A subunit vaccine for bovine viral diarrhea: lipid-based formulation containing bovine viral diarrheas virus E2 protein in combination with adjuvants to improve immunogenicity

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INTRODUCTION

Bovine viral diarrhea virus (BVDV) contributes to a respiratory disease complex in cattle and is an important pathogen in the cattle industry. Effective vaccines are essential for reducing the economic and animal welfare impact of BVDV. Fabrication and physical characterization of a cationic lipid-based delivery system for intranasal administration of the E2 protein as a BVDV vaccine was performed. A previously developed triple adjuvant (TriAdj) comprised of innate defense regulator IDR-1002 peptide, poly(I:C), and a polyphosphazene, which self-assembles into particles, has shown improvement in the immunogenicity of the antigenic E2 protein of BVDV[1]. Cationic lipidic nanoparticle vaccines are anticipated to bind to the negatively charged nasal epithelium leading to increased retention time of the formulation[2] and to enhance immunogenicity via activation of mucosal immunity. The intranasal route of administration offers a significant benefit for vaccines against respiratory pathogens at the route of entry.

HYPOTHESIS

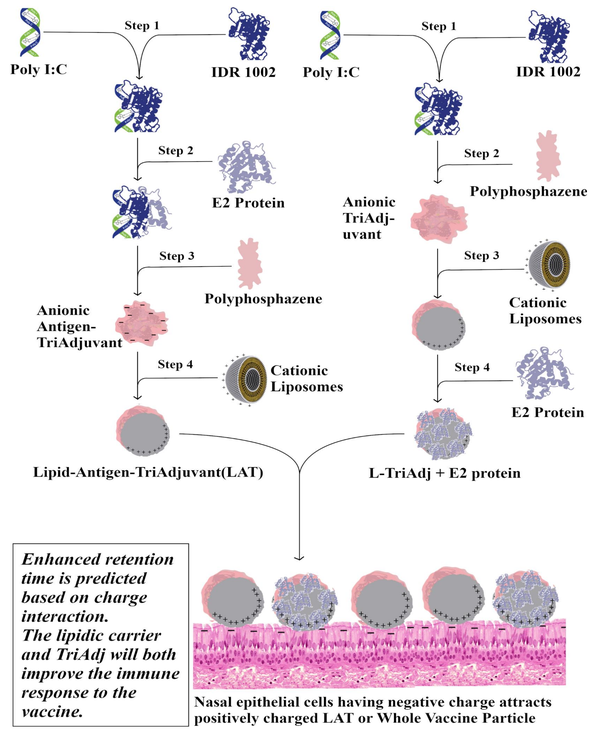
1. Cationic lipid nanoparticles can be formulated for TriAdj by electrostatic condensation and microfluidics–enabled self-assembly.
2. A cationic lipid nanoparticle formulation of TriAdj will be an effective adjuvant for BVDV E2 protein vaccines when administered intranasally.

OBJECTIVES

- To optimize cationic lipid formulations of the adjuvant TriAdj (L-TriAdj) co-formulated with acellular antigens (E2 protein) based on physical characterization.
- To demonstrate the feasibility of lyophilizing the vaccine and maintaining submicron mean diameter and cationic charge upon reconstitution.

MATERIALS AND METHODS

To prepare the triple adjuvant TriAdj, polyphosphazene, IDR-1002 peptide and poly (I:C) were mixed in 1:2:1 weight ratio which was previously optimized in vivo. Cationic liposomes comprised of DDAB and DOPE (50:50 mol:mol) were prepared and combined at a stoichiometric ratio with TriAdj to make cationic lipid nanoparticles (L-TriAdj). Two preparations of whole vaccine were prepared: 1) A simple mixture of L-TriAdj + E2 protein; 2) Lipid-Antigen-TriAdj (LAT) delivery system. In the second method, the E2 protein was incorporated into the poly (I:C)-IDR-1002 peptide comixture followed by addition of polyphosphazene to obtain Antigen-TriAdj (AT), then the lipid was added to form an electrostatically condensed cationic complex (LAT). The effect of lyophilization on the physical properties was explored using 5% w/v dextrose, 2.5% w/v dextrose and 5% w/v trehalose as lyoprotectants for L-TriAdj with various drying times.



RESULTS

Table 1: Characterization data of L-TriAdj + E2 particles and LAT particles.

Particles	Size (nm)	Zeta Potential (mV)
Liposomes	100+/- 10	+ 50 +/- 2.5
TriAdjuvant	550+/- 32	- 14 +/- 1.1
L-TriAdjuvant	150+/-13.5	+ 34 +/- 2.2
L-TriAdj + E2	232.7+/38.68	+ 42 +/- 7.78
Antigen (E2) -TriAdjuvant	377.16 +/- 9.4	- 11.7 +/- 0.3
LAT (Lipid-Antigen-TriAdjuvant	225.4 +/- 113.7	+ 41.5 +/- 0.9

- L-TriAdj particles lyophilized with 5% w/v trehalose then reconstituted showed consistent mean diameters (300+/-25 nm) irrespective of lyophilization time (Figure III).
- Lyophilized and reconstituted LAT particles had a significantly greater mean diameter (337.7+/-260.8 nm) than non-lyophilised LAT particles (225.4+/-113.7 nm).
- Currently, cationic liposome fabrication is being optimized using a microfluidics platform, obtaining a monodisperse formulation with mean diameter of 78.15 +/- 30.2 nm.

Figure I. Mean Size of L-TriAdj in 2.5% (w/v) Dextrose in Water by Lyophilization Time (n=3, mean ± STD, p<0.05).

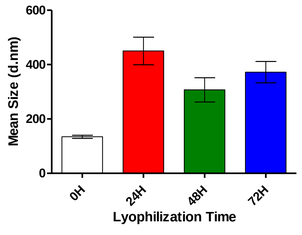
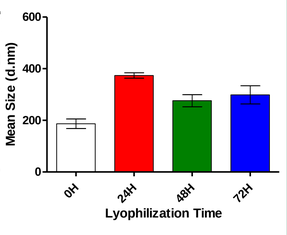


Figure II. Mean Size of L-TriAdj in 5% (w/v) Dextrose in Water by Lyophilization Time (n=3, mean ± STD, p<0.05).



RESULTS (cont…)

Figure III: Mean diameter of L-TriAdj in 5% (w/v) trehalose in water vs lyophilization time (n=3, mean ± SD, p<0.05).

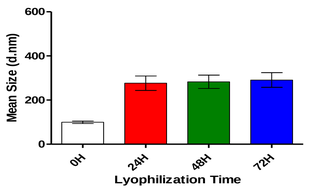
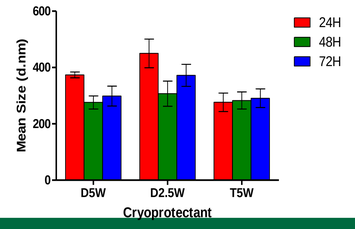


Figure IV. Overall Mean Size by Lyophilization Time for Each Cryoprotectant (n=3, mean ± STD).



CONCLUSION

- At this stage trehalose appears to be the best lyoprotectant irrespective of lyophilization time for L-TriAdj and will be evaluated for L-Antigen-TriAdj.
- Characterization data of the particles prepared using LAT procedures where mean diameter is <400nm and cationic is in the desirable range for intranasal vaccination.
- In vivo efficacy of the vaccine particles will be determined in the near future.

REFERENCES

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