and 10% carbon dioxide by using an anaerobic jar and Campy Pak II (BBL Microbiology Systems, Cockeysville, Md.). These harvested stocks were stored as described above, and titrations were conducted by serial 10-fold dilution of the stocks prior to storage and at the time of infection.

Bacteriologic methods. Rectal swabs and stomach contents were plated on modified Skirrow *Campylobacter* agar (24) containing 5% defibrinated sheep blood and antibiotics, including cefoperazone (20 mg/liter), vancomycin (10 mg/liter), and amphotericin B (2 mg/liter) (Prepared Media Laboratories, Renton, Wash.). Plates were incubated at 42°C for up to 96 h under microaerobic conditions as described above. Colonies with the typical appearance of *C. jejuni* or *C. coli* were stained to examine for gram-negative curved rods and then subcultured onto brucella anaerobic agar with 5% sheep blood. Identification as *C. jejuni* was confirmed by positive oxidase, catalase, and hippuricase tests and susceptibility to nalidixic acid (30 μg per disk). Hippuricase-negative, nalidixic acid-sensitive, thermophilic *Campylobacter* isolates were classified as *C. coli* (19). Before experimental inoculation, juveniles 5 to 8 in the nursery had also been naturally infected with a nalidixic acid-resistant, hippuricase-negative, thermophilic *Campylobacter* sp. (M. A. Bronsdon, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, C130, p. 321) as well as with *C. jejuni* and *C. coli*.

Bacteriologic culture for *Shigella* spp. was conducted by inoculation of XLD and MacConkey agar with the rectal swab. Plates were incubated aerobically at 37°C and were examined at 24 and 48 h. Selected gram-negative organisms were subcultured on tryptic soy agar. *Shigella* isolates were identified by the API 20E system (Analytab Products, Inc., Plainview, N.Y.) and were serotyped using commercial antisera (Difco Laboratories, Detroit, Mich.).

To determine the concentration of *C. jejuni* organisms in feces, 0.1-g samples of fresh feces were suspended in 1 ml of sterile saline and vortexed vigorously, and serial 10-fold dilutions in sterile saline were inoculated onto *Campylobacter* agar plates. The colonies were counted after 48 h of incubation. In addition, the numbers of *Campylobacter* colonies on the primary isolation plate inoculated with the rectal swab or with stomach contents were recorded by a semiquantitative score. The swab was inoculated onto half the plate, and three dilution streaks were made without flaming the loop. Scoring was as follows: 1+, <10 colonies in initial half-plate area; 2+, ≥10 colonies in the initial half-plate area; 3+, ≥10 colonies in the first streak; +4, ≥10 colonies in the second streak; 5+, ≥10 colonies in the third streak.

Blood culture was conducted by inoculation of Columbia broth VACUTAINER tubes (Becton Dickinson Vacutainer Systems, Rutherford, N.J.) with 3 ml of heparinized blood. The cap was replaced with a sterile cotton plug, and the tubes were incubated at 42°C under microaerobic conditions. Subcultures were inoculated onto brucella anaerobic agar and examined at 24 to 96 h.

Experimental challenge. *Campylobacter*- and *Shigella*-free infants 1 to 4 were experimentally inoculated when they were 3.5 (infants 3 and 4) or 4.5 (infants 1 and 2) months old (age differences resulted from availability of animals). The infants were fasted for 2 h, cimetidine (4 mg/kg of body weight; Tagamet hydrochloride; SKF Lab Co., Carolina, P.R.) was administered 90 min before inoculation, and 15 ml of 1.2% sodium bicarbonate was instilled into the stomach via a nasogastric tube 20 min before inoculation. At the time of challenge, stomach contents were withdrawn via a stomach tube for pH measurement; pHs were from 6.5 to 7.6 (normal pH, 4.2). The inoculum of 3×10^{11} *C. jejuni* 81-176 organisms was administered in 10 ml of brucella broth via a nasogastric tube. The tube was flushed with 5 ml of brucella broth. The feeding bottles with SMA-R infant milk formula were replaced 2 h postchallenge (PC).

Rectal swabs were collected from all four infants for culture on days 1, 2, 5, 6, 11, 13, 17, 20, and 30 PC. Titration of stool specimens was conducted on days 2, 5, 6, 11, 17, and 20 PC. Vomited stomach contents were cultured on days 2 to 4 PC from infants 1, 2, and 4. Blood was cultured on day 2 PC. Feces were examined for fecal leukocytes with new methylene blue stain and for occult blood (Hematest; Miles Laboratories, Elkhart, Ind.) on days 2 to 5 PC. Feces were weighed, and the fecal dry matter of preinfection specimens and specimens from days 2 to 4 PC were determined by drying fecal samples to constant weight in an oven at 200°F. Daily temperature, body weight, and milk intake were recorded. Hematologic examinations for total erythrocyte and leukocyte counts were conducted on days 3 and 10 preinfection and on days 2, 3, and 10 PC. Hematology was not conducted on infants 2 and 3 on day 3 or on any infant on days 4 to 9 PC because any alterations may have been due in part to the effects of surgery.

Intestinal biopsies (2 by 1 mm) were obtained from the ascending and midcolon (5 to 15 cm from the ileocecal junction) and jejunum or ileum (6 to 10 cm proximal to the ileocecal junction) via laparatomy of the infants under general anesthesia with aseptic surgical technique. Administration of butorphanol tartrate (0.15 mg/kg) (Stadol; Bristol Laboratories, Syracuse, N.Y.) every 8 h for analgesia for 2 days after surgery relieved discomfort and helped the animals maintain voluntary milk intake. The biopsies were obtained 25 days preinfection and at 48 h PC (infants 2 and 3) or 72 h PC (infants 1 and 4). Preinfection and PC biopsy sites were adjacent in individual animals. Tissues were fixed in 10% neutral buffered Formalin for light microscopy. Serial sections cut at 5 μm from paraffin-embedded tissues were stained with hematoxylin and eosin or with a combination stain containing hematoxylin, eosin, Alcian blue, and Saffron.

Experimental rechallenge. Two SPF infants (2 and 3) were rechallenged with 3×10^{10} *C. jejuni* 81-176 organisms at 30 days after the initial challenge. Infants 1 and 4 were rechallenged with 3×10^{10} cells of the heterologous strain (79-168) of *C. jejuni*. At the same time, four 12-month-old juvenile *M. nemestrina* monkeys (5 to 8) from an infant primate research laboratory were challenged with 3×10^{10} *C. jejuni* 81-176 cells.

Premedication of all eight infants followed the procedures for initial experimental infection described above. Bacterio-

logic culture of rectal swabs and titration of *Campylobacter* organisms in feces were conducted on days 1 to 4, 7, 9, 12, and 15 PC. Complete blood cell counts were obtained 6 days before and 3 days after rechallenge.

Polyacrylamide gel electrophoresis (PAGE). The isolates from the challenge and rechallenge experiments were subcultured onto brucella anaerobic agar, harvested in 1.5 ml of sterile distilled water, centrifuged at 3,000 × *g* for 15 min, and washed twice, and the pellets were suspended in 0.5 M Tris hydrochloride buffer, pH 6.8. Bacterial cells on ice were disrupted by sonication for 10 min at 50% efficiency with 2.5-s burst cycles with a cup probe, and the whole-cell preparations were boiled for 10 min in 200 μl of lysing buffer (2% sodium dodecyl sulfate [SDS], 25% glycerol, 4 g of 2-mercaptoethanol, 0.03% bromophenol blue in 100 ml of 0.5 M Tris hydrochloride buffer, pH 6.8). Analysis by SDS-PAGE was by the method of Laemmli (17), using 4.5% stacking and 10.0% running gels. Samples of 100 μl per lane were electrophoresed at a constant current of 20 mA per gel. Proteins were visualized with Coomassie blue stain and compared with molecular weight markers (Sigma Chemical Co., St. Louis, Mo.).

Serotyping. Rabbit antisera were prepared as described previously against 15 *C. jejuni* and 5 *C. coli* Penner serotypes commonly found in humans (14). For the passive hemagglutination test, the titers of reactivity of the sera were determined and adjusted to a concentration four times the endpoint titer. Saline-extracted antigens from test strains were used in passive hemagglutination assays against each antiserum (20). Isolates of *C. jejuni* recovered from experimentally infected monkeys were serotyped to confirm that infection was with the strain inoculated.

Immune response. Plasma was collected from SPF infants 1 to 4 prior to experimental challenge; on days 10 and 20 PC; 3 days before rechallenge (day 27); and on days 37, 44, and 53 PC (i.e., days 7, 14, and 23 after rechallenge). The latter four specimens were also obtained from the 12-month-old juveniles (5 to 8) undergoing challenge with *C. jejuni* 81-176.

Antibody titers in serum were measured by enzyme-linked immunosorbent assay using a protocol similar to that described previously, with minor modifications (4). Briefly, the enzyme-linked immunosorbent assay used an acid-glycine antigen preparation of strains of Penner 1, 2, and 3 serotypes constituting 2.5 μg of group-specific surface proteins per ml, 1:1,000 dilutions of human immunoglobulins A, M, and G (IgA, IgM, and IgG), peroxidase conjugates (Dako Corporation, Santa Barbara, Calif.), and 0.04% *o*-phenylenediamine substrate (Sigma Chemical Co., St. Louis, Mo.). A 100-μl sample of diluted serum was incubated for 2 h at 37°C. The wells were aspirated and washed three times with phosphate-buffered saline–0.1% Tween 20. A 100-μl sample of the appropriate peroxidase conjugate was added, and the plates were incubated for 2 h at 37°C. After the plates were washed, 100 μl of *o*-phenylenediamine substrate was added and incubated for 30 min at room temperature. The reaction was stopped by adding 50 μl of 2.5 M H_2SO_4 and read immediately. The titers were measured by optical density at 490 nm on sera diluted 1:40 for IgG and 1:10 for IgA and IgM. The tests were repeated three times on each infant. Statistical analysis of slopes was performed by the multivariate general linear model for comparison of titers at different time intervals in infants challenged with strain 81-176 and rechallenged with homologous or heterologous strains of *C. jejuni*.

TABLE 1. Summary of clinical signs in four infant *M. nemestrina* monkeys inoculated with 10^{11} *C. jejuni* 81-176 cells

Clinical signs	Experimental results
Onset of diarrhea (h after inoculation)	34 ± 5.0[a]
Peak of diarrhea (days after inoculation)	4 ± 0.1
Duration (days after inoculation)	9 ± 0.9
Fecal dry matter	
Prechallenge (%)	14 ± 1.5
PC (%)	2 ± 0.6[b]
Presence of (no. positive/total):	
Occult blood	4/4
Bacteremia	3/4
Fecal leukocytes	1/4
Vomition	3/4

[a] Mean ± standard error of the mean.
[b] Significantly different from prechallenge values ($P < 0.01$).

RESULTS

Experimental challenge of SPF *M. nemestrina* with 3×10^{11} *C. jejuni* 81-176 cells. Beginning at 24 h PC, infants 2 and 4 were depressed and exhibited abdominal discomfort. The onset, peak, and duration of diarrhea in all infants are shown in Table 1. Infant 3 had mucoid bloody diarrhea on day 3 and had fecal leukocytes on days 2 and 3. All infants had occult blood on days 2 to 4 PC. Three infants (1, 2, and 4) vomited between days 2 and 4 PC. Corresponding to the diarrhea was a reduction in fecal dry weight in all infants at 48 to 96 h PC compared with the normal preinfection fecal dry weight measurements (Table 1). The numbers of *C. jejuni* cells in the feces showed peak titers of 10^{10} per g on day 2 PC and declined progressively thereafter (Fig. 1). Fecal cultures no longer yielded *C. jejuni* by days 17 to 30 PC. There was a significant correlation between fecal bacterial counts and the scores on primary plate isolations ($r = 0.72$; $P < 0.01$). The lower limit of sensitivity by rectal swab culture was 10^2 to 10^3 organisms per g. *C. jejuni* was isolated from vomited stomach contents of infant 1 on day 3 PC, but cultures of vomited milk from infants 2 and 3 between days 1 and 4 were negative. Blood cultures were positive from three of the four infants (Table 1). All daily body temperature measurements were normal.

Blood cell counts for all infants at all times sampled between days 2 and 10 PC were normal. The infants weighed 1.2 ± 0.2 kg preinfection. They lost 12 ± 2.2% of body

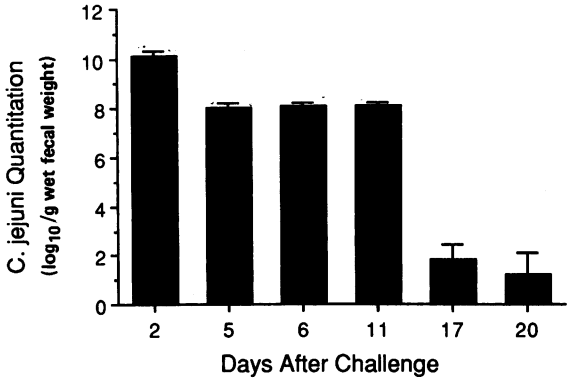

FIG. 1. Quantitation of *C. jejuni* 81-176 in feces of four SPF infant *M. nemestrina* monkeys by titration on days 2 to 30 PC. Error bars indicate standard error of the mean.

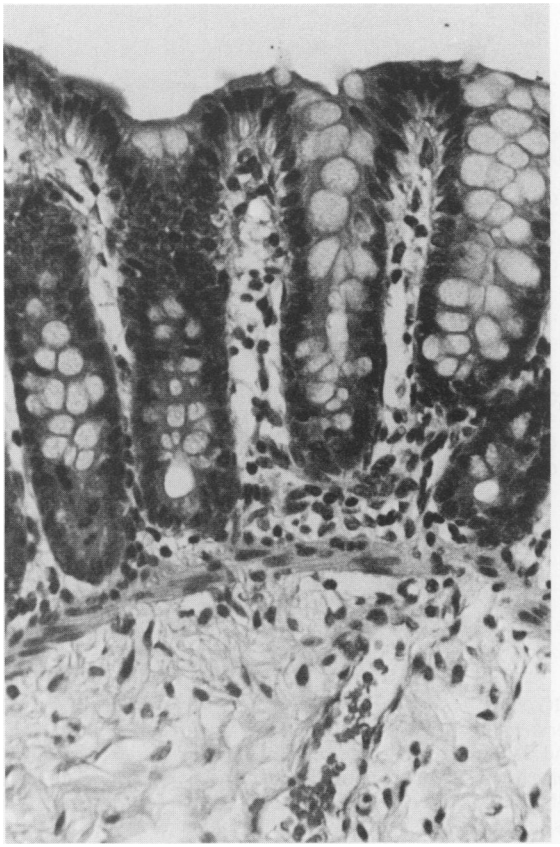

FIG. 2. Histology of preinfection normal colon from infant 2. Hematoxylin and eosin stain; magnification, ×250.

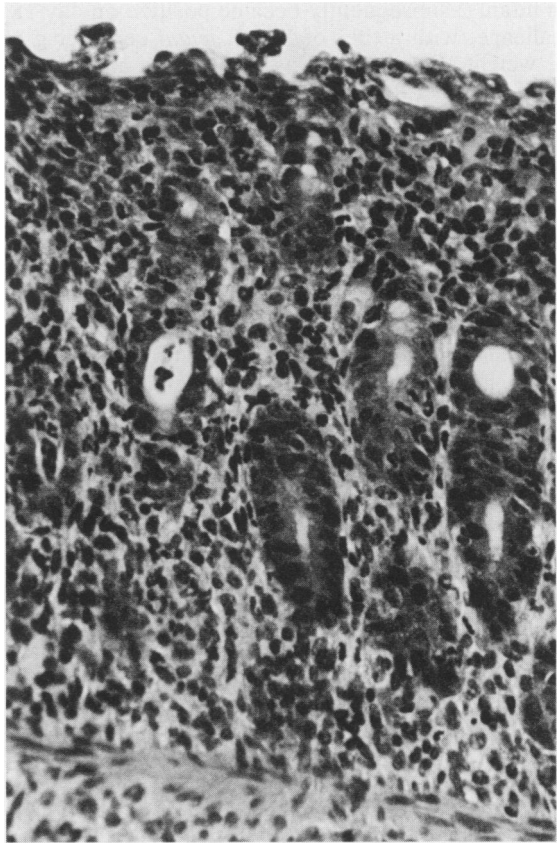

FIG. 3. Colitis in infant 3 on day 2 PC showing infiltration of large numbers of neutrophils and lymphocytes in the lamina propria and cryptitis. Hematoxylin and eosin stain; magnification, ×250.

weight at 24 to 72 h PC and regained normal weight by days 4 to 6 PC. The weight losses immediately PC and postsurgery correspond to reduced milk intake, and the weight was regained as milk intake returned to normal levels.

Histopathologic findings. Prior to *C. jejuni* challenge, the small intestine and colon histology of infants 1 to 4 was normal (Fig. 2). Biopsies from infants 2 and 3 on days 2 PC and from infants 1 and 4 on day 3 PC showed colitis with edema and diffuse infiltration of large numbers of neutrophils, plasma cells, and lymphocytes in the colonic lamina propria. Neutrophils were locally intense around glands, infiltrated between the epithelial cells in glands, and accumulated in the crypt lumen, resulting in cryptitis (Fig. 3). Goblet cells in the crypts were reduced in number. In the submucosa immediately beneath the muscularis mucosa, the lymphatics were dilated and there was mild to moderate diffuse and perilymphatic infiltration of mononuclear cells. The small intestine was normal by microscopic examination.

Experimental rechallenge. (i) SPF infants inoculated 30 days previously with strain 81-176 and rechallenged with 3×10^{10} homologous *C. jejuni* 81-176 cells. Beginning at 24 h after rechallenge, infant 2 developed soft unformed stools which became formed but still soft at 48 to 64 h, and stools became normal 72 h after rechallenge. Infant 3 developed soft, semiformed yellow feces at 24 h, soft but formed feces at 72 h, and normal stools at 96 h after rechallenge. Infant 3 vomited at 48 and 72 h after rechallenge. The diarrhea was mild in both infants. Fecal leukocyte and occult blood examinations were negative. Body temperatures were normal.

Infants 2 and 3 had normal fecal dry weights of 11.8 to 14.6% at 48 to 96 h after rechallenge. Bacterial counts were +3 on days 1 and 2 after rechallenge, and both infants were negative by day 9 (Fig. 4). SDS-PAGE protein profiles of the isolates recovered were identical to that of strain 81-176, and serotyping confirmed that isolates were Penner 23/26. Stools

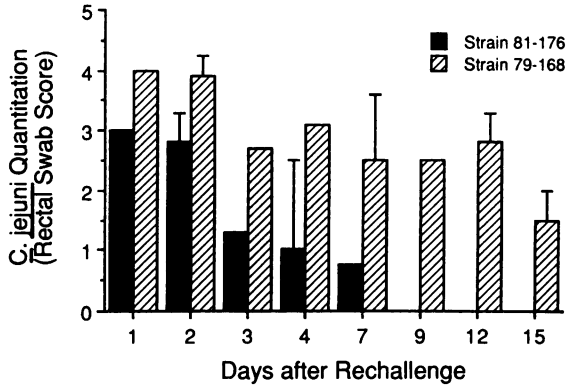

FIG. 4. Quantitation of *C. jejuni* in feces from rectal swab scores (see Materials and Methods) after rechallenge of four *M. nemestrina* monkeys with *C. jejuni* 81-176 (homologous) and *C. jejuni* 79-168 (heterologous). On day 15, infant 2, which had been rechallenged with strain 81-176, was found to be excreting strain 79-168, indicating that cross-infection had occurred (data not shown). Error bars indicate standard error of the mean.

from infant 2 subsequently became positive on day 15 after rechallenge, with a titer of 10^7 *C. jejuni* cells per g of wet fecal weight. The isolate was serotype 3, and SDS-PAGE showed an SDS-PAGE profile identical to that of strain 79-168, indicating cross-infection from infants infected with the heterologous strain of *C. jejuni*.

(ii) Rechallenge with 3×10^{10} heterologous *C. jejuni* 79-168 cells. Infant 1 had soft, unformed stool at 48 to 96 h after rechallenge. It was normal 5 days after rechallenge. Infant 4 had soft, formed stool at 24 h after rechallenge and was normal by 48 h after rechallenge. Fecal leukocyte and occult blood examinations were negative, and body temperatures remained normal.

The fecal dry weight was reduced to 8% in infant 4 at 48 h after rechallenge. There was insufficient sample from infant 1 for measurement. *C. jejuni* 79-168 was reisolated from both infants on days 1 to 15 after rechallenge. The bacterial counts remained high on day 15 (Fig. 4) with titers of 6.8×10^6 to 7.5×10^6 per g of wet fecal weight. The SDS-PAGE profiles and serotyping confirmed that the isolates recovered were strain 79-168.

Experimental challenge of four 1-year-old *M. nemestrina* monkeys with 3×10^{10} *C. jejuni* 81-176 cells. Three of the juveniles (6 to 8) had been infected with multiple serotypes during the 12 months in the infant nursery. The Penner serotypes included 5 and 34/4 (all three juveniles); 24 (juveniles 6 and 7); 30, 4, and 30/34 (infant 8); 8 (juveniles 7 and 8); 30, 4/5/34, and 4/30 (juvenile 6); 46 (juvenile 7); and 38 and 34 (juvenile 8). There were also untyped isolates in all three juveniles. Each juvenile had been infected with at least six to eight different serotypes of *C. jejuni* and *C. coli*. The isolates from juvenile 5 were not serotyped, but infection with multiple serotypes are likely to have occurred. In addition, all of the juveniles were infected with nalidixic acid-resistant, hippuricase-negative *Campylobacter* spp. reported previously in this colony (M. A. Bronsdon, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985). *C. coli* cells were isolated from all four juveniles on days 2 and 9 prechallenge and in various numbers PC from juveniles 6 to 8 (Fig. 5) which had titers of 3×10^2 to 2.3×10^8 per g of wet fecal weight.

After challenge with *C. jejuni* 81-176, none of these juveniles showed any signs of illness. Stools were normal in juveniles 5, 6, and 8, but soft unformed feces prechallenge continued in juvenile 7 throughout the observation period. This was attributed to *Shigella flexneri*, which was isolated 9 days before challenge and throughout the 15 days PC. *Shigella flexneri* was not isolated during earlier periods of weekly monitoring when the stool was of normal consistency. Blood cultures obtained 48 h PC were negative. Daily body weight, temperature, and blood counts were normal. The experiment was terminated on day 15 PC.

Low numbers of *C. jejuni* 81-176 (six colonies) and *C. coli* (+2) were cultured from the rectal swab of juvenile 7 on day 1 PC (Fig. 5). *C. jejuni* 81-176 was also isolated from juvenile 8, cultured, and titrated on day 5 PC when the rectal swab showed +1 *C. coli* (Fig. 5), and titration plates found 10^3 *C. jejuni* 81-176 cells per g of wet fecal weight and 7×10^3 *C. coli* cells per g.

Serological response. The serological responses to inoculation and challenge are shown in Fig. 6. After challenge with *C. jejuni* 81-176, the IgG level rose significantly in all SPF infants (1 to 4) ($P < 0.01$). In infant 1, IgM and IgA titers were increased on day 10, and the IgM remained elevated through day 21 PC. Other infants did not exhibit an IgA or IgM response following the initial inoculation.

After rechallenge with the homologous strain of *C. jejuni*

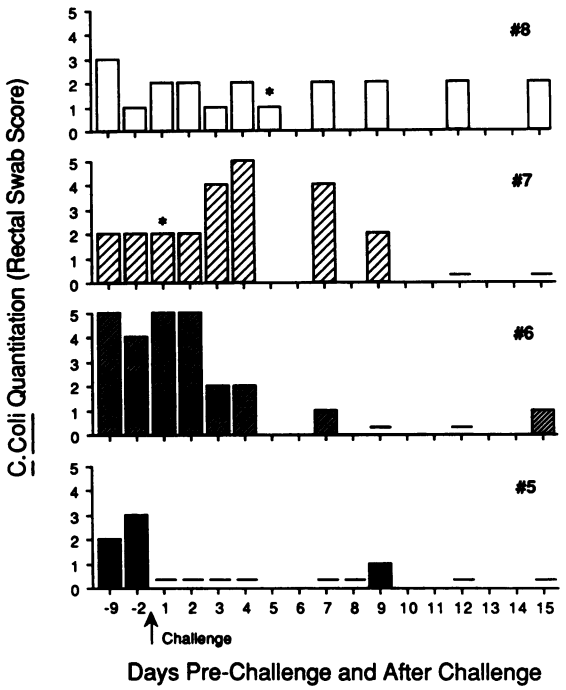

FIG. 5. *Campylobacter* excretion by four 1-year-old juvenile *M. nemestrina* monkeys (5 to 8). Each of the juveniles excreted *C. coli* 9 and 2 days prechallenge and for various durations PC with *C. jejuni*. Rectal swab quantitation scoring is described in Materials and Methods. Juveniles 7 and 8 were positive for *C. jejuni* 81-176 on days 1 and 5 PC, respectively (*) (infant numbers are indicated at the top right of each graph). None of the juveniles exhibited diarrhea. No culture was conducted on days 5, 6, 10, and 14 PC except for juvenile 8 on day 5 PC.

on day 30 postinfection, infants 2 and 3 showed a further rise in IgG titer ($P < 0.01$). Before rechallenge with the heterologous strain of *C. jejuni* (79-168), infants 1 and 4 showed a drop in the IgG titer beginning on day 21 PC and continuing to day 38. After rechallenge, the secondary IgG immune response in both infants increased at a significantly faster rate (slope = 0.067 ± 0.002) than in infants challenged with the homologous strain (slope = 0.031 ± 0.008) ($P < 0.05$) reaching statistically similar levels as after homologous rechallenge. Thus, the increases of IgG titers determined by optical density at 490 nm throughout the entire experiment (slope = 0.033 ± 0.003) were similar in all infants irrespective of the strain of rechallenge and age. The IgA and IgM titers increased in infants 1, 2, and 4 but not in infant 3. The IgM response was not affected by age or the strain of *C. jejuni* used for rechallenge. The IgA response to rechallenge was higher in infants 4.5 months old (slope = 0.197 ± 0.005) compared with 3.5-month-old infants (slope = 0.006 ± 0.003) ($P < 0.05$).

Juveniles 5 to 8 had prechallenge optical density at 490 nm readings for IgG, IgM, and IgA of 1.0 ± 0.2, 0.6 ± 0.1, and 0.4 ± 0.2, respectively, and the levels were not elevated after challenge with *C. jejuni* 81-176.

DISCUSSION

In this study, infection of four SPF infant *M. nemestrina* monkeys with *C. jejuni* 81-176 produced an acute diarrheal illness. The findings of bloody stools, fecal leukocytes, and tissue inflammation closely resembled those for human *Cam-*

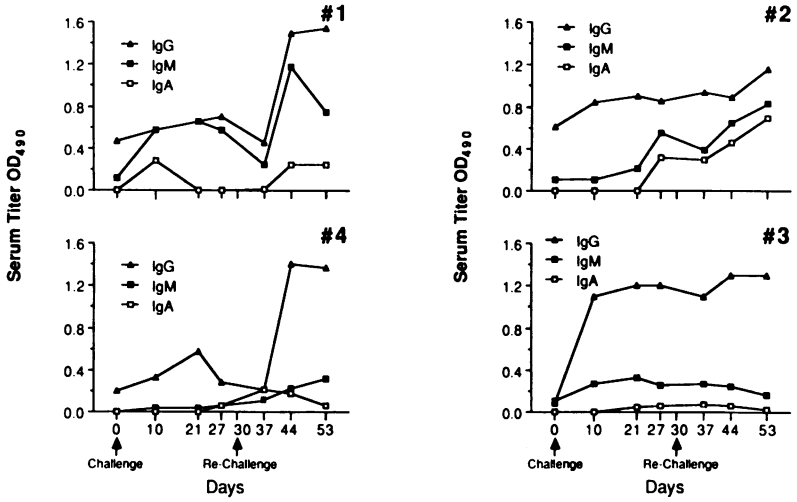

FIG. 6. Plasma antibody levels in SPF infants 1 to 4 PC with *C. jejuni* 81-176 and after rechallenge with homologous *C. jejuni* 81-176 (infants 2 and 3) or heterologous *C. jejuni* 79-168 (infants 1 and 4). Infant numbers are indicated at the top right of each graph. Antibody levels represent the optical density of enzyme-linked immunosorbent assay plates read at 490 nm with serum dilutions of 1:40 for IgG and 1:10 for IgA and IgM.

pylobacter infection (6, 8, 10, 12, 18; Cover and Blaser, in press), including the natural (16) and experimental (2) acute colitis observed with *C. jejuni* 81-176 infection. Despite the very high challenge dose, the quantitation and duration of *C. jejuni* excretion were similar to those observed in humans (8; Cover and Blaser, in press). Bacteremia in the infants 48 h PC was not found in experimentally infected humans (2), a difference that may reflect the higher challenge dose, age of the subjects, species differences, or more sensitive culture methods. In humans, *C. jejuni* bacteremia is associated with infection in young, aged (8), or immunocompromised patients (7). The organism was recovered from vomited gastric contents. The peak of the diarrhea lasted 1 to 2 days, and all infants were back to normal by 7 to 11 days PC; these findings also resemble those for human infections (10). The serologic responses to challenge were consistent with the humoral immunity to natural and experimental infections in humans (4), although elevations in IgA and IgM were not seen in three infants after initial challenge. This may be because of individual animal variation, technical factors in the enzyme-linked immunosorbent assay with anti-human immunoglobulin reagents, or differences in the immune response between monkeys and humans. Thus, by a variety of criteria, infection of SPF infant *M. nemestrina* monkeys is apparently a good model of *C. jejuni* infection in humans. The availability of an animal model in which symptoms and pathology are similar to those seen in the human disease may facilitate future studies of the pathogenesis and immunity to infection with *Campylobacter* spp.

Initial necropsy studies in affected humans suggested that *Campylobacter* infection might primarily involve the small intestine (8), whereas sigmoidoscopy studies indicated that colitis was a common finding (6, 18). The localization of inflammation in the colon and the absence of small intestinal involvement in these animals supports the conjecture that *C. jejuni* infection primarily causes a colitis and that small intestinal involvement is less common.

In many developing countries where *C. jejuni* is endemic, symptomatic infections may occur in very young children, but beyond an early age, infections frequently are asymptomatic (15, 21). Clinical, epidemiologic, and serologic studies suggest development of immunity following repeated exposure to these organisms (3, 9, 15). That monkeys raised under conventional laboratory conditions and known to have been infected with various *Campylobacter* strains and species showed no clinical signs and brief duration of excretion of *C. jejuni* after challenge supports the hypothesis that immunity due to previous infections is responsible for the mild or inapparent symptoms associated with recurrent infections of humans in developing countries. This information is also useful for an understanding of the possible etiologic role of *Campylobacter* spp. in monkeys, since the agent is commonly isolated from laboratory-housed primates with diarrhea, but the significance has not been established (23). These observations suggest that *C. jejuni* is enteropathogenic in monkeys, as it is in humans, and that acquired immunity may be protective under the enzootic conditions of laboratory housing for monkeys.

The rechallenge studies in SPF monkeys are also consistent with the hypothesis that immunity protects against disease. The rechallenge with the homologous *C. jejuni* 81-176 resulted in a much milder illness than the initial inoculation, paralleling the findings in experimentally infected humans (2). Since nonimmune SPF monkeys were not challenged with the heterologous strain 79-168, the extent of illness produced by this organism in monkeys cannot be properly evaluated. Nevertheless, the mild nature of the findings observed after rechallenge with this strain is consistent with the hypothesis of heterologous immunity as well. However, the longer duration of excretion of the heterologous strain after rechallenge suggests that heterologous immunity may not be as complete as homologous immunity. The excretion of significantly larger numbers of heterologous *C. jejuni* cells than of the homologous strain following rechallenge of infant *M. nemestrina* monkeys is consistent with the results of studies of colonization in mice after vaccination with live or heat-killed organisms (1). From studies of vaccinated mice rechallenged with a homologous or heterologous strain of *C. jejuni*, it was suggested that antigens on the surface of the bacterial cell or flagella may be important immunologically (1).

Specific antibody levels in serum were boosted after rechallenge, especially in the IgG class. Differences in the rate of increase of IgG antibodies in heterologous compared

with homologous rechallenge may be related to the strain differences of *C. jejuni* but could also be explained by the absence of a clear drop in the IgG level in infants 2 and 3 after challenge and before rechallenge. Elevation of IgA after rechallenge was higher in 4.5-month-old infants than in 3.5-month-old infants. The conventionally raised juvenile *M. nemestrina* monkeys which had experienced recurrent infections with *Campylobacter* spp. had elevated levels of specific plasma IgA, IgM, and IgG prior to challenge, similar to levels seen PC in the SPF animals and also similar to levels in older children in human populations in developing countries (3, 9). The absence of clinical signs following challenge of these juveniles suggests that the high levels of humoral antibodies may be an indicator of protection.

In conclusion, SPF *M. nemestrina* monkeys provide a suitable animal model for studies of the pathogenesis of *C. jejuni* diarrhea. Additional studies are required to characterize the mechanisms causing colitis and to define the intestinal immune responses to *C. jejuni* infection. This model could facilitate an understanding of this disease in humans and in monkeys.

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