

Title: OTII OVA-specific Tregs can be Induced to Traffic into the Mouse Eye and Modulate Experimental Autoimmune Uveoretinitis via Intravitreal Injection of Ovalbumin Peptide.

Purpose: Antigen specific T cell trafficking allows targeted delivery of immune modulating cell therapies into sites of ongoing immune dysregulation. Here we examine the effects of antigen specific Tregs on in vitro T cell proliferation and explore their use as a cell therapy in experimental autoimmune uveoretinitis (EAU), a mouse model of uveitis, to test the hypothesis that antigen specific Treg cells can be induced to suppress immune mediated inflammation.

Methods: CD4⁺ CD25⁺ Tregs were isolated using magnetic activated cell sorting from OTII mice with T-cell receptors specific for ovalbumin peptide (OVA). In-vitro proliferation assays were performed with varying concentrations of OTII Tregs co-cultured for 96 hours with a fixed number of irradiated splenocytes and CD4⁺ T cells (Tconv) in the presence of OVA. EAU was induced in CD45.2 C57/BL6 mice with RBP 629-643 peptide in addition to CFA and pertussis toxin was adoptively transferred into recipient C57/BL6 mice. On day 6 of adoptive transfer EAU induction, CD45.1 OTII Tregs were injected intraperitoneally into recipient C57/BL6 mice. Concurrently mice were also injected intravitreally with OVA or a vehicle control peptide (L144). OCT imaging was performed on day 8, 10, 12 to monitor inflammation. On day 13 flow cytometry analysis was performed for retinal and spleen samples stained for CD3, CD4, CD8, CD25, CD11b, CD45.1, CD45.2.

Results: In-vitro results identified increasing suppression of proliferation in response to higher ratios of Treg:Tconv cells with a 4-fold reduction in cell number at the highest concentrations of Tregs. Of eyes injected with OVA or L144, increased inflammation was evident on OCT imaging from day 8 onwards compared to eyes from mice induced with adoptive transfer EAU alone. Comparing inflammation scores of OCT images, OVA treated eyes manifested mild inflammation when compared to moderate inflammation in eyes treated with vehicle control peptide L144.

Conclusions: In addition to providing evidence of antigen specific OTII Treg suppression in vitro on T cell proliferation in the presence of OVA we show Treg driven reduction in inflammation in EAU in eyes receiving ovalbumin peptide versus control peptide alone. This provides evidence of antigen specific Treg as treatment of ocular inflammation.

