Lipid nanoparticle formulation of a triple adjuvant for intranasal vaccines: RNA sequencing identifies the immune system reactome pathways

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ABSTRACT

Mucosal vaccines constitute a promising non-invasive approach to improve vaccine effectiveness. However, there are challenges related to local tolerance and the stability of antigens. A **novel triple adjuvant** system (TriAdj) comprised of poly(I:C), innate defense regulator peptide IDR-1002 and polyphosphazene (polymer) has been formulated as cationic lipid nanoparticles (L-TriAdj) to provide enhanced mucoadhesion and immune stimulation for intranasal vaccination. To understand the impact of the lipid components of L-TriAdj on cellular viability, vaccine uptake, and its effect on gene expression patterns, we have conducted in vitro experiments using macrophage cells. L-TriAdj was created by combining anionic TriAdj with cationic lipid nanoparticles formed by self-assembly of DDAB/DOPE or DDAB/DOPE/DSPC/cholesterol. Different ratios of lipid to TriAdj were used, and inactivated porcine reproductive and respiratory syndrome virus (PRRSV) was added to the L-TriAdj. RNA was isolated and transcriptomic sequencing was performed to analyze the immune response of the cells to stimulation. We also assessed cytotoxicity of the cationic lipid nanoparticles and observed cellular uptake using flow cytometry and fluorescence imaging. The results showed that TriAdj up-regulates key immune pathways, such as pro-inflammatory innate signaling, FCGRI signaling, ER-phagosome pathway, and antigen presentation, while lipid nanoparticles without TriAdj lack these effects, highlighting the crucial role of TriAdj in modulating the immune response. Furthermore, specific L-TriAdj formulations not only reproduce the effects of TriAdj but also enhance antiviral immune mechanisms. Formulations containing DDAB/DOPE/DSPC/cholesterol had the highest cellular viability, exceeding 80%. These formulations also had over 90% cellular uptake.

PURPOSE

- To achieve effective mucosal adhesion and induce both mucosal and systemic immune responses by;
  - investigating the impact of lipid composition and the lipid:TriAdj ratio on the characteristics of cationic lipid nanoparticles formulated with TriAdj.
  - Relate vaccine composition to cell viability and uptake.
  - Evaluate differences in immune system reactome pathways stimulated by varying vaccine adjuvant composition.

METHODS

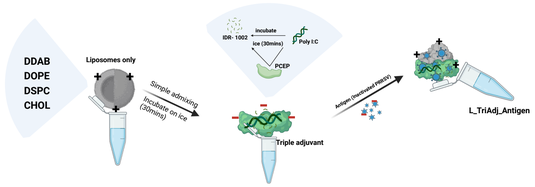

Figure 1: Schematic representation of the components of TriAdj and the preparation of the lipid nanoparticle adjuvant formulation.

- L-TriAdj was prepared in two steps: liposomes were made by the thin-film extrusion method, followed by addition of pre-formed TriAdj by simple mixing for self-assembly of L-TriAdj particles (Fig. 1) .The lipid components of L-Triadj consisted of appropriate molar ratios of DDAB, DOPE, DSPC, and CHOL.
- L-Triadj ratios were varied. L-TriAdj A corresponds to a ratio of 15:1(mol:vol), L-TriAdj B corresponds to a ratio of 20:1 (mol:vol), and L-TriAdj C corresponds to a ratio of 5:1(mol:vol). PRRSV was added to the L-TriAdj formulation at an amount corresponding to a desired dosing level of 1ug per 20uL L-TriAdj.
- RAW 264.7 mouse macrophage cells were used to carry out all in-vitro analyses. This cell line is commonly used for preclinical vaccine nanoparticle screening.
- Cytotoxicity was measured using MTT assays while cellular uptake was observed by flow cytometry and fluorescence microscopy (EVOS®) using CY 7- PE tagged lipids.
- Cells were stimulated for 12 hours with TriAdj or varying L-TriAdj ratios, and the latter also tested with addition of the PRRSV antigen. RNA was isolated using RNeasy Mini Kit (Qiagen). Sample quality was assessed using a 2100 BioAnalyzer (Agilent) and Quanti-iT (ThermoFisher) to quantify the amount of RNA. Differential gene expression analysis was performed using DESeq2 version 1.26.0.

RESULTS

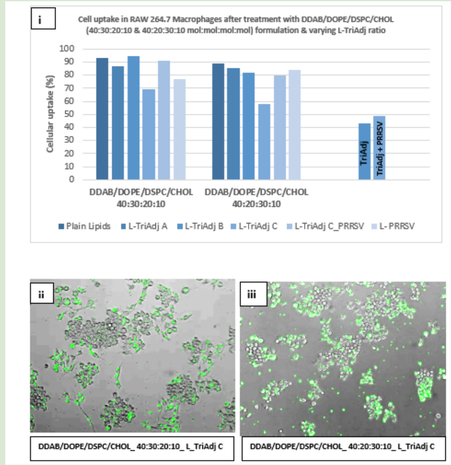

Figure 2: Percentage cellular uptake of CY 7-PE labeled lipid nanoparticle formulations in RAW 264.7 cells analyzed through flow cytometry (i) and fluorescent microscope (ii & iii).

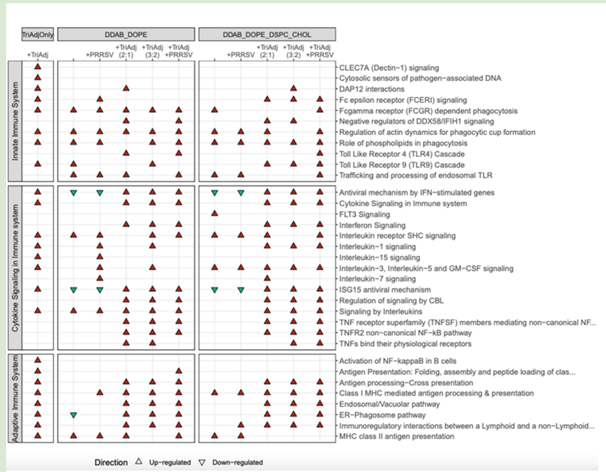

Figure 3: Enriched immune system pathways that differ across vaccine formulations.

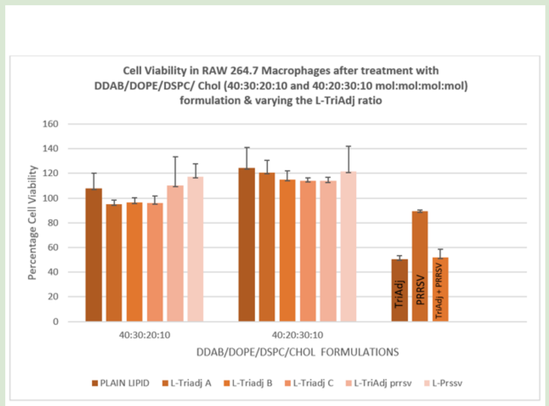

Figure 4: Percentage cell viability of lipid nanoparticle formulations in RAW 264.7 macrophage cells after 12 hours treatment.

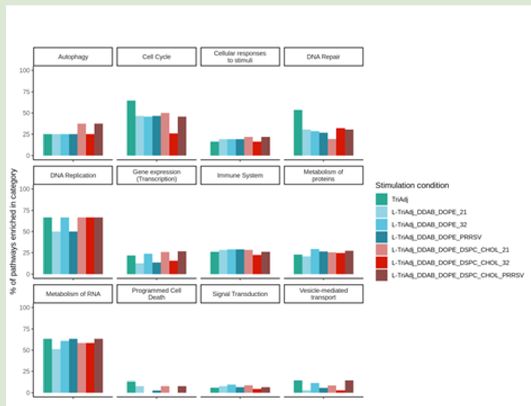

Figure 5: Percentage of pathways enriched in select Reactome pathway categories.

RESULTS

- The liposome particle sizes varied between 130nm and 160nm, and the zeta potential varied between 44mV and 48mV. A positive correlation was noted between lipid content and a decrease in particle size, alongside a rise in cationic lipid nanoparticle charge.
- Irrespective of the proportion of lipid used in the TriAdj complex, there appears to be a marked decrease in the toxicity of TriAdj, and all tested liposome vaccine preparations displayed a robust protective effect against cell death, exhibiting >80% cell viability (Fig 4).
- Uptake studies revealed CY 7 labeled L-TriAdj formulations were effectively internalized in the cytosol of the cells exhibiting over 85% cellular uptake (Fig 2).
- Higher proportion of cell cycle, DNA repair, and programmed cell death pathways were enriched upon TriAdj stimulation (Fig 5).
- L-TriAdj formulations generated immune responses similar to TriAdj in RNA-Seq study. There was an up-regulation of antiviral immune mechanisms, TLR4 cascade, and TNF receptor superfamily members . Pro-inflammatory interleukin signaling pathways (eg. IL1, IL3, IL5) are not consistently up-regulated by L-TriAdj formulations lacking DSPC and cholesterol (Fig 3).

CONCLUSIONS

The cellular viability and uptake are significantly influenced by both the lipid compositions and the lipid:TriAdj ratio. Analysis of RNASeq data indicated that the combination of the triple adjuvant with lipid formulations effectively reduces the cytotoxic effects of TriAdj while eliciting distinct immune system pathways. However, although L-TriAdj formulations activate adaptive immune pathways, they do not fully replicate the effects of TriAdj. Consequently, additional investigations are required to validate these results. The use of lipid carriers to improve the safety and effectiveness of vaccine adjuvants highlights the immense potential of liposome and lipid nanoparticle therapeutics.

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