Understanding the Impact of Antigen-Specific Regulatory T cells on Anergy Induction *in vivo*

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Abstract: In order to properly function, the immune system must achieve a fine balance between attacking foreign invaders while avoiding damage to healthy (self) tissue. One way it maintains this balance is through the induction of T cell anergy. Anergy is a key tolerance mechanism that renders self-reactive T cells hyporesponsive, thereby preventing the development of autoimmune diseases. T cell anergy is typically induced under steady state conditions when antigen recognition occurs without co-stimulation, such as in the absence of inflammation. Regulatory T (Treg) cell-mediated suppression of self-reactive T cells may contribute to anergy induction, but their specific role remains poorly defined. In this study, we investigate how antigen-specific Tregs influence the generation and accumulation of anergic CD8 T cells *in vivo*. To test this, we generated Tregs *ex vivo* from OVA-specific OT-II CD4 T cells. We injected OVA-specific OT-I CD8 T cells with or without OT-II Tregs into mice expressing OVA as a model self-antigen. After fourteen days, we observed an increase in the proportion of OT-I T cells in the spleen of mice co-adoptively transferred with OT-II Tregs as compared to mice injected with OT-I T cells alone. We confirmed, via *ex vivo* re-stimulation, that OT-I T cells induced in the presence of OT-II Tregs remained anergic. Interestingly, single-cell RNA-sequencing revealed that levels of antigen-specific Tregs correlate with transcriptional heterogeneity among anergic CD8 T cells *in vivo*. These findings suggest that antigen-specific Tregs promote the accumulation of anergic CD8 T cells. This work contributes to a broader effort to define mechanisms of peripheral tolerance, providing a foundation for their potential use in therapeutic interventions to prevent autoimmune disease.