Immune Correlates Of Cell-Associated HIV RNA In Infected Individuals On Long-Term Antiretroviral Therapy

Chuen-Yen Lau[1], Thuy Nguyen[1], Matthew Adan[1], Robert Gorelick[2,8], Jeannette Higgins[3,8], Jessica Earhart[1], Ariana Savramis[1], Catherine Rehm[4], Robin Dewar[5,8], Deborah McMahon[6], Anuradha Ganesan[7,8,9], Brian Luke[10], Frank Maldarelli[1]

[1]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health; [2]AIDS and Cancer Virus Program; [3]AIDS Monitoring Laboratory; [4]National Institute of Allergy and Infectious Disease, National Institutes of Health; [5]Virus Isolation and Serology Laboratory; [6]Division of Infectious Diseases, University of Pittsburgh; [7]Infectious Disease Clinical Research Program, Uniformed Services University of the Health Services; [8]Walter Reed National Military Medical Center; [9]Henry M Jackson Foundation for the Advancement of Military Medicine, Inc.; [10]Frederick National Laboratory for Cancer Research

Background

- Chronic low-level immune activation in people living with HIV (PLWH) on suppressive antiretroviral therapy (ART) is associated with elevated morbidity and mortality.
- Sources or immune activation are uncertain, but may be related to ongoing HIV RNA production from persistently infected cells.
- Characterizing the relationship between persistence of infected cells and immune inflammatory state is essential for eradication and control of HIV.
- To investigate the role of HIV RNA levels in immune activation during ART, we used multi-modal statistical analyses to identify cellular immune subsets associated with HIV RNA levels.

Hypothesis

- Analysis of clinical, viral, and immune characteristics will identify factors associated with HIV persistence.

Study Participants

- 74 people living with HIV (PLWH) were recruited from the National Institutes of Health, Walter Reed National Naval Medical Center and University of Pittsburgh.
- All were virally suppressed below commercial assay limit of detection for at least 3 years.

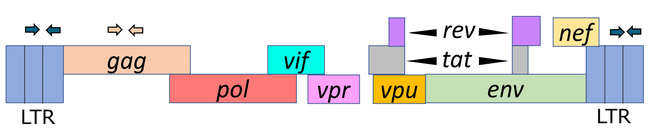
Methods

- Peripheral blood mononuclear cells (PBMC) were obtained by phlebotomy or pheresis.
- HIV LTR and *gag* RNA and DNA measured by multiplexed droplet digital PCR (ddPCR)

Table 1: ddPCR HXB2 coordinates[1]:

Gene	Forward	Reverse
LTR (R-U5)	517-539	576-597
gag (p24)	1299-1324	1358-1377

Figure 1: ddPCR Primer combinations to amplify LTR R-U5 (➡ ⬅)or *gag* (⇨ ⇦) sequences



- CD4 and CD8 subsets determination
  - Flow cytometry for peripheral blood immunophenotyping
  - Memory/naïve subsets (CD45RA+, CD27+), activation (CD38+, HLA-DR), NK (CD16+CD56+), regulatory T cells (CD25+)
- Statistical Analysis
  - Identify immune parameters most frequently associated with HIV *gag* production
  - 24 statistical tests including linear, parametric, and non-parametric correlation, regression, and classification.
  - Varying dataset structure assumptions
  - Factors significant by many tests likely to be robust correlates

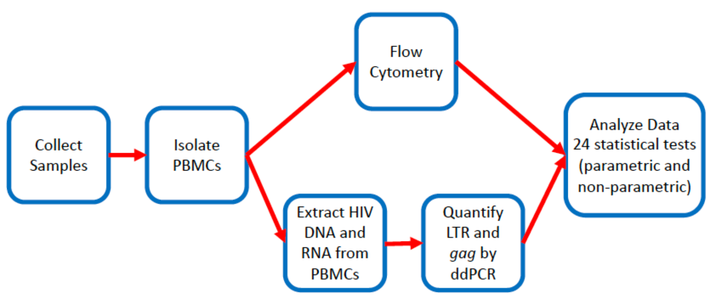


Figure 2. Methods Flow Chart
* PBMCs = peripheral blood mononuclear cells

Results

Table 2. Participant Characteristics (n=74)

Characteristic	Value
Age, years (range)	49.96 (20-70)
Sex, female, percent	11
Race / Ethnicity	
White, percent	51
Black / African-American, percent	38
Latinx, percent	12
Min duration HIV infection, years (range)	17.2 (3.9-34.2)
Pre-ART HIV RNA, median Log_{10} copies /mL	5
Nadir CD4, cells /μL (range)	265 (1-870)
CD4 at sample timepoint, cells /μL (range)	687 (250-1765)

Table 2. All 74 participants had DNA values. 60 of the 74 participants also had RNA values.

Figure 3. Provirus Features After Long-Term ART

A. Most Proviruses are Deleted in *gag*

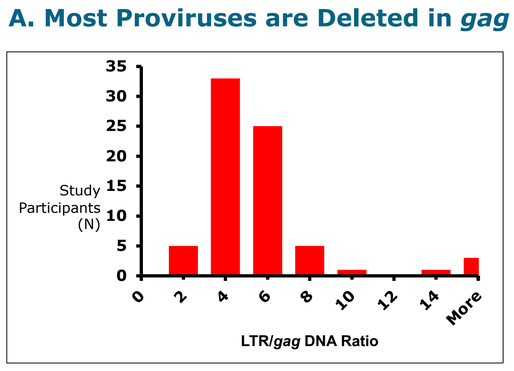


Figure 3A. Histogram analysis of HIV LTR:*gag* DNA Ratios

B. Levels of Proviruses That Still Have *gag* Vary Over 1000-fold

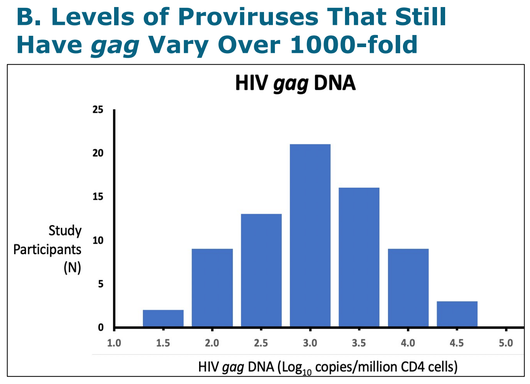


Figure 3B. Histogram analysis of HIV *gag* DNA copy numbers

Figure 4. Levels of *gag* Containing Proviruses Associate With Composite of Nadir CD4 and CD8 Populations

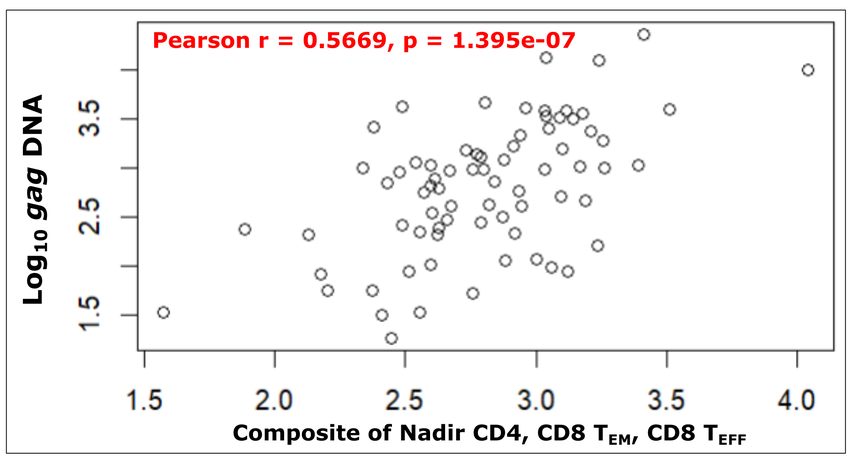


Figure 4. Best fit 3-term model. Composite=3.0229–0.0013765 (Nadir CD4) + 0.02283 (CD8+27-RO+) – 0.01792 (CD8+27-RO-). Nadir CD4 had a significant negative association in 21/24 tests. Memory CD8 had a significant positive association in 15/24 tests. Effector memory CD8 had a significant positive association in 14/24 tests. T_{EM} = Effector Memory T cells, T_{EFF} = Effector T cells.

Figure 6: HIV *gag* RNA levels are low relative to HIV DNA levels

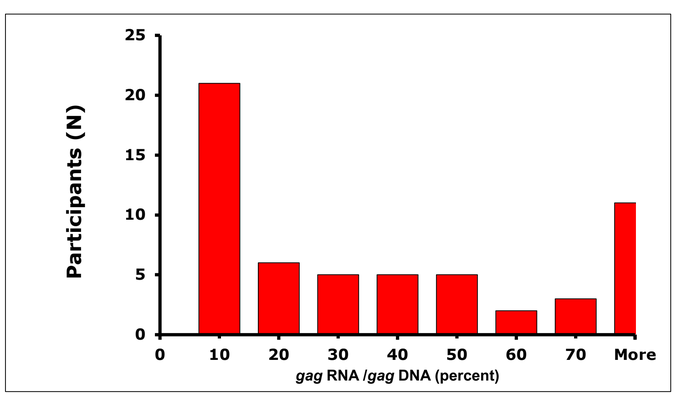


Figure 6. Histogram analysis of HIV gag RNA / gag DNA percentages

Figure 7: Ratio of HIV *gag* RNA:*gag* DNA Associates With Composite of Age and Natural Killer Cells

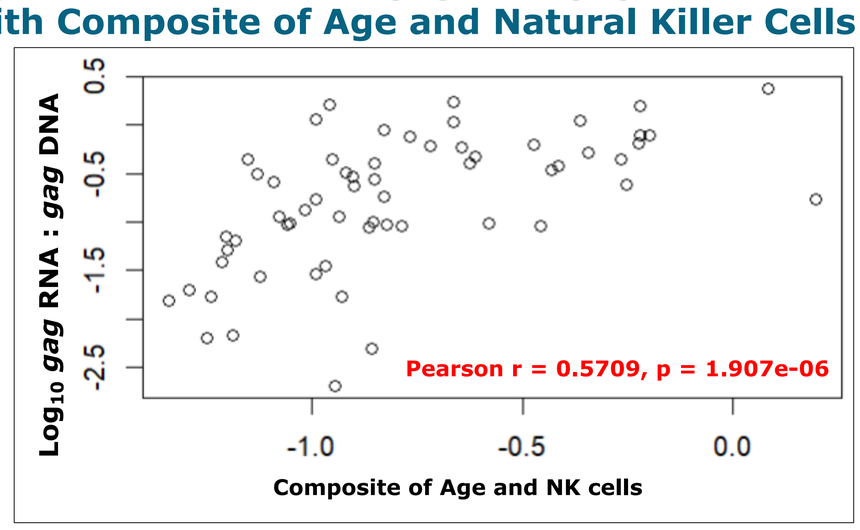


Figure 7: Best fit 2-term model. Composite = -0.02331 (Age) + 0.03064 (CD16+56+). Natural killer (NK) cells had a significant positive association in 13/24 tests. Age had a significant negative association in 20/24 tests. Age may be a proxy for duration of infection or duration of ART.

Summary

- HIV provirus populations vary in size and composition after long-term ART
- Levels of HIV *gag* DNA correlate with Nadir CD4, T_M, T_{EM}, and infection duration.
- Only a portion of *gag*-containing cells have *gag* RNA.
- HIV *gag* RNA /*gag* DNA correlates with proportion of NK cells
- HIV gag RNA may be a driver of innate immune activation
- Comprehensive statistical approaches can identify composite markers for additional study

Implications

- Higher Nadir CD4 may indicate better immune reserve and ability to control provirus.
- Longer infection may indicate lower immune reserve and ability to control provirus.
- Antigen-specific memory CD8 cells may be stimulated by intact genomes.
- NK cells may be sensing HIV RNA-containing cells.
- Expression from proviruses may stimulate innate immunity.

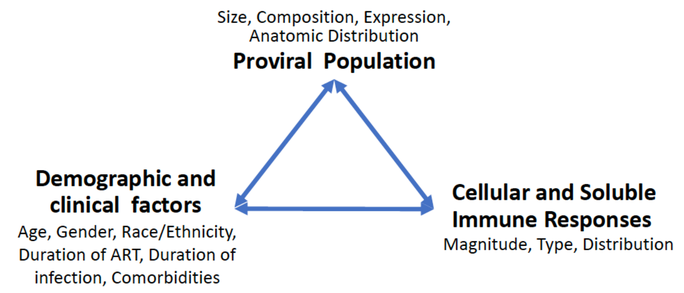


Figure 8: Conceptual Model of HIV Persistence and Immune Activation

Future Directions

- Functional evaluation of innate immune factors that interact with the HIV provirus during suppressed infection.
- Longitudinal assessments to understand the dynamics of provirus levels and their transcription.
- Characterization of changes in the provirus and immune responses during treatment interruption.
- Comparison of provirus and immune dynamics in different anatomic locations.

References

1. Anderson and Maldarelli, Curr Protoc Microbiol. 2018; 51(1):e62
2. Anderson, et al. Viruses. 2020; 12(2):136
3. Lau, et al. Viruses. 2021; 13(12): 2512

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For additional information:
Chuen-Yen Lau
lauc@mail.nih.gov

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