Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Lambda display phage as a mucosal vaccine delivery vehicle for peptide antigens

Patricia González Cano [a], Lakshman N.A. Gamage [a,1], Kristen Marciniuk [a,c], Connie Hayes [b], Scott Napper [a,c], Sidney Hayes [b], Philip J. Griebel [a,d,*]

[a] VIDO-InterVac, University of Saskatchewan, Saskatoon, SK S7N 5E3, Canada
[b] Department of Microbiology and Immunology, College of Medicine, University of Saskatchewan, Saskatoon, SK S7N 5E5, Canada
[c] Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5E5, Canada
[d] School of Public Health, University of Saskatchewan, Saskatoon, SK S7N 2Z4, Canada

ARTICLE INFO

Article history:
Received 1 July 2017
Received in revised form 26 October 2017
Accepted 7 November 2017
Available online xxxx

Keywords:
IgA
Lambda display phage
Mucosal immune responses
Peptide antigens
Peyer's patches

ABSTRACT

Bacteriophage are structurally stable in the gastro-intestinal tract and have favorable traits of safety, stability, ease of production, and immunogenicity. These attributes make them potential candidates as oral vaccine delivery vehicles but little is known about their capacity to induce mucosal immune responses in the small intestine. Whole body imaging of mice confirmed lambda bacteriophage (LP) were distributed throughout the gastro-intestinal tract 24 h after oral delivery. In newborn calves, targeted delivery of LP within the small intestine confirmed LP were immunogenic in a dose-dependent manner and were taken up by Peyer's patches. LP-specific IgA responses were induced within both Peyer's patches and draining mesenteric lymph nodes. A lambda display phage (LDP) was constructed to present three immunogenic disease specific epitopes (DSE) from cervid prion protein (amino acids 130–140 [YML]; 163–170 [YRR]; and 171–178[YRR]) fused to phage capsid head protein D (LDP-DSE). Targeted delivery of purified LDP-DSE to intestinal segments induced IgA responses to all three peptide epitopes. Further, delivery of bacteria expressing soluble D-DSE also induced epitope-specific IgA responses in the targeted Peyer's patches. These are the first studies to report use of LDP to induce epitope-specific IgA responses in the small intestine and confirm Peyer's patches function as a site for LP uptake. Furthermore, IgA responses to peptide epitopes on LDP were observed in the absence of a mucosal adjuvant. These observations confirm LDP have the capacity to function as a mucosal delivery vehicle with protein D as an effective carrier for peptide epitopes.

1. Introduction

Bacteriophage (phage) [1] are viruses [2] that infect bacteria and phage are members of the microbiome [3,4] present in the oral cavity, respiratory tract, [5] and digestive tract of humans [6–8] and animals [9–11]. Phage have also been identified in the blood of healthy human donors [12]. They play a role in shaping the microbiome [11] and are considered a part of the normal microflora [13]. Bacteriophage have been used for antimicrobial therapy but also have potential as vaccine delivery vehicles [14–17].

Since phage replicate in bacteria, they are considered safe for eukaryotic hosts but as foreign antigens they interact with the mammalian immune system [18]. Immune recognition of phage may involve both mucosal and systemic immune systems since phage have been isolated from blood and other tissues following oral administration [19]. There was concern that phage may elicit adverse local or systemic inflammatory immune responses but phage and phage proteins failed to induce pro-inflammatory cytokine production [20,21]. Also, as an oral vaccine delivery system phage must survive the harsh conditions in the digestive tract. *In vitro* experiments demonstrated phage could survive extremes of pH with 68–77% recovery following exposure to a pH less than 2 [22].

Our previous studies confirmed lambda display phage (LDP) could be employed for parenteral immunization [16]. We found that 4 porcine *Circovirus 2* (PCV2) coat protein (CAP) epitopes could

Abbreviations: ASC, antibody secreting cells; PP, Peyer's patch; LDP, Lambda display phage; LP, lambda particles.

* Corresponding author at: VIDO-InterVac, University of Saskatchewan, Saskatoon, SK S7N 5E3, Canada.
E-mail address: philip.griebel@usask.ca (P.J. Griebel).
[1] Current address: CFIA, 534-East Cordova St, Vancouver, BC V6A 1L7, Canada.

https://doi.org/10.1016/j.vaccine.2017.11.010
0264-410X/

be expressed as a single recombinant protein fused to lambda protein D (D-CAP) to generate LDP. Purified LDP-D-CAP, when injected intra-dermally, induced both antibody and T-cell mediated immune responses. Vaccine immunogenicity was confirmed by PCV2 neutralization with sera from LDP-D-CAP vaccinated pigs and a PCV2-specific DTH reaction [16]. The immunogenicity of LDP, in the absence of an adjuvant, led us to hypothesize that purified LDP may also be used as a mucosal vaccine delivery vehicle.

LDP used in this investigation were designed to incorporate multiple peptide epitopes previously optimized for the induction of prion protein (PrP)-specific antibody responses [23,24]. Three disease specific epitopes (DSE) from cervid PrP were fused to phage capsid head protein D (D-DSE). We first investigated uptake of lambda particles (LP) and LDP from the intestinal lumen and then evaluated the capacity of D-DSE and LDP-DSE to induce mucosal immune responses in the absence of an adjuvant. We provide evidence that LDP are taken up from the small intestine by Peyer's patches (PPs) and that multiple B cell epitopes are immunogenic when fused to lambda D-protein. These studies also confirm that LDP were immunogenic and induced IgA antibody responses to displayed peptide epitopes in the absence of a mucosal adjuvant.

2. Material and methods

2.1. Animal experiments

All animal experiments were conducted at the University of Saskatchewan in accordance with guidelines approved by the Canadian Council on Animal Care and endorsed by the University of Saskatchewan Animal Care Committee (Protocol 2002105 for intestinal surgeries; Protocol 19940212 for mouse imaging studies).

2.1.1. Lambda phage labeling and imaging in mice

LP were labeled with AF750 (Alexa fluor 750®succinimidyl ester, (Thermo Fisher Scientific, Mississauga ON, Canada) as described previously [25]. Briefly, 1×10^{12} purified LP particles (1.13×10^{10} particles/μg phage DNA) were re-suspended in 300 mM $NaHCO_3$, pH 8.6, containing AF750 at a final concentration of 1 mg/mL and incubated for 1 h in the dark. Non-conjugated AF750 was removed by dialyzing twice for 2.5 h each in a 0.5 mL 3.5 K MWCO Slide-A-Lyzer™ mini dialysis device (ThermoFisher Scientific), against 300 mM $NaHCO_3$, pH 8.6. The concentration of labeled phage was determined by DNA concentration and adjusted to a final concentration of 1×10^{10} particles/ml in PBSA. Three Balb/c mice were orally gavaged with 1×10^9 particles delivered in 100 μL $Ca^{++}Mg^{++}$-free phosphate buffered saline (PBSA). Mice were euthanized 24 h later and the gastrointestinal tract was removed from the abdominal cavity before scanning with an IVIS® Lumina II *in vivo* Imaging System (PerkinElmer, Waltham MA, USA).

2.1.2. Intestinal segments for targeted delivery of lambda phage

The surgical procedure used to prepare intestinal segments was previously published [26]. For the current study jejunal segments were prepared in 10–14 day old calves and subdivided with silk ligatures into 10–15 cm long compartments that either contained or excluded PPs. Details of intestinal segment preparation and immunization protocols are provided with Supplementary Fig. 1.

2.2. Tissue collection and cell isolation

Calves were euthanized with 20 mL/45 kg Euthanyl (Bimeda-MTC, Cambridge ON, Canada) and tissues were collected within 20 min and placed in ice-cold AIM V (Thermo Fisher Scientific. Single cell suspensions were isolated from PPs as previously described ([27,28]). Single cell suspensions were prepared from LNs by finely mincing tissues with a scalpel blade and passing cell suspensions through a 40 μm nylon cell strainer (BD Falcon™, Mississauga ON, Canada). Viable cell concentrations were adjusted to 5×10^6 cells/mL for ELISPOT assays.

2.3. ELISPOT assay for IgA and IgG antibody-secreting cells (ASCs)

ELISPOT assays were performed as described previously [39] with the following modifications. Multiscreen-HA ELISPOT 96-well plates (EMD Millipore, Oakville ON, Canada) were coated with100 μL/well of LP (1×10^{10} pfu/mL) or 100 μL/well containing 8 μg/ml of either YYR, YML or RL peptide [23,29]. Bound immunoglobulins were detected with either biotin-conjugated anti-bovine IgG (AbDSerotec/Bio-Rad, Raleigh NC, USA) or anti-bovine IgA (AbDSerotec). An image of each well was captured using the AID Elispot Reader ELR07 (AutoimmunDiagnostika GmbH, Strassberg, Germany) and the number of spots enumerated using AID Elispot Reader Software V7.0 (AutoimmunDiagnostika GmbH). Antigen-specific ASCs per million cells was calculated using the formula: [(average number of ASCs for cells in triplicate wells with an antigen – average number of ASCs for cells in triplicate wells cultured with medium alone) × 2].

2.4. D-DSE and D-His fusion protein purification

E. coli strain 594 = R594 *lac*3350 *galK*2 *galT*22 *rpsL*179 IN(*rrnD-rrnE*1 [30] was transformed with either the pcIpR-D-[DSE]-mcs-timm or pcIpR-His-Dcoe-timm plasmids encoding the D-fusion proteins as described in [31] and transformed bacteria were used to analyze expression of the D-fusion proteins. D-His protein was purified by following the manufacturer's instructions for Ni-NTA His binding Resin (Novagen, Madison WI, USA) and eluted protein was purified with an ÄKTA Explorer FPLC System (Amersham Pharmacia Biotech, Uppsala, Sweden). Protein was loaded onto a size exclusion column (HiPrepSephacryl 100 xk26/65, GE Healthcare, Mississauga ON, Canada). Positive fractions were pooled, concentrated with Amiconultra NMWL, 10 KDa (Millipore), and filtered through a 0.22 μm low protein binding filter (Millipore). Final protein concentration was determined using the Pierce BCA protein assay kit (Thermo Fisher Scientific).

2.5. Gene expression plasmids for phage display

Genes corresponding to the three epitopes were synthesized to enable either amino or carboxy-terminal fusion to the lambda D protein and were ordered from Integrated DNA Technologies (IDT; Coraville IA, USA). Genes were obtained either as insertions cloned within their pIDTSMART-Amp plasmids or as synthetic gene blocks. These were amplified by PCR, or cloned directly to produce thermally inducible expression versions of ColE1-based pcIpR-D-fusion-timm plasmids (Fig. 1A) and these were used to prepare lambda display particles (LDP). Dcoe is a codon optimized (55 codon changes) version of the wild type gene, Dwt [31]. Polypeptides fused to D (Fig. 1B) were optimized for expression in *E. coli* and to avoid problematic restriction sites. Construction of pcIpR-D-fusion-timm plasmids is described in Supplemental Methods File 1.

2.6. Preparation of phage lysates of LDP with D-fusions

LP displaying D-fusion proteins were prepared as described in Sections 2.4 and 2.5 of [31], however, the infecting phage λ*imm*434(*18*,*12*)P22 replaced the previous λ*imm*434*cI*$^-$ lysate #5 which encodes the rep(*O*,*P*)λ genes, since *P* expression can rapidly