Developing Amphiphilic Diblock Co-Polymers for the Delivery of Cytosolically Active Immunostimulants

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A significant challenge facing synthetic vaccine development is inefficient delivery of antigens and immunostimulatory adjuvant molecules to the cytosol of antigen presenting cells. In order to overcome this challenge, we are developing pH-responsive diblock copolymers that can be utilized as nano-carriers for these adjuvants. We postulate that a successful carrier must form sub-100 nm particles under physiological conditions as well as have endosomal escape capabilities to promote delivery to cytosolic pathogen recognition receptors. A library of amphiphilic diblock copolymers synthesized with a poly(ethylene glycol) (PEG) first block and a pH-responsive and hydrophobic 2nd block composed of dimethylaminoethyl methacrylate (DMAEMA) and either butyl methacrylate (BMA) or hexyl methacrylate (HMA). The second blocks were designed to have compositions of 20%, 30%, and 40% BMA or HMA, and the effect of block composition and alkyl chain length on micellar nanoparticle assembly and intracellular delivery were evaluated. To determine the ability of polymer chains to form micellar nanoparticles, dynamic light scattering (DLS) was performed over a range of pH 5.8-7.4, representing acidification during endo/lysosomal trafficking. Polymers containing 30% HMA, 40% HMA, and 40% BMA successfully formed 13-18 nm diameter micelles at pH 7.4 and transitioned to unimeric polymer chains with decreasing pH. Next, a red blood hemolysis assay was performed across the same pH range to evaluate the pH-dependent membrane destabilizing activity of polymers. Results showed that polymers composed of 40% BMA displayed the greatest membrane disruptive characteristics at pH 5.8 and pH 6.2, however, at other pH values less than pH 7.4 polymers containing 20% HMA and 30% HMA were significantly more hemolytic than their BMA counterparts. Finally, a cell viability assay was carried out to determine the cytotoxicity of polymeric carriers. The results showed an average of 80% or higher cell viability at concentrations ranging from 0.01-10 μg/ml, with the 40% HMA polymer demonstrating the least cytotoxicity. In conclusion, initial results suggest that PEG-*bl*-DMAEMA-*co*-BMA and PEG-*bl*-DMAEMA-*co*-HMA are promising cytosolically active nano-carriers and that the endosomolytic activity of such carriers can be tuned through both composition and the alkyl chain length of hydrophobic monomers. Ongoing evaluations are focused on utilizing DMAEMA residues to electrostatically complex nucleic acid adjuvants and evaluating drug delivery efficacy in cell culture.