Molecular mechanisms of the CD8+ T cell-mediated non-cytolytic silencing of HIV-1 transcription

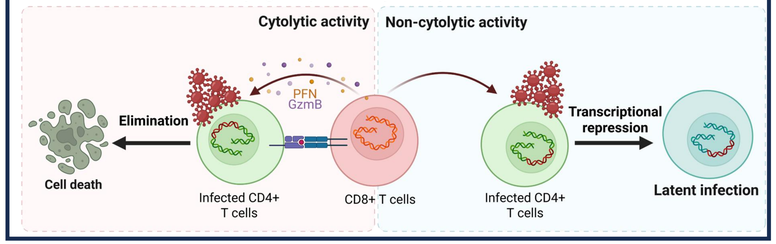
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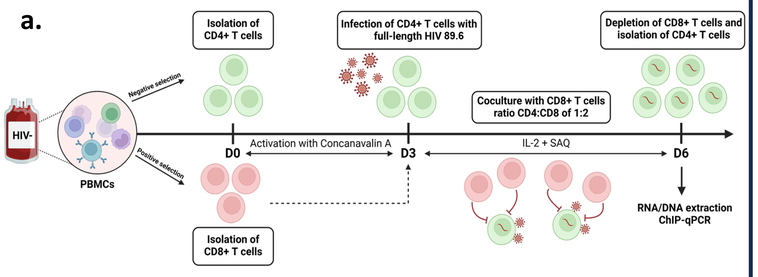
BACKGROUND

Despite ART, HIV-1 persistence in latently-infected reservoirs remains the major challenge of HIV-1 infection. Therefore, a deeper understanding of the molecular mechanisms governing HIV-1 latency is essential to achieve a functional cure. In this context, the shock-and-kill HIV cure strategies aim at reactivate viral reservoirs using latency reversing agents (LRAs). However, the clinical efficacy of current LRAs has been limited so far notably due to the in vivo of CD8+ T cells. Indeed, studies have demonstrated that beyond their cytotoxic activity, CD8$^+$ T cells paradoxically contribute to the maintenance of latent reservoirs by promoting HIV-1 transcriptional silencing in infected CD4$^+$ T cells, notably by enhancing their survival, stemness and quiescence. However, the precise molecular mechanisms underlying this pro-latency activity remain unknown.



METHODS

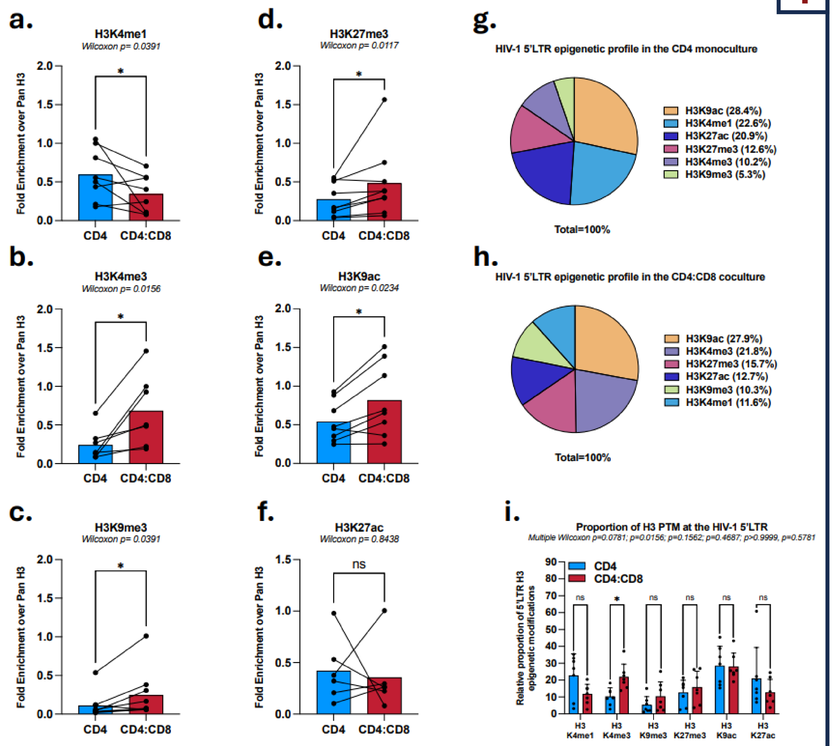
To accurately capture the complexity of the mechanisms involved in the non-cytotoxic, pro-latency activity of CD8$^+$ T cells in a physiological context, we established an *in vitro* primary cell-based model.



Development of a primary cell model to study the effect of CD8+ T cells on the establishment of HIV-1 latency.

(a) Experimental workflow schematic. Briefly, CD4$^+$ and CD8$^+$ T cells were enriched from PBMCs of HIV-naïve donors and activated with concanavalin A for 3 days. CD4$^+$ T cells were infected with HIV-1 strain 89.6 by spinoculation at day 3, then cultured alone (monoculture) or with CD8$^+$ T cells at a 1:2 ratio (co-culture) in medium containing IL-2 and saquinavir for 3 additional days. CD8$^+$ T cells were depleted at day 6 prior to CD4$^+$ T-cell isolation for downstream investigations (including RNA/DNA extraction and ChIP-qPCR). **(b)** Flow cytometry analysis of CD8 surface expression in co-cultured cells before and after CD8$^+$ T-cell depletion. **(c)** Flow cytometry assessment of CD4$^+$ T-cell viability in monoculture and coculture conditions using live/dead staining that stains dead cells. **(d)** Quantification of HIV-1 unspliced RNA levels in CD4$^+$ T cells from monoculture and co-culture conditions by RT-qPCR. Data are representative of results from at least three independent experiments. Statistical analysis methods and corresponding p values are indicated above each graph.

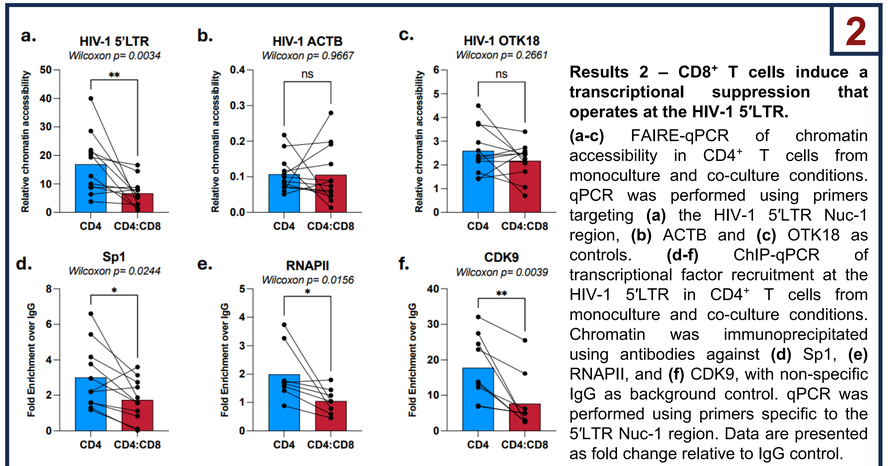
RESULTS 1



Results 1 – CD8$^+$ T cell pro-latency activity is associated to a shift from permissive to repressive chromatin at the HIV-1 5'LTR.

(a-f) ChIP-qPCR quantification of histone modifications at the HIV-1 5'LTR in CD4$^+$ T cells from monoculture and co-culture conditions. Chromatin was immunoprecipitated using antibodies against H3K4me1, H3K4me3, H3K9me3, H3K27me3, H3K9ac, and H3K27ac, with Pan-H3 as control. qPCR was performed using primers specific to the 5'LTR Nuc-1 region. Data are presented as fold change relative to Pan-H3 control. **(g-h)** Relative proportion of each histone modification at the 5'LTR calculated from ChIP-qPCR data in **(g)** monoculture and **(h)** co-culture conditions. **(i)** Statistical comparison of the magnitude of change for each histone modification between monoculture and co-culture conditions.

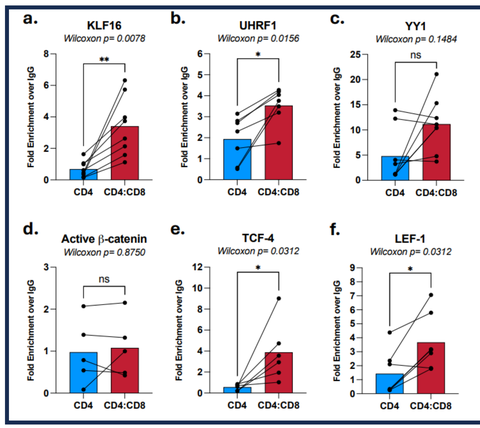
RESULTS 2



Results 2 – CD8$^+$ T cells induce a transcriptional suppression that operates at the HIV-1 5'LTR.

(a-c) FAIRE-qPCR of chromatin accessibility in CD4$^+$ T cells from monoculture and co-culture conditions. qPCR was performed using primers targeting **(a)** the HIV-1 5'LTR Nuc-1 region, **(b)** ACTB and **(c)** OTK18 as controls. **(d-f)** ChIP-qPCR of transcriptional factor recruitment at the HIV-1 5'LTR in CD4$^+$ T cells from monoculture and co-culture conditions. Chromatin was immunoprecipitated using antibodies against **(d)** Sp1, **(e)** RNAPII, and **(f)** CDK9, with non-specific IgG as background control. qPCR was performed using primers specific to the 5'LTR Nuc-1 region. Data are presented as fold change relative to IgG control.

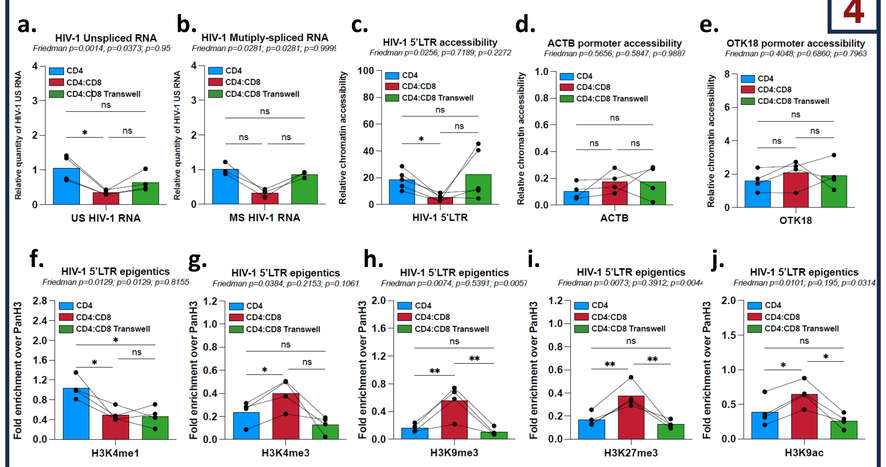
RESULTS 3



Results 3 – CD8$^+$ T cells-mediated HIV-1 silencing involves KLF16, UHRF1, and β-catenin-independent TCF/LEF recruitment to the 5'LTR.

(a-c) ChIP-qPCR quantification of transcription factors recruitment at the HIV-1 5'LTR in CD4$^+$ T cells from monoculture and co-culture conditions. Chromatin was immunoprecipitated using antibodies against **(a)** KLF16, **(b)** UHRF1, **(c)** YY1, **(d)** active β-catenin, **(e)** TCF-4, and **(f)** LEF-1, with non-specific IgG as background control. qPCR was performed using primers specific to the 5'LTR Nuc-1 region. Data are presented as fold change relative to IgG control. Data are representative of results from at least three independent experiments.

RESULTS 4



Results 4 – CD8$^+$ T cell-mediated HIV-1 silencing require close proximity for maximal suppression

(a-c) RT-qPCR quantification of HIV-1 RNA classes in CD4$^+$ T cells from three culture conditions: monoculture, direct co-culture with CD8$^+$ T cells, and transwell separation from CD8$^+$ T cells. **(a)** unspliced (US) and **(b)** multiply spliced (MS) HIV-1 RNA levels were measured. **(c-e)** FAIRE-qPCR analysis of chromatin accessibility in CD4$^+$ T cells using primers targeting **(c)** the HIV-1 5'LTR Nuc-1 region, **(d)** ACTB promoter, and **(e)** OTK18 promoter. **(f-j)** ChIP-qPCR quantification of histone modifications at the HIV-1 5'LTR in CD4$^+$ T cells. Chromatin was immunoprecipitated using antibodies against **(f)** H3K4me1, **(g)** H3K4me3, **(h)** H3K9me3, **(i)** H3K27me3, and **(j)** H3K9ac, with Pan-H3 as control. qPCR was performed using primers specific to the 5'LTR Nuc-1 region. Data are presented as fold change relative to Pan-H3 control. Data are representative of results from at least three independent experiments.

CONCLUSION

Our results constitute the first direct demonstration that CD8+ T cells trigger mechanisms that lead to known critical features of epigenetic and transcriptional HIV silencing in CD4+ T cells. This is of major interest for more effective HIV cure strategies : (1) by increasing the CTL function of CD8+ T cells to reduce the size of the HIV reservoir, and (2) by inhibiting the CD8 pro-latency activity to improve HIV reactivation by LRA and translate in next-generation shock-and-kill clinical trials.

PLAIN LANGUAGE SUMMARY

We identified cellular factors that enable CD8$^+$ T cells to keep HIV dormant inside infected CD4$^+$ T cells, contributing to HIV-1 long-term persistence. Targeting these factors could help counteract this CD8$^+$-driven HIV persistence and ultimately improve the effectiveness of future HIV-1 cure strategies.

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