HIV Proviral Populations Differ by Sex and Immune Activation Levels During Antiretroviral Therapy

Chuen-Yen Lau[1], Thuy Nguyen[1], Matt Adan[1], Jessica Earhart[1], Danielle Konlian[1], Lindsey Adams[1], Mary Zipparo[1], Robin Dewar[2], Jeanette Higgins[3], Catherine Rehm[4], Seble Kassaye[5], Anuradha Ganesan[6], Deborah McMahon[7], Robert J. Gorelick[8], Brian Luke[8], Frank Maldarelli[1]

[1]HIV Dynamics and Replication Prog, NCI, NIH; [2]Virus Isolation and Serology Lab, NCI, NIH; [3]AIDS Monitoring Lab, NCI, NIH; [4]Lab of Immunoregulation, NIAID, NIH; [5]Div. of ID, Dept. of Medicine, Georgetown; [6]Walter Reed and Henry M Jackson Foundation; [7]Div. of ID, Dept. of Medicine, Univ. of Pittsburgh; [8]Frederick National Lab for Cancer Research

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BACKGROUND

Persistence of HIV infected cells during antiretroviral therapy (ART) is a barrier to cure. Infected cells contain proviruses that can express RNA and HIV proteins even if defective for replication, leading to immune activation. Intact and defective proviruses decline more slowly in female than in male people with HIV (PWH)[1] , though males show higher cell associated and plasma HIV RNA levels[2]. Roles of sex, age, and immune status in persistence and expression of HIV proviral populations during ART are not well understood. To investigate determinants of persistence and expression of HIV proviruses, we analyzed levels of total and *gag*-deleted proviruses and their RNA expression in the context of clinical and immune parameters in male and female PWH undergoing long-term ART.

HYPOTHESIS

Investigating population correlates of HIV DNA and RNA levels in a cohort with a substantial proportion of women will allow comparison of characteristics between men and women

METHODS

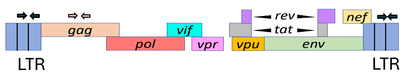
Study Participants

- 103 PWH were recruited from protocols at the National Institutes of Health, Walter Reed National Naval Medical Center, University of Pittsburgh, and Women's Interagency HIV Study
- All had plasma HIV RNA levels below commercial assay limit of detection for at least 3 years.
- Demographic and clinical information was obtained from medical records
- Immunophenotyping
  - Peripheral blood mononuclear cells (PBMC) were obtained by phlebotomy or pheresis
  - CD4 and CD8 subsets were determined
    - Memory/naïve subsets (CD45RA+, CD27+), activation (CD38+, HLA-DR), NK (CD16+CD56+), Treg (CD25+)
- Virologic Analyses
  - HIV LTR and *gag* RNA and DNA were measured in PBMC-derived nucleic acid by multiplexed droplet digital PCR (ddPCR) using targets in HIV LTR and *gag*
  - Single genome sequencing of HIV LTR and gag was performed to evaluate ddPCR primer/probe matching
  - Single copy HIV RNA in plasma was determined

Table 1: ddPCR HXB2 coordinates[3]

Gene	Forward	Reverse	Probe
LTR (R-U5)	517-539	576-597	552 - 570
gag (p24)	1299-1324	1358-1377	1325 - 1354

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



- Statistical Analysis
  - Univariate evaluation of relationship between immunophenotype or clinical variable and HIV DNA or RNA parameter (n=31) using 12 statistical tests
  - Variables passing (p<0.05) at least 4/12 tests evaluated in regression models
  - "BEST" model for each HIV DNA and RNA parameter: Lowest Bonferroni-corrected Pearson p-value among models where all variables contributed significantly AND an uncorrected Pearson p-value < 0.05 for selected models
  - Exploratory evaluation of interaction between sex and age < or >50 years
    - 50 years selected based on typical changes in hormone status

RESULTS

Participant Characteristics

Characteristic	Total (n=103)	Females (n=38)	Males (n=65)	p-value
Age in years, mean (range)	50 (27-80)	49 (27-80)	50 (28-70)	0.3071
Race, n (%)				
White	46 (45)	13 (34)	33 (51)	0.15
Black	44 (43)	19 (50)	25 (38)	0.30
Asian	1 (1)	0 (0)	1 (2)	1
Multiple	5 (5)	3 (8)	2 (3)	0.355
Unknown	7 (7)	3 (8)	4 (6)	1
Ethnicity, n (%)				
Non-Hispanic or Latino	87 (84)	31 (82)	56 (86)	0.58
Hispanic or Latino	16 (16)	7 (18)	9 (14)	0.58
Minimum duration of HIV infection at sample time in years, mean (range)	18 (4-38)	19 (5-38)	17 (4-34)	0.2854
Duration of ART at sample time in years	15 (3-31)	15 (3-31)	14 (3-29)	0.8717
Nadir CD4 in cells/μL, mean (range)	233 (0-665)	187 (0-550)	259 (1-665)	**0.0287**
Nadir CD4 < 200 cells/μL, n (%)	38 (37)	17 (45)	21 (32)	0.195
Absolute CD4 at sample time point in cells/μL, mean (range)	737 (250-1765)	837 (380-1646)	679 (250-1765)	**0.0058**
CD4% of total lymphocytes at sample timepoint, mean (range)	38 (10-57)	40 (26-57)	36 (10-56)	0.0679

Table 2: Participant Characteristics. All participants had DNA values. 87 (36 females and 51 males) of the 103 participants also had RNA values. Characteristics of the subset with RNA values reflected those of the full cohort.

RESULTS

HIV Provirus Levels Vary >1000-fold After Long-term ART
Approximately Half of All Proviruses are *gag*-Defective
Females Have More Deleted Proviruses

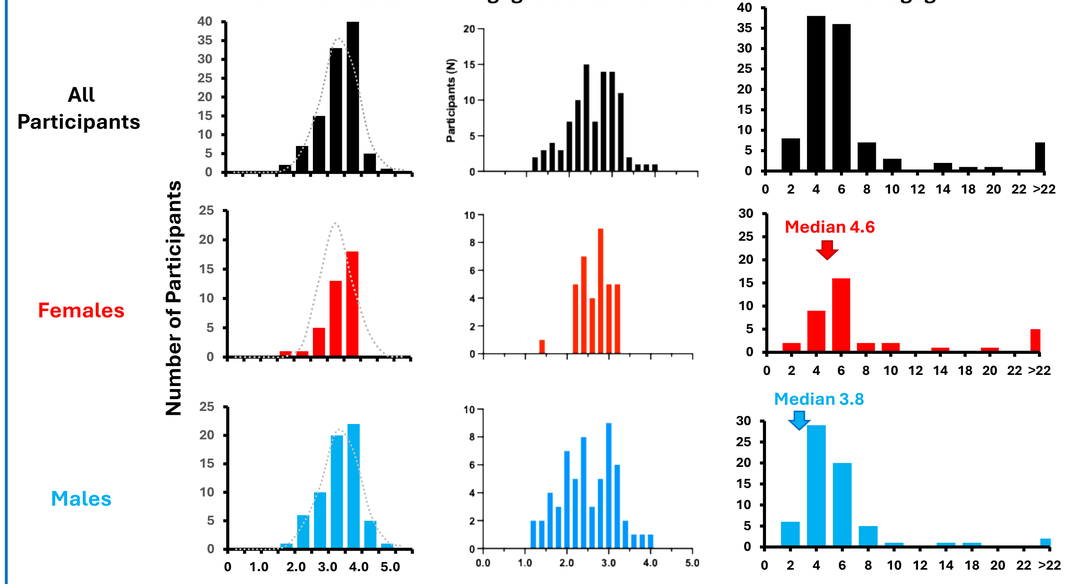


Figure 2: Across highly variable total provirus levels (left column), a majority of proviruses are *gag*-defective (middle column). Females have a higher proportion of *gag*-defective proviruses than males (right column). Non-parametric statistics were used to assess differences due to rightward skewing of the data, demonstrated by the dotted line indicating a normal distribution for total proviruses. The LTR/*gag* ratio (right column) reveals differences between males and females not apparent from analyses of log transformed LTR DNA or *gag* DNA.

Levels of Cell Associated HIV RNA Per Provirus are Higher in Females than in Males

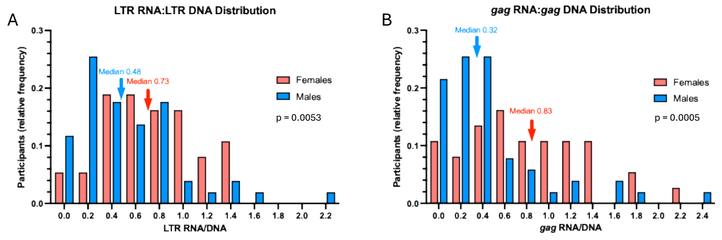


Figure 3: RNA per respective provirus. No differences in total HIV LTR or *gag* RNA levels between females and males were detected (data not shown). Proportion of RNA:DNA for both total proviruses represented by LTR (A) and for *gag*-containing proviruses (B) is higher for females compared to males, suggesting higher levels of expression in females. Mann-Whitney p-values are shown.

Strong Correlation Between Levels of HIV Proviruses and Percent CD8 Memory Cells

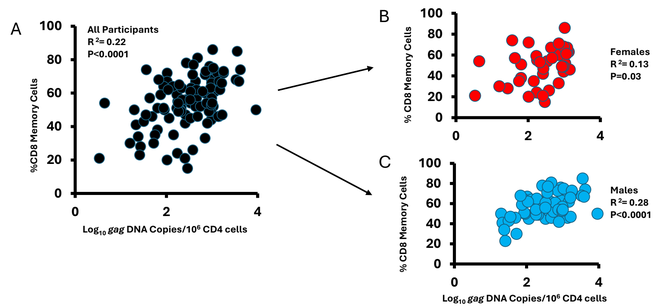


Figure 6: CD8 memory cell percentage and *gag* DNA levels strongly correlate. The correlation is significant for the full cohort (A), females (B) and males (C). While there is no difference in slopes between females and males (Mann-Whitney p-value = 0.7228), the elevations are different (Mann-Whitney p-value = 0.0051). This may be related to differences in CD8 memory cells percentages among males and females (Female median 51.5%, Male median 59.0%, Mann-Whitney p-value = 0.0074)

HIV DNA and RNA Levels Correlate With Immunophenotypes

HIV Proviral Analysis	Group	HIV Nucleic acid	Age	Time on ARV	Nadir CD4	CD4	CD4 38	CD4 38 dr	CD4 Ro+	CD8 25	CD8 27+ Ro+	CD8 Ro+	CD16 56
	All	log(LTR DNA)	0.22			-0.24	-0.22	0.24	0.27		0.28	0.43	
	All	log(gag DNA)				-0.26	-0.22	0.24	0.25		0.32	0.47	
	Females	log(LTR DNA)					-0.32			0.36	0.42	0.47	
	Males	log(LTR DNA)	0.36	0.26	-0.26	-0.31		0.31	0.32			0.46	
	Males	log(gag DNA)	0.42	0.32	-0.28	-0.35		0.34	0.3		0.28	0.53	
HIV RNA Expression Analysis	**Group**	**HIV Nucleic acid**	**Age**	**Time on ARV**	**Nadir CD4**	**CD4**	**CD4 38**	**CD4 38 dr**	**CD4 Ro+**	**CD8 25**	**CD8 27+ Ro+**	**CD8 Ro+**	**CD16 56**
	All	LTR RNA/DNA	-0.32	-0.24									
	Males	log(gag RNA)			-0.35	-0.39		0.29				0.42	
	Males	LTR RNA/DNA	-0.5	-0.38									0.36
	Males	gag RNA/DNA	-0.44	-0.3									0.29

Table 3: Immunophenotyping variables with ≥3 significant Pearson correlations or appearances in best models for HIV DNA and RNA parameters. Pearson r shown for variables with significant Pearson correlations. Black = Positive correlations. Red = negative correlations. Cells for "best model" variables are outlined.

HIV Proviral Levels Vary by Sex and Age

Comparison	All Females and All Males	Females and Males ≥ 50	Females and Males < 50
Log_{10} *gag* DNA Copies/10^6 CD4 cells	p=0.2564	p=0.0059	P=0.1829
LTR:*gag* DNA	p=0.0137	p=0.0005	P=0.9072
LTR RNA:LTR DNA	p=0.0053	p=0.0057	p=0.8254
gag RNA:*gag* DNA	p=0.0005	p=0.0095	p=0.2596

Table 4: HIV RNA and DNA parameters vary by sex and age. Differences are present between males and females >50 years but not <50 years. Mann-Whitney p-values are shown for parameters with at least one significantly different parameter by sex. Medians are listed below the p-values. F=Female, M=Male.

Summary

- HIV proviral levels vary >1000-fold during long-term ART in males and females
- *gag*-deleted proviruses are common in males and females
  - Females have more *gag*-deleted provirus than males
- Females have more non-*gag*-defective and *gag*-defective RNA per provirus than males
- Immune correlates are multifactorial
  - Provirus levels correlate with percent CD8+Ro+ memory T cells in both sexes
- Differences in HIV DNA and RNA levels between sexes are significant in PWH ≥ 50 years old

Implications

- **Study Design:** Large range in proviral levels indicates need for study designs with careful matching to appropriately power comparisons of HIV populations in females and males
- **Persistence Mechanisms**: Differences in levels of HIV RNA and DNA in males and females after age 50 suggest potential roles for hormonal status and age in determining proviral populations
- **Interventional Studies:** Age and sex should be considered in HIV eradication and control strategies

Future Directions

- New Study participant groups
  - Stratify by age and sex
- Analysis of Intact Provirus DNA (IPDA)
- Longitudinal assessments to understand the dynamics of HIV DNA and RNA
- Analyses of additional biomarkers, such as soluble markers of inflammation
- Comparison of provirus and immune dynamics in during reactivation

Thank you to our amazing study participants!

For more information, contact Chuen-Yen Lau at lauc@mail.nih.gov

References

1. Gianella, Clinical Infectious Diseases. 2022; 75(1):73-80
2. Scully, Journal of Infectious Diseases. 2019; 219:1084-94
3. Anderson and Maldarelli, Curr Protoc Microbiol. 2018; 51(1):e62

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