Construction of a dual subunit vaccine against *Campylobacter jejuni*

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INITIAL HYPOTHESIS AND OBJECTIVES

The main objective is the construction of a dual subunit vaccine against *Campylobacter jejuni*, which stimulates the generation of specific antibodies and provides protection against heterologous strains of the same microorganism.

The initial hypothesis is that the methodology used in the construction of vaccine is feasible and successful, as well as that exposure of selected subunits (PorA and PEB1) generates a specific protective immune response against *Campylobacter jejuni* in individuals who was administered.

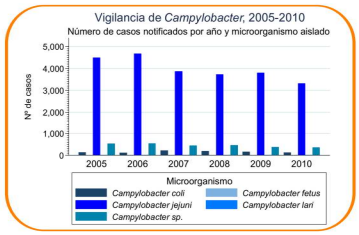

Figure 1. Incidence of campylobacteriosis in Spain during 2010.

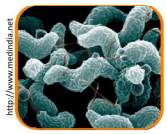

Figure 2. Scan EM micrography of *C. jejuni.*

Figure 3. Chickens in a ecological poultry farm located in EE.UU.

BACKGROUND

Campylobacter jejuni, is a Gram-negative, microaerophilic, spiral-shaped, motile by flagella and encapsulated bacterium. It is a zoonotic food-borne pathogen, which is attributed to be the major cause of bacterial diarrhea worldwide [1]. In Spain 6,340 cases of *Campylobacter* infection were reported during 2010 to SIM (*Sistema de Información Microbiológica*), of which 83.4% of the isolates belonged to *C. jejuni*, according to the *Instituto de Salud Carlos III* [2]. In other developed countries, the estimated incidence of *C. jejuni* diarrhea has been reported to be as high as 40,000/100,000 [1, 3, 4]. The main source of infection is thought to be the consumption and handling of undercooked poultry meat among other food, including water [1]. The majority of campylobacteriosis cases result in uncomplicated acute gastroenteritis, but the symptoms range from mild non-inflammatory diarrhea, watery and self-limiting, to severe inflammatory enteritis [5, 6]. In addition, infection with *C. jejuni* is associated with post-infectious complications such as reactive arthritis, Reiter's syndrome and Guillain-Barré syndrome (GBS) [7]. The significance described of *C. jejuni* infection as well as increasing of the antibiotic resistant strains isolation, highlights the need for effective methods of prevention against campylobacteriosis, notably vaccination.

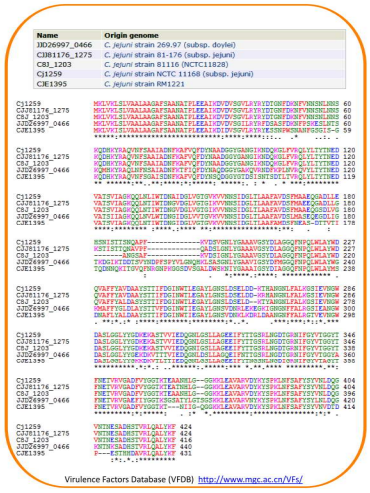

Figure 4. Multi-alignment of *porA* related to MOMP of *Campylobacter sp.*

One of the main problems to develop effective vaccines against *C. jejuni* is that the acquisition of immunity is serotype specific [8, 9], and there are numerous serotypes of *C. jejuni* according to the Penner serotyping scheme, based on the polysaccharide capsule [10]. These problems could be solved if vaccine is based on conserved antigens. Two good representatives of these antigens in *C. jejuni* are PorA [11], and PEB1 [12].

PEB1 is the major cell adherence molecule of *C. jejuni* and *C. coli*, and is exposed on the cell surface [13]. It is encoded by *peb1A* gene and its main recognized function is colonization of intestine, but may also be involved in amino acid transport [14].

PorA, the major outer membrane protein (MOMP) of *C. jejuni*, is encoded by *porA* gene and is found in abundant quantity in this organism [11]. It is involved in ion transport and adhesion to the intestinal mucosa [15].

PorA and PEB1 are commonly recognized by convalescent sera from patients with *C. jejuni* sporadic diarrhea [16, 17, 18], indicating that both are a good target for the immune system. Given the presumed validity of these two antigens as vaccine subunits, the design of a dual subunit vaccine is proposed with heat-labile *E. coli* enterotoxin as an adjuvant.

Figure 5. Multi-alignment of *peb1A* related to PEB1/CBF1 of *Campylobacter sp.*

MATERIALS AND METHODS

OBTAINING OF VACCINE SUBUNITS rPorA and rPEB1

PCR amplification of ***porA*** and ***peb1A*** from ***C. jejuni 81-176*** genome

Digestion of vector and PCR products with **NdeI** and **XhoI**

Cloning *porA* and *peb1A* genes, independently, into **pET-16b** vector, making a translational fusion of **6His-Tag** with both genes

Separately **transformation** of pET-16b-*porA* or pET-16b-*peb1A* into ***E. coli*** **BL21 (DE3)**

Selection of *E. coli* BL21 (DE3) clons by plating in **LB-ampicillin** plates. Confirmation by **colony PCR**.

A confirmed pET-16b-*porA* or pET-16b-*peb1A* clone will be **grown** at 37ºC in constant agitation

Culture will be **IPTG-Induced** at an OD^{600nm} of 0,65

Centrifugation 10' at 5000 rpm

✖ Supernatant

Resuspension of pellet in purification buffer and treatment with **lysozyme** 15 minutes at 4ºC

Several rounds of **sonication** and centrifugation 10' at >5000 rpm

✖ Supernatant

Resuspension of pellet with solubilization buffer that contains **Urea** and **Triton-X**

Centrifugation 10' at >5000 rpm

6His-PorA and 6His-PEB1 will be confirmed by **SDS-PAGE** and **Western-blot** analysis

Purification by **nickel IMAC**

Cleavage of 6His-Tag by treatment with **Factor Xa**

Purification by Gel Filtration **Chromatography**

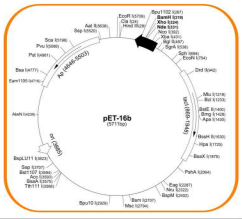

Figure 6. pET-16b vector genetic map

ORAL VACCINATION

BALB/c mice will be used. Adult mouse does not suffer diarrhea as a result of *C. jejuni* infection, but the microorganism can colonize the intestine and isolate in the stool [13].

Group 1 (Control)
300 µl PBS

Group 2 (adjuvant control)
5 µg *E. coli* heat-labile enterotoxin (HLET) and 300 µl PBS

Group 3
150 µg rPorA + 150 µg rPEB1 and 300 µl PBS

Group 4 (complete vaccine)
150 µg rPorA + 150 µg rPEB1 + 5 µg *E. coli* HLET and 300 µl PBS

Oral inoculations will be performed twice at week during 4 weeks.

3 weeks post vaccination, mice will be orally inoculated with a mixture of *C. jejuni* strains to a final concentration of 2 x 10^9 CFU/ml

Over the next 10 days samples of blood and feces will be collected to evaluate the reduction of *C. jejuni* colonization and the generation of a specific antibody response by ELISA

EXPECTED RESULTS

A specific **Th1/Th17** cellular immune response and especially a **specific humoral immune response** are tried as a result of the administration of the oral vaccine. Thus, generation of specific antibody against exposed antigens, specifically **IgA** isotype, is sought to provide heterologous and protective immune response against *Campylobacter jejuni*.

DISSEMINATION PLAN

A patent application will be submitted to a patent agency such as *Oficina Española de Patentes y Marcas (OEPM)* or European Patent Office (EPO). Having gained the patent, the project will be disseminated through a paper publication in a high-impact journal such as **Clinical and Vaccine Immunology** (CVI) or **Vaccine** journal.

PROJECT BENEFITS

This approach stands out from other similar projects by usage of a **dual subunit vaccine** based on **two conserved antigens** that provides protection to heterologous strains of *C. jejuni.*

Vaccination could be the **most effective prophylactic mechanism** for campylobacteriosis control, because hygienic measures established in the industry are not very effective at preventing this disease due to most infections occur at home by mishandling of food and the low Minimum Infectious Dose (MID) of this organism. Thus, human or veterinary vaccination could be very interesting.

[1] Friedman, Neimann, Wegener and Tauxe. 2000. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations, p. 121–138. In I. Nachamkin and M. J. Blaser (ed.), Campylobacter, 2nd ed. ASM Press. **[5] Instituto de Salut Carlos III.** Resultados de la vigilancia epidemiológica de las enfermedades transmisibles. Informe anual. Año 2010. http://www.isciii.es/. **[3] Coker, Isokpehi, Thomas, Amisu and Obi.** 2002. Emerging Infectious Diseases Journal, 8:237-244. **[4] Oberhelman and Taylor**. 2000. Campylobacter infections in developing countries, p. 139-54. In I. Nachamkin and M. J. Blaser (ed.), Campylobacter, 2nd ed. ASM Press. **[6] Nielsen, Luijkx, Vegge, Johnsen, Nuijten, Wren, Ingmer and Krogfelt.** 2012. Clinical Vaccine Immunology, 20:113-19. **[7] Elmi, Watson, Sandu, Gundogdu, Mills, Inglis, Manson, Imprie, Bajaj-Elliott, Wren, Smith and Dorrel.** 2012. Infection and Immunity, 80:4089-98. **[8] Mølbak and Havelaar**. 2008. Burden of illness of campylobacteriosis and sequelae, p. 151-62. In I. Nachamkin, C. Szymanski, and M. J. Blaser (ed.), Campylobacter, 3rd ed. ASM Press. **[10] Burr, Caldwell, Bourgeois, Morgan, Wistar Jr. and Walker**. 1988. Infection and Immunity, 56:99-105. **[11] Pavlovskis, Rollins, Haberberger, Green, Habash, Strocko and Walker.** 1991. Infection and Immunity, 59:2259-64. **[12] Karlyshev, Linton, Gregson, Lastovica and Wren.** 2000. Molecular Microbiology, 35:529-41. **[13] Islam, Raghupathy and Albert.** 2010. Clinical and Vaccine Immunology, 17:1666-71. **[14] Du, Li, Tang, Huang, Sun.** 2008. World journal of Gastroenterology, 14(40): 6244-48. **[16] Kervella, Pages, Pei, Grollier, Blaser and Fauchere.** 1993. Infection and Immunity, 61:3440-48. **[15] Pei and Blaser.** 1993. The Journal of Biological Chemistry, 268:18717-25. **[19] Moser, Schroeder and Salnikow.** 1997. FEMS Microbiol Lett. 15; 157(2), 233-8. **[17] Pei, Ellison and Blaser.** 1991. The Journal of Biological Chemistry, 266:16363-69. **[20] Blaser and Duncan**. 1984. Infection and Immunity. Vol. 44, Nº 2, 292-98. **[21] Huang, Sahin and Zhang.** 2007. FEMS Microbiol Lett. 272(2), 137-43.