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Latex bead-based artificial antigen-presenting cells induce tumor-specific CTL responses in the native T-cell repertoires and inhibit tumor growth

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ABSTRACT

Cell-free artificial antigen-presenting cells (aAPCs) were generated by coupling H-2K^b/TRP2 tetramers together with anti-CD28 and anti-4-1BB antibodies onto cell-sized latex beads and injected intravenously and subcutaneously into naïve mice and antigen-primed mice (B6, H-2K^b). Vigorous tumor antigen-specific CTL responses in the native T-cell repertoire in each mouse model were elicited as evaluated by measuring surface CD69 and CD25, intracellular IFN-γ, tetramer staining and cytolysis of melanoma cells. Furthermore, the aAPCs efficiently inhibited subcutaneous tumor growth and markedly delayed tumor progression in tumor-bearing mice. These data suggest that bead-based aAPCs represent a potential strategy for the active immunotherapy of cancers or persistent infections.

1. Introduction

Antigen-specific T lymphocytes can be primed and amplified ex vivo or in vivo by antigen-presenting cells (APCs). An increasing number of experimental and clinical studies have demonstrated the therapeutic role of dendritic cells (DCs) for cancer immunotherapy [1–3]. However, DC vaccines are currently limited by the need to derive large scale of autologous or allogeneic DCs from patients or compatible healthy donors. As an alternative strategy, artificial APCs (aAPCs) have been developed for the establishment of therapeutic cellular immunity. They encompass both cell-based and acellular technologies [4]. Cellular aAPCs include mouse fibroblasts, human leukemia cell lines or insect cells that are genetically engineered to overexpress target antigens and costimulatory molecules [5]. Cell-free aAPCs are made by covalently coupling MHC/peptide complex (pMHC) and CD28-ligand onto cell-sized magnetic beads or latex beads [6].

Numerous researchers around the world have already documented that magnetic bead or latex bead-based aAPCs are a powerful tool to expand antigen-specific T cells ex vivo for adoptive immunotherapy of cancers and infectious diseases; they are as good as, or even better than, classical peptide-pulsed DCs [7–11]. However, few studies have investigated the potential of cell-free aAPCs for active immunotherapy in vivo. A significant concern is that the lack of both adhesion molecules and membrane fluidity on the acellular aAPCs would lead to the failure of the immune synapse during aAPCs trafficking in vivo. Caserta has developed CD4$^+$ T cell-targeted latex bead-based aAPCs coated with MHC class II/peptide dimers (I-A^d/LACK) and anti-CD28 antibodies. In vivo administration of the aAPCs led to antigen-specific CD4$^+$ T-cell activation and proliferation in secondary lymphoid organs, conferred partial protection against subcutaneous tumors and prevented the establishment of lung metastases [12]. Ugel has also generated aAPCs by coupling pMHC-Ig dimers (H-2K^b/TRP2) and B7.1-Ig molecules to magnetic beads. The mice models of subcutaneous melanoma or lung melanoma metastases were infused with the low-affinity CTLs specific for mouse TRP2 antigen followed by in vivo administration of the aAPCs. The aAPCs injection augmented the activity of the adoptively transferred CTLs and significantly delayed tumor growth. The clearance of metastases correlated with the in situ proliferation of the transferred CTLs [13]. These data suggest that aAPC administration can potentially be used to overcome current problems related to low-avidity antitumor CTLs, therefore increasing the efficiency of the adoptive immunotherapy of cancer.

In this report, whether the bead-based aAPCs injection could elicit the proliferation and cytotoxicity of native CTLs in a native T-cell repertoire will be determined. Here, mouse TRP2, a melanoma-associated self-antigen, was used as a target tumor

Abbreviations: APCs, antigen-presenting cells; MHC, major histocompatibility complex; DCs, dendritic cells; PE, R-phycoerythrin; APC, allophycocyanin; CFSE, carboxyfluorescein succinimidyl ester; FITC, fluorescein-5-isothiocyanate; CTLs, cytotoxic T cells; mAb, monoclonal antibody; PBS, phosphate buffered saline.

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antigen. Naïve mice and antigen-primed mice (B6, H-2K^{b+}) were injected with aAPCs made by co-coupling H-2K^{b}/TRP2 tetramers, anti-mouse CD28 and anti-mouse 4-1BB antibodies to latex beads. The resulting antigen-specific CTL responses were evaluated and compared across the mice models. Furthermore, the effects of aAPCs on subcutaneous melanoma growth were investigated in preventative mode of therapy. This report initially demonstrates the presentation of bead-based aAPCs to expanding CTLs in the native T-cell repertoires in naïve mice, antigen-primed mice and tumor-bearing mice and, thus, highlights the potential strategy for active immunotherapy.

2. Materials and methods

2.1. Mice and cell lines

Male C57BL/6 (B6, H-2K^{b}) mice were purchased from the Comparative Medicine Center of Yangzhou University (Yangzhou, Jiangsu, China) and typically used in experiments at 6–8 weeks of age. They were maintained in plastic cages at 21 ± 2 °C with free access to pellet food and water and kept on a 12-h light/dark cycle. Animal welfare and experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006) and were approved by the animal ethics committee of Southeast University.

Mouse melanoma cell line B16 (H-2K^{b+}, TRP2^{+}), mouse myeloma cell line SP2/0 (H-2K^{b-}/TRP2^{-}) and mouse fibrosarcoma cell line S180 (H-2K^{b+}/TRP2^{-}) were purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China) and maintained in RPMI-1640 medium with 10% fetal bovine serum (FBS) at 37 °C in a 5% CO_2 atmosphere.

2.2. Preparation of H-2K^{b}/peptide monomers and tetramers

TRP2$_{180–188}$(SVYDFFVWL) is an H-2K^{b}-restricted, dominant T cell epitope derived from mouse melanoma antigen. OVA$_{257–264}$(SIINFEKL) is a well-known T cell epitope from ovalbumin and is presented by H-2K^{b} molecules. H-2K^{b}/TRP2$_{180–188}$ monomers and H-2K^{b}/OVA$_{257–264}$ monomers and their tetrameric forms were generated in-house, as our previously described [14–16]. Briefly, recombinant H-2K^{b} heavy chain and peptide-fused mouse β2 microglobulin (β2m) produced in *Escherichia coli* were solubilized in urea and folded in a guanidine refolding buffer. After size exclusion purification in fast performance liquid chromatography (FPLC) (Pharmacia Fine Chemicals, Uppsala, Sweden), the monomers were biotinylated with the enzyme, BirA (Avidity, LLC, Denver, CO, USA). Tetrameric forms were prepared by the stepwise addition of PE-conjugated streptavidin or PE-free streptavidin (one-fourth of the total every 20 min; Molecular Probes, Inc., Eugene, OR, USA) to achieve a 1:5 molar ratio of avidin to monomer.

Of note is that we linked the peptide of interest to the N-terminus of mouse β2m via a Gly-Gly-Ser motif-containing linker, and a His6 tag was linked to the C terminus of mouse β2m [15]. The structural and functional characterization of pMHC monomers and tetramers was performed as our previously described [16].

2.3. Preparation of aAPCs and control beads

Similar to our previous reports [17,18], sulfated polystyrene latex beads with a diameter of 5 μm (Interfacial Dynamics, Portland, OR, USA) were coated with a 1:1:1 mixture of anti-His$_6$ mAb (Amersham Biosciences, Piscataway, NJ, USA), anti-mouse CD28 mAb (Functional, clone37.51, eBioscience Inc., San Diego, CA, USA) and anti-mouse CD137 (4-1BB) mAb (Functional, clone17B5, eBioscience). The antibodies were incubated with 75 μg/10^{8} beads in phosphate buffered saline (PBS) overnight at 4 °C on a rotator. Beads were then washed and resuspended in blocking buffer (0.1 M PBS, 3% mouse serum, 0.05% sodium azide) for 1 h at 4 °C. After washing, beads were incubated with H-2K^{b}/TRP2 tetramers (25 μg/10^{8} beads) for an additional 1 h at 4 °C. The beads were then washed with PBS. The resulting beads (aAPCs) were stored in PBS at 4 °C and used within 24 h. All buffers and reagents were sterilized. Two types of control beads were prepared in an identical fashion but without the H-2K^{b}/TRP2 tetramers (anti-CD28/4-1BB/His beads) or without anti-CD28 mAb, anti-4-1BB mAb and H-2K^{b}/TRP2 tetramers (anti-His beads) (Fig. 1).

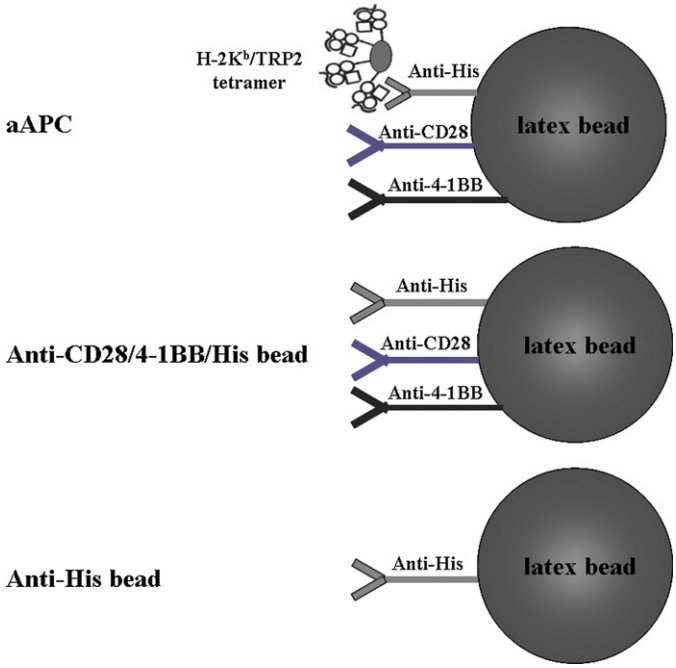

Fig. 1. Schematic diagram of aAPC and control beads.

2.4. Administration of aAPCs in naïve mice and antigen-primed mice

Naïve B6 mice were randomly assigned to 1 of 3 groups and infused with aAPCs, anti-CD28/4-1BB/His beads and anti-His beads, respectively. Beads were administered to each group three times; the first and second injections were administered 7 days apart via i.v. (intravenous, tail vein) injection (5×10^{6} beads/mouse/time point), and the third injection was administered via s.c. (subcutaneous) injection 14 days after the first injection (3×10^{6} beads/mouse). Animals were sacrificed 5 days after the final administration. Their splenocytes were harvested, seeded into 96-well round-bottom plates (BD Falcon, Bedford, MA, USA) (2×10^{5} cells/well) and then co-cultured for 0–3 days with TRP2$_{180–188}$ peptide (2 μg/ml) and mouse IL-2 (100 IU/ml, R&D System, Minneapolis, MN, USA) in RPMI-1640 medium with 10% FBS at 37 °C in a 5% CO_2 atmosphere.

To generate target antigen-primed mice, naïve B6 mice were injected s.c. two times with B16 melanoma cells, which are derived from B6 mice and express TRP2 melanoma antigen, at a dose of 3000 cells per mouse per time point with an interval of 10 days. On day 7 after the final injection of B16 cells, the antigen-primed B6 mice were randomly assigned to 1 of 3 groups and infused with aAPCs, anti-CD28/4-1BB/His beads or anti-His beads, respectively, in a fashion similar to the regimen for naïve mice treatment.