IMMUNOCORE

0509

Labelled High Affinity TCRs for Detection of HIV Epitopes on Infected Cells: How Low Can We Go?

Zoë Wallace[1], Jonathan Chamberlain[1], Esra Guc[1], Praveen K. Singh[1], Lucy Dorrell[1]

[1]Immunocore Ltd, 92 Park Drive, Milton Park, Abingdon, Oxfordshire, OX14 4RY, UK

Background

- HIV persists in a non-productive state in CD4+ T cells during ART, presenting a major barrier to HIV eradication.
- A single cell surface biomarker to uniquely identify these infected cells would allow further characterisation of the reservoir and facilitate assessment of potential curative interventions.
- Growing evidence suggests that a substantial proportion of the reservoir is transcriptionally and translationally active, leading to presentation of viral T cell epitopes[1, 2].
- High affinity HIV-specific TCRs that can detect viral peptides at low copy numbers are potential tools for identification and isolation of reservoir cells for further analysis.
- We engineered soluble, high affinity TCRs (pM affinity)[3,4] to investigate the level of expression of viral peptides presented by HLA class I molecules on the surface of HIV-infected cells (Figure 1).

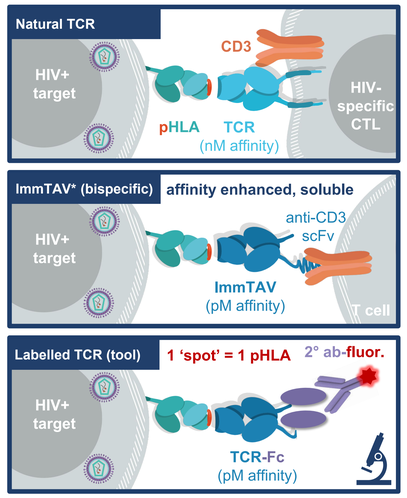
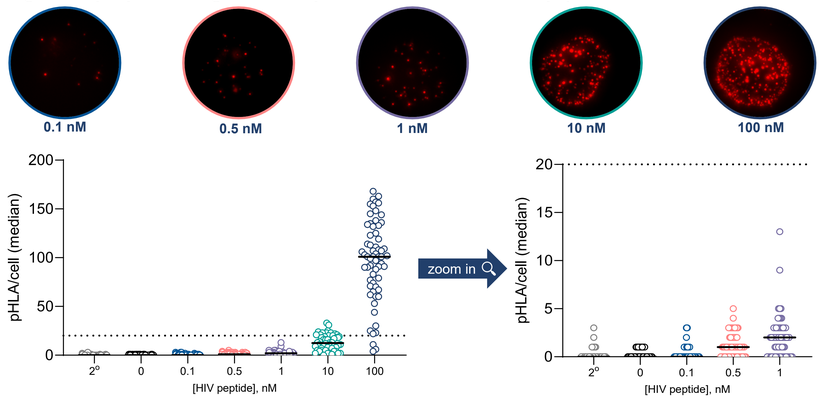


Figure 1. Transformation of natural, HIV-specific TCR into high affinity soluble molecules for T cell redirection or labelling

*Immune mobilising monoclonal TCR Against Viruses (ImmTAV)

Results: Peptide pulsed model demonstrates exquisite sensitivity (<10 pHLA copies/cell) of high affinity TCR

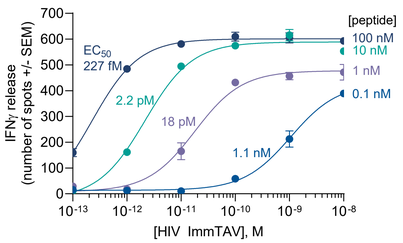
Figure 2. Epitope counting of T2 cells pulsed with a titration of HIV peptide



T2 cells pulsed with a titration of HIV Gag peptide were labelled with a high affinity TCR-Fc construct and stained with a fluorescent secondary antibody for imaging by TIRF microscopy (maximum intensity projections of Z-stack images shown in pictures above for each peptide titration). One circle represents one cell at the pHLA/cell count. At least 40 cells imaged/quantified per condition.

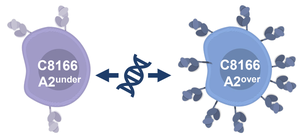
Figure 3. ImmTAV-redirected T cells secrete IFNγ when cultured with peptide pulsed T2 targets expressing <10 pHLA / cell (mean)

T2 cells pulsed with titrated HIV peptide (0.1 nM – 100 nM) were cocultured with an HIV-specific ImmTAV (100 fM – 10 nM) and HIV-naïve donor PBMC as effectors (1:1 E:T) overnight before assessing IFNγ release by ELISpot. Half-maximal effective concentration (EC_{50}) for each concentration of peptide shown for one representative donor.



Results: TCR bispecific (ImmTAV) detects infected cells expressing <15 pHLA copies/cell

Figure 4. HLA-A2 expression on CRISPR-modified CD4+ T cell lines compared to primary T cells



CRISPR was used to manipulate HLA-A2 expression in the C8166 T cell line. HLA-A2 was over-expressed to amplify pHLA signal ($A2^{over}$), while knock-down was used to mimic expected in vivo conditions of low pHLA resulting from Nef mediated downregulation ($A2^{under}$).

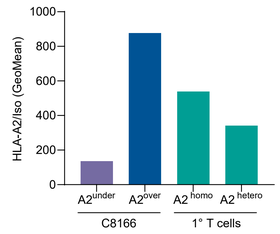
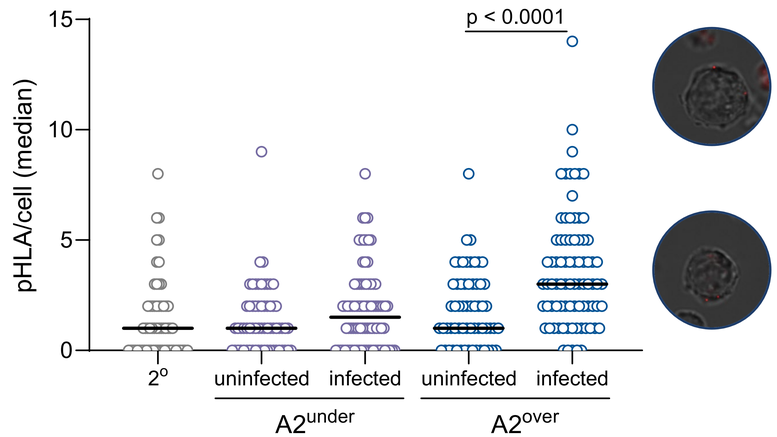
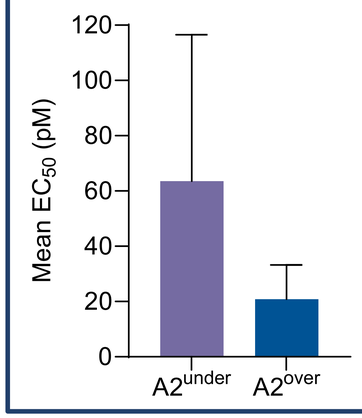


Figure 5. < 15 pHLA/cell detected on HIV-infected T cell line under or over-expressing HLA-A2



C8166 under or overexpressing HLA-A2 were infected with HIV IIIB (> 90% p24+ by flow cytometry) or left uninfected before staining for epitope counting with high affinity HIV-specific TCR (representative images of one Z-slice of TCR staining (red dot) on phase-contrast shown for infected $A2^{over}$). One circle represents one cell at the pHLA/cell count. At least 40 cells imaged/quantified per condition. Statistical significance of difference between uninfected and infected cells shown for $A2^{over}$ only (Mann-Whitney test).



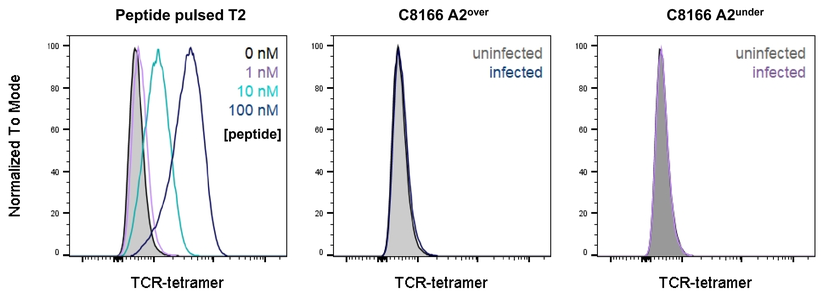
- Viral pHLA complexes were detected on the surface of an HIV-infected cell line with a high affinity TCR
- Loss of signal with infected cells with sub-physiological HLA-A2 expression indicates that lower limit of detection is ~10 pHLA/cell
- Despite low pHLA/cell counts, killing of infected cells ($A2^{over}$ and $A2^{under}$) was observed with the same high affinity TCR fused to anti-CD3 scFv (ImmTAV) in coculture assay with CD8+ T cells from an HIV-naïve donor

Figure 6. ImmTAV-mediated killing of HIV-infected T cell lines

C8166 under or overexpressing HLA-A2 were infected with HIV and cocultured with HIV-naïve donor CD8+ T cells and titrated HIV-specific ImmTAV for 7 days before determining the % elimination of Gag+ cells by flow cytometry. Mean EC_{50} shown.

Results: Flow cytometry is less sensitive than TIRF microscopy for detection of antigen-positive cells by TCR staining (LLOD ~10-30 pHLA copies/cell)

Figure 7. TCR labelling of HIV antigen-positive cells for flow cytometry



HIV antigen+ cells (and antigen- controls; grey) were stained with a high affinity TCR-tetramer for flow cytometry. From left to right: peptide pulsed T2s (0 – 100 nM HIV peptide), HIV-infected C8166 $A2^{over}$ and HIV-infected C8166 $A2^{under}$.

- Biotinylated high affinity HIV-specific TCRs were multimerised and labelled with streptavidin-PE to enable flow cytometric analysis of HIV antigen+ cells (peptide pulsed or in vitro infected cell lines).
- TCR-tetramers were able to detect T2 cells pulsed with ≥10 nM peptide, equivalent to 10s+ pHLA/cell (Figure 2). However, neither T2 cells pulsed with 1 nM peptide (equivalent to ≤ 10 pHLA/cell) nor infected cells yielded a signal above background levels.
- Further signal amplification strategies are needed to enable detection of HIV-infected cells within a mixed population using labelled TCR staining for flow cytometric analysis.

Conclusions

Table 1.

	Peptide pulsed T2 cells						HIV+ C8166	
pHLA/cell	**0 nM**	**0.1 nM**	**0.5 nM**	**1 nM**	**10 nM**	**100 nM**	**$A2^{under}$**	**$A2^{over}$**
Range	0 – 1	0 – 3	0 – 5	0 – 13	0 – 33	4 – 164	0 – 8	0 – 14
Median	0	0	1	2	12.5	101	1.5	3
Mean	0.3	0.4	1.1	1.9	13	97.3	1.8	3.6

- A high affinity HIV-specific TCR detects cell surface pHLA complexes at levels as low as ~10 copies/cell, as quantified by high resolution microscopy.
- The same TCR in anti-CD3 bispecific format redirects killing of HIV-infected cells expressing low pHLA copies on the cell, thus corroborating the microscopy findings.
- Infected cells could not be specifically identified by flow cytometry using the labelled TCR, indicating that further signal amplification is needed for this technique to be suitable for cell sorting.

References

1. Astorga-Gamaza A, Buzon MJ. The active human immunodeficiency virus reservoir during antiretroviral therapy: emerging players in viral persistence. Curr Opin HIV AIDS. 2021 Jul 1;16(4):193-199. doi: 10.1097/COH.0000000000000685. PMID: 33973900.
2. Dubé M, et al. Spontaneous HIV expression during suppressive ART is associated with the magnitude and function of HIV-specific CD4+ and CD8+ T cells. Cell Host Microbe. 2023 Sep 13;31(9):1507-1522.e5. doi: 10.1016/j.chom.2023.08.006. PMID: 37708853; PMCID: PMC10542967.
3. Wallace, Z., Singh, P.K., and Dorrell, L. (2022). Combination strategies to durably suppress HIV-1: Soluble T cell receptors. J Virus Erad 8, 100082. 10.1016/j.jve.2022.100082.
4. Yang, H. et al. (2016). Elimination of Latently HIV-infected Cells from Antiretroviral Therapy-suppressed Subjects by Engineered Immune-mobilizing T-cell Receptors. Mol Ther 24, 1913–1925. 10.1038/mt.2016.114.