Poster 166

Detection and Characterization of Cells expressing HIV RNA by Flow Cytometry

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Background

- Our current knowledge of HIV-infected cell populations is largely derived from flow cytometry studies using antibodies to p24
- p24 requires artificial activation for its detection, especially when analyzing rare HIV+ cells from ART-treated individuals.
- Much less is known about cell populations that express both p24 and HIV RNA, despite their potential importance to cure efforts.

Hypothesis And Aims

- **Simultaneous detection of HIV RNA and p24 expression induced by Latency Reversing Agents (LRAs) will reveal how latent CD4 T cell reservoirs vary in their capacity to reactivate HIV expression.**
- **Aim 1**: Characterize HIV p24 and RNA expression following *in vitro* HIV infection.
- **Aim 2**: Evaluate HIV RNA and p24 expression in PBMCs from ART treated people with HIV, following stimulation with LRAs.

Methods

- For **Aim 1**, primary human CD4 T cells from HIV-negative blood donors were isolated by negative selection, activated with α-CD3/CD28 beads and infected with HIV_{IIIB} for 4-5 days.
- For **Aim 2**, frozen PBMC from 3 ART-suppressed donors were cultured in RPMI, IL-2, IL-7 and IL-15, and were treated with SAHA (10uM), Romidepsin (10uM), or α-CD3/CD28 for 48 hours.
- PrimeFlow™ probes bind specifically to target RNA sequences, and are added after conventional antibody staining. Proprietary fluorescence amplification technology permits detection of target RNAs by flow cytometry.
- Live, $CD3^+CD4^+$ singlets from infected samples and uninfected controls were analyzed for their expression of immunological markers, HIV p24 and HIV RNA. The PrimeFlow™ probe set used here spans the entire HIV genome.
- Significant differences in expression of lymphocyte markers were identified using the Wilcoxon rank test.

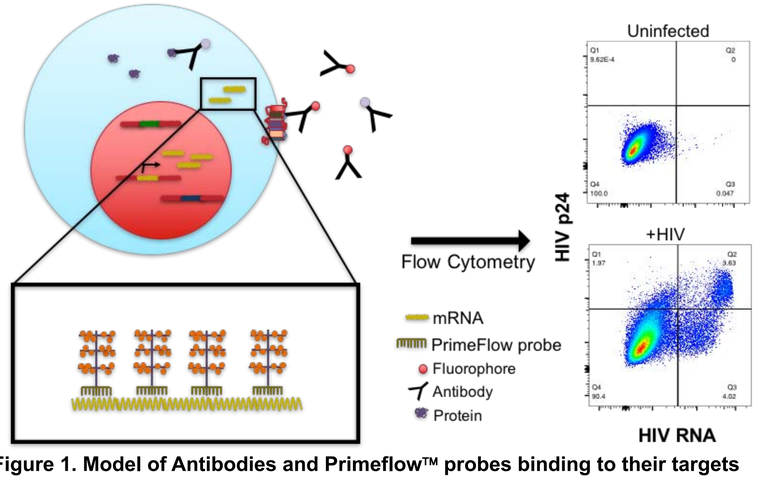


Figure 1. Model of Antibodies and Primeflow™ probes binding to their targets

Aim 1 Results: Cells infected *in vitro*

- We readily detected cells that expressed HIV RNA or p24 in *in vitro* infected cells from 7 donors (Figure 2).
- A substantial proportion of *in vitro* infected cells expressed HIV RNA, but not p24 (mean=2.4% of total cells, Figure 2C).
- Over a third (mean=35%) of cells that expressed HIV RNA did not co-express p24.
- Except for background signal, these populations were absent in uninfected controls (mean<0.25% for RNA+p24-, <0.002% for RNA+p24+, <0.07% for HIV RNA^- $p24^+$, Figure 2C).

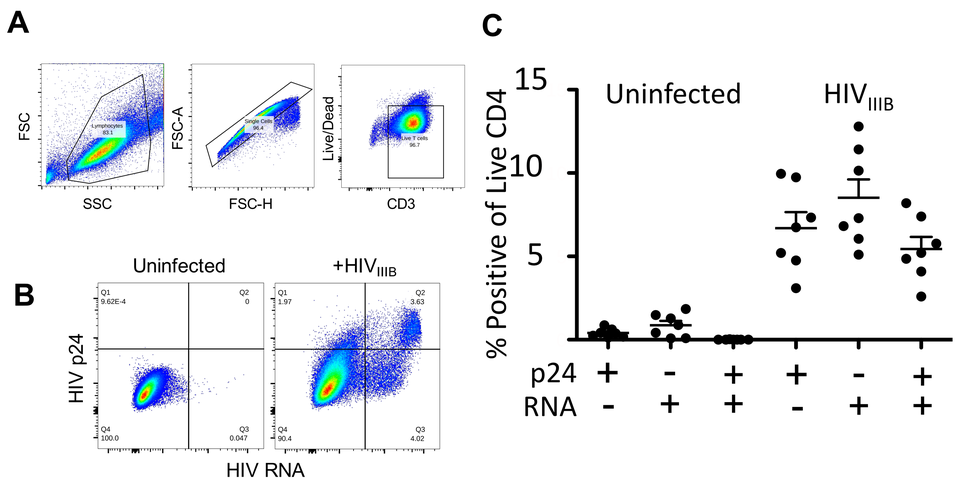


Figure 2. Gating Strategy and Background detection of p24 or HIV RNA

- *In vitro* HIV-infected cells, but not uninfected controls, had a population of cells with decreased CD45RA (2.1% vs 0.007%, p<0.02), and this population of $CD45RA^{low}$ cells was enriched in both HIV RNA^+p24^+ cells (31.4% vs. 5.4% of total, p<0.008) and RNA+p24- cells (9.2% vs. 2.6% of total, p<0.03).
- $CD27^+$ cells were diminished in HIV RNA^+ $p24^-$ cells from infected samples, but not in HIV RNA^+ $p24^+$ dual-positive cells (p<0.04 and p=.17, representative plots shown in Figure 3)

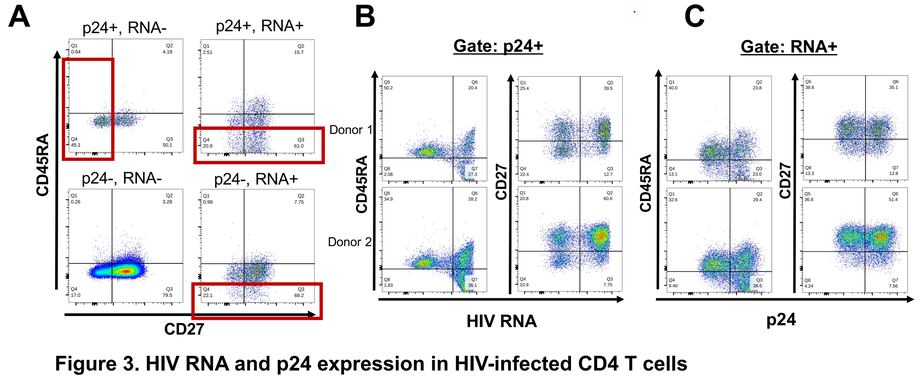


Figure 3. HIV RNA and p24 expression in HIV-infected CD4 T cells

Aim 2 Results: Cells from ART suppressed people

- SAHA and Romidepsin treatment produced measurable increases in p24+HIV RNA+ dual positive cells (Figure 4)

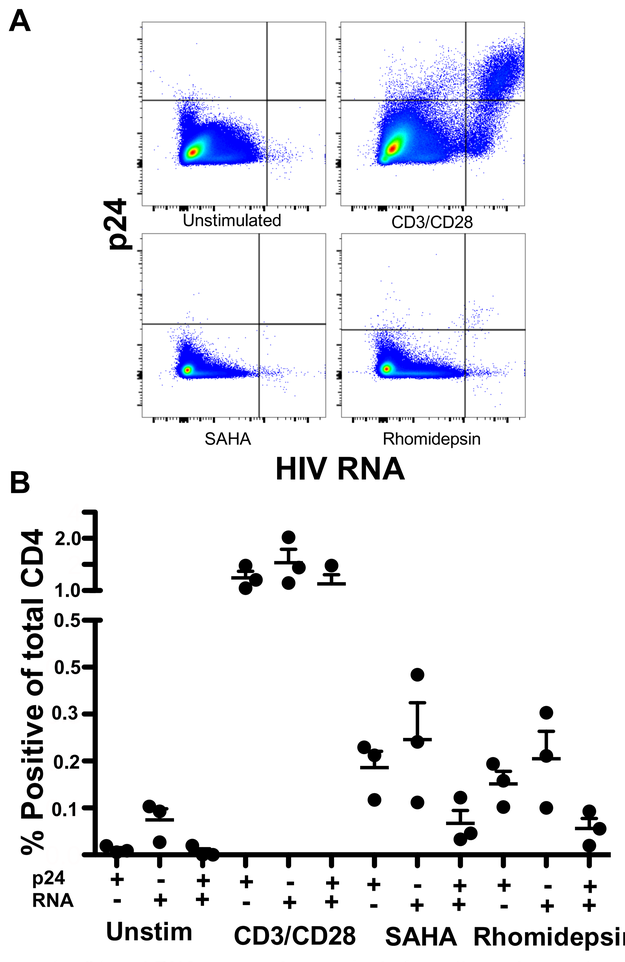


Figure 4. p24 and RNA expression analysis from three donors treated with LRAs

Conclusions

- **PrimeFlow™ detected HIV RNA and p24 on a per-cell basis by flow cytometry.**
- **Different T cell subsets exhibit unique expression patterns of HIV RNA and p24.**
- **LRAs vary in their ability to induce HIV expression from latent reservoirs.**
- **HIV RNA detection allows a more detailed characterization of cells producing HIV than p24.**
- **Differences between cells expressing HIV RNA vs. p24 may have important biological consequences and for evaluating replication competent reservoirs.**