Polyanyhydride Nanoparticle-based Influenza Vaccine Elicits Viral Neutralizing Titers and Enhances Cell-Mediated Immunity

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Statement of Purpose: Highly pathogenic avian influenza (HPAI) H5N1 has long been monitored by viral epidemiologists as a potential pandemic strain due to the immunological naivety of humans to the H5 hemagglutinin (HA). A major public health concern with HPAI is the associated morbidity and mortality associated with infection. Therefore, great emphasis has been placed on preventive immunization [1-3]. Polyanhydrides based on copolymer combinations of aromatic 1,6-bis(*p*-carboxyphenoxy) hexane (CPH), and 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) are unique amphiphilic biomaterials that improve protein stability and sustained release of encapsulated protein [4-7]. Polyanhydride nanoparticles exhibit inherent immunomodulatory activity in vitro by activating antigen presenting cells [8, 9] and in vivo by increasing monocyte chemotactic cytokine production (MCP-1 and IP-10) as well as inducing mild inflammatory activity with minimal adverse events at the administration site [10].

Methods: Polyanhydride nanoparticle-based H5 influenza vaccines were formulated using combinations of free and encapsulated recombinant stabilized trimeric H5 A/Mongolia/Whooper Swan/1203/05 protein (H5-T) using the 20:80 CPTEG:CPH (20:80 C:C) formulation as well as with or without the addition of viral pathogen associated molecular patterns (PAMPs), Poly I:C and Poly dA:dT, which are known to ligate conserved innate immune receptors. Control immunizations consisting of H5-T adjuvanted with monophosphoryl lipid A (MPLA) or soluble antigen alone (sH5-T) were also administered subcutaneously. Cohorts of animals were administered only a primary immunization while a separate set of mice received a prime/boost immunization regimen on days zero, 21, and 42. H5-specific antibody responses were evaluated using Luminex based conjugated bead analysis. Antigen recall response was evaluated by CFSE-labeling of lymphocytes recovered from the draining lymph node and culturing with H5-T for 96 hours followed by FACS analysis. Lastly, H5-specific neutralizing antibody responses were evaluated using a H5 HA pseudotyped reporter virus and results reported as the inverse serum dilution that neutralized 50% of virus infectivity (ID50).

Results: Compared to the other nanoparticle formulations, 20:80 C:C nanoparticles containing Poly I:C induced greater anti-H5 viral neutralizing antibody titers after a single dose regimen (Figure 1A). Regardless of formulation, neutralizing antibody titers were more effectively generated using a prime/boost immunization regimen compared to single dose (Figure 1B). $CD4^+$ T cell antigen recall responses were also increased by inclusion of Poly I:C when compared to other immunization regimen (Figure 1C).

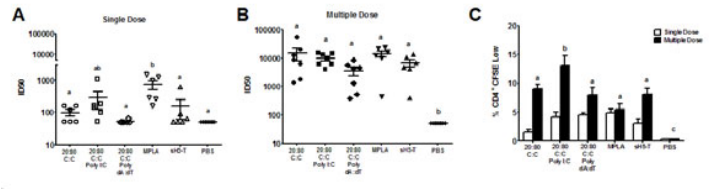

Figure 1. Enhanced CD4 cellular immunity is generated by polyanhydride nanoparticle vaccine containing Poly I:C. Neutralizing antibody evaluated at 63 days post-primary immunization (A). Neutralizing antibody evaluated at 63 days post-primary immunization and boost immunizations on day 21 and day 42 (B). Percent of $CD4^+$ $CFSE^{low}$ T cells after 96 hour H5-specific re-stimulation. T cells from mice receiving the prime/boost regimens are depicted by closed histograms and those from mice receiving single dose regimens are depicted by the open histograms (C). One-way ANOVA with Tukey's post-test statistical analysis of multiple dose regimens is shown with different letters indicating differences among groups. $p < 0.05$.

Conclusions: Polyanhydride nanoparticle-based vaccines incorporating H5 trimer were able to elicit a robust neutralizing antibody response. No statistical differences in neutralizing antibody titer were identified among the different prime-boost vaccine formulations. However, a polyanhydride nanoparticle vaccine containing a dsRNA mimic, Poly I:C, induced enhanced $CD4^+$ recall responses indicating well-established, long-lived memory populations. These data indicate that the polyanhydride nanoparticle-based delivery platform is versatile and can be adapted to increase adaptive immune efficacy, while representing a safe biomaterial nano-adjuvant alternative for subunit vaccines.

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