In vivo targeting of dendritic cells in lymph nodes with poly(propylene sulfide) nanoparticles

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Statement of Purpose: The ability to deliver biodegradable nanoparticles to dendritic cells can be a valuable immunotherapeutic approach. In this study we investigate the lymphatic delivery of poly(propylene sulfide) (PPS) nanoparticles to dendritic cells residing in lymph nodes. These nanoparticles are ideal for lymphatic delivery because they offer long-term stability *in vivo*, can be synthesized to small sizes (<45nm), and are covered with a layer of hydrophilic poly(ethylene glycol) (PEG) to prevent clearance by the mono-nuclear phagocyte system.

Methods: PPS-PEG nanoparticles with diameters of 20, 45, and 100 nm were synthesized by emulsion polymerization by following a protocol reported earlier [1]. We use a previously developed technique, microlymphangiography for analyzing the effects of size on the lymphatic capillary uptake of fluorescently labeled PPS-PEG nanoparticles in mouse tail injections [2]. Additionally we assess the nanoparticle retention time and localization within lymph nodes following bolus injections in a mouse tail. Finally we determine the response of dendritic cells to nanoparticle internalization. Following injections, lymph nodes are dissected, cryo-sectioned, stained for specific immune cells, and analyzed with fluorescence microscopy or FACS.

Results / Discussion: It was found that 20 nm particles most readily entered into the lymphatic vessels. The 20 and 45 nm nanoparticles had significant retention in the lymph nodes, displaying a strong presence at 24, 72, 96 and surprisingly up to 120 hrs post-injection. The nanoparticles were not co-localized with either the T cell or B cell zones of the lymph node. Strong co-localization of nanoparticles with the antigen presenting cells, macrophages and dendritic cells was demonstrated. Additionally it was shown that there is significant endocytosis/phagocytosis of the nanoparticles by not only macrophages but also dendritic cells. Finally, it was determined that following internalization of nanoparticles, dendritic cells matured as demonstrated by increased expression of the co-stimulatory receptors CD80 and CD86.

Conclusions: We are the first to report that macrophages are not the only cell type that internalizes particles in lymph nodes; resident dendritic cells in the lymph nodes have also endocytosed the PPS-PEG nanoparticles. Additionally we demonstrate that nanoparticles caused dendritic cells to mature. Future studies will focus on using the PPS-PEG nanoparticles to deliver immunomodulatory agents such as peptides or proteins to dendritic cells in the lymph node.

References: [1] A. Rehor, J.A. Hubbell, and N. Tirelli (2005) *Lang* **21**: 411-17. [2] M.A. Swartz, D.A. Berk, and R.K. Jain (1996) *Amer Jour of Phys* **39**: H324-H329.