# PHENOTYPE OF SIV-INFECTED CELLS IN BLOOD AND TISSUES USING A NEW SINGLE CELL APPROACH

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## BACKGROUND

- Characterizing the populations which sustain SIV replication at single-cell level may lead to the identification of key mechanisms of SIV persistence.

CD4+ T cells are the main cellular targets of both HIV and SIV. While the phenotype of HIV-infected cells has been extensively reported, the cellular subsets in which SIV actively replicates in blood and tissues from non-human primates (NHPs) remain largely unidentified. We developed a novel flow-based approach to identify the phenotype of SIV-infected cells during untreated SIV infection. By using two different antibodies specific for the p27 SIV protein (2F12 and 4B7) we enumerated and phenotyped productively infected cells with high specificity.

## METHODS

We analyzed infected cells in multiple tissues from 9 male chronically SIVmac239-infected ART-naïve rhesus macaques (ranging from 4 to 6 years old), including blood, spleen, axial, mesenteric and inguinal lymph nodes. CD4+ T cells were isolated by negative magnetic selection and stained with a combination of antibodies to obtain the phenotype of p27+ cells (CTLA-4, PD-1, Ki67, CD69, CD127, CD45RA, CCR7, CD27, CD95, CD3, CD4, CD8).

The assay (SIV-Flow) can detect a single infected cell in a million CD4+ T cells and has a large dynamic range (1 to 100,000 infected cells/$10^6$ CD4+ T cells). We also quantified SIV DNA and cell-associated SIV RNA (LTR-Gag) in these samples.

In viremic SIV-infected macaques, **productively infected cells** are highly enriched in the **stem-cell** and **effector memory** subsets and express high levels of **Ki67**, **PD-1** and the integrin **α4β1**.

## RESULTS

Figure 12

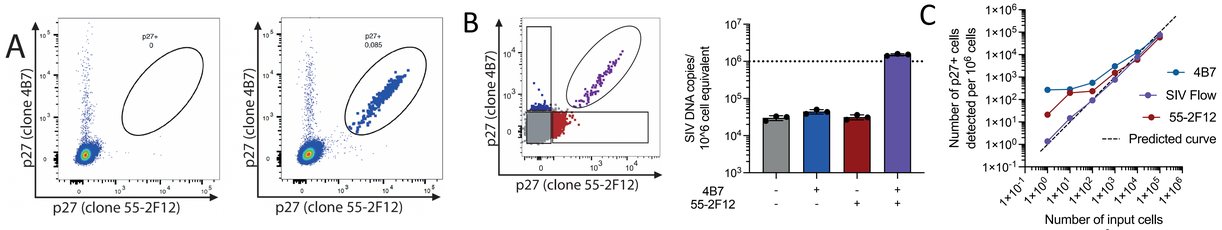



**Figure 1. Specific and sensitive detection of SIV infected cells by combining two p27 antibodies. (A)** Combination of clones 4B7 and 55-2F12 in flow cytometry for SIV-infected CD4 T cells identification in SIV-infected macaques (right panel) and uninfected control (left panel). **(B)** SIV-Flow specificity. We sorted by flow cytometry the four populations obtained in the SIV-Flow staining (double negative; 4B7+; 55-2F12+; double positive) and quantified SIV DNA in the extracted DNA from these cells. Results show that the p27+ population is enriched in SIV DNA (approximately 1 SIV genome per cell) compared to the other three populations analyzed. **(C)** SIV-Flow linearity and limit of detection. Single staining with each p27-recognizing antibodies or double staining (SIV-Flow) were performed in cellular cultures of HUT 78 SIV-infected cells spiked into non-infected PBMCs at different frequencies. The SIV-Flow assay detected a single SIV-infected cell per million of non-infected cells with a wide linearity range compared to the single staining.

Figure 2

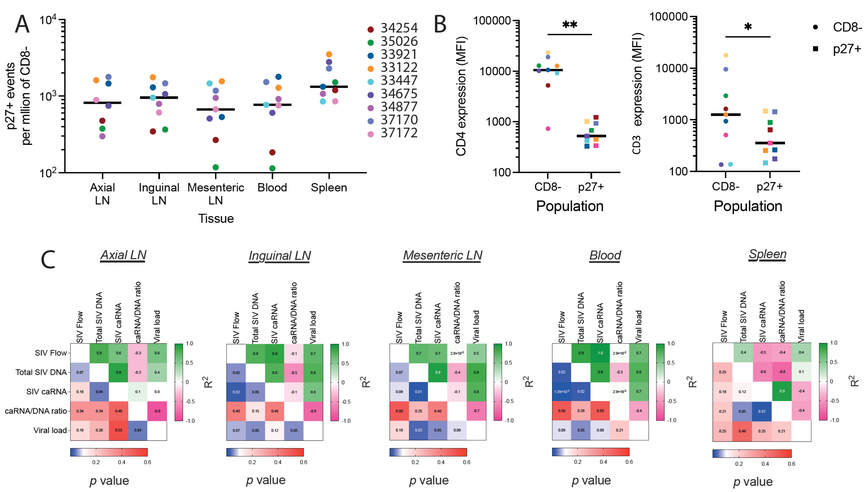



**Figure 2. SIV-Flow detects productively infected cells in blood and tissues and reveal CD4 and CD3 downregulation. (A)** SIV-Flow quantification in tissues. Among all the compartments evaluated, the spleen showed the highest frequency of productively infected cells, although this did not reach statistical significance **(B)** p27+ cells express lower cell surface levels of CD3 and CD4 molecules than their uninfected counterparts **(C)** SIV-Flow correlates with viral load and conventional SIV reservoir markers in the blood. In lymph nodes, there were strong associations between SIV-Flow and reservoir markers, but no correlation was observed in the spleen.

Figure 3

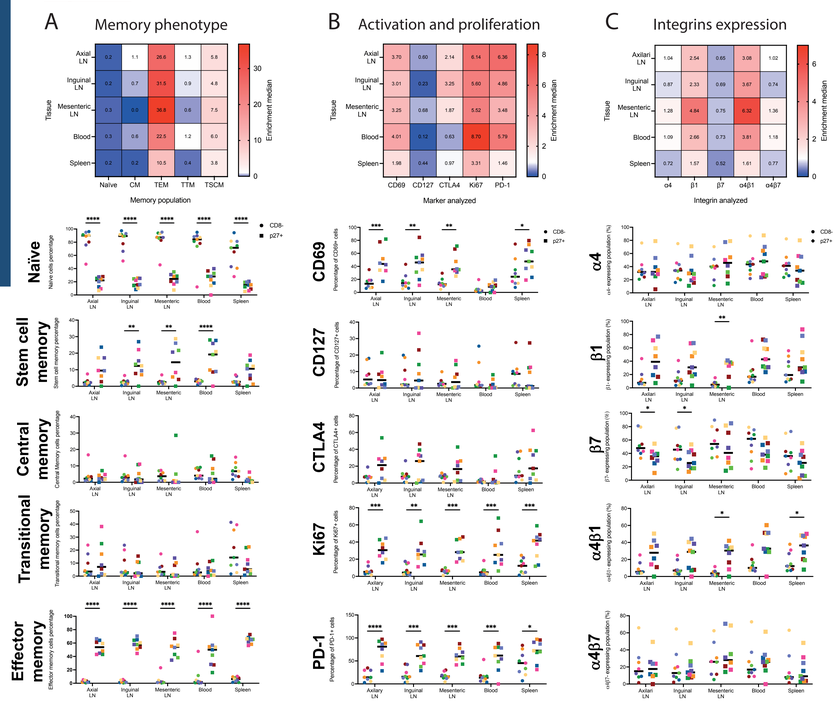



**Figure 3. SIV-Flow reveals that productively infected cells mainly display stem-cell and effector memory phenotypes and express high levels of Ki67, PD-1 and the integrin α4β1.** The median enrichment (enrichment matrix, top) and the percentage of cells expressing each phenotype within the bulk CD4 T cells or p27+ population (scatter plots, bottom) for the 9 animals analyzed. **(A)** Memory phenotype. **(B)** Expression of CD69, Ki67, CD127, CTLA-4 and PD-1. **(C)** Expression of the integrins α4β1, α4β7 and their subunits in each population.

## CONCLUSIONS

We designed a specific and sensitive single-cell approach to identify SIV-infected. SIV-Flow values correlate with viral DNA and caRNA in the blood and in most lymph nodes.

In viremic SIV-infected NHPs, productively-infected cells are strongly enriched in the effector memory subset, and to a lesser extent in the stem cell memory population. Productively infected cells display a highly activated and proliferative phenotype. Our results suggest that activated and proliferating CD4+ T cells are important contributors to SIV replication in chronically infected NHPs, and that the stem cell compartment may also contribute to viral burden.