Immune Clearance Proteomic Signatures Linked to Lower Completed HIV RNA Levels

357

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INTRODUCTION

- In ART-suppressed people with HIV (PWH), subsets of infected cells continue to express various viral products, which likely contribute to chronic inflammation/immune activation and organ damage.
- During suppressive ART, incomplete HIV transcripts far outnumber completed or multiply spliced HIV RNA.
- It is unclear to what degree various HIV transcripts contribute to immune activation or mechanisms that may help clear infected cells.
- Efavirenz (EFV) may induce pyroptosis of HIV-expressing cells, but it is unclear if this process reduces levels of different HIV transcripts.

Hypotheses:

1. **Compared to early/incomplete HIV RNA, completed and multiply spliced HIV RNA will correlate more strongly with plasma markers of immune activation**
2. **Use of EFV will correlate with lower levels of completed HIV RNA**

METHODS

- PBMC and plasma were obtained from a cohort of 50 well-characterized ART-suppressed PWH.
  - Median values: age 58, 10 yrs VL suppression, current/nadir CD4: 590/100
  - 88% male, 30% on EFV
- Levels of cell-associated HIV transcripts in PBMC were quantified by RT-ddPCR: 1) total initiated (TAR); 2) 5' elongated (LTR-pre-gag); 3) mid-transcribed, unspliced (pol); 4) 3' or distal (nef); 5) completed (U3-polyA); and 6) multiply spliced (tat-rev).
- Levels of human plasma proteins were measured using the Olink Inflammation Explore platform (358 proteins) and the Olink Reveal platform (1034 proteins) [total of 1392 proteins]
- HIV RNA and human protein levels were log transformed and normalized to standard deviations (z-scored).
- Linear regression models used to assess all associations, adjusted for age, sex, and CD4 count.
- Unadjusted P-values were corrected for multiple comparisons and expressed as Storey's Q values.

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- **HIV transcripts** in PBMC correlate with levels of different human **plasma proteins**. Most correlations are negative.
- These **proteomic signatures** may reflect immune responses that help to **sense and clear HIV-expressing cells.**

RESULTS

We observed multiple correlations between levels of each HIV transcript and various human plasma proteins. Most associations (78-99%) were negative (inverse).

	HIV transcript						
	Initiated	5' Elongated	Pol (mid)	Nef (3')	Completed	Multiply Spliced	Avg HIV Z-score
# (%) of proteins associated with each HIV RNA (nominal P<0.05)	126 (9.1%)	169 (12.1%)	59 (4.2%)	79 (5.7%)	82 (5.9%)	133 (9.6%)	169 (12.1%)
# (%) of negative associations	125 (99%)	160 (95%)	46 (78%)	77 (97%)	78 (95%)	123 (92%)	165 (98%)

Table 1: Numbers and percent of plasma proteins associated with each HIV transcript

Some plasma proteins were associated with multiple HIV transcripts. CD40LG correlated negatively with 5' elongated HIV RNA (Q<0.05).

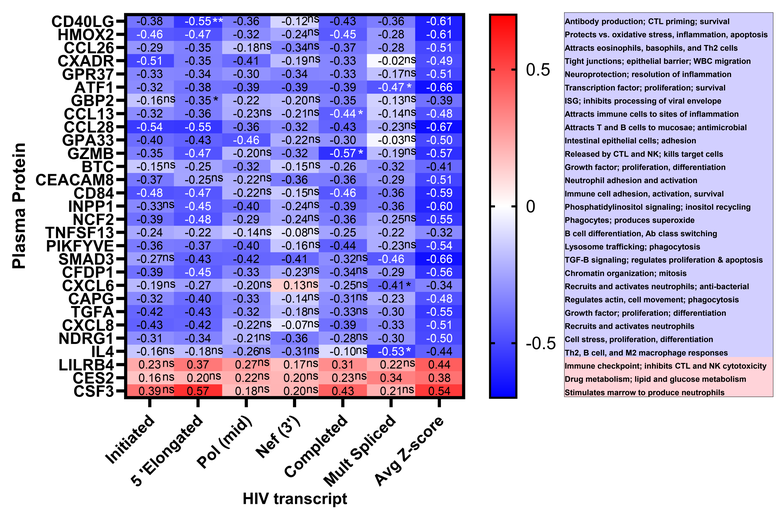


Figure 1: Plasma proteins associated with multiple HIV transcripts. Heat map shows the standardized beta coefficients, indicating the change in expression (in standard deviations [SD]) of each plasma protein (rows) per one SD change in the level of each HIV RNA (columns 1-6) or the average z-score across all HIV transcripts (last column). Proteins were included in the table if they showed low Q values or consistent associations with different HIV transcripts (≥4 negative or 2 positive associations). Unadjusted P values are significant unless indicated by ns(=not significant). *Indicates 0.05<Q<0.125. **Q<0.05. Protein functions are indicated in the table to the right.

RESULTS

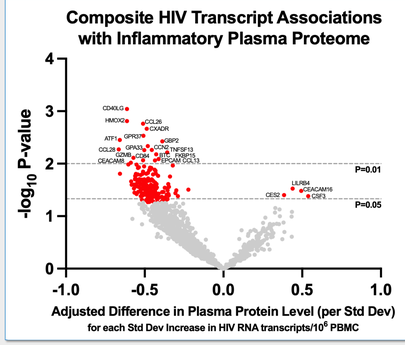


Figure 2: Volcano plot showing associations between levels of human plasma proteins and the average of HIV transcript z-scores. The x-axis shows the standardized beta coefficient (strength of association). The y-axis shows the $-\log_{10}$ unadjusted P value. Individual proteins with low P values have been annotated.

Proteins negatively associated with HIV RNA are enriched for cell killing and chemotaxis pathways (Q<0.05). Proteins with positive associations tend to be enriched for T cell anergy and myeloid differentiation (Q=0.16).

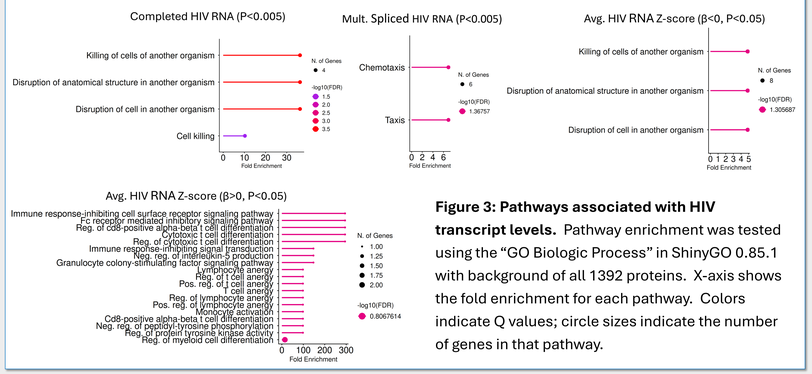


Figure 3: Pathways associated with HIV transcript levels. Pathway enrichment was tested using the "GO Biologic Process" in ShinyGO 0.85.1 with background of all 1392 proteins. X-axis shows the fold enrichment for each pathway. Colors indicate Q values; circle sizes indicate the number of genes in that pathway.

The NK cell marker KLRD1 tended to correlate negatively with the ratio of completed to 5' elongated HIV transcripts (β = -0.46, Q = 0.056).

EFV use no longer significantly associated with completed HIV RNA levels (-0.48 std deviations, P=0.16) in the updated n=50 analysis (n=15 on EFV)*

*Differs from preliminary results from first 32 PWH (n=11 on EFV), which suggested that EFV use correlates with lower levels of completed HIV RNA

CONCLUSIONS

1. In ART-treated PWH, significant and typically consistent correlations were observed between levels of different HIV transcripts in PBMC and levels of human proteins in plasma, including CD40LG.
2. Most (78-99%) significant associations were negative. Proteins negatively associated with completed and overall HIV RNA levels were enriched for pathways involving immune response and cell killing.
3. Few proteins correlated positively with overall HIV RNA levels. These tended to be involved in T cell anergy and myeloid differentiation.