Vaccines against *Campylobacter jejuni*

Daniel A. Scott

Enteric Diseases Program, Naval Medical Research Institute, Bethesda, Maryland

*Campylobacter jejuni* is one of the most common causes of diarrhea worldwide. The gastrointestinal manifestations of *Campylobacter* infection range from watery diarrhea to severe dysentery. *Campylobacter* infection has also been linked to the postinfectious sequelae of reactive arthritis and Guillain-Barré syndrome. Evidence from epidemiologic and volunteer studies suggests that development of a vaccine to prevent gastrointestinal disease and limit colonization is possible. Efforts to develop live attenuated or subunit vaccines are limited by the finite knowledge of *Campylobacter* pathogenesis and lack of a conserved protective antigen, respectively. An oral killed, whole-cell vaccine has been shown to be safe and effective in animal models and is currently being tested in phase 1 volunteer studies.

*Campylobacter jejuni* is currently thought to be the most common cause of bacterial diarrhea in the United States and, on a global basis, probably ranks just behind enterotoxigenic *Escherichia coli* (ETEC) as a leading bacterial cause of both endemic and traveler’s diarrhea (TD) [1–6]. Because *C. jejuni* is an important cause of TD, the US Department of Defense has actively sponsored a *Campylobacter* research program headed by the US Navy. This program stresses basic pathogenesis research, and more recently it has expanded to applied vaccine research and army-navy collaboration on clinical and epidemiologic studies. This program is now testing a prototype inactivated, whole cell (WC) vaccine in volunteers.

Epidemiology

Studies by the Centers for Disease Control and Prevention and a Seattle health maintenance organization found that *Campylobacter* species were isolated from diarrhea stools two to four times as frequently as were *Salmonella* or *Shigella* species [2, 7–9]. In developing countries, where hygiene and sanitation are lacking, an average child may have several severe diarrhea episodes per year, and the incidence of *Campylobacter* enteritis in children <5 years of age is as high as 40,000/100,000, or 0.4 episodes per child per year [10, 11]. On the basis of regional estimates of incidence, the annual number of patients developing *Campylobacter* diarrhea worldwide is a staggering 400 million [12].

As noted above, there is growing evidence that *Campylobacter* species are second only to ETEC as a cause of TD [1, 13–18]. Recent studies of Finnish travelers in Morocco found that during the winter months, 28% of TD cases were attributable to *Campylobacter* infection, compared with only 8% attributable to ETEC [14]. In other studies of civilian travelers, the percentage of TD cases with *Campylobacter* organisms isolated from their stools ranges from lows of 1%–3% in Mexico and 11%–15% in travelers to the Indian subcontinent to highs of 29% and 39% in travelers to Morocco and Thailand, respectively [1].

Several studies have shown the importance of *Campylobacter* species as a cause of TD in the US military. Navy vessels visiting port cities in South America, West Africa, and the Middle East found *Campylobacter* species to be associated with 10%–20% of the overall diagnosed diarrhea episodes, with isolation frequencies ranging as high as 39% among cases following port visits to some Latin American cities [19] (Bourgeois AL, personal communication). During exercises in Thailand in 1990, 6% of US military participants developed diarrhea and *Campylobacter* organisms were isolated from 41% of 137 cases [1, 20]. After a 1994 exercise, 155 (47%) of 330 participants who responded to a questionnaire reported having diarrhea at least once during their 1-month stay in Thailand [5]. Of 104 patients who sought medical attention during that exercise, 57 (55%) had *Campylobacter* organisms isolated from their stools.

The threat posed by *Campylobacter*-associated TD is exacerbated by the large percentage of isolates resistant to erythromycin, the newer macrolides, and the fluoroquinolones [5, 20–22] and by the fact that *Campylobacter*-associated TD appears to be more severe than TD caused by other enteropathogens [15].

Clinical Spectrum of Disease

*Campylobacter* enteritis can present with symptoms ranging from watery diarrhea to dysentery. There is usually an accom-
panying fever and abdominal cramps, which can be severe. Untreated acute infection will most often run its course in 3–5 days, followed by convalescent shedding of the organism for several days to weeks [23, 24].

Like Salmonella, Shigella, and Yersinia enteritis, Campylobacter enteritis has been associated with development of a reactive arthritis or arthropathy (RA) [25–34]. Studies of outbreaks [27, 32] and case series [28, 34] have found an incidence of rheumatic complaints ranging from about 1% to 14% in subjects with evidence of C. jejuni infection. However, these studies did not use a consistent definition of reactive arthritis and included patients with joint symptoms, which in some cases lasted only 3–7 days [28]. In most reported cases, symptoms have resolved completely.

A less common but potentially more devastating complication of Campylobacter infection is Guillain–Barre syndrome (GBS) [35–37]. The association between GBS and Campylobacter infection is supported by both serologic and culture data and will be discussed in other sections [35]. The occurrence of these postinfectious sequelae must be taken into account in the development of potential Campylobacter vaccines.

**Campylobacter jejuni as a Target for Vaccine Development**

Data from epidemiologic and volunteer studies suggest development of an effective Campylobacter vaccine is feasible. In the developing world, where Campylobacter infection is hyperendemic, disease occurs primarily in young children. Although infection rates remain high throughout childhood, symptomatic infection rates are highest during the first year of life, followed by a marked decline in the ratio of symptomatic to asymptomatic infection [10, 38, 39]. Most infections are asymptomatic in children ≥5 years old. In Mexico, Bangladesh, Africa, and Thailand, this apparent early acquisition of immunity during the first 2 years of life has been shown to be accompanied by rising titers of Campylobacter-specific antibodies [39–41].

In a volunteer study, 7 people were made ill via ingestion of C. jejuni 81–176. One month later, the 7 subjects and 12 naive controls were challenged with the same isolate. None of the previously infected volunteers became ill, compared with 6 of the 12 controls [42, 43]. The organism was, however, able to colonize both naive and previously challenged volunteers.

Development of a vaccine may be complicated by the tremendous antigenic diversity of the organism and by the fact that the protective epitopes are not clearly defined. The most common typing schemes are the Lior system, which includes 108 serotypes, and the Penner system, which has >60 serotypes [44–46]. The Penner or O serotyping system is based on lipopolysaccharide and lipooligosaccharide antigens; the serodeterminant of the Lior scheme is a heat-labile antigen, which was originally thought to be flagellin. Genetic analyses with site-specific flagellin mutants, however, have indicated that in most serotypes examined, flagellin is not the Lior serodeterminant [47]. Animal studies have indicated that protection against intestinal colonization, in at least one animal model, is Lior serotype specific [48, 49]. The lack of specific information on the nature of the Lior serodeterminant and the large numbers of serotypes complicate vaccine development. However, several studies indicate that a limited number of Lior serotypes predominate in different regions of the world [44]. For example, in Thailand, ~75% of all isolates belong to 5 Lior serotypes (Bourgeois AL, personal communication).

Campylobacter vaccine development programs must also consider the ability of this organism to cause RA and GBS. However, epidemiologic studies to determine whether prior exposure to Campylobacter species will increase or decrease the risk of RA or GBS after a subsequent infection are lacking. Therefore, it is difficult to assess the impact that vaccination will have on the incidence of these diseases. An effective vaccine will by definition prevent the clinical syndrome of Campylobacter enteritis but may not prevent colonization. Volunteer studies have shown clearly that colonization without disease can induce both local intestinal and serum antibody responses [43], which could potentially trigger postinfectious sequelae. However if illness and intestinal tissue damage are required for the infection to result in RA or GBS, then a vaccine that prevents illness would decrease the risk of RA and GBS. Also of concern is the potential for a Campylobacter vaccine, developed using any of the strategies discussed below, to cause RA or GBS. For RA, no bacterial factors have been identified as being causative. The risk of GBS after Campylobacter infection appears to be increased after infection with certain lipopolysaccharide serotypes and may be the result of human ganglioside mimicry [50–55]. If the bacterial structures involved in inducing GBS can be clearly delineated, then it would be possible to construct even live attenuated vaccines that could be given without a risk of GBS.

**Campylobacter Vaccine Strategies**

It is widely believed that for a vaccine to be effective against an enteric agent, it must be able to stimulate intestinal immunity [56–59]. For this and logistical reasons, the oral route of immunization has been identified as the preferred approach in the Children’s Vaccine Initiative [60]. It takes advantage of the tremendous amounts of lymphoid tissue in the oropharynx and intestine and is simpler to administer than parenteral immunization. Several approaches to developing an oral vaccine are possible.

Live attenuated vaccines. Live attenuated, oral vaccines against 2 bacterial pathogens (Salmonella typhi and Vibrio cholerae) effectively stimulated mucosal immunity and provided excellent protection in field or volunteer challenge studies [61–63]. Using genetics to develop a live attenuated Campylobacter vaccine [64] is an attractive approach. This is complicated again by the paucity of information on pathogenesis and
physiology of the organism. However, mutants of *C. jejuni* 81–176, which are nonvirulent in the ferret model because of either defects in invasion or pilus biosynthesis, are being evaluated in navy or Navy Medical Research Institute (NMRI) laboratories for their protective efficacy in animal models. The inclusion of a *recA* mutation [64] would preclude reversion to wild-type by this naturally transformable enteropathogen [65]. In addition to the attenuation of the organism, more must be learned about the mechanisms by which *Campylobacter* organisms induce reactive arthropathy or GBS before the large-scale use of a live attenuated strain would be practical.

**Subunit vaccines.** Two campylobacter antigens, flagellin and a protein called PEB1, have been suggested as subunit vaccine candidates for use either as purified recombinant proteins or by expression in carrier vaccine strains, such as live attenuated *Salmonella* or *Shigella* species. Flagellin has long been recognized as an immunodominant antigen recognized during infection, and numerous studies have suggested a role for the protein in protection [66, 67]. The overall structure of campylobacter flagellins is similar to those of the Enterobacteriaceae, in that the amino and carboxy ends, which function in the transport and assembly of the monomers into the filament, are highly conserved among different *Campylobacter* isolates, and the central region appears antigenically diverse [68]. Power et al. [68] have studied the antigenicity of *Campylobacter coli* VC167 flagellin in detail and have shown that the major immune response seems to reside in the highly conserved amino and carboxy ends of the protein, areas which are not surface-exposed in the flagellar filament. In fact, the only surface-exposed epitopes identified were those that react with antibodies directed against posttranslational glycosyl modifications [68–70]. Moreover, a mutant defective in the ability to synthesize these glycosyl modifications loses the ability to protect against strains of the same Lior serotype in the RITARD model [48, 71], suggesting a role in the protective immune response for the modified flagellin but not the unmodified primary amino acids. The antigenic diversity of campylobacter flagellins, coupled with the fact that these eubacterial proteins are glycosylated, may make the development of flagellin subunit-based vaccines highly problematic.

The other protein that has been suggested as a potential subunit-based vaccine target is PEB1, a highly immunogenic protein conserved among *C. jejuni* isolates [72] that has also been suggested to function as an adhesin to eukaryotic cells [72]. As more is understood about the pathogenesis of *Campylobacter* species, it is likely that additional conserved subunit target proteins will be identified. One such candidate that was recently described are peritrichous pilus–like appendages whose expression is induced in the presence of several bile salts [73].

**Killed WC vaccines.** Inactivated microorganisms offer several advantages as potential vaccines for mucosal immunization. Physically, they are naturally occurring microparticles, which should enhance interactions between their surface and mucosal lymphoid tissues. As vaccines, they are inexpensive to produce and possess multiple antigens that can be important for protection. Formulations can be modified to offer protection against prevalent serotypes over time or in different geographic regions, as is done for influenza virus vaccines. Presentation of multiple antigens may be particularly important for pathogens like *Campylobacter* species, for which protective antigens are not known or not available in recombinant forms. Although less appealing for parenteral administration, these WC preparations are generally safe for mucosal immunization.

Several killed WC oral vaccines are under development and marketed in Europe. The best studied is an oral inactivated cholera vaccine composed of heat- and formalin-killed WCs of *V. cholerae* of different biotypes and serotypes plus the nontoxic B subunit of cholera toxin (B subunit purified from *V. cholerae* [BS]). A randomized, double-blind, placebo-controlled field trial involving 63,000 persons in rural Bangladesh established the safety, immunogenicity, and efficacy of the WC-BS vaccine against cholera [74–77]. Two or three doses of the WC-BS vaccine conferred 85% protection against cholera for the first 6 months in all age groups tested and 51% overall protection after 3 years [77]. More recently, a new formulation of the WC-BS cholera vaccine containing a recombinant (r) BS (WC-rBS) was also found to be safe and give high levels of protection (protective efficacy = 86%) against symptomatic cholera in Peruvian military recruits [78, 79] and 86% protection against ETEC diarrhea in Finnish travelers [80]. Although not as immunogenic as live attenuated *V. cholerae* vaccines [81, 82], these vaccines have proven that an orally administered WC vaccine can be safe and provide reasonable protection against an enteric pathogen.

The Enterics Program at NMRI has studied the hypothesis that a killed WC vaccine against *Campylobacter* species could be safe, immunogenic, and protect against disease, particularly if combined with an effective mucosal adjuvant, such as *E. coli* heat-labile toxin (LT) [83]. Initial studies using *C. coli* VC167 showed that when sonicated cells were combined with 25 μg of LT and given orally to mice, the mucosal immune response was equal to live infection with the same strain [84]. The duration of intestinal colonization after live challenge could also be significantly shortened in mice or rabbits immunized with sonicates plus LT but not by immunization with sonicates alone.

More recent work using a mixture of heat- and formalin-killed *C. jejuni* 81–176 in mice has shown that LT enhances the mucosal immune response over a wide range of vaccine doses [85]. Mice were orally immunized with three doses (at 48-h intervals) of either 10^5, 10^7, or 10^9 vaccine particles alone or in combination with 25 μg of LT. Significant *Campylobacter*-specific IgA and low levels of IgG were detected in intestinal lavage fluid only after immunization with adjuvanted vaccine. In contrast, similar levels of *Campylobacter*-specific serum antibody levels were stimulated by WCs with or without LT. The optimal secretory immune response was induced following vaccination with 10^7 of the *Campylobacter* killed WC
(CWC)-LT formulation, suggesting the ratio of adjuvant to inactivated cells may be important. When challenged orally with live C. jejuni 81–176, mice immunized with either 10^5, 10^7, or 10^9 WCs plus LT showed colonization resistance, whereas only the highest dose of CWC alone (10^9 cells) gave comparable protection. Both vaccine formulations provided similar levels of protection against systemic spread of challenge organisms [85].

In follow-on experiments, the relative protective efficacy and duration of immunity induced by CWC or CWC-LT vaccines were compared in an oral immunization–intranasal challenge model [86]. Both CWC and CWC-LT formulations provided varying degrees of protection against illness and intestinal colonization for up to 4 months after completion of the two-dose (14-day intervals) primary immunization series. However, the adjuvanted preparation appeared superior to formalin-inactivated WCs alone in that immunity in this vaccination group was still evident in mice 8 months after immunization (Baqar S, personal communication). In vitro T cell proliferative responses to C. jejuni antigens were also measurably enhanced by the addition of LT.

Two doses of orally administered CWC vaccine with or without LT were well tolerated in rhesus monkeys [87]. Elevated Campylobacter-specific IgA and IgG antibody-secreting cells were detected in the peripheral blood of most animals after vaccination, but the IgA antibody-secreting cell response was significantly enhanced by coadministration of LT in a dose-dependent manner. LT also significantly enhanced the serum Campylobacter-specific IgA and IgG responses.

These results suggest that both killed WCs alone and LT adjuvanted preparations are promising oral Campylobacter vaccine candidates. The addition of LT in different animal models enhances the mucosal and serum immune response to Campylobacter antigens, enhances the protective efficacy of the CWC at lower vaccine doses, and prolongs the duration of immunity more than CWC alone. Given these data, my laboratory felt that a CWC vaccine warranted further evaluation in human volunteers.

Currently, my colleagues and I are studying a monovalent, formalin-inactivated, WC vaccine (CWC) made from C. jejuni 81–176 (Penner serotype 23/36; Lior serogroup 5). This strain is invasive in cell culture [64, 88] and was originally isolated from the feces of a 9-year-old girl with diarrhea who became ill during a milkborne outbreak in Minnesota [89]. Although the full lipopolysaccharide structure of C. jejuni 81–176 has not been determined, antibody- and lectin-binding assays have not shown mimicry of any gangliosides associated with GBS (Moran AP, personal communication). In 1984, the strain was used in a human volunteer study at the Center for Vaccine Development, University of Maryland, described above [42]. The organism used to make the vaccine was recovered from a challenged volunteer with diarrhea.

The vaccine currently under study was prepared by the Walter Reed Army Institute of Research, Department of Biologics Research, and Antex Biologics (formerly MicroCarb), Gaithersburg, MD, through a cooperative research and development agreement. The cells used for vaccine preparation were grown under conditions that attempted to maximize motility and flagella expression and ability to invade eukaryotic cells in vitro; the cells were inactivated by use of 0.2% formalin. The final preparation had intact flagella, as determined by electron microscopy, and retained its ability to agglutinate in Lior 5 antisera.

Volunteer studies of this vaccine are ongoing. They have shown that the vaccine is well-tolerated and moderately immunogenic (Scott D, unpublished data). Studies combining the vaccine with a mucosal adjuvant have recently been completed, and a volunteer challenge study is planned for the near future.

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References


