Poster #277

TCF-1 expression is associated with HIV-specific CD8+ T cell proliferative capacity

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Introduction

- HIV is a chronic viral infection for which there is no cure.
- HIV-specific CD8+ T cells play an important role in controlling infection, including in individuals who naturally control viral replication to undetectable levels in the blood without ART ("**controllers**").[1-3]
- In comparison, HIV-specific CD8+ T cells isolated from non-controllers (both off – "**viremic**" – and **on ART**) are exhausted in that they demonstrate impaired regenerative (i.e., proliferative/effector differentiation) capacity.[4-7]
- Several HIV "cure" strategies (e.g., therapeutic vaccination) seek to generate functional/non-exhausted HIV-specific CD8+ T cell responses with regenerative capacity in HIV-infected individuals on ART.[8]
- However, little is known in HIV-specific CD8+ T cells about what pathways promote regenerative capacity in **controllers** or maintain exhaustion in the setting of **ART**.

Questions

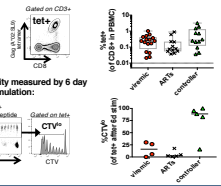
1) *Do the molecular pathways that support the regenerative capacity of antigen-specific CD8+ T cells in other contexts (e.g., TCF-1) also support the regenerative capacity of HIV-specific CD8+ T cells in controllers?*

2) *What aspects of exhaustion are maintained in HIV-specific CD8+ T cells isolated from individuals on suppressive antiretroviral therapy (ART, i.e., HIV is undetectable in the blood)?*

Study Design

- 10-20 x 10^6 cryopreserved PBMCs from individuals in 3 groups enrolled in SCOPE*
- **HIV-specific CD8+ T cells identified by MHC Class I tetramer staining** using HLA-A*02, *03, *24, or -B*07-restricted tetramers specific for peptides derived from HIV proteins Gag, Pol, Nef***
- Phenotyping + proliferative capacity of **tetramer (tet)+** cells by flow cytometry. Markers (evaluated pre-stim) include:
  live/dead, CD3, CD4, CD8, tetramer
  CCR7, CD45RA, CD127
  TCF-1, Tbet, Eomesodermin,
  PD-1, CD127, Granzyme B, Perforin
- Analysis performed using non-parametric statistical testing
  (lines = median; NSD = no significant difference)

GROUP	n**	CD4 count (cells/mm³) median (range)	HIV VL (copies/mL) median (range)
VIREMIC (no ART)	16	484 (249-875)	66,381 (8,000-986,000)
ART-suppressed (ARTs)	13	490 (249-894)	all <40 (prior non-controller, >2y on ART)
CONTROLLER (no ART)	13	675 (558-1098)	all <100



Proliferative capacity measured by 6 day *in vitro* peptide stimulation:

* San Francisco-based cohort of >2,500 HIV-infected individuals
** not all parameters assessed for all individuals
*** MHC Class I peptide monomers from Rafick-Pierre Sékaly

Fig. 1: Controller tet+ cells (vs viremic and ARTs): Similar %effector-memory subsets

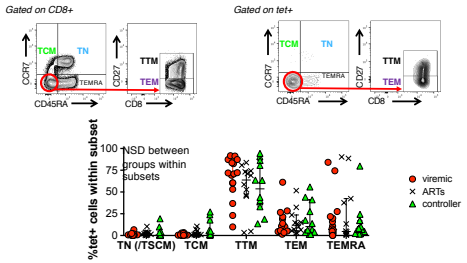


Fig. 2: Controller tet+ cells (vs viremic):

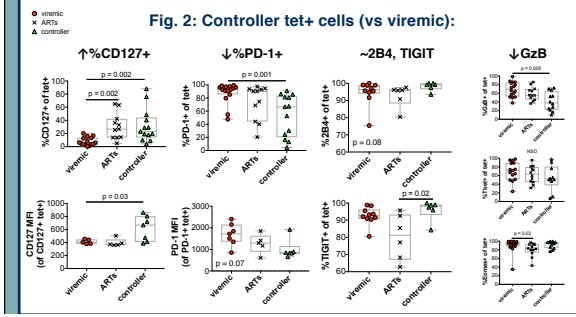
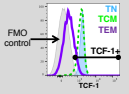


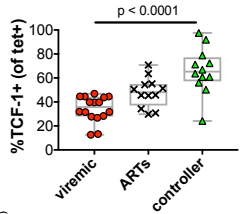
Fig. 3: HIV-specific CD8+ T cells from controllers express higher levels of TCF-1 compared to cells from viremic or ARTs non-controllers

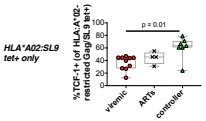
Background: TCF-1 ("T cell factor 1")

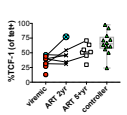
- Gene name: TCF7
- Wnt-signaling transcription factor
- High expression in bulk CD8 TN, TCM, TSCM:[9]



- Controls regenerative capacity of ag-specific memory CD8+ in acute infection and CAR T cells[10-11]
- Necessary + sufficient for proliferative burst of ag-specific CD8s after anti-PD1 therapy in chronic infection (animals)[12-14]
- Little known about role in human virus-specific CD8s[15]







Controllers have ↑ TCF-1 expression across effector-memory subsets in both tetramer+ cells (LEFT) and bulk CD8+ T cells (RIGHT)

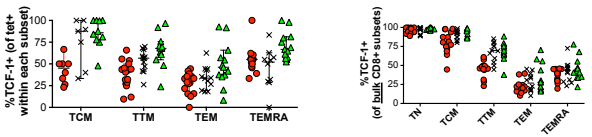


Fig. 4: High expression of TCF-1 in tet+ cells correlates with high proliferative capacity and lower HIV VL

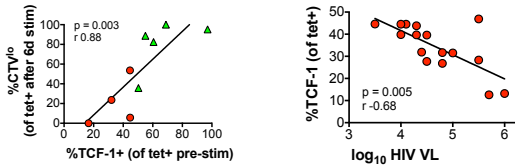


Fig. 5: TCF-1+ HIV-specific (tet+) CD8+ T cells are less terminally differentiated and have modestly lower PD-1 expression

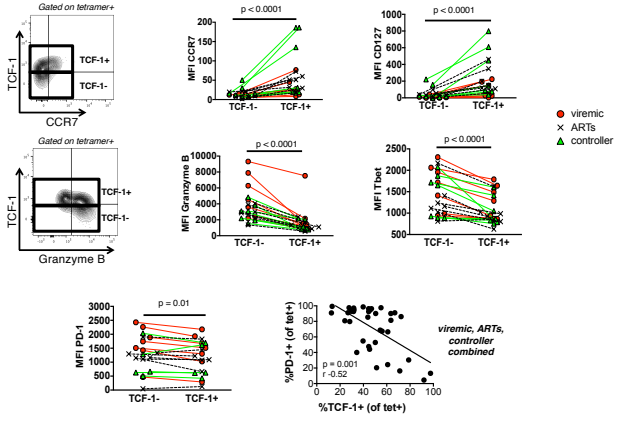


Fig. 6: 6d *in vitro* peptide stimulation => divided tet+ cells from controllers downregulate TCF-1 expression

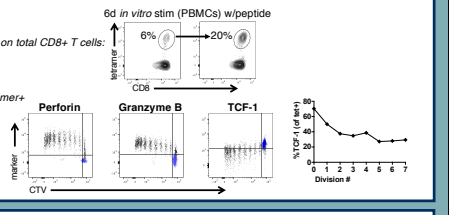
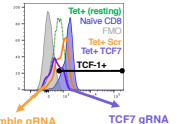


Fig 7: CRISPR/Cas9-mediated knockout of TCF7 gene in HIV-specific CD8+ T cells

Questions: Is TCF-1 expression required for enhanced proliferative capacity in controllers? Does expression actively repress an effector differentiation program?



PBMCs from controller with known tetramer response

- Rest PBMCs o/n
- Nucleofect Cas9+gRNA
- Rest 6d

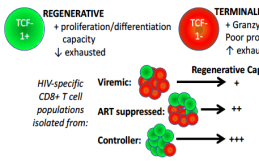
scramble gRNA — TCF7 gRNA

tet+ cells	tet+ cells	tet+ cells
45% TCF-1+	70% TCF-1+	26% TCF-1+
MFI 674	MFI 968	MFI 461

No change in PD-1, CD127, GzB, Tbet, Eomes, Perforin expression (in tet+ or bulk CD8)

With Joe Hiatt, Alex Marson.

Model: TCF-1 and HIV-specific CD8+ T cell exhaustion



These data suggest that the regenerative capacity of the HIV-specific CD8+ T cell population is related to the size of the TCF-1+ subpopulation (and TCF-1 may regulate this capacity).

"HIV cure" interventions (and other CD8+ T cell therapies, e.g., CAR T cells, other cancer immunotherapy, CD8-based vaccines) would ideally target/elicit this subpopulation to enhance the antigen-specific CD8+ T cell response.

Future Directions

- Is TCF-1 necessary/sufficient to support regenerative capacity of HIV-specific CD8+ T cells?
  - CRISPR/Cas9 deletion followed by proliferation assay
- What transcriptional programs associate with TCF-1 expression in HIV-specific CD8+ T cells?
  - Single-cell RNA sequencing
- How is regenerative capacity of antigen-specific CD8+ T cells regulated in tissues?
  - Parallel analysis of tetramer+ cells from LN, gut
- Can therapeutic interventions elicit a greater proportion of TCF-1+/regenerative HIV-specific CD8+ T cells?
  - Study tetramer+ cells from more individuals treated with ART very early in HIV infection
  - Study tetramer+ cells post-therapeutic vaccination (new vs old responses)

Acknowledgements

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