Non-spherical Artificial Antigen Presenting Cells for Tumor Immunotherapy

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Introduction: Synthetic, acellular artificial antigen presenting cells (aAPCs) have broad applications.[1] Previous work developing aAPCs has focused exclusively on spherical platforms. Yet, the geometry of the interaction between an activated antigen presenting cell and a T cell is quite different from that of two spheres. While two spheres have a small surface area of interaction, a large interface between an antigen presenting cell and a T-cell is required for immune synapse formation post T-cell receptor triggering.[2] To explore the role of shape in this process, we altered the shape of PLGA microparticles to generate ellipsoidal PLGA microparticles with varying long axis lengths and aspect ratios (ARs). We compared aAPCs (**Fig. 1a**) generated from these ellipsoidal particles (**Fig. 1b, 1e**) with aAPCs generated from spherical PLGA microparticles (**Fig. 1c**) with respect to their ability to activate T-cells in both an antigen-dependant fashion *in vitro* and in a tumor-prevention model *in vivo*.

Methods: PLGA microparticles were made by single-emulsion and embedded in a PVA/glycerol film which was stretched in an oven at 90°C to the desired extent. Stretched particles were recovered by film dissolution. aAPC were fabricated from stretched and non-stretched microparticles by coupling MHC-IgG dimer (with either cognate or non-cognate peptide in the MHC pocket) and anti-CD28 mAb onto the surface of the particles via EDC-sulfo NHS chemistry. These aAPCs were added to CD8+ pmel T-cells (from mice with transgenic T-cell receptors (TCRs)) that were stained with carboxyfluorescein succinimidyl ester (CFSE). After exposure to aAPCs, proliferation was analyzed by flow cytometry looking for dilution of the CFSE dye and by manual cell counting at day 7. For the *in vivo* melanoma tumor prevention study, mice were preinjected i.v. with naïve pmel T cells (d -4), s.c. with particles (d -1), then injected with tumor in the hindlimb (d 0). Responses were boosted with subsequent s.c. injection of a second particle batch (d 3), and tumor growth was followed by measurement with external calipers. For confocal studies, PLGA microparticles with encapsulated 5(6)-carboxy-tetramethylrhodamine dye were made into aAPCs, mixed with CFSE-labeled T-cells, and imaged together to examine cognate formation.

Results: The "stretched" biomimetic non-spherical aAPCs were successful at stimulating T-cells in an antigen-specific fashion. Interestingly, at sub-saturating doses, high aspect ratio aAPCs show significantly enhanced activity (enhanced T-cell proliferation by CFSE dilution on flow cytometry and by cell counting) beyond spherical aAPCs with particle volume and antigen content held constant. In addition, aAPCs show improved activity with increasing particle aspect ratio (**Fig. 1f, g**). Significantly, high-aspect ratio aAPCs improve survival in a s.c. melanoma-prevention mouse model compared to non-cognate aAPCs ($p=0.004$) as well as cognate spherical aAPCs ($p=0.05$) (**Fig. 1i**). Confocal imaging indicates that the proliferative advantage seen by stretched aAPCs may be due to improved interaction along that long axis, as T-cells will form more contacts with stretched aAPC and will dynamically migrate to the long axis of the particle.

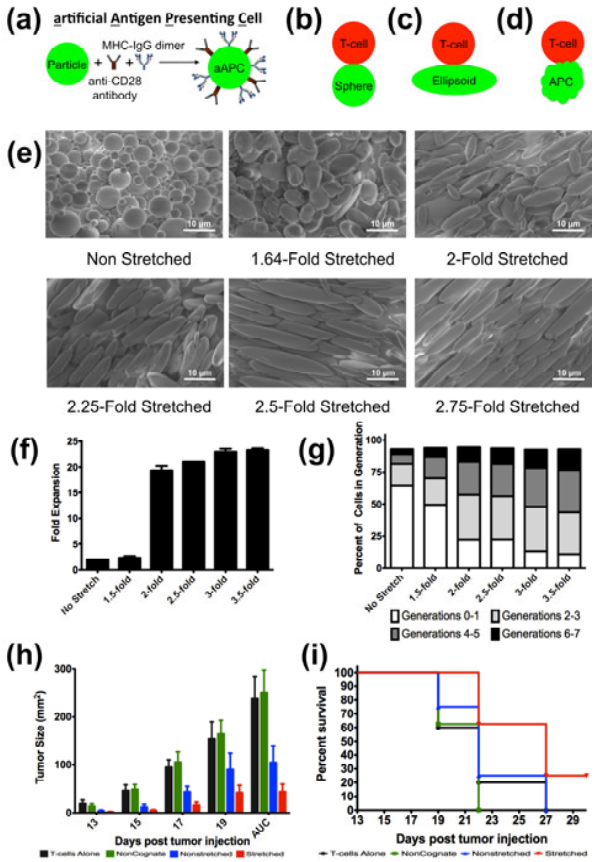

Fig. 1: (a) Synthesis of aAPC. (b-d) Schema of T-cell interacting with a sphere, an ellipse, or an APC. (e) SEM of non-stretched and stretched particles. (f) Fold expansion of pmel T cells 7d after exposure to 0.1 mg particles / 10^5 cells. (g) Generational analysis from CFSE dilution, 3d post exposure. (h,i) *in vivo* melanoma prevention: Tumor size (h) and survival (i) for T cells alone, non-cognate, non-stretched, and stretched aAPC.

Conclusions: Particle geometry is a critical design criterion to consider in the generation of aAPCs, and may offer insights into the essential role of geometry involved in the interaction between T-cells and biological APCs. aAPCs thus may not only be an enabling tool for antigen-specific immunotherapy, but also for studying basic aspects of T-cell biology.

References: 1) Ugel, S, et al. Cancer Res. 2009;69:9376-9384. 2) Monks, CR, et al. Nature. 1998;395:82-86