Intracellular Trafficking of Polyanhydride Nanospheres by Antigen Presenting Cells

[1]BD Ulery, [2]Y Phanse, [2]SA Sarkar, [1]M Torres, [2]B Bellaire, [2]MJ Wannemuehler & [1]B Narasimhan
Departments of [1]Chemical and Biological Engineering and [2]Vet Microbiology and Preventive Medicine, Iowa State Univ

Statement of Purpose: While vaccines are a vital method to prevent disease, improvements in their effectiveness and patient compliance are necessary. It has been shown that biodegradable polyanhydride particles possess the capacity to function both as adjuvants and single dose delivery vehicles.[1] Currently, it is unknown how particles enhance antigen uptake and processing by antigen presenting cells (APCs). By elucidating the interactions between polyanhydride nanospheres and APCs, we can better predict their success as vaccine delivery systems. It is hypothesized that the ability of tailored polyanhydride chemistries to modulate antigen processing and presentation will provide mechanistic insights leading toward the ability to rationally designing efficacious vaccines.

Methods: Random copolymers of 1,6-bis(*p*-carboxyphenoxy)hexane and sebacic acid (CPH:SA) and copolymers of 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane and 1,6-bis(*p*-carboxyphenoxy)hexane (CPTEG:CPH) were tested as potential vaccine delivery vehicles. Polymers were synthesized by melt polycondensation under vacuum to obtain molecular weights ~10,000 Da and were characterized by ^{1}H nuclear magnetic resonance, gel permeation chromatography and differential scanning calorimetry. Nanospheres were fabricated using an anti-solvent nanoencapsulation method modified from Mathiowitz et al.[2] Particle morphology and size distribution were measured by scanning electron microscopy and quasi-elastic light scattering, respectively. THP-1 human monocytic cells and primary murine bone marrow derived dendritic cells (BMDCs) were cultured in the presence of polyanhydride nanospheres to evaluate the effect of particle chemistry on uptake, intracellular localization and APC activation. Cellular responses were measured by flow cytometry and laser scanning confocal microscopy (LCSM).

Results: Polanhydride nanospheres between 80 and 800 nm in diameter possessing spherical shape were consistently fabricated regardless of polymer chemistry. Scanning electron micrographs of 20:80 and 50:50 CPH:SA nanospheres are shown in Figure 1.

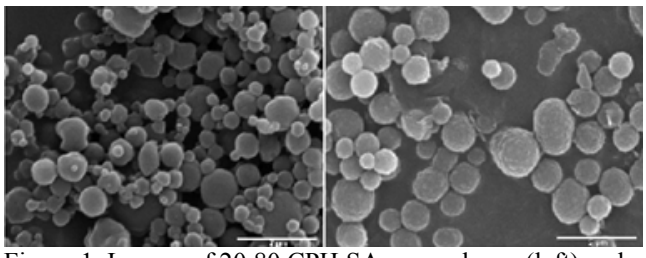

Figure 1. Images of 20:80 CPH:SA nanospheres (left) and 50:50 CPH:SA nanospheres (right)

Following the addition of nanospheres of varying chemistry to cultures of murine BMDCs, flow cytometry was utilized to assess enhanced cell surface expression of MHC I, MHC II, CD40 and CD209 (DC-SIGN). An increase in the percentage of BMDCs expressing both histocompatability complexes and co-stimulatory markers was observed for 20:80 CPH:SA, poly(SA), 50:50 CPTEG:CPH and poly CPTEG. Cell surface marker expression on BMDCs cultured in the presence of poly(CPH), 50:50 CPH:SA, 10:90 CPTEG:CPH and 20:80 CPTEG:CPH was unchanged. The greatest increase in expression was seen with the least hydrophobic polymers (poly(SA) and poly(CPTEG)). In addition, the enhanced presentation of MHC I and CD209 were not observed with a MPLA positive control. Nanospheres loaded with FITC-dextran (3% w/w) were added to THP-1 monocytes and murine BMDCs and the uptake and intracellular localization of the nanospheres were analyzed by LCSM. Shown in Figure 2 is an example of the confocal images generated for THP-1 monocytes.

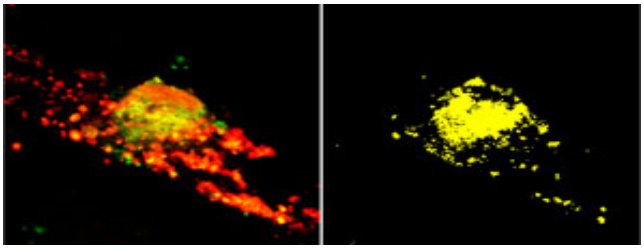

Figure 2. LSCM Photomicrographs of THP-1 cells following the addition of FITC-dextran loaded 20:80 CPH:SA (green), Alexa 555 CTx (Molecular Probes, Eugene, OR) labeled lipid rafts (red) and co-localization (yellow) on the left. The right image shows only regions of significant overlap of the two fluorescent markers.

By systematically altering cellular component markers we have observed variances in the handling of different polyanhydride nanospheres. The data show that less hydrophobic polymers are more readily uptaken by phagocytic cells.

Conclusions: The increased expression of cell surface markers on APCs and the uptake of less hydrophobic polyanhydrides provide insight into the mechanism by which specific polymer chemistries exert adjuvant activity as vaccine delivery vehicles.

Acknowledgment: The authors acknowledge financial support from the DOD–ONR (Award no. N00014-06-1-1176).

References:

[1]Kipper MJ, et al. J Biomed Mater Res A. 2006;76:798-810; [2]Mathiowitz E, et al. Nature. 1997;386:410-414