Induction of Regulatory T Cell Phenotypes for Applications in Multiple Sclerosis

Rebeca T. Stiepel[1], Sean Simpson[1], Christopher J. Genito[2], Eric M. Bachelder[1], Kristy M. Ainslie[1,2,3]

[**1**] Division of Pharmacoengineering and Molecular Pharmaceutics, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599 [**2**] Division of Microbiology & Immunology, University of North Carolina School of Medicine, Chapel Hill, NC 27599 [**3**] Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill and North Carolina State University, USA

Statement of Purpose: Multiple sclerosis (MS) is a debilitating autoimmune disorder characterized by the destruction of myelin in the central nervous system. Current treatments include symptom management and immune suppression, but there is no cure to date. In order to improve upon current treatment options, mechanisms of immune tolerance are being explored. In this work, microparticles (MPs) loaded with antigen and a tolerizing agent are utilized to passively target uptake by dendritic cells (DCs). Antigen presentation from tolerized DCs can then induce regulatory T cell phenotypes (e.g. FOXP3+ T-regs) to counteract self-reactive T cells in an antigen specific manner, offering potential to alleviate autoimmunity without blanket immunosuppression[1]. The goal of this work is to validate a strategy for the induction of T regulatory type 1 (TR1) cells for further applications in an MS model. As such, the induction of TR1 cells is evaluated *in vitro* and *in vivo* with OT-II mice.

Methods: To evaluate TR1 induction *in vitro*, bone marrow derived dendritic cells (BMDCs) were isolated from C57Bl/6 mice and treated with dexamethasone (DXM) for 24h. Treated BMDCs were then co-cultured with labeled CD4+ T cells isolated from OT-II mice. Cells were co-cultured for 4 days in the presence of cognate antigen, ovalbumin MHC-II peptide ($OVA_{323-339}$), then stained for flow cytometry. To evaluate TR1 induction *in vivo*, MPs were made with acetalated dextran (Ace-DEX). Ace-DEX was synthesized according to the established protocol[2], and MPs were made via spray drying. Blank MPs were made as well as MPs loaded with 1% wt/wt DXM and 1% wt/wt $OVA_{323-339}$. On day 0 and day 2, MPs were administered subcutaneously to OT-II mice. One group received blank MPs as a control, and the other group received OVA/DXM MPs at a dose of 20µg DXM and 10µg $OVA_{323-339}$. On day 9, spleens, inguinal lymph nodes (LNs), and perigonadal fat (fat) were harvested and processed for flow cytometry. For each tissue, unstimulated cells were prepared as well as cells stimulated with phorbol myristate acetate (PMA) and ionomycin.

Results: Increasing DXM dose for BMDCs subsequently results in higher expression of IL-10 in CD4+ T cells, consistent with a TR1 phenotype (Fig. 1). After OVA/DXM MP treatment in OT-II mice, a higher count of dead T cells was observed in the spleen, and an increased frequency of IL-10 producing CD4+ T cells was observed in the spleen and fat (Fig. 2).

Conclusions: BMDCs tolerized with DXM appear to induce TR1 cells. Similarly, treatment with MPs loaded with DXM and $OVA_{323-339}$ resulted in increased frequency of CD4+ T cells expressing IL-10, indicating Ace-DEX MPs can effectively deliver DXM and antigen *in vivo* to achieve a higher proportion of regulatory T cell phenotypes. Future work is planned to further optimize induction of TR1s in OT-II mice and to evaluate the clinical benefit of DXM and antigen loaded MPs in a mouse model for MS.

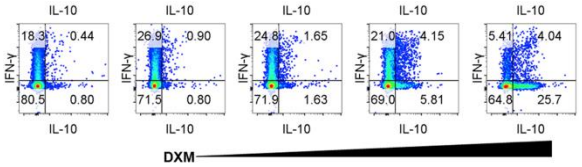

Figure 1: Measurement of IL-10 and IFN-γ in CD4+ T cells after co-culture with DXM-treated BMDCs. DXM doses left to right are as follows: no treatment, 0.1, 1, 10, and 100µM.

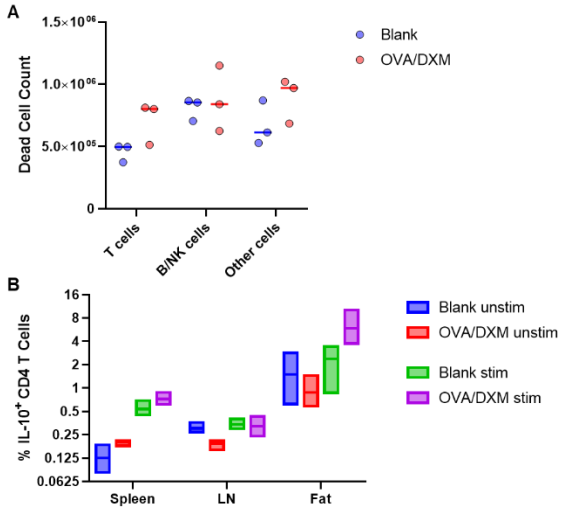

Figure 2: A) Dead cell counts from OT-II mouse spleens. B) Percentage of CD4+ T cells expressing IL-10 isolated from OT-II mouse spleens, inguinal LNs, and perigonadal fat on day 9 after the first MP dose. Blank and OVA/DXM refer to MP groups. Unstim and stim refers to unstimulated cells and cells stimulated with PMA and ionomycin, respectively.

References: [**1**] Cifuentes-Rius A, et al. *Nature Nanotechnology* (2021), 16, 37–46 [**2**] Kauffman KJ, et al. *ACS Appl Mater Interfaces* (2012), 4 (8), 4149–4155.