

PD-1⁺ melanocortin receptor dependent-Treg cells prevent autoimmune disease

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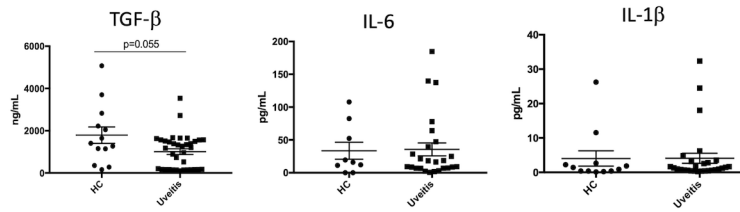
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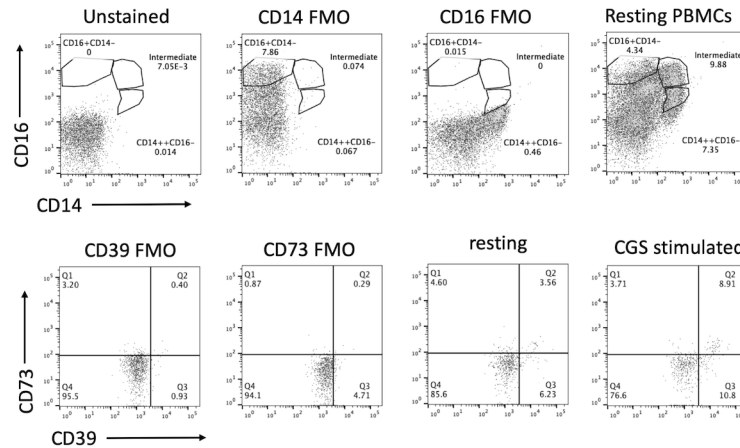
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Supplementary Materials



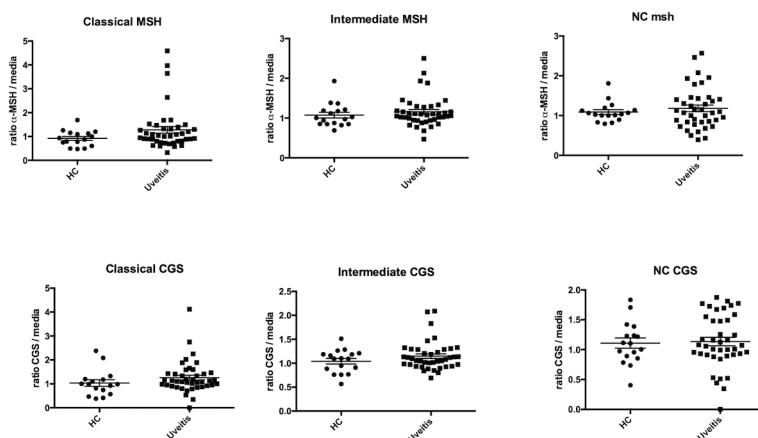
Supp Figure 1

Supplementary Figure 1. Cytokine profile in PBMCs from uveitis patients. PBMCs from uveitis patients or healthy donors were cultured for 48 hours in serum free media. After the 48-hour culture the supernatants were collected and cytokines were measured by Bioplex. The cytokines that were not significantly different between PBMCs from health controls (HC) and uveitis patients (uveitis) is shown. The regulatory cytokine TGF-β is shown HC (n = 13) and uveitis (n = 36), the proinflammatory cytokines IL-6 HC (n = 9) and uveitis (n = 25), and IL-1β HC (n = 12) and uveitis (n = 28) are shown. Significance was assessed by nonparametric Mann-Whitney U test.



Supp Figure 2

Supplementary Figure 2. Representative flow cytometry graph of PBMCs stained for CD14 and CD16. PBMCs from healthy donors were cultured for 48 hours in serum free media (SFM). Cells were stained for CD14 and CD16 and analyzed by flow cytometry. CD14 and CD16 expression was used to identify classical monocytes as CD14⁺CD16⁻, intermediate monocytes as CD14⁺CD16⁺, or non-classical monocytes as CD14⁻CD16⁺.



Supp Figure 3

Supplementary Figure 3. Monocyte distribution following melanocortin or A2Ar

stimulation. PBMCs were collected from healthy controls (controls) or uveitis patients (uveitis) and incubated in SFM for 48 hours. Cells were incubated with 1 ng/mL α -MSH (MSH) to stimulate the melanocortin receptors or cells were incubated with 1 mM CGS21680 (CGS) to stimulate A2Ar. The percentage of monocyte subtype, classical, intermediate, or non-classical (NC) was determined and because of variation from person to person, each treated sample for controls or patients were normalized against the untreated sample (media) for that same control or patient. Therefore, each panel shows the ratio of treated over untreated for controls (n = 16-17) and patients (n = 40-42) for each monocyte subtype as indicated for either α -MSH or CGS treatment. Significance was assessed by nonparametric Mann-Whitney U test.