SUMMARY
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Immunogenicity of polymer entrapped antigen depends on many parameters associated with polymer characteristics, formulation and immunization methods. It needs a multidisciplinary research approach involving, polymer chemistry, protein/antigen stability, pharmaceutics and immunology. In this study, an attempt was made to optimize the immune response from polymer entrapped TT particles while studying various formulation factors. Hydrophobic biodegradable polymer PLA was used for preparation of TT entrapped particles. Excipients were incorporated both in the internal and external aqueous phases to protect the immunoreactivity of TT during particle formulation using solvent evaporation method. Parameters associated with particle characteristics were optimized to achieve long lasting anti-TT antibody response from single point intramuscular immunization. Both primary and memory antibody response were evaluated immunizing with single dose of polymer entrapped TT particles. The following conclusions were observed from the current study:

1. Use of excipients not only influenced the encapsulation efficiency of TT but also affected the emulsion stability and polymer particle characteristics in major ways.

2. Rat serum albumin was found to protect the immunogenicity of microencapsulated TT. Increasing TT concentrations in internal aqueous phase during particle formulation helped in protecting the immunoreactivity of TT during particle formulation.

3. Addition of sucrose in the internal aqueous phase (IAP) was found to reduce the encapsulation efficiency drastically. This loss in encapsulation efficiency on addition of solutes was attributed to swelling of IAP as it becomes osmotically different from external aqueous phase (EAP).

4. Restoring osmotic balance of EAP by addition of sucrose/solutes helped in countering the influx of water from EAP to IAP and prevented the swelling of IAP. This resulted in reduction of loss of IAP droplets to EAP and improved the encapsulation efficiency of TT in PLA particles.
5. Addition of sucrose in EAP was found to increase the burst release of TT from PLA particles. The overall antigen released from microparticles was also enhanced. Formulations without sucrose in IAP did not exhibit similar burst release phenomenon.

6. Incorporation of NaHCO₃ in IAP led to ten fold reduction in burst release. However, TT released during the entire phase of study was more uniform than other formulations.

7. Optimization of excipients in both in internal and external aqueous phase improved the encapsulation efficiency of TT from 20-30 % to > 65 % in PLA particles.

8. Presence of alum resulted in reduction of in vitro released amount of TT from microparticles.

9. Kinetics of TT adsorption on alum showed that 90 % TT was adsorbed on alum within a few seconds of vortexing at room temperature. It seems possible that TT released during burst phase from microparticles gets adsorbed on alum and help in enhancement of high antibody titer.

10. Reduction in particle size led to the enhancement in the burst release as well as over all release of antigen from microparticles. This was attributed to the increase in total surface area with decrease in particle size.

11. As the particle size was decreased from 99 μm to 4 μm, antibody titers elicited by particles increased from single point immunization. Particles with submicron size range < 2 μm (0.50-1.98) gave titers lower than that elicited by particle with 2-8 μm size.

12. Anti-TT antibody titers generated from particle based immunization was enhanced considerably when an admixture of particle and alum was used for immunization.

13. Anti-TT antibody titer was found to be dependent on the antigen load. Higher TT load resulted in higher antibody titers from single dose immunization of PLA entrapped TT particles.

14. Primary antibody titers generated by immunization with microparticles were dependent on the dose of microencapsulated TT.

15. Single doses of PLA entrapped TT formulation with alum were found to be more immunogenic than the single dose of the same amount of alum adsorbed TT.

17. Tetanus toxoid entrapped PLA particles immunized with alum enhanced antibody titers, which were comparable to two doses of alum adsorbed vaccine. At lower doses, the immunogenicity of the polymer entrapped TT was found to be higher than two divided doses of alum adsorbed TT.

18. Single dose immunization with polymer entrapped TT elicited memory antibody response upon re-exposure to very small amount of soluble TT, whose magnitude and sustainability varied for particles with different characteristics and correlated with primary antibody response.

19. Enhanced memory response was also found to be dependent on the factors affecting primary antibody response. Higher memory antibody response was exhibited by particles with size range 2-8 μm, high antigen load and with particles releasing higher amount of TT during burst phase.

20. Memory response was also dependent on the dose of TT immunized in the form of polymer particles.

21. Memory response generated by particle based immunization was significantly higher than that induced by equivalent dose of alum adsorbed TT, which exhibited early and small peaks. Very low doses of microencapsulated TT administered with alum also exhibited improved memory response from single point immunization.

22. The affinities of antibody during memory response in case of microencapsulated TT were similar to that generated by alum adsorbed vaccine.

23. Memory antibody response generated using particle based immunization sustained at a higher level over a longer period of time as compared to antibody response generated by two doses of alum adsorbed TT.

24. Antibodies generated during memory response using polymer particle based immunization had similar IgM titers but higher IgG titers than that observed with conventional two doses of alum adsorbed TT.

25. Immunization of admixture of polymer entrapped TT and alum improved both the primary and secondary antibody response from single point immunization.
The present work helped in optimizing the parameters associated with particle characteristics to improve the immune response from single dose vaccine. However mechanisms working towards such improved immune response at cellular level need further investigation. There is scope for further improvement and some of the areas which need attention are:

1. Cellular uptake studies of different sized particles and elucidation of the role of particular sized particles suitable either for generation of cellular response or humoral response.

2. Cooperative effect of adjuvant at cellular level for improvement in immune response from polymer entrapped antigen. It needs to delineate the role played both by the particle and alum in presence of each other for augmented antibody response from single point immunization.

3. Immune response from polymer entrapped particles using other routes such as intra-dermal or intra-peritonial injection.

4. Mechanism of improved immunological memory from single point particle based immunization at cellular level. It is of considerable interest to evaluate what parameters associated with polymer particle based formulation results in improved immunological memory.