Intra-lymph node delivery of biomaterial depots prevents and reverses Experimental Autoimmune Encephalomyelitis

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Statement of Purpose: Recent studies have explored the potential of therapeutic vaccination to control the chronic attack of "self" molecules that occurs in autoimmune diseases, such as multiple sclerosis (MS). Importantly, these studies have highlighted the critical link between the combinations and delivery kinetics of signals (e.g., self antigen, regulatory drugs or cytokines) and the immune response elicited. Most of these studies employed systemic delivery of soluble factors, necessitating frequent dosing to generate therapeutic effects. Several recent approaches have focused on targeting lymph nodes (LNs), the tissues that coordinate immune function. In LNs, T cells are activated and differentiated in response to a cognate antigen, and the cues received during these processes impact how T cells develop. Thus, locally modulating T cell phenotype in LNs during differentiation could polarize systemic immune response away from inflammatory populations and toward beneficial regulatory T cells (T_{REGS}). We proposed an approach based on intra-lymph node controlled release from depots co-loaded with a hallmark autoantigen of MS (myelin peptide, MOG) and a drug known to promote T_{REG} development, rapamycin (Rapa). We hypothesize that targeted delivery of these depots in LNs could promote myelin-specific T_{REGS} that control autoimmunity in mouse models of MS, without the broad suppression of immune function associated with many current MS therapies.

Methods: An emulsion/solvent evaporation technique was used to generate biodegradable, poly(lactide-co-galactide) microparticles encapsulating MOG and/or Rapa. Particle size, as well as cargo loading and release kinetics were quantified by laser diffraction and UV/Vis spectrophotometry, respectively. Fluorescently-labeled depots were incubated with CD11c+ dendritic cells (DCs) isolated from the spleens of mice and used to assess particle uptake by microscopy and flow cytometry (FACS). *In vivo* studies were conducted by inducing mice with experimental autoimmune encephalomyelitis (EAE), a mouse model of progressive MS. Mice then received a single treatment of various depot formulations either i) one day prior to the typical onset of clinical symptoms (i.e., progressive paralysis) to test the potential of depots to *halt* the progression of EAE, or ii) at the approximate peak of disease to investigate whether depots *reverse* established disease (i.e., reverse paralysis).

Results: Microparticles were readily synthesized with control over the loading of MOG and/or Rapa depending on cargo input. *In vitro* testing demonstrated that depots were phagocytosed by primary antigen presenting cells (**Fig. 1A**). Early stage treatment with particles co-encapsulating MOG and Rapa (MOG/Rapa depots) resulted in a significantly decreased clinical EAE score (**Fig. 1B**) and a reduced incidence of disease, with only 50% of mice developing symptoms of paralysis,

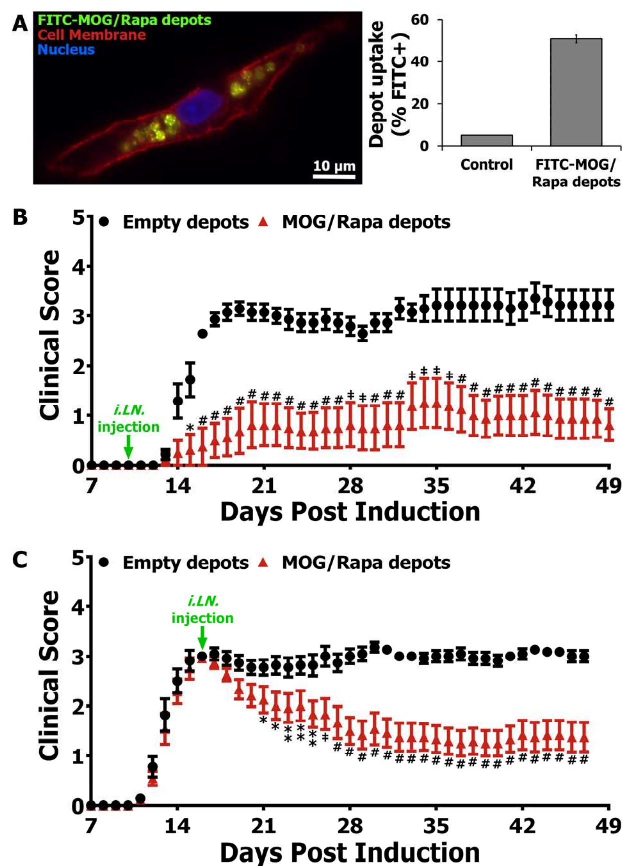

Figure 1: A) Depot uptake by primary DCs was verified by microscopy (left) and flow cytometry (right). A single dose of MOG/Rapa depots, administered *i.LN.*, was able to halt (B) or reverse (C) EAE in mice. (*$p \leq 0.05$, **$p \leq 0.01$, ‡$p < 0.001$, #$p \leq 0.0001$).

compared to 100% in the vehicle control group. When administered to mice with established EAE, MOG/Rapa depots reduced mean clinical score from 3.0 on the day of treatment to 1.4 at day 47 of the study (ongoing). Conversely, mice treated with Empty depots had a mean score of 3.0 at the time of treatment and at day 47 (**Fig. 1C**). Functionally, MOG/Rapa depots restored partial or full motor function to the hind limbs of mice, reversing disease-induced paralysis.

Conclusions: A single dose of MOG/Rapa depots, injected directly to the inguinal LNs of mice, was able to halt or reverse EAE. Ongoing studies aim to identify the mechanism of action, including testing depot effect on DC activation and T cell phenotype (e.g., T_{REGS}, T_H17) *in vitro*. Future *in vivo* studies will test immune cell frequency and phenotype, particularly in the LN microenvironment, via FACS and immunohistochemistry. Finally, we aim to test the antigen specificity of this tolerogenic effect to evaluate the potential to control autoimmunity without broad immunocompromise.