Deposition and Persistence of Polyanhydride Nanoparticle Vaccines upon Intranasal Administration

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Statement of Purpose: The pulmonary immune system is unique in that it must balance elimination of antigen and regulation of inflammation while maintaining the fragile gas-exchange interfaces that must be taken into account when designing intranasal vaccines [1, 2]. Nanoparticles based on polyanhydride copolymers of sebacic acid (SA), 1,6-bis(*p*-carboxyphenoxy) hexane (CPH), and 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) have been previously demonstrated to serve as an effective vaccine delivery platform to induce protection against *Yersinia pestis*, the causative agent of pneumonic plague [3]. The protection induced by the nanoparticle vaccine formulation can be attributed to its ability to stabilize protein antigens, increase internalization by antigen presenting cells (APCs), and sustain release of the antigen resulting in long-lived antibody titers with high avidity [3, 4]. Here, we examine the role of size and chemistry on the interactions between intranasally administered polyanhydride particles and lung tissue via studies on deposition, uptake and internalization by antigen presenting cells, and persistence within the lung. Vaccine formulations utilizing polyanhydride nanoparticles were compared with traditional adjuvants to gain insight into the protective mechanism.

Methods: Monodisperse poly(SA) particles of 260 nm, 470 nm, and 2.4 μm diameter were synthesized with encapsulated rhodamine dye. The clearance and uptake of these particles were compared with monodisperse non-degradable polystyrene particles of similar sizes via ex vivo imaging of the lung and flow cytometry. Vivo Tag 680 (Perkin Elmer)-conjugated F1-V (antigen for *Yersinia pestis*) was administered intranasally alone (soluble), with MPLA, or encapsulated into polydisperse 50:50 CPTEG:CPH nanoparticles (~400 nm). Lungs were excised at 2 and 48 hours after particle administration and analyzed via ex vivo imaging, flow cytometry, and high throughput microscopy to determine the distribution of F1-V, percentage of cells that had internalized F1-V, and cell type.

Results: Greater particle deposition within the lung was observed for the 470 nm poly(SA) and 390 nm polystyrene particles when compared to larger or smaller particle sizes, as measured by ex vivo imaging of the lungs 6 hours post-administration (Fig 1A). Further analysis showed a chemistry-dependent trend, as poly(SA) nanoparticles were cleared from the lungs within 48 hours, while CPTEG:CPH nanoparticles persisted in the lungs (Fig 1B). All vaccine formulations were found to distribute uniformly within the lung. Internalization of F1-V nanoparticles by dendritic cells was sustained over 48 hours, and was increased in macrophages, unlike MPLA formulations where detection of cells that had internalized F1-V drastically decreased by 48 hours (Fig 1B).

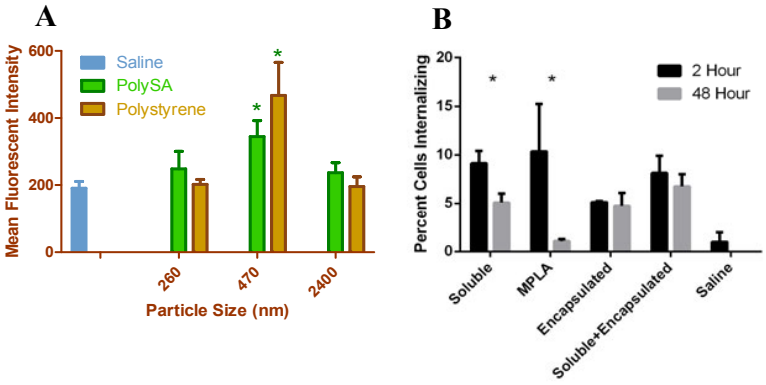

Figure 1. Polyanhydride particle size and chemistry affect deposition and internalization by antigen presenting cells. **(A)** Fluorescent imaging of excised lungs was used to determine deposition and distribution of particles at 6 hours. * indicates $p<0.05$ in comparison to saline mice. **(B)** High throughput microscopy demonstrated that percentage of lung cells internalizing F1-V delivered alone or with MPLA decreased rapidly over 48 hours, while the number of cells that had internalized F1-V when encapsulated in nanoparticles persisted. * indicates $p<0.05$.

Conclusions: Particle size was shown to significantly impact deposition within the lungs upon intranasal administration, with the monodisperse 470 nm nanoparticles having the greatest deposition. The persistence of the nanoparticles in the lung is dependent upon polymer chemistry, with the more readily erodible poly(SA) particles being cleared within 48 hours of administration. In contrast, the slower degrading 50:50 CPTEG:CPH nanoparticles persisted longer and greater biodistribution was observed. The delivery of the antigen in conjunction with traditional adjuvants like MPLA was quickly cleared from the lungs. The persistence of polyanhydride nanoparticles sustains antigen release, resulting in continuous recruitment of APCs. The greater persistence of the 50:50 CPTEG:CPH nanoparticles in the lungs may explain the previously observed long-lived (23 weeks) anti-F1-V antibody titer and protective immunity against pneumonic plague in mice immunized with F1-V encapsulated into polyanhydride nanoparticles [3].

References:

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