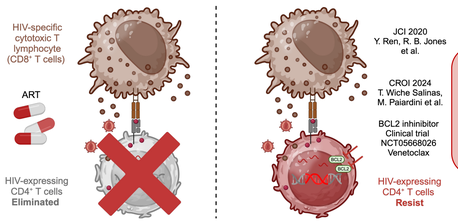
The Soft Escape: HIV alters Host Cell Cytoskeleton as a mechanism of resistance to CTL Killing

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1. Background

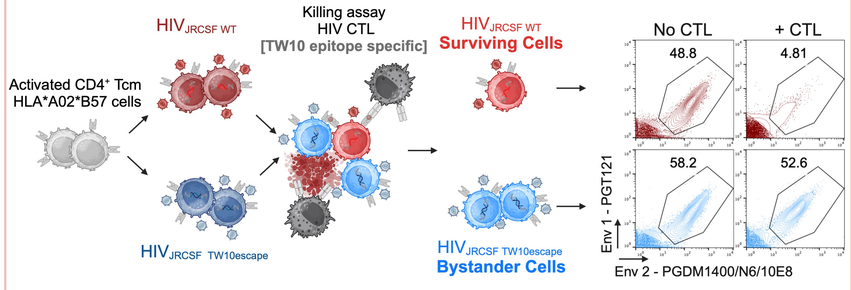
- HIV-expressing CD4⁺ T cells persist under long-term antiretroviral therapy (ART) and there is evidence of on-going antigen stimulation of HIV-specific CTL.
- We hypothesized that a selection of survivors with CTL-resistant properties may limit the elimination of infected cells
- Cancer cells resistance to CTL elimination is well known in the oncology field. In HIV, we showed that BCL-2 (inhibiting apoptosis) antagonists sensitize CTL resistant HIV reservoirs to elimination



Question : What additional mechanisms confer resistance to CTL-mediated killing in HIV-expressing CD4+ T cells?

2. Methods

Central memory CD4+ T cells (Tcm) from each of 4 HIV-neg donors were divided and infected with either 'WT'JRCSF or 'TW10esc'JRCSF with an escape mutation in the Gag-TW10 CTL epitope. These were labeled with CTFR (WT) or CFSE (TW10esc), mixed and cultured with or without TW10-specific CTL clones.



3. Results

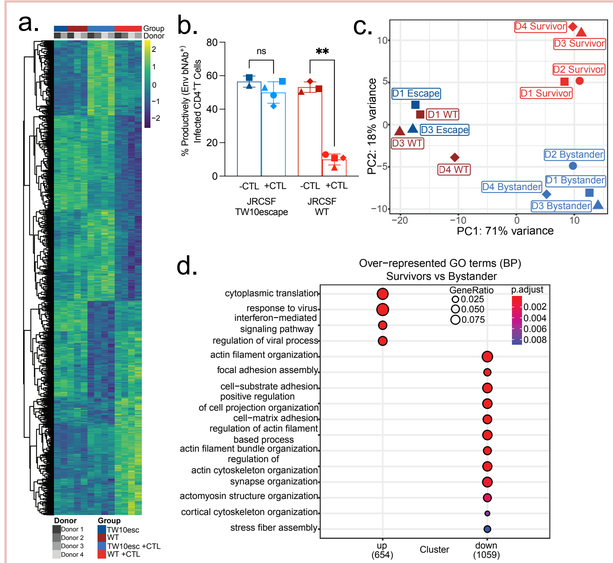


Figure 1: A subset of HIV-expressing cells resist CTL-mediated killing through distinct transcriptional signatures, including cytoskeleton genes downregulation

a. Heatmap: differentially expressed genes from bulk RNAseq comparing HIV JRCSF TW10escape and HIV JRCSF WT before and after CTL killing. n= 4 donors

b. Killing assay: percentage of Env+ productively infected cells before and after killing with HIV JRCSF TW10escape and WT in the same wells. n=4 donors, One-way ANOVA was performed (p<0.0001).

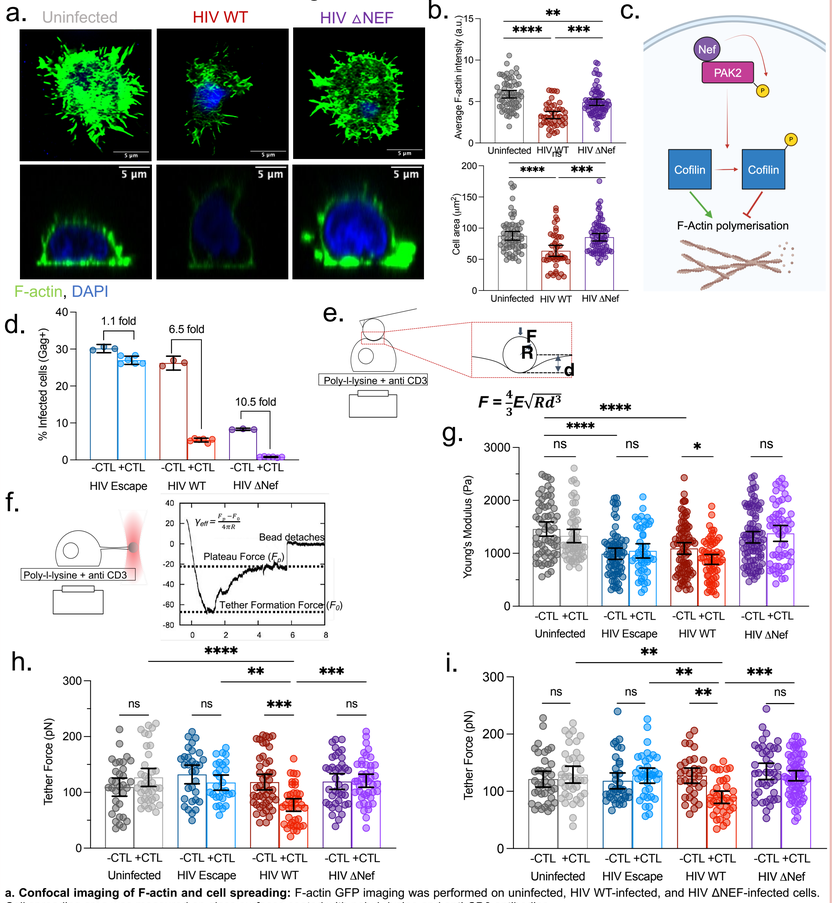
c. Principal Component Analysis (PCA) plot: illustrating RNA transcriptional profiles of: HIV-infected cells (WT or TW10escape) before CTL challenge (dark blue and red), HIV WT-infected surviving cells post-CTL killing (red) and HIV TW10escape-infected bystander cells post-CTL killing (blue)..

d.Gene Ontology analysis of differential gene expression
Pathway analysis highlighting upregulated and downregulated transcriptional profiles in survivors (pCTL WT) compared to bystander cells (pCTL TW10).

e.Schematic of enrichment and depletion of the WASP/WAVE pathway. Sharing indicating log2FC, with cytoscape.

c.Schematic of HIV-infected cells heterogeneity in stiffness : lower stiffness target known to be harder to kill by CTL

Figure 2: HIV-Nef deceases the average stiffness of HIV-infected cells, enabling the softest fractions of cells to resist CTL killing



a. Confocal imaging of F-actin and cell spreading: F-actin GFP imaging was performed on uninfected, HIV WT-infected, and HIV ΔNEF-infected cells. Cell spreading area was measured on glass surfaces coated with poly-L-lysine and anti-CD3 antibodies.

b.. F-actin quantification & Cell spreading area quantification : Uninfected cells (grey), HIV WT-infected cells (red), and HIV ΔNEF-infected cells (purple). Each point represents an individual cell. One-way ANOVA was performed (p<0.0001).

c.Schematic: HIV Nef alters cellular cytoskeleton through PAK2 phosphorylation

d. Competitive killing assay: Percentage of surviving HIV JRCSF TW10-, WT-, and ΔNEF-infected cells after 16h co-culture with TW10 CTLs (E:T 1:1). n=3 donors.

e. Schematic: Atomic force microscopy for cell stiffness measurement (Pa) in fixed cells.

f. Schematic: Optical tweezers for membrane tension measurement (pN) in live cells.

g. Cell stiffness in infected Tcm CD4+ T cells, before and after CTL-mediated killing. One-way ANOVA, p<0.0001. n=2 donors, two experiments per condition. Each data point represents a single cell.

h. & i. Membrane tension in Tcm CD4+ T cells uninfected or infected with HIV JRCSF WT, ΔNEF, or TW10escape viruses, before and after CTL-mediated killing. One-way ANOVA, p<0.0001. n=2 donors, two experiments per condition. Each data point represents a single cell.

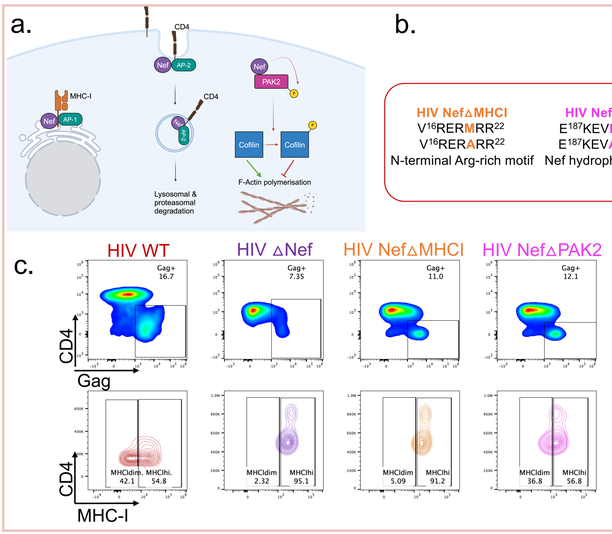


Figure 3: HIV-Nef enhances CTL resistance by modulating cellular cytoskeleton via PAK2, reducing cell stiffness and membrane tension.

a. Schematic representation of the interactions between the viral protein Nef and host cell proteins AP-1, AP-2, and PAK2.

b. Single amino acid mutagenesis: A mutation in Nef designed to prevent MHC-I downregulation or interaction with PAK2.

c. Flow cytometry analysis of HIV-expressing cells showing MHC-I downregulation with various mutants.

d. & e. Competitive killing assay: Percentage of surviving cells infected with HIV. JRCSF WT, NEFΔMHCI, or NEFΔPAK2 after 16 hours with TW10 CTLs (E:T ratio 1:1). n=2 donors.

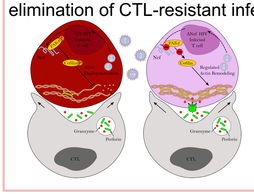
f. Cell stiffness in infected Tcm CD4+ T cells before and after CTL-mediated killing. One-way ANOVA, p<0.0001, two experiments per condition

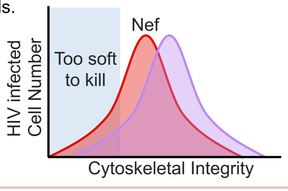
g. Membrane tension in Tcm CD4+ T cells infected with HIV JRCSF WT, NEFΔMHCI, ΔNEF, or NEFΔPAK2, before and after CTL-mediated killing. One-way ANOVA, p<0.0001; two experiments per condition.

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4. Conclusion

Our findings reveal a collaboration between host-cell biophysical heterogeneity and HIV-Nef in promoting immunevasion. Nef reduces infected CD4+ T-cell stiffness and membrane tension by altering the cytoskeleton, allowing the softest cells to evade CTL-mediated killing. Therapeutics targeting cell stiffness, either by inhibiting Nef's cytoskeletal effects or modulating host factors, may enhance elimination of CTL-resistant infected cells.





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