Campylobacter jejuni Strain CG8421: A Refined Model for the Study of Campylobacteriosis and Evaluation of Campylobacter Vaccines in Human Subjects

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Background. A robust human challenge model for Campylobacter jejuni is an important tool for the evaluation of candidate vaccines. The previously established model conveys a potential risk of Guillain-Barré syndrome attributable to lipooligosaccharide ganglioside mimicry. This work establishes a new C. jejuni human challenge model that uses a strain (CG8421) without ganglioside mimicry and that applies Campylobacter-specific cellular immunity screening to achieve high attack rates at lower inoculum doses.

Methods. Healthy Campylobacter-naïve adults participated in an open-label challenge trial. Participants were dosed with C. jejuni CG8421 and followed as inpatients. Pattern of illness, bacterial shedding, and immunologic responses were determined.

Results. Following screening, 23 subjects received 1 × 10⁶ or 1 × 10⁵ colony-forming units of C. jejuni, with attack rates (percentage of patients who became ill) of 100% (1 × 10⁶ colony-forming units) or 93% (1 × 10⁵ colony-forming units). Every subject shed CG8421; the median time to diarrhea onset was 72.3 h (interquartile range, 53.9–99.9 h). Symptoms included abdominal cramps (74%), nausea (65%), and fever (39%). No major safety concerns occurred, including bacteremia, hypotension, or postinfectious sequelae. Unexpectedly, recrudescent infection occurred in 2 subjects (1 subject without Campylobacter-specific adaptive immune responses and 1 with azithromycin resistance acquired in vivo); both infections cleared after receipt of additional antibiotics. Cumulative Campylobacter-specific immune responses were as follows: serologic response occurred in 87% (IgA) and 48% (IgG) of subjects, in vitro interferon-γ production occurred in 91% of subjects, and 96% of subjects had IgA antibody–secreting cells and fecal IgA detected.

Conclusions. The C. jejuni CG8421 challenge model provides a safe and effective tool, without the risk of Guillain-Barré syndrome. The model demonstrates high attack rates after lower doses of challenge inoculum, provides further understanding of immunologic responses, and permits future investigation of candidate Campylobacter vaccines.

Campylobacter species, predominantly Campylobacter jejuni, are a leading cause of food- and water-borne disease [1, 2]. Campylobacter infections are also a significant cause of diarrhea in children in resource-poor countries, as well as in travelers and military personnel [3–5]. Increasing antibiotic resistance and the associ...
ation of Campylobacter infection with serious postinfectious sequelae, such as Guillain-Barré syndrome (GBS), have brought attention to the importance of vaccine development [6, 7]. Human challenge models that use a well-characterized strain, have a predefined dose of inoculum, and have a known attack rate (i.e., the percentage of persons who become ill) are invaluable tools for controlled assessment of early-stage candidate Campylobacter vaccines in small numbers of subjects. C. jejuni strain 81–176 has been extensively studied in other challenge models of C. jejuni, but 2 major obstacles limit its future use [8–10]. First, a high dose (1 × 10^9 colony-forming units [CFUs]) of C. jejuni has been required to achieve a ≥75% attack rate [10]. Second, the outer lipooligosaccharide (LOS) core of the 81–176 strain synthesizes ganglioside mimics, a mechanism by which C. jejuni infection is thought to induce GBS [11–14].

Strains of C. jejuni, including CG8421, that lack ganglioside mimicry have been characterized via structural and genome sequencing for use in human challenge models [15]. Post-hoc analysis of data from studies of the 81–176 strain also suggests that inclusion of cell-mediated immunity assays (i.e., analysis of in vitro induction of interferon [IFN]-γ) to subject screening may improve the selection of Campylobacter-naïve subjects and permit the use of lower a lower inoculum dose in challenge [10]. To develop a new challenge model and to expand understanding of human immune response to infection, we describe the administration of C. jejuni strain CG8421 to Campylobacter-naïve, healthy subjects.

Figure 1. Characteristics of screened subjects and subjects who participated in the Campylobacter jejuni CG8421 challenge study. IFN, interferon; Ig, immunoglobulin.

METHODS

The study was an open-label, dose-ranging inpatient trial of oral inoculation of C. jejuni strain CG8421. After a comprehensive screening evaluation, sequential groups of subjects were enrolled to receive a dose of 1 × 10^8 CFUs of C. jejuni, on the basis of experimental studies with C. jejuni strain 81–176 reported elsewhere [8–10]. The study was designed to permit escalation or de-escalation of the inoculum dose by 1 log_{10}, with a target attack rate of ≥75% of subjects meeting the primary end point of campylobacteriosis. The clinical protocol was performed under an Investigational New Drug Application and was approved by all Institutional Review Boards. A 4-member independent data safety and monitoring board was convened.

Subject recruitment and eligibility. Subjects were healthy adults aged 18–50 years. All were counseled, provided informed consent, and passed a test of comprehension. Excluded subjects included those with chronic or active illnesses, including arthritis, gastritis, and neurologic disease (including GBS). Also excluded were subjects who were food-handlers and those with susceptible contacts (i.e., young children and elderly or immunocompromised persons). Neurologic and joint examinations were performed at screening and throughout the study. Laboratory exclusions included positive tests for human leukocyte antigen B27, hepatitis B surface antigen, human immunodeficiency virus type 1, and hepatitis C antibodies; im-
Table 1. Clinical and Microbiologic Characteristics of Subjects Challenged with *Campylobacter jejuni* CG8421

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cohort 1, 1 x 10^6 CFUs (n = 8)</th>
<th>Cohort 2, 1 x 10^6 CFUs (n = 7)</th>
<th>Cohort 3, 1 x 10^5 CFUs (n = 8)</th>
<th>Cohorts 2 and 3, 1 x 10^5 CFUs (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacteriosis</td>
<td>8 (100)</td>
<td>6 (86)</td>
<td>8 (100)</td>
<td>14 (93)</td>
</tr>
<tr>
<td>Any diarrhea</td>
<td>8 (100)</td>
<td>6 (86)</td>
<td>8 (100)</td>
<td>14 (93)</td>
</tr>
<tr>
<td>Severe diarrhea</td>
<td>6 (75)</td>
<td>3 (43)</td>
<td>8 (100)</td>
<td>11 (73)</td>
</tr>
<tr>
<td>Time to first diarrheal stool, median h (IQR)</td>
<td>60 (40–92)</td>
<td>72 (55–94)</td>
<td>86 (72–99)</td>
<td>73 (71–98)</td>
</tr>
<tr>
<td>Time to campylobacteriosis,a median h (IQR)</td>
<td>64 (35–91)</td>
<td>76 (71–95)</td>
<td>86 (65–100)</td>
<td>76 (71–100)</td>
</tr>
<tr>
<td>Diarrheal stool, median no (IQR)</td>
<td>13 (5–16)</td>
<td>8 (4–12)</td>
<td>11 (10–17)</td>
<td>10 (7–17)</td>
</tr>
<tr>
<td>Maximum diarrhea volume, median mL per 24 h (IQR)</td>
<td>806 (429–1381)</td>
<td>711 (562–1060)</td>
<td>784 (550–960)</td>
<td>752 (562–1038)</td>
</tr>
<tr>
<td>Diarrheal stool, median no (IQR)</td>
<td>1279 (396–2263)</td>
<td>908 (748–1220)</td>
<td>1157 (714–1746)</td>
<td>1026 (748–1590)</td>
</tr>
<tr>
<td>Receipt of early antibiotic treatment</td>
<td>7 (88)</td>
<td>5 (71)</td>
<td>8 (100)</td>
<td>13 (87)</td>
</tr>
<tr>
<td>Duration of inpatient stay, median days (IQR)</td>
<td>6.5 (6.0–8.5)</td>
<td>9.0 (7.0–9.0)</td>
<td>7.5 (7.0–8.0)</td>
<td>8.0 (7.0–9.0)</td>
</tr>
<tr>
<td>Gastrointestinal symptom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>2 (25)</td>
<td>5 (71)</td>
<td>8 (100)</td>
<td>13 (87)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0 (0)</td>
<td>2 (29)</td>
<td>3 (38)</td>
<td>5 (33)</td>
</tr>
<tr>
<td>Dysentery</td>
<td>2 (25)</td>
<td>1 (14)</td>
<td>0 (0)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Occult blood positive (faecal)</td>
<td>7 (88)</td>
<td>3 (43)</td>
<td>7 (88)</td>
<td>10 (67)</td>
</tr>
<tr>
<td>Abdominal cramping, any</td>
<td>4 (50)</td>
<td>7 (100)</td>
<td>6 (75)</td>
<td>13 (87)</td>
</tr>
<tr>
<td>Abdominal cramping, severe</td>
<td>1 (13)</td>
<td>1 (14)</td>
<td>2 (25)</td>
<td>3 (20)</td>
</tr>
<tr>
<td>Systemic symptom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>4 (60)</td>
<td>2 (29)</td>
<td>3 (38)</td>
<td>5 (33)</td>
</tr>
<tr>
<td>Headache</td>
<td>6 (75)</td>
<td>2 (29)</td>
<td>8 (100)</td>
<td>10 (67)</td>
</tr>
<tr>
<td>Myalgias</td>
<td>4 (50)</td>
<td>2 (29)</td>
<td>3 (38)</td>
<td>5 (33)</td>
</tr>
<tr>
<td>Chills</td>
<td>5 (63)</td>
<td>1 (14)</td>
<td>2 (25)</td>
<td>3 (20)</td>
</tr>
<tr>
<td>Microbiologic feature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to shedding, median h (IQR)</td>
<td>22.9 (21–47)</td>
<td>42.3 (25–55)</td>
<td>27.4 (26–28)</td>
<td>27.5 (26–51)</td>
</tr>
<tr>
<td>Time to clearance, median h (IQR)</td>
<td>20.9 (16–30)</td>
<td>35.0 (13–52)</td>
<td>17.9 (16–22)</td>
<td>21.3 (15–35)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no (%) of subjects, unless otherwise indicated. CFU, colony-forming unit; IQR, interquartile range.

*a* Campylobacteriosis diagnosis met either by diarrhea criteria or by fever with gastrointestinal symptoms.

munoglobulin (Ig) A deficiency, pregnancy, and hematology or serum chemistry abnormalities.

Subjects were also excluded if they had a clinical history of *Campylobacter* infection (ie, any diarrheal episode while traveling to a country where *Campylobacter* is endemic or a culture-confirmed infection). Subjects were also excluded on the basis of immunological results; immunological standards for exclusion were defined as a serologic response to glycine-extracted (GE) antigens of *C. jejuni* CG8421 (IgA end point titer >1:2000) or a cell-mediated response to *C. jejuni* (IFN-γ level >400 pg/mL after in vitro stimulation of peripheral blood mononuclear cells [PBMCs] with formalin-fixed whole-cells of *C. jejuni* CG8421) [9, 10]. Participants in prior *C. jejuni* vaccine or challenge studies were also excluded.

**Challenge strain.** *C. jejuni* strain CG8421 (serotype HS 23, 36) was isolated from a United States soldier in Thailand who presented with 4 days of dysentery, fever, nausea, and body aches. The strain was susceptible to nalidixic acid, ciprofloxacin, and azithromycin and was resistant to tetracycline. Chemical analysis of the LOS core indicated a structure that lacked all glycolipid mimicry [15]. Moreover, complete genome sequencing confirmed the absence of genes for synthesis of N-acetyl neuraminic acid [15]. The strain invades INT407 and Caco-2 cells at levels <5% that of the highly invasive 81–176 strain [11, 15]. The genome sequence confirmed genes encoding cytolethal distending toxin [15]. The *C. jejuni* CG8421 master seed lot was grown under Good Manufacturing Practice (Charles River Laboratories) conditions and frozen at −70°C. A master seed lot vial was thawed for each cohort, was plated on Mueller-Hinton media, and was grown for 20–22 h at 42°C under microaerobic (10% CO₂, 5% O₂, 85% N₂) conditions. After confirmation of identity, plates were flooded with phosphate-buffered saline, and bacterial lawns were harvested. Bacterial suspensions were diluted using optical density growth curves measured with an ultraviolet spectrometer. Final 1-mL aliquots were added to 29 mL of sterile 1.3% bicarbonate solution im-
Figure 2. Comparison of immunoglobulin (Ig) G, IgG and IgA responses in plasma and fecal samples between cohorts dosed with 1 × 10⁵ and 1 × 10⁶ colony-forming units of Campylobacter jejuni CG8421.

Immediatedly prior to dosing. Challenge inoculum verification was performed by enumeration, in triplicate, of viable counts on Mueller-Hinton agar.

On the day of dosing (day 0), subjects fasted for 90 min, drank 120 mL of sterile USP-grade bicarbonate buffer solution, and were dosed 1 min later with the bacterial challenge inoculum, within 30 min of preparation. The subjects were observed, and vital signs were measured 30 min after dosing. Subjects fasted for an additional 90 min after dosing.

End points and definitions. The study’s primary end point was campylobacteriosis, defined as a clinical illness with documented C. jejuni infection occurring within 144 h (6 days) after dosing, which included either diarrhea or a febrile illness (temperature, ≥38°C) without diarrhea but with at least 2 associated gastrointestinal symptoms.

All stools passed were documented for time, weight, and presence of gross or occult blood. Specimens were graded 1–5 as described elsewhere, with grades 3–5 defined as diarrhea [16]. Diarrhea was defined as mild (1 loose/liquid stool ≥200 g in any 48-h period, or ≥3 loose/liquid stools in a 24-h period), moderate (4–5 diarrheal stools in 24 h or 401–800 grams within 24 h), or severe (≥6 loose/liquid stools in 24 h or ≥800 grams of loose/liquid stools in 24 h). Dysentery was defined as ≥2 episodes of gross blood in a loose stool. All symptoms were classified as mild (noticeable, short-lived, not requiring intervention or change in activities), moderate (interrupting some activities), or severe (interrupting all activities).

Clinical monitoring/management. Subjects were admitted to the inpatient research ward. Before dosing, stool sample cultures excluded bacterial and protozoan pathogens. Vital signs were monitored, including orthostatic blood pressure measurements. All diarrheal losses were replaced with oral rehydration solution. Intravenous fluids were administered if subjects met criteria for abrupt onset of voluminous diarrhea (≥300 g single stool or ≥400 g over 2 h), hypovolemia, or at physician discretion. Blood cultures were performed for subjects with a fever (temperature, ≥38°C). Electrolyte tests were performed if intravenous fluids were used.

Subjects were treated with strain-sensitive antibiotics no later than 144 h after challenge. Antibiotics were administered early for any 1 of the following reasons: moderate or severe diarrhea and diarrhea of any severity with either ≥2 severe symptoms (abdominal pain/cramps, nausea, myalgias, arthralgias, or gross blood in stool), fever (temperature, ≥38°C), or any vomiting. Antibiotics included 500 mg of azithromycin twice daily for 3 days in cohort 1 and 5 days in cohort 2. Subjects in cohort 3 received 500 mg of oral ciprofloxacin 500 twice daily and 500 mg of azithromycin twice daily for 5 days.

Subjects were discharged after antibiotics were started, symptoms were resolved, and 2 stool cultures (≥12 h apart) were negative for C. jejuni. After discharge, stool cultures for C. jejuni were performed on day 14, 21, 28, and 35 after dosing. Subjects were followed up for safety for 6 months.

Stool microbiology. The first 2 stools of each day were cultured within 4 h. After subjects started antibiotics (cohort 2 and 3), enhanced culturing techniques were used (per specimen, 0.5 g stool was homogenized into 1 mL of phosphate-buffered saline and was plated onto 6 plates) to maximize detection.

Immunological studies. Peripheral blood samples were collected before and after dosing (day 0, 1, 3, 5, 7, 9, 14, and 28) in EDTA tubes (Becton Dickinson). Stool samples were collected for detection of fecal IgA before and after (days 4, 7, 9, 14, and 28) infection and were frozen at −70°C, within 2 h after sample collection.

To determine systemic humoral immune responses, antigen-specific serum IgA and IgG levels were determined by enzyme-linked immunosorbent assay with use of GE antigens.
from homologous strains, as described elsewhere [17, 18]. IgA antibody-secreting cell (ASC) responses to C. jejuni GE antigens (3 μg/mL) were enumerated as described elsewhere [19].

To analyze mucosal humoral responses, levels of total and antigen-specific fecal IgA to C. jejuni CG8421 GE antigens were determined by enzyme-linked immunosorbent assay, as described elsewhere [17, 20]. Antigen-specific fecal IgA titers were adjusted to 1000 μg/mL of total IgA. Cellular (IFN-γ) responses were determined as previously described [10] in PBMCs collected at screening, prior to infection (day 0), and at 28 days after infection. Inflammatory responses were measured using kits for detection of lactoferrin (Leuko-Test; Inverness Medical Professional Diagnostics) and C-reactive protein (CRP) (RapiTex; Dade Behring). Undetectable levels of CRP were assigned a value of 3 (detection limit, 6 μg/mL). Reported values are the maximum CRP levels detected after C. jejuni dosing.

Statistics. Data were recorded on case report forms, then moved into an electronic database with 100% verification. Kruskal-Wallis 1-way analysis of variance (ANOVA) was used for continuous variables, and a Fisher’s exact test was used for nominal variables. Time-to-event analyses were performed for the time to diarrhea onset after dosing with use of a Cox proportional hazards model.

For serologic and fecal IgA responses, reciprocal end point titers were natural-log converted for comparisons across study groups by a repeated measures ANOVA, with study group as the between-subject factor and sample collection time points as the repeated factor. Seroconversion and fecal IgA response were denoted as a ≥4-fold increase over the baseline titer and reciprocal titer >10 after challenge. Maximum ASC levels and the amount of IFN-γ production after dosing were compared using a Kruskal-Wallis 1-way ANOVA. A positive ASC response was defined as ≥5 antigen-specific ASCs per 1 × 10⁶ peripheral blood mononuclear cells. Median (IQR) changes in IFN-γ from baseline (day 0) to day 28 after challenge were 555.8 (347.9–1039.5) pg/mL for cohort 1 and 476.2 (319.1–679.2) pg/mL for cohorts 2 and 3.

### Table 2. Antigen-Specific and Inflammatory Immune Responses in Subjects Challenged with Campylobacter jejuni CG8421

<table>
<thead>
<tr>
<th>Assay</th>
<th>Cohort 1, 1 × 10⁶ CFUs (n = 8)</th>
<th>Cohorts 2 and 3, 1 × 10⁶ CFUs (n = 15)</th>
<th>Severe diarrhea, response ratea</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (serum) b</td>
<td>37.5 (8)</td>
<td>53.3 (7)</td>
<td>Yes (n = 17)</td>
</tr>
<tr>
<td>IgA (serum) b</td>
<td>87.5 (8)</td>
<td>86.7 (13)</td>
<td>No (n = 6)</td>
</tr>
<tr>
<td>ASC (IgA) c</td>
<td>87.5 (62)</td>
<td>100 (90)</td>
<td>94.1 (96)</td>
</tr>
<tr>
<td>IgA (feces) d</td>
<td>87.5 (48)</td>
<td>100 (54)</td>
<td>94.1 (53)</td>
</tr>
<tr>
<td>IFN-γ f</td>
<td>87.5 (5)</td>
<td>93.3 (8)</td>
<td>89.2 (6)</td>
</tr>
<tr>
<td>Lactoferrin (fecal)</td>
<td>87.5 (2)</td>
<td>66.7 (2.5)</td>
<td>50.0 (2.5)</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>87.5 (12)</td>
<td>86.7 (24)</td>
<td>66.7 (24)</td>
</tr>
</tbody>
</table>

**NOTE.** ASC, antibody-secreting cell; CFU, colony-forming unit; IFN, interferon; Ig, immunoglobulin.

- a Response rates are calculated as the percentage of subjects meeting the definition of response. With the exception of ASC responses, the number in parentheses is the median peak fold increase from baseline titer among responders. For ASC response, the number in parentheses represents the median maximum number of ASCs detected among responders.

- b Response defined as a ≥4-fold increase in baseline titer and a reciprocal titer >10 after challenge.

- c Response defined as ≥5 ASCs per 1 × 10⁶ peripheral blood mononuclear cells.

- d Response defined as a ≥4-fold increase in total IgA–adjusted baseline titer and a total IgA–adjusted reciprocal titer >10 after challenge.

### RESULTS

One hundred twenty-six healthy adult subjects were screened (Figure 1). Fifty-seven subjects passed eligibility screening; 23 subjects were entered into the study and received a challenge inoculum of C. jejuni CG8421 in 3 sequential cohorts. Table 1 summarizes clinical and microbiological findings. Subjects in the first cohort (n = 8) received 0.97 × 10⁶ CFUs, yielding a 100% attack rate. Subjects in the second cohort (n = 7) re-
received $0.84 \times 10^5$ CFUs, with an 86% attack rate, and those in the third cohort received $0.54 \times 10^5$ CFUs, with an attack rate of 100% ($n = 8$). The attack rate did not vary significantly between inoculum levels and met the predefined target of $\geq 75\%$. Of the 23 subjects, all but 1 subject (in cohort 2) met the campylobacteriosis end point, leaving an overall attack rate of 96% for all doses.

The challenge model was safe with no serious adverse events reported, including bacteremia or hypotension. Symptoms for most subjects were consistent with moderate to severe campylobacteriosis. Subjects experienced diarrheal illnesses beginning a median of 72.3 h after dosing, and it consisted of watery, moderate- to large-volume diarrhea (Table 1). Diarrheal episodes were also characterized by frequent stools, with a median of 6.3 stools per 24 h. Dysentery was uncommon, occurring in 3 (13%) subjects, but occult blood was detected for 17 (74%) subjects. Other gastrointestinal symptoms included abdominal cramps ($n = 17$; 74%) and nausea ($n = 15$; 65%). Fever (temperature, $>38.0^\circ C$) was observed in 9 subjects (39%); temperature range, 38.2$^\circ C$–39.5$^\circ C$; 2 (8.7%) had higher fever (temperature, $\geq 39^\circ C$). Headache (53.3%) and myalgias (39.1%) were also common. Intravenous fluids was administered to 8 (35%) persons on the basis of the protocol-driven criteria for diarrhea volume losses or abrupt onset of diarrhea, although none of the subjects showed signs or symptoms of hypovolemia, and all ingested oral fluids without difficulty.

All subjects who were undergoing primary infection shed the challenge strain of C. jejuni a median of 27.2 h after dosing. After antibiotics were started, stool cultures cleared rapidly and were negative for C. jejuni a median of 21.3 h later.

Clinical and microbiologic differences between cohorts of patients who received $0.84 \times 10^5$ or $0.54 \times 10^5$ CFUs (the $10^5$ cohorts) and those who received $0.97 \times 10^6$ CFUs (the $10^6$ cohort) were not statistically significant. The median time from dosing to meeting the end point definition of Campylobacteriosis (vs onset of illness or first diarrheal stool) was 63.5 h for the $10^5$ cohort and 75.5 h for the two $10^6$ cohorts. The $10^5$ cohorts trended toward a later onset of disease, less-severe symptoms, and lower diarrhea volumes. No postinfectious neurologic, gastrointestinal, or rheumatologic sequelae were noted in any subject.

Eighty-seven percent of subjects mounted an IgA response to C. jejuni GE antigens after infection, with a median 8-fold ($10^5$ cohort) and 13-fold ($10^6$ cohorts) increase in IgA (Figure 2 and Table 2). A less prominent IgG response was observed (48%); a lower fold increase was observed in fewer subjects than for IgA (8-fold and 7-fold increases for the $10^5$ and $10^6$ cohorts, respectively).

All but 1 subject demonstrated fecal IgA and IgA ASC responses, which were slightly higher in the $10^6$ cohorts than in the $10^5$ cohort (54-fold vs 48-fold increase; Figure 2). ASC counts were also higher in the $10^6$ cohorts, compared with the $10^5$ cohort (median, 90 vs 52 ASCs per $1 \times 10^6$ PBMCs; Table 2 and Figure 3).

As part of inclusion criteria, all subjects had IFN-$\gamma$ levels <400 pg/mL prior to receiving the C. jejuni CG8421 inoculum. All subjects except 2 had a $>2$-fold IFN-$\gamma$ increase after challenge (91%). The IFN-$\gamma$ levels in the $10^5$ cohort demonstrated a median IFN-$\gamma$ increase of 555.8 pg/mL, compared with an increase of 476.2 pg/mL in the $10^6$ cohort (Table 2 and Figure 4).

Increases in the levels of inflammatory markers CRP and fecal lactoferrin were also observed in most subjects and are stratified by diarrhea severity in Table 2. Subjects with severe diarrhea had a 2-fold higher CRP level, whereas subjects without severe diarrhea had higher fecal and serum IgA levels.

Two subjects experienced recrudescence of C. jejuni shedding after a complete course of antibiotics (azithromycin) therapy and $\geq 2$ negative stool cultures for C. jejuni infection. The relapse strain was confirmed to be CG8421 by pulsed-field gel electrophoresis.

![Figure 3](image.png)

**Figure 3.** Comparison of antigen-specific antibody-secreting cell (ASC) responses between cohorts dosed with $1 \times 10^5$ and $1 \times 10^6$ colony-forming units of Campylobacter jejuni CG8421. ASC counts were 0 ASCs per $1 \times 10^6$ peripheral blood mononuclear cells (PBMCs) in all subjects prior to challenge.

![Figure 4](image.png)

**Figure 4.** Comparison of pre- and post-dose in vitro Campylobacter-specific interferon $\gamma$ (IFN-$\gamma$) production in cohorts dosed with $1 \times 10^5$ and $1 \times 10^6$ colony-forming units of Campylobacter jejuni CG8421.
trophoresis and polymerase chain reaction (data not shown). One subject (cohort 1; recrudescence day 28) lacked antigen-specific immunologic responses to Co. jejuni infection [21]. The second subject (cohort 2; recrudescence day 21) remained asymptomatic, but shed C. jejuni CG8421 that had developed resistance to macrolides. Both subjects remained clinically well and had documented eradication of C. jejuni after further treatment with antibiotics to which the strains were sensitive.

DISCUSSION

Human challenge models of C. jejuni are critical tools to evaluate the efficacy of investigational human Campylobacter vaccines and to enhance our understanding of pathogenesis and immunity. C. jejuni strain 81–176 had been used as the standard reference strain for human infection studies of campylobacteriosis for over 2 decades, predating the current understanding of the pathogenesis of C. jejuni–associated GBS. No neurologic sequelae have occurred after exposures to strain 81–176, which include infections from the original outbreak, use in 2 human challenge studies, and use as a killed whole-cell vaccine component [8–10]. Nevertheless, strain 81–176 synthesizes an LOS core composed of GM2 and GM3 gangliosides with the potential for LOS phase variation, which may cause GBS through molecular mimicry [11]. Moreover, after human infection with 81–176, some subjects mount transient anti-ganglioside (GM1) antibodies [14]. Collectively, these data suggest that continued use of strain 81–176 in human studies is an unacceptable safety risk.

This work has built on the lessons learned from studies of 81–176 to refine an experimental human challenge model of campylobacteriosis with use of a recently characterized strain of C. jejuni, CG8421, which lacks ganglioside mimicry, including sialylation of LOS [15]. It has been suggested recently that sialylation of LOS cores of C. jejuni is required for in vitro invasion of epithelial cells, and strain CG8421 is markedly less invasive in vitro than 81–176 [22, 23]. However, the data described here clearly demonstrate that sialylated LOS cores are not required for human diarrheal disease.

In addition to mitigating the risk of GBS, our CG8421 model was also safe, with a consistently high attack rate and robust immune responses when using a low concentration of inoculum (1 × 10^6 CFUs). Most importantly, serious adverse events, such as hypotension/shock or postinfectious sequelae, did not occur. Recrudescent infection occurred after receipt of antibiotics in 2 subjects, after the subjects had become asymptomatic and stool cultures were negative, which may reflect the epidemiology of Campylobacter in light of protracted subject monitoring. Shedding was eliminated with further antibiotic treatments and modifications to the microbiological surveillance techniques (increased volume and number of cultures per specimen). Antibiotic treatment permitted continued safe application of the model.

Clinical disease after CG8421 infection consisted of moderate- to large-volume diarrhea with symptoms of headache, myalgias, and abdominal cramping. Fever occurred in 9 (39%) of our subjects and was severe (temperature, ≥39°C) in 2 persons (9%). No subject had small-volume mucoid stools suggesting colitis, which may be because of the prompt use of antibiotics. Significant clinical differences were not found between patients who received 1 × 10^6 and 1 × 10^7 CFUs of C. jejuni, although subjects who received the lower dose had a slightly longer incubation period and a lower total volume of loose stools. After initiation of antibiotics in subjects, symptoms resolved and stool cultures cleared rapidly.

The immunologic data obtained enhance our understanding of the natural human immune response to C. jejuni. Almost all subjects demonstrated significant IgA responses to infection in serum and stool specimens and as IgA ASCs in circulation. Similarly, IFN-γ responses to infection were observed in all subjects except 1, who mounted no detectable antigen-specific immune responses and experienced a recrudescence of infection [21]. Although quantitative and qualitative immune correlates of protection from campylobacteriosis remain largely unknown, additional insights may be provided by a detailed evaluation of this subject.

This new model contributes to refinements made to C. jejuni challenge models since the 1980s [8–10]. With the addition of an exclusion criteria based on cellular immunity (IFN-γ) as a marker of C. jejuni exposure, an attack rate >75% was accomplished at a 4-log10 lower dose than that required with strain 81–176 [10]. Screening for IFN-γ has likely yielded a more susceptible subject population, but this hypothesis remains to be directly tested. Standardized Food and Drug Administration definitions of diarrhea were also used to ensure consistency across future C. jejuni models and for comparison of diarrhea severity with other enteric infections (ie, Shigella) [24].

The CG8421 challenge model provides an important tool to evaluate human Campylobacter vaccines and will advance our understanding of how to prevent campylobacteriosis and its significant postinfectious sequelae.

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