Post-treatment controllers exhibit distinct CD8+ T cell features before and after ART interruption

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Background

Rare individuals, termed post-treatment controllers (PTCs), can exhibit prolonged periods of very low level viremia upon ART withdrawal, but the mechanisms by which this occurs remain poorly understood. In this study, we assessed whether T cells from PTCs exhibit unique properties.

Methods

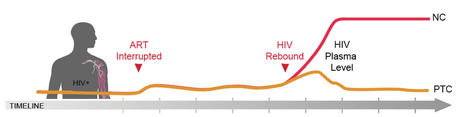
A 40-parameter CyTOF cell panel was developed to characterize the differentiation, activation, and other phenotypic features of T cells. The panel was applied to PBMCs collected on ART prior to treatment interruption, and at multiple timepoints follow treatment interruption, from clinically-matched PTC (n=20) and non-controller (NC) (n=32) participants. CyTOF datasets were gated on T cells, and clustered and mixed effects models were used to identify associations with PTC/NC status.

Results Summary

By implementing cluster-resolution optimization on all samples, we identified nine distinct T cell clusters. Prior to ART interruption, one cluster (cluster 2) was significantly less abundant in PTCs as compared to NCs. This cluster consisted of memory CD8+ T cells expressing high levels of PD1 and TIGIT, suggesting exhaustion. After ART interruption, a separate cluster (cluster 3) comprised of activated CD8+ Temra cells expressing high levels of the pro-survival factor BIRC5 increased in abundance over time in PTCs, while it exhibited the opposite pattern in NCs. Finally, we found that post-ART, NCs harbored a larger proportion of a cluster (cluster 6) comprised of CD4-CD8- T cells expressing high levels of Tfh marker CXCR5 and activation marker CD30, both previously shown to be increased on HIV-infected cells.

Study Design

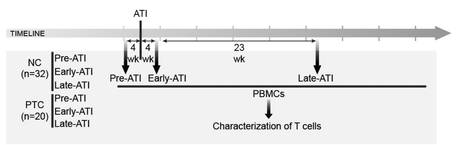
Post-Treatment Controllers (PTCs) control HIV viremia following analytical treatment interruption



NC: Non-controller
PTC: Post-treatment controller

Study Design: Longitudinal PTC vs. NC samples from CHAMPS cohort

ATI: Antiretroviral Therapy Interruption



Purpose

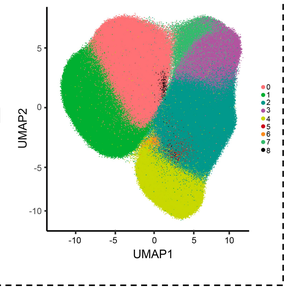
To characterize T cell determinants of control in PTCs as compared to NCs

Results

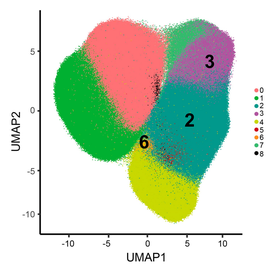
CyTOF panel for T cell phenotyping

Lineage Markers	Phenotyping Markers				
T Cells: CD3 CD4 CD8	Differentiation State: CD45RO CD45RA CD62L CD57 CD127 CD27 RORγt Tbet CRTH2 CD73 Blimp1 CD7	Activation State: CD69 CD25 CD28 CD30 HLADR CD38 ICOS OX40	Homing Receptors: CCR5 CCR6 CCR7 CXCR4 CXCR5	Checkpoint Moleculars: PD1 TIGIT CTLA4	Adhesion and integrins: CD49d(α4) CD103 CD29(β1) α4β7
B Cells: CD19					

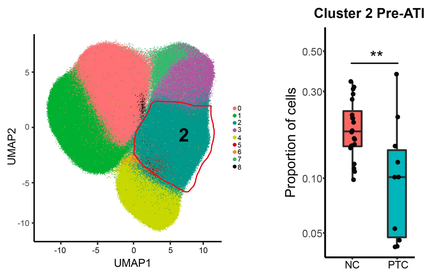
Nine clusters of T cells were identified from our cohort of PTCs and NCs



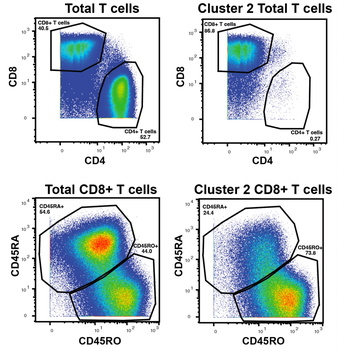
Three clusters (2, 3, and 6) were differentially abundant between PTCs and NCs



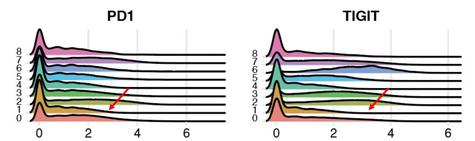
Cluster 2 was significantly more abundant in NCs than PTCs at the pre-ATI timepoint



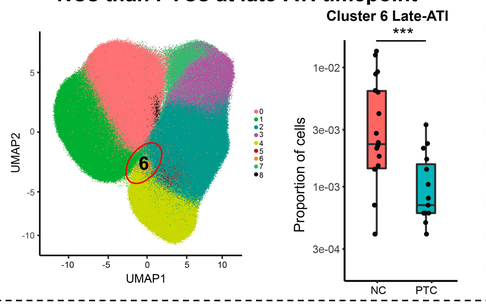
Cluster 2 consists mostly of memory CD8+ T cells



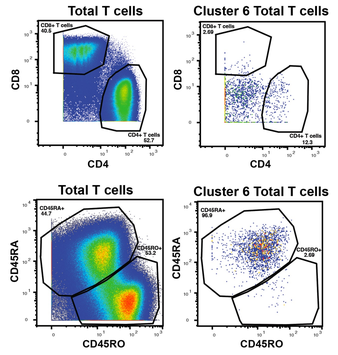
Cluster 2 expresses high levels of checkpoint/exhaustion markers PD1 and TIGIT



Cluster 6 was significantly more abundant in NCs than PTCs at late-ATI timepoint

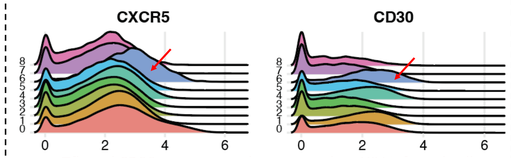


Cluster 6 consists mostly of CD4-CD8-CD45RA+ T cells



Results

Cluster 6 expresses high levels of Tfh marker CXCR5 and activation marker CD30

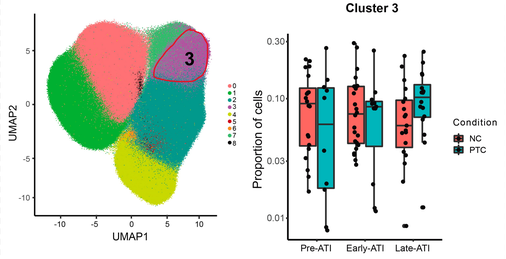


Tfh and CD30 are commonly expressed on HIV-infected cells

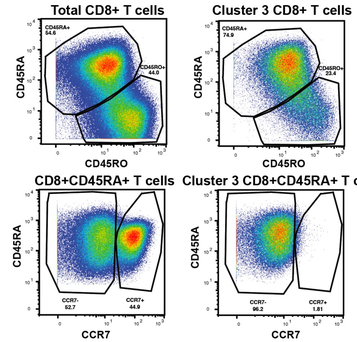
CD4 is downregulated by HIV during infection

Cluster 6 may include productively-infected cells that have downregulated CD4?

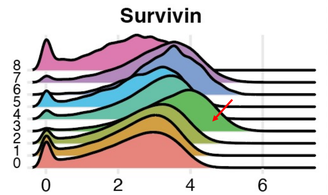
Cluster 3 increased in abundance after ATI in PTCs, while it exhibited the opposite pattern in NCs



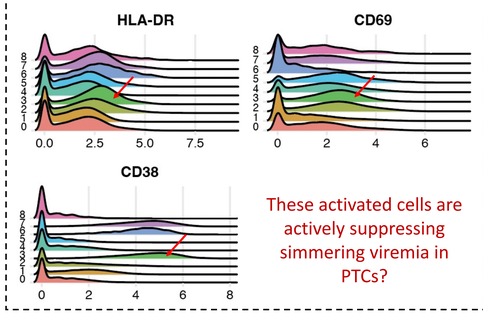
Cluster 3 consists mostly of CD8+ Temra cells



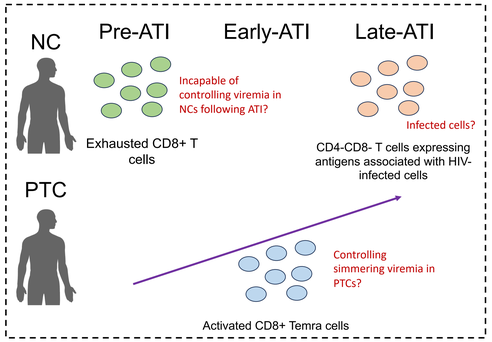
Cluster 3 expresses high levels of pro-survival factor Survivin (Birc5)



Cluster 3 expresses multiple activation markers



These activated cells are actively suppressing simmering viremia in PTCs?



Summary

- **9 clusters of T cells were identified from PTC/NC PBMCs**
- **3 clusters were significantly differentially abundant between PTC and NC samples**
  - Pre-ATI
    - NCs have more exhausted CD8+ T cells (PD1+ TIGIT+)
  - Late post-ATI
    - NCs have more CD4-CD8- T cells expressing CXCR5 and CD30
  - Longitudinal changes
    - In PTCs only, a population of activated CD8+ Temra expands over time

Conclusions

Our data suggest that prior to interrupting ART, people who subsequently control HIV exhibit lower frequencies of exhausted CD8+ T cells, which may enable these individuals to better control HIV replication in the absence of ART. After ART interruption, a population of activated CD8+ Temra expressing BIRC5 expands in PTCs but not NCs; theoretically, these cells might persist longer and be able to maintain more direct virus control. Post-ATI viremia in NCs is associated with a population of CD4-CD8- T cells expressing antigens associated with HIV-infected cells; ongoing work seeks to determine whether these cells are productively-infected with HIV.

Acknowledgements

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