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Subclinical CMV and EBV DNA increases HIV DNA Molecular Diversity in Peripheral Blood during Suppressive Antiretroviral Therapy (ART)

Antoine Chaillon, Masato Nakazawa, Gemma Caballero, Brianna Scott, Christy Anderson, Sara Gianella.

University of California San Diego, San Diego, CA, USA

Sara Gianella, MD
University of California, San Diego
9500 Gilman Drive
San Diego, CA 92093-0679, USA
E-mail: gianella@ucsd.edu
Web: http://gianella.ucsd.edu

Background

Detectable Cytomegalovirus (CMV) DNA is associated with increased activation of CD4⁺ T cells and with a slower decay of HIV DNA in people starting ART during early HIV infection (1, 2).

Objective

To investigate the impact of CMV and Epstein-Barr Virus (EBV) DNA on HIV DNA molecular diversity in the setting of early ART.

Cohort and Sampling

We included at least 3 longitudinal PBMCs from 37 persons starting ART during early HIV infection and who reached virologic suppression (<50cp/ml, no viral blips) within a median of 3 months from the estimated date of HIV infection (EDI) (IQR: 2.6-6.8 months).

Table 1. Demographics and clinical metrics of the 37 study participants

Characteristics	Values
CMV Shedding Status, n (%)	
Non-Shedder	18 (48.6)
Shedder	19 (51.4)
EBV Shedding Status, n (%)	
Non-Shedder	19 (51.4)
Shedder	18 (48.6)
Men who have sex with men (MSM), n (%)	37 (100.0)
Age at enrollment (year,) median (IQR)	35 (29-44)
Length of follow up (months), median (IQR)	23 (22-28)
Months from EDI to ART initiation, median (IQR)	3.1 (1.5-6.5)
Peak HIV RNA, (log_{10} copies/ml), median (IQR)	5.7 (4.9-6.4)
Baseline CD4⁺ cell count (cells/µl), median (IQR)	548 (394-710)
CD4/CD8 ratio, median (IQR)	0.5 (0.4-0.7)
HIV DNA levels (log_{10} copies/10^6 CD4⁺), median (IQR)	2.7 (1.9-3.4)
HIV RNA levels (log_{10} copies/ml), median (IQR)	5.2 (4.7-5.6)
Normalized Entropy levels (*env* region), entropy/Ln($n_{haplotype}$), median (IQR)	3.0 (2.6-3.8)

Data Generated and Statistical Analysis

- In each PBMC sample (N=120), levels of HIV, CMV and EBV DNA were measured by digital droplet (dd)PCR.
- Deep Sequencing of HIV DNA C2-V3 *env* was performed using the MiSeq Illumina platform.
- Cleaned mapped reads were obtained after iterative read mapping and quality filtering using an in-house pipeline) (3). The HIV DNA molecular diversity (Shannon Entropy/log($n_{Haplotype}$)) was computed (4).
- A linear mixed-effect regression model was used to analyze the effect of CMV or EBV DNA on HIV DNA molecular diversity and its change from ART initiation (baseline) to the end of follow-up (up to 53 months).

Results

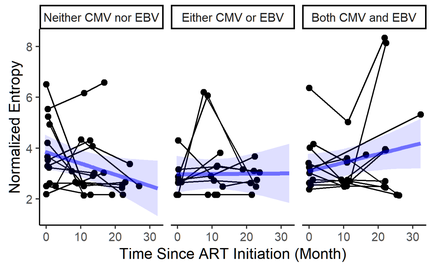
Table 2. Subclinical CMV and EBV DNA is associated with increase in HIV DNA diversity.

	Univariable Models			Multivariable Model		
Predictor	**Coef**	**95% CI**	**p**	**Coef**	**95% CI**	**p**
Intercept	3.41	(2.86, 3.96)	0	3.84	(3.16, 4.52)	0
Time (Month Since ART Initiation)	0.00	(-0.02, 0.03)	0.969	-0.04	(-0.09, 0)	**0.047**
Viremic Shedding			0.412*			
Neither CMV nor EBV (Reference Category)						
Either CMV or EBV	-0.43	(-1.24, 0.39)	0.294	-0.87	(-1.87, 0.13)	0.089
Both CMV and EBV	0.06	(-0.73, 0.84)	0.886	-0.74	(-1.71, 0.23)	0.132
Viremic Shedding by Time						
Neither CMV nor EBV by Time (Reference Category)						
Either CMV or EBV by Time				0.05	(-0.02, 0.11)	0.156
Both CMV and EBV by Time				0.08	(0.02, 0.14)	**0.009**
Covariates						
Age at enrollment	0.01	(-0.02, 0.05)	0.400			
Peak Viral Load (log_{10} copies /ml)	0.11	(-0.21, 0.44)	0.482			
CD4 ($\sqrt{cell\ counts}$ /µl)	-0.05	(-0.12, 0.01)	0.107			
CD4/CD8 Ratio	-0.61	(-1.7, 0.48)	0.260			
Early ART Initiation (≤90 Days)	0.32	(-0.35, 0.99)	0.335			

Legend. Study participants were classified as shedders if their average CMV/EBV level (i.e., the mean across all samples) was greater than the median of the average CMV (0 copies/106 cells) or EBV (64.1 copies/106 cells) level, respectively.

Coef: regression coefficient, 95% CI: Confidence Interval, CMV: Cytomegalovirus, EBV: Epstein-Barr Virus.

Figure 1. Dynamic of HIV diversity (*env*) since ART initiation by CMV or EBV shedding status.



Legend. Participants were classified into one of the "Neither CMV nor EBV", "Either CMV or EBV", and "Both CMV and EBV" groups depending on their shedding patterns. Black dots and line indicate entropy values and participants, respectively. Blue lines and shaded areas indicate model-predicted values and corresponding 95% confidence intervals, respectively.

RESULTS

- **Significant interaction between HIV DNA diversity and viral shedding status over time (p=0.028, Table 2).**
- **Normalized entropy levels tended to increase over time among participants with detectable CMV and EBV DNA (0.03±0.02, p=0.089), while it significantly declined among participants with no viral shedding (-0.04±0.02, p=0.047). The difference in slope between these two groups was significant (p=0.009, Figure 1).**

Conclusions

- **These observations suggest that subclinical CMV and EBV DNA might affect HIV persistence by promoting HIV replication at low levels during early ART.**
- **Future studies with anti-CMV therapeutics could help determine the underlying mechanisms and if causal associations exist.**

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