Inducible HIV reservoirs display a senescent-like phenotype and are sensitive to senolytics

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Introduction

Identifying the mechanisms contributing to the persistence of HIV reservoirs during antiretroviral therapy (ART) is essential to the development of strategies aimed at eradicating them. Since senescent T cells accumulate with age and infection, we hypothesized that the HIV reservoir may preferentially persist in these cells after prolonged ART, and could therefore potentially be sensitive to senolytic drugs.

Phenotyping the inducible, translation competent reservoir

Protocol

1) PBMCs are thawed and enriched in CD4⁺ T cells

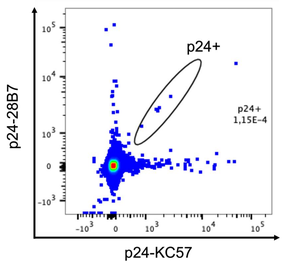
2) CD4⁺ T cells are stimulated 24h:
12.5µg/mL anti-CD, 3.5µg/mL anti-CD28, 10ng/mL IL-2

3) Cells are harvested and stained :
- CD3, CD4, CD8, CD45RA, CCR7
- Live/Dead
- p24 (x2)
- TIGIT, PD-1, CTLA-4,
- IL-2, IL-17, TNFα, IFNγ
- Granzymes A, B and K
- CD57, KLRG1

Materials & Method

Strategy

HIV-Flow : 2 antibodies targeting p24



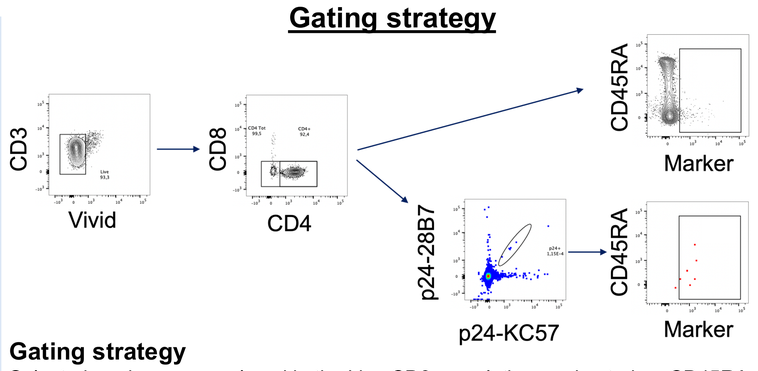
Example of p24 staining
Two antibodies are used, targetting two different epitopes of the p24 protein. For added specificity, only the double positive cells (inside the diagonal gate) are considered p24⁺.

Participants

11 ART treated PWH
All males
Median age : 50 years old
Median time on ART : 11.5 years

3 untreated PWH
All males
Median age : 45 years old

Gating strategy

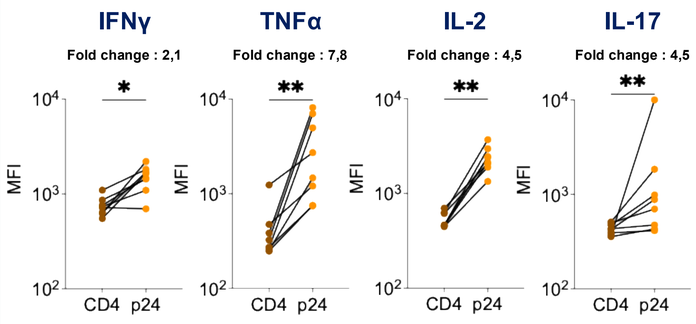


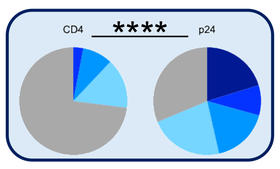
Gating strategy
Selected markers are analyzed in the Live CD8⁻ population, and gated on CD45RA

HIV reservoir cells express higher levels of specific cytokines, granzymes, immune checkpoints and senescence markers than uninfected

We measured the level of expression of several markers to find which ones were increased in the **memory p24⁺ cells** compared to the **memory CD4⁺ T cells**

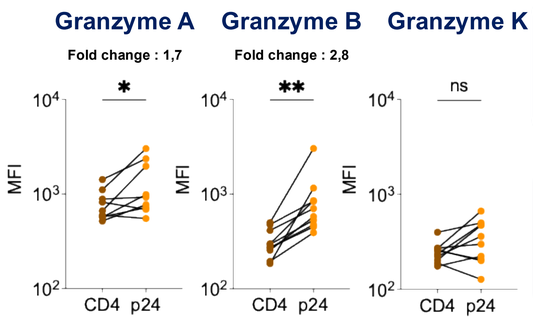
Cytokine production

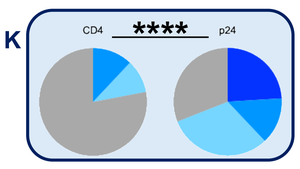




p24⁺ cells express higher levels of cytokines, and express several cytokines simultaneously

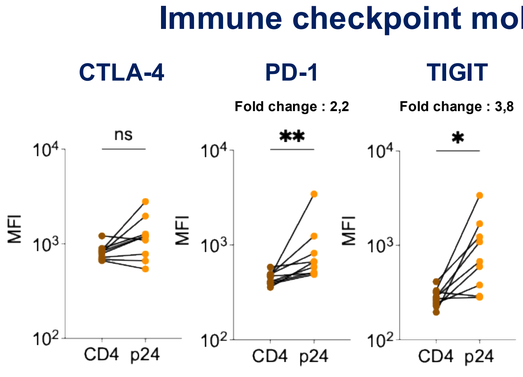
Granzymes

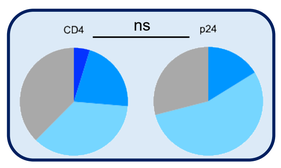




p24⁺ cells express higher levels of granzymes A and B, and express several granzymes simultaneously

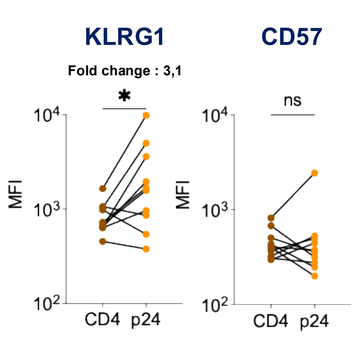
Immune checkpoint molecules

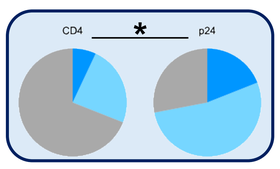




p24⁺ cells express higher levels of PD-1 and TIGIT

Senescence markers





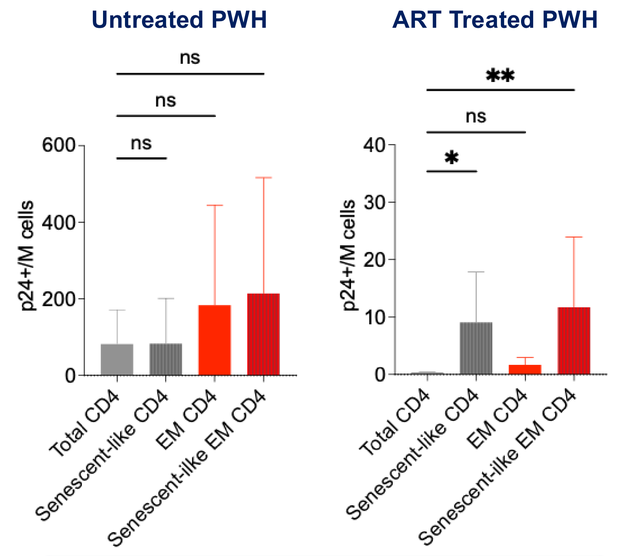
p24⁺ cells express higher levels of KLRG1

Graphs show the level of expression of each marker, represented by the MFI. We compared these levels between the memory CD4⁺ T cells (in **brown**) and the memory p24⁺ cells (in **orange**).
Each ● represents a participant.
Statistical differences were determined using the Mann-Whitney test.

Pie charts show the co-expression of the markers in the memory CD4+ T cells, compared to the memory p24+ cells. 4 3 2 1 0
Statistical differences were determined using Fisher's exact test.

HIV reservoir cells are enriched in subsets of senescent-like cells

We characterized a « senescent-like phenotype » as being KLRG1⁺ Granzyme⁺ Cytokine⁺.
We compared the levels of p24⁺ cells found in cells harboring this phenotype to the levels in the total CD4⁺ T cell population



The p24⁺ population is enriched in cells expressing simultaneously KLRG1, granzymes and cytokines, and this enrichment is independant of their effector memory status.
This enrichment is not found in untreated PWH, suggesting that prolonged ART may be necessary for this phenotype to emerge.
Longitudinal studies will be required to confirm this hypothesis.

Histograms show the frequency of p24⁺ cells found in each marker.
Statistical differences were determined using one-way ANOVA.

Can senescent-like p24+ cells be eliminated with senolytics?

Materials & Method

Participants

5 ART treated PWH
All males
Median age : 59 years old
Median time on ART : 17 years

Protocol

1) PBMCs are thawed and enriched in CD4⁺ T cells

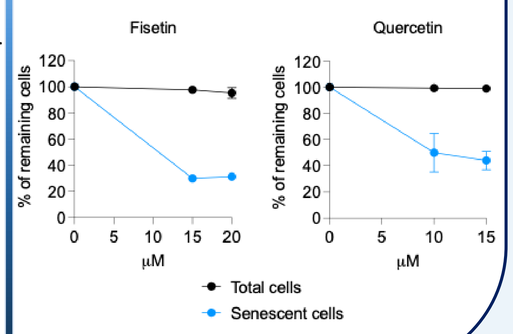
2) Cells are treated with 20µM of the pancaspase inhibitor Q-VD-Oph and rested for 1h

3) Cells are stimulated with 162nM of PMA and 1µg/mL of Ionomycin, and treated with senolytic drugs for 24h : 15µM of Quercetin or 15µM of Fisetin

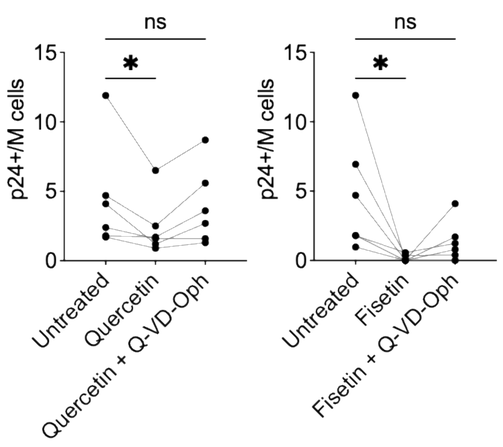
4) The frequency of p24⁺ cells is determined by HIV-Flow, with the following markers: CD3, CD4, CD8, Live/Dead, p24 (x2)

Treatment

Fisetin and Quercetin were used at 15µM, a dose determined to be able to eliminate more than 50% of senescent cells (characterized as being CD28⁻ KLRG1⁺ CD57⁺), without altering the global CD4⁺ T cell population.



Senolytics eliminate HIV reservoir cells



Treatment with Quercetin or Fisetin leads to a reduction of p24⁺ cells (2-fold and 30-fold respectively).
When Q-VD-Oph, a cell death inhibitor, is added, the level of p24⁺ cells is rescued, indicating that the p24⁺ cells are indeed being killed by the drugs, and not just unable to express the p24 protein

Graphs show the frequency of p24⁺ cells obtained for each condition.
Each ● represents a participant.
Statistical differences were determined using one-way ANOVA

Conclusion

Our data suggest that cells of the inducible, translation competent reservoir are enriched in a senescent-like phenotype defined as being KLRG1⁺ Granzyme⁺ Cytokine⁺, and that these cells can be specifically eliminated by senolytics.

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Conflict of Interest Disclosure "I have no conflicts of interest"