

Pump-Priming Grant

Report

Title Exploring CD1 Antigen Presentation Pathways: A Potential Therapeutic Target in Vascularised Composite Allograft Rejection

Objective

Vascularised composite allotransplantation (VCA), the transplantation of multiple tissue types as a functional unit, is a transformative technique with wide-ranging benefits in the field of reconstructive surgery. The predominate obstacle to the progress of the field remains the high rates of allograft rejection, far exceeding those observed in solid organ transplants.

The purpose of this study was to define the involvement of CD1 antigen presentation pathways in acute T cell mediated rejection of VCA. We aimed to explore how activation of this pathway correlates with local immune activation and severity of rejection, with a longer term vision that defining the detailed mechanisms of rejection will benefit both the transplantation and wider cutaneous pathology field.

Method

Thirteen skin biopsy samples from eight patients were profiled using a pre-designed 370 gene multi-tissue panel, supplemented with a 50 gene custom panel selected from a literature review and results from the GeoMx VCA study. Biopsies across rejection grades were selected to facilitate mechanistic understanding of changes across disease severity.

After processing, data quality parameters were assessed, including the median transcripts detected per cell, transcripts per 100um² and total high quality decoded transcripts. In total 13 samples encompassing 421,143 cells were captured for downstream quality control assessment and analysis. All samples were manually assessed for tissue integrity and transcript quality, with any underperforming areas excluded. Despite a variation in the age of the FFPE samples, a factor associated with RNA degradation and potential reduction in assay performance, no significant correlation was observed between sample age and median detected transcripts per cell, or total high-quality decoded transcripts.

Results

Single-cell spatial transcriptomics approaches provide single cell resolution without the need for sample dissociation, thereby preserving tissue architecture and spatial context to better understand immune responses in situ. To assess skin rejection mechanisms at single-cell resolution, a subset of biopsies from VCA recipients were analysed using the Xenium platform (10X Genomics). Samples were carefully selected to cover the spectrum of rejection phenotypes, ranging from stable function to high grade rejection. Borderline cases, where features cannot reliably be ascribed to rejection, were also included as these cases are typically challenging for both pathologists and clinicians.

Identifying and annotating cell types

Cells from all tissue samples were merged and empty cells expressing no unique genes removed. A larger sample containing significantly more cells was down sampled to 50,000 cells, resulting in a total of 217,860 cells being retained. After normalisation, dimensionality reduction through PCA then UMAP plotting aided in evaluating the influence of any technical factors on variance within the dataset.

Automated cell type annotation was conducted using an inflammatory skin reference atlas. Annotation performance was assessed against known canonical marker expression and accuracy of mapping to UMAP-identified neighbourhoods. Manual curation was used to refine the automated annotations and arrive at consensus annotations.

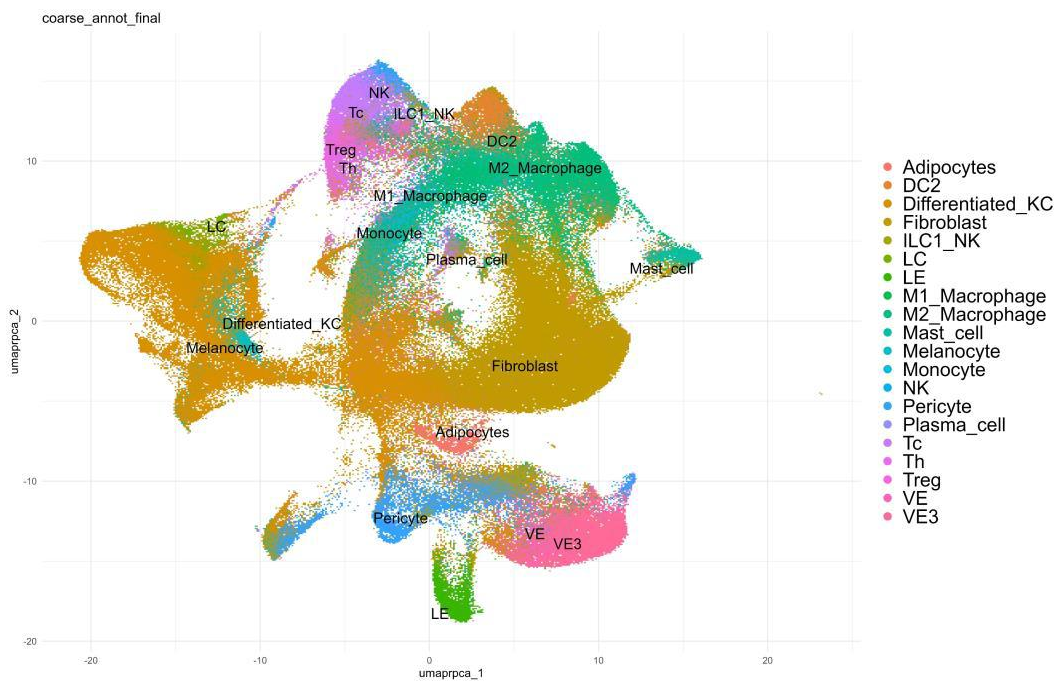


Fig. 1. Consensus cluster annotations. UMAP plot demonstrating consensus cell type annotations after automated annotation and manual curation.

Additionally, canonical marker expression of cell subtypes used to validate cluster annotations.

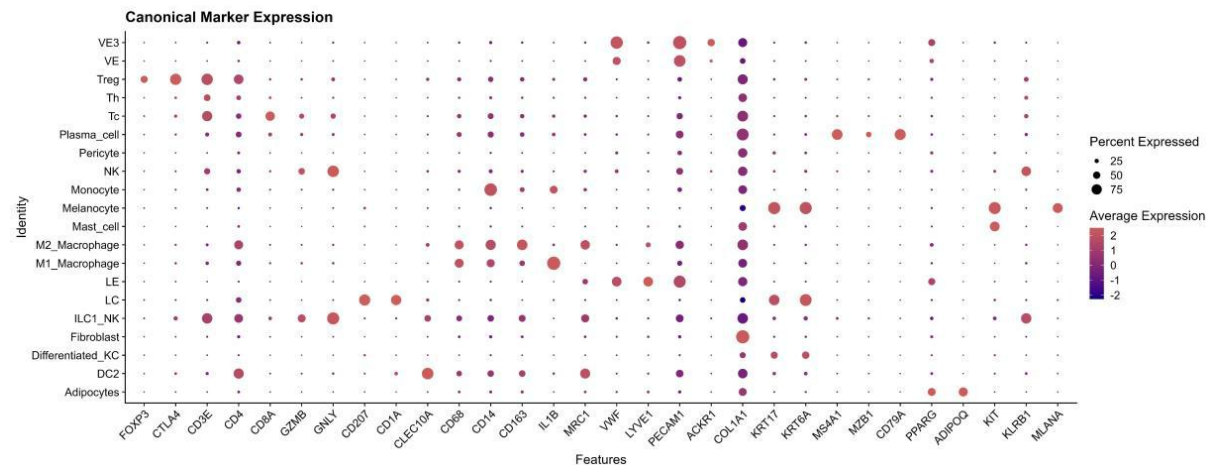


Fig. 2. Canonical marker expression of cell subtypes used to validate cluster annotations.

Comparison between sample groups

The composition of the samples between groups was subsequently compared to identify cell types associated with rejection states (Fig. 3A-B). Although not significant, a trend towards increased M2 macrophages and decreased Langerhans cells was observed with increasing rejection severity (Fig. 3C).

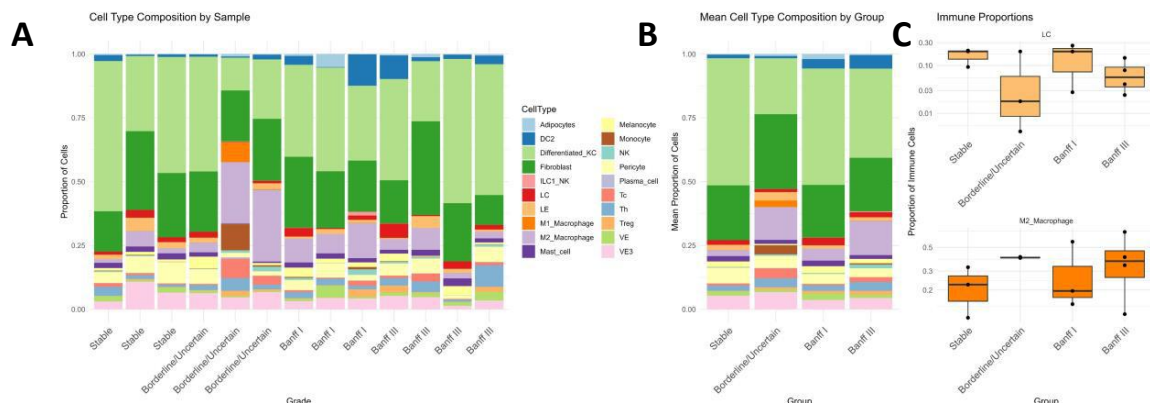


Fig. 3. Cell type composition for each sample, illustrating variability across rejection states (A). Mean cell type composition stratified by Banff grade (B). Macrophage and Langerhans cell density as a proportion of all immune cells across groups (C).

The data generated in this project provide an overview of the immunological landscape of VCA. The initial analysis did not reveal a direct link with CD1, further analysis will assess its link on a pathway level. Advanced analysis of the dataset is ongoing, with the aim of identifying significant cellular interactions during rejection that could be targeted to specifically disrupt the development of acute skin rejection. The key objectives of future analysis include a receptor-ligand analysis to identify the signalling networks driving rejection and characterisation of the spatial distribution of infiltrating immune cells within the tissue to determine whether any patterns are associated with rejection severity. Finally, cell-cell interactions will be mapped using a nearest-neighbour analysis to evaluate the influence of rejection on cell communication.

Outputs (publications/presentations)

1. Results obtained within this grant are included in Sarah Short DPhil thesis "A spatially resolved, in depth analysis of tissue signatures of tolerance and rejection in transplantation", University of Oxford, submitted Hillary term 2025
2. British Society of Immunology 2023 congress presentation "Characterising the immune niche of vascularised composite allografts and barrier organ transplants during stable function and active rejection."
3. International Transplantation Society Meeting (ITS) 2023 presentation and travel award "A spatially resolved, in depth analysis of tissue signatures of tolerance and rejection in transplantation"

4. The TTS congress 2024 oral presentation and mentor-mentee award “Spatial analysis of vascularised composite allografts reveals distinct transcriptional pathways and immune niche dynamics associated with rejection.”

Next Steps (what is it leading to)

This study represents the first of its kind to be completed in a cohort of skin transplant recipients and the results are being prepared for publication upon completion of a more advanced data analysis in collaboration with a biostatistician. Additionally, some of the results obtained with this grant will provide preliminary data for application for further funding, both by the TRIG group and by Sarah in Australia.