Polyanhydride Nanoparticle Adjuvants for Anthrax Vaccine

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Statement of Purpose: A need currently exists for improved vaccines against anthrax, a significant public health concern due to its potential as a bioterrorist and biowarfare agent. Use of the currently licensed anthrax vaccine (AVA) is hindered by poor public perception, high reactogenicity, and a five immunization vaccine schedule followed by yearly boosters. Developing a novel anthrax vaccine comprised of polymeric adjuvants capable of controlling antigen delivery and enhancing immune activation would offer the benefit of a single immunization with a reduced antigenic dose [1]. Polyanhydrides are a class of biomaterials with excellent biocompatibility and have shown much promise as vaccine delivery vehicles and adjuvants [1-3]. Specifically, copolymers based on sebacic anhydride (SA), 1,6-bis(p-carboxyphenoxy) hexane (CPH), and 1,8-bis(p-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) have been evaluated as adjuvants [1-3]. By rationally modifying the chemical composition, tunable antigen release kinetics [4] can be achieved that can provide an antigen depot in vivo. Furthermore, they have antigen stabilization capabilities as well as immunomodulatory properties [2]. The goal of this work was to identify a nanoparticle-based vaccine formulation capable of encapsulating and stably releasing the recombinant protective antigen (rPA) of Bacillus anthracis in order to induce an avid, neutralizing antibody response.

Materials/Methods: Polyanhydrides and rPA-loaded nanoparticles (20:80 and 50:50 CPH:SA and 20:80 and 50:50 CPTEG:CPH) were fabricated as previously described [3,4]. Release kinetics of rPA from nanoparticles were assessed over 60 days.

Protein immunogenicity was determined in vivo by subcutaneously injecting released rPA adjuvanted with alum into A/J mice. After two weeks, an antigenic challenge was administered and after three weeks serum was collected. Antibody titers and avidity were assessed with an ELISA. Figure 1 describes the treatment groups and the protocol for this in vivo study.

Figure 1: Immunization regimen, treatment groups, and downstream analysis for the in vivo rPA stability study.

The stability and function of rPA was assessed in vitro using a macrophage cytotoxicity assay. RAW 264.7 macrophages were cultured with rPA-loaded nanoparticles and soluble lethal factor (LF) to measure the functionality of rPA upon release. In this biological assay, cytotoxicity is induced only if functional rPA is released from the nanoparticles. Toxin neutralization (TN) assays were also performed using serum from mice immunized as described in Figure 1. In the TN assay, macrophages are protected from the lethal effects of rPA and LF if neutralizing antibodies are present in the mouse serum.

Results/Discussion: Nanoparticles composed of 50:50 CPTEG:CPH and 20:80 CPH:SA released protein the most rapidly, followed by 50:50 CPH:SA nanoparticles, and 20:80 CPTEG:CPH nanoparticles displayed the slowest rPA release kinetics.

Figure 2: A) Antibody titers from mice immunized as described in Figure 1. B) Cell viability following incubation with select rPA-loaded nanoparticle formulations (cytotoxicity assay).

Additional analyses demonstrated that mice immunized with rPA released in vitro from either 50:50 and 20:80 CPTEG:CPH produced the highest antibody titers with the greatest avidity, similar to mice immunized with native rPA adjuvanted with alum. Lower antibody titers were induced by mice immunized with rPA released from CPH:SA nanoparticles. This observation is likely a result of a loss of immunogenicity because of the acidic microenvironment produced by the degradation of the SA-containing nanoparticles [4]. Results from the cytotoxicity assay demonstrated that functional rPA was released from CPTEG:CPH nanoparticles. Specifically, rPA released from 50:50 CPTEG:CPH nanoparticles induced macrophage death as well as native rPA. In contrast, rPA released from CPH:SA nanoparticles was significantly less active in the cytotoxicity assay. Using the TN studies, similar neutralizing antibody titers were observed from mice immunized with rPA released from CPTEG:CPH nanoparticles and mice immunized with native rPA.

Conclusions: This biological approach to investigating rPA stability upon release from polyanhydride nanoparticles has provided strong evidence that 50:50 CPTEG:CPH nanoparticles are excellent candidates for delivery of functional immunogens, capable of eliciting neutralizing antibody.