Vaccine 36 (2018) 388–394

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Oral administration of PLGA-encapsulated CpG ODN and *Campylobacter jejuni* lysate reduces cecal colonization by *Campylobacter jejuni* in chickens

Khaled Taha-Abdelaziz [a,b], Douglas C. Hodgins [a], Tamiru Negash Alkie [a,c], Wanderely Quinteiro-Filho [a], Alexander Yitbarek [a], Jake Astill [a], Shayan Sharif [a,*]

[a] Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada
[b] Pathology Department, Faculty of Veterinary Medicine, Beni-Suef University, Al Shamlah, 62511 Beni-Suef, Egypt
[c] Department of Biology, Wilfrid Laurier University, 200 University Ave W, Waterloo, ON N2L 3G1, Canada

ARTICLE INFO

Article history:
Received 31 March 2017
Received in revised form 14 November 2017
Accepted 28 November 2017
Available online 7 December 2017

Keywords:
Chicken
Campylobacter
Toll-like receptor ligand
CpG ODN
Nanoparticle
PLGA

ABSTRACT

Campylobacter jejuni (*C. jejuni*) is a major cause of bacterial food-borne illness in humans. It is considered a commensal organism of the chicken gut and infected chickens serve as a reservoir and shed bacteria throughout their lifespan. Contaminated poultry products are considered the major source of infection in humans. Therefore, to reduce the risk of human campylobacteriosis, it is essential to reduce the bacterial load in poultry products. The present study aimed to evaluate the protective effects of soluble and PLGA-encapsulated oligodeoxynucleotides (ODN) containing unmethylated CpG motifs (E-CpG ODN) as well as *C. jejuni* lysate as a multi-antigen vaccine against colonization with *C. jejuni*. The results revealed that oral administration of a low (5 μg) or high (50 μg) dose of CpG resulted in a significant reduction in cecal *C. jejuni* colonization by 1.23 and 1.32 $\log_{10}$ ($P < .05$) in layer chickens, respectively, whereas E-CpG significantly reduced cecal *C. jejuni* colonization by 1.89 and 1.46 $\log_{10}$ in layer and broiler chickens at day 22 post-infection (slaughter age in broilers), respectively. Similar patterns were observed for *C. jejuni* lysate; oral administration of *C. jejuni* lysate reduced the intestinal burden of *C. jejuni* in layer and broiler chickens by 2.24 and 2.14 $\log_{10}$ at day 22 post-infection, respectively. Moreover, the combination of E-CpG and *C. jejuni* lysate reduced bacterial counts in cecal contents by 2.42 $\log_{10}$ at day 22 post-infection in broiler chickens. Anti-*C. jejuni* IgG antibody (Ab) titers were significantly higher for broiler chickens receiving a low or high dose of E-CpG or a low dose of *C. jejuni* lysate than for chickens receiving the placebo. Furthermore, a positive correlation was observed between serum IgG Ab titers and cecal counts of *C. jejuni* in these groups. These findings suggest that PLGA-encapsulated CpG or *C. jejuni* lysate could be a promising strategy for control of *C. jejuni* in chickens.

1. Introduction

Campylobacter jejuni (*C. jejuni*) is considered a commensal bacterium in chickens [37,18]. Colonization of the chicken intestine with *C. jejuni* commonly occurs by 3 weeks of age and infected chickens shed bacteria throughout their lifespan. Although both layer and broiler chickens become infected with *Campylobacter*, broiler chickens play a more important role in transmission of this bacterium to humans [25]. *C. jejuni* bacteria cause severe enteritis in humans [3], but chickens are usually not clinically affected by colonization [11,18]. It is unclear whether this distinction is due to differences in the structures and functions of the immune system or to the ability of *C. jejuni* to evade or subvert the immune system of chickens [8]. Contaminated chicken meat is considered the most important means of *C. jejuni* infection in humans [37]. Therefore, to reduce the risk of human campylobacteriosis, it is essential to reduce the *C. jejuni* load in poultry products by reducing the intestinal *C. jejuni* burden in poultry flocks.

Various intervention strategies have been applied to reduce colonization rates, including on-farm biosecurity measures, vaccination, genetic selection, dietary manipulation, and the use of antimicrobial alternatives [9,20]. However, these strategies have been only partially effective in reducing the burden of *C. jejuni*. Among the above strategies, immunological interventions such as vaccines are of note. The limited protective efficacy of the existing

* Corresponding author.
E-mail address: shayan@uoguelph.ca (S. Sharif).

https://doi.org/10.1016/j.vaccine.2017.11.073
0264-410X/

vaccines against *Campylobacter* [36,24,16], signifies the need for more efficacious vaccination strategies.

A large body of experimental evidence suggests that Toll-like receptor- (TLR) ligands (TLR-L) can be exploited as adjuvants within vaccine formulations or as stand-alone antimicrobial agents to enhance adaptive immune responses as well as innate responses systemically and in the intestine [22,32,34]. In chickens, parenteral and *in ovo* administration of CpG ODN (oligodeoxynucleotides (ODN) containing unmethylated CpG motifs) has been shown to be efficacious in preventing or reducing bacterial infections, such as *Escherichia coli* and *Salmonella enterica* serovar Typhimurium [14,33]. This suggests that CpG could serve as a potent mucosal adjuvant in chickens, especially against enteric infections. However, the oral delivery of CpG remains a challenge due to the short half-life and potential degradation by gastric acid and digestive enzymes [32]. Efforts have been made to overcome these shortcomings and increase the intracellular availability of CpG at target sites by formulation in protective delivery systems, such as poly (D, L-lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) [6,15]. Recent studies have shown that PLGA NPs formulations enhance the stability of the ligand by protecting it from enzymatic degradation and rapid clearance and also extend the duration of immune system stimulation by releasing the ligand in a slow and controlled manner [12,26,2]. Moreover, PLGA particles are readily taken up via intestinal Peyer's patches in chickens [17] and intestinal M cells [10].

The aim of the present study was to evaluate the protective effects of soluble and PLGA-encapsulated CpG (E-CpG) as well as *C. jejuni* lysate as a multi-antigen vaccine against colonization with *Campylobacter*, with the goal of reducing intestinal colonization in chickens with this bacterium.

2. Materials and methods

2.1. Chickens and housing

One-day-old commercial broiler chicks (Ross 708, Maple Leaf Foods, New Hamburg, Ontario) were randomly housed in floor pens on dry, clean wood shavings and one-day-old specific-pathogen-free (SPF white leghorn, Canadian Food Inspection Agency, Fallowfield, Ontario) layer chicks were housed in the isolation facility of the Ontario Veterinary College, University of Guelph (Guelph, ON). Chickens were fed antibiotic- and feed additives-free diets ad libitum. During the experimental period, chickens were monitored for weight gain and general health problems. There were no signs of morbidity, mortality and chickens remained healthy throughout the experiment. This research was approved by the University of Guelph Animal Care Committee in compliance with the guidelines of the Canadian Council on Animal Care.

2.2. Preparation of Campylobacter culture

Campylobacter jejuni strain 81–176 was cultured as previously described [7]. To confirm challenge dose, bacterial inocula were plated onto Mueller–Hinton (MH) agar plates, containing Preston *Campylobacter* Selective Supplement (Oxoid, Basingstoke, Hampshire, UK), before and after challenge (for details see Supplemental materials).

2.3. Preparation of outer-membrane proteins (OMPs)

A crude mixture of cellular OMPs of *C. jejuni* was extracted according to the method of McCoy et al. [23] (for details see Supplemental materials).

2.4. Preparation of Campylobacter lysate

A mid-log culture of *C. jejuni* was centrifuged and washed with Dulbecco phosphate-buffered saline (DPBS), and diluted in DPBS to an OD 600 of approximately 0.01 corresponding to approximately 2.0×10^7 CFUs/ml. The suspended bacteria were lysed as previously described [8]. Briefly, the bacteria were heat-killed at 65 °C for 30 min and then sonicated on ice (six 15-s pulses interspersed with 30-s pauses). Sonication was verified by separation on a 10% SDS-PAGE gel, followed by staining with Coomassie blue. The protein concentration was measured using a BCA Protein Assay Kit (Thermo Fisher Scientific, Rochester, USA). One milliliter of lysate (2.0×10^7 CFUs/ml) contained approximately 8.6 μg protein. The lysate was stored at −80 °C until use.

2.5. CpG ODN

A synthetic class B CpG ODN 2007 [5′-TCGTCGTTGTCGTTTTGTCGTT-3′] (Sigma, St. Louis, USA) with a phosphorothioate backbone, was reconstituted in endotoxin-free water and diluted to working concentrations in DPBS. CpG-loaded PLGA NPs were prepared as previously described [31] (for details see Supplemental materials).

2.6. Vaccination and challenge

2.6.1. Comparing administration routes and doses of CpG

One hundred and fifty one-day-old SPF layer chicks were randomly divided into 5 groups. At 13 days of age, six birds from each group were euthanized. Blood samples were collected to test for the presence of maternal IgG Abs. On day 14, two groups were injected intramuscularly with a low (5 μg) or high (50 μg) dose of soluble CpG in a 200 μl volume, two groups were orally treated with a low (5 μg) or high (50 μg) dose of CpG in a 500 μl volume, and one group received PBS as a placebo. All groups were orally challenged with 10^7 CFUs of *C. jejuni* 24 h after treatment with CpG or PBS. Approximately 0.5–1 g of cecal contents were collected at 2, 5 and 8 days post-infection (n = 6–8 per time point), and 10-fold serial dilutions in PBS were plated onto MH agar containing Preston *Campylobacter* Selective Supplement. Plates were incubated in microaerophilic conditions at 41 °C and CFUs of *C. jejuni* were enumerated after 40–48 h and expressed as $\log_{10}$ *Campylobacter*/g of cecal content.

For all experimental trials, a non-challenged, non-treated group was kept in a separate room and four birds were euthanized at each sampling time point; cecal contents were plated onto MH agar to monitor for unintended contamination with *Campylobacter*.

2.6.2. Oral administration of soluble CpG, E-CpG and C. jejuni lysate in SPF layer chickens

One hundred and thirty-eight one-day-old SPF layer chicks were randomly divided into 4 groups. At 14 days of age, chickens were orally treated with 5 μg CpG, or 5 μg E-CpG, or *C. jejuni* lysate (containing 4.3 μg protein), or PBS 24 h prior to oral challenge with 10^7 CFUs of *C. jejuni*. Cecal contents were collected at 8, 15 and 22 days post-infection (n = 8–11 per time point) and CFUs of *C. jejuni* were enumerated as described above.

2.6.3. Oral administration of E-CpG and C. jejuni lysate in broiler chickens

One hundred and sixty-eight one-day-old broiler chicks were randomly divided into 5 groups. At 14 days of age, chickens were orally treated with a low dose of E-CpG (5 μg), or a high dose of E-CpG (25 μg), or a low dose of *C. jejuni* lysate (4.3 μg protein) or a high dose of *C. jejuni* lysate (21.5 μg protein), or PBS 24 h before challenge with 10^7 CFUs of *C. jejuni*. Cecal contents were collected