

**Differential Regulation of
Activation and Differentiation Processes
in CD4⁺ T Cell Subsets
Revealed by Multidimensional Analysis**

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Alla BRADLEY

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Activation and Differentiation Processes
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Alla BRADLEY

ABSTRACT

T cells and B cells originate from the same common lymphoid precursor in the bone marrow. Unlike B cells, which complete their maturation process in the bone marrow, T cells leave the bone marrow and migrate to the thymus, where they undergo T cell lineage commitment and complete their maturation. CD4⁺ and CD8⁺ T cells comprise two major classes of T cells.

T cells recognise and respond to self- and foreign antigens using the T cell receptors (TCRs). In T cells, TCR signalling initiates downstream transcriptional mechanisms for T cell activation and differentiation. Activated T cells further differentiate into various effector T cell subsets, which mount a specific immune response towards the antigen. It is essential to control the magnitude of the ensuing immune response in order to prevent excessive inflammation and autoimmunity.

Negative regulatory mechanisms exist to control the activities of effector T cells. Key negative regulatory mechanisms include CTLA-4 and PD-1 signalling and Foxp3-mediated suppression of activated T cells. Foxp3-expressing regulatory T cells (Tregs) require TCR signals for their suppressive function and maintenance in the periphery. It is, however, unclear how TCR signalling controls the transcriptional programme of Tregs. Since most of studies identified the transcriptional features of Tregs in comparison to naïve T cells, the relationship between Tregs and non-naïve T cells

including memory-phenotype T cells (Tmem) and effector T cells (Teff) is not well understood.

The first part of the project aimed to dissect the transcriptomes of various T cell subsets from independent datasets using the multidimensional analysis method Canonical Correspondence Analysis (CCA). Firstly, I showed that at the cell population level, resting Tregs share gene modules for activation with Tmem and Teff. Importantly, Tmem activate the distinct transcriptional modules for T cell activation, which are uniquely repressed in Tregs. In addition, I analysed the T cell subset data and *Tcra* KO data, and showed that the activation phenotype of Tregs at the transcriptomic level was dependent on TCR signals, and was more actively operating in activated Tregs.

The second part of the project used a new CCA-based method, Single-Cell Combinatorial CCA (SC4A), and thereby analysed unannotated single cell RNA-seq data from tumour-infiltrating T cells. This analysis revealed that *FOXP3* expression occurs predominantly in activated T cells. Further analyses identified FOXP3-driven and T follicular helper (Tfh)-like differentiation pathways in tumour microenvironments, and their bifurcation point, which was enriched with recently activated T cells.

In conclusion, the project showed that the activation mechanisms downstream of TCR signals are operating in resting Tregs and activated Tregs, and also identified the bifurcation of Tregs and Teff differentiation and their maturation processes by single cell analysis

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