Validation of Differential Gene Expression in Subtypes of CD4+ T Cells in Uveitis

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Alumni and Friends

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Introduction

- Uveitis represents a heterogenous group of diseases characterised by inflammation within the eye and is the cause of 10-15% of cases of blindness in high income countries[1].
- Non-infectious forms of uveitis are driven by immune processes and are often mediated by Th17 and Th1 subtypes of CD4+ T cells.
- Experimental autoimmune uveoretinitis (EAU) is a mouse model of human non-infectious uveitis that can be induced using active immunisation or adoptive transfer methods.
- A CD4+ T cell subtype called ThP can induce EAU in recipient mice and can then be isolated for mechanistic analysis after disease onset.

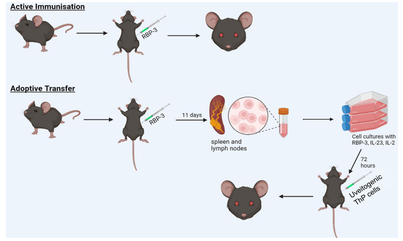


Figure 1: Methods of EAU Induction. Active immunisation involves intraperitoneal injection of immunising peptide RBP-3. Adoptive transfer involves producing ThP cell cultures from spleens and lymph nodes of actively immunised mice and injecting these cells into naïve recipient mice.

Naïve CD4+ T cells differentiate into Th1, Th17 and ThP subtypes which are implicated in EAU pathogenesis

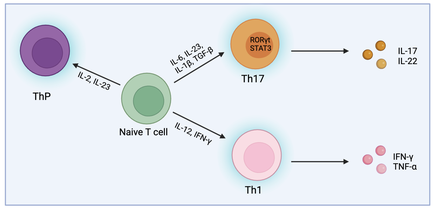


Figure 2: Differentiation of naïve T cells into Th1, Th17 and ThP subtypes. Naïve T cells encounter various cytokines which results in differentiation into Th1, Th17, or ThP subtypes that are known to drive EAU. ThP cells are are injected into recipient mice in adoptive transfer.

Hypothesis

Genes that are upregulated in pathogenic CD4+ T cells in EAU encode proteins that can be found on the cell surface of specific CD4+ T cell subtypes that drive disease and these proteins can therefore be considered as markers of pathogenicity.

Upregulation of *Cd226, Klrk1,* and *Tnfsf14* in pathogenic CD4+ ThP Cells from EAU mice

- 119 significantly upregulated genes were found in pathogenic CD4+ ThP cells harvested from eyes and spleen of EAU mice.
- *Cd226, Klrk1 and Tnfsf14* were chosen for further analysis because the proteins that they express (CD226, CD314 and TNFSF14) are implicated in autoimmunity.

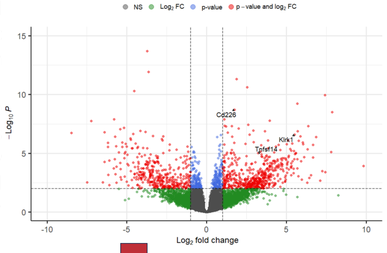


Figure 3: Volcano plot displaying genes in pathogenic CD4+ T cells compared to endogenous CD4+ T cells from mice with EAU induced by adoptive transfer. RNA-Seq data was analysed using differential gene expression analysis. Significant differential gene expression is shown in red. *Cd226*, *Klrk1* and *Tnfsf14* (log2FC: 1.79, 3.15 and 5.53. p<0.05.

Methodology for investigating genes of interest at the protein level

Flow cytometric analysis was performed to identify the proportion of CD4+ T cells that expressed CD226, CD314, or TNFSF14 *in vitro* and *in vivo.*

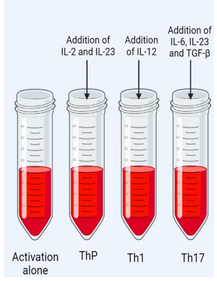


Figure 4: Splenocyte cell culture polarising conditions. CD4+ T cells from naïve mice were activated using anti-CD3/CD28. Cytokines were added to create Th1, Th17 and ThP polarising conditions.

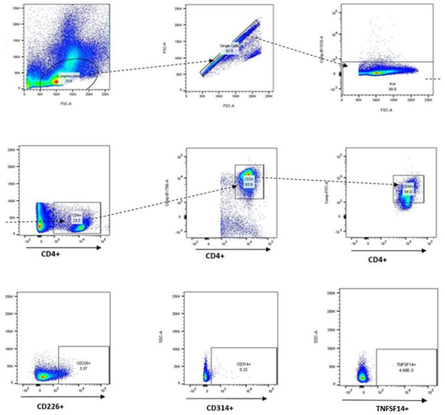


Figure 5: Flow cytometry gating strategy. Antibodies including antibodies to CD4, CD226, CD314 and TNFSF14 were used to stain samples. CD226, CD314 and TNFSF14 were gated in a viable, singlet CD4+ T cell population.

CD226, CD314, or TNFSF14 are found on the surface of CD4+ T cells under Th1, Th17 and ThP polarising conditions *in vitro*

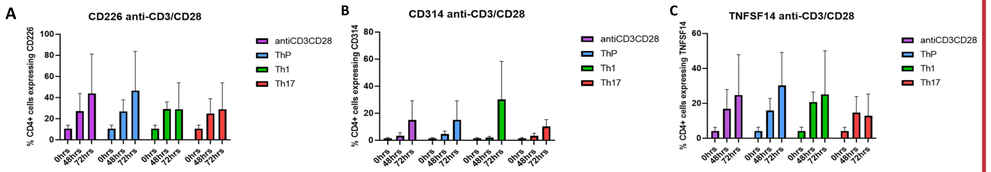


Figure 6: Increase in the average proportion of CD4+ T cells that express proteins of interest over time of differentiation. Samples were stained using antibodies to CD4, CD226, CD314 and TNFSF14 and analysed with flow cytometry at 0, 48 and 72 hours following addition of cytokines. T cells were activated using anti-CD3/CD28. Graphs show mean + SEM. Mixed effects analysis found statistically significant differences between time points in CD314 data. p<0.0.5.

Upregulation of the proportion of CD4+ T cells that express CD226, CD314, or TNFSF14 in active sites of disease *in vivo*

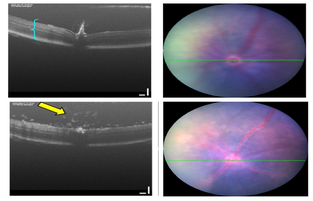


Figure 7: Naïve vs inflamed eye with EAU. OCT (LHS) and corresponding fundus photograph images (RHS) from the right eye of a B10 mouse post active immunisation.
A: Day 9, defined retinal layers (blue bracket) indicate lack of disease.
B: Day 11, white precipitates (yellow arrow) indicate severe disease.

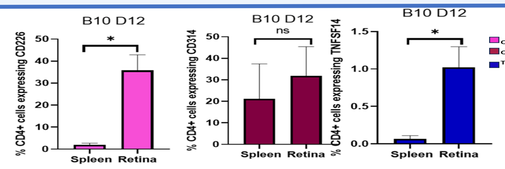


Figure 8: Average % of CD4+ T cells that express proteins of interest in retinas compared to spleen of B10 mice with EAU during active disease. EAU was induced using active immunisation. Retinas are active sites of disease; spleen controls were used. Graphs show mean + SEM. Statistical analysis: Mann Whitney U test. (n=4)*p<0.05. ** p<0.01.

Conclusions and Future Directions

- **Proteins (CD226, CD314, TNFSF14) encoded by upregulated genes in pathogenic CD4+ T cells in EAU mice can be considered markers of pathogenicity in uveitis.**
- **CD226, CD314 and TNFSF14 could have future use as prognostic markers in patients with uveitis.**
- **CD226, CD314 and TNFSF14 are known therapeutic targets, therefore future work could involve repurposing therapies that target these proteins for treatment of uveitis.**

References and Acknowledgements

1. Jolitov KA, Lobo-Chan A-M. Epidemiology and risk factors in non-infectious uveitis: a systematic review. Frontiers in Medicine. 2021:1541.