Degradation Kinetics Determine Intrinsic Immune Activity of Rapidly Degradable Polycations

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Statement of Purpose: Recent studies reveal that ubiquitous materials used in vaccination, such as poly(lactic-co-glycolic acid) (PLGA) and polystyrene, are able to activate antigen presenting cells and inflammatory immune pathways (e.g., inflammasomes), even in the absence of other immunogenic signals. Physicochemical properties play a role in influencing the type and degree of these effects and include characteristics such as charge, chemical functionality, hydrophobicity, and molecular weight (MW). Studies with hyaluronic acid for example indicated that high MW polymers fail to activate dendritic cells (DCs), while low MW fragments activate DCs at levels comparable to strong adjuvants such as lipopolysaccharide (LPS). A new class of rapidly degrading, pH responsive polymers called poly(beta-amino esters) (PBAEs) have been widely used in drug delivery, and in particular, for delivery of DNA vaccines. Compared with PLGA - which generally degrades over weeks or months - PBAEs degrade over hours or days, allowing for fast release of cargo that could be advantageous for vaccine design. However, how these polymers and the low MW fragments generated as the polymers degrade impact immune response remains unclear. Using a primary cell co-culture system, we tested the hypothesis that the intrinsic immune activity of PBAEs depends on the evolving MW of fragments as the polymers degrade at physiological conditions.

Methods: PBAEs were synthesized using a Michael-type addition reaction and characterized by nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC). Polymers were degraded in buffer for one week to generate fragments with decreasing MWs. Polyplexes were prepared by complexing the cationic PBAEs with a model polyanion, poly(styrene sulfonate) (SPS). The size and zeta potential of these particles were determined by laser diffraction and electrophoretic migration, respectively. CD11c+ DCs were purified from spleens of mice. Each fragment pool, in soluble or particle form, was then incubated with DCs in the presence or absence of the inflammatory toll-like receptor (TLR) agonists PolyIC (TLR3) or LPS (TLR4). Flow cytometry and ELISA were used to quantify the toxicity, surface activation, and cytokine secretion of the DCs as a function of PBAE MW and formulation. To assess antigen presentation, DCs were incubated with a model antigen (SIINKEFL), along with each of the PBAE MWs and formulations. Antigen presentation was quantified using an antibody that binds SIINFEKL peptide presented in the context of major histocompatibility complex I (MHC-I). T cell proliferation was evaluated by treating DCs with PBAEs then co-culturing the cells with T cells isolated from OT-I transgenic mice expressing T cell receptors specific to SIINFEKL (displayed in MHC-I).

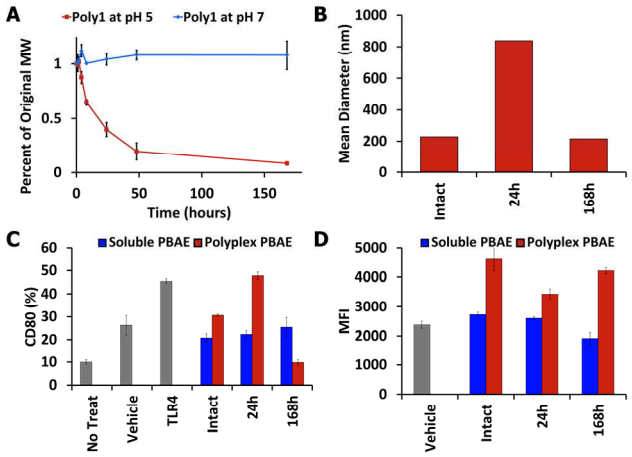

Figure 1. A) PBAEs degrade over 168 hours. B) Particles formed from fragments of different MWs exhibit different sizes that drive C) DC activation and D) antigen presentation compared to soluble polymer fragments.

Results: PBAEs were synthesized with an average MW of 3.9kDa, then incubated in buffer at either pH 5 or pH 7. PBAEs incubated at pH 7 were stable over 7 days, whereas PBAEs rapidly degraded at pH 5 over the same interval (**Fig 1A**). Polyplexes formed from different MW fragments exhibited zeta potentials ranging from -45mV to +30mV and sizes of ~200nm to ~1um (**Fig 1B**). In soluble form, low MW fragments had no effect on DC activation (**Fig 1C,** blue) and co-incubating DCs with PBAE fragments and a TLR3 or TLR4 agonist provided no synergistic effect compared to the TLR agonist alone. In contrast, PBAE polyplexes activated DCs to varying degrees depending on fragment MW (**Fig 1C**, red). Additionally, polyplexes increased antigen presentation at all fragment MWs compared to soluble fragments, likely due to the ability of DCs to efficiently internalize particulate materials (**Fig 1D**). Ongoing mechanistic studies reveal that DC activation by PBAE particles is not mediated by IL-1β-dependent inflammasome activation.

Conclusions: Rapidly degrading polymers such as PBAEs degrade in acidic conditions similar to those in tumor microenvironments and in endosomes of DCs following uptake. Of note, our results demonstrate that as PBAEs degrade, the size of the particles made from degraded fragments changes and that these larger particles are able to more efficiently activate DCs. Ongoing studies will determine the link between rapidly degrading polymers and *in vivo* function of DCs and T cells. Understanding the link that physicochemical properties of biomaterials (e.g., MW, hydrophobicity) have on immune cells as these materials degrade could allow for more rational design of future vaccine and immunotherapy carriers that elicit particular immune response characteristics based on carrier properties.