Novel Vaccine Strategies Against *Y. pestis*

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Statement of Purpose: *Yersinia pestis*, the causative agent of pneumonic plague, has been designated by the CDC as a Category A bioterrorism agent due to its ease of transmission and high mortality rate.[1] A recombinant fusion protein, rF1-V, has been shown to induce protective immunity against plague.[2] One of the major drawbacks of this vaccine is that delivery of multiple doses are required to be efficacious. Utilizing the long-term delivery and adjuvant capabilities of biodegradable polyanhydride particles, as shown with tetanus toxoid[3], a single dose anti-plague vaccine may now be feasible.

Methods: Random 20:80 and 50:50 copolymers of 1,6-bis(*p*-carboxyphenoxy)hexane and sebacic acid (CPH:SA) and copolymers of 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane and 1,6-bis(*p*-carboxyphenoxy)hexane (CPTEG:CPH) were tested as potential plague vaccine adjuvants. Polymers with molecular weights ~ 10,000 Da were synthesized by melt polycondensation under vacuum and characterized by ^{1}H nuclear magnetic resonance, gel permeation chromatography and differential scanning calorimetry. An anti-solvent nanoencapsulation method adapted from Mathiowitz *et al.*[4] was used to fabricate nanospheres. Scanning electron microscopy and quasi-elastic light scattering were employed to evaluate particle morphology and size distribution, respectively. Vaccines were delivered subcutaneously (100 µL) or intranasally (40 µL) to C57BL/6 mice. Antigen-specific immune responses were assessed by ELISA titers and ^{3}H-TdR lymphocyte proliferation. Stimulation indices for the lymphocyte proliferation assays were generated by dividing the level of ^{3}H-TdR incorporated by lymphocytes stimulated with 5 µg rF1-V compared to the amount incorporated by unstimulated cells. Mice were challenged with 10^4 cfu of *Y. pestis* CO92 and survival after intranasal challenge was monitored for 20 days post-infection as a measure of protection from disease.

Results: An rF1-V dose titration study was performed using 5-fold dosing from 0.2 µg to 125 µg delivered subcutaneously. Mice immunized with 25 µg of rF1-V produced an IgG antibody titer of 6400 at 21 days post-immunization, a level that was predicted to confer protection. Further experiments using 25 µg rF1-V delivered intranasally twice, three weeks apart, followed by intranasal challenge with the virulent CO92 strain confirmed this hypothesis . Overall, 67% of mice vaccinated with antigen alone survived whereas all of the mice survived when antigen was delivered with multiple doses of IL-12. The fully protected mice had an average IgG antibody titer of ~ 9000 before challenge.

Polyanhydride nanospheres between 80 and 800 nm in diameter were consistently fabricated regardless of polymer chemistry. In order to determine the adjuvanticity of the nanospheres, 500 µg of blank particles of varying chemistry were co-delivered with 25 µg soluble rF1-V. At 21 days post-immunization, mice receiving 20:80 CPH:SA and 50:50 CPTEG:CPH nanospheres had IgG antibody titers of 25,600, whereas mice receiving 20:80 CPTEG:CPH and 50:50 CPH:SA nanospheres had titers of 1600 and 800, respectively. The adjuvant activity of 20:80 CPH:SA and 50:50 CPTEG:CPH nanospheres were also demonstrated by enhanced lymphocyte proliferation as shown in Figure 1. The results showed that 20:80 CPH:SA and 50:50 CPTEG:CPH caused statistically significant increases in the lymphocyte proliferative response to rF1-V.

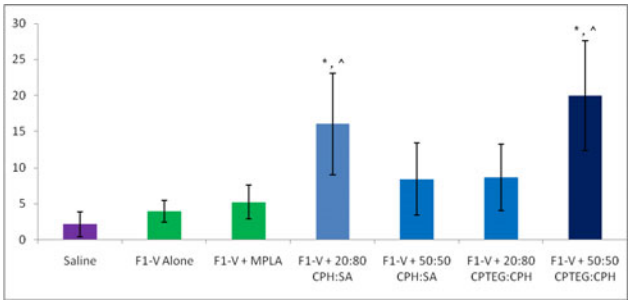

Figure 1. *In vitro* stimulation indices of lymphocytes cultured in the presence of 5 µg/mL rF1-V for 72 hours. * = $p < 0.025$ to saline and ^ = $p < 0.025$ to F1-V+MPLA

Conclusions: The data provided evidence of the potential for 20:80 CPH:SA or 50:50 CPTEG:CPH nanospheres to be used as an adjuvant for a single dose anti-plague vaccine. Current antigenic and pathogenic challenge studies are underway to evaluate the effectiveness of polyanhydride nanospheres with encapsulated rF1-V as a vaccine.

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