Design and in-vivo evaluation of lipid nanoparticles with a triple adjuvant to achieve enhanced immunity against *Bordetella pertussis*.

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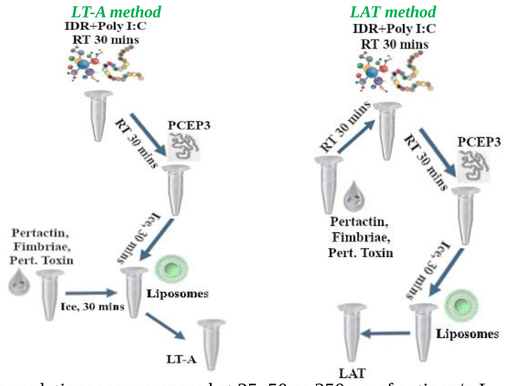
Purpose

- Pertussis is a respiratory infection caused by the pathogen *Bordetella pertussis*.
- Recent outbreaks affected 24 million people worldwide of which most mortality was observed in infants less than one year old.
- Potential causes of re-emergence are inadequate protection in infants, failing of acellular vaccines to induce mucosal immunity and waning of immunity over time.
- Need to redesign the current vaccine for balanced Th1/Th2 response and both mucosal and systemic immunity.
- Vaccine adjuvants enhance immunogenicity of vaccines by inducing stronger, faster and long-lasting immune responses.
- Intranasal vaccines achieve mucosal immunity directly at the portal of entry.

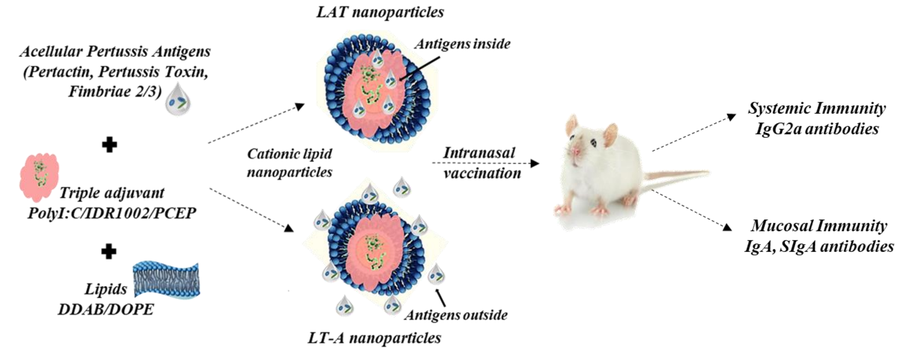
Methods

Formulation: Acellular pertussis antigens were formulated with a triple adjuvant system (TriAdj) composed of: poly(I:C) a TLR agonist, innate host defense regulator peptide (IDR 1002) and a polyphosphazene [PCEP, a carrier and for pathogen-associated molecular pattern (PAMP) recognition], created into lipid nanoparticles with DDAB:DOPE (1:1 mol:mol) by 2 methods:

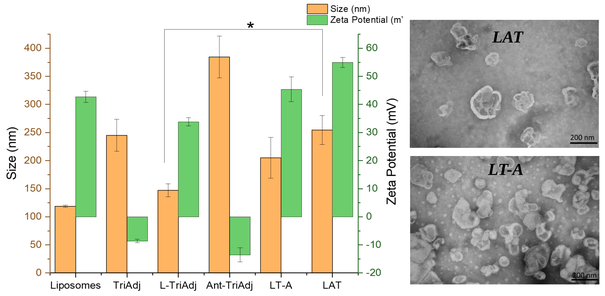


Formulations were prepared at 25, 50 or 250 µg of antigen/mL.

For in-vivo immunogenicity studies in mice, 20µl containing 1 or 5 µg of antigens were administered intranasally with a booster dose at 28 days. Mice were terminated at 56 days. Serum levels of IgA, IgG1 and IgG2a as well as secreted IgA from nasal wash were measured by ELISA. Statistical differences were assessed by ANOVA and Tukey's post-hoc test.

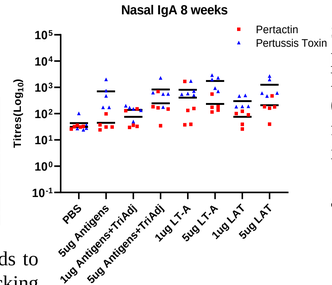


Results

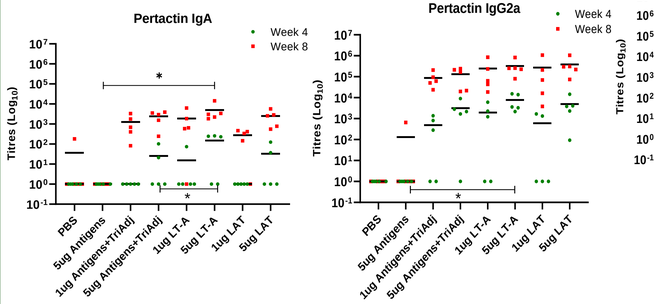


Changes in zeta potential from negative to positive after addition of lipids to the TriAdj confirms their interaction. TEM images indicate different packing arrangements for LT-A and LAT

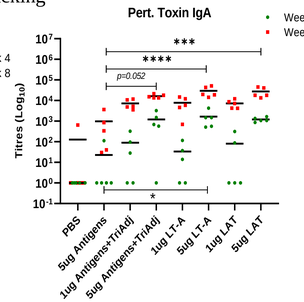
All mice responded to the lipid vaccine for pertactin and pertussis toxin.

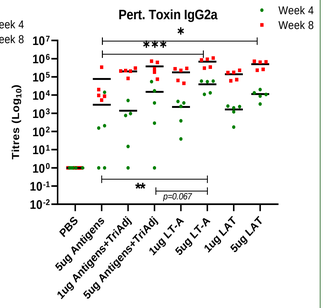


SIgA titres of 5µg LT-A for pertussis toxin were 2.5-fold higher compared to unformulated (plain) antigens. The lipid formulation showed a trend* to improving the IgA responses at 1µg dose compared to TriAdj alone. **but p>0.05 due to variability*



5.7-fold higher pertactin IgA for 5µg LT-A and 5.5-fold higher pertactin IgG2a titres compared to antigens alone at 4 weeks.





70-fold higher pertussis toxin IgA for 5µg LT-A at 4 weeks maintained at 30-fold at 8 weeks and IgG2a levels 10-fold higher compared to antigens alone.

Key messages

- Intranasal vaccines can have far-reaching impacts by improving mucosal immunity, particularly for respiratory diseases.
- LT-A method with antigens on the exterior were more immunogenic compared to the LAT with antigens on the interior.
- LT-A vaccines produced high IgA, IgG1, IG2a and SIgA titres indicating that the vaccine contributes to a balanced Th1/Th2 type response.
- LT-A vaccines provide much needed early onset of immunity after one vaccine dose.
- The antigen dose was relatively high, thus a strong response was noted in all adjuvant groups; the lipid formulations may have an advantage at lower doses. A dose-response study will follow.
- Lipid-based triple adjuvant nanoparticles can be utilized for intranasal vaccine delivery and have broad applications for various therapeutic and vaccine formulations.

References

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Acknowledgements

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