IrsiCaixa

Institut de Recerca de la Sida

CD8⁺ SUBSET-DEPENDENT OVEREXPRESSION OF TIGIT⁺ AND TIGIT⁺TIM-3⁺ BY HIV-1 DESPITE ART

0302

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BACKGROUND

The expression of inhibitory Receptors (iRs) blocks CD8⁺ T-cell activity in HIV-1 infection. The control of the expression of iRs is critical for recovering CD8+ T-cell function. However, the alterations of iR expression in CD8+ T-cells by HIV-1 infection are not fully characterized but are essential to identify future immunotherapeutic targets to regain antiviral immunity.

AIM

To delineate alterations in the expression of iRs in association with lineage markers in CD8+ T cell that are not normalize in HIV-1 infected individuals despite long-term and fully suppressive cART.

STUDY GROUPS

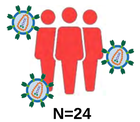
Chronically HIV-1 infected



N=24

Group		S1	S2
Age (years)		40 (36 - 45)	49 (44 - 54)
Gender	Male, (%)	18 (75%)	
	Female, (%)	6 (25%)	
CD4⁺ T-cell count/µl		573 (464 - 992)	910 (773 - 1111)
VL (log copies/ml)		Undetectable	Undetectable
Years undetectable		2.29 (1.83 - 2.8)	10.25 (7.65 - 12.8)

Early HIV-1 infected



N=24

Ei
43 (41 - 46)
24 (96%)
1 (4%)
625 (552.5 - 754.5)
4.61 (4.3 -5.19)
0

Healthy uninfected



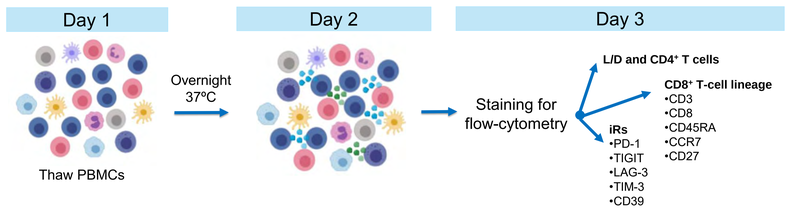
N=24

HC
43 (38.5 - 52)
16 (67%)
8 (33%)

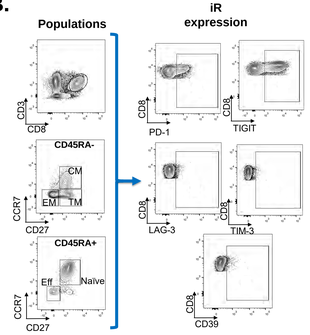
The study was carried out retrospectively taking into account clinical data and biological sample availability. All the study groups were balanced by age. S1 and Ei individuals were also balanced based on CD4⁺ T-cell counts.

IMMUNOPHENOTYPE OF CD8⁺ T CELLS

A.



B.



C.

1. Previous analysis using FlowJo
   - Gating strategy until the population of interest
   - Select samples you want analyze
   - One single-cell data (csv file) per sample

2. Data preprocessing
   - Select randomly n cells or all the cells from each sample
   - Define markers of interest
3. t-SNE
   - t-SNE using a random cell sample
4. Clustering: Phenograph algorithm
   - Define clusters and visualize them
   - Calculate % or counts in graphs
5. Heatmap
   - Heatmap association between clusters and markers.

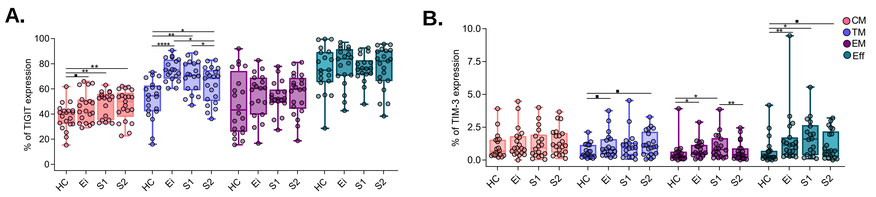
6. Visualization and analysis
   - Interesting markers for visualization, don't use default scale.
   - Take clusters of interest

A. Cryopreserved PBCMs were thawed, co-stimulated and surface stained using a 15-colour panel of antibodies. We included live/dead probe, markers of CD8⁺ T-cell lineage (CD3, CD4, CD8, CD45RA, CD27 and CCR7), iRs expression and the metabolic marker CD39. **B.** Gating strategy for iR expression in total CD8⁺ T cells and subpopulations. **C.** Pipeline for single-cell analysis of multidimensional data of flow cytometry. CM: Central Memory, TM: Transitional Memory, EM: Effector memory, Eff: Effector.

HIV-1 infection drives irreversible overexpression of TIGIT alone or co-expressed with TIM-3 and LAG-3 or CD39 in a CD8⁺ T-cell subset-dependent manner

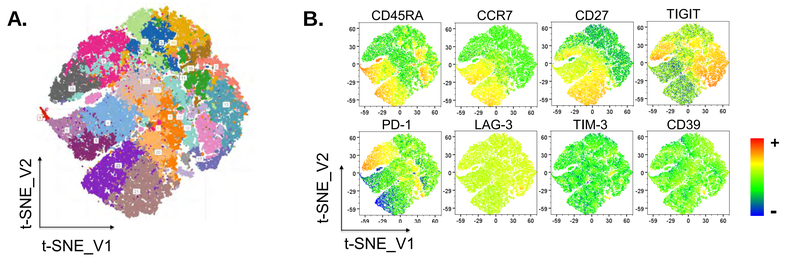
RESULTS

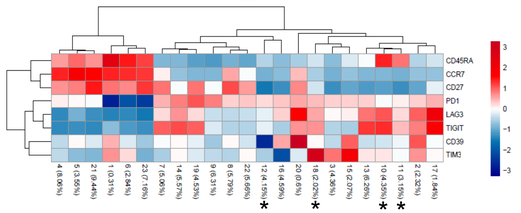
• CD8⁺ T-cell subset-specific differences in iR expression and impact of cART

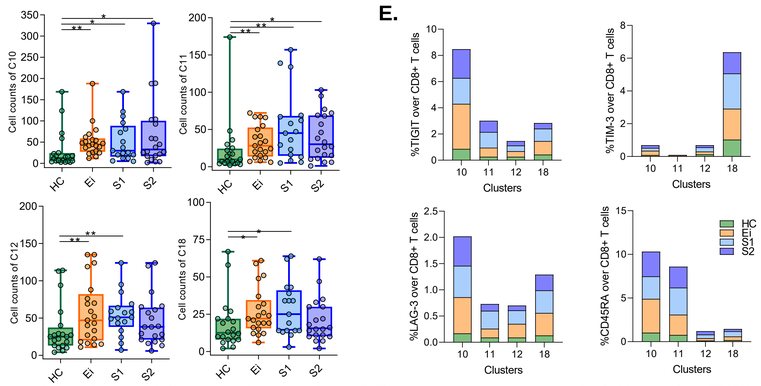


Expression of TIGIT and TIM-3 according to CD8⁺ T-cell subsets. **A.** TIGIT expression. **B)** TIM-3 expression. Horizontal lines denote the median values within each group. For statistical analysis, we used Mann-Whitney for unpaired samples and Wilcoxon test for paired samples. p values: *<0.05, **<0.005, ***<0.0005, ****<0.00005. • ≈0.05 indicates trends.

• Four differentiated clusters with CD8⁺ memory and effector-like features alter during long-term cART

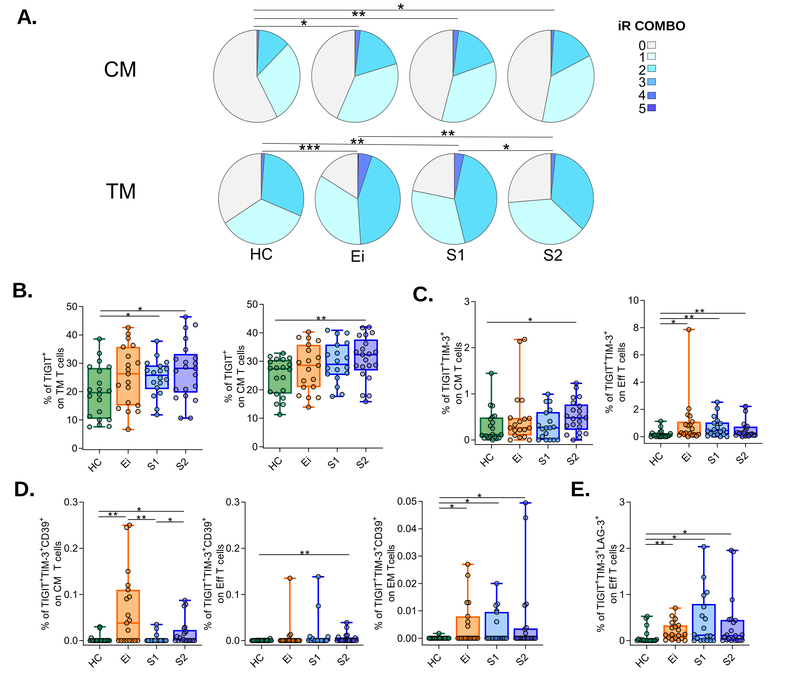






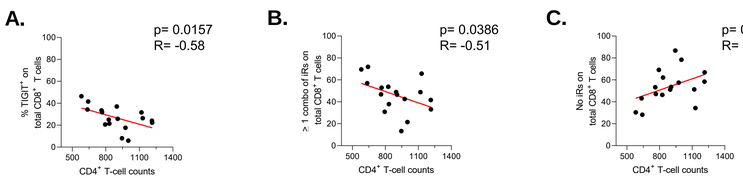
Dimensionality reduction using t-SNE and cluster composition of CD8⁺ T cells based on single-cell data. **A.** t-SNE plots of 60,636 random cells from total CD8⁺ T cells of the four analyzed groups and unsupervised KNN algorithm for clustering. Cells are colored according to their cluster. **B.** Representative visualization of iR expression and lineage markers by t-SNE. The color gradient displays the relative marker expression. **C.** Heatmap representation of the median biexponential-transformed marker expression normalized to a -3 to 3 range of respective markers within the 23 cellular clusters. **D.** Representation of S1 and S2 differentially expressed clusters 10, 11, 12 and 18. **E.** Distribution percentages of TIGIT, TIM-3, LAG-3 and CD45RA distribution for all groups in clusters 10, 11, 12 and 18. For statistical analysis, we used Mann-Whitney for unpaired samples and Wilcoxon test for paired samples. p values:*<0.05, **<0.005.

• Cluster decomposition identify overexpression of TIGIT⁺ alone or combined with TIM-3⁺, LAG-3⁺ and CD39⁺ in CM and TM CD8⁺ T-cells during long-term cART



A. SPICE distribution on iR polyexpression grouped by TIGIT, PD-1, LAG-3, TIM-3 and CD39. Pie chart indicates number of combinations from 0 to 5 iRs. Each pie slice represents the median proportion of one combination in CM and TM CD8⁺ T cells. **B.** TIGIT⁺ at single level on CM and TM. **C.** TIGIT⁺TIM-3⁺ in CM and Eff. **D.** TIGIT⁺TIM-3⁺CD39⁺ combinations on CM, Eff and EM. **E.** TIGIT⁺TIM-3⁺LAG-3⁺ combinations in Eff. For statistical analysis, we used Mann-Whitney for unpaired samples and Wilcoxon test for paired samples. P values: *<0.05, **<0.005.

• Negative correlation between TIGIT⁺ and combinatorial expression of iR and CD4⁺ T-cell counts



Correlations between CD4⁺ T-cell counts and iR expression in CD8⁺ T cells in long-term treated individuals. **A.** Percentage of single TIGIT⁺ on total CD8⁺ T cells as a function of CD4⁺ T-cell counts. **B.** One or more iRs expressed on total CD8⁺ T cells as a function of CD4⁺ T-cell counts. **C.** Absence of iR expression on total CD8⁺ T cells as a function of CD4⁺ T-cell counts.

CONCLUSIONS

- iR expression on CD8⁺ T cells is subset specific and normalized in a time dependent fashion in cART.
- Single-cell analyses identified four clusters of cells irreversible altered despite long-term cART.
- Clusters showed memory and effector-like features and specific combinations of iRs.
- Cluster decomposition by SPICE analysis confirmed lack of normalization of the expression of TIGIT⁺ alone or combined in minor proportion with others iRs.
- CD4⁺ T-cell counts were negatively correlated with TIGIT⁺ or combinatorial expression of iRs on total CD8⁺ T cells

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CROI Conference on Retroviruses and Opportunistic Infections

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