Available online at www.sciencedirect.com

ScienceDirect

Procedia in Vaccinology 9 (2015) 38 – 43

Procedia in Vaccinology

www.elsevier.com/locate/procedia

8th Vaccine & ISV Congress, Philadelphia, USA, 2015

The application of a proteoliposome adjuvant system in the development of a *Campylobacter jejuni* vaccine

Barbara Rickaby[a], Nelson F. Eng[b], Annika Flint[c], Alain Stintzi[c], Francisco Diaz-Mitoma[b]

[a]Laurentian University, 935 Ramsey Lake Rd, Sudbury, P3E 2C6, Canada
[b] Advanced Medical Research Institute of Canada, 41 Ramsey Lake Rd, Sudbury,P3E 5J1, Canada
[c]University of Ottawa, 451 Smyth Rd, Ottawa, K1H 8M5, Canada

Abstract

The high incidence of *Campylobacter jejuni*-associated diarrhoea, the increase in the frequency of drug resistance, and the correlation between infection and Guillain-Barré syndrome, has heightened the need to develop effective anti-*Campylobacter* vaccines. Due to the risk of auto-immunity, vaccinations using whole-cells or attenuated cells are not clinical candidates. Subunit vaccines are viable alternatives and have been shown to be effective against other enteric pathogens. This research tested the efficacy of the catalase (KatA) from *C. jejuni* as a potential vaccine candidate against the pathogen. The protein was found to be immunogenic following an intramuscular murine immunization. A balanced IgG1/IgG2a response was generated when KatA was co-administered with monophosphoryl lipid A and alum. The antibodies elicited reduced the adhesion and invasion of *C. jejuni* to human epithelial cells (IECs) *in vitro*. The next phase of testing will be in combination with a proteoliposome (PL) derived from the outer membrane of *V. cholerae*. PL nano-vesicles were generated with an average size of ≈70 nm and had a zeta potential of ≈-40 mV.

© 2015 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license
(http://creativecommons.org/licenses/by-nc-nd/4.0/).
Selection and peer-review under responsibility of the 8th Vaccine Conference Organizing Committee.

Keywords: Campylobacter jejuni; catalase; KatA; proteoliposome, vaccine development; mucosal adjuvant

1. Background

Every year, diarrhoeal diseases claim the lives of over 5 million children living in conditions where the lack of food and water sanitation fosters the transmission of enteric pathogens [1]. *Campylobacter jejuni* is the most frequently reported cause of bacterial gastroenteritis in humans [2] and the second leading cause of traveller's diarrhoea following *Escherichia coli* [3]. The high incidence of *C. jejuni* infections and increasing antibiotic resistance [4] has prompted the development of vaccines against this pathogen. The recent association of *C. jejuni*

1877-282X © 2015 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license
(http://creativecommons.org/licenses/by-nc-nd/4.0/).
Selection and peer-review under responsibility of the 8th Vaccine Conference Organizing Committee.
doi:10.1016/j.provac.2015.05.007

infections and Guillain-Barré syndrome (GBS) [5] has created an unusual dilemma by eliminating the possibility of developing a whole-killed or attenuated vaccine. GBS is a neurological disease characterized by ascending paralysis that can lead to respiratory muscle compromise and even death [6]. It is the leading cause of acute neuromuscular paralysis in the developed world and is believed to be the result of a post-infection autoimmune response [6]. The outer membrane of *C. jejuni* contains lipopolysaccharide (LPS) and lipooligosaccharide (LOS) that closely resemble human gangliosides on the myelin sheath of neurons triggering antibody-mediated demyelination [7].

The catalase A (KatA) protein is a homotetrameric protein approximately 55 kDa is size. It has been suggested to be an excellent vaccine candidate against enteric bacteria such as *C. jejuni* [8]. Palyada *et al*. (2009) confirmed the necessity of the KatA protein for host colonization *in vivo* as KatA deficient *C. jejuni* mutants were unable to colonize the cecum of baby chicks [9]. The antigenicity of the KatA protein, however, is yet to be tested and the functionality of the antibodies verified.

In the effort to develop a novel *C. jejuni* vaccination strategy, a proteoliposome delivery system will be tested. The proteoliposome (PL) is a new concept and has recently been employed as highly effective vaccine technology [10,11]. PLs are nanoparticles derived from the outer membranes of bacteria and contain numerous pathogen-associated molecular patterns (PAMPs) that effectively stimulate the innate immune system [12]. Recent research has demonstrated the ability of PLs to stimulate enhanced mucosal immunity to a secondary unrelated antigen co-administered with the PL[10]. This preliminary work involved the production and characterization of PLs in house from *Vibrio cholerae*. In the second phase of this research, the PL will be tested with KatA in a mucosal vaccine administration.

2. Materials and Methods

KatA Protein

Overexpression of KatA was performed in *E. coli* BL21 cells using the protein expression vector pGST and purification was performed as described previously [13]. In brief, *E. coli* cells were pelleted and resuspended in 100 mM NaCl,10 mM Tris pH 7.3 buffer containing protease inhibitor (Roche, Mississauga, Canada). The cell membranes were disrupted via sonication and debris was removed by centrifugation at 13,000 rpm for 15 min. The cell lysate containing the GST-KatA fusion protein was then purifed using glutathione sepharose 4B resin (GE Healthcare, Baie D'Urfe, Canada). Clevage of the GST tag from KatA was performed on the resin with the addition of TEV protease and gentle shaking overnight. The KatA protein was washed from the resin using 100 mM NaCl, 20 mM Tris pH 7.3 and concentrated using a 30 kDa cut-off centrifugal filter (EMD Millipore, MA, USA). The concentrated KatA protein was further purified by sized exclusion chromatography using the AKTA fast protein liquid chromatography (FPLC) system equipped with a Superdex-200 column (GE Healthcare).

Immunization

The vaccine was administered intramuscularly at time 0, 3 and 6 weeks to female BALB/c mice (Charles River Laboratories, Sherbrooke, Canada). Four mice were housed per cage and food and water was provided *ad libitum*. Eight mice were in each immunization group. All experiments were conducted according to the Guidelines for the Care and Use of Laboratory Animals and was approved by the Animal Care Committee of Laurentian University (Sudbury, Canada). Adjuvants, added where required included alum (Invivogen, San Diego, CA) and monophosphoryl lipid A (MPL, Invivogen) (Table 1).

Table 1. Vaccine formulations administered via intramuscular injection to mice.

Group	Vaccine Formulations
A	PBS
B	5 µg KatA
C	5 µg KatA + 100 µg Alum
D	5 µg KatA + 5 µg MPL
E	5 µg KatA + 100 µg Alum + 5 µg MPL
F	10^8 inactivated/killed *C. jejuni* in PBS

Download English Version:

https://daneshyari.com/en/article/2473696

Download Persian Version:

https://daneshyari.com/article/2473696

Daneshyari.com