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Effect of HIV Suppression on Cytokines in Blood and Seminal Plasma

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BACKGROUND

- HIV infection disrupts the cytokine network and it remains disrupted after antiretroviral therapy (ART) [1]
- Cytokines in genital secretions are important for understanding the mechanisms of HIV sexual transmission and may influence systemic inflammation [2,3]

METHODS

- 34 cytokine/chemokines were measured by multiplex-bead assay (as in [2])
- 20 men were included (1 excluded for abnormal lab values). Cytokines were measured in longitudinal semen and blood samples before and after ART
- We used Partial Least Squares Discriminant Analysis (PLS-DA) to visualize the difference in cytokine pattern between the time points
- We ranked the relative importance of cytokines for determining suppression status
- Any cytokines with Variable Importance on PLS Projections (VIP) scores exceeding 1 were deemed important in predicting suppression status
- These were subsequently tested using Wilcoxon Signed Rank Tests

Table 1: Demographics and clinical metrics of 19 patients included for analysis.

Gender/Orientation; n (%)	MSM	19 (100.0)
Race/Ethnicity; n (%)	White (non-hispanic)	14 (73.7)
	Hispanic/Latino	2 (10.5)
	Other/Multiracial	3 (15.8)
HIV Diagnosis Timeline; n (%)	Acute (Ab neg)	7 (36.8)
	Early (< 70 days)	12 (63.2)
HIV Treatment Timeline; median (range)	EDI to ART Initiation (wk)	12.9 (2.7,401.4)
	ART Initiation to Suppression (wk)	14.0 (2.1,91.9)
Study Timeline; median (range)	Weeks between time points	29.1 (3.1,515.1)
Viral RNA (log10 copies/mL); median (range)	Peak Viral RNA	5.67 (3.77,7.44)
	Viral RNA at Pre-ART tp	4.66 (2.75,6.58)
Lymphocytes (cells/µL); median (range)	Nadir CD4 count	553 (143,913)
	CD4 at study entry	702 (324,997)
Days to suppression; median (range)	EDI to suppression	203 (73, 3453)
	ART Start to suppression	98 (15, 643)

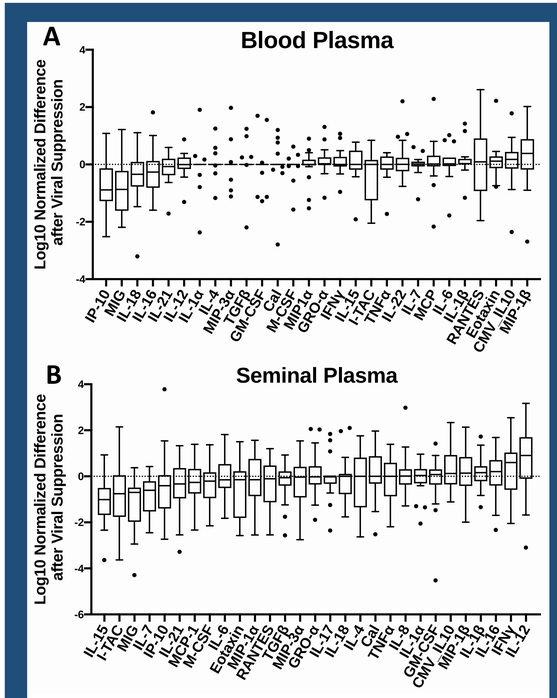
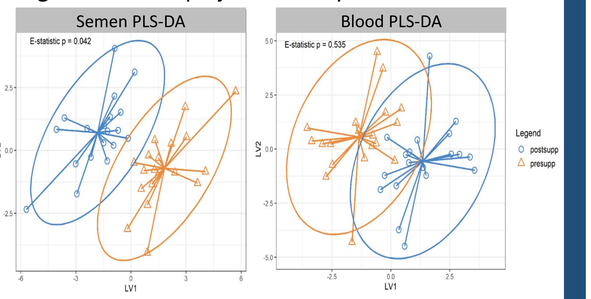


Figure 1: Differences in cytokine concentrations in blood (A) and semen (B) after suppression by ART. Boxplots graph the differences in the log10 normalized values across suppressed and non-suppressed time points.

Figure 2: PLS-DA projections of cytokine networks



Legend: Partial Least Squares Discriminant Analysis (PLS-DA) was used to visualize the difference in cytokine pattern between the time points (orange = pre-suppression; blue = post-suppression.

Table 2: Results of Wilcoxon Signed Rank Test for cytokines with VIP > 1

Sample Type	Cytokine	VIP	W	P	Adjusted P	Romano-Wolf Rejection (Y/N)
Genital Secretions	MIG	1.76	184	<0.001*	<0.001*	Y
	IL-15	1.86	182	<0.001*	<0.001*	Y
	IL-7	1.79	180	<0.001*	<0.001*	Y
	I-TAC	1.50	159	0.008*	0.019*	Y
	IP-10	1.04	138	0.087	0.112	N
	M-CSF	1.33	130	0.169	0.169	N
	Eotaxin	1.24	117	0.056	0.084	N
	CMV_IL-10	1.49	39	0.141	0.158	N
	IL-12	1.56	34	0.045	0.081	N
Blood	IP-10	2.14	169	0.002*	0.006*	Y
	MIG	2.15	169	0.002*	0.006*	Y
	IL-18	1.40	142	0.06	0.14	N
	IL-16	1.22	131	0.156	0.182	N
	I-TAC	1.77	101	0.093	0.164	N
	MIP-1β	1.00	57	0.134	0.182	N
	MCP-1	1.29	53	0.464	0.464	N

Legend: Cytokines with Variable Importance on PLS Projections (VIP) scores >1 were tested using Wilcoxon Signed Rank Tests for significance. The p-values of these multiple comparisons were adjusted using false discovery rate, and results were then verified using Romano Wolf-controlled error rates for multiple testing.

RESULTS

Significant overlap of the PLS-DA projections (Fig. 2) in blood suggested no significant difference in the overall cytokine network after suppression of viremia, even though individual cytokines changed in line with published findings from other studies (Fig. 1). However, the projections are significantly different in seminal plasma, highlighting the importance of immune activation in this compartment. When tested individually (Table 2), four cytokines were significantly different across time points in semen (MIG, IL-15, and IL-7: all $p < 0.001$; I-TAC: $p = 0.019$), while only two were significantly different across time points in blood (MIG and IP-10, both $p = 0.006$).

CONCLUSIONS

- Viral suppression with ART has the most significant decrease in the inflammatory milieu in seminal plasma
- Effect on cytokines in the blood is weaker, but consistent with prior analysis
- Highlight the utility of novel statistical methods in the analysis of large data sets of cytokine measurements
- Intriguingly, levels of MIG (aka CXCL9), IP-10 (aka CXCL10), and ITAC (aka CXCL11), which are ligands for the chemokine receptor CXCR3, were all impacted by ART
- Decreases in seminal plasma of IL-7 and IL-15 with ART suggests that the need for homeostatic proliferation and anti-apoptotic function is no longer present—perhaps because resident lymphocytes populations are restored into the genital compartment once HIV suppression is achieved
- Consistently, decreases of the CXCR3 ligands are indicative of reduced need for trafficking and recruitment of lymphocytes between lymphoid and peripheral (e.g. male genital tract) tissues after HIV suppression

ADDITIONAL INFORMATION

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