Immunomodulatory Polyanhydride Particle Vaccines to Provide Protection Against Plague

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Statement of Purpose: The causative agent of pneumonic plague, *Yersinia Pestis*, has been labeled by the CDC as a Category A bioterrorism agent due to its ease of transmission and high mortality rate.[1] A recombinant fusion protein, F1-V, has shown effectiveness as a vaccine antigen against plague.[2] The major drawbacks of this vaccine are that it must be delivered via multiple doses to be effective and induces a overwhelmingly antibody-mediated immune response. Utilizing the long-term delivery, adjuvant and immunomodulatory capabilities of polyanhydride particles, a single dose plague vaccine can be realized.

Methods: Random copolymers of 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane and 1,6-bis(*p*-carboxyphenoxy)hexane (CPTEG:CPH) were tested as adjuvants for a potential plague vaccine. Polymers with molecular weights ~ 10,000 Da were synthesized via melt polycondensation under vacuum and were characterized by ^{1}H nuclear magnetic resonance, gel permeation chromatography and differential scanning calorimetry. An anti-solvent precipitation method was used to fabricate microparticles and nanoparticles. Scanning electron microscopy was employed to evaluate particle morphology and size distribution. Vaccines were delivered intranasally to C57BL/6 mice and were evaluated with an antigenic or pathogenic challenge. Antigenic challenge was assessed by IgG antibody titers, cytokine secretion, ^{3}H-thymidine lymphocyte blastogenesis and carboxyfluorescein succinimidyl ester incorporation. Fourteen day post-challenge survival was considered a success for pathogenic protection.

Results: Spherically shaped, F1-V loaded 50:50 CPTEG:CPH polyanhydride nanoparticles between 50 and 250 nm in diameter and microparticles between 1 and 20 µm in diameter were fabricated. Scanning electron micrographs of the particles are shown below in Figure 1.

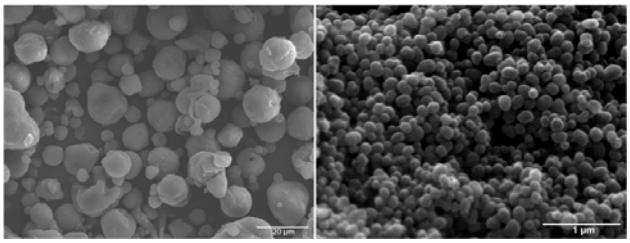

Figure 1. Images of 50:50 CPTEG:CPH microparticles (6.8 ± 3.9 µm, scale bar = 20 µm) (Left) and nanoparticles (130 ± 32 nm, scale bar = 1 µm) (Right)

Pathogenic protection experiments using 25 µg F1-V delivered intranasally twice with multiple doses of an adjuvant, IL-12, were conducted with 62% of mice surviving challenge. Protected mice had an average 35-day total IgG antibody titer of ~ 10,000. In order to determine the adjuvant and immunomodulatory capacity of polyanhydride particles, 500 µg of 2% loaded F1-V particles (10 µg encapsulated F1-V) were co-delivered with 15 µg soluble F1-V were delivered intranasally (40% encapsulated). MPLA, monophospholipid A, was utilized as a control and supplemental adjuvant. 25 µg of F1-V alone induced a 35-day total IgG antibody titer of 350. 25 µg of F1-V delivered with 10 µg MPLA gave a titer of 6700. 50:50 CPTEG:CPH microparticles alone induced a titer of 500 and a titer of 2100 when coupled with MPLA. 50:50 CPTEG:CPH nanoparticles stimulated titers of 1500 and 20,000, alone and with MPLA, respectively. Figure 2 shows the IgG subtype antibody titers.

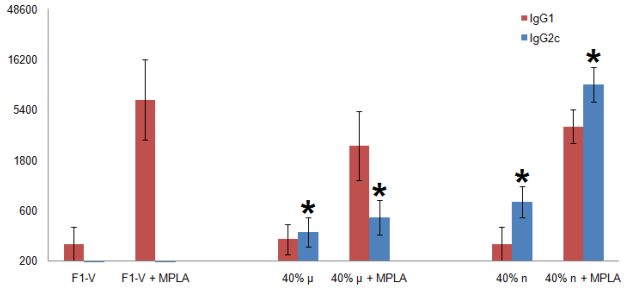

Figure 2. IgG subtype antibody titers.
* = $p < 0.0001$ to F1-V and F1-V + MPLA

The presence of IgG2c provides evidence of a Th1 mediated immune response, which is helpful in fighting intracellular infections due to the stimulation of cytotoxic T-cells. This finding is supported by the 40% nanoparticle + MPLA vaccine's ability to induce splenocytes to have very responsive CD4 and CD8 T-cells is evident by high levels of cellular expansion in response to F1-V protein in-vitro. Also, these cells secreted high levels of the cytokines, chemical signals, IL-6, IL-12 and TNF-α, which are essential in facilitating the immune response.

Conclusions: The data provided reveals the potential for 50:50 CPTEG:CPH particles to be used as adjuvants as well as a delivery systems for a single dose, aerosol plague vaccine. The ability for polyanhydride particles to both enhance and modulate the immune response is a highly desired adjuvant function since subunit vaccines alone often do not induce a robust enough response or correct immunological profile to protect against disease.

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References:

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[2]DuBois AB, et al. Vaccine. 2007;25:4747-4754