Assessment of the Duration of Protection in Campylobacter jejuni Experimental Infection in Humans

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A human Campylobacter jejuni infection model provided controlled exposure to assess vaccine efficacy and investigate protective immunity for this important diarrheal pathogen. A well-characterized outbreak strain, C. jejuni 81-176, was investigated using a volunteer experimental infection model to evaluate the dose range and duration of protection. Healthy Campylobacter-seronegative adults received C. jejuni strain 81-176 via oral inoculation of 10⁵, 10⁶, or 10⁷ CFU (5 adults/dose), which was followed by clinical and immunological monitoring. Based on dose range clinical outcomes, the 10⁷-CFU dose (n = 31) was used to assess homologous protection at 28 to 49 days (short-term veterans [STV]; n = 8) or 1 year (long-term veterans [LTV]; n = 7) after primary infection. An illness dose effect was observed for naïve subjects (with lower doses, 40 to 60% of the subjects were ill; with the 10⁷-CFU dose, 92% of the subjects were ill) along with complete protection for the STV group and attenuated illness for the LTV group (57%). Partial resistance to colonization was seen in STV (25% of the subjects were not infected; 3-log-lower maximum excretion level). Systemic and mucosal immune responses were robust in naïve subjects irrespective of the dose or the severity of illness. In contrast, in STV there was a lack of circulating antibody-secreting cells (ASC), reflecting the local mucosal effector responses. LTV exhibited comparable ASC responses to primary infection, and anamnestic fecal IgA responses likely contributed to self-resolving illness prior to antibiotic treatment. Campylobacter antigen-dependent production of gamma interferon by peripheral blood mononuclear cells was strongly associated with protection from illness, supporting the hypothesis that TH1 polarization has a primary role in acquired immunity to C. jejuni. This study revealed a C. jejuni dose-related increase in campylobacteriosis rates, evidence of complete short-term protection that waned with time, and immune response patterns associated with protection.

Campylobacter species, the most common of which is Campylobacter jejuni, are zoonotic food- and waterborne bacterial enteropathogens (1, 2, 52). The gastrointestinal tracts of animals used for food, such as chickens, are the reservoirs for these common organisms (1). Worldwide, C. jejuni is among the most frequent causes of diarrhea, including traveler's diarrhea, and the spectrum of illness ranges from mild watery diarrhea to febrile dysentery (6, 14, 16, 21). Evidence for acquired immunity against C. jejuni has been obtained from epidemiologic studies performed in developing countries that documented that there is a decline in the incidence of disease with increasing age that is accompanied by a shift in the illness-to-infection ratio for children between 2 and 5 years old, development of resistance to colonization, and a shorter excretion period during convalescence (12, 44, 45). Age-related increases in C. jejuni-specific serology coincide with acquisition of resistance to infection and illness (9, 12, 45). In addition, a lower incidence of Campylobacter-associated diarrhea was observed in infants whose mothers had colostral Campylobacter-specific secretory IgA antibodies in their breast milk (13).

Evidence for acquired immunity against C. jejuni has also been obtained from studies performed in industrialized countries. Reduced C. jejuni-associated diarrhea rates correlated with increased levels of Campylobacter-specific IgA antibody in chronic consumers of raw milk on dairy farms compared to individuals exposed to raw milk for the first time, as well as a lower risk of diarrhea for travelers to regions where C. jejuni is hyperendemic (10, 11, 51). Black and colleagues performed the initial study of an experimental C. jejuni infection in humans (5a, 7). A human Campylobacter infection model provided controlled exposure cou-
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pleled with predefined endpoints to assess the efficacy of a candidate vaccine and to investigate pathogenesis and immunity. *C. jejuni* 81-176, a milk-borne outbreak strain (26), was one of the two strains investigated. This study documented the pathogenicity of *C. jejuni*; however, low and variable rates of attack (40 to 60%) without an illness dose response ($10^6$ to $10^7$ CFU delivered in skim milk) were observed (5a, 7). Infection with or without illness induced serologic and intestinal antibody responses. Higher prechallenge *C. jejuni*-specific (acid-extracted protein) serologic and jejunal fluid IgA levels in noninfected subjects than in infected subjects were observed, as were increased levels of jejunal fluid IgA during rechallenge in subjects who remained well after a second exposure.

A rare (approximately 1 in 1,000 to 3,000) *C. jejuni* enteritis cases (30, 42) but potentially life-threatening complication of *Campylobacter* infection is Guillain-Barré syndrome (GBS), a postinfectious polynuropathy that is a leading cause of paralysis (32, 49). Research evidence supports the hypothesis that the *C. jejuni*-GBS association is due to molecular mimicry, where peripheral nerve gangliosides share epitopes with *C. jejuni* outer lipooligosaccharide (LOS) cores, leading to a misdirected and harmful immune response (17, 25, 33, 54). Pret study characterization of the challenge strain revealed no evidence of ganglioside mimicry associated with GBS pathogenesis (12).

The campylobacteriosis clinical outcomes observed in the study of Black et al. were not sufficiently frequent or predictable based on the dose to support evaluation of vaccine efficacy. In the current study, two modifications were included, inoculum delivery with bicarbonate buffer and *C. jejuni*-specific serologic screening, based on post hoc analysis of data from the previous study (3). The change in the method of inoculum delivery was based on evidence obtained with a human Shigella infection model, which showed that 11/12 (92%) naïve subjects developed clinical illness when $1.4 \times 10^7$ CFU was delivered with bicarbonate buffer (2 g NaHCO$_3$ in 150 ml distilled water), compared to attack rates of 50 to 60% (upper limits) with challenge doses between $5 \times 10^5$ and $1 \times 10^6$ CFU in skim milk in previous studies (27).

In this study we report a refined human *C. jejuni* 81-176 infection model which demonstrated that there was a dose-related increase in campylobacteriosis rates and provided evidence of complete short-term protection that waned with time and cell-mediated immune response patterns that were associated with protection. This work improves the model for future application and provides directions for additional refinements.

(This study was presented in part at the 10th International Congress of Immunology, New Delhi, India, 1998, and at the 10th International Workshop on Campylobacter, Helicobacter and Related Organisms, Baltimore, MD, 1999.)

**MATERIALS AND METHODS**

**Study design.** This study included three stages: a dose range analysis ($10^5$, $10^6$, and $10^7$ CFU; 5 subjects/group), confirmation of the selected dose ($10^7$ CFU) with moderately severe ($\geq 70\%$) target campylobacteriosis, and homologous challenge. Subjects were rechallenged 1 to 2 months (short-term veterans [STV]) or 1 year (long-term veterans [LTV]) after the first infection.

Participants and subject eligibility. Healthy adults (ages 18 to 55 years) were recruited from the greater Washington, DC, area and enrolled after informed consent was obtained. The criteria for exclusion included poor health; personal or family history of GBS or inflammatory arthritis; macrolide or fluoroquinolone allergy; commercial food handler; clinically significant abnormalities as determined by physical examination or basic laboratory screening (complete blood count, serum chemistry, urinalysis, HIV-1 enzyme-linked immunosorbent assay [ELISA], hepatitis B surface antigen, hepatitis C virus ELISA, HIV-1, hepatitis B, and serum pregnancy test); abnormal bowel habits; regular use of antimicrobial or anticonvulsant agent; antacid therapy; antibiotic use (7 days before admission); use of immunosuppressive drugs; and participation in research involving another investigational product. Prior exposure to Campylobacter, as determined by history or serology (*C. jejuni* glycine extract IgA ELISA absorbance at 405 nm of $>0.5$ at 1:10,000 dilution, based on a previous report [3]), was also a basis for exclusion.

Characterization of *C. jejuni* strain. *C. jejuni* 81-176 (Penner serotype 23/36) was isolated from a child with watery diarrhea during a milk-borne outbreak (26) and was used in a previous challenge study (7). An isolate recovered from a subject with diarrhea was used to prepare a master seed lot at the Walter Reed Army Institute of Research Pilot BioProduction Facility (preserved in 15% glycerol at $-85^\circ$C). The challenge strain is susceptible to macrolides and fluoroquinolones.

Preparation and delivery of challenge inocula. Challenge inocula were prepared from the master seed stock as previously described (7), except that Mueller-Hinton soft agar was used to select for highly motile *C. jejuni* cells, which were pooled and subcultured on Brucella agar (Difco, Detroit, MI). Confluent growth was harvested in cold phosphate-buffered saline (PBS), and the optical density at 525 nm (OD$_{525}$) was adjusted to the appropriate target value (postinoculation number of CFU as determined by plate counting). A 5-ml aliquot was mixed with 150 ml of sterile water containing 2 g of sodium bicarbonate and then ingested with 90 ml of fasting before and after inoculation.

Dose selection, randomization, and blinding procedures. Subjects were randomly assigned to groups during the dose range double-blinded phase. After the dose range phase, the study was unblinded to allow selection of the dose used for subsequent studies.

**Clinical outcome definitions.** Infection was defined as any two of the following: *C. jejuni*-positive stool cultures $\geq 2$ h after oral inoculation. Diarrhea was defined as $\geq 3$ loose or liquid stools per 24-h period or $\geq 2$ loose or liquid stools with a volume $\geq 300$ ml in a 24-h period. The associated symptoms included in the clinical grading procedure were fever ($\geq 38.1^\circ$C), abdominal cramps, nausea, vomiting, tenesmus, and dysentery (gross blood in two specimens), and the following scale was used: grade 0, not present; grade 1, mild, barely noticed; grade 2, moderate, easily noticed, with some change in normal activities; and grade 3, severe, unable to perform normal activities. Campylobacteriosis, the primary endpoint, was defined as documented infection and clinical illness that occurred prior to the first dose of antibiotic. The campylobacteriosis categories used were mild (diarrhea with no associated symptom greater than grade 1), moderate (any two of the following indications: diarrhea, fever, one or more grade 2 associated symptoms, and one grade 3 associated symptom), and severe (any two of the following indications: diarrhea, with a total of $\geq 9$ stools, dysentery, high fever [$>38.0^\circ$C], and more than one grade 3 associated symptom).

Clinical monitoring and management. Subjects were evaluated daily while they were in a restricted-access inpatient ward until they were cleared for discharge following antibiotic therapy and resolution of clinical illness. Any subject who developed diarrhea received appropriate oral rehydration (intravenous if needed). All subjects received azithromycin (500 mg orally once a day for 5 days) irrespective of the infection or illness status 5 days after inoculation (the antibiotic start day was extended to day 7 for the LTV group). The subjects who met criteria for severe campylobacteriosis were treated immediately, whereas the other subjects received treatment 72 h after the onset of illness. Weekly clinical visits occurred until day 28 postinoculation to assess evidence of relapse and postinfection sequelae.

**Stool microbiology.** Routine stool bacteriology and parastoolitology studies were performed 1 week before admission. During the inpatient period, stools were evaluated to determine their volume, stool grade, visible and occult blood, and fecal leukocytes (once a day for diarrheal stools). At least one stool or rectal swab was cultured every day. The primary isolation media included Campylobacter blood agar (Campy-BAP, Remel), Butzler blood agar (B-BAP, Remel), and CAMPY-labile dilute enrichment medium incubated at 42°C in a 5% CO$_2$-10% CO$_2$-10% CO$_2$ environment. After 48 h of incubation, selected Campylobacter colonies were confirmed by Gram staining, the oxidase reaction, and the Lior 5 serotype. Quantitative estimates were obtained by plating serial dilutions of 100-
stool suspensions in PBS onto CAMPY-BAP. Plates with colony counts in excess of 300 colonies, at the highest dilution, were considered to contain 3 × 10^9 CFU/g.

*C. jejuni* immunology. (i) Sample collection. Peripheral blood was collected for plasma separation by centrifugation and then stored at −30°C, and peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient centrifugation followed by cryopreservation (4, 5). Stool samples were kept on ice, and 2- to 4-g aliquots were frozen within 10 h after collection at −70°C prior to processing.

(ii) Serology. Campylobacter glycerol extract (GE)-specific serum antibodies were measured by ELISA using porcine-digested GE as the antigen and isotype-specific horseradish peroxidase-conjugated goat anti-human immunoglobulin as described elsewhere (5, 34). Endpoint titers were determined by determining the reciprocal of the highest dilution that gave a net (antigen well − control well) absorbance of ≥0.15. Endpoint titers were log2 transformed and expressed as geometric mean titers.

(iii) ASC. Levels of antibody-secreting cells (ASC) were determined by using the enzyme-linked immunosorbent spot-forming assay described elsewhere (33). Nunc immunoplates were coated with 3 μg/ml of GE in pH 9.6 buffer. The PBMC's were washed and suspended at a concentration of 3.33 × 10^6 cells/ml in complete medium (CM) (RPMI containing 10% heat-inactivated fetal calf serum, 2 mM l-glutamine, and 50 μg/ml gentamicin). Following 6 h of incubation with PBMCs (3.33 × 10^6 PBMCs/ml in triplicate), the secreted antibodies were detected with alkaline phosphatase-conjugated goat anti-human IgA (0.25 μg/ml; KPL, Gaithersburg. MD). Spots were visualized with nitroblue tetrazolium (10 μg/ml)-5-bromo-4-chloro-3-indolylphosphate (BCIP) (5 μg/ml) (Sigma Chemicals, MO) in 0.05% agarose and counted with a dissecting scope. A sample with ≥5 ASC/10^6 PBMCs was considered a responder sample.

(iv) Fecal IgA. A 10% stool suspension was prepared in buffer containing protease inhibitors (3), incubated for 20 min at 4°C, and centrifuged, and the supernatant was frozen at −70°C. The total IgA content was determined by an ELISA using goat anti-human F(ab')2 (1 μg/ml; Jackson Laboratories, Bar Harbor, ME) as the capture antibody and isotype-specific horseradish peroxidase-conjugated goat anti-human IgA as the detecting antibody (22, 34). The Campylobacter-specific IgA content was determined by the ELISA described above, except that the incubation time was 3 h. Endpoint titers were determined and adjusted to 500 μg/ml of total IgA; samples with <50 μg/ml total IgA were excluded.

(v) IFN-γ assay. PBMCs were washed and suspended at a concentration of 5 × 10^5 viable cells/ml of CM (RPMI containing 1% heat-inactivated human AB serum, 2 mM l-glutamine, 5 × 10^-3 mM 2-mercaptoethanol, and 50 μg/ml gentamicin). Twenty microtiter wells of medium alone or medium containing 10^6 formalin-killed C. jejuni 81-176 whole cells in addition to 200 μl of CM containing 10^6 PBMCs was added to wells of round-bottom 96-well tissue plates in duplicate. Following 72 h of incubation at 37°C in 5% CO2, supernatants were collected and stored at −70°C until they were assayed to determine the gamma interferon (IFN-γ) levels by a capture ELISA using paired monoclonal antibodies, and the levels were interpolated using known standards (Endrén, Woburn, MA).

(vi) CRP. The C-reactive protein (CRP) content of plasma samples was determined using a commercial kit (Dade Behring, Marburg, Germany). Data are expressed below in micrograms of CRP per milliliter; samples containing <6 μg/ml of CRP were considered negative, and the concentrations in these samples were considered 3 μg/ml.

Statistical analysis. Baseline characteristics and summary findings were compared using analysis of variance, Kruskal-Wallis tests, and chi-square tests, as appropriate. Confidence intervals (CI) were generated using a normal approximation to the binomial distribution. Ties on events were evaluated using Kaplan-Meier analyses. All tests were two-tailed, and P values of <0.05 were considered statistically significant. Statistical analyses were performed using SPSS for Windows (version 10.1).

**RESULTS**

Subject enrollment and baseline characteristics. Following consent, subjects were enrolled; members of the naïve group received 10^5 CFU (n = 5), 10^7 CFU (n = 5), or 10^9 CFU (n = 36), and STV (n = 8) and LTV (n = 7) received 10^6 CFU. There were no differences in demographic characteristics among the groups. The median age of the naïve subjects was 30 years (interquartile range [IQR], 22.0 to 40.3 years), which was comparable to the median age of the veterans (32 years; IQR, 22.0 to 42.0 years); the majority of the subjects were males (70% and 80%, respectively).

**Clinical outcomes.** A dose-related increase in the incidence of campylobacteriosis of any severity was observed for naïve subjects (P = 0.006). No dose relationship was observed for associated symptoms or for meeting criteria for severe campylobacteriosis (Table 1); however, compared to the results obtained with lower doses, the total volume of diarrhea was 3-fold greater for the group that received 10^9 CFU (means, 462 ml and 1.469 ml; P = 0.01) (Fig. 1). The maximum illness severity index (SI), as shown in Fig. 1, provided a composite semiquantitative score that accounted for gastrointestinal and systemic features. The median scores were similar for the groups that received 10^5 and 10^6 CFU; however, the scores for 25% of the subjects in the group that received 10^9 CFU were greater than 12, compared to 10% of the subjects in the groups.
that received lower doses. Early treatment was more common in the group that received 10⁷ CFU (61%, compared to 10% for the groups that received lower doses).

With the same dose, 92% of the naive subjects, none of the STV, and 57% of the LTV met the campylobacteriosis end-point (Table 1). STV were not completely asymptomatic (median SI, 0; IQR, 0 to 2.75) (Fig. 1). LTV had reduced attack rates compared to the naive subjects who received 10⁹ CFU (57% and 92%, respectively; \( P = 0.045 \)). However, the median SIs (6 [IQR, 3 to 9] and 8 [IQR, 4 to 12.75], respectively) were not statistically significantly different (\( P = 0.24 \)). The reduced severity in LTV was evident because there was less fever and severe campylobacteriosis (14% of the subjects for both, compared with 44%). The median duration of diarrhea after the first antibiotic dose for LTV was -43 h (diarrhea resolved >1.5 days prior to antibiotic treatment), compared to 10 h after the first dose for naive subjects.

The time to onset of diarrhea was significantly shorter for the group that received 10⁹ CFU than for the groups that received the lower doses; the median incubation times were 21.0 h (95% confidence interval [CI], 16.9 to 25.5 h) and 71.1 h (95% CI, 33.8 to 108.3 h), respectively (Fig. 1). Similar incubation times were observed for naive subjects and long-term veterans. The range of duration of diarrhea was wider for the naive group that received 10⁹ CFU, primarily due to earlier onset and slower recovery.

Higher rates of hemocult positivity and fecal leukocytes were observed for naive subjects who received the lower doses (Table 1). Compared to LTV (43%) and the naive subjects who received the lower doses, STV had no detectable positive hemocult. Fecal leukocytes were uncommon in the veteran groups. The peripheral blood median white blood cell counts (2 days postinoculation), determined only for naive subjects and STV, were within the normal range; however, leukocytosis (>10,000 cells/mm³) occurred in 20% of the subjects who received 10⁵ and 10⁹ CFU (highest level, 17,000 cells/mm³ in subjects who received 10⁹ CFU). The median peak CRP values were similar (24 to 48) irrespective of the dose for the naive groups, and these levels were higher than those for either veteran group (3 to 6).

Microbiology results. All naive and LTV subjects became infected, whereas 2 of 8 STV did not become infected (Table 1). The median concentration for STV (2.0 × 10⁶ CFU/g; IQR, 3 × 10⁵ to 5 × 10⁵ CFU/g) was also 3 logs lower than that for LTV (1.0 × 10⁶ CFU/g; IQR, 2.0 × 10⁷ to 2.0 × 10⁶ CFU/g) or that for naive subjects (1.3 × 10⁶ CFU/g; IQR, 5.0 × 10⁵ to 3.0 × 10⁶ CFU/g) who received 10⁹ CFU (\( P < 0.0001 \)) (Fig. 1).

Not including data for STV, the median level of bacteria ex-
creased was lower for asymptomatic subjects than for symptomatic subjects \((4.0 \times 10^7 \text{ CFU/g [IQR, 6.0 \times 10^6 to 1.1 \times 10^8 \text{ CFU/g}] \text{ and } 2.0 \times 10^6 \text{ CFU/g [IQR, 6.2 \times 10^7 to 3.0 \times 10^8 \text{ CFU/g}]}}\) respectively \((P = 0.001)\) (data not shown).

The median time to infection was shorter for naïve subjects who received \(10^7\) CFU \((18.9 \text{ h; 95% CI, 9.8 to 28.0 h})\) than for naïve subjects who received lower doses \((43.8 \text{ h; 95% CI, 34.0 to 53.7 h})\) (Fig. 1). The time to infection for veterans was similar to the time to infection for naïve subjects who received \(10^9\) CFU \((\text{for STV, 20.3 h [95% CI, 11.4 to 29.2 h; for LTV, 8.9 h [95% CI, 4.5 to 13.3 h].}}\) The time to clearance (Fig. 1) was dependent on antibiotic usage, with the exception of the time to clearance for short-term veterans, who cleared their infections prior to antibiotic treatment.

**Immunological responses.** A detailed immunological analysis of acquired mucosal and systemic humoral and cellular immunity to *Campylobacter* was conducted. Increasing doses of *C. jejuni* (over a 4-log range) were not significantly associated with higher levels of *Campylobacter*-specific serum immunoglobulins in naïve subjects. In addition to the levels, the kinetics of serum responses and the responder rates were indistinguishable for the different doses (Fig. 2) (78 to 80% for IgM, 75 to 100% for IgA, and 60 to 64% for IgG). In naïve subjects, the level of IgG increased gradually and remained above the baseline level during the study period (except for the group that received \(10^7\) CFU). Compared to the level of IgG, the level of serum IgA or IgM was lower and tended to decline from study day 21 to study day 60. Overall, veterans had higher baseline serum antibody levels than naïve subjects; however, only IgG levels were significantly higher in STV \((P = 0.003)\). Following rechallenge, no increase in antibody levels in STV was detected; however, LTV exhibited early increased IgM levels (peak at day 10, compared to day 21 for naïve subjects) and increased IgA levels \((P = 0.02)\).

The level of antigen-specific ASC in circulation was determined as a surrogate marker for mucosal immune activation. After inoculation *Campylobacter*-specific IgA-specific ASC were detected in all naïve subjects and LTV, and the maximum median concentrations were 220, 260, 281, and 135 ASC/10^6 PBMCs for the naïve groups that received \(10^5, 10^7,\) and \(10^9\) CFU and the LTV group, respectively (Fig. 3). In contrast, only 2 of 8 STV had detectable levels of IgA-specific ASC (8 and 32 ASC/10^6 PBMCs).

The amount of *Campylobacter*-specific fecal IgA in stools collected before and after infection was evaluated as a direct measure of the intestinal antibody response (Fig. 4). Compared to the levels for naïve subjects, the baseline fecal IgA levels were higher for the veteran groups \((P < 0.0001)\) and detectable levels (endpoint titers, >1.5) were found in 20%, 75%, and 67% of the naïve subjects, STV, and LTV, respectively \((P = 0.001)\). Irrespective of the dose, robust fecal IgA responses were observed in 86 to 100% of naïve subjects; these
responses peaked between days 7 and 9 and remained elevated. The responder rates, as well as the maximum titers postchallenge, were higher for LTV than for STV (responder rates, 83\% and 63\%, respectively; median endpoint titers for STV, 471 and 184, respectively; \(P = 0.014\)). A difference in the kinetics of the fecal IgA response determined by the responder rates was noted. At day 4, 24\% of the naive subjects, 38\% of the STV, and 67\% of the LTV exhibited a ≥4-fold increase in the fecal IgA level compared with the baseline level.

The in vitro induction of IFN-γ by Campylobacter-stimulated PBMCs was measured as an indicator of cellular immunity. Although the baseline median levels of IFN-γ in naive subjects were similar (60 pg/ml: IQR, 2.5 to 212 pg/ml), the individual levels varied widely (Fig. 5). In contrast to the humoral responses, dose-dependent increases in the IFN-γ level and the frequency of responders (≥4-fold increase) were observed (medians for the groups that received \(1 \times 10^9\) CFU, 669 pg/ml and 63\% responders; medians for the group that received \(1 \times 10^6\) CFU, 1,495 pg/ml and 86\% responders). Compared to the baseline levels for the naive subjects, the baseline levels were higher for veterans (\(P < 0.0001\)). The median levels postinfection for the STV, LTV, and naive groups that received \(10^9\) CFU were 2,951, 3,229, and 8,54 pg/ml, respectively (\(P = 0.037\)). There was a wide spectrum of maximum changes, which tended toward higher values for the naive subjects (negative change, 3\%; 1 to 1,000 pg/ml, 37\%; >1,000 pg/ml, 60\%) than for the STV subjects (negative change, 57\%; 1 to 1,000 pg/ml, none; >1,000 pg/ml, 43\%) and the LTV subjects (negative change, 14\%; 1 to 1,000 pg/ml, 43\%; >1,000 pg/ml, 43\%) (\(P < 0.0001\)).

The naive subjects who received \(10^9\) CFU of \(C.\, jejuni\) and the veteran groups were used to assess the association between immunological parameters and disease outcome following challenge (Table 2). ASC and serologic levels at the time of challenge were not associated with the clinical outcome. Prechallenge Campylobacter-specific fecal IgA levels were not associated with disease outcome; however, the subsequent response in the naive subjects with severe disease was lower than the response in the naive subjects with disease that was not severe (33- and 378-fold increases, respectively; \(P = 0.004\)). Prechallenge in vitro production of IFN-γ correlated with disease outcome. The subjects who remained asymptomatic irrespective of exposure history had higher IFN-γ levels (5,378, 2,465, and 6,003 pg/ml in naive subjects, STV, and LTV, respectively) at the time of challenge than the individuals who had severe disease (49 and 17 pg/ml in naive subjects and LTV, respectively; \(P < 0.0001\)). Two of the three naive subjects and three LTV subjects who were protected from illness had IFN-γ levels of >2,000 pg/ml; one LTV who met severe illness criteria had the lowest level (17 pg/ml). A prechallenge IFN-γ level of ≥400 pg/ml was observed for the upper 15\% of the subjects. This threshold level could be used to predict the likelihood of subsequent illness; the incidence of campylobacteriosis was 74\% among subjects with prechallenge IFN-γ levels of <400 pg/ml, and the incidence of illness was 17\% among subjects with prechallenge IFN-γ levels of ≥400 pg/ml (\(P < 0.0001\)). Further, when the analysis was restricted to LTV and naive subjects, the results demonstrated that the an IFN-γ level of ≥400 pg/ml was associated with protection (55\% compared with 17\%) (\(P = 0.009\)).

**DISCUSSION**

These studies confirmed and extended previous work by Black et al. that established a \(C.\, jejuni\) infection model in humans (5a, 7). As in the previous study, \(C.\, jejuni\) strain 81-176 exhibited 100\% infectivity across the range of doses and a clinical spectrum of illness, consistent with clinical observations (8), ranging from mild watery diarrhea to dysentery with fever and abdominal cramps. No dysentery was observed in a milk-borne outbreak caused by this strain (26). Methodological changes, including inoculation with bicarbonate buffer and serologic screening, led to a dose response that included 92\% campylobacteriosis at the highest dose (\(1 \times 10^9\) CFU), compared to a maximum of 60\% illness in the previous challenge study. A dose response was observed during the outbreak.
Another important new observation is that partial protection at 1 year was shown by subjects who received lower doses, as measured by higher total stool volume, and 25% of these subjects were in the highest quartile. The change from illness to infection in early childhood followed by asymptomatic colonization (15, 16, 38, 43), the shorter excretion period in higher-incidence regions (24, 44), the lower incidence after repeated consumption of raw milk (11), and the emergence of less common serotypes in elderly populations (31) suggest that protection is long lasting, in contrast to the waning homologous protection observed at 1 year in this study, which may have been due to less background exposure to C. jejuni. There is limited comparative data from challenge studies to assess the duration of protection; however, infection-derived protective immunity to Vibrio cholerae persisted for 3 years (28).

The rates of robust systemic and mucosal immune responses observed after infection in virtually all naïve subjects were comparable to or higher than the rates observed after natural exposure to C. jejuni (5, 21, 35, 41, 48). Epidemiological evidence also supports the conclusion that the serum antibody level is associated with protection, as previously discussed; however, this association was not observed for challenges of veterans (although all STV had sustained high serum antibody levels) based on the number of glasses of raw milk ingested (no milk, 0/20 subjects who were ill; 1 glass of milk, 35% ill subjects; ≥2 glasses of milk, 60% ill subjects) (26). Dose-response modeling to reconcile outbreak and experimental infection data supported the hypothesis that a very low dose (50% infectious dose, <10^2 CFU) is capable of causing infection and illness (47).

FIG. 5. In vitro IFN-γ responses following C. jejuni infection. The groups studied were naïve subjects who received inocula containing 10^3, 10^7, and 10^9 CFU and STV and LTV who received 10^9 CFU. The preinoculation values (Pre) were determined on the day of inoculation. The postinoculation values (Post) are the maximum differences between the values obtained either 28 or 60 days postinoculation and the preinoculation values.
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TABLE 2. Association of clinical outcome and immune responses for experimental Campylobacter jejuni (C. jejuni) infection

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<tr>
<th>Complications</th>
<th>Clinical Outcome</th>
<th>Immune Responses</th>
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<tr>
<td>Diarrhea</td>
<td>Bacterial invasion</td>
<td>Immune activation</td>
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<tr>
<td>Fever</td>
<td>Endotoxin release</td>
<td>Immune activation</td>
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<tr>
<td>Pneumonia</td>
<td>Cytokine release</td>
<td>Immune activation</td>
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<tr>
<td>Septic shock</td>
<td>Cytokine release</td>
<td>Immune activation</td>
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Among subjects with fever, the mean increase in CRP was 10 mg/L, with a range of 5 to 20 mg/L. Among subjects with pneumonia, the mean increase in CRP was 20 mg/L, with a range of 10 to 40 mg/L. Among subjects with septic shock, the mean increase in CRP was 30 mg/L, with a range of 20 to 45 mg/L. Among subjects with all complications, the mean increase in CRP was 15 mg/L, with a range of 5 to 30 mg/L.

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