Polyanhydride Nanovaccines Elicit Protective Virus Neutralizing Titers and Cell-mediated Immunity Against Influenza

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Statement of Purpose: The pandemic potential of highly pathogenic H5N1 avian influenza has generated great interest in the development of preventive vaccines. Traditional vaccine technologies have limited storage capabilities and relatively long production times that are unsuitable for pandemic preparation and response [1]. Polyanhydride nanovaccines encapsulating subunit antigens offer an alternative platform for the development of efficacious pandemic vaccines. Previously, polyanhydride nanoparticles have been shown to stabilize and sustain the release of subunit proteins, resulting in long-lived protective antibody titers [2, 3]. This immunomodulatory platform has also been noted to enhance cell-mediated immunity and the generation of T cell memory populations [4, 5] that are often associated with broader protective immunity against influenza.

Methods: A recombinant H5 hemagglutinin trimer ($H5_3$) was expressed and encapsulated into 20:80 CPTEG:CPH polyanhydride nanoparticles. Mice received a single dose or prime/boost regimen (3 doses, 21 days apart) of subcutaneous immunizations containing a combination of soluble $H5_3$, $H5_3$-loaded nanovaccine, and poly I:C. Control immunizations consisting of soluble $H5_3$ alone as well as adjuvanted with MPLA or blank 20:80 CPTEG:CPH nanoparticles were also performed. H5-specific neutralizing antibody responses were evaluated using a H5 HA pseudotyped reporter virus 42 and 63 days post-immunization. In addition, lymphoctyes were harvested from the draining lymph nodes at 63 days post-immunization and stimulated ex vivo to examine T cell proliferation. Finally, prime/boost immunized mice were challenged intranasally with low pathogenic, PR-8 reassortant virus containing H5N1 genes. The viral load and presence of inflammatory cytokines within the lung were examined three days post-challenge with PCR and a fluorescent-based multiplex assay, respectively. Additionally, the body weight of all mice was monitored over the course of 14 days post-challenge.

Results: Regardless of formulation, single dose $H5_3$ vaccine formulations required 63 days to obtain neutralizing antibody titers equivalent to the MPLA-adjuvanted control. In contrast, prime/boost immunization demonstrated antibody titers equivalent to the control at 42 days post-immunization. Prime/boost immunization regimens, especially those containing nanovaccine and poly I:C, were found to significantly enhance $CD4^+$ T cell proliferation upon *ex vivo* stimulation 63 days post-immunization. Finally, all $H5_3$ vaccine formulations were found to be protective against viral challenge. Immunized mice gained or maintained body weight through the duration of the study, similar to naïve, non-challenged mice (Figure 1). In contrast, saline immunized controls lost approximately 20% body weight before recovering. $H5_3$-vaccinated mice also demonstrated reduced viral load (Figure 1) and little to no inflammatory cytokines present in the lungs.

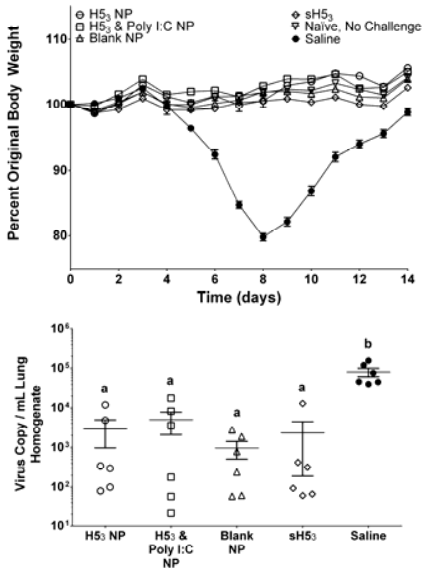

Figure 1. $H5_3$-vaccination protective against low pathogenic, viral challenge. $H5_3$-immunized weight maintain body weight (top) and demonstrate reduce viral load (bottom) post-challenge.

Conclusions: The studies herein demonstrate the strong immunogenic properties of the $H5_3$ antigen. All formulations displayed neutralizing antibody titers similar to a MPLA-adjuvanted control, while the poly I:C + nanovaccine formulation enhanced $CD4^+$ T cell proliferation. Upon challenge with a live virus, $H5_3$-vaccinated mice were protected laying a foundation for the polyanhydride nanovaccine platform in developing influenza vaccines.

References:

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