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Improved immune response from biodegradable polymer particles entrapping tetanus toxoid by use of different immunization protocol and adjuvants

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Abstract

Poly lactide-co-glycolide (PLGA) and polylactide (PLA) particles entrapping immunoreactive tetanus toxoid (TT) were prepared using the solvent evaporation method. The effect of different formulation parameters such as polymer hydrophobicity, particle size and use of additional adjuvants on the generation of immune responses in experimental animals was evaluated. Immune responses from hydrophobic polymer particles were better than those from hydrophilic polymer. Immunization with physical mixtures of different size particles resulted in further improvement in anti-TT antibody titers in Wistar rats. Physical mixture of nano and microparticles resulted in early as well as high antibody titers in experimental animals. Immunization with polymer particles encapsulating stabilized TT elicited anti-TT antibody titers, which persisted for more than 5 months and were higher than those obtained with saline TT. However, antibody responses generated by single point immunization of either particles or physical mixture of particles were lower than the conventional two doses of alum-adsorbed TT. Immunization with nanoparticles along with alum resulted in very high and early immune response: high anti-TT antibody titers were detected as early as 15 days post-immunization. Use of a squalene emulsion along with the particles during immunization enhanced the level of anti-TT antibody titers considerably. Single point immunization with admixtures of PLA microparticles and alum resulted in antibody response very close to that achieved by two injections of alum-adsorbed TT; the antibody titers were more than 50 μg/ml over a period of 6 months. These results indicated that the judicious choice of polymer and particles size, protecting the immunoreactivity of the entrapped antigen and the appropriate design of immunization protocol along with suitable adjuvant can lead to the generation of long lasting immune response from single dose vaccine formulation using polymer particles.

Keywords: Polymer particles; Tetanus toxoid; Single dose vaccine; Adjuvant; Hydrophobicity; Anti-TT antibody titers

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1. Introduction

Use of poly lactide-co-glycolide (PLGA) and polylactide (PLA) polymers for entrapping vaccine/antigens provides a viable alternative to multi dose vaccine injection schedules for immunization (Hanes et al., 1997; Cleland, 1999). Studies on the use of PLGA particles carried out to explore the possibilities of developing a single shot vaccine for tetanus toxoid (TT) have met with little success (Alonso et al., 1994; Kersten et al., 1996; Audran et al., 1998; Tobio et al., 2000; Johansen et al., 2001). The low antibody response generated by PLA or PLGA encapsulated TT particles in most of the reports has been attributed to antigen instability inside the polymer particles (Alonso et al., 1994; Chang and Gupta 1996; Raghuvanshi et al., 1998; Sasiak et al., 2001; Wreet et al., 2000). Results of antibody titers obtained from groups immunized with two injections of 5 Lf TT each on alum and equivalent doses of encapsulated TT showed that the conventional immunization schedule gives significantly higher antibody titer than immunization with polymer encapsulated TT (Kersten et al., 1996; Higaki et al., 2001; Diwan et al., 2001; Johansen et al., 2001). Even though the antibody titers persisted for long time in the case of immunization with TT particles, a desired sustained antibody response in vivo matching that of two single injection of 5 Lf TT given at an interval of 4–6 weeks needs further investigation. It necessitates the formulation of polymer particles containing immunopotent TT on one hand and understanding of the antigenic response generated in vivo on the other.

Development of a single dose vaccine using biodegradable polymer needs the optimization of parameters necessary for the generation of an improved immune response. It is essential that the particles should entrap immunoreactive antigens (Raghuvanshi et al., 2001) and that the release of antigen should mimic the conventional vaccination schedule, thus providing in vivo auto boosting to elicit the desired antibody response. Polymer particle should be less than 5 μm in diameter to be efficiently taken by antigen presenting cells (Eldridge et al., 1991a) and preferably be made from hydrophobic polymer for better antigen presentation (Kreuter et al., 1988). Apart from this, it is also desirable that antigens released from polymer particles are immunogenic. This is most important as polymer particles release soluble antigen and it is widely documented that soluble antigens are weak immunogens. Hence, in spite of the adjuvant effect, immune responses from polymer entrapped antigen are generally low in comparison to the alum adsorbed counterpart. Thus to develop a single dose vaccine, it is not only imperative to release immunoreactive antigen from polymer particles but also essential to make them more immunogenic either by delivery to macrophages or immunizing along with an adjuvant for better presentation to antigen presenting cells. Probably, because of the failure to meet these requirements, many aiming at developing a single dose vaccine using polymer particle based immunization have not been successful. The earlier reports on TT were on large size particles (10–100 μm) and continuous release of TT from PLGA particles was shown for 1-month time (Alonso et al., 1994; Johansen et al., 1998; Sachwendeman et al., 1998; Tobio et al., 2000). There are reports that decreasing particle size (Eldridge et al., 1991a) and increasing hydrophobocity of polymeric particles both improve the immunogenicity of entrapped antigen (Conway et al., 1997). However, very little has been reported on the combined effect of parameters such as hydrophobiocity, particle size and effect of additional adjuvant on immune response from polymer entrapped antigen.

We have recently reported the preparation of immunoreactive TT polymer particles, taking care of possible organic solvent induced antigen denaturation during particle formulation (Raghuvanshi et al., 1998, 2001). In the present study, the immunogenicity of nanoparticles (<1 μm diameter) and microparticles (<5 μm diameter) entrapping TT prepared from PLA and PLGA polymer was evaluated. Adjuvant activity associated with polymer particles was further improved using a combination of particles or an additional adjuvant. Immunization with hydrophobic polymer particles along with alum as an adjuvant resulted in antibody responses very close to those achieved by two injection of alum adsorbed TT.

The role of hydrophobic polymer particles and the use of additional adjuvant on potentiating the immune response from polymer particles based immunization are discussed in this paper.

2. Materials and methods

2.1. Materials

Poly D,L-lactide-co-glycolide, [PLGA, 50:50, 45 kD, high mol. weight (HMW)] and Poly D,L-lactide (PLA, 45 kD) were purchased from Birmingham Polymer Inc. USA, PLGA, [50:50, 14 kD low mol. weight (LMW)] were prepared as reported earlier (Mehta et al., 1995). TT (3000 Lf/ml, protein content 8 mg/ml) was purchased from Serum Institute of India, Pune. Rat serum albumin (RSA) [A-6272] and Polyvinylalcohol, MW 30,000 (PVA) were from Sigma Chemicals USA. Micro BCA protein assay kit was from Pierce, USA. Polyclonal antibodies to TT and goat anti-TT HRPO conjugate were from Reagent Bank of National Institute of Immunology, New Delhi, India.

2.2. Methods

2.2.1. Preparation of polymer particles

PLA and PLGA polymers particles entrapping TT were prepared using a multiple emulsion solvent evaporation method (Raghuvanshi et al., 2001). The primary emulsion between the internal aqueous phase (TT in PBS, pH 7.4) and the organic phase (polymer solution in DCM) was prepared by sonication (20 W, 80% duty cycle, 20 cycles) (Branson, Sonifier 450, USA). Organic to aqueous phase volume ratio during primary emulsification step was 40. Secondary emulsification was carried out in 1% PVA solution either by sonication (20 W, 80% duty cycle, 20 cycles) (Branson, Sonifier 450, USA) to obtain nanoparticles or by homogenization (10,000 rpm for 10 min) (Virtis, Cyclone I.Q., USA) for microparticles. The volume of the secondary emulsion was four times that of the primary emulsion. The resulting polymeric particles were washed twice with cold PBS and lyophilized to obtain free flowing powder. The size of the particles was measured using GALAI-CIS-1 particle size analyzer. All particles were prepared using RSA as a stabilizer in the internal aqueous phase during the primary emulsification step.

2.2.2. Estimation of protein content of polymer particles

To measure the protein content of particles, accurately weighed polymer particles were dissolved in acetonitrile to solubilize the polymer while precipitating the encapsulated protein. The precipitated protein was pelleted by spinning at 5000 rpm for 10 min and then dissolved in 1% SDS solution. Total protein content of the solution was determined by micro BCA assay. Extracted proteins were run on SDS-PAGE gel, and density of gel bands was scanned to quantify the amount of TT in the protein mixture. TT loading was calculated as the per cent weight of protein per unit weight of polymer.

2.2.3. In vivo animal studies

Immunogenicity of the encapsulated TT in particles was evaluated in an animal model using Wistar rats (six animal in each group). Rats were injected intramuscularly with nanoparticles or microparticles of TT made from PLGA polymer. Particles made from PLA and PLGA were also immunized to evaluate the suitability of polymer for single dose vaccination. In each case, 30 μg of TT containing particles were immunized. A physical mixture of polymer particles of different size was also used for immunization. One group of rats was immunized with a 1:1 mixture of nanoparticles and microparticles made from PLGA 50:50 (LMW/14 kD). This immunization was carried out to achieve an early response from nanoparticles and a late response from microparticles, which could result in sustained antibody response. Another group was immunized with a 1:1 mixture of nanoparticles and microparticles, both made from PLA, 45 kD polymer. The total dose of TT was 30 μg for the physical mixture groups. Particles were immunized along with adjuvant such as squalene and alum. Fifty microliters of squalene emulsion (prepared by using 3 ml of saline, 30 μl of squalene and 30 μl of polysorbate 80) was used for each rat

during immunization. Two injections of TT (5 Lf each, total 30 μg) adsorbed on alum was given at 4 weeks interval and was compared with PLGA 14 kD particles as: (a) a single dose injection of 15 Lf encapsulated TT; (b) a single dose injection of 15 Lf encapsulated TT along with 50 μl of alum; and (c) a single dose injection of a mixture of 5 Lf TT on alum and 10 Lf TT in encapsulated form. For the admixture of alum and particles, 50 μl of 2% alum (Superfos Biosector a/s, Denmark) per animal was mixed with the particles prior to immunization.

Animals were maintained according to the guidelines established by the Institute Animal Ethics Committee of the National Institute of Immunology, New Delhi. Animals were bled at different time interval through retro-orbital plexus and ELISA for analyzed serum anti-TT antibody titers. Anti-TT antibody titers were defined as μg of antibody/ml of the sera employing affinity purified antibody as references in ELISA. Antibody titers of individual animals ($n = 6$) were estimated in duplicate and their concentrations (μg/ml) were determined as geometric means. To analyze the statistical significance of the antibody titers student's t-test at 95% confidence level was carried out.

2.2.4. ELISA protocol

Anti-TT antibodies in rat sera were estimated as reported previously (Raghuvanshi et al., 1998). Briefly 1 μg of TT in 100 μl of PBS (50 mM, pH 7.4) was coated in each well of 96 well flat bottom Nunc immunoplates. The plates were then washed with PBS-T (1% v/v polysorbate 20 in PBS) and different dilutions of rat serum in PBS-T was added to the wells and incubated for 1 h at 37 °C. After washing thrice with PBS-T, 100 μl of goat-*anti*-rat-HRPO conjugate diluted in PBS-T was added into each well and incubated for 1 h at 37 °C. Finally, 100 μl of *O*-phenyl diamine (OPD) dissolved in citrate–phosphate buffer (pH 4.5) along with H_2O_2 was added to each well and incubated for 20 min at room temperature. The reaction was stopped by adding 5 N H_2SO_4 (50 μl/well) and the absorbance was measured at 492 nm.

3. Results and discussion

3.1. Preparation and evaluation of immune response from polymer particles

PLA and PLGA polymer particles were prepared using a multiple emulsion solvent evaporation method incorporating 2.5% RSA in the internal aqueous phase as stabilizer along with TT (Raghuvanshi et al., 2001). Nanoparticles and microparticles entrapping TT were formulated from a variety of polymers, the mean size of microparticles and nanoparticles being 4 μm and 630 nm, respectively. Details of the characteristics of TT particles prepared from different polymer are presented in Table 1. Continuous release of TT from particles made from PLGA and PLA particles were observed after an initial burst phase (20%) and TT released from polymer particles showed immunoreactivity in in vitro ELISA tests. Immune responses from polymer entrapped TT particles were better than saline TT and was in accordance with the in vitro release of TT from polymer particles (Raghuvanshi et al., 2001). These observations suggested that polymer particles encapsulating TT have adjuvant properties due to their particulate nature and their smaller size helps in better uptake by the antigen presenting cells.

3.2. Effect of particle size and polymer composition on immunogenicity

Polymer particles of two size ranges, one with an average diameter of 0.5 μm (nanoparticles) and another with an average diameter of 3.9 μm (microparticles) were prepared from three different polymers viz. PLGA (50:50, LMW, 14 kD), PLGA (50:50, HMW, 45 kD) and PLA (45 kD) and were used for immunization. No significant difference in the kinetics as well as the extent of anti-TT antibody titers generated from PLGA (50:50, LMW, 14 kD) nanoparticles and microparticles were observed (Fig. 1). Maximum of 60 μg/ml of antibody titers could be achieved from PLGA 14 kD particles, which was higher than the titers observed with saline TT. Anti-TT antibody titers from PLGA (50:50/45 kD) microparticles were

Table 1
Characteristics of PLGA and PLA polymer particles

Polymer	% Encapsulation		Mean diameter (μm)	
	Nanoparticles	Microparticles	Nanoparticles	Microparticles
PLGA 50:50 (45 kD)	72	64	0.67	3.75
PLGA 50:50 (14 kD)	69	76	0.52	3.42
PLA (45 kD)	76	69	0.69	4.0

slightly higher than PLGA (50:50/45 kD) nanoparticles. However, the antibody titers from 14 kD PLGA particles were higher than that from 45 kD polymer particles. LMW PLGA particles elicited higher antibody titers than HMW PLGA particles probably because of its low glass transition temperature (Tg) (around 39 °C). Low Tg causes softening of the polymer matrix at 37 °C and thus releases entrapped TT near the periphery of the particle matrix more rapidly. Rapid degradation of low molecular weight polymer particle also helped in presenting the antigens quickly to the antigen-presenting cells (APC). Both these effects associated with LMW polymer particles results in eliciting high antibody responses.

Immunization with PLA particles elicited significantly higher anti-TT antibody titers ($P < 0.03$) as compared to PLGA particles (Fig. 2). There was little difference in anti-TT antibody titers generated from nano or microparticles. Peak anti-TT antibody titers were almost 4–5 times higher and at the end of 5 months, and antibody titers from PLA particles were twice higher than PLGA particles. These observations can be explained on the basis that PLA is more hydrophobic compared to PLGA due to the presence of an additional

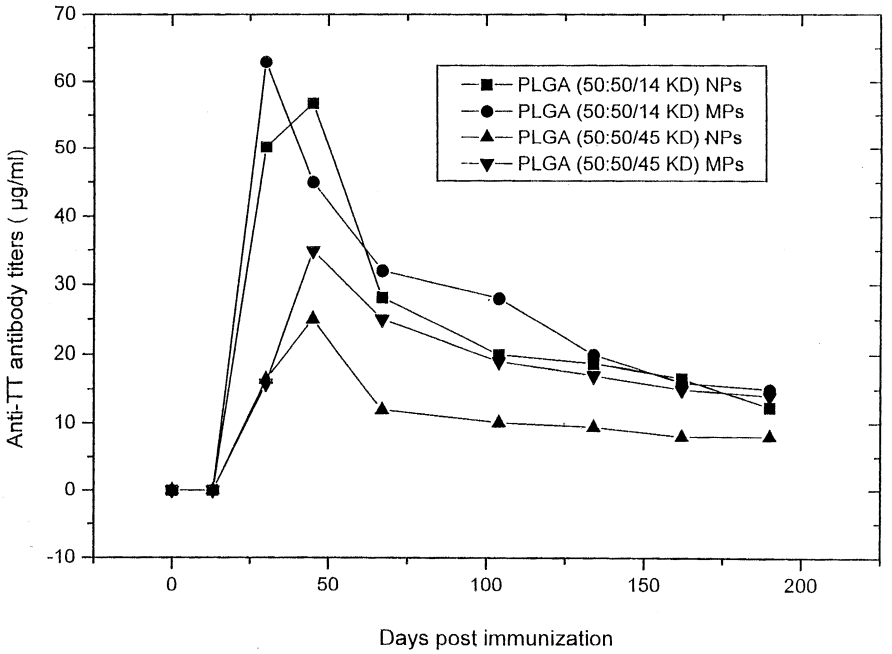

Fig. 1. Anti-TT antibody titer from group of rats immunized with 15 Lf TT encapsulated in PLGA (50:50,14 kD nanoparticles (-■-) and microparticles (-●-) and in PLGA (50:50, 45 kD nanoparticles (-▲-)and microparticles (-▼-).

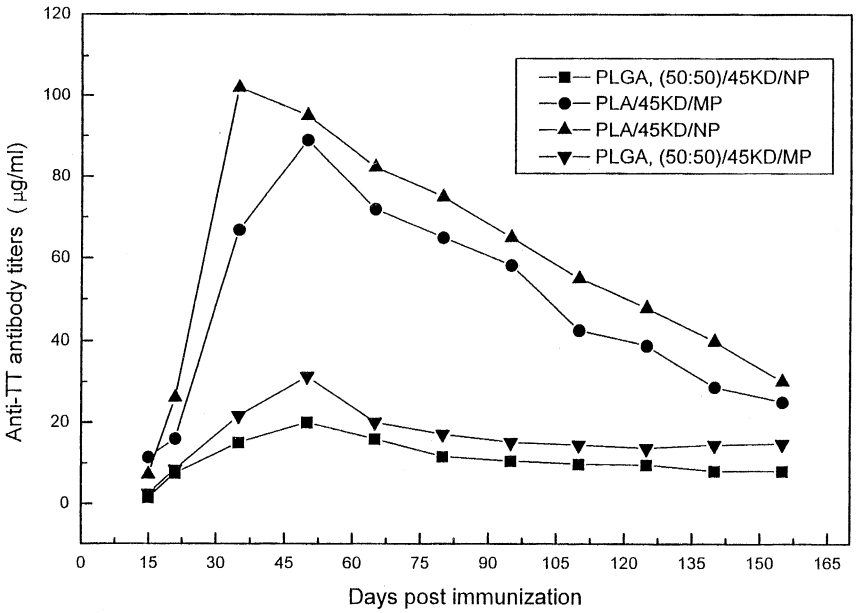

Fig. 2. Anti-TT antibody titer from group of rats immunized with 15 Lf TT encapsulated in PLA, 45 kD nanoparticles (-▲-) and microparticlers (-●-) and from PLGA 50:50, 45 kD nanoparticles (-■-) and microparticles (-▼-).

$-CH_3$ group in lactic acid. Because of this hydrophobicity factor, APC are preferentially attracted towards PLA particles and this helps in eliciting improved immune response. The superiority of hydrophobic polymer particles in improving immunogenicity of polymer entrapped antigen has been reported for Influenza A vaccine (Hilbert et al., 1999), for ovalbumin (Nakaoka et al., 1996) and TT (Raghuvanshi et al., 2001). These observations are consistent with the earlier reports demonstrating that the adjuvant effect of particulate polymeric particles is increased on increasing the hydrophobicity of the polymer (Kreuter et al., 1988).

These results also indicate that, even though these particles have adjuvant action, particles alone are not sufficient to elicit high antibody titers as observed from two doses of alum adsorbed TT (Peak titers from two dose alum adsorbed TT was more than 300 μg/ml). Such low anti-TT antibody titer from polymer entrapped TT in comparison to two alum adsorbed TT vaccination schedule has been reported by many groups (Kersten et al., 1996; Higaki et al., 2001; Diwan et al., 2001). The observed anti-TT antibody titers were as follows: PLA, 45 kD nanoparticles (peak titer: 105 μg/ml of rat serum) > PLA, 45 kD microparticles (peak titer: 85 μg/ml of rat serum) > PLGA (50:50, LMW, 14 kD) nanoparticles (peak titer: 65 μg/ml of rat serum) > PLGA (50:50, LMW, 14 kD) microparticles (peak titer: 58 μg/ml of rat serum) > PLGA (50:50, HMW, 45 kD) microparticles (peak titer: 35 μg/ml of rat serum) > PLGA (50:50, HMW, 45 kD) nanoparticles (peak titer: 22 μg/ml of rat serum) > saline TT (peak titers 3 μg/ml of rat serum). However, anti-TT antibody titers elicited by the present formulations were much better in comparison to reports of many groups, a fact which can be attributed to the protection of immunoreactivity of the entrapped antigen (Raghuvanshi et al., 2001) and use of smaller size particles for efficient uptake by macrophage cells (Men et al., 1999).

3.3. Effect of physical mixture of particles on immunogenicity

To further improve the immunogenecity of the entrapped TT, physical mixture of particles were

used for immunization. Immunization with mixture of nanoparticles and microparticles made from PLGA, 14 kD polymer elicited high antibody titer in comparison to the single use of particular particles (Fig. 3). Two distinct antibody titer peaks, one at day 30 (100 μg/ml) and other at day 105 (65 μg/ml) were observed with immunization of physical mixture of PLGA, 14 kD nano and microparticles (Fig. 3). The first peak could be due to the nanoparticles, which are suitable for uptake by APCs. The second peak could be because of microparticles, which are also made from LMW PLGA, which degrades fast and releases the encapsulated TT responsible for second peak. The response from this immunization schedule was high and sustained for a longer period.

Anti-TT antibody titers from physical mixtures of nano and microparticles made from PLA polymer also showed improved immune response in comparison to the single type of particles (Fig. 4). The antibody titers from physical mixture of nano and microparticles were around 160 μg/ml on day 75, after which the immune response decreased. However, anti-TT antibody response from physical mixture of PLA particles was not only high but also remained at a higher level over a period of 150 days. Physical mixture of PLA particles elicited higher and sustained antibody titer than the physical mixture of PLGA particles ($P < 0.01$). Such improvements in antibody titers by using a physical mixture of antigen containing polymer particles has been reported for TT (Gander et al., 1993; Men et al., 1995) and Staphylococcal enterotoxin B toxoid (Eldridge et al., 1991b).

Improvement in immune response from physical mixture of different particles can be attributed to the combined adjuvant effect of both types of particles. The presence of more particles provide a higher surface area which may help in adsorbing the burst-released antigens from the particles (Calis et al., 1995) and present these surface adsorbed antigens through MHC class II pathways for improved immune response (Men et al., 1999). Mixtures of PLA polymer particles, because

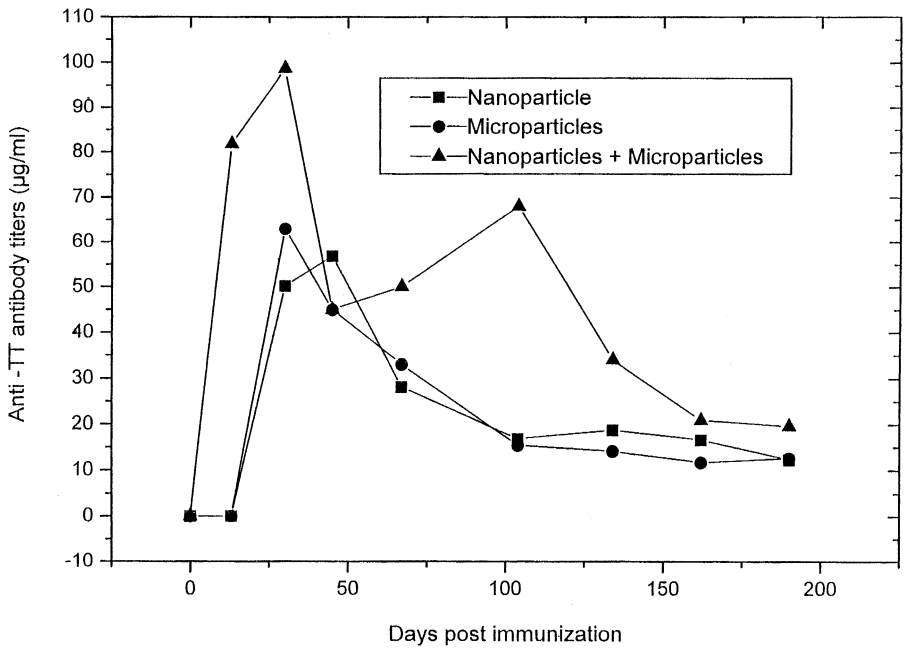

Fig. 3. Anti-TT antibody titer from group of rats immunized with 15 Lf TT encapsulated in PLGA 14 KID microparticles (-●-) and naoparticles (-■-) and physical mixture of nano and microparticles together equivalent to 15 Lf TT (-▲-).

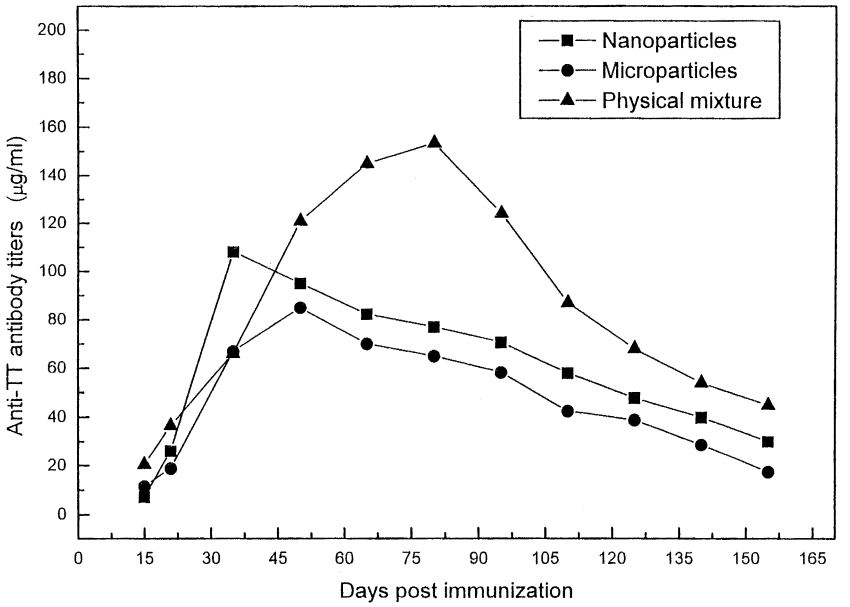

Fig. 4. Comparison of anti-TT antibody titers from rats immunized 15 Lf TT entrapped in PLA 45 kD microparticles (-●-) and nanoparticles (-■-) and physical mixture of both the particles together equivalent to 15 Lf TT (-▲-).

of the high adsorption of protein onto the surface and higher hydrophobicity, elicited much higher antibody response.

3.4. *Effect of adjuvants on the immunogenicity of particles encapsulating TT*

In order to improve the immunogenicity of the encapsulated TT particles, additional adjuvants and different immunization protocols were tried during immunization. Two injections of 5 Lf alum adsorbed TT was still found to be the best among the four immunization protocols (Fig. 5). The antibody response observed was in the following order: conventional two injection schedule > 15 Lf TT in particles along with alum > 5 Lf TT on alum + 10 Lf TT in particles > 15 Lf TT in particles ($P < 0.05$). It was found that use of alum along with polymer particles during immunization improved the antibody titers significantly (Fig. 5). Combination of particles along with alum not only enhanced the immune response but also changed the kinetics of the response. High antibody titers were detected as early as 15 days post-immunization using both PLGA nanoparticles and alum. The high initial antibody response could be due to synergistic adjuvant effect of both the small size particles and alum used for immunization. Better performance of group immunized with 5 Lf on alum along with 10 Lf in microparticles in comparison to the group immunized with 15 Lf in particles could be because of better priming of the immune system by alum adsorbed TT. Proper and efficient priming of the immune system is essential for a healthy and mature antibody response, which it probably lacks when 15 Lf TT is given in polymer particles without alum. TT released from the particles in the burst phase was in soluble form and hence was comparatively less immunogenic than alum adsorbed TT. Because of this, the released antigens are not able to prime the system efficiently and resulted in eliciting weak immune response. Improved immune response from admixture of polymer entrapped antigen and adjuvant have been reported for TT (Tobio et al., 2000) and Influenza A vaccine (Hilbert et al., 1999). These finding are in accordance with the earlier reports suggesting that sustained presentation of surface adsorbed protein to the immune system was a major factor in the induction and long term

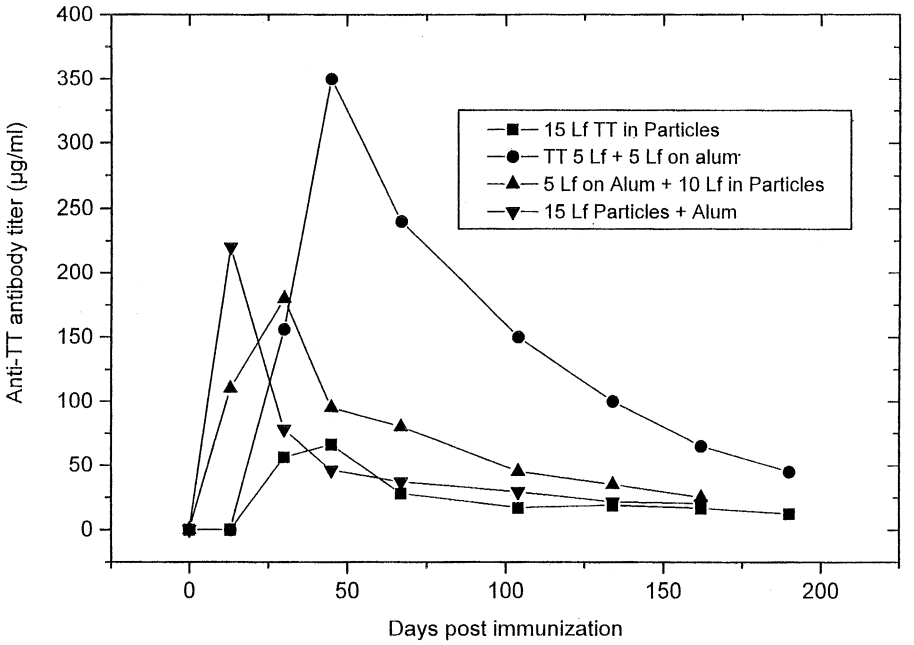

Fig. 5. Anti-TT antibody titer from group of rats immunized with conventional two injection schedule of 5 Lf TT adsorbed on alum at interval of 30 days (-●-), 15 Lf TT entrapped in PLGA 14 kD nanoparticles (-■-), 15 Lf PLGA nanoparticles mixed with alum (-▼-) and 10 Lf encapsulated in PLGA 14 kD particles along with 5 Lf adsorbed on alum (-▲-).

maintenance of high antibody titer (Coombes et al., 1996).

Squalene in the form of an oil and water emulsion has been reported to have better adjuvant action than alum with some of the antigens (O'Hagan et al., 1997). Immunization with polymer particles encapsulating 15 Lf TT suspended in squalene elicited very high antibody response in comparison to the particle immunization ($P < 0.02$) (Fig. 6). High antibody titer (260 µg/ml) was observed with immunization of particles along with the squalene emulsion. However, the antibody titers was lower than the two conventional injection schedule of TT immunization, peak antibody titers 360 µg/ml (Fig. 6). Immunization of particles along with squalene not only enhanced peak antibody titers (260 µg/ml) but also showed higher antibody response over 180 days in comparison to TT encapsulated polymer particles ($P < 0.04$).

3.5. Immune response from PLA microparticles along with alum

To further improve the antibody titers, PLA particles were immunized along with alum as additional adjuvant. Single dose immunization of PLA particles along with alum improved the anti-TT antibody titers very close to that achieved by two doses of alum adsorbed TT. The antibody titers were not only high but also were maintained above 40 µg/ml for almost 150 days (Fig. 7). The immune response from the admixture of PLA particles and alum elicited very high anti-TT antibody titers in comparison to particles given alone ($P < 0.01$). Improvement in immune response from polymer particles with additional adjuvant has been reported for ovalbumin (O'Hagan et al., 1991), TT (Tobio et al., 2000) and for Diptheria toxoid (Diwan et al., 2001). Such improvement in immune response with admixture

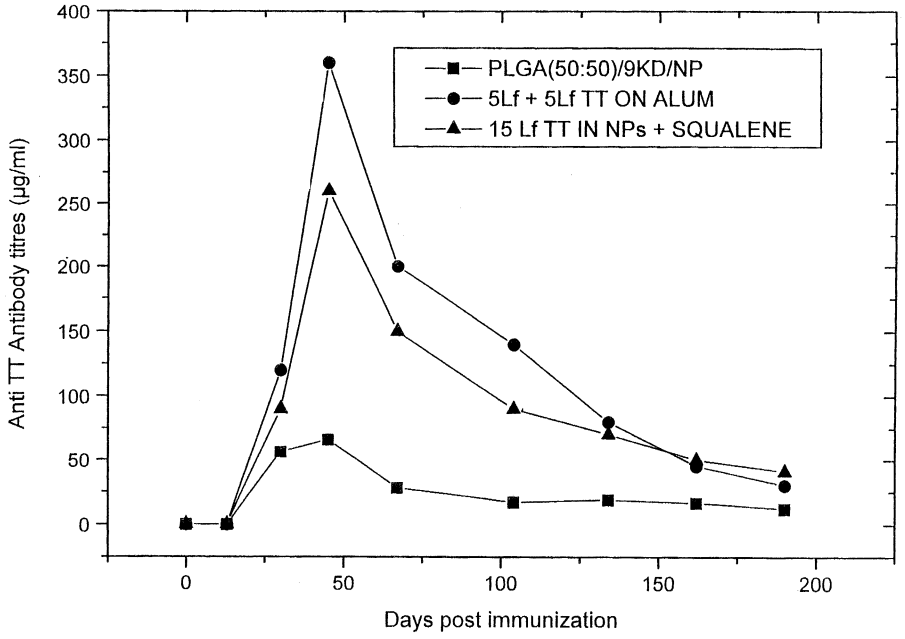

Fig. 6. Anti-TT antibody titer from group of rats immunized with 15 Lf TT entrapped in PLGA 14 kD nanoparticles (-■-), conventional two injection schedule of 5 Lf TT adsorbed on alum at interval of 30 days (-●-) and 15 Lf TT encapsulated in PLGA 14 kD particles suspended in squalene emulsion (-▲-).

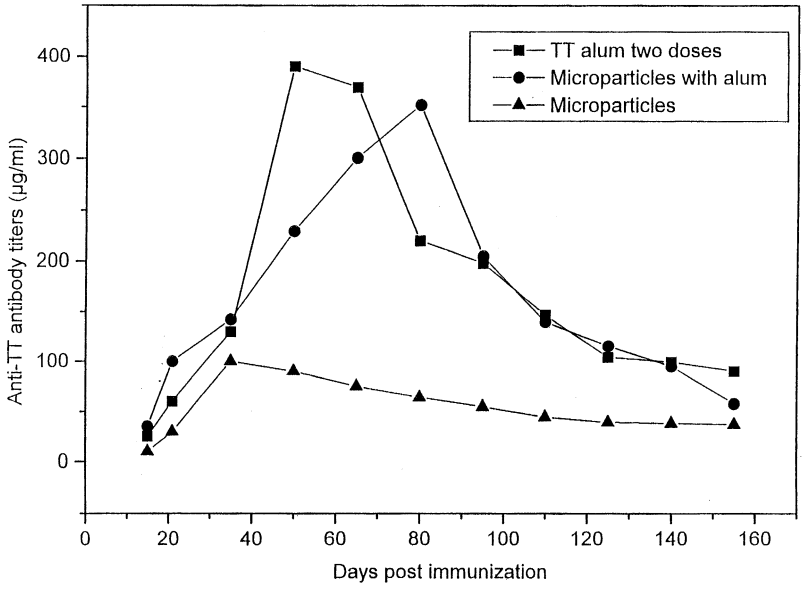

Fig. 7. Comparison of anti-TT antibody titers from rats immunized 15 Lf TT entrapped in PLA 45 kD microparticles (-▲-) and microparticles mixed with alum (-●-) with that of conventional two injection schedule of 5 Lf TT on alum at interval of 30 days (-■-).

of particle and alum have been reported recently for TT (Johansen et al., 2001). However, in spite of using alum as an additional adjuvant along with the particles, anti-TT antibody titers were significantly lower than two doses of alum adsorbed TT (Johansen et al., 2001). Whereas in the present formulation, PLA particle immunization along with alum resulted in significant enhancement of antibody titers in comparison to the PLA particles and anti-TT antibody titers were very close to that achieved from two doses of alum adsorbed TT (Fig. 7). This is because, polymer particles were immunized along with alum rather than lyophilized along with alum as reported by Johansen et al. (2001) where the use of lyophilized alum must have reduced the immunogenicity of the antigen as reported previously (Alving et al., 1993). Improved immunogenicity of TT particles in presence of alum may be due to the additional adjuvant property of alum, or may be due to adsorption and better presentation of antigens from the polymer particles. It has been observed that in vitro release of TT from polymer particles in to release medium is reduced drastically in presence of alum (Katare and Panda, 2001) and probably, the burst released antigen from the particles is adsorbed on to the alum and is presented to the immune system resulting in high antibody response. Further, it has been reported that polyvalent cation such as Al^{3+} neutralize the negatively charged protein and thereby make them more hydrophobic (Naim et al., 1997). Thus use of alum apart from activating macrophages will help in improving the hydrophobicity of the particulate antigen delivery system. Increased hydrophobicity of the polymeric delivery system resulted in improved immune response from PLA particle. Immunization with PLA particles along with alum probably provided all the requirements for the elicitation of high antibody response, the hydrophobicity of the polymer helped in enhanced uptake by antigen presenting cells, protection of immunoreactivity during particle formulation helped in the continuous release of immunoreactive antigen from the polymer particles, lower size particles and presence of alum helped in better presentation of the released antigen to the immune system. All these above factors probably acts synergistically to elicit high and sustained anti-TT antibody response from a single point immunization.

4. Conclusion

Extensive studies involving PLA and PLGA polymer particles have been carried out for the development of single dose vaccine using TT, diphtheria toxoid, Hepatitis B surface antigen and many other potential vaccine candidates with little success. The fact that entrapment and continuous release of the antigen provide better immune response than the soluble antigen have raised the hope for developing single dose vaccine formulation using polymer particles. However, unlike drug delivery systems, which often provide maximum therapeutic index with controlled release formulations, similar objectives with vaccine candidates have not been achieved. This is because, drugs are more robust molecules than protein antigen and more importantly, drugs act themselves whereas in most of the cases protein antigens need adjuvant for immune response. Unless these above two issues are addressed carefully while using polymer based controlled release system for single dose vaccine, the outcome will be futile. What is needed is the formulation of polymer particles entrapping immunoreactive antigen and its release in a manner mimicking the conventional vaccination schedule and, more importantly, devising immunization protocol for better presentation of the entrapped antigen for generation of long lasting immune response. In this report, we investigated different parameters associated with the immune response generated from polymer entrapped TT. It was observed that by judicious choice of the polymer and particle size, protecting the immunoreactivity of the entrapped antigen in the polymer particles and more importantly using an immunization protocol along with additional adjuvant, high and long lasting antibody response can be generated from single dose vaccines using polymer particles.

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