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Vaccine 23 (2005) 4315–4321
Vaccine
www.elsevier.com/locate/vaccine

Prevention of disease in ferrets fed an inactivated whole cell *Campylobacter jejuni* vaccine

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Received 10 November 2004; received in revised form 28 March 2005; accepted 29 March 2005
Available online 3 May 2005

Abstract

Ferrets were used to demonstrate the potential of a killed whole cell vaccine prepared from *Campylobacter jejuni* to protect against disease. *C. jejuni* strain 81–176 was grown in BHI broth, formalin-fixed, and resuspended in PBS to a concentration of 10^{10} cells per ml. This vaccine (CWC) or live organisms were delivered orally with a nasogastric tube into anesthetized animals treated to reduce gastric acidity and intestinal motility. When 5×10^{10} CFU of the vaccine strain (Lior serotype 5) or one of two other serotypes, CGL-7 (Lior 4) or BT44 (Lior 9), was used to challenge the ferrets, all of the animals developed a mucoid diarrhea. If the animals had been challenged with 5×10^{9} CFU of the homologous strain 1 month before challenge with 10^{10} CFU, 80–100% protection against disease was seen. This protection was also obtained after an initial exposure to the 81–176 strain followed by challenge with either of the heterologous strains. CWC was used to see if protection demonstrated with the live organisms could be produced with the non-living preparation. When 10^{9} cells of CWC was given as two doses 7 days apart with or without 25 μg of a coadministered mucosal adjuvant, LT_{R192G}, only 40–60% of the animals were protected. If the regimen was changed to four doses given 48 h apart, 80% of the animals were free of diarrhea after subsequent challenge. Increasing the number of cells in the four dose regimen to 10^{10} cells did not improve protection. Animals given four doses of 10^{10} cells combined with LT_{R192G} were subsequently challenged with 10^{10} cells of the homologous strain or the heterologous strain CGL-7. The CWC protected against both strains. Serum IgG antibody titers determined by ELISA showed little increase following the CWC four dose vaccination regimen, compared to animals given one dose of the live organism. On subsequent challenge, however, both CWC vaccinated and live-challenged ferrets showed comparable antibody titer increases above those obtained following the initial challenge or vaccination. Western blots were used to show that the immunodominant antigen in vaccinated animals was a 45 kDa protein, while in ferrets challenged with live organisms the immunodominant antigen was a 62 kDa protein. These data show that the CWC can be used to protect against disease caused by *Campylobacter*. They also show that protection and serum IgG responses do not depend upon the use of the mucosal adjuvant and that cross protection among some of the major serotypes of *Campylobacter* responsible for human disease is possible.
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Keywords: *Campylobacter*; Vaccine; Diarrhea

1. Introduction

Campylobacter jejuni is now recognized as a leading cause of foodborne disease in the United States, as well as worldwide [1–4]. It is likely that an effective vaccine can be developed against disease caused by *Campylobacter*. Prospective epidemiological and human challenge studies suggest that protective immunity develops after a prior *C. je-*

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0264-410X/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.vaccine.2005.03.038

juni infection [4–6]. Human volunteers fed *C. jejuni* become infected and respond with the development of specific serum IgG, IgM, and IgA to *Campylobacter* [6]. In developed countries, a peak incidence of disease is seen in children below 1 year of age and in young adults [7–10]. In developing countries, where campylobacters are hyperendemic, symptomatic disease occurs in young children and persistent carriage occurs in adults without symptomatic disease [11,12]. It appears that a high level of endemic disease (i.e., hyperendemicity) results in the development of specific serum and secretory antibodies and less severe disease [13–15]. Following exposure, specific serum and secretory antibodies develop that provide strain-specific immunity and protection from disease caused by the homologous strain, although recolonization may occur [5,6].

Vaccines have not been developed for use against *Campylobacter*. Orally-administered inactivated whole cell *Campylobacter* vaccines offer a potentially useful approach towards immunization against *C. jejuni*. Physically, inactivated organisms are naturally-occurring microparticles that should enhance interactions between the antigens they carry and the mucosal lymphoid tissues. As vaccines, they are safe when given orally and inexpensive to produce and administer. Whole cells possess multiple antigens that can be particularly important to protection, particularly when protective antigens are not known.

When grouped according to a serotyping scheme based on heat labile antigens [16] only about a dozen of the over 100 Lior serotypes are associated with disease in humans. Strain 81–176 of *C. jejuni* was selected to make an inactivated whole cell vaccine since it is one of the clinically important serotypes, Lior 5. Further, this strain has been used in clinical challenge studies [5,6] and does not show mimicry of any gangliosides associated with Guillian Barre syndrome (GBS) (A.P. Moran, personal communication).

An important question is whether a single serotype, such as Lior 5, can protect against disease induced by other clinically important serotypes. Due in part to the difficulty in doing cross serotype protection studies in humans, as well as the problem of trying to be sure no challenge strains may induce GBS, an animal model is needed to show cross-strain protection against disease.

Colonization, rather than disease, models have been used to show that the CWC vaccine does protect against *C. jejuni*. Mice have been orally immunized with a three-dose primary series of particles of CWC (48 h intervals) at doses of 10^5, 10^7, or 10^9 cells [17]. The vaccine was given to mice with or without the mucosal adjuvant consisting of the heat-labile enterotoxin of *Escherichia coli* (LT) [18]. These studies showed that the *Campylobacter*-specific intestinal IgA response was dependent on the use of LT, whereas serum immunoglobulin responses were not. Colonization resistance was induced over a broad range of vaccine doses when LT was included. However, only the highest dose (10^9) of CWC alone gave comparable levels of protection against colonization. Both the adjuvanted and unadjuvanted formulations given at the intermediate dose (10^7) provided equivalent protection against systemic spread of challenge organisms.

Colonization has also been studied in the Removable Intestinal Tie Adult Rabbit Diarrhea, or RITARD, model for *Campylobacter* infection [19]. This model relies on a surgical procedure, in which the challenge organism is introduced into the bowel of immunized and nonimmunized rabbits to model intestinal colonization: no disease is produced in animals old enough to have completed immunization. Protection against colonization was seen when three doses of CWC were given by gavage at weekly intervals [20]. Protection was not obtained in this model if LT was not present. This protection against colonization was Lior serotype specific [16,20].

Ferrets are one of the few animals that develop a *Campylobacter*-induced diarrheal disease similar to that seen in humans [21–23]. Ferrets develop enterocolitis associated with natural *C. jejuni* infection and following experimental inoculation with pure cultures, develop mild to moderate diarrhea characterized by the presence of mucus, fecal leukocytes and blood in the stool. Diarrhea was more severe in younger (6–7 week old) animals than older (11–12 week old) animals, with more fluid stools often accompanied by anorexia for a day or two. Ferrets fed live ferret-derived strains of *Campylobacter* develop an immune response making these animals resistant to disease following a second challenge with the same strain [22]. For both adult and young ferrets, infection and disease occur without any prior manipulation of the animals.

The purpose of the present study was to use the ferret to provide evidence that protection against enteric disease caused by *Campylobacter* could be obtained in a natural host and that this protection might be relatively conserved. Protection against disease was found to be at least somewhat conserved among serotypes of *Campylobacter.* These studies showed, furthermore, that protection against disease was associated with vaccine dose and, in contrast to colonization, was not enhanced by use of adjuvant. Further, following vaccination with the killed preparation, a strong serum immune response was obtained in protected animals with a 45 kDa OMP as the immunodominant antigen.

2. Materials and methods

2.1. Bacterial culture

C. jejuni strain 81–176 (Lior serotype 5) was isolated during a 1981 foodborne outbreak. Strains CGL7 (Lior serotype 4) and BT44 (Lior serotype 9) were isolated during military field exercises in Thailand from the stools of patients with acute diarrhea. Frozen stocks were thawed, inoculated onto tryptic soy blood agar plates and incubated at 42 °C in polybags (Levin Bros Paper Co., Chicago, IL) with an atmosphere of 85%N_2–10% CO_2–5%O_2. Cells were first passed through Mueller Hinton motility agar to confirm colonies remained motile and then plated to Muller Hinton agar. After