**Homing Of Antigen-specific Engineered Regulatory T Cells to Human Pancreatic Islets**

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The therapeutic application of regulatory T cells (Tregs) for the treatment of autoimmune disorders, although efficacious, has been limited by the scarcity of antigen-specific Tregs. If antigen-specific Tregs, capable of reaching the desired autoimmune target, could be produced on demand, antigen-specific immune suppression of autoimmune diseases would be achievable. One approach to endow T cells with a desired antigen-specificity uses chimeric T cell antigen-receptors (CAR) with antibody-type specificity. Adoptive cell transfer therapies with CAR-re-directed cytotoxic T cells, have shown impressive efficacy in the treatment of hematologic malignancies. Accordingly, employing such technology to re-direct Tregs to sites of autoimmune attack may be a useful therapeutic approach to alleviate a broad spectrum of diseases in which uncontrolled auto and alloimmune responses play a major role. We recently developed pancreatic beta-cell, antigen-specific, CAR Tregs (1) and explored their therapeutic potential against T1D in our humanized mouse model (2). Ours is the first successful, antigen-specific CAR-Treg treatment of T1D in a humanized mouse model that closely resembles the human disease. Based on our mice data, we believe treatment with pancreatic beta-cell, antigen-specific CAR-Tregs will allow for recovery and reconstitution of beta cells in human T1D patients as well. The purpose of this study was to determine if antigen-specific human CAR Tregs could also identify target and home to human pancreatic islets in culture as they do in mice. The study involved drawing 10 cc of blood 1-2 weeks prior to pancreas surgery; followed by collection of a small piece of pancreas (5 cc wedge) once the pancreas was removed for a clinical indication (cancer, pancreatitis). We first isolated Tregs from peripheral blood of these human donors and expanded them in vitro. Treg cells were genetically modified to express either a beta-cell antigen-specific (GAD65) CAR or an irrelevant (EPCAM) CAR construct together with GFP marker. CAR Tregs were selectively expanded in the presence of rhGAD65 antigen. Once pancreas tissue became available, it was processed for islet separation using the collagenase method. Pancreatic islets were then co-cultured with syngeneic CAR Tregs for 7 days. IncuCyte S3 Live-cell System (Sartorius) is a real-time system that automatically acquires and analyzes HD, phase and fluorescent images of cell cultures, around the clock, for 7 days, while cells remain undisturbed. Live immunofluorescence microscopy demonstrated the distinct homing of GAD65 CAR Tregs to islets as compared to control EPCAM CAR Tregs as early as 24 hours of co-culture. Importantly, proliferation of GAD65 CAR Tregs was clearly demonstrated within 72 hours. GAD65 CAR Treg cytokine profile from co-culture supernatant was characteristic of activated Tregs. 

**Presentation Type**: Oral
**Presentation Date**: Monday, June 13
**Presentation Time**: 11 AM - 12:30 PM