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Low-dose controlled release of mTOR inhibitors maintains T cell plasticity and promotes central memory T cells

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ABSTRACT

An important goal for improving vaccine and immunotherapy technologies is the ability to provide further control over the specific phenotypes of T cells arising from these agents. Along these lines, frequent administration of rapamycin (Rapa), a small molecule inhibitor of the mammalian target of rapamycin (mTOR), exhibits a striking ability to polarize T cells toward central memory phenotypes (T_{CM}), or to suppress immune function, depending on the concentrations and other signals present during administration. T_{CM} exhibit greater plasticity and proliferative capacity than effector memory T cells (T_{EFF}) and, therefore, polarizing vaccine-induced T cells toward T_{CM} is an intriguing strategy to enhance T cell expansion and function against pathogens or tumors. Here we combined biodegradable microparticles encapsulating Rapa (Rapa MPs) with vaccines composed of soluble peptide antigens and molecular adjuvants to test if this approach allows polarization of differentiating T cells toward T_{CM}. We show Rapa MPs modulate DC function, enhancing secretion of inflammatory cytokines at very low doses, and suppressing function at high doses. While Rapa MP treatment reduced – but did not stop – T cell proliferation in both $CD4^+$ and $CD8^+$ transgenic T cell co-cultures, the expanding $CD8^+$ T cells differentiated to higher frequencies of T_{CM} at low doses of MP Rapa MPs. Lastly, we show in mice that local delivery of Rapa MPs to lymph nodes during vaccination either suppresses or enhances T cell function in response to melanoma antigens, depending on the dose of drug in the depots. In particular, at low Rapa MP doses, vaccines increased antigen-specific T_{CM}, resulting in enhanced T cell expansion measured during subsequent booster injections over at least 100 days.

1. Introduction

Most vaccines amplify immune responses elicited against foreign molecules ("antigens") associated with a pathogen, exploiting the exquisite specificity the immune system harnesses to destroy pathogens without targeting host cells. Despite the importance of these technologies, one of the arising themes for new vaccines and immunotherapies is the need for approaches that not only generate large responses, but that also program specific functions and phenotypes of these responses [1,2]. In this study we used peptide antigen and common molecular vaccine adjuvants to study how controlled release of low doses of modulatory drugs during T cell expansion alters immune cell differentiation and enhances immune function in cells and mice.

Our studies are motivated by fascinating recent work revealing that regular, low doses of immunosuppressive drugs can be used to enhance vaccination and immunotherapy by maintaining plasticity – that is, limiting the differentiation – of T cells. Rapamycin (Rapa), and analogs of this drug, are some of the most-well studied along these lines [3,4]. Rapa has historically played a significant role as an immunosuppressant, but this drug class targets the mTOR pathway, which is intimately involved in metabolism, cell growth, and differentiation [5,6]. Several seminal studies demonstrate regular, systemic administration of Rapa increases the durability of expanding antigen-specific $CD8^+$ T cells, and in particular, that the phenotypes of these cells are polarized away from effector T cells (T_{EFF}) and toward central memory T cells (T_{CM}) [7,8]. While T_{EFF} are needed to combat pathogenic cells, these cells become less proliferative as they differentiate from naïve T cells. In contrast, T_{CM} are more plastic, exhibiting the highly proliferative capacity needed to rapidly generate large numbers of antigen-specific T_{EFF}. Thus, expanding T_{CM} against a target antigen might provide a route to generate large, durable populations of antigen-specific cells

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that mount potent responses by maintaining a highly proliferative characteristic.

Gaining control over the processes above to direct immune cell differentiation could have a transformative impact in combating infectious disease, immune dysfunction, and cancer. Cancer, for example, is difficult to treat due to the heterogeneity of disease and the ability of tumors to metastasize, evade and suppress the immune system, and cause relapse [9]. Over the past decade, adoptive cell therapies – in which tumor-primed T cells are transferred to animals or patients – have led to the revelation that T_{CM} are important drivers of anti-tumor immunity [10]. The high proliferation rates of T_{CM} support rapid response against established tumors and improved protection against relapse [11–14]. Unfortunately, as T_{CM} expand, the differentiation to effector memory (T_{EM}) or effector (T_{EFF}) T cells results in reduced numbers of cells and cytokines (e.g., IL-2) to overcome the immunosuppressive tumor environment [15,16].

Adoptive cell therapy, cancer vaccination, and checkpoint blockade are some of the most studied emerging technologies in cancer research, and Rapa is also already being implemented clinically as a route to suppress the excess metabolic and proliferative function of cancer cells [17–19]. Newer pre-clinical studies are exploring modulation of low-doses of Rapa and other drugs to polarize T cell function during cancer vaccination in melanoma, glioblastoma, and thymoma models [20–23]. However, inducing T_{CM} in animals or people with Rapa or other drugs is hindered by short half-lives ($t_{1/2}$ = 1–2 h), the need for frequent injections (1–5×/day), drug hydrophobicity, and the challenge of co-delivering drug cues with other vaccine components to target immune tissues such as lymph nodes (LNs), where tumor-specific T_{CM} are primed by antigen-presenting cells (APCs) [24]. Overcoming these hurdles might enable cancer vaccines that achieve adoptive transfer-like potency without need for isolation, expansion, and reinfusion of cells from a patient.

In LNs, APCs process and present antigens to activate T and B cells. Thus, vaccines must reach LNs to generate antigen-specific responses. Importantly, the specific type of response depends intimately on the soluble factors and surface molecules encountered by immune cells during antigen presentation. This characteristic has created enormous interest in harnessing biomaterials for vaccines against disease and cancer immunotherapies by targeting LNs, controlling delivery kinetics, and minimizing toxicity [25–28]. In this report, we combined common candidate vaccine formulations with polymer particles that slowly release minute doses of Rapa to test if this immunomodulator enhances immune response. We hypothesized that controlled release of low doses of Rapa during vaccination with peptide antigens and molecular adjuvants would moderate co-stimulation by APCs, alter cytokine secretion, and polarize differentiating T cells toward T_{CM} that enhance the size and quality of vaccine response. We show that release of Rapa from polymer depots during vaccination preserves plasticity in differentiating T cells, allowing generation of antigen-specific T cells, but biasing these populations toward a central memory phenotype that enhances response in primary cell co-culture models and in mice.

2. Materials and methods

2.1. Peptides

Trp2 peptide (SVYDFFVWL) formulated as a disodium salt and MOG peptide (MEVGWYRSPFSRVVHLYRNGK) were synthesized by Genscript.

2.2. Particle synthesis and characterization

PLGA microparticles were synthesized using a double emulsion/solvent evaporation method previously described [26]. Briefly, an organic phase consisting of 80 mg of PLGA (Durect) dissolved in 5 mL dichloromethane was prepared. In samples encapsulating Rapa, polymer solution was added to a vial containing 2 mg of dried drug (LC Laboratories). A primary emulsion was formed by adding 500 μL of water with the organic phase followed by sonication for 30 s at 12 W. This primary emulsion was then added to 40 mL of 2% *w*/*v* polyvinyl alcohol (PVA, Sigma) and homogenized for 3 min at 16,000 rpm. Excess organic solvent was evaporated overnight under stirring. Particle formulations were passed through a 40 μm cell strainer and collected via centrifugation (5000*g*, 5 min, 4 °C). Particles were then washed three times in in 1 mL water, with centrifugation steps (5000*g*, 5 min, 4 °C) to collect particles in between, and resuspended in water. Particle diameter was determined using laser diffraction (Horiba LA-950). Yield was quantified by drying a known volume of particle suspensions under air, recording the weight, and back-calculating a total mass yield for each batch. To quantify Rapa loading, known masses of dried particles were dissolved in DMSO, and the absorbance at 278 nm was determined using UV/VIS spectrophotometry. Absorbance values were compared with a standard curve of known concentrations of Rapa to calculate drug loading per mass of particles.

2.3. Dendritic cell uptake, activation and cytokine secretion

$CD11c^+$ cells were isolated from the spleens of 4–6 week old C57BL/6 mice (The Jackson Laboratory) using magnetic isolation according to the manufacturer's protocol (Miltenyi). Cells were plated in 96 well plates at 10^5 cells/well for uptake and activation studies, or at 2.5×10^5 cells/well for studies to analyze cytokine secretion, in RPMI 1640 media (Lonza), supplemented with 10% fetal bovine serum (Corning), 2 mM L-glutamine (Gibco), 55 μM β-mercaptoethanol (Sigma-Aldrich), 1× non-essential amino acids (Fisher Scientific), 10 mM HEPES (Fisher Scientific), and 1× Penn/Strep (Gibco). With the exception of indicated control wells, cells were stimulated with lipopolysaccharide (LPS) (Invitrogen) at 1 μg/mL; cells were treated with decreasing doses of soluble Rapa, matched-doses of encapsulated Rapa (Rapa MPs), or equivalent masses of empty MPs as a control. Soluble Rapa was dissolved in DMSO, and the final v/v% of DMSO in the well was 0.2%. After 4, 18, or 48 h of culture, cells were collected for analysis by flow cytometry and supernatants were collected for analysis of IL-6, IL-12p70 and IFNγ concentrations by ELISA (BD Biosciences). Briefly, cells were collected, washed in FACS buffer (1% bovine serum albumin in 1× PBS), and either resuspended in 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI) for analysis of particle uptake by flow cytometry, or blocked with anti-CD16/CD32 (BD Biosciences). After blocking, cells were then stained with anti-CD40, anti-CD80, and anti-CD86 (BD Biosciences) for 20 min at room temperature, washed two more times as above, and resuspended in DAPI for analysis by flow cytometry. All flow cytometry data was collected on a Canto II (BD Biosciences) and analyzed using Flowjo software (Tree Star). In studies to analyze particle uptake by microscopy, $CD11c^+$ cells were isolated, as above, and 1×10^6 cells were plated in glass-bottom dishes with No. 1.5 thickness cover slips (MatTek). After 2 h of incubation of cells with fluorescent particles, cells were washed twice with PBS, fixed in 4% paraformaldehyde, and washed with PBS and additional three times. Fixed cells were stained with Wheat Germ Agglutinin, Texas Red-X Conjugate (Thermo Fisher) at 5 μg/mL for 10 min, washed twice with PBS, and resuspended in Hoescht (Thermo Fisher) at 4 μg/mL for imaging.

2.4. Transgenic T cell co-culture studies

In order to characterize the effects of Rapa MPs on CD4 and CD8 T cell responses, co-cultures consisting of wild type dendritic cells (DCs) and T cells from transgenic mice were performed. T cells were from 2D2 mice C57BL/6-Tg(Tcra2D2,Tcrb2D2)1Kuch/J (The Jackson Laboratory) which have $CD4^+$ T cell receptors specific for MOG, and from Trp2-clone 37 mice (National Cancer Institute, National Institutes of Health), which have $CD8^+$ T cell receptors specific for Trp2. Splenic DCs from C57BL/6 mice were first isolated and plated at 10^5 cells/well,