Lymph Node Injection of Adjuvant-loaded Microparticles Elicits Viral Vector-level T-cell Response Without Boosting

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Statement of Purpose: Materials-based vaccination strategies often rely on intramuscular (i.m.) or intradermal (i.d.) injection of microparticles (MPs) or nanoparticles (NPs) containing antigens and adjuvants. While such strategies have demonstrated promise, a major limitation hindering these approaches is inefficient lymph node (LN) draining, where as much as 99% of the injected dose remains at the injection site. Intranodal (i.n.) injection of soluble antigen to LNs has recently shown impressive utility in vaccination, permitting efficient targeting to LNs. Unfortunately, LN vaccination often requires surgical methods and involves complex injection regiments. Further, to date the incorporation of biomaterials in these approaches has not been investigated. Thus we proposed a novel strategy for vaccination by coupling direct and non-surgical LN delivery with biomaterial carriers loaded with antigens and adjuvants. We hypothesized that this approach would 1) dramatically improve the efficiency with which antigens and adjuvants reach the LN and 2) allow high levels of control over the delivery of bioactive cargo through tuning of carrier properties. Since LN vaccination is clinically-feasible in humans, this approach could provide a broadly-applicable route for enhancing vaccination, with increased potency and dose sparing.

Methods: Hybrid lipid-polymer particles were synthesized using emulsion-based processes, and particles were stabilized with amphiphilic lipids that provide a facile route for incorporation of specific chemical moieties (e.g., poly(ethylene glycol)). Using a non-toxic tracer, we identified conditions that allowed visualization of target injection sites after LN drainage of dye injected at the tailbase in mice. Fluorescently-labeled MPs injected at dye drainage sites, in conjunction with whole animal imaging, were used to confirm the presence of particles in LNs. Flow cytometry and histological analysis were employed to characterize particle uptake by LN-resident antigen presenting cells (APCs) and the distribution of MPs/NPs within the LN. Mice were immunized with soluble ovalbumin (OVA) antigen and poly(I:C) – a TLR3 agonist – in soluble form or encapsulated in MPs. Vaccines were administered i.m. or i.n., and immune response was monitored via SIINFEKL tetramer staining.

Results: Polymeric MPs and NPs were readily synthesized, and the properties of these materials (size, surface charge, cargo loading) were tunable by controlling synthesis conditions. We used a non-toxic tracer dye in conjunction with particles encapsulating model cargo or immunogenic agents to develop a potent, biomaterial-mediated LN delivery system. We first identified conditions permitting visual identification of inguinal LNs 24 hours after tailbase injection of tracer dye. Using the dye drainage site as a "target" for i.n. injection (Fig. 1A), fluorescent molecules were administered in either soluble or MP form, and signal intensity was monitored for 1 week (Fig 1B, left). Soluble fluorophore quickly diffused

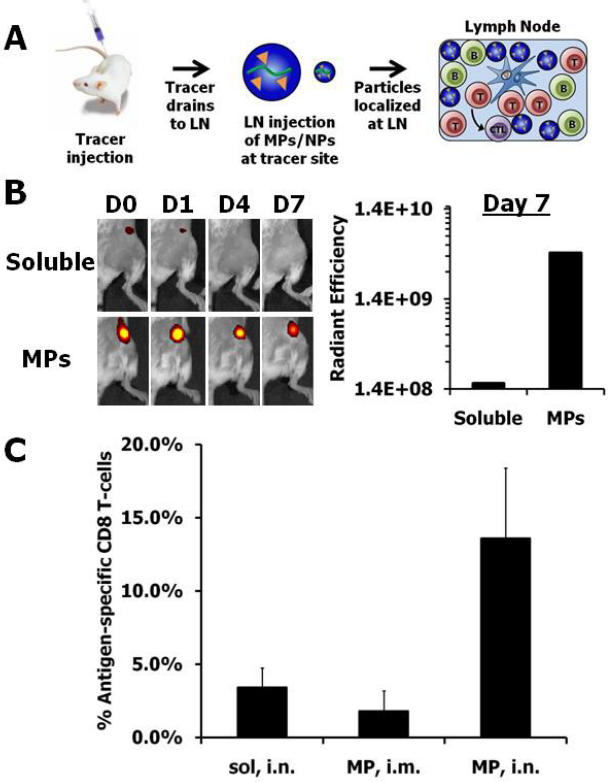

Figure 1. i.n. injection of MPs (A) mediates increased persistent of model cargo (B). Immunization with OVA and poly(I:C) MPs elicits a synergistic T-cell response (C)

from the injection site, whereas fluorophore encapsulated in MPs exhibited a marked increase in persistence, exhibiting a 30-fold higher signal compared with soluble fluorophore on day 7 after injection (Fig. 1B, right). Histological and flow cytometric analysis confirmed that i.n. injections permitted significantly increased localization of particles to LNs (10-30x) compared with i.m. injections and that up to 25% of LN-resident APCs (dendritic, macrophage, and B-cells) internalized particles following i.n. injection. Finally, mice immunized i.n with soluble poly(I:C) or i.m. with poly(I:C) MPs exhibited modest antigen specific T-cell responses (2-4%), while poly(I:C) MPs injected i.n. "armed" up to a shocking 23% of T-cells against OVA (mean=14%, Fig 1C).

Conclusions: Tailbase injections of tracer dye permits non-surgical i.n. injection in a small animal model, and cargo encapsulated in MPs or NPs shows significantly increased persistence following i.n. injections. Importantly, significantly higher levels of LN-resident APCs internalize these particles when administered i.n., and injection of adjuvant-containing MPs synergistically enhances antigen-specific T-cell response, reaching levels associated with some viral vectors after only a single dose. Together, these data suggest that LN injection of biomaterial vaccine candidates could permit substantial improvements in targeting, potency, and dose sparing.