Polyanhydride nanoparticle adjuvants for development of a single dose anthrax vaccine

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Statement of Purpose: Anthrax is a significant public health concern because of its potential as a bioterrorist and biowarfare agent. The current anthrax vaccine is slow to generate a protective immune response, has considerable side effects and requires a complex vaccination schedule of multiple immunizations. Thus, a safe and effective anthrax vaccine is required for protecting the armed forces and civilians. Biodegradable polyanhydrides have been studied for ~25 years as drug delivery vehicles (**1**). Recent studies have shown that polyanhydride particles can be used as antigen delivery agents for vaccines (**2**). This is further supported by their antigen stabilization and immunomodulatory properties. Two polyanhydride chemistries have been shown to be of particular interest for designing new adjuvants: poly(sebacic anhydride) (SA) and 1, 6-bis (*p*-carboxyphenoxy)hexane (CPH). In this study, we tested the ability of CPH:SA-based copolymer nanoparticles delivering the recombinant protective antigen (rPA) of *Bacillus anthracis* to induce humoral and cell-mediated immune responses against the recombinant protective antigen (rPA) of *B. anthracis* using a single dose vaccine regimen.

Methods: Nanoparticle fabrication: CPH:SA copolymers were synthesized from the corresponding prepolymers via a melt polycondensation reaction in glass vials utilizing a multiplexed robotic deposition apparatus (**3**). The synthesis took place at 180°C and 0.3 torr vacuum for 1.5 h. rPA-encapsulated nanoparticles were fabricated by dissolving the antigen and copolymer in methylene chloride, sonicating the solution, and precipitating it into pentane (nanoprecipitation).

Animal Studies: Eleven week-old female A/J mice were subcutaneously immunized with rPA encapsulated CPH:SA nanoparticles. All mice were "antigenically challenged" with rPA at day 64 and necropsied at 69 days post-vaccination. At necropsy, serum was collected to measure the anti-rPA antibody response. Draining lymph nodes (DLN) were harvested to characterize the lymphocyte population and to assess rPA antigen-specific lymphocyte proliferation.

Results/Discussion: During an in vitro recall response, the most robust antigen-specific proliferative response was observed from DLN cells harvested from mice receiving rPA-containing 20:80 nanoparticles (Fig 1). This proliferative response was greater than that observed from DLN cells from mice immunized with alum-adjuvanted rPA (positive control). An evaluation of humoral immunity showed that mice immunized with rPA-containing 50:50 CPH:SA nanoparticles had a more robust anti-rPA antibody response than mice immunized with rPA-containing 20:80 CPH:SA nanoparticles (Fig 2).

At 69 days post-vaccination, there was an expansion of $CD19^+$ B cells observed in the DLN of mice receiving either formulation of rPA-containing nanoparticles that was comparable to that observed in mice immunized with alum-adjuvanted rPA. $CD19^+$ B cells from mice immunized with nanoparticles also demonstrated enhanced antigen-specific proliferation when re-stimulated with rPA antigen in vitro (data not shown).

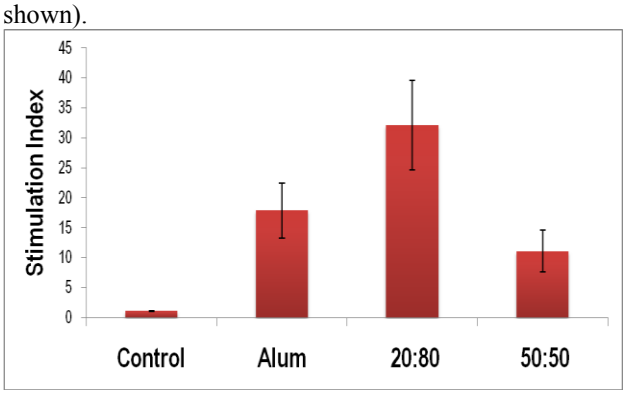

Fig 1. Mice immunized with rPA-containing 20:80 CPH:SA nanoparticle adjuvants show an enhanced antigen-specific proliferative response in vitro as compared to mice immunized with other vaccination regimens.

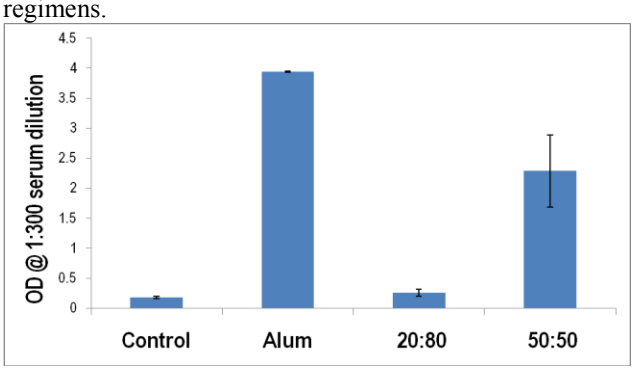

Fig 2. Mice immunized with rPA-containing 50:50 CPH:SA nanoparticles had a greater anti-rPA antibody response compared to non-immunized animals or to those immunized with 20:80 CPH:SA nanoparticles.

Conclusions: DLN cells from mice immunized with rPA-continaing 20:80 CPH:SA nanoparticles demonstrated enhanced antigen-specific proliferation in vitro. In contrast, mice immunized with rPA-containing 50:50 CPH:SA nanoparticles had a greater anti-rPA antibody response. These data demonstrate that polyanhydride nanoparticle formulations have the potential to be tailored to induce specific immune repsonses and can be used as effective adjuvants in developing a single dose anthrax vaccine.

References: 1. Leong K et al, 1986 J Biomed Mater Res, 51-64, 2.Vogel et al 2005 Biomaterials 721-28. 3. Petersen L et al 2009 Biomaterials 5131-42.