Ultra-Low Level HIV p24 Production as a Driver of Immune Activation in Individuals Treated with ART

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Background

Despite antiretroviral therapy (ART) residual immune activation has been observed in people living with HIV and is associated with ongoing morbidity. Especially low levels of Gag-specific CD8 T-cell responses are seen despite the absence of detectable HIV replication. We are hypothesizing that the driver for persistent Gag-specific CD8 T cell responses is low level HIV protein production.

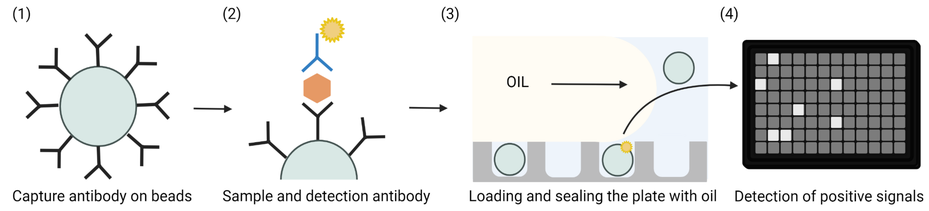
Material and Methods

Here, we optimized an ultrasensitive p24 single-molecule array from (SIMOA™) to detect p24 in plasma at a 1000-fold lower concentration compared to previous assays. This advancement allowed us to analyze chronically infected people with HIV who are undergoing treatment (with undetectable viral load) and compared it to immune correlates measured by a multicolor immune assay.

- **Ultra-low level HIV p24 protein is continuously produced in 26% of ART-treated people living with HIV, despite the fact that all participants had no detectable HIV viral load.**
- **Age, gender, ART regimen and cell-associated HIV RNA showed no association with p24 levels.**
- **Ultra-low level HIV p24 production is associated with immune activation and antigen-specific CD4/CD8 T cell responses.**

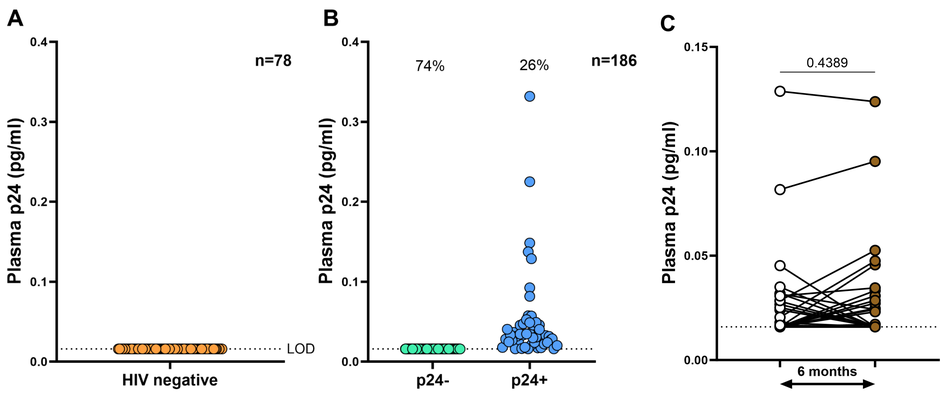
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Figure 1: Single Molecule Array



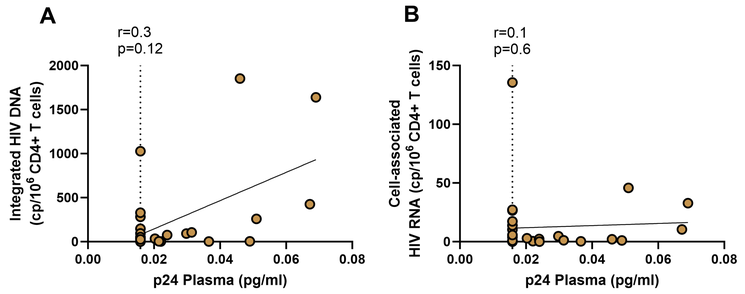
After the beads, sample, and detection antibody have been mixed together (1)(2), the solution is applied to a cartridge with over 235,000 microwells. Each microwell can hold only one bead. Oil is then added to the cartridge and forms a liquid-tight seal of the wells (3). The resulting signals are captured by using microscope optics (4).

Figure 2: p24 is detectable in 26% of individuals with long-term ART



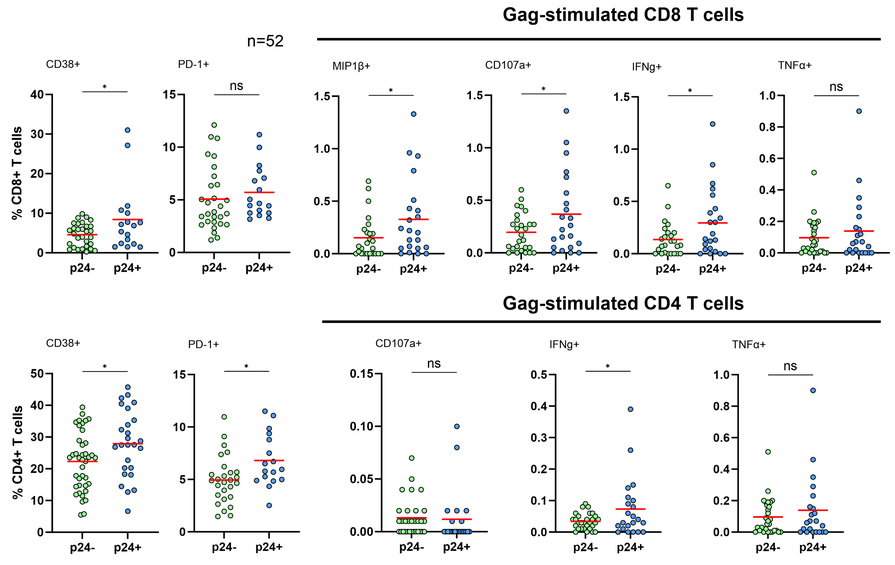
We studied 186 people living with HIV who were on suppressive ART for at least four years, and we measured their p24 levels in the plasma. (A) While p24 was undetectable in individuals without HIV, (B) low level p24 [range 16.0 fg/ml – 330 fg/ml] was detectable in 49 out of 186 people (26%) with chronic, and ART-treated HIV and were stable over time (C). All individuals had no detectable viral load and similar time on ART.

Figure 3: No correlation with integrated HIV DNA or cell-associated HIV RNA



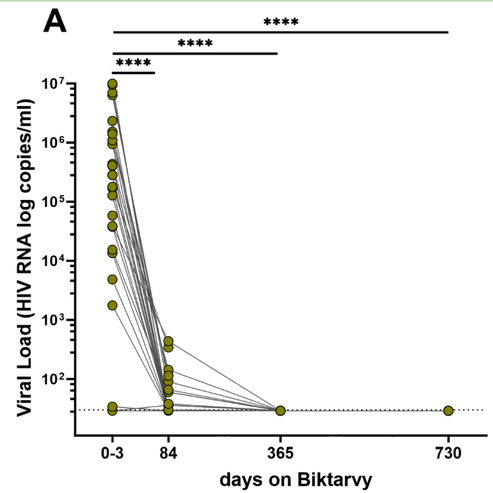
(A) Integrated HIV DNA copy number in CD4 T cells from ART-treated individuals with different levels of un- or detectable p24 levels. (B) Cell-associated RNA copy number in CD4 T cells from ART-treated individuals with different levels of un- or detectable p24 levels.

Figure 4: Association of p24 with activation and HIV-specific T cell responses



CD8 and CD4 T cells from p24+ individuals showed significantly increased expression of activation marker and antigen-specific responses compared to p24- individuals (P < 0.05).

Figure 5: Changes of p24 protein production from acute to chronic infection



To determine changes of p24 protein production from acute to chronic infection, we studied 43 people who initiated suppressive ART during acute HIV and remained virally suppressed over a two year period. (A) Despite HIV-1 RNA that declined to undetectable levels (<30 copies/mL), (B) p24 levels remained detectable in 25% of individuals two years after ART start.

Conclusion

Despite continuous ART, we were able to detect ultra-low levels of p24 production, as well as immune activation. This suggests that either ongoing viral replication (and protein production) or active transcriptional HIV integration sites could be drivers of HIV immune activation..