Controlled Release Formulations for Increasing Local Numbers of Regulatory T Cells

Siddharth Jhunjhunwala, Giorgio Raimondi, Erin Nichols, Stephen Thorne, Angus Thomson, Steven Little
University of Pittsburgh

Statement of Purpose: Currently, aberrant inflammation (responsible for autoimmune disease and transplant rejection) affects over 10 million people in the US. It is well documented that a potential treatment option for these diseases is to increase the presence of regulatory T cells (Treg) at local sites, which leads to reduced severity of immune responses [1]. At present, the only method of achieving increased local numbers of Treg is through systemic infusion *ex vivo* cultured cells (a method plagued with problems). Herein, we discuss the development and testing of 2 distinct (but inter-related) biomimetic formulations that can be used to dramatically increase local populations of Treg, with the potential to ameliorate autoimmunity and transplant rejection.

Methods: Controlled release formulations for various cytokines and drugs were prepared and characterized as described [2-4]. For the *in vivo* migration and tumor regression assays, live animal imaging was performed using the IVIS 200 (Caliper Life Sciences, Hopkinton, MA). For the in vitro Treg induction assay, naïve CD4+ cells were cultured in the presence of soluble and/or encapsulated factors and at specified time points, cells were extracted from culture for cell phenotype analysis using flow-cytometry.

Results: The first biomimetic formulation that we prepared was a microparticle based system for the release of CCL22. Recently, it has been demonstrated that one of the mechanisms tumors employ to evade the immune system is the recruitment of Treg through secretion of CCL22 [5]. We hypothesized that controlled release of this CCL22 from a transplant site would delay rejection through the recruitment of Treg. CCL22 was successfully encapsulated into degradable PLGA microparticles (CCL22MP), thereby achieving the initial goal of sustained, long term release of the chemokine (Fig. 1).

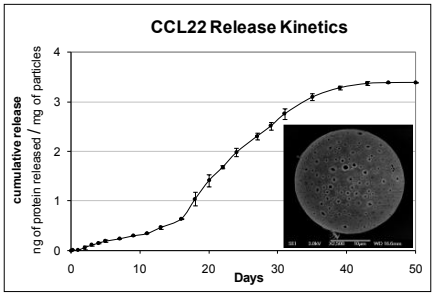

Figure 1. In vitro release kinetics of CCL22 from particles as measured in serum (10%) containing media. Release was measured in triplicates and error bars indicate standard error. Inset is a scanning electron micrograph of a single CCL22MP.

In order to test the ability of CCL22MP to recruit Treg in vivo, we used a novel *in vivo* migration assay based on tracking a given cell population using live animal imaging. Through this assay, we were able to determine that Treg migrated towards and co-localized specifically with CCL22MP and not control particles (Fig. 2a). Additionally, implantation of allogeneic cell transplants at the sites of particle injection significantly prolonged the survival of these cells (Fig. 2b).

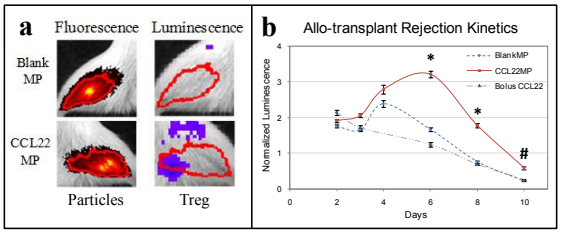

Figure 2. **a.** Live animal imaging demonstrating co-localization of Treg (lumiscence) with CCL22MP (fluorescence) but not control particles. **b.** Rejection kinetics of allogeneic cell transplants implanted at the site of particle injections (n≥ 10 for particles, and n=5 for bolus CCL22 groups). * - $p<0.05$ comparing CCL22MP to other groups, and # - $p<0.05$ comparing CCL22MP to BlankMP.

As another distinct strategy to increase local numbers of Treg, we have identified a specific set of factors to expand these cells as well as to induce conversion of naïve T cells to Treg. Tolerogenic antigen presenting cells are known to increase the numbers of Treg through effective antigen presentation in the presence of an immunosuppressive milieu. We propose to mimic the function of these cells using microparticles that can activate T cells while simultaneously releasing cytokines and drugs that maintain an immunosuppressive milieu. As a first step towards this, we have identified the exact combination of factors required for induction and expansion of Treg (Fig. 3). Each of these factors has previously been encapsulated in microparticles by us and others [2-4]. We are in the process of testing the efficacy of the factor-encapsulating formulations in aiding the induction and expansion of Treg *in vivo*.

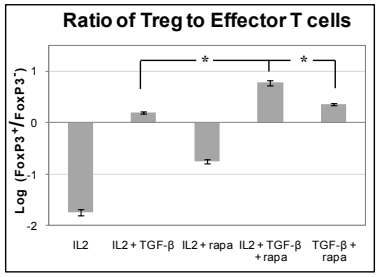

Figure 3. In vitro Treg induction and expansion in the presence of various factors. Treg measured as $FoxP3^+$ cells and effector T cells as $FoxP3^-$ cells. n≥3, * - $p<0.001$.

Conclusions: We have successfully fabricated, for the first time (to the best of our knowledge), chemokine releasing microparticles with the ability to attract Treg *in vivo*. Our preliminary results strongly suggest that these microparticles are capable of markedly prolonging the survival of transplants. Further, we have successfully identified a combination of factors that can significantly increase numbers of Treg and are in the process of testing the controlled release formulations for these factors. Each of these formulations, either independently or in combination, has the potential to act as therapeutic in a variety of autoimmune diseases and transplant rejection.

References: 1. Wing K. Nat. Immunol. 2010;11:7-13.
2. Jhunjhunwala S. J. Con. Rel. 2009;133:191-197.
3. Thomas TT. Pharm. Sci. 2004;93:1100-1109.
4. Defail AJ. Biomaterials. 2006;27:1579-1585.
5. Curiel TJ. Nat. Med. 2004;10:942-949.