Sex Differences in Cytokine Profiles During Suppressive Antiretroviral Therapy

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BACKGROUND

Despite lower HIV RNA levels, women progress faster to AIDS than men.

The reasons for these observations are not clear

- might be a consequence of heightened inflammatory response in women.
- might also contribute to sex differences in HIV persistence.

Overarching Goal: To investigate the sex differences in plasma cytokine profiles in men and women with chronic HIV on suppressive ART.

METHODS

Data Generated

Concentrations of 36 cytokine/chemokines by Luminex in blood plasma of 50 women and 49 men (sex at birth) with chronic HIV on suppressive therapy (**Figure 1**).

Statistical Analysis

Cytokines with >70% undetectable values were excluded from analyses. We initially performed a principal component analysis to see if participants clustered by sex, and then fit a PLS-DA model where we used cytokines to predict sex at birth. The significance of the difference in 9 cytokines with VIP>1 was further tested using a Wilcoxon rank-sum test. False Discovery Rate (FDR) correction to p-values were applied to adjust for multiple comparisons. To adjust for differences in baseline characteristics we fit linear regression models for each cytokine and individually included each baseline characteristic as a covariate.

- Mediators of innate immunity, inflammation and chemotaxis (interleukin [IL]-1α, IL-1β, IL-6, IL-17, IL-18, IL-21, IL-22, IL-8 (CXCL8), MIG (CXCL9), IFN-inducible-protein [IP]-10 (CXCL10), I-TAC (CXCL11), TNF-α, monocyte-chemotactic-protein [MCP]-1 (CCL2), macrophage-inflammatory-protein [MIP]-1α (CCL3), MIP-1β (CCL4), regulated-on-activation-normally-T-cell-expressed-and-secreted [RANTES, CCL5], MIP-3α (CCL20), MIP-3β (CCL19), CXCL13, GRO-α (CXCL1), TNF-related apoptosis-inducing ligand (TRAIL), and Vascular Endothelial Growth Factor (VEGF).
- Mediator of hematopoiesis (macrophage-colony-stimulating-factor [M-CSF] and granulocyte-macrophage-colony-stimulating factor [GM-CSF]).
- Anti-inflammatory cytokine (IL-10, IL-13, transforming-growth-factor [TGF]-β, PD-L1).
- Mediators of lymphocytes activation, proliferation, and differentiation (IL-2, IL-4, IL-7, IL-12, IL-15, IL-16, CCL3, CCL4, CCL5, CCL20, IFN-γ).
- Soluble receptor antagonist (IL-1RA).

Figure 1 Cytokines/chemokines panel divided by immunological function.
Legend: We measured the concentrations of 36 cytokines/chemokines involved in different immunological functions. In consideration of their pleotropic effects, CCL3, CCL4, CCL5, CCL20 were included in two different functional groups, both as mediators of chemotaxis and as mediators of activation, proliferation, and differentiation of lymphocytes. Data were collected using a Bio-Plex Suspension Array Reader (Bio-Rad Laboratories Inc., Hercules, CA) and Bio-Plex manager software (version 4). Lower limit of Detections were determined using 5P logistic regression and the curve optimization feature. Curves between all plates were compared between batches for consistency.

- Plasma cytokines/Chemokine profiles are significantly different between men and women (sex at birth).
- Women have significantly higher concentrations of pro-inflammatory cytokines and the T-cell homeostatic factor IL-7.
- Careful experimental design and systemic inclusion of sex (and gender) as a research variable is imperative.

RESULTS

Median age was 53 years for women and 46 years for men; 26 (52%) women were post-menopausal and 43 (86%) acquired HIV through heterosexual contact. All men reported sex with men as risk factor.

Both groups had suppressed plasma HIV RNA. Median CD4⁺ T cell counts were 721 cells/μl (range: 300-1362) for women and 625 (range: 282-1149) for men (**Table 1).**

Overall, PCA analysis on cytokine profile shows distinct clustering of men (orange triangles) and women (blue circles) (**Figure 2, Panel A**).

We were able to predict sex at birth in the PLS-DA model with an error rate of 13% (**Figure 2, Panel B**).

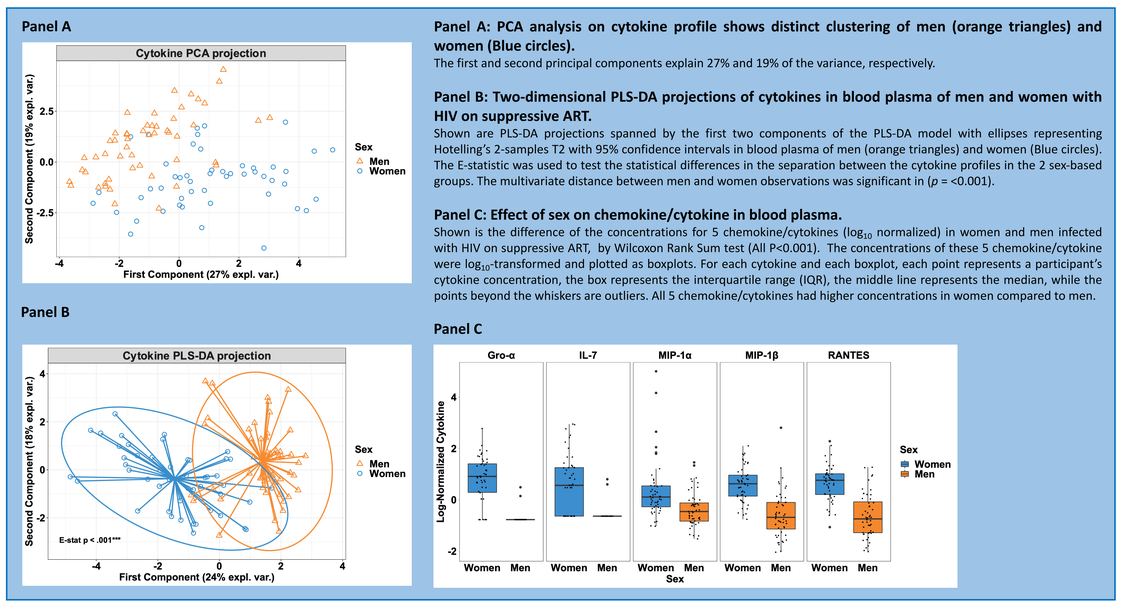
We further identified 5 cytokines which were all significantly higher (in women compared to men):

- Four inflammatory chemokines, namely Gro-α, RANTES, MIP-1α, MIP-1β, as well as the T-cell homeostatic factor IL-7 (**Figure 2, Panel C**).
- The effect of sex remained significant after adjusting for CD4⁺ Count, age, ethnicity, and race for all cytokines, except for MIP-1 α and race.

Characteristic	Male (n = 52)	Female (n = 50)	Overall (n = 102)	p-value
Age	46 (30, 68)	53 (22, 65)	50.00 (22, 68)	< .001***
Race				
White	39 (75.0%)	8 (16.0%)	47 (46.1%)	<.001***
African American	11 (21.2%)	37 (74.0%)	48 (47.1%)	
Asian	2 (3.8%)	1 (2.0%)	3 (2.9%)	
Native American	0 (0.0%)	1 (2.0%)	1 (1.0%)	
Other	0 (0.0%)	3 (6.0%)	3 (2.9%)	
Ethnicity				
Non-Hispanic	32 (61.5%)	43 (86.0%)	75 (73.5%)	0.010*
Hispanic	20 (38.5%)	7 (14.0%)	27 (26.5%)	
CD4 count	635 (282, 1149)	721 (300, 1362)	660 (282, 1362)	0.046*
Nadir CD4 count	215 (0, 667)	172 (0, 600)	195 (0, 667)	0.128
Menopause				
Pre	(-)	24 (48.0%	24 (48.0%	-
Post	(-)	26 (52.0%)	26 (52.0%)	

Mean (sd) for continuous variables, count (percentage) shown for categorical variables.
T-test was used to compare continuous variables and a Chi-square test or Fisher's exact was used to compare categorical variables.

Table 1: Baseline Characteristics and Demographics



Panel A: PCA analysis on cytokine profile shows distinct clustering of men (orange triangles) and women (Blue circles).
The first and second principal components explain 27% and 19% of the variance, respectively.

Panel B: Two-dimensional PLS-DA projections of cytokines in blood plasma of men and women with HIV on suppressive ART.
Shown are PLS-DA projections spanned by the first two components of the PLS-DA model with ellipses representing Hotelling's 2-samples T2 with 95% confidence intervals in blood plasma of men (orange triangles) and women (Blue circles). The E-statistic was used to test the statistical differences in the separation between the cytokine profiles in the 2 sex-based groups. The multivariate distance between men and women observations was significant in (p = <0.001).

Panel C: Effect of sex on chemokine/cytokine in blood plasma.
Shown is the difference of the concentrations for 5 chemokine/cytokines (log_{10} normalized) in women and men infected with HIV on suppressive ART, by Wilcoxon Rank Sum test (All P<0.001). The concentrations of these 5 chemokine/cytokine were log_{10}-transformed and plotted as boxplots. For each cytokine and each boxplot, each point represents a participant's cytokine concentration, the box represents the interquartile range (IQR), the middle line represents the median, while the points beyond the whiskers are outliers. All 5 chemokine/cytokines had higher concentrations in women compared to men.

CONCLUSIONS

The observed sex-based differences in cytokines might contribute to maintain higher immune activation in women compared to men despite suppressive therapy and may explain why women progress faster to AIDS and experience more HIV-related complications.

Increased levels of IL-7 in women suggest that homeostatic proliferation may have a differential contribution to HIV reservoir maintenance in women than men.

Our study emphasizes the importance of sex-specific studies of viral pathogenesis.

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