Screening and Co-Delivery of Tolerogenic Adjuvants and Antigen for the Creation of Antigen-Specific Immunotherapy for Multiple Sclerosis
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Purpose
Autoimmune diseases result from the breakdown of immune tolerance to self-antigens, which leads to the failure of the host’s immune response to distinguish self from non-self. Currently available therapies for autoimmunity, including multiple sclerosis (MS), often result in non-specific immunosuppression. The success of vaccines may hold the key to creating effective antigen-specific immunotherapy for autoimmune diseases. Vaccine dogma suggests that co-delivery of two signals; immunogenic antigen and immune stimulator (i.e. adjuvant), can promote the development of an antigen-specific immune response. We hypothesize that by co-delivering disease causing self-antigen and a tolerance inducing compound, or ‘tolerogenic adjuvant’, we can create an antigen-specific immunotherapy to treat autoimmunity. To this end, we systematically screened potential tolerogenic adjuvants in vitro using a relevant murine model of MS and formulated an emulsion system to co-deliver both antigen and ‘tolerogenic adjuvant’.

Methods
Using a murine model of MS, experimental autoimmune encephalomyelitis (EAE), induced with the myelin sheath associated self-antigen proteolipid protein peptide (PLP); antigen-specific splenocytes were obtained to screen potential tolerogenic adjuvants. The potential tolerogenic adjuvants, including dexamethasone, rapamycin, FK-506, propargylglycine, simvastatin, andrographolide, curcumin, acetylsalicylic acid, ibrutinib, and dimethyl fumarate were tested in the splenocytes system both with and without co-administration with self-antigen, PLP. Following treatment of the splenocytes for 120hrs, cell metabolic activity and levels of the cytokines TNF-α, IFN-γ, IL-2, and IL-10 were measured. Several promising treatments and informative controls were also analyzed for cell population changes by epi-fluorescence microscopy and flow cytometry. The most successful treatments were then formulated for co-delivery with PLP use an emulsion system. In particular, emulsions for co-delivery were created with incomplete freunds adjuvant (IFA), as IFA has previously been shown to have tolerogenic properties.

Results
Analysis of immune responses in EAE splenocytes has shown that this system can be used to effectively screen for antigen-specific responses to tolerogenic adjuvants. In vitro screening has also demonstrated that dexamethasone has promise as a potential tolerogenic adjuvant. Dexamethasone was found to decrease TNF-α production while simultaneously increasing IL-10 production as compared to other compounds tested. It was also shown by flow cytometry that co-delivery of PLP and dexamethasone result in a decrease in T-cell populations; which are believed to play a major role in EAE pathogenesis. Due to the promising results of dexamethasone in the screening assays, it has been formulated for co-delivery with the PLP antigen in IFA. Co-delivery in the IFA emulsions has been shown to result in an extended release of both components.

Conclusion
We have demonstrated that EAE splenocytes may be used to effectively test the immune response to potential antigen-specific immunotherapy for autoimmune disease. Our studies indicate that dexamethasone may be used as a tolerogenic adjuvant when co-delivered with antigen. An effective antigen-specific immunotherapy for autoimmunity will require the co-delivery of self-antigen and tolerogenic adjuvant for in vivo treatment. Initial studies have demonstrated that dexamethasone and PLP antigen can be successful formulated for co-delivery in an emulsion system. Future work will involve testing the co-delivery of tolerogenic adjuvant and antigen in vivo in the EAE model.