Sex Based Differences in HIV Reservoir Activity and Residual Immune Activation

Eileen P. Scully[1,2,3], Monica Gandhi[4], Rowena Johnston[5], Robert Gorelick[6], Jeffrey Lifson[6], Sharon Lewin[7], Jonathan Karn[8], Nicolas Chomont[9], Peter Bacchetti[10], Steven G. Deeks[4]

[1]Johns Hopkins University School of Medicine, Baltimore, MD, United States [2]Ragon Institute of MGH, MIT, and Harvard, Massachusetts General Hospital, MA, United States, [3]Division of Infectious Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States [4]Department of Medicine, University of California, San Francisco, CA, United States, [5]amfAR, the Foundation for AIDS Research, New York, NY, United States, [6]AIDS and Cancer Virus Program, Leidos Biomedical Research, Frederick National Laboratory, MD, United States, [7] The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Victoria, Australia, [8]Case Western Reserve University, Cleveland, OH, United States, [9]Research Centre of the Centre Hospitalier de l'Université de Montréal (CRCHUM) and Université de Montréal, Montreal, QC, Canada, [10]Department of Epidemiology and Biostatistics, University of California, San Francisco, CA, United States

Background and Rationale

Biological sex modulates immune responses[1,2], with clear sex differences in susceptibility to and clinical severity of several infectious diseases[3,4]. HIV RNA levels are lower in women than men in the absence of antiretroviral therapy (ART), particularly early after seroconversion[5,6]. Less is known about sex differences in HIV during ART. Recent data has demonstrated that estrogen can directly modulate HIV transcription in the setting of latency reversal (Karn et al, IAS 2015, Vancouver). Here, we sought to define sex differences in the size and activity of the HIV reservoir and measures of immune activation in a cohort of premenopausal women and matched men on ART.

Methods

Cohort: Premenopausal women on ART with ≥1 year of viral suppression were prospectively enrolled (n=26) and matched with men (n=26) on age, duration of viral suppression, CD4 count/nadir and unusual clinical phenotypes. All participants were enrolled through the SCOPE cohort at UCSF and provided written informed consent.

Measures of reservoir and activity: Integrated HIV DNA (iDNA) was measured in resting CD4 T cells. Residual plasma viremia was determined on 7mL of plasma using a single copy assay (SCA) for HIV gag (HMMCgag) as previously described[12]. Cell associated (CA) multiply spliced (ms) and unspliced (us) HIV RNA in resting CD4 T cells was measured and normalized to 18S RNA input. The frequency of CD4 T cells producing tat/rev RNA after activation was measured by the Tat/rev Induced Limiting Dilution Assay (TILDA) in a subset of subjects[13]. T cells were phenotyped by flow cytometry.

Statistical analysis: Virologic data were assessed using negative binomial regression to generate estimates of the effect of female sex on the outcome variable with a measure of input as the exposure variable (e.g. 18s RNA for HIV RNA measures, plasma volume for HMMC gag measures) as previously described[14]. Multivariate models were built by stepwise addition of predictor variables, with sex forced as a covariate, until no remaining candidate predictor had p<0.05. Immune subsets were compared by Mann Whitney statistics. Relationship of T cell parameters to virologic parameters was assessed with Spearman rank correlations and P-values for differences in correlations between men and women were obtained by standard calculations using the Fisher z-transformation (http://vassarstats.net/rdiff.html)

Results

Table 1.

Cohort Characteristics	Men	Women
Age in years, median (IQR)	43 (33-48)	41 (35-48)
CD4 nadir cells/uL, median (IQR)	270 (131-442)	214 (111-317)
CD4 at sampling cells/uL, median (IQR)	646 (544-825)	677 (530-861)
Duration of infection years, median (IQR)	7 (4.0-11.5)	8 (4.8-14.3)
Duration of viral suppression years, median (IQR)	3.3 (2.1-6.7)	2.8 (1.8-4.3)
Max pretreatment viral load, median (IQR)	4.74(4.4-5.4)	4.61(3.8-5.2)
CMV positive, *n* (%)	26 (100)	21 (81)
Active HCV infection, *n* (%)	2 (7.7)	1 (3.8)
IDU, *n* (%)	3 (12)	5 (19)
Race, *n* (%)		
White	9 (35)	8 (31)
Black	7 (27)	6 (23)
Hispanic	4 (15)	4 (15)
Asian	2 (8)	3 (12)
Native American	1 (4)	0
Mixed/Multiracial/Other	3 (11)	5 (19)

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Figure 1:
Comparison of virologic markers by sex.

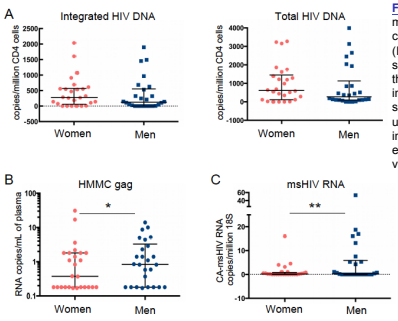


Figure 1. (A) Integrated and total DNA measured in isolated CD4 cells were comparable between men and women. (B) Low level viremia measured by single copy assay was lower in women than in men. (C) msHIV RNA was lower in women. (D) There was no statistically significant difference in the level of usHIV RNA. For all values median and interquartile range are shown, fold effect estimates, confidence intervals and p values in Table 2.

Table 2

The effect of female sex on virologic measures. Negative binomial regression in univariate and multivariate models to assess the quantitative influence of female sex on virologic measures.

HIV reservoir measure	Female fold effect	Confidence Interval	p value
iHIV DNA	1.39	0.57-3.37	0.47
tHIV DNA	1.38	0.67-2.74	0.39
SCA (HMMCgag) adjusted for: - duration of suppression - treatment interruptions	0.23	0.08-0.72	0.011
HMMCgag:iHIV DNA	0.43	0.20-0.91	0.027
CA msHIV RNA	0.16	0.05-0.51	0.002
CA msHIV RNA adjusted for: - CD4nadir - controller phenotype	0.25	0.09-0.71	0.009
CA msHIV RNA:iHIV DNA	0.29	0.13-0.64	0.002
CA usHIV RNA	*0.65*	*0.29-1.43*	*0.280*
CA usHIV RNA adjusted for: - max pretreatment viral load - CD4 nadir - race - early treatment initiation - controller phenotype	*0.68*	*0.35-1.32*	*0.253*
CA usHIV RNA:iHIV DNA	*0.52*	*0.25-1.07*	*0.08*

Figure 2:
Comparison of the inducible reservoir by sex in isolated CD4⁺ T cells.

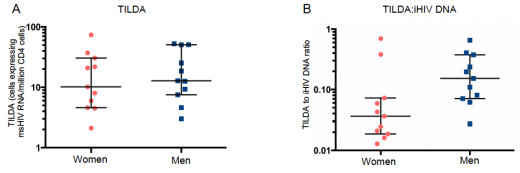


Figure 2. (A) TILDA values were comparable between men and women with an estimated female fold effect of 0.81 with a confidence interval of 0.33-2.01 and p=0.63 (negative binomial regression). (B) The ratio of TILDA:integrated HIV DNA was estimated as approximately 2 fold lower in women (fold effect of female sex 0.45, CI 0.16-1.21, p=0.11) using a customized maximum likelihood modeling of the well-by-well TILDA results together with the detailed iHIV DNA data.

Figure 3:
Comparison of markers of T cell activation and exhaustion by sex.

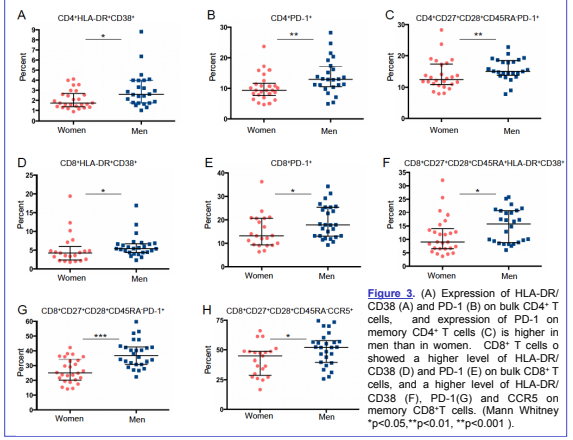


Figure 3. (A) Expression of HLA-DR/CD38 (A) and PD-1 (B) on bulk CD4⁺ T cells, and expression of PD-1 on memory CD4⁺ T cells (C) is higher in men than in women. CD8⁺ T cells o showed a higher level of HLA-DR/CD38 (D) and PD-1 (E) on bulk CD8⁺ T cells, and a higher level of HLA-DR/CD38 (F), PD-1(G) and CCR5 on memory CD8⁺T cells. (Mann Whitney *p<0.05,**p<0.01, **p<0.001).

Figure 4:
Relationships between T cell parameters and virologic measures vary by sex.

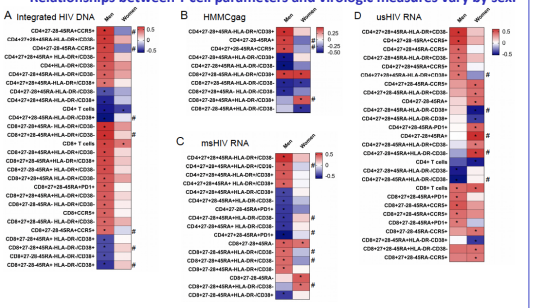
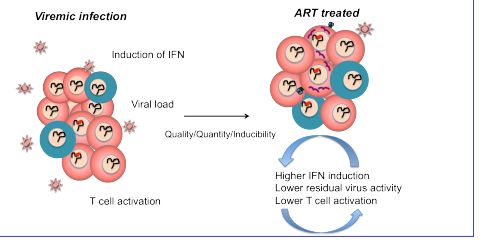


Figure 4. (A) Spearman's rho associations between T cell parameters and iHIV DNA (A), HMMCgag (B) msHIV RNA (C), and usHIV RNA (D). * indicates associations with rho with a p value <0.05, # symbols indicate where the difference between rho values for men and women were significantly different (p<0.05)

Conclusions

In a well-matched cohort of ART-treated, virally suppressed women and men, multiple measures of virus activity and immune activation/exhaustion were lower in women despite comparable frequencies of CD4+ T cells harbouring HIV DNA. These data support sex differences in control of HIV latency. Biologic sex may impact the efficacy of curative interventions and manipulation of sex hormones may play a role in cure strategies.



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