# Characterising the immune response in Herpes Keratitis

Sana Arshad[1,2], Damien Hunter[1,2], Kirstie Bertram[2,3], Andrew White[1,2], Constantinos Petsoglou[2,4], Anthony Cunningham[2,3], Nicole Carnt[1,2,5]

[1]Centre for Vision Research, The Westmead Institute for Medical Research, Westmead, NSW, Australia [2]University of Sydney, Sydney, NSW, Australia [3]Centre for Virus Research, The Westmead Institute for Medical Research, Westmead, NSW, Australia, [4]NSW Tissue Banks, Sydney, NSW, Australia [5]School of Optometry and Vision Science, University of New South Wales, Kensington

## PURPOSE

- Herpes Keratitis is caused by herpes simplex virus and is the leading cause[1] of infectious-related blindness in developed countries[2].
- As there is no cure, a greater understanding of the limited knowledge on the immune response is necessary to develop better treatments.
- Our aim is to characterise the immune cell subsets in human herpes keratitis tissue, localise them and compare them to healthy control tissue

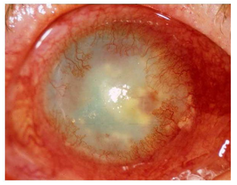
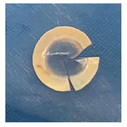


**Figure 1**. Slit lamp photograph of patient presenting with herpes keratitis in the stroma demonstrating corneal opacity[3].


## METHODS: Outline



**Tissue was collected from NSW Tissue Banks and Sydney Eye Hospital**

| | |
|---|---|
| HT | Healthy control tissue donated for research |
| RT | Corneal tissue from donors rejected for transplantation due to factors such as corneal ulceration or low endothelial cell count |
| HK | Herpes keratitis tissue from patients undergoing corneal transplant surgeries |

¾ of the sample's epithelium and stroma was enzymatically digested

Cells isolated were stained with fluorescently labelled antibodies

Acquisition on BD FACSymphony™

¼ of the sample was fixed, paraffin embedded and sectionsmT94bR8B

Section stained with fluorescent antibodies

Tissue is imaged on Olympus VS120

**Figure 2. Flow diagram of methods outline**

## METHODS: Flow Cytometry Gating Strategy

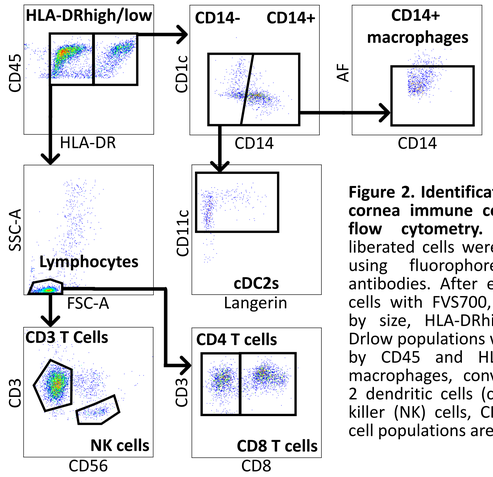



**Figure 2. Identification of human cornea immune cell subsets by flow cytometry.** Enzymatically liberated cells were identified by using fluorophore conjugated antibodies. After excluding dead cells with FVS700, and doublets by size, HLA-DRhigh and HLA-Drlow populations were identified by CD45 and HLA-DR. CD14+ macrophages, conventional type 2 dendritic cells (cDC2s), natural killer (NK) cells, CD3/CD4/CD8 T cell populations are shown.

## RESULTS: Immune cells cluster closely together in Herpes Keratitis tissue

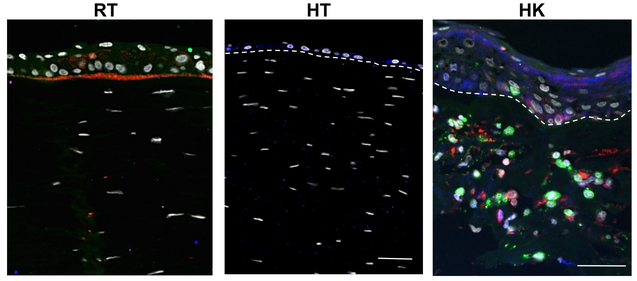



**Figure 5. Representative immunofluorescent images** of healthy tissue (HT), rejected for transplantation tissue (RT) and herpes keratitis tissue (HK). Scale bar = 50µm

## REFERENCES

[1]KOUJAH, L., SURYAWANSHI, R. K. & SHUKLA, D. 2019. Pathological processes activated by herpes simplex virus-1 (HSV-1) infection in the cornea. *Cell Mol Life Sci,* 76, 405-419.
[2]KARSTEN, E., WATSON, S. L. & FOSTER, L. J. 2012. Diversity of microbial species implicated in keratitis: a review. *Open Ophthalmol J,* 6, 110-24.
[8]ROWER, A., ST. LEGAR, A., JEON, S., DHALIWAL, D., KNICKELBEIN, J. & HENDRICKS, R. 2013. Herpes keratitis. *Progress in Retinal and Eye Research*, 32, 88-101.

## RESULTS: Epithelium of herpes keratitis tissue shows a decrease in myeloid cells and an increase in lymphocytes, most notably natural killer cells

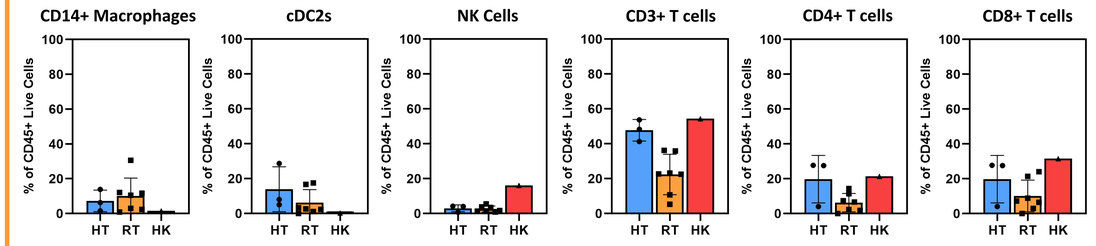



**Figure 3. Proportions** of myeloid cells (CD14+ macrophages and conventional type 2 dendritic cells (cDC2s) and lymphocytes (natural killer (NK) cells, CD3+, CD4+ and CD8+ T cells) in the epithelium of healthy tissue (HT), rejected for transplantation tissue (RT) and herpes keratitis tissue (HK).

## RESULTS: The stroma shows a decrease in myeloid cells and lymphocytes in herpes keratitis tissue compared to HT and RT controls.

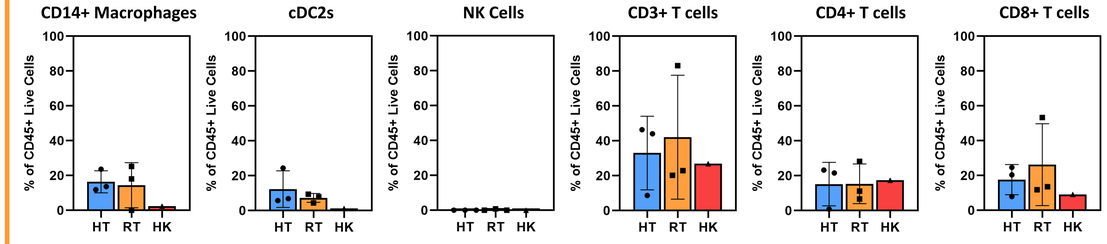



**Figure 4. Proportions** of myeloid cells (CD14+ macrophages and conventional type 2 dendritic cells (cDC2s) and lymphocytes (natural killer (NK) cells, CD3+, CD4+ and CD8+ T cells) in the stroma of healthy tissue (HT), rejected for transplantation tissue (RT) and herpes keratitis tissue (HK).

## RESULTS: NK cells constitute a large proportion of the lymphocyte infiltrate in Herpes Keratitis epithelium

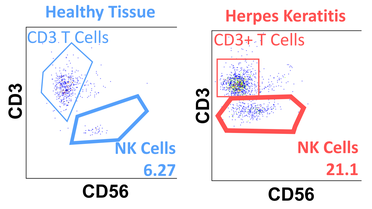



**Figure 5.** Representative flow cytometry plot shows populations of T cells (CD3+CD56-), and natural killer cells (NK) cells (CD3-CD56+) in healthy and herpes keratitis tissue.

## CONCLUSIONS

- Our interim results suggest that T cells and particularly natural killer cells have a key role in the inflammation seen in herpes of keratitis
- To confirm validate and confirm these results, we will continue to collect cases of herpes keratitis

**e**: sana.arshad@sydney.edu.au
For more, please scan the QR code