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Antigen recognition-triggered drug delivery mediated by nanocapsule-functionalized cytotoxic T-cells

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ABSTRACT

Cytotoxic T-Lymphocytes (CTLs) kill pathogen-infected or transformed cells following interaction of their T-cell receptors (TCRs) with foreign (e.g. virus-derived) peptides bound to MHC-I molecules on the target cell. TCR binding triggers CTLs to secrete perforin, which forms pores in the target cell membrane, promoting target death. Here, we show that by conjugating drug-loaded lipid nanoparticles to the surface of CTLs, their lytic machinery can be co-opted to lyse the cell-bound drug carrier, providing triggered release of drug cargo upon target cell recognition. Protein encapsulated in T-cell-bound nanoparticles was released following culture of CTLs with target cells in an antigen dose- and perforin-dependent manner and coincided with target cell lysis. Using this approach, we demonstrate the capacity of HIV-specific CTLs to deliver an immunotherapeutic agent to an anatomical site of viral replication. This strategy provides a novel means to couple drug delivery to the action of therapeutic cells *in vivo*.

1. Introduction

The ability to precisely control the spatial and temporal delivery of therapeutic agents *in vivo* would revolutionize the treatment of human disease. This overarching goal has motivated the development of stimuli-responsive nanoparticles designed to release drug cargos in response to the chemical properties of a target tissue environment, such as the low pH of tumors; or in response to physical stimuli such as light, heat, or magnetic fields applied to an anatomical target site (reviewed in Refs. [1,2]). A promising strategy is to interface drug delivery technologies with cell therapy, by conjugating or loading therapeutic cells with drug delivery payloads [3–10] (reviewed in Ref. [11]). In such approaches, programmed or environment-responsive drug release provided by a synthetic drug carrier can be married with the precision tissue homing properties of living cells. We previously demonstrated that cytotoxic T-lymphocytes (CTLs) can carry drug-loaded nanoparticles through the covalent attachment of lipid-based nanocapsules to cell surface proteins [6,7,11,12]. These nanocapsule-CTL conjugates (NC-CTL) exhibited unimpaired abilities to kill target cells and trafficked normally *in vivo*, accumulating in tumors that expressed cognate antigen [6,7,11,12]. In this work we used NC-CTL technology to focus drug delivery on the CTLs themselves, through a pseudo-autocrine mechanism [6], or to deliver drugs to tumors by passive release [7]. However, it would be attractive to control the timing and location of drug payload release by linking it to cell function, and in the case of therapeutic T-cells, to link drug delivery to antigen recognition – which would localize payload release to sites of antigen expression (e.g., infected tissues or tumors) and avoid premature drug release at other sites like blood and lymphoid tissues.

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Here we demonstrate that one of the key functions of cytotoxic lymphocytes, lytic granule exocytosis, can be used as a trigger to release therapeutic payloads from CTL-bound nanoparticles. We show that, as CTLs recognize antigen displayed on the surface of target cells, lipid-based nanoparticles chemically conjugated to the surface of these cells are trafficked to the T-cell/target cell contact site (the immunological synapse). Subsequent release of the membrane pore-forming protein perforin at the synapse leads to both disruption of the target cell membrane and the membrane of the lipid drug carrier, leading to rapid cargo release (Fig. 1). In an *in vivo* model of HIV infection, we demonstrate that HIV-specific CTLs carrying nanoparticles loaded with an immunotherapeutic agent (the interleukin IL-15), can specifically release this cytokine in tissues where infected cells are detected, resulting in enhanced elimination of infected cells as compared to HIV-specific CTLs with empty nanoparticles. This approach provides a general platform for achieving time- and space-regulated drug delivery, by linking drug release to the highly sensitive and specific sensing of antigens by CTLs.

2. Materials and methods

2.1. Ethics statement

HIV-infected individuals were recruited from the Maple Leaf Medical Clinic in Toronto, Canada through a protocol approved by the University of Toronto Institutional Review Board. Secondary use of the samples from Toronto was approved through the Massachusetts General Hospital (MGH) Institutional Review Board. All subjects were adults, and gave written informed consent. Animal experiments were performed under a protocol approved by the MGH and MIT IACUCs.

2.2. Generation of T-cell clones

Peripheral blood mononuclear cells (PBMCs). were stimulated with optimal CD8 T-cell epitopes for 6-h, enriched for antigen-specific cells using the IFN-γ secretion Detection and Enrichment Kit (Miltenyi), and cloned at limiting dilution on irradiated feeder cells as has been previously described [13]. Clones were selected from 96-well plates at dilutions where no more than 1 in 5 wells displayed growth and screened for specificity by IFN-γ ELISPOT. Specific clones were expanded on irradiated feeder cells.

2.3. Generation of ICMV nanocapsules

ICMVs were generated as previously described. Briefly, 0.1 mg of DOPG 0.4 mg of DOPC and 0.65 mg of maleimide containing lipid MPB were combined in chloroform and dried to lipid films (lipids from Avanti Polar Lipids). These films were resuspended in a buffer of 20 mM Bis-tris propane pH 7.2 containing 10.0 mg/ml Alexa647-OVA (Life Technologies), or 0.7 mg/ml of the IL-15Sa ALT-803 (Altor Bioscience Corporation), by multiple rounds of vortexing. Mixtures were sonicated for 5 min to form unilamellar nanoparticles and then $CaCl_2$ was added to induce the fusion of particles into multilamellar structures. Particles were then crosslinked by treating with DTT, resulting in the formation of covalent bonds between maleimide functional groups in neighboring lipid bilayers. Particles were then pelleted, washed twice with water, and resuspended in XVIVO-10 serum-free medium (Lonza). IL-15Sa encapsulation was determined by lysing particles and performing enzyme linked immunosorbent assay (ELISA), and calculated as 2.46 ± 0.10 μg of IL-15Sa per 1.15 mg of lipid (0.21%).

2.4. Generation of NC-CTLs

CTL clones were used 2–3 weeks after the most recent restimulation with irradiated feeder cells. A full batch of ICMVs as described above was combined with 50×10^6 CTLs and incubated at 37 °C for 1 h with constant agitation. Cells were then pelleted at 1200 × g for 5 min, washed twice with 1 ml each of XVIVO-10 medium, and resuspended in 200 μL of 10 mg/ml polyethylene glycol thiol (PEG-SH) in PBS for 10 min at 37 °C to quench free maleimide groups. Resulting NC-CTLs were then resuspended in RPMI +10% FBS + penicillin/streptomycin + L-glutamine (R-10) supplemented with 50 U/ml IL-2 (Hofmann La Roche) (R10-50), and used in downstream applications. For the cells used in the *in vivo* experiment depicted in Fig. 5, IL-15Sa cargo loading was calculated as 216 ± 4 ng/10^6 NC-CTLs by lysis and ELISA.

2.5. Assessing NC-CTL polarization

Alexa-647 OVA NC-CTLs were co-cultured with peptide-pulsed CFSE-labeled autologous $CD4^+$ T cells (targets) for 20 min at 37 °C. Cells were then fixed with 2% paraformaldehyde (PFA), stained with Phalloidin Alexa-658 and DAPI in 0.005% Triton-X100 for two hours, and imaged by confocal microscopy. Maximum intensity projects were generated for 56 conjugate images. For each

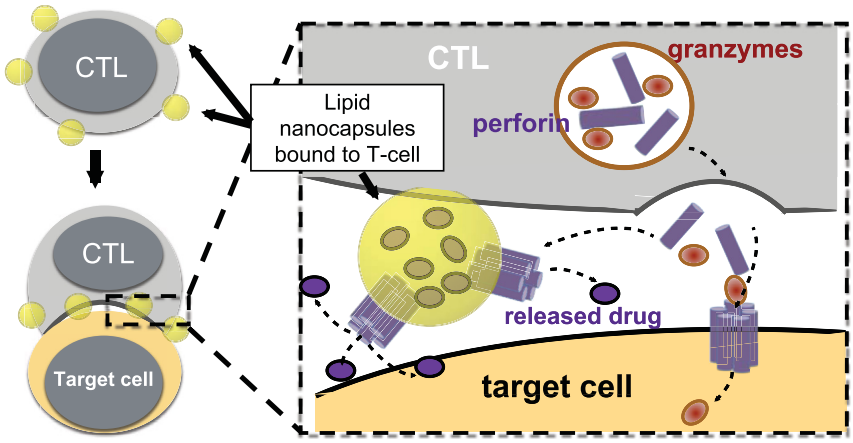

Fig. 1. Strategy for CTL-triggered drug release from lipid nanocapsules. CTLs encountering target cells release perforin and granzymes into the immunological synapse formed between the CTL and target cell. Lipid nanocapsules (NCs) covalently anchored to CTL surface proteins traffic into the synapse, where they are exposed to perforin released by the CTL, enabling disruption of the NC and release of encapsulated drug in tandem with lysis of the target cell.