Providing new blood monitoring tools for the prognosis and prevention of transplant rejection

Understanding the role of cross- reactive antiviral T cells

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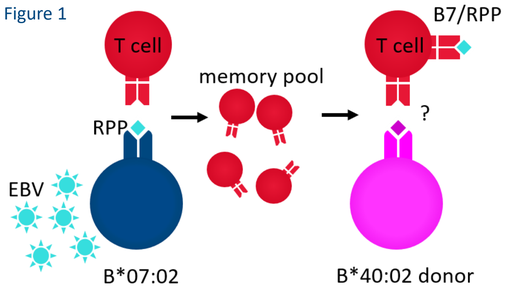
Introduction

From the first encounter with an antigen our immune system moulds specific T cells to establish a memory pool capable of defending the individual from subsequent attack. In some cases, memory T cells whilst specific for the original antigen presented by their own human leukocyte antigen (HLA), can also recognise alternative unrelated HLA-antigen complexes; termed **heterologous immunity**.

Figure 1



Here we examine the ability of certain **virus-specific memory T cells** to cross-react with allogenic HLA complexes, such as observed in the setting of solid organ transplantation. Memory CD8+ T cells from lung transplant recipients were identified as specific for **HLA-B*07:02** presenting the Epstein Barr Virus epitope EBV_{RPP} and cross-reactive with **HLA-B*40:02.**

Aims

- To confirm the cross-reactivity of this TCR model by expressing the TCR in a cell line (SKW3.hCD8) in order to perform functional tests
- using **stimulator cell lines** expressing the cognate (HLA-B*07:02 with and without the EBV_{RPP} peptide) and cross-reactive (HLA-B*40:02) HLAs
- and measuring activation of the SKW3.hCD8.TCR cells by **CD69 expression** on flow cytometry.

Methods

Manipulating TCR into pMIG vector

4X TCR sequences were analysed from 2 donors; lung transplant recipient (LTR) 54.1/54.2 and 119.2/119.2a

1. The TCR constructs and pMIGII vector were digested using restriction enzymes EcoRI and BglII to create 'sticky ends'
2. The TCRs were then ligated into the vector *(containing ampicillin resistance and GFP tag)* and transformed into DH5α competent *E.coli* cells to amplify and purify DNA
3. The DNA was isolated from selected colonies for sequencing. *If the sequence was correct, the plasmid was used for retroviral transduction into the **SKW3.hCD8 cells**.*

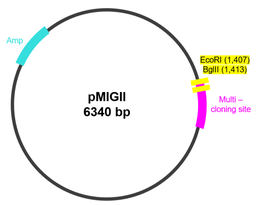
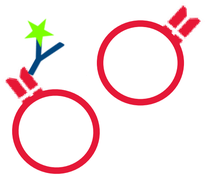


Figure 2: *pMIGII plasmid retroviral vector map adapted from Holst, Nature Protocols 2006; 1:406 figure 2a.*



Surface staining to confirm expression of HLA/TCR

- SKW3.hCD8 cells were stained with conjugated antibodies PECy7 CD3 and PerCPCy5.5 CD8 to confirm expression of TCR.
- The two stimulator cell lines (K562 (SAL) and C1R) were stained with primary antibodies ME-1 *(specific for HLA-B7)* and Bw6 *(specific for a serological group of HLA-B alleles)* to confirm HLA expression. Bw6+ ME-1+ indicated HLA-B7 expression, Bw6+ ME-1- is-indicative of HLA-B40 expression.

CD69 FACS assay to monitor T cell activation

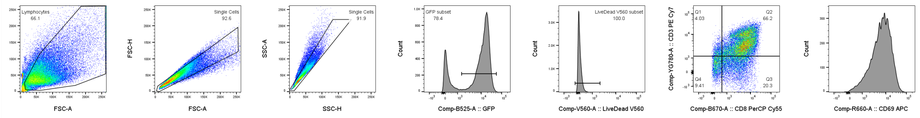
note: functional assay performed on different TCR within the same model; LTR117. SKW3.hCD8 cells expressing an alternative TCR (LTR5) were used as a negative control.

Day 1:
SKW3.hCD8.LTR117 and LTR5 cells were cultured with different stimulation conditions (media alone and CD3/CD28 beads then APCs: K562, SAL.B7, SAL.B7/EBV_{RPP}, SAL.B4001, SAL.B4002, C1R, C1R.B7, C1R.B7/EBV_{RPP} and C1R.B4002) at a 1:1 ratio for 17hrs at 37°C.

Day 2:
An antibody cocktail containing CD3, CD8, CD69 and Live/Dead Dye was added and the cells were acquired by flow cytometry.

Results

Figure 3 (below): *Gating was set up on the + control to show to steps of analysis by flow cytometry*



Level of CD69 upregulation was used to measure activation; media alone was used as a negative control and CD3 CD28 was used as a non-specific activator for a positive control. Two different cell lines were tested each expressing three different HLAs + parental alone. All conditions were compared on a histogram (*figure 5*) and converted to a graph (*figure 4*) showing a fold increase in mean fluorescence intensity from the media alone stimulation condition.

Figure 4

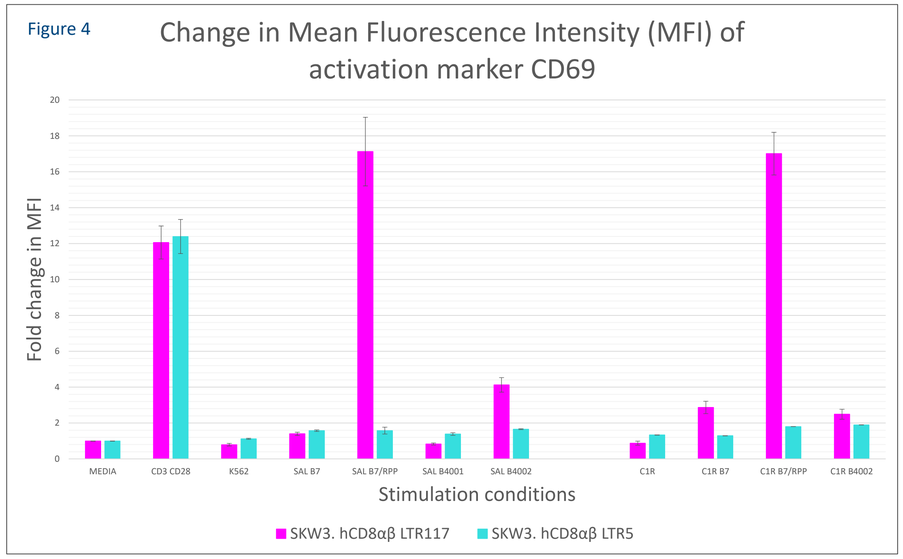
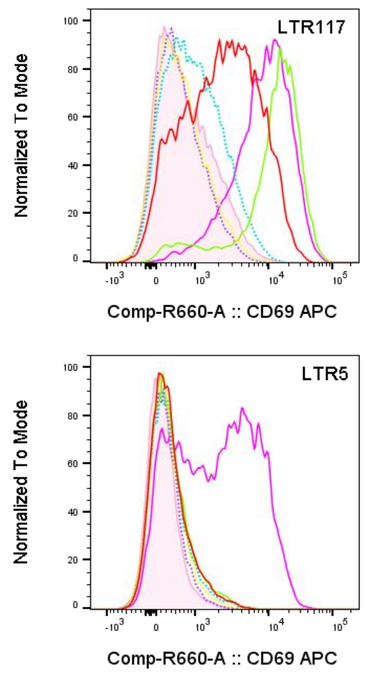


Figure 5: *all samples displayed are from the CD8 (PerCP Cy55+) and CD3 (PE Cy7+) subset*



Conclusion

We **did manage to show cross reactivity** within the SAL cell line as the B4002 CD69 MFI is greater than that of the B7 alone and parental (K562) by over 4 fold However, the activation in the C1R cell line has to be disregarded due to the B7 alone having a greater response.
This does fortunately ask a different question: **is the peptide of importance narrowed down to one cell line?**

Discussion

This step within the project has confirmed cross-recognition, however, further investigations are needed to establish the specific peptide being presented by the alloreactive HLA and the immune response to this recognition (whether or not the target cell is destroyed) in order to bring this investigation closer to clinical applications.

Supervised by: Dr Nicole Mifsud and Dr Louise Rowntree

References

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2. Van den Heuvel H, Heutinck KM, van der Meer-Prins EP, et al. Detection of Virus-Specific CD8+ T Cells With Cross-Reactivity Against Alloantigens: Potency and Flaws of Present Experimental Methods. Transplantation Direct 2015
3. Rowntree L, Kotsimbos T, Mifsud N, et al. Deciphering the clinical relevance of allo-human leukocyte antigen cross-reactivity in mediating alloimmunity following transplantation. Current Opinion in Organ Transplantation. 2016;21(1):29-39.

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