“Kick and Kill” of Latent HIV-1 Infection in Naïve and Central Memory CD4⁺ T cells

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ABSTRACT

Background: A “kick and kill” strategy, which involves the administration of a latency reversing agent (LRA) to induce HIV-1 out of latency and promote death of the HIV-1-infected cells by cytolytic or viral cytopathic effects, may eradicate the latent HIV-1 reservoir in resting CD4⁺ T cells. However, the resting CD4⁺ T cell population is heterogeneous and is composed of distinct subsets that differ in lifespan, proliferative capacity, antigen response time, and in CCR5 and CXCR4 expression levels. It is not known whether the “kick and kill” strategy will be effective in each of the T cell subsets. Therefore, we investigated latency reversal in purified CD4+ naïve (T_N) and central memory (T_{CM}) T cells, which together constitute ~ 70-80% of the resting CD4⁺ T cell population.

Methods: We developed primary cell models of both R5- and X4-tropic HIV-1 latency in T_N and T_{CM} CD4⁺ T cells, using a direct infection protocol as described previously (Saleh et al. Blood 2007;110:4161). Latency reversal (assessed by extracellular HIV-1 RNA) and concomitant virus-induced cell death was evaluated after exposure to anti-CD3/CD28 antibodies, PMA/PHA, prostratin or SAHA.

Results: Consistent with previously published data, both R5- and X4-tropic viruses infected T_N cells less efficiently than T_{CM} cells. Consequently, the frequency of latently infected T_N cells was significantly lower than in the T_{CM} cells. Stimulation with anti-CD3/CD28 antibodies, PMA/PHA or prostratin, effectively reactivated latent HIV-1 in both T cell subsets. When the data were normalized for infection frequency more extracellular virus production was observed in X4-tropic (but not R5) infected T_N cells compared to T_{CM} cells. Interestingly, we found a strong correlation between the number of latently infected cells and extracellular virus production in T_{CM} cells, but not in T_N cells. HIV-1 DNA in both T_N and T_{CM} cells decayed with half-lives ranging from 2.5 to 5.4 days after exposure to anti-CD3/CD28 antibodies, PMA/PHA or prostratin. In comparison, SAHA was unable to reactivate latent HIV-1 in either T_N or T_{CM} cells, and consequently the latently infected cells persisted after exposure to drug.

Conclusions: We have developed robust primary cell models of HIV-1 latency in CD4⁺ T_N and T_{CM} cells that can be used to better understand the establishment and reversal of latency in these key reservoirs of HIV-1 infection. Importantly, we demonstrate decay of the latent HIV-1 reservoir in both T cell subsets when the LRA effectively stimulates virus production.

METHODS

Purification of resting CD4⁺ T_N and T_{CM} cells from blood

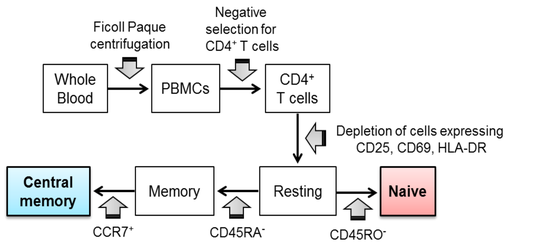


Figure 1: Isolation of purified resting CD4⁺ T_N and T_{CM} cells. PBMCs were isolated from whole blood followed by negative selection for resting CD4⁺ T cells. The resting CD4⁺ T cells were then divided into two tubes for negative selection of memory cells (CD45RA⁻, CD45RO⁺) and T_N cells (CD45RO⁻, CD45RA⁺). The memory CD4⁺ T cells were further purified into T_{CM} cells by positive selection for cells expressing CCR7.

Primary cell model of HIV-1 latency in resting CD4⁺ T_N and T_{CM} cells

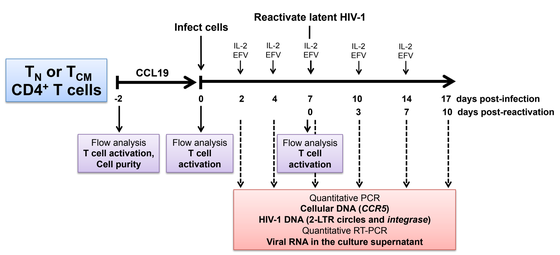


Figure 2: Primary cell model of HIV-1 latency in resting CD4⁺ T_N and T_{CM} cells. Purified T_N and T_{CM} cells were exposed to CCL19 (100nM) for 2 days, before being infected with HIV-1 (MOI=1). Latency was established over 7 days. Cell purity and cellular activation were assessed by flow cytometry. After the 7 day infection, cells were treated with different latency reversing agents for 10 days.

RESULTS

Effect of CCL19 treatment on T cell activation, the F-actin density and HIV-1 co-receptor expression

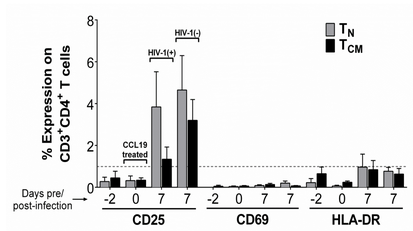


Figure 3: CCL19 and HIV-1 infection do not induce T cell activation. Expression of the activation markers CD25, CD69 and HLA-DR were measured by flow cytometry.

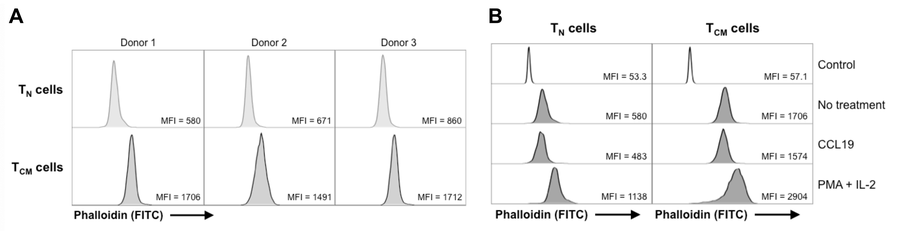


Figure 4: CCL19 has no effect on actin polymerization. (**A**) Filamentous actin (F-actin) was measured by phalloidin staining of purified resting CD4⁺ T_N and T_{CM} cells by flow cytometry. (**B**) To determine if CCL19 treatment had an effect on actin polymerization, cells were stained with phalloidin 48 hours after CCL19 treatment and were compared to untreated cells. Cells stimulated with PMA and IL-2 were used as a positive control. MFI = mean fluorescence intensity. Representative data from N = 3.

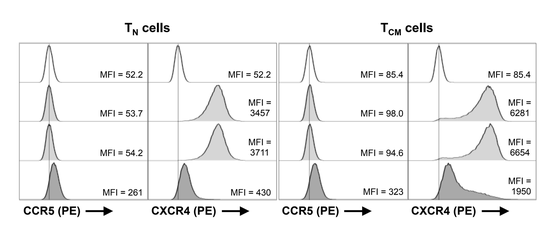


Figure 5: CCL19 has no significant effect on the HIV-1 co-receptors CCR5 and CXCR4. To determine if CCL19 treatment had an effect on CCR5 or CXCR4 expression levels, cells were stained with either CCR5-PE or CXCR4-PE 48 hours after CCL19 treatment compared to untreated control cells. Cells stimulated with anti-CD3/CD28 were used as a positive control. Representative data from N = 3.

HIV-1 infection of CD4⁺ T_N and T_{CM} cells

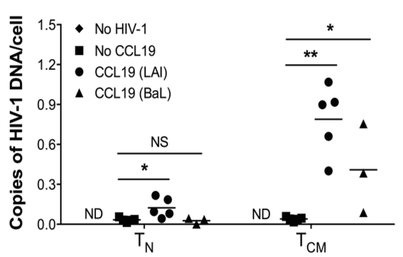


Figure 6: Measure of total HIV-1 DNA 7 days post-infection with HIV-1_{LAI} (X4-tropic) or HIV-1_{BaL} (R5-tropic) after CCL19 treatment compared to untreated cells. NS = not significant. * = $p < 0.05$. ** = $p < 0.005$.

Virus production from latently infected T_N and T_{CM} cells post-stimulation with anti-CD3/CD28, PMA+PHA, prostratin, and SAHA

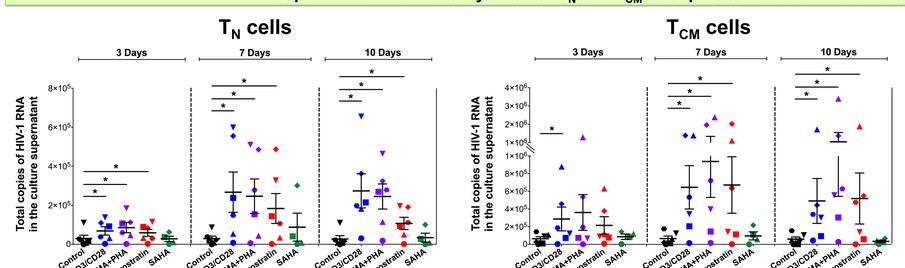


Figure 7: Virion production post-treatment with anti-CD3/CD28 antibodies, PMA+PHA, prostratin, or SAHA. Total HIV-1 RNA post-treatment is compared to untreated controls. Each symbol represents a different donor. Horizontal bars represent the mean +/- the standard error of the mean (SEM).

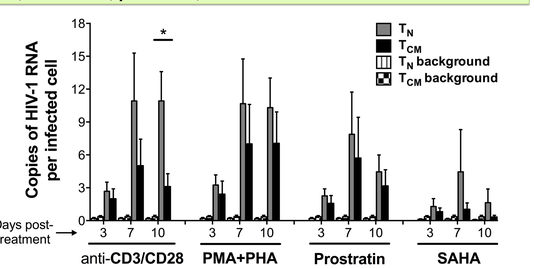


Figure 8: HIV-1 RNA per infected cell. Copies of HIV-1 RNA in the culture supernatant normalized to copies of HIV-1 RNA per infected cell at day 0. N=6 except for SAHA data where N=4. * = $p < 0.05$. All data are presented as the mean; error bars represent SEM.

Correlation between level of infection and virus production post-treatment

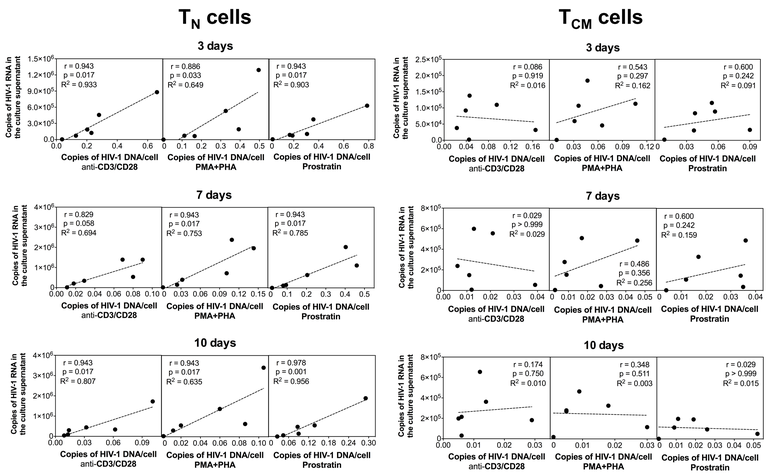
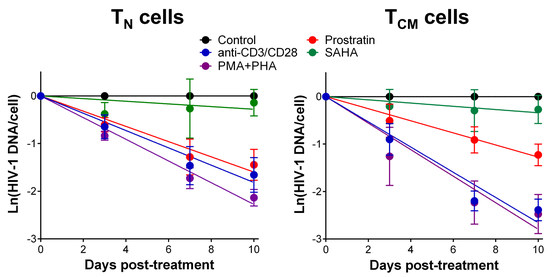


Figure 9: Correlation analyses between level of infection and virus production post-treatment from T_N and T_{CM} cells latently infected with HIV-1_{LAI}. Correlation analysis of T_N cells and T_{CM} cells 3, 7 and 10 days post-treatment with anti-CD3/CD28 antibodies, PMA +PHA, or prostratin. Spearman correlation coefficients (r) are shown. Each dot represents a different donor. p value > 0.05 is considered significant.

Measure of HIV-1 DNA decay post-treatment



Treatment	T_{CM} $T_{1/2}$ (days)	T_{CM} p value	T_N $T_{1/2}$ (days)	T_N p value
αCD3/CD28	2.61	< 0.001	3.82	< 0.001
PMA + PHA	2.48	< 0.001	3.05	< 0.001
Prostratin	5.44	< 0.001	4.34	< 0.001
SAHA	20.35	0.1322	24.85	0.2776

Figure 10: Decay of HIV-1 DNA following treatment of latently infected T_N and T_{CM} cells. Decay of HIV-1 DNA following treatment with anti-CD3/CD28 antibodies, PMA+PHA, prostratin, or SAHA compared to untreated controls. Data are presented as the mean; error bars represent the SEM. N=6 except for SAHA data where N=4.

T cell activation and proliferation post-treatment

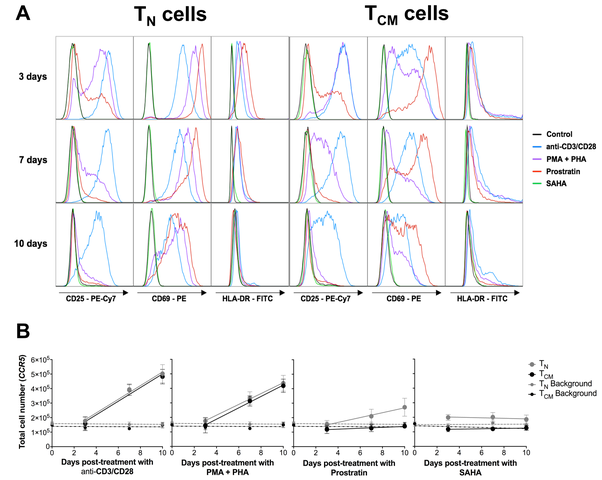


Figure 11: Cellular activation and cell proliferation post-treatment. (**A**) Measure of cellular activation over a 10 day treatment of uninfected T_N and T_{CM} cells with anti-CD3/CD28 antibodies, PMA+PHA, prostratin, or SAHA compared to untreated controls. Representative data from N = 2. (**B**) Cellular proliferation, measured by qPCR of the CCR5 gene, post-treatment of latently infected T_N and T_{CM} cells.

CONCLUSIONS

We have developed a robust primary cell model of HIV-1 latency in purified resting CD4⁺ T_N and T_{CM} cells that can now be used to better understand the establishment and reversal of latency in these key reservoirs. Using this model, we have shown:

- Treatment with CCL19 and HIV-1 infection do not alter the resting phenotype of purified T_N and T_{CM} cells.
- Treatment with CCL19 does not effect F-actin density or HIV-1 co-receptor expression.
- T_{CM} cells are infected more efficiently than T_N cells following treatment with CCL19.
- T_N cells produce as much or more virus per infected cell post-treatment than T_{CM} cells.
- There is a strong correlation between infection frequency and virus production post-treatment for TCM cells but there is no correlation for TN cells.
- HIV-1 DNA decays similarly in T_N and T_{CM} cells post-treatment.
- Decay of the latent reservoir is only observed when the LRA effectively stimulated virus production.
- All of the LRAs that reactivated latent HIV-1 induced global T cell activation.

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