Duration of Viremia Modulates Avidity of HIV-specific T Cell Receptors

400

Funsho J. Ogunshola[1,2,6], Nishant K. Singh[1,2,6], Vincent Butty[2], Anurag R. Mishra[1,2], Liza Vecchiarello[1], Kate B. Juergens[1,2], Sophia Cheever[1,2], Zachariah Habte[1], Marius Allombert[1], Anabelle Webber[1], Alicja Piechocka-Trocha[1], Anusha Nathan[1], Ahmed Fahad[1], Xiaolong Li[1,4], Kavidha Reddy[3,5], Musie Ghebremichael[1], Gaurav Gaiha[1], Krista Dong[1,5], Brandon J. Dekosky[1,2], Thumbi Ndung'u[1,3,5], Michael Birnbaum[1,2] and Bruce D. Walker[1,2,5,6]

[1]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, 02169, USA. [2]Massachusetts Institute of Technology, Cambridge, MA 02169, USA. [3]Africa Health Research Institute, 4013, Durban, South Africa. [4]University of Science and Technology of China, Hefei 230001, Anhui, China. [5]HIV Pathogenesis Programme, Doris Duke Medical Research Institute, University of KwaZulu-Natal, 4013 Durban, South Africa. [6]Howard Hughes Medical Institute, Chevy Chase, MD, USA.

BACKGROUND

Virus-specific CD8⁺ T cells contribute to control of chronic viral infections such as HIV-1, but the impact of duration of antigen exposure on the establishment of T cell memory is not well understood.

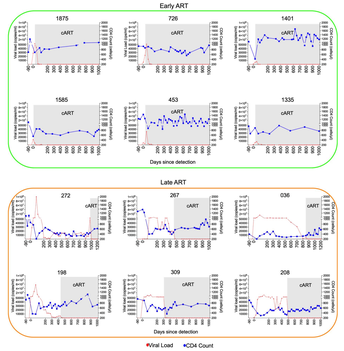
METHODS

In this study, we examined epitope-specific CD8⁺ T cell repertoires in people living with HIV-1, where duration of antigen exposure was modulated by the time of initiation of continuous suppressive antiretroviral therapy (ART) following hyperacute infection. After ART-induced undetectable viremia, we analyzed the impact of early (n=6) versus delayed (n=6) ART initiation on the clonotypic composition, clonotypic avidity and cross-reactivity of the HIV-specific T cell repertoire. Using barcoded tetramers, we assessed T cell receptor (TCR) clonotypes directed against HLA-B*58:01 epitopes and their variants in persons selected for expressing this class I allele.

FINDINGS

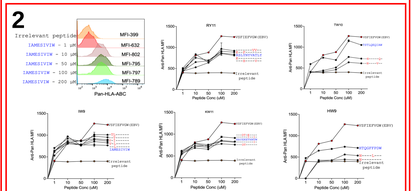
Polyclonality and cross-reactivity were detected in both early and late-treated participants. For each epitope, there were clonotypes able to cross-recognize all autologous variants, but these were rarely induced. Early ART initiation led to a significant enrichment of high-avidity TCRs compared to those with prolonged exposure before ART, a finding independent of specific TCR V gene usage. Our results underscore the importance of limiting antigen exposure to promote the development of highly avid T cell clonotypes in a chronic viral infection, and that differences in duration of antigenic exposure do not modulate the limited cross-reactivity observed. These findings have important implications for guiding T cell-based vaccine strategies and therapeutic interventions aimed at achieving a functional HIV-1 cure.

1



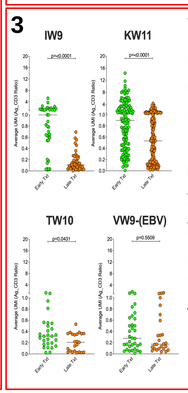
Longitudinal analysis of plasma HIV-1 RNA levels (red) and absolute CD4+ T cell counts (blue) in 12 participants expressing the HLA-B*58:01 allele. Gray shading represents periods of intensive ART treatment.

2



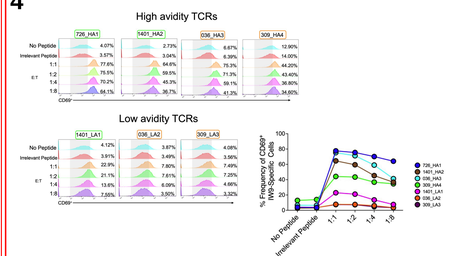
Representative histograms for HLA MFI for consensus and variant IW9 peptides (1–200 µM) in a TAP-deficient, mono-allelic cell line engineered for HLA-B*58:01 expression. MFI values represent pan-HLA staining, with each data point showing the mean of technical triplicates from experiments performed twice.

3



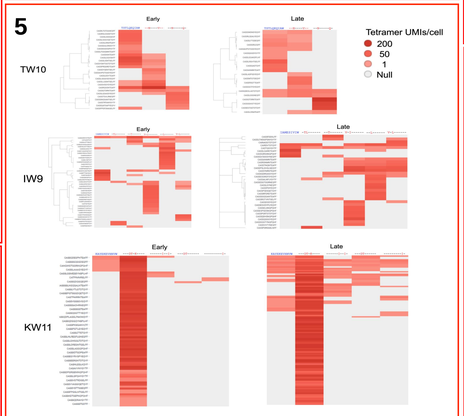
TCR clonotype avidity was estimated based on CDR3 sequences, calculated as the ratio of bound tetramers to CD3 expression per cell. Statistical analysis was performed using a non-parametric t-test.

4



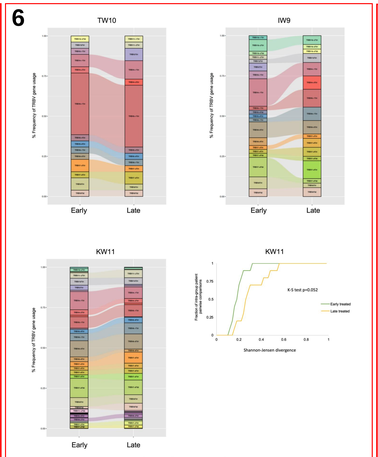
The histogram illustrates the mean fluorescence intensity (MFI) of CD69 on IW9-specific monoclonal following stimulation with IW9 presented by a mono-allelic cell line expressing the HLA-B*58:01 allele. Different colors represent various experimental conditions, including no stimulation, irrelevant peptide, and varying effector-to-target ratios. The experiments were conducted in triplicate to ensure the reliability of the data. Dose response (monoclonal T cells) to target (antigen-presenting cells) ratio, illustrating the dynamics of CD69 expression when a constant number of effector cells were co-cultured with varying numbers of target cells.

5



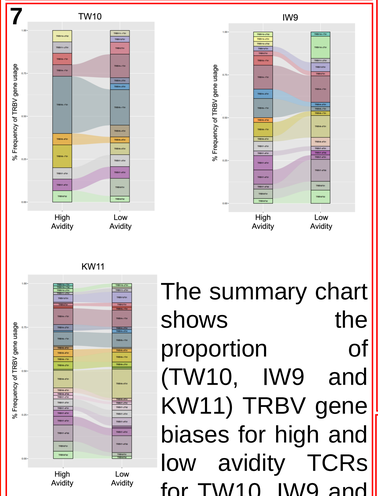
Heatmaps illustrating type-specific and cross-reactive clonotypes within the TW10, IW9 and KW11 antigen families, comparing individuals with immediate vs. delayed ART treatment initiation.

6



Summary of shared TRBV gene usage for TW10, IW9, and KW11-specific TCR clonotypes in early- and late-treated participants, with distinct color codes representing different TRBV genes. A cumulative distribution function (CDF) plot illustrates intra-participant KW11-specific TCR repertoire changes in response to varying durations of antigenic stimulation. TCR diversity was quantified using Shannon-Jensen divergence (JSD).

7



The summary chart shows the proportion of (TW10, IW9 and KW11) TRBV gene biases for high and low avidity TCRs for TW10, IW9 and KW11 respectively.

CONCLUSIONS

- Limited antigen exposure during the early Fiebig stages is sufficient to induce HIV-specific T cell responses.
- Most autologous epitopes in these participants, restricted by the HLA-B*58:01 allele, are capable of stabilizing the HLA-B*58:01 molecule.
- The frequent use of the *TRBV5-1*01* gene in response to the TW10 antigen, as well as in IW9 and KW11-specific T cells, suggests a potential association between this V gene and *HLA-B*58:01* restriction.
- Broadly cross-reactive clonotypes encompassing all defined variants are inducible but never the dominant clonotypes
- Although prolonged antigen exposure can increase V gene diversity, biases in TRBV gene usage appears to be independent of antigen exposure duration.
- Early treated participants show a significant increase in high-avidity T cell clones, which exhibit greater antigen sensitivity compared to those treated later.

ACKNOWLEDGEMENTS AND FUNDING SOURCES

We thank the FRESH participants, as well as the FRESH and HIV pathogenesis programme (HPP) laboratory staff. This work was support by the Howard Hughes Institute (HHMI), the Bill and Melinda Gate Foundation, Center For AIDS Research (CFAR)

REFERENCES

1. Lichterfeld, M., Yu, X. G., Le Gall, S. & Altfeld, M. Immunodominance of HIV-1 specific CD8(+) T-cell responses in acute HIV-1 infection: at the crossroads of viral and host genetics. *Trends Immunol* **26**, 166-171, doi:10.1016/j.it.2005.01.003 (2005).
2. Straub, A. *et al.* Recruitment of epitope-specific T cell clones with a low-avidity threshold supports efficacy against mutational escape upon re-infection. *Immunity* **56**, 1269-1284.e1266, doi:10.1016/j.immuni.2023.04.010 (2023).
3. Schober, K. *et al.* Reverse TCR repertoire evolution toward dominant low-affinity clones during chronic CMV infection. *Nat Immunol* **21**, 434-441, doi:10.1038/s41590-020-0628-2 (2020).
4. Tscharke, D. C., Croft, N. P., Doherty, P. C. & La Gruta, N. L. Sizing up the key determinants of the CD8(+) T cell response. *Nat Rev Immunol* **15**, 705-716, doi:10.1038/nri3905 (2015).

AUTHORS' CONTACTS:

Funsho J. Ogunshola, fogunshola@mgh.harvard.edu
Bruce D. Walker, bwalker@mgh.Harvard.edu
Michael Birnbaum, mbirnb@mit.edu