Dendritic cells and IL-7 synergize to increase latent-infected CD4⁺ T cell populations

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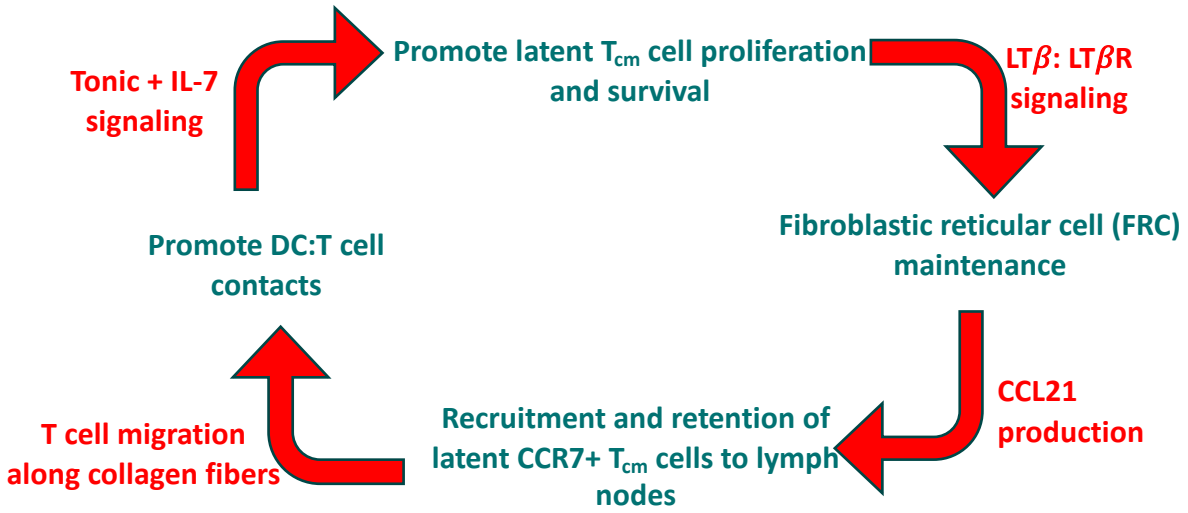
1. BACKGROUND

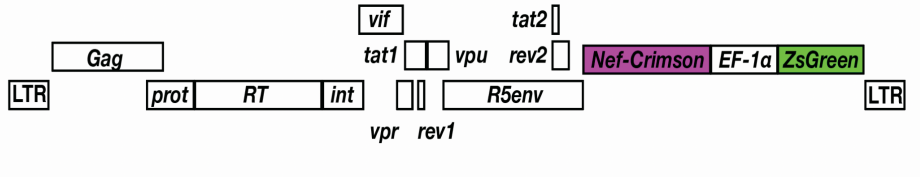
- Viral latency is a state of reversibly nonproductive infection of HIV reservoirs (R.F. Siliciano and W.C. Greene, 2011)
- HIV-1 latent reservoir is a cell type or anatomical site that allows persistence of replication competent HIV-1 for long periods of time in patients on optimal ART (R.F. Siliciano and W.C. Greene, 2011)
- **Establishment of the latent reservoir is a critical barrier to HIV cure** (Matthew D Marsden & Jerome A Zack, 2019

2. LYMPHOID ORGANS AS A "HUB" FOR MAINTAINING SURVIVAL OF NAÏVE AND MEMORY T CELLS



3. METHOD TO VISUALIZE LATENTLY-INFECTED T CELLS

- **Generate a full-length HIV latency reporter to follow latently-infected T cells in real-time.**



AIM

- To investigate the effect of dendritic cells and IL-7 on latently infected CD4⁺T cell populations.

HYPOTHESIS

- IL-7 and signalling thorough DC:T cell interactions contribute to the maintenance of HIV latently infected CD4⁺T cells *in vivo*

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RESULTS

(1.) $HIV_{Nef\text{-}CRMZY}$ delineates between productive and latent HIV infections in CD4⁺ T cells

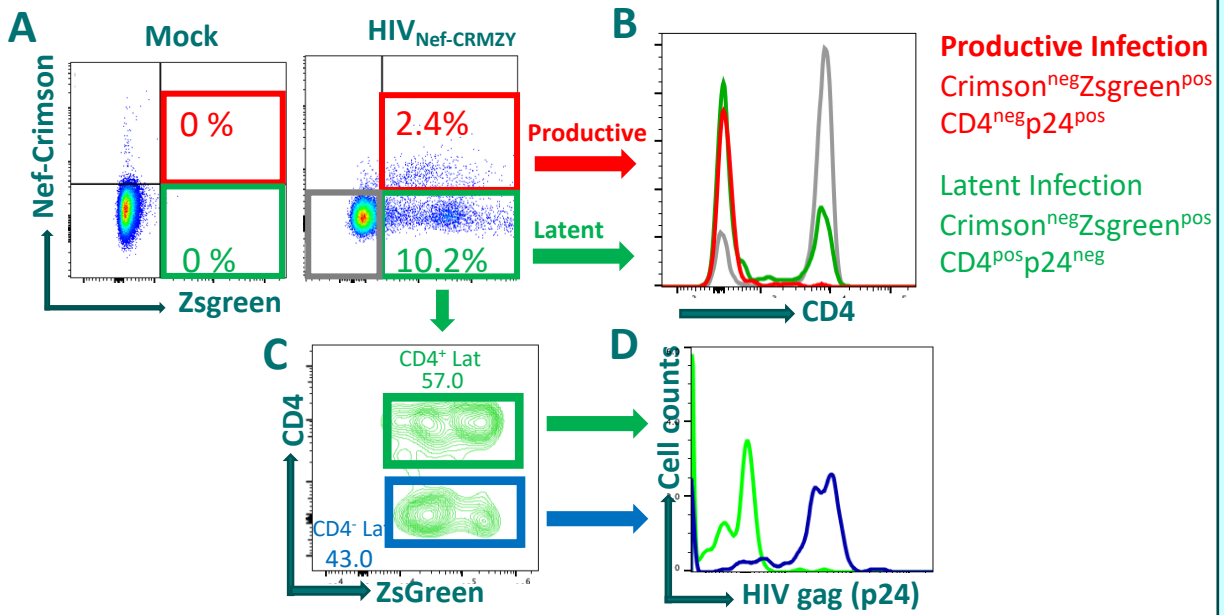

Figure 1:HIV $_{Nef\text{-}CRMZY}$ delineates between productive and latent HIV infections in CD4⁺ T cells
Naïve CD4+ T cells were isolated from PBMC and infected with HIV $_{Nef\text{-}CRMZY}$ at an MOI of 0.1. 5days post infection, productively infected cells (red, E2Crimson⁺ZsGreen1⁺) and latently infected cells (green,E2Crimson⁻ZsGreen1⁺) were visualized by flow cytometry (A). CD4 downregulation in productively infected cells (red, E2Crimson⁺ZsGreen1⁺) and latently infected cells (green,E2Crimson⁻ZsGreen1⁺) was analyzed (B). latently infected cells (green,E2Crimson⁻ZsGreen1⁺) was analyzed for their CD4 expression (C). CD4⁺ latent T cells and CD4⁻ Latent T cells were analyzed for HIV gag (p24) (D). Results are representative of 5 independent experiments.

(2.) Latent $HIV_{Nef\text{-}CRMZY}$ infected CD4⁺ T cells can be reactivated

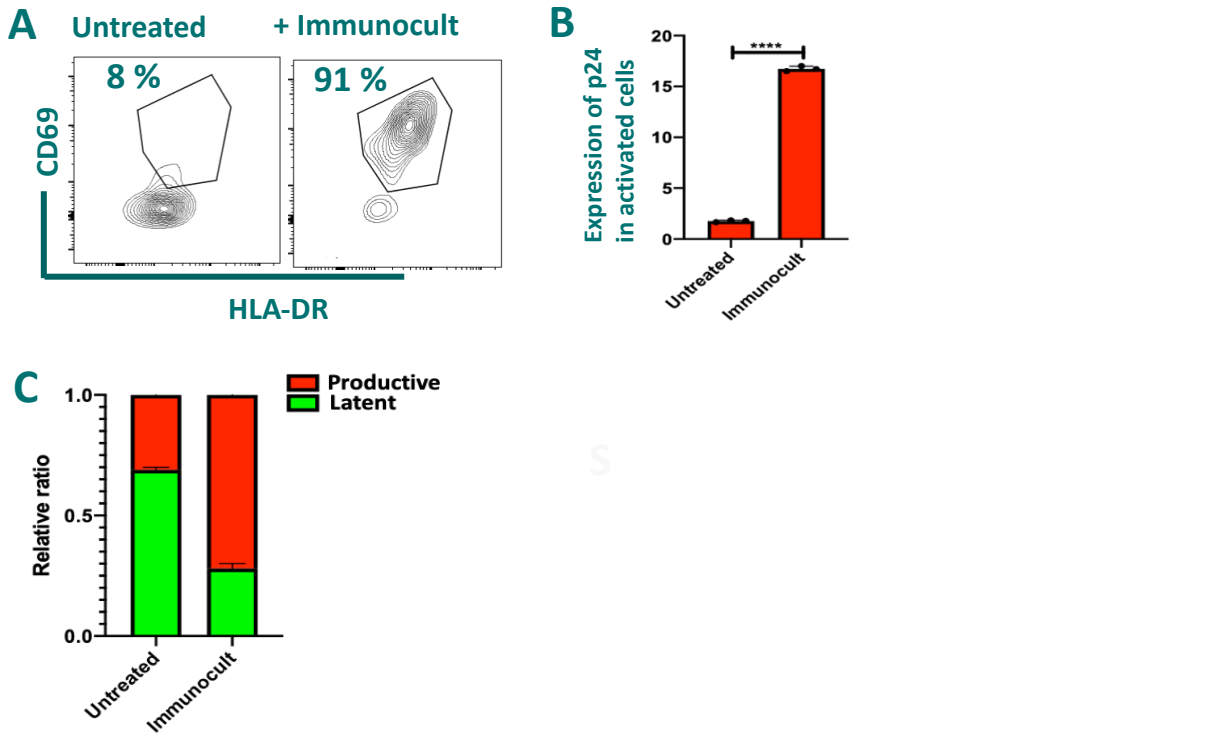

Figure 2: Latent HIV $_{Nef\text{-}CRMZY}$ infected CD4⁺ T cells can be reactivated
CD4⁺ T cells were infected with HIV $_{Nef\text{-}CRMZY}$ for 5days. After 5days, immunocult (CD3/CD28) cell activator was used to reactivate latently infected cells for 24hrs, activation status was confirmed by assessing CD69⁺HLA-DR⁺) (A). Activated latent cells were assessed for evidence of productive infection by evaluating p24 staining (B). Relative ratio of productive infected cells and latently infected cells were analysed (C).****, $p < 0.001$.

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(3.) Dendritic cells and IL-7 synergize to maintain CD4⁺ Latent (P24⁻) T cells after infection

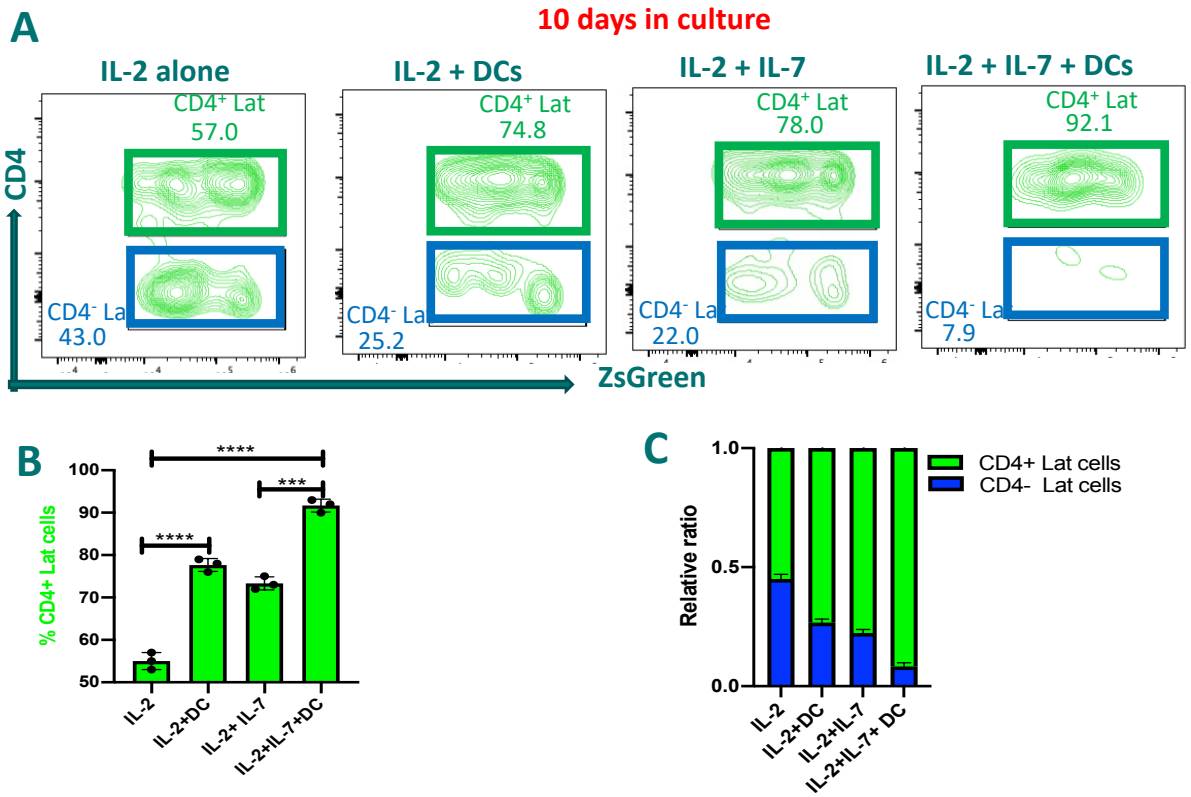

Figure 3: Addition of autologous Dendritic cells and IL-7 synergize to expand the proportion of CD4⁺ p24⁻ ZsGreen1⁺ latently infected T cells

CD4⁺T cells were infected with $HIV_{Nef\text{-}CRMZY}$ for 5 days. At 5 days post-infection, IL-7 (50ng/ml) and DCs (1 DC: 3 T cells) were added to the culture. 10 days later, the proportion of latently infected cells were assessed by flow cytometry (A). Percentage of CD4 expressing latently infected T cells were graphically represented (B). Relative ratio of latently infected (CD4+ and CD4- T cells) were assessed (C). ***, $p < 0.001$. ****, $p < 0.0001$.

(4.) Dendritic cells and IL-7 promotes proliferation (Tag-IT ^{neg}) and survival (BCL2 ^{Pos}) of CD4⁺ Latent (P24⁻) T cells after infection

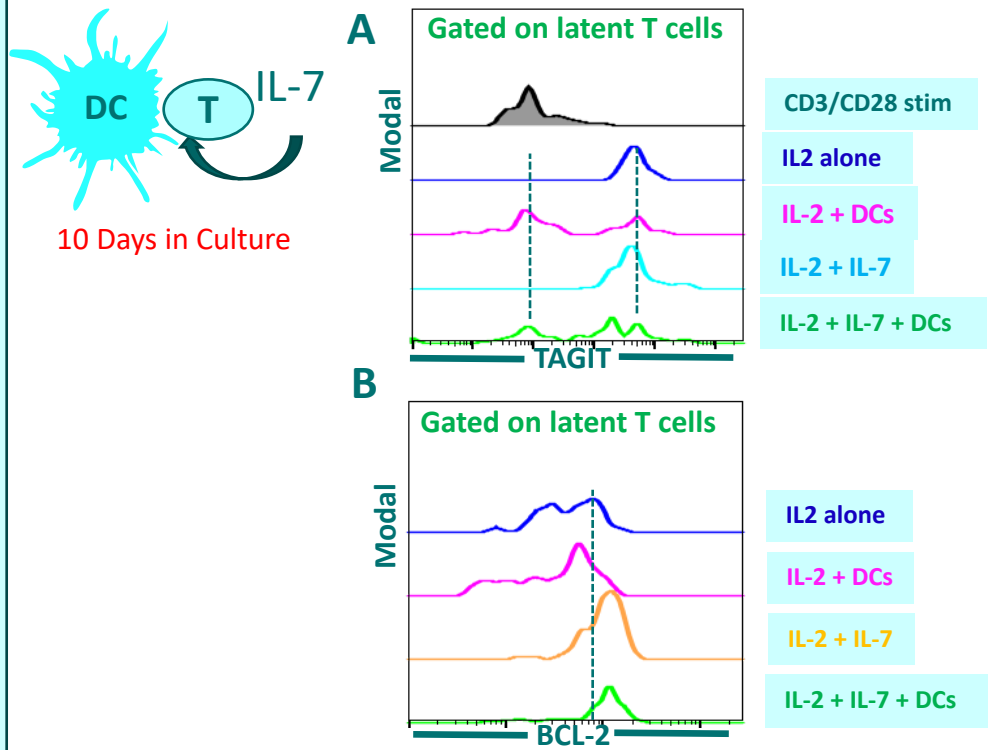

Figure 4: Addition of autologous Dendritic cells and IL-7 synergize to expand/maintain the proportion of CD4⁺ p24⁻ZsGreen1⁺ latently infected T cells

CD4⁺T cells were infected with $HIV_{Nef\text{-}CRMZY}$ for 5 days. At 5 days post-infection, IL-7 (50ng/ml) and DCs (1 DC: 3 T cells) were added to the culture. 10 days later, the population of proliferated (TagIT ^{neg}) latently infected cells were assessed by flow cytometry (A). Population of latently infected T cells expressing pro-survival molecule (BCL-2) were assessed by flow cytometry (B).

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SIGNIFICANCE AND CONCLUSIONS

- We have developed a dual reporter HIV that encodes for all HIV proteins and allows us to follow latently infected T cells for up to 15 days in culture.
- Importantly, the expression of full-length Nef allows us to use CD4 downregulation as a sensitive marker of Nef expression and define latently-infected cells using our system as ZsGreen1$^+$CD4^{high}p24^{neg}.
- We show that physiological DC:T cell interactions in the lymph node, along with IL-7 signaling, that help promote homeostatic proliferation of central memory T cells, may also be co-opted by latently infected T cells for their own survival and proliferation.
- This may help explain why latent T cells can persist for many years and represents a major barrier to achieve HIV cure in infected individuals, unless these mechanisms are disrupted.

RELEVANCE OF OUR FINDING TO HIV STUDY

- Our data suggest that dendritic cells and IL-7 may synergize to facilitate latently-infected T cell expansion by promoting cell survival and/or proliferation.
- Our *in vitro* model system will help define cellular and molecular mechanisms that may lead to the expansion of the viral reservoir, with the possibility of testing whether antigen recognition also play a role in this process.

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FUTURE DIRECTIONS

- Evaluate the mechanism of DC mediated maintenance of CD4⁺ latent T cells (MHC-II blockade, CD80 and CD86 blockade)
- Visualize latently infected CD4⁺ T cells in the lymph node of humanized mice.
- Assess whether, antigens (e.g. flu, gut bacteria) preferentially expands latent T cells in the presence of dendritic cells and IL-7 without causing reactivation of latent T cells

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CanCURE
Together, we Can CURE HIV

CIHR IRSC
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