Armed Sentinels of the Eye

Identifying conjunctival-resident memory T cells for eye-drop vaccines

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Background

The conjunctiva is a highly vascular membrane susceptible to pathogen entry[1]. It forms a part of the **ocular mucosa**[2] as it contains the **conjunctiva-associated lymphoid tissue (CALT)** that is well connected to all other lymphatic tissue within the body[3] (Figure 1).

Tissue resident memory T-cells (**TRMs**) found in the mucosa can be **reactivated substantially more rapidly** than other circulating memory T-cells and have an additional advantage of providing **continuous protection** to peripheral tissues[4]. This continuous protection can be utilised to advance research on **human eye-drop vaccines**. However, the presence of TRMs in the various layers of the human conjunctiva has not been established yet (figure 2).

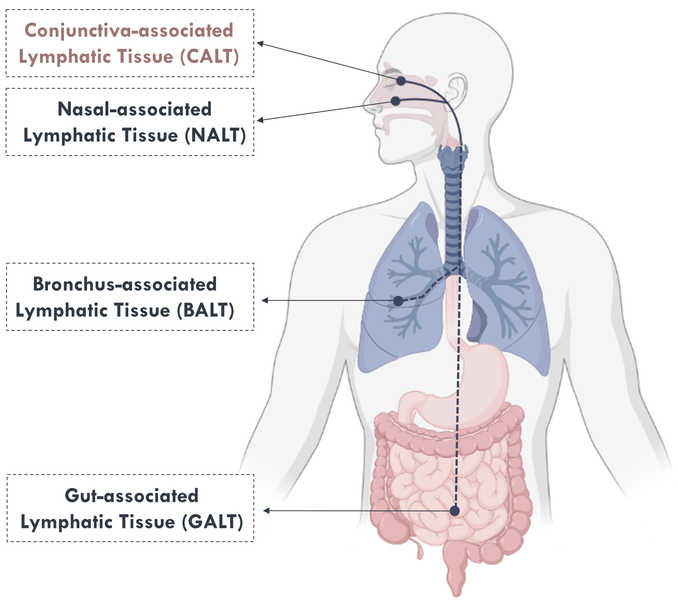


Figure 1. The conjunctiva-associated lymphatic tissue (CALT) is connected via circulating lymphatic cells to all the other lymphatic tissues of the body.

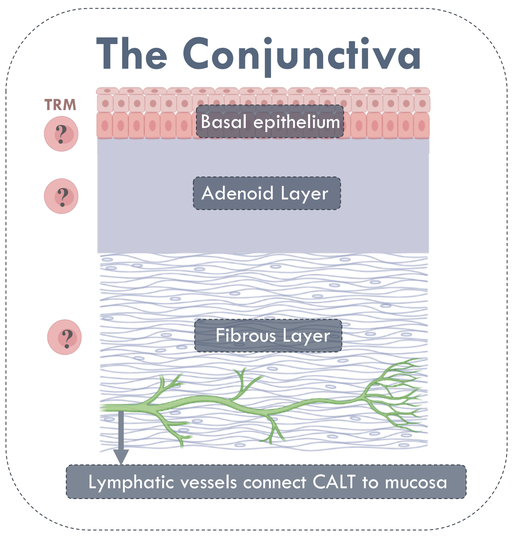


Figure 2. Histological layers of the conjunctiva of the eye. The presence of TRMs is yet unestablished in the basal epithelium and the adenoid and fibrous layers.

Aim

To determine the **localisation** of TRMs in healthy human conjunctivas **within the layers and relative to other immune cells** of the conjunctiva.

Hypothesis

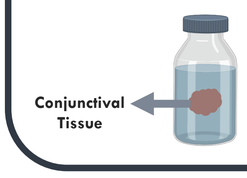
TRM subsets will be distributed in **all the layers** of the conjunctiva, **in close proximity to other immune cells,** similar to other mucosal tissues of the body.

Research Plan

Enrol and consent healthy cataract surgery patients n=17

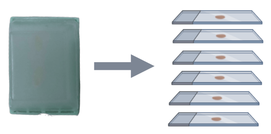
Tissue Collection

1. Optimise a collection method for the conjunctival tissue



Histology

2. Fix the collected tissue, embed using paraffin then section serially

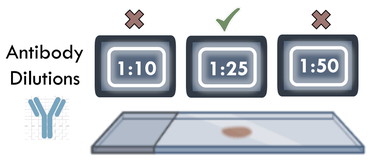


Immunofluorescent Microscopy

3. Design Immunofluorescent microscopy (IF) panels to identify TRMs

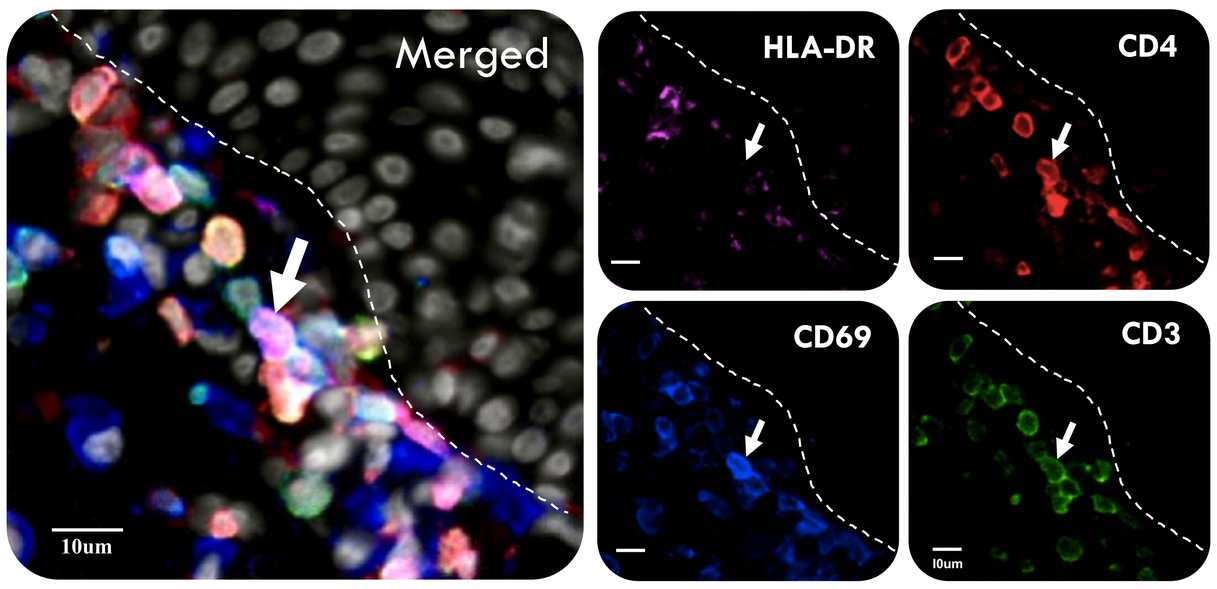
Channel	1	2	3	4	5	6
FITC	rCD4	rCD4	rCD69	rHLA-DR	rCD14	rCD69
TRITC	rCD3	rCD3	rCD3	rCD69	mDC-SIGN	rCD11c Biotinylated
Cy-5	rCD8	mCD56	mCD103	rCD4	-	gLangerin
Cy-7	rCD69	rCD69	rCD4	mCD3	-	mDC-SIGN

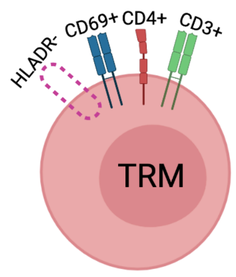
4. Titrate and optimise antibodies using IF and a positive control tissue (abdominal skin)



5. Image tissue sections using fluorescence microscopy and analyse using ImageJ

Result 1 — CD69+ TRMs were identified in all layers of the conjunctiva





Target cell	Marker
Myeloid	rHLA-DR
TRMs	rCD69
CD4+T-cell	rCD4
T-cells	mCD3

Figure 3. Optimised immunofluorescent staining panel for identifying CD69+ TRMs. Merged and separate channels are shown for the adenoid layer of a representative conjunctiva. The white arrow is indicating a HLA-DR-, CD69+, CD4+, CD3+ TRM cell. HLA-DR is used to exclude CD69+ myeloid cells. DAPI is shown in grey. Scale bar = 10um

HLADR- CD3+ CD69+ TRMs

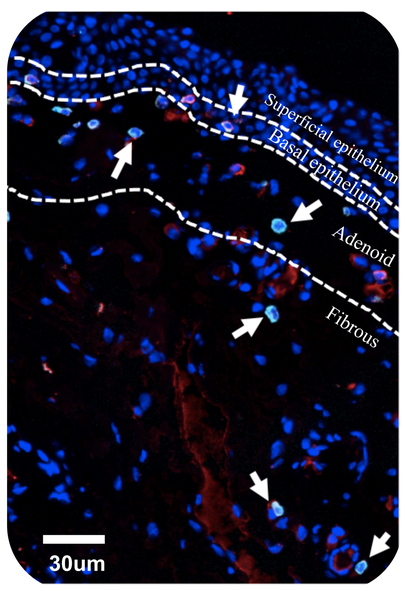
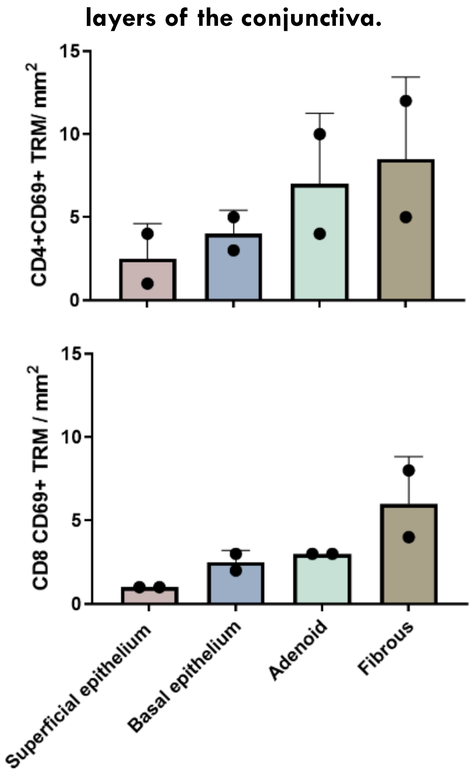
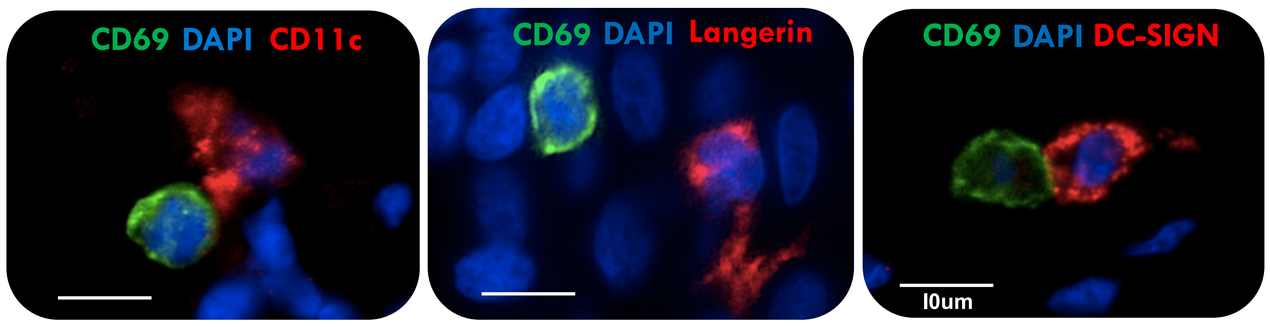


Figure 4. Immunofluorescent staining showing CD69+ TRMs (white arrows) in the various layers of the conjunctiva.

Figure 5. CD69+ TRM subsets increase in density in the deeper layers of the conjunctiva.



Result 2 — CD69+ TRMs are localised in close proximity to antigen presenting cells



Target cell	Marker
CD69+ TRMs	rCD69
Dendritic cell	Biotin CD11c
Langerhans	gLangerin
Macrophages	mDC-SIGN

Figure 6. IF staining using panel shown on the right of CD69+ TRMs are in close proximity to all antigen presenting cells.

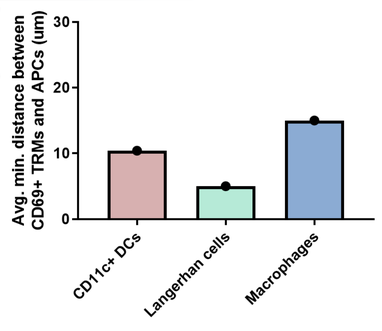


Figure 7. A large number of antigen presenting cells were situated in close proximity to TRMs (n=1)

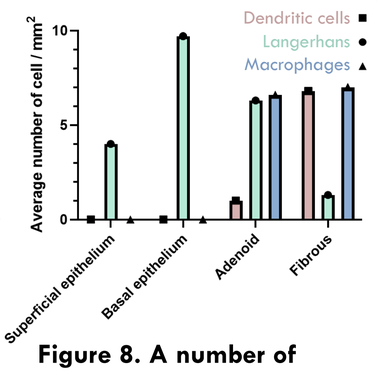


Figure 8. A number of antigen presenting cells populate in the various layers of the conjunctiva. (n=1)

Conclusions

- CD69+ TRMs were identified in **all conjunctival layers** displaying a graded distribution in their arrangement within the tissue increasing in density with tissue depth.
- CD69+ TRMs were frequently located in **close proximity to antigen presenting cells** of the conjunctiva. Hence, these cells rely on each other for pathogen clearance.

Significance

- Since TRMs are able to be established in all layers of the human conjunctiva, an **eye drop vaccines can establish** them there for protection against viral pathogens.
- Determining the localisation of TRMs relative to antigen presenting cells allows for further development of a **human conjunctival explant model** for **high throughput vaccine screening**. Example target pathogens include SARS-CoV-2.

Future Steps

- Establish the localisation of TRMs in the healthy human conjunctiva with respect to **B cell populations.**

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