Viral Suppression and Marijuana Modulate Transcriptome Bioprofile In Youth With HIV

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BACKGROUND

Viral suppression by antiretroviral therapy (ART) modulates many inflammatory pathways perturbed by HIV, but does not completely normalize inflammation. Recreational use of marijuana may affect pro-inflammatory pathways. A systems biology approach was applied to define peripheral blood cell transcriptome profiles associated with ART-dependent viral suppression and recreational marijuana use in youth with HIV (YWH).

METHODS

The study included 60 youth [median age 22 to 24 years], including **Group I**, HIV-seronegative no substance use (n = 25), and three groups of YWH on ART (n = 35), including **Group II**, no substance use and failed viral control (n = 7); **Group III**, no substance use and achieved complete viral suppression (n = 20); **Group IV**, viral suppressors who used marijuana (THC, n = 8) **(Table 1)**.

Table 1. Demographic and clinical characteristics of study participants (n = 60).

Characteristics	HIV-seronegative	HIV-seropositive with ART		
	Group I	Group II	Group III	Group IV
	(n = 25)	VL > 50 (n = 7)	VL ≤ 50 (n = 20)	VL ≤ 50 (n = 8)
Substance use	No	No	No	Marijuana[c]
Age (years)[a]	22 [20, 23]	24 [23, 26]	24 [23, 26]	24 [22, 26]
Male[b]	68	71	85	75
African American[b]	80	86	80	100
Days on ART[a]	NA	917 [801, 1012]	1047 [900, 1061]	932 [518, 1018]
HIV-1 plasma RNA (copies/mL)[a]	NA	2379 [123, 17400]	≤ 50	≤ 50
CD4 T-cells (number/μL)[a]	751 [462, 864]	431 [276, 777]	748 [582, 958]	624 [522, 862]

[a] **Median** [25th and 75th quartile range]; [b] **%**; [c] **Marijuana use** based on toxicology assay for THC, Δ9-tetrahydrocannabinol, a primary ingredient in marijuana.

Peripheral blood cell mRNA was profiled using Affymetrix HG-U133 Plus 2.0 Arrays. Probe signals were normalized and background corrected using Robust Multiarray Averaging (RMA). Variation in expression data was analyzed by principal component analysis (PCA). Differentially expressed genes (DEGs) were identified by Significance Analysis for Microarrays (FC ≥ 1.3) and (FDR ≤ 0.05). Pathway enrichment analysis was performed using Ingenuity Pathways Analysis to identify enriched pathways (p ≤ 0.001). Comparison among study groups was performed using nonparametric One-way ANOVA in GraphPad Prism 7.04 with P < 0.05 as significant.

MAIN FINDINGS

- **Sustained plasma viral suppression by ART for up to three years among YWH, compared to YWH with detectable virus, reduces inflammatory-related gene expression by about 80%.**
- **Recreational marijuana use among virally suppressed YWH significantly reduces inflammatory-related gene expression.**

RESULTS

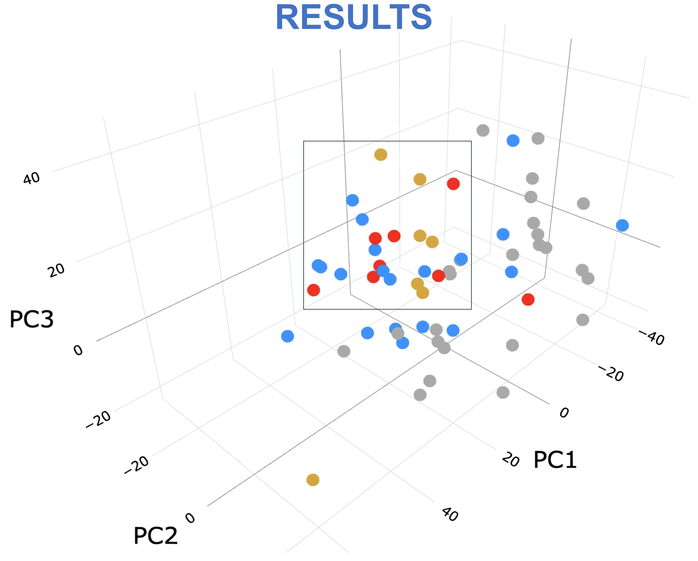


Figure 1. Principal component analysis (PCA). To visualize relatedness of study populations, PCA was based on normalized expression values of the top 5% of highly variable probes (2,734 of 54,675) from all 60 individuals. **Colors:** grey, **Group I** (n = 25); orange, **Group II** (n = 7); blue, **Group III** (n = 20); or red, **Group IV** (n = 8). Viral infection clearly separates YWH from HIV-uninfected youth.

23 of 35 YWH (66%) cluster together distinct from most seronegative individuals (boxed area), including: 6/7 Group II, ~85%; 10/20 Group III, 50%; 7/8 Group IV, ~87%; but only 2/25, 8% Group I. Viral infection groups II, III, and IV are more closely related independent of marijuana use or extent of viral replication.

Table 2. DEGs and significantly perturbed pathways in HIV-seropositive study groups compared to Group I.

Study groups		II	III	IV
DEGs	Total number	2448	440	430
	Up/Down	1118/1330	150/290	156/274
Perturbed pathways		127	25	1
DEGs in perturbed pathways		602	70	14
Common pathways		22	22	0
Mean DEGs in common pathways		31	10	NA

- Compared to transcriptome profiles among seronegative participants, detectable viral replication (Group II) dysregulated almost 2500 genes. Suppressed viral replication (Group III) reduced perturbed genes almost 80%.
- Active viral replication (Group II) perturbed 127 pathways. Sustained viral suppression (Group III) reduced the perturbed pathways to 25, of which 22 pathways were shared by Group II.
- Marijuana use (Group IV) normalized all pathways perturbed in Groups II and III, but perturbed one new pathway, the **protein ubiquitination pathway**.
- 22 inflammatory pathways were perturbed in Groups II and III.
  - 74% of DEGs within the perturbed pathways were normalized by viral suppression (Group III).
  - 18 of 22 perturbed pathways included the same DEGs:
    - **RAP2A**, **PIK3C2A**, **PIK3CB**: shared by Groups II and III
    - **RAP2B**: Group III only
  - 7 DEGs were observed specific to Group II,
    - Upregulation of **RAP2B**, **ITGB1**, **KLRD1** and **KLRC3**
    - Down-regulation of **SLC8A1**, **E2F3** and **COL4A3BP**

CONCLUSIONS

HIV replication and long-term viral suppression display unique blood transcriptome bioprofiles. Surprisingly recreational marijuana use normalized many pathways and genes dysregulated by HIV.