EE282

Project Analysis Proposal Revision

9-19-25

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Topic: Immunology

Analysis type: Microarray based profiling of differential gene expression between Conventional and Regulatory CD4 T-cells in spleen and CNS tissues across homeostatic and inflammatory states.

Dataset from National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO)

Background

Myelin is the structural component of neuron sheaths that facilitate signal transduction in the central nervous system. The auto immune disorder multiple sclerosis (MS) results from an initial failure of the adaptive immune response to distinguish self from non-self, where native myelin antigens inappropriately trigger immune response meant for foreign antigens.

For those afflicted, the resulting damage to myelinated neurons can lead to a multitude of sensorimotor, vision and cognitive impairments.

The role of T regulatory cells (Tregs) in MS is unique and complex. While autoimmune inflammation is initiated by failure of Treg mediated self-tolerance, paradoxically, it is the performance of a distinct emergency responder population of Tregs that determines disease progression or remission. As such, the gatekeepers of immune tolerance can remediate their own initial failure. The key to such remediation is the plasticity of the adaptive immune response.

The plurality of strategies Tregs employ to resolve the inflammatory response consists of a shared core repertoire secreted (IL-10, TGF-β) or contact mediated (CTLA-4, checkpoint inhibition) factors that appear to vary by inflammation source and affected physiological niche[1,2]. This highlights the necessity of delineating which transcriptional and functional changes lead to differential outcomes of Tregs in resolving autoimmune neuroinflammation.

Multiple in vivo studies implicate a distinct phenotype shared by Tregs most effective in resolving CNS inflammation.

My rotation project focuses on characterizing Treg mediated immune regulation in the murine homologue of MS, Experimental Autoimmune Encephalitis (EAE). This includes leveraging the deep tissue penetrative capacities of multiphoton imaging to construct a spatiotemporal profile of Treg migration and infiltration into CNS tissues in situ, and flow cytometry-based phenotyping to comprehensively census relative proportions of pro and anti-inflammatory immune cells present in the inflamed CNS at defined time points throughout the disease course.

An apt complement to these approaches lies in profiling the transcriptomic changes that occur in CD4+ populations that give rise to competent emergency responder CD4+ Treg populations.

Thus, an optimal survey of genes differentially expressed between Naïve and CNS Treg populations integrates and leverages spatiotemporal, phenotypic *and* genomic characterization of anti-inflammatory Tregs active in the CNS and is essential to understanding the phenomenology that gives rise to emergence of CNS specific anti-inflammatory Tregs. Proximally, such characterizations hold great potential for development of targeted cell-based treatments for MS, while broadly, unraveling the temporal transcriptional dynamics in disease specific heterogeneity of Treg phenotypes may inform targeted treatment of other autoimmune disorders.

Project

My analysis will be of the RNA microarray sequencing dataset GSE164460:

Transcriptome analysis of Tregs (CD4+Foxp3+) and Tcons (CD4+Foxp3-) from spleen and CNS of naïve mice and at the peak of EAE, uploaded to NCBI Gene Expression Omnibus and originally published by Pohar et al, in "Antigen receptor-engineered Tregs inhibit CNS autoimmunity in cell therapy using nonredundant immune mechanisms in mice."

Severity and recovery outcomes in auto immune inflammatory states such as MS and EAE are governed by relative proportion and function of regulatory and conventional CD4+ T-cells. In order to comprehensively capture extant and emergent phenotypes, Pohar et.al examine differential gene expression in 1) homeostasis and CNS inflammation and 2) in each respective disease state, between spleen and CNS tissues.

Comparison of genes differentially expressed between normal and EAE Treg transcriptomes aligns with and enriches our lab's goal in the comprehensive characterization of the immune regulation capacities of Tregs in the context of auto immune neuroinflammation.

Using a series of tools from RStudio and the dataset from the Pohar et al study, I will perform analyses of differentially expressed Treg genes between normal and EAE inflamed CNS tissues in an exercise intended to practice microarray data analyses and independently test the replicability of the original study's findings.

I will then narrow the focus of my analyses on quantifying the 5 most differentially expressed Treg genes. Of particular interest to me are expression of genes for canonical Treg immunosuppressive cytokines *IL-10* and *TGF-β*, and tissue repair promoting factor *amphiregulin*. I will then corroborate this list with published Treg transcriptomic profiles found in literature facilitate distillation of the most indispensable components of competent Tregs and will contribute to the robustness of our lab's ongoing profile of their role in regulating auto immune neuroinflammation.

Method

I will download software packages required for R analysis of microarray data from Affymetrix gene chip mouse platform (mouse 4302) including "GEOquery" "affy", and "oligo" from Bioconductor.

Data "unpacking" and "cleaning" will include unpacking .tar files into .CEL format ("Untar") and data quality control will include normalization and background signal noise correction using Affymetrix native "Robust multichip average" (RMA) method.

I will analyze genes differentially expressed and perform gene set enrichment analyses (GSEA) between wild type and EAE Tregs in CNS tissues (brain and spinal cord) and spleen tissues at the peak of disease in R using the "limma" R package. ~~Variance between replicates will be shown in dispersion plot~~ and I will analyze the top 10 ~~and top 5~~ DEGs using volcano plot "ggplot2"/ "ggcorrplot" MA plot "ggmaplot" and PCA "FactoMineR" respectively.

Rationale

Many of the necessary software tools and applications are available as open-source freeware. A few of the proposed analyses (ggplot2) have been previously covered in class as part of homework 3.

Working with an extant data set with known outcomes provides adequate framework and context for me to explore, learn and practice procurement, manipulation and analysis of genomic data in the R studio environment, uncoupled from time and resource constraints of 1) acquisition of biological samples 2) operational expertise and instrument availability for microarray analyses.

Reference

1) Pohar, J., O'Connor, R., Manfroi, B., El-Behi, M., Jouneau, L., Boudinot, P., Bunse, M., Uckert, W., Luka, M., Ménager, M., Liblau, R., Anderton, S. M., & Fillatreau, S. (2022). Antigen receptor-engineered Tregs inhibit CNS autoimmunity in cell therapy using nonredundant immune mechanisms in mice. *European journal of immunology*, *52*(8), 1335–1349. https://doi.org/10.1002/eji.202249845
2) Ito, M., Komai, K., Mise-Omata, S. *et al.* Brain regulatory T cells suppress astrogliosis and potentiate neurological recovery. *Nature* **565**, 246–250 (2019). https://doi.org/10.1038/s41586-018-0824-5