

Lack of Homologous Protection Against *Campylobacter jejuni* CG8421 in a Human Challenge Model

Beth D. Kirkpatrick,¹ Caroline E. Lyon,¹ Chad K. Porter,² Alex C. Maue,² Patricia Guerry,² Kristen K. Pierce,¹ Marya P. Carmolli,¹ Mark S. Riddle,² Catherine J. Larsson,¹ Douglas Hawk,² Elizabeth A. Dill,¹ A. Fingar,¹ Frederic Poly,² Kelly A. Fimlaid,¹ Fahmida Hoq,² and David R. Tribble³

¹University of Vermont College of Medicine, Unit of Infectious Diseases and Vaccine Testing Center, Burlington, Vermont; ²Naval Medical Research Center, Enteric Diseases Department, Silver Spring, Maryland; and ³Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences, Bethesda, Maryland

Background. *Campylobacter jejuni* is a common cause of diarrhea and is associated with serious postinfectious sequelae. Although symptomatic and asymptomatic infections are recognized, protective immunity is not well understood. Previous data suggests that interferon γ (IFN- γ) may be associated with protection. To better define the clinical and immunologic development of protective immunity to *C. jejuni*, we assessed the ability of an initial infection to prevent clinical illness after a second experimental infection.

Methods. Subjects with no clinical or immunologic evidence of prior infection with *C. jejuni* received an initial challenge with *C. jejuni* CG8421 with rechallenge 3 months later. The primary endpoint was campylobacteriosis, as defined by diarrhea and/or systemic signs. Close inpatient monitoring was performed. Serum immunoglobulin A (IgA) and immunoglobulin G (IgG), fecal IgA, IgA antibody-secreting cells (ASCs), and IFN- γ production were evaluated. All subjects were treated with antibiotics and were clinically well at discharge.

Results. Fifteen subjects underwent a primary infection with *C. jejuni* CG8421; 14 (93.3%) experienced campylobacteriosis. Eight subjects received the second challenge, and all experienced campylobacteriosis with similar severity. Immune responses after primary infection included serum IgA, IgG, ASC, and IFN- γ production. Responses were less robust after secondary infection.

Conclusions. In naive healthy adults, a single infection with CG8421 did not protect against campylobacteriosis. Although protection has been demonstrated with other strains and after continuous environmental exposure, our work highlights the importance of prior immunity, repeated exposures, and strain differences in protective immunity to *C. jejuni*.

Clinical Trials Registration. NCT01048112

Keywords. Campylobacteriosis; human challenge model; *Campylobacter jejuni*; homologous protection.

Campylobacter jejuni is among the most common causes of enteric infection worldwide, and its complex relationship with the human host is just starting to be understood. *C. jejuni* causes inflammatory enteritis

manifested by diarrhea or dysentery, fever, and abdominal cramping. Asymptomatic infection/colonization is also common, as described in children after repeated exposure to *C. jejuni* in resource-poor countries [1, 2]. Recently, incidence estimates of 1 symptomatic or asymptomatic *C. jejuni* infection every 2 years have also been reported in adults in developed countries [2].

C. jejuni infections have strong associations with postinfectious sequelae, strain variability, and increasing resistance to antibiotics. These include the demyelinating neurologic syndrome Guillain-Barré, chronic gastrointestinal symptoms, and postinfectious arthritis [3–6].

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Correspondence: Beth D. Kirkpatrick, MD, University of Vermont College of Medicine, Given Bldg, Beaumont Ave, Burlington, VT 05405 (beth.kirkpatrick@uvm.edu).

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The kinetics and composition of the human immune response to *C. jejuni* are poorly understood and difficult to evaluate in field settings because of the inability to know onset of infection, strain differences, and previous exposures. Human challenge models, in contrast, provide a controlled method to understand and define immunologic responses to infection and/or correlates of protection [7–9].

We and others have described the human challenge model development of *C. jejuni*, most recently using strain CG8421 [9]. This strain, which lacks ganglioside mimicry in its lipo-oligosaccharide, replaced strain 81-176, which expresses ganglioside 2 and ganglioside 3 and was epidemiologically linked to Guillain-Barré [7, 10]. Previous human challenge studies with 81-176 and A3249 demonstrated that *Campylobacter*-specific immunoglobulin A (IgA) and interferon γ (IFN- γ) are associated with resistance to clinical disease, suggesting these components might be important markers of protective immunity [7, 8].

To better define protective immunity to *C. jejuni* and to further develop the model, we challenged healthy, immunologically naive adults with *C. jejuni* CG8421 and then rechallenged subjects 3 months later with the same strain. As with previous challenge trials, this study was performed with the expectation that a primary infection would afford significant, if not complete, clinical protection after rechallenge [7, 8].

METHODS

The study was an open-label, inpatient trial of oral inoculation of *C. jejuni* strain CG8421. After comprehensive screening, naive subjects received 5.5×10^5 colony-forming units (CFUs) of *C. jejuni* CG8421, based on previous experimental studies [9]. Three months later, the same veteran individuals were chosen to receive a second inoculation of CG8421, at the same dose, and with identical follow-up. Three additional naive subjects were challenged with the veteran group. The clinical protocol was approved by all institutional review boards (Clinical Trials.gov: NCT01048112). An independent data safety and monitoring board was convened.

Endpoints and Definitions

The primary study endpoint was campylobacteriosis, defined as a clinical illness with documented *C. jejuni* infection, occurring within 144 hours (6 days) of dosing. Clinical illness included either diarrhea or a febrile illness ($\geq 38^\circ\text{C}$) without diarrhea but with at least 2 associated gastrointestinal symptoms (vomiting, abdominal cramping, tenesmus). Infection with *C. jejuni* was defined as a positive stool culture occurring >24 hours after dosing regardless of symptoms. All stools passed were documented for time, weight, and blood. Specimens were graded 1–5 as described, with grades 3–5 defined as diarrhea [9].

Diarrhea was defined as mild (one loose/liquid stool ≥ 300 g, or ≥ 2 loose/liquid stools ≥ 200 g in any 48-hour period, or ≥ 3 loose/liquid stools in a 24-hour period), moderate (4–5 diarrheal stools in 24 hours or 401–800 g within 24 hours) or severe (≥ 6 loose/liquid stools in 24 hours or >800 g of loose/liquid stools in 24 hours). Dysentery was ≥ 2 episodes of gross blood in a loose stool. All symptoms were classified as mild (noticeable, short-lived, not requiring intervention or changing activities); moderate (interrupting some activities), or severe (interrupting all activities). An additional index, which uses both measures of systemic illness (eg, fever) and gastrointestinal symptoms (eg, diarrhea severity, cramping), was used to measure severity of the overall clinical illness [8].

Subject Recruitment/Eligibility

Subjects were healthy, aged 18–50 years, with no evidence of prior *C. jejuni* exposure. Extensive screening procedures have been described [9]. Exclusions included gastrointestinal, neurologic, or rheumatologic disease. Immunologic exclusions were a serologic response to *C. jejuni* CG8421 glycine extracted antigens (IgA $>1:2000$ by reciprocal endpoint titer) or IFN- γ >400 pg/mL after in vitro stimulation of peripheral blood mononuclear cells (PBMCs) with formalin-fixed whole-cells of *C. jejuni* CG8421 [8]. Volunteers meeting the endpoint of campylobacteriosis in the first inoculation were eligible to receive the second. This group was rescreened using parameters above (except *C. jejuni* immuno-assays) to confirm continued eligibility. Any volunteer experiencing a serious adverse event or recrudescence of infection was ineligible for the second challenge.

Challenge Strain and Dosing Procedures

C. jejuni strain CG8421 (serotype Penner heat-stable 23, 36) was isolated and characterized as previously described. The lipo-oligosaccharide core of this strain lacks all ganglioside mimicry and the genes needed for synthesis of *N*-acetyl neuraminic acid, necessary for glycolipid mimicry associated with Guillain-Barré [11]. The master seed lot was grown under Good Manufacturing Practice, conditions for growth and preparation of the challenge inoculum, as described [9]. On the day of dosing (day 0), subjects fasted for 90 minutes, then drank 120 mL of sterile USP-grade bicarbonate solution, followed 1 minute later by the inoculum in 30 mL of bicarbonate solution. Subjects fasted for an additional 90 minutes after dosing. The dosing and follow-up procedures were the same for both inpatient periods [9].

Clinical Monitoring/Management

Subjects were continuously monitored as inpatients, as described, for either a single challenge episode or two identical challenge episodes [9]. All diarrheal losses were replaced with oral rehydration solution. Intravenous fluids were used if subjects met criteria for abrupt onset of voluminous diarrhea

(>300 g single stool or >400 g over 2 hours) or hypovolemia or at physician discretion. Blood cultures were performed for fever >38°C. Electrolyte levels were monitored if intravenous fluids were used. Stool microbiology to detect *C. jejuni* shedding was performed on the first 2 stools of each day, as described [9].

Subjects were treated with ciprofloxacin (500 mg twice daily) and azithromycin (500 mg daily) for 5 days, starting no later than 144 hours (6 days) after challenge. Two antibiotics were given, as per US Food and Drug Administration guidance from past challenge models [9, 12]. Antibiotics were given earlier for moderate or severe diarrhea or for diarrhea of any severity with either ≥ 2 severe symptoms of abdominal pain/cramps, nausea, myalgias, arthralgias, or gross blood in stool or fever $\geq 38^\circ\text{C}$ or any vomiting. Subjects were eligible for discharge after antibiotics had been started, 2 stool cultures (≥ 12 hours apart) were negative for *C. jejuni*, and symptoms were resolved. Stool cultures for *C. jejuni* were additionally performed on days 14, 21, 28, 35, 60, and 90 after dosing. Subjects were followed for safety for 6 months.

Any volunteer experiencing recrudescence of infection was confirmed to be shedding the challenge inoculum by polymerase chain reaction and had confirmation of antibiotic susceptibilities by minimal inhibitory concentration, as described [12, 13]. Unless antibiotic resistance had developed, oral ciprofloxacin and azithromycin were given for an additional 10 days. Subjects were monitored clinically for an additional 6 months and microbiologically for 5 weeks after shedding ended [12].

Immunological Studies

Sample Collection

For both dosing episodes, peripheral blood was collected in ethylenediaminetetraacetic acid tubes before and after dosing. Stool samples for fecal IgA were collected before and after infection (days 0, 4, 7, 9, 14, 28, 90) and frozen at -70°C within 2 hours of collection.

Assays methods have been described [9, 14]. Antigen-specific serum IgA and IgG were determined by enzyme-linked immunosorbent assay using homologous-strain glycine-extracted antigens. Total and antigen-specific fecal IgA levels to *C. jejuni* CG8421 glycine-extracted antigens were determined by enzyme-linked immunosorbent assay. Antigen-specific fecal IgA is represented as a ratio of specific/total fecal IgA. Total IgA was determined using monoclonal IgA1/A2 antibodies. Antibody-secreting cell (ASC) responses were enumerated, as described [8]. Briefly, plates were coated with *C. jejuni* glycine-extracted antigens (3 $\mu\text{g}/\text{mL}$) and incubated with 3.3×10^5 PBMCs per well in complete media. Secreted antibodies were detected with goat antihuman antibodies (0.25 $\mu\text{g}/\text{mL}$) and visualized with nitroblue tetrazolium (10 $\mu\text{g}/\text{mL}$)-5-bromo-4-chloro-3-indoylphosphate (5 $\mu\text{g}/\text{mL}$). Plates with spot counts $\geq 5/10^6$ PBMCs were considered positive [8]. For systemic cytokine responses, peripheral mononuclear cells (screening and 0, 28, 69, 90 days after

infection) were incubated for 48 hours; supernatants were used to determine IFN- γ .

Data Analysis

All data were entered into a Microsoft Access database with 100% verification. Proportion of subjects meeting the primary endpoint, campylobacteriosis, was compared across baseline *Campylobacter* status, naive versus veteran. Analyses of secondary clinical outcomes included comparisons of the proportion with clinical outcomes as well as the distribution of diarrhea severity, stool frequency, stool volume, maximum temperature, time to illness, and time to infection across study groups.

Immunologic response definitions were consistent with prior studies of this strain [9]. Briefly, serologic and cell-mediated immunity responses were defined as a ≥ 4 -fold and ≥ 2 -fold increase over reciprocal baseline titers, respectively, whereas ASC response was defined as ≥ 5 ASCs per 10^6 PBMCs. Comparison between naive and veteran subjects were made using nonparametric tests (Kruskal–Wallis, continuous data) and Fisher exact test (categorical data) unless assumptions were fulfilled for Student *t* or Pearson χ^2 . Paired *t* tests were also used to compare postinoculation responses to baseline. All statistical analyses were performed using SAS version 8.2 and were interpreted in a two-tailed fashion using $\alpha = 0.05$.

RESULTS

As shown in Figure 1, 70 healthy adult subjects were screened. Thirty-two subjects passed; 15 were enrolled and received a challenge inoculum of 5×10^5 CFUs *C. jejuni* CG8421. Twelve naive subjects were dosed during the first inpatient period. Eight veteran subjects and 3 naive subjects were dosed (or redosed) during the second inpatient period. The median age of the subjects was 23.2 years (interquartile range [IQR], 19.9–28.4). Twelve of 15 subjects were male. No subject experienced bacteremia, and all shed *C. jejuni* CG8421 after each dose.

Of the naive subjects, all met the campylobacteriosis endpoint except a single volunteer who shed *C. jejuni* but remained asymptomatic (92%). As shown in Table 1, clinical characteristics of naive volunteers, whether dosed in the first or second inpatient period, were similar. For this group, the median time to the campylobacteriosis endpoint was 52.3 (range, 32–62) hours. In addition to diarrhea (median volume, 1122 mL; range, 493–1621 mL), most volunteers experienced headache and abdominal cramping. Dysentery was seen in 2 (13.3%) subjects and fever in 8 (53%). Eight naive volunteers (53%) had severe diarrhea, and the median disease severity score was 9.5 (range, 0–14) [8]. All volunteers were asymptomatic at discharge and healthy upon readmission for the second dose.

Unexpectedly, the attack rate of campylobacteriosis in veteran subjects was unchanged. Despite previous exposure to *C. jejuni*

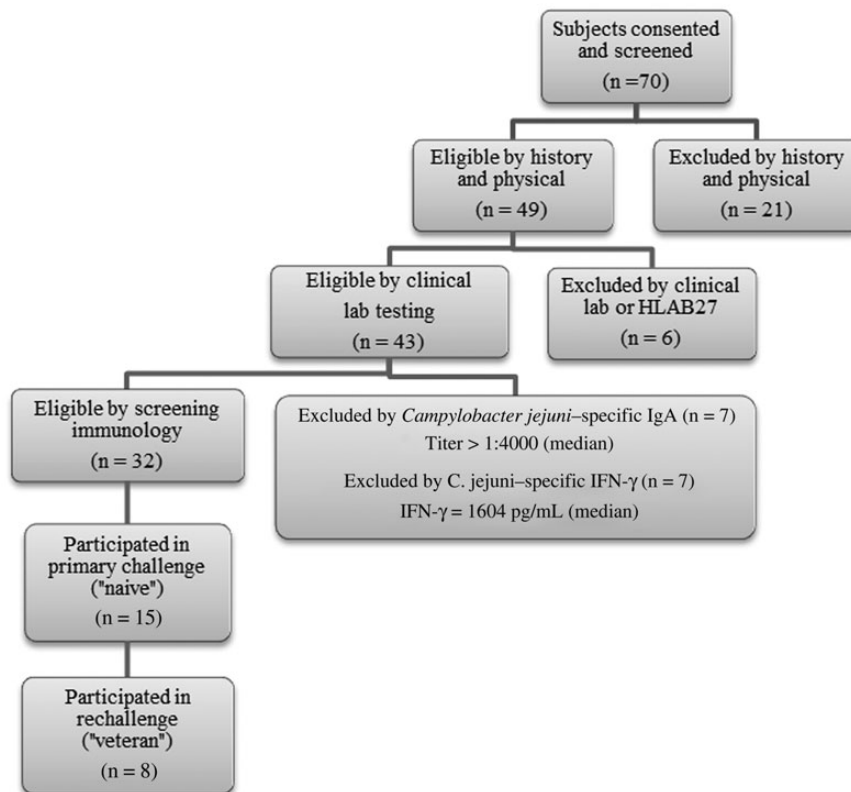


Figure 1. Screening eligibility and enrollment of volunteers participating in the *Campylobacter jejuni* challenge and rechallenge trial. Abbreviations: IFN- γ , interferon γ ; IgA, immunoglobulin A.

CG8421, volunteers had neither protection from reinfection nor an attenuated clinical illness (Table 1). The median time to first diarrhea stool was slightly longer in veteran subjects (51.1 hour; IQR, 37.0–67.3) than in naive subjects (45.9 hours; IQR, 43.7–51.2) as was the time to reach the endpoint of campylobacteriosis (naive subjects, 52 hours; veteran subjects, 54 hours). Nevertheless, all (100%) veterans met the clinical endpoint of campylobacteriosis, and more veteran subjects experienced severe diarrhea (n = 7; 88%) than during primary infection. Total stool volume remained largely unchanged (median, 1122; range, 900–1669).

A few differences in clinical symptoms were noted; nausea, in particular, was more common in naive subjects (73% vs 25%; $P = .039$). Systemic complaints of fever and headache were also less frequent in veterans; however, numbers were small. As noted, more veteran subjects met the criteria for severe diarrhea (n = 7; 87.5%) than those receiving their first dose (n = 8; 53.3%), although this difference was not statistically different. When naive and veteran subjects were compared using a diarrheal disease severity index, no differences were evident [8].

One naive subject during the first inpatient period experienced an asymptomatic recrudescence on day 28; stool culture

and polymerase chain reaction confirmed the CG8421 strain. Resolution was documented after additional antibiotics. No postinfectious complications were identified after 6 months of observation.

Immunologic responses were generally more robust after the first exposure to CG8421 (Table 2). Seroconversion (4-fold rise) was found in 67% (n = 10) and 53% (n = 7) of naive subjects after initial exposure to CG8421. After rechallenge, no veteran subjects had additional 4-fold rises. Of note, as seen in Figure 2A and 2B, most IgA responses had returned to baseline within 3 months, and responses to rechallenge were low (<4 fold-rises). In contrast, IgG levels did not fall to baseline after primary challenge but had minimal increases after the second dose and fell thereafter. For IgA ASCs, (Figure 3), 67% (n = 10) of naive subjects had a positive response, with a median spot number of 62 (Figure 3). Although most veterans had an ASC response, the maximal spot count was substantially lower (median, 14. 2). Similarly, as seen in Figure 4 and Table 2, approximately half of both naive and veteran subjects had a >2-fold rise in IFN- γ levels from baseline ($P = .04$), but no additional enhancement of IFN- γ response was seen after second intervention. IFN- γ levels fell close to baseline before the

Table 1. Clinical Outcomes of Subjects After Challenge With *Campylobacter jejuni* CG8421

Outcome	All Naive	Veteran
No.	15	8
Met primary endpoint of campylobacteriosis, No. (%)	14 (93)	8 (100)
Any diarrhea, No. (%)	14 (93)	8 (100)
Severe diarrhea, No. (%)	8 (53)	7 (88)
Time in hours to campylobacteriosis, median (IQR)	52.3 (46.9–56.1)	53.7 (48.3–73.7)
Time in hours to first diarrheal stool, median (IQR)	45.9 (43.7–51.2)	51.1 (37.0–67.3)
Number of diarrhea stools, grade 3–5, median (IQR)	14 (7–17)	13 (11–16)
Highest 24-h stool output number, grade 3–5, median (IQR)	7 (4–10)	8 (6–10)
Highest 24-h stool output volume, mL, grade 3–5, median (IQR)	661 (406–754)	710 (574–859)
Total diarrhea volume, mL, median (IQR)	1122 (493–1621)	1122 (900–1669)
Time in hours to first antibiotic dose, median (IQR)	57.0 (48.0–86.0)	58.1 (46.7–76.4)
Gastrointestinal symptoms, No. (%)		
Nausea	11 (73) ^a	2 (25) ^a
Vomiting	0 (0.0)	1 (13)
Abdominal pain/cramping, any	14 (93)	6 (75)
Abdominal pain/cramping, severe	3 (20)	0 (0)
Dysentery	02 (13.3)	1 (13)
Systemic symptoms, No. (%)		
Fever	8 (53)	2 (25)
Headache	12 (80)	5 (63)
Diarrheal disease Severity index, ^b median (range)	9.5 (01–14)	8 (4–11)

Abbreviation: IQR, interquartile range.

^a No associations were statistically significant, except nausea. $P = .04$.

^b See [8].

second dose in most subjects. Interestingly, despite a screening level of <400 pg/mL, one volunteer had an IFN- γ level at day 0 (before dosing) of 6000 pg/mL (suggesting exposure between screening and dosing), a level which would have excluded him from participation. This person (subject 011), was the only asymptomatic subject after dosing with *C. jejuni* CG8421.

DISCUSSION

We performed a rigorously monitored inpatient study in which primary infection with *C. jejuni* was followed 3 months later by

Table 2. Core Immunogenicity Assays After Challenge and Rechallenge With *Campylobacter jejuni* CG8421

<i>C. jejuni</i> CG 8421 Antigen	Assay	Study Group	
		Initial Challenge, % (Peak Fold Rise) (n = 15)	Rechallenge, % (Peak Fold Rise) (n = 8)
Glycine extract	ASC (IgA) ^a	66.7 (62.1)	87.5 (14.2)
	IgA (serum) ^b	66.7 (10.3)	0.0
	IgG (serum) ^b	53.3 (9.9)	0.0
Glycine extract	IgA (Fecal) ^c	66.7 (81.1)	37.5 (16.4)
Whole cells	Interferon γ ^d	57.2 (4.2)	50.0 (4.3)

Response rates are denoted by the percentage of volunteers meeting the responder definition. The number in parentheses is the median peak fold rise from baseline titer among responders. Antibody-secreting cell (ASC) responses are summarized as percentage of responders and then median maximum number of ASC among responders in parentheses. Response rates for the rechallenge are based on study day 90 “baseline” values.

Abbreviations: ASC, antibody-secreting cell; IgA, immunoglobulin A; IgG, immunoglobulin G.

^a Responder defined as ≥ 5 spots per 10^6 peripheral blood mononuclear cells.

^b Responder defined as ≥ 4 -fold rise in baseline titer and a postchallenge reciprocal titer > 10 .

^c Responder defined as ≥ 4 -fold rise total IgA adjusted baseline titer and a postchallenge total IgA adjusted reciprocal titer > 10 .

^d Responder defined as ≥ 2 -fold rise in interferon γ (pg/mL) per 10^6 peripheral blood mononuclear cells.

an identical second challenge. Our data confirm the safety and reproducibility of the CG8421 challenge model; however, the most important finding was a complete absence of homologous protection after second challenge. Rechallenge was associated with diarrheal illnesses clinically indistinguishable from primary infection. Although the onset of illness was slightly delayed in the rechallenge subjects, the attack rate (percentage with campylobacteriosis), severity of infection, diarrhea volume, and duration of shedding were not different.

Immunologically, we demonstrated responsiveness to primary infection, with increases from baseline in serum IgG and IgA, fecal IgA, IgA ASCs, and *C. jejuni*-specific IFN- γ . With the exception of serum IgG, all immune parameters fell toward baseline in the 3 months between doses in most volunteers. After the second dose, “boosting” or enhancement of these immune responses was minimal; median IgG responses remained elevated but did not rise after second challenge, and serum IgA and fecal IgA measurements demonstrated only minimal increases. Although the ASC pattern was not surprising because these cells are home to the gut mucosa, the unresponsiveness of systemic and fecal antibody responses and the lack of clinical protection make it clear that there is much to be learned about the development of protective immunity, including the optimal timing and dose of subsequent infections, the role of antigenic tolerance,

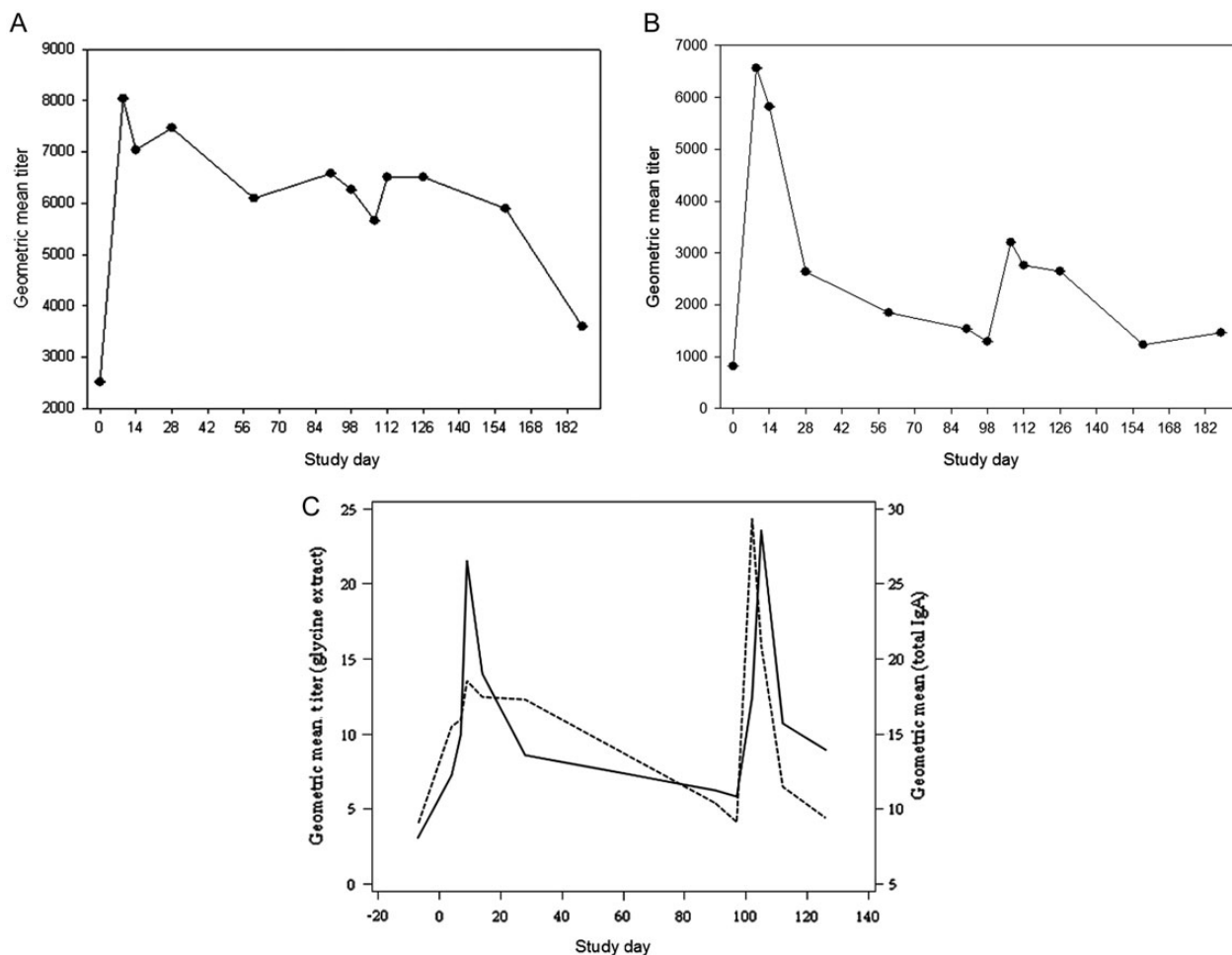


Figure 2. Serum immunoglobulin G (IgG), immunoglobulin A (IgA), and fecal IgA responses after challenge (day 0) and secondary challenge (day 98) with *Campylobacter jejuni* CG8421. *A*, Serum IgG responses after primary (day 0) and secondary (day 98) challenge with *C. jejuni* CG8421. *B*, Serum IgA responses after primary (day 0) and secondary (day 98) challenge with *C. jejuni* CG8421. *C*, Fecal IgA responses after primary (day 0) and secondary (day 98) challenge with *C. jejuni* CG8421. Hatched lines represent total fecal IgA. Solid lines represent *C. jejuni* antigen-specific fecal IgA. Abbreviation: IgA, immunoglobulin A.

and which components of the innate and adaptive responses contribute to protection.

We expected that measures of *C. jejuni*-specific IFN- γ would yield informative data reiterating its role in protection from clinical disease, as suggested from a prior challenge model with 81-176 [8]. To explore this question, only subjects without evidence of exposure to *C. jejuni* were enrolled. IFN- γ levels increased after primary challenge, but most fell toward baseline before redosing and were not significantly changed after the rechallenge. Thus, although we cannot confirm whether high predose levels of IFN- γ are associated with protection, it is clear that a single primary infection with *C. jejuni* CG8421 at the 10^5 CFUs dose will not reliably prompt elevated and sustained IFN- γ levels. Evaluation of the contribution and kinetics of IFN- γ -producing T lymphocytes is ongoing (K. Fimlaid, unpublished

data) and deserves further study in subjects with a range of IFN- γ levels at challenge and in subjects known to have multiple exposures.

Our data can be directly compared with 2 previous *C. jejuni* homologous strain re-challenge models using strains A3249 and 81-176: both demonstrated some protection from clinical disease [7, 8]. In the A3249 model, 2 volunteers were rechallenged with 10^6 CFUs 1 month after primary infection; neither developed illness. In a larger 81-176 trial, subjects were rechallenged with a higher dose of *C. jejuni* (10^9 CFUs) either 1–2 months (short-term veteran subjects) or 12 months (long-term veteran subject) after primary challenge. All short-term veteran subjects ($n = 7$) and 38% of long-term veteran subjects ($n = 7$) did not develop illness [9]. Notably, at similar doses (10^{5-6} CFUs) the campylobacteriosis attack rates for primary infection for

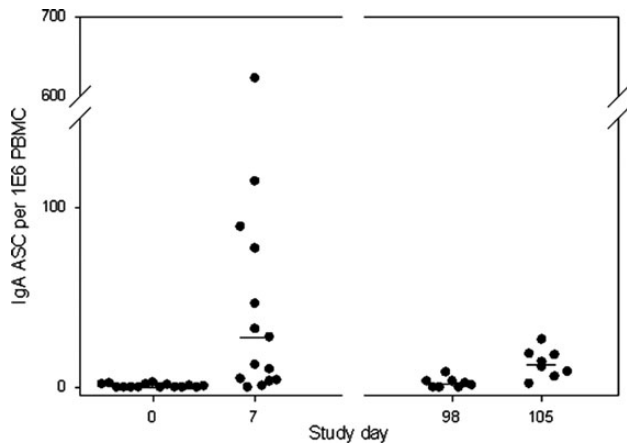


Figure 3. Immunoglobulin A antibody-secreting cell responses after primary and secondary challenge with *Campylobacter jejuni* CG8421. Horizontal lines denote the median number of antibody-secreting cells for each day. Abbreviations: ASC, antibody-secreting cell; IgA, immunoglobulin A; PBMC, peripheral blood mononuclear cell.

both A3249 and 81-176 are lower (19% and 60%, respectively) than at the 10^5 CFUs dose for CG8421 (93%). Taken together, although neither model excluded volunteers based on predosing IFN- γ (as in our work), these studies reiterate the significance of timing of subsequent infections in the development of protective immunity [15].

Our work contributes to recent contributions from large field trials, epidemiologic modeling studies, and experimental models [2, 16–18]. In addition to *Campylobacter*'s known importance as a cause of symptomatic disease in children in

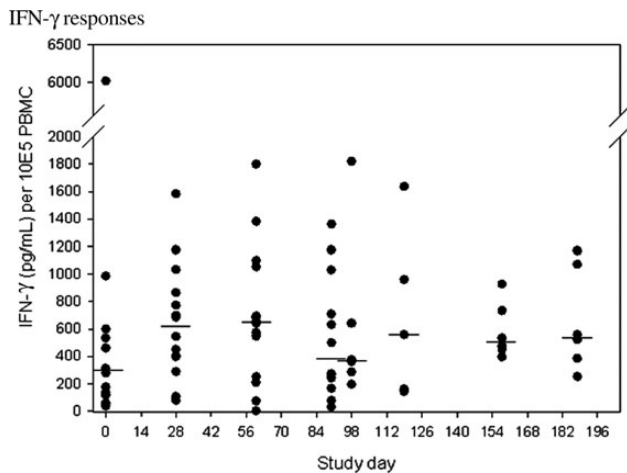


Figure 4. Interferon γ produced from peripheral blood mononuclear cells after primary and secondary challenge. Horizontal lines denote median interferon γ levels for each study day. Abbreviations: IFN- γ , interferon γ ; PBMC, peripheral blood mononuclear cell.

resource-poor regions, the frequency of asymptomatic infection in all populations is becoming more recognized (Karen Kotloff [Global Enteric Multicenter Study] and William Petri Jr [Malnutrition and Enteric Diseases], personal communication) [1, 2, 19, 20]. Symptomatic disease wanes with age and repeated exposures, although protection is likely short-lived in the absence of continuous exposure [2]. In contrast, and as illustrated here, naive adults in developed countries without prior exposure in childhood may experience a severe clinical disease, akin to the first infection in childhood in resource-poor settings [1]. In this population, with less frequent exposures, it seems unlikely that long-lived protective immunity would be expected after a single exposure to *C. jejuni*.

Recent evaluations have described the innate and adaptive immune components of campylobacteriosis in a variety of models. Human ex vivo intestinal epithelial and dendritic cell coculture systems show expansion of T helper 1 and T helper 17T cells after *C. jejuni* infection at the mucosal surface and suggest the importance of IFN- γ , interleukin 22, and the interleukin 17 family in the acute and effector phases of *C. jejuni* infection [18]. *C. jejuni* also induces dendritic cell maturity and induces proinflammatory cytokines and STAT3 activation [16, 17]. Our observations from this human homologous rechallenge model caution that despite clinical and laboratory markers of inflammation, innate responses, and evidence of immunogenicity, clinical protection from disease (and corresponding adaptive immune responses) cannot be assumed to follow. Thus, it is increasingly important to identify which components of the innate immune response directly contribute to future protective responses.

Our data reiterate that protection from campylobacteriosis may require multiple (symptomatic or asymptomatic) infections; this was particularly evident when naive volunteers were chosen. Future investigations to better understand protective immunity, should include an evaluation (challenge model or birth cohort in endemic setting) of repeated exposures. Impact of strain differences on immune responses is also important; data show that capsule variations modulate intestinal colonization and cytokine expression [21]. Finally, detailed immunologic evaluations (systemically and mucosal) should confirm the significance of T-cell populations, dendritic cell maturation, and STAT activation. We were fortunate to work with a highly controlled human population and hope these observations provide avenues of inquiry toward a comprehensive understanding of protective immunity to *C. jejuni* in human populations.

Notes

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The study protocol was approved by the University of Vermont Committees on Human Research and the Naval Medical Research Center institutional review boards in compliance with all applicable federal regulations governing the protection of human subjects.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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