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Biomimetic biodegradable artificial antigen presenting cells synergize with PD-1 blockade to treat melanoma

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ABSTRACT

Biomimetic materials that target the immune system and generate an anti-tumor responses hold promise in augmenting cancer immunotherapy. These synthetic materials can be engineered and optimized for their biodegradability, physical parameters such as shape and size, and controlled release of immune-modulators. As these new platforms enter the playing field, it is imperative to understand their interaction with existing immunotherapies since single-targeted approaches have limited efficacy. Here, we investigate the synergy between a PLGA-based artificial antigen presenting cell (aAPC) and a checkpoint blockade molecule, anti-PD1 monoclonal antibody (mAb). The combination of antigen-specific aAPC-based activation and anti-PD-1 mAb checkpoint blockade induced the greatest IFN-γ secretion by CD8+ T cells *in vitro*. Combination treatment also acted synergistically in an *in vivo* murine melanoma model to result in delayed tumor growth and extended survival, while either treatment alone had no effect. This was shown mechanistically to be due to decreased PD-1 expression and increased antigen-specific proliferation of CD8+ T cells within the tumor microenvironment and spleen. Thus, biomaterial-based therapy can synergize with other immunotherapies and motivates the translation of biomimetic combinatorial treatments.

1. Introduction

Biomimetic materials that target the immune system hold promise for cancer immunotherapy [1]. Synthetic immunotherapies can be designed with defined characteristics and therefore often outperform their cell-based counterparts. These platforms can be engineered in terms of biodegradability [2], controlled release of immuno-modulators [3], and physical parameters including shape and size [4]. Biomimetic materials can be customized to incorporate combination therapies in an all-in-one therapeutic and are therefore an exciting platform for the future of cancer immunotherapy. Despite their potential, current development of combinatorial immunotherapies utilizing biomaterials has been limited as their interaction with other existing therapeutics must first be understood.

Synthetic artificial antigen presenting cells (aAPC), a biomaterial-based immunotherapy, have shown success in generating an anti-tumor immune response *in vitro* and *in vivo* [5–8]. aAPC are three-dimensional platforms that minimally express the two signals required for T cell activation – a signal 1, peptide-MHC (pMHC) to provide T cell receptor (TCR) specificity, and a signal 2,

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such as anti-CD28 monoclonal antibody (mAb) to provide the co-stimulatory "go" signal. aAPC can be functionalized with tumor-specific pMHC to activate a patient's immune system against cancer antigens and mediate tumor rejection [9–11]. They can be utilized in adoptive cell transfer (ACT) of *ex vivo* activated autologous T cells [9,12,13] or directly administered intravenously (IV) for *in vivo* anti-tumor T cell activation [14,15]. Synthetic aAPC platforms have distinct advantages over cellular systems in terms of long-term storage and the ability to optimize T cell activation and biocompatibility [16]. Unlike biological antigen presenting cells used as cellular therapy, biomaterial-based aAPC have the advantage of being able to maintain an "always on" state that cannot be down-regulated by the microenvironment as well as flexibility for manufacturing as an acellular product. Compared to PLGA-based drug delivery particles for cancer therapy, the anti-cancer drugs must reach and destroy every cancer cell to ultimately be effective. In contrast, poly (lactic-co-glycolic acid) PLGA-based aAPC particles for immunotherapy need only reach tumor specific T cells that can recognize the tumor antigen for the aAPC to then be able to direct a robust systemic immunotherapy response against the cancer cells. Biomimetic modifications of PLGA-based aAPC materials that greatly enhance their effector capacity, including controlling the shape of the aAPC [4,17] or slowly releasing pro-inflammatory cytokines from their core [18,19], have demonstrated the benefit of bringing novel materials engineering concepts to the development of immunotherapeutics.

In addition to amplifying positive regulators of the immune system, inhibiting negative regulators has also shown success in generating anti-tumor immune responses. Checkpoint molecules, including programmed death 1 (PD-1) and CTLA-4, are negative regulators of T cell function. These molecules are upregulated on tumor infiltrating lymphocytes and on activated T cells expanded during ACT, being described as a rheostat of the immune system [20]. PD-1 signaling inhibits CD8+ T cell effector function upon ligation with its ligand, programmed death ligand 1 (PD-L1), and is one of the methods by which tumors escape immune surveillance. Checkpoint blockade with monoclonal antibodies against PD-1 and PD-L1 delay tumor growth in murine tumor models [21,22], and FDA approved monoclonal anti-PD-1 and anti-CTLA-4 antibodies have shown significant overall response rates and long-term survival benefits. However, clinical responses only reach approximately 30% [23–26] indicating that there is a necessity for improvement.

Single-targeted approaches have limited efficacy because cancerous cells utilize multiple mechanisms to avoid immune surveillance and the immune system internally suppresses prolonged strong activation [27]. The combination of checkpoint inhibitors with other immunotherapies that boost T cell effector functions or promote cancer cell recognition by the immune system have potential to increase anti-tumor effectiveness. Checkpoint blockade in conjunction with T cell costimulatory antibodies resulted in tumor regression in multiple murine tumor models [28–30] and increased effector functions of exhausted CD8+ T cells by forcing them out of quiescence [31]. These studies suggest that checkpoint blockade can boost the effects of other immune-stimulatory approaches, although their interaction with biomaterial-based antigen-specific T cell stimulation has not been studied.

Here, we investigate the synergy between a biomimetic material, biodegradable PLGA-based aAPC, and anti-PD-1 monoclonal antibody treatment for the activation of tumor-specific CD8+ T cells. Combinatorial treatment enhances CD8+ T cell effector functions *in vitro* and significantly delays tumor growth *in vivo*. These results demonstrate the effectiveness of PLGA-based aAPC in combination immunotherapy, and identify a molecule that could potentially be incorporated and released from polymeric aAPC for increased effectiveness.

2. Materials and methods

2.1. Artificial antigen presenting cell synthesis and characterization

Artificial antigen presenting cells were synthesized in a two-step core particle formation and functionalization. Particles cores were synthesized from poly (lactic-co-glycolic acid) (PLGA 50:50 lactic acid to glycolic acid ratio, MW 34,000–58,000 Da) that was purchased commercially (Sigma Aldrich; St. Louis, MO). For a typical microparticle synthesis, 100 mg of PLGA was dissolved in 5 mL dichloromethane and homogenized into a 50 mL, 1% poly vinyl alcohol (PVA) solution by an T-25 digital ULTRA-TURRAX IKA tissue homogenizer at a speed of 5000 rpm (IKA Works; Wilmington, NC). The resulting microparticle emulsification was then added to 100 mL of 0.5% PVA solution. The dichloromethane was then allowed to evaporate over the course of 4 h. After particle hardening, the particles were washed three times in water through centrifugation at 3000*g* for 5 min. The washed microparticle solution was flash frozen in liquid nitrogen and lyophilized for 1 day prior to characterization and use.

Functionalization was achieved through EDC/NHS chemistry to conjugate carboxylic acid terminated PLGA to amines on the proteins of interest. Lyophilized particles were dissolved in 0.1 M MES buffer at pH 6.0 at a concentration of 2 mg/mL 100 μL of EDC/NHS (Sigma Aldrich; St. Louis, MO) stock solution at 40 mg/mL and 48 mg/mL respectively were added to each sample and the particles were activated for 30 min. The resulting surface activated particles were washed in PBS through centrifugation at 5000 g for 5 min. The particles were resuspended in PBS at 2 mg/mL 8 μg MHC IgG dimer loaded with the antigen of choice and 10 μg anti-CD28 monoclonal antibody (mAb) (BD Biosciences; San Jose, CA) was added to each sample and the particles were allowed to react with the proteins overnight at 4 °C. The resulting aAPC were washed 3x in PBS through centrifugation at 5000*g* and then dissolved in 400 μL of 100 mg/mL endotoxin free sucrose. The resulting suspension was then lyophilized overnight.

Particle imaging was conducted with a Leo FESEM scanning electron microscope. To prepare samples for analysis, lyophilized particles were mounted onto carbon tape (Nisshin EM Co.; Tokyo, Japan) and placed upon aluminum tacks (Electron Microscopy Services; Hatfield, PA). The excess particles were removed by blowing air across the surface of the tack and the sample was then sputter coated with a 20 nm thick layer of gold-palladium. The samples were then loaded into the microscope and imaged. The images were processed in ImageJ to obtain size information.

To determine the amount of protein on the surface, conjugated aAPC microparticles were stained with Alexa Fluor 647 goat anti-mouse IgG for the dimer and Alexa Fluor 546 goat anti-hamster IgG for the anti-CD28 (Life Technologies; Grand Island, NY) for 1 h at 4 °C. The particles were subsequently washed with PBS three times and fluorescence readings of particles were evaluated for fluorescence with a BioTek Synergy 2 plate reader (Biotek; Winooski, VT). The mass of protein on the particle was calculated to evaluate conjugation efficiency. Conjugation efficiency was calculated as (Protein Calculated on Particles)/(Protein Added to Conjugation Media) *100%.

2.2. Anti-PD1 monoclonal antibody synthesis

Anti-PD-1 mAb clone G4 was grown from the G4 hybridoma cell line. Hybridoma cells were grown in hybridoma serum free media supplemented with L-glutamine. After one week, the supernatant