Does The Human Uvea Contain a Previously Uncharacterised Population of T Resident Memory Cells?

Ian R. Reekie, Lakshanie Wickramasinghe, Andrew D. Foers, Tom Thomas, Ananya Bhalla, Dylan Windell, Robert Hedley, Dave Copland, Colin Chu, Sarah Coupland, Andrew Dick, Srilakshmi Sharma, Jonathan Sherlock, Mark C. Coles, Christopher D. Buckley

1. Background

The eye is often cited as an immune privileged site, and it is widely believed that there are no cells of the lymphoid lineage within the globe (1). Resident memory T-cells (T-RM) are a subset of antigen-experienced T-lymphocytes which are widespread in bodily tissues (2). These cells play a key role in host defence and have also been implicated in disease states such as psoriasis.

Single cell RNA sequencing (scRNAseq) is a technique which allows the identification of cells by transcriptome at single cell resolution, and can be used to identify novel and rare cell populations within tissues of interest.

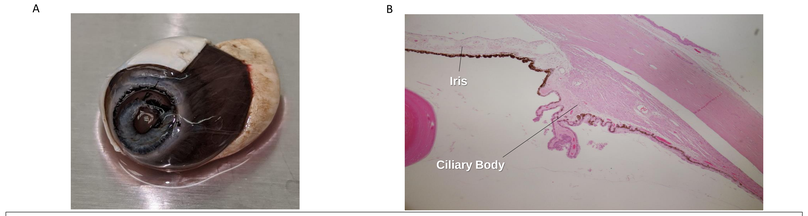


Figure 1:
A- Globe with sclera reflected to show the uveal tract.
B- Haematoxylin and eosin stained segment showing the iris and ciliary body of an adult human eye.

2. Objectives

- To develop a 'Cellular Atlas' of the healthy adult and fetal human uvea, showing the spatial organisation of bioinformatically defined cell populations.
- To determine whether the human uveal tract contains diverse populations of lymphocytes.

3. Methods

- Single cell RNA sequencing (10X Chromium) of enzymatically dissociated whole tissue specimens of adult and foetal uveal tract.
- Analysis of human uveal specimens by immunohistochemistry to spatially localise cell populations.
- Flow cytometry to delineate the lymphocyte populations of the uveal tract.

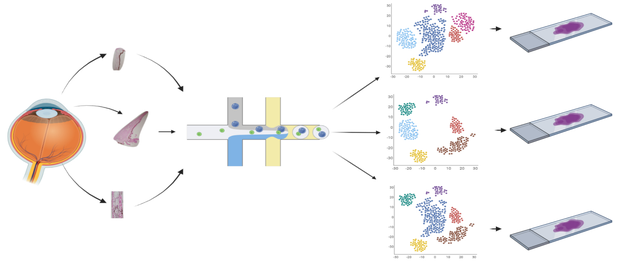


Figure 2
Schematic of experimental design.

4. Results: Single cell atlas of the adult human uvea demonstrates a distinct T-cell population

Single cell RNA sequencing of healthy adult iris, ciliary body and choroid tissue reveals cell type clusters defined by canonical markers. The largest clusters within each tissue are made up of stromal cells. Within each uveal tissue there is a distinct population of T-cells (CD3+).

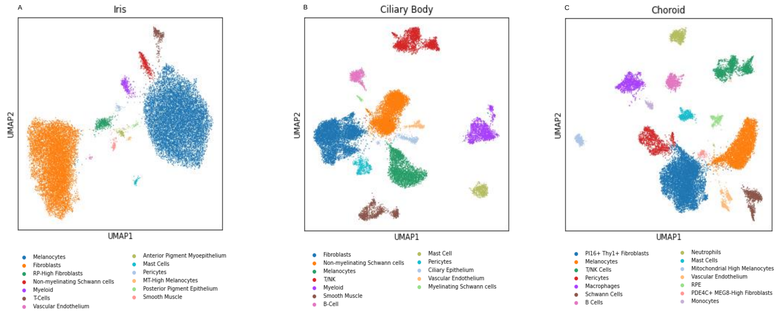


Figure 3: A,B and C - UMAPs of adult iris, ciliary body and choroid scRNAseq datasets, showing all cell types present within the tissues as identified by canonical makers. N=3 adult donors with no history of eye disease.

5. Results: Resident T cell populations of the uveal tract shown by flow cytometry

Flow cytometry allows identification of cell types by well-defined protein markers. Dissociated adult iris, ciliary body and choroid tissue demonstrated distinct T-cell populations. These were larger in the ciliary body and choroid than the iris. Many of these cells express CD69, a marker of T-RM status.

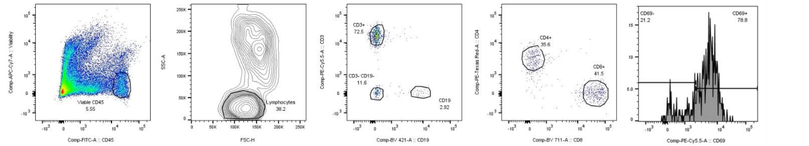


Figure 4: Representative flow cytometry plots demonstrating a substantial T-cell population within the human ciliary body. The majority are cytotoxic CD8+ T-cells. A large proportion of CD3+ T-cells are also CD69+. Representative of N=6 biological repeats.

6. Results: T cells within the ciliary body and choroid express T-RM markers

CD69 is a marker of T-RM status. The T-cell clusters of the ciliary body and choroid datasets also express several other markers of T-RM status such as CD69, CCL5, CD44, RUNX3 and PDRM1. Importantly, markers of naïve T-cell status such as SELL, S1PR1 and CCR7 are not highly expressed, nor are markers of T-cells exhaustion such as CTLA4 and PDCD1, supporting the hypothesis of T-RM identity.

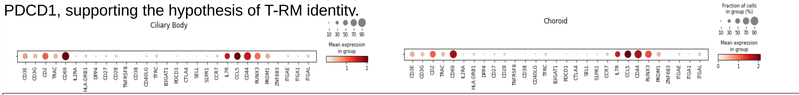


Figure 5: Dot-plots of key markers of T-cell identities in the ciliary body and choroid.

7. Results: T cells within the uveal tract are located outside of blood vessels

CD3+ CD69+ T cells can be localised within human uveal tissues by immunofluorescent staining. Importantly, they lie outside of blood vessels, marked by CD31 staining. This demonstrates that these T cells are not contaminants from the vasculature, and instead may have a role in tissue homeostasis and disease states.

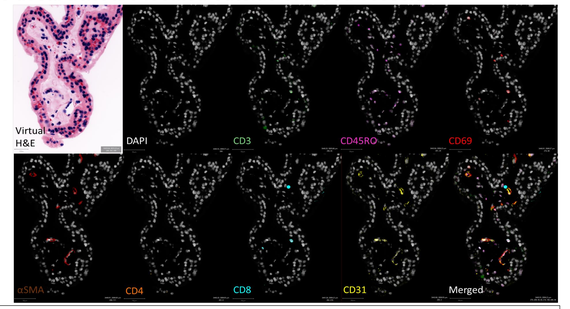


Figure 6: Immunofluorescence image of a ciliary process stained for T-RM markers and localising markers for blood vessels.

8. Results: T-RM cells are not detectable in human fetal uveal tract

In many tissues T-RM are thought to be seeded following antigenic challenge, e.g. following infection. Human fetal uveal tissue contain a substantial NK cell population, but few T-cells which do not express T-RM markers. This supports the hypothesis that these cells are generated in post-natal life.

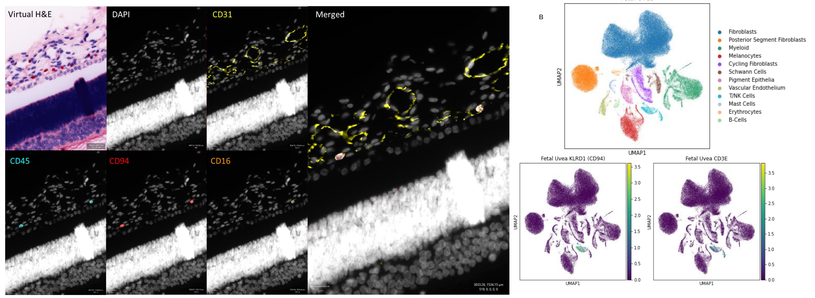


Figure 7: A - Immunofluorescence image of the fetal choroid and retina stained for NK cell markers and localising markers for blood vessels.
B – UMAPS of cluster annotations in the fetal uvea and markers of T-cell (CD3E) and NK cell (CD94) status.

9. Conclusions

- The human uveal tract contains previously uncharacterised population of tissue resident T cells.
- In the adult ciliary body and choroid, T cells express canonical markers of T-RM, including CD69.
- T cells with markers of T-RM are not seen in foetal tissues, supporting the hypothesis that these cells are seeded in adult life in response to immune challenge.

References
1. Forrester JV, Xu H. Good news-bad news: the Yin and Yang of immune privilege in the eye. Front Immunol. 2012;3:338.
2. Thome JJ, Farber DL. Emerging concepts in tissue-resident T cells: lessons from humans. Trends Immunol. 2015;36(7):428-35.

Disclosures
LW, ADF, AB and CDB received financial support from Janssen Pharmaceuticals
JS is an employee of Janssen Pharmaceuticals