HIV antibody and T cell responses on ART are associated with HIV DNA but not RNA

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Background

- HIV-specific immune responses decline after initiation of antiretroviral therapy (ART). Infected cells that persist during ART are thought to be invisible to the immune system, but if there is intermittent antigen expression and recognition, HIV antibody (Ab) and T cell responses may correlate with measures of HIV persistence.
- Current methods to quantify the HIV reservoir in individuals on ART use large volumes of blood or tissue biopsies. Measuring immune responses to HIV may be an alternative, more sensitive and efficient way to assess HIV persistence in blood and tissue.
- ELISPOT responses can be used to monitor magnitude and specificity of T cell responses. Quantitative antibody (Ab) assays can be performed with a few microliters of serum or plasma, are reproducible, and results can be obtained within hours.
- We assessed whether HIV antibody and T cell responses correlated with measures of HIV persistence in people on long-term ART.

Methods

- Longitudinal peripheral blood mononuclear cells (PBMC) and plasma samples were obtained from an ART-treated cohort of chronically infected individuals (ACTG A5321). All participants had well documented and sustained virologic suppression: HIV RNA levels <50 copies/mL with no blips or ART interruption (≥21 consecutive days) from year 1 of follow-up (Gandhi et al. PLoS Path 2017).
- Cell-associated (CA) HIV DNA and unspliced RNA levels (using qPCR targeting pol) was performed on stored PBMC and plasma samples from pre-ART (n=101), year 1 (n=101), year 4 (n=101), and years 6-15 (n=66) on ART.
- Less-sensitive (LS) VITROS (S/Co) and Avidity VITROS (AI) Ab measurements to Env/p24 were performed on plasma from all time points except pre-ART. VITROS, a chemiluminescent assay to detect HIV-1/2 Ab, is cleared by FDA for diagnosis of infection.
  - To measure anti-HIV Ab quantity and binding ability, the assay was modified to be LS by using dilution (1:400 dilution in buffer) and increasing the cutoff
  - To measure avidity by treatment with guanidine (guanidine-incubation/buffer-incubation; was calculated to yield an avidity index).
- Plasma HIV RNA was measured by single copy assay.
- T cell responses (IFN-g ELISPOT) against Gag, Pol, Env, Nef/Tat/Rev, Vpr/Vpu/Vif were measured at the last time point (yr 4-15; median 7 yr of ART).

Results I: Antibodies decline with time on ART

The Ab assays measure antibody evolution in titer and avidity.

We compared the overall results for the antibody assays used, looked for decline in reactivity over time on treatment and compared this with HIV reservoir measurements.

Table 1

		Years on ART		
		1	4	6-5
		N=101	N=101	N=46
Antibody level: LS-VITROS Signal:Cutoff ratio (S/Co)	**Median**	**32.4**	**16.6**	**9.8**
	Q1, Q3	17.5, 59.3	7.7, 34.8	4.2, 21.8
Antibody Avidity (VITROS Avidity Index)	**Median**	**0.7**	**0.56**	**0.52**
	Q1, Q3	0.54, 0.80	0.41, 0.72	0.35, 0.65

Both HIV Ab measurements showed a statistically significant decline between yr 1 and yr 4 on ART with median participant-specific decline of **19%/yr for S/Co and 5.6%/yr for avidity index (AI)** (p<0.001 for each; signed rank test) (Figure 1). Only one and four participants were found to have increases in Ab as measured by LS-VITROS and Avidity VITROS, respectively (Table 2); the rest had declines in Ab.

Figure 1

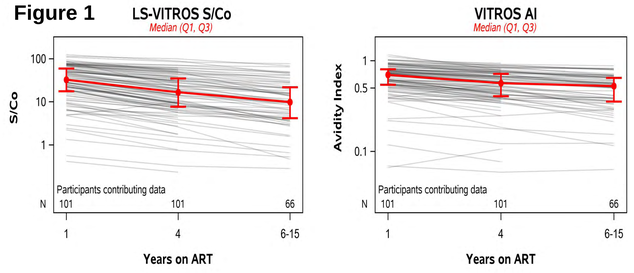


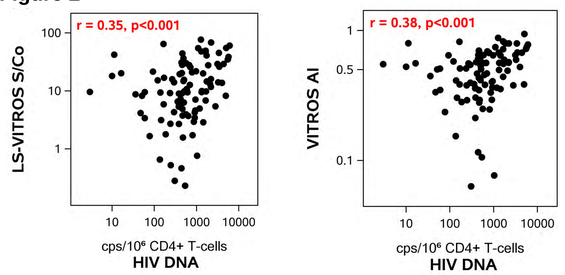
Table 2

Slope/yr from year 1 to year 4 on ART		Total (N=101)	P-Value
Antibody level: LS-VITROS log_{10} (S/CO)	Median	-0.09	<0.001
	Q1, Q3	-0.12, -0.06	
Negative Slope	Yes	100 (99%)	
	No	1 (1%)	
Antibody Avidity Vitros log_{10} (Avidity Index)	Median	-0.03	<0.001
	Q1, Q3	-0.04, -0.02	
Negative slope	Yes	97 (96%)	
	No	4 (4%)	

Results II: Antibody levels and avidity correlate with HIV DNA on ART

S/Co and AI were positively associated with HIV DNA after 1 year of ART (Spearman r= 0.24 and 0.27, p≤0.02) and at last time point (median 7 years on art; Figure 2). There was no correlation between Ab and CA-RNA.

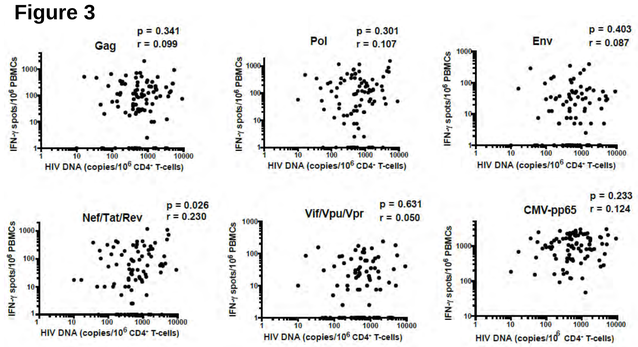
Figure 2



Results III: ELISPOT responses correlate with HIV DNA

ELISPOT responses were measured for HIV-specific responses including Gag, Pol, Env, Nef/Tat/Rev and Vpr/Vpu/Vif and non-specific proteins EBV and CMV (Figure 3; Thomas et al. Plos Path 2017). There were no correlations to HIV RNA (data not shown).

Figure 3



ELISPOT responses to Nef/Tat/Rev, but not responses to other antigens, were found to correlate with HIV DNA (r=0.23; p=0.03) (Table 3).

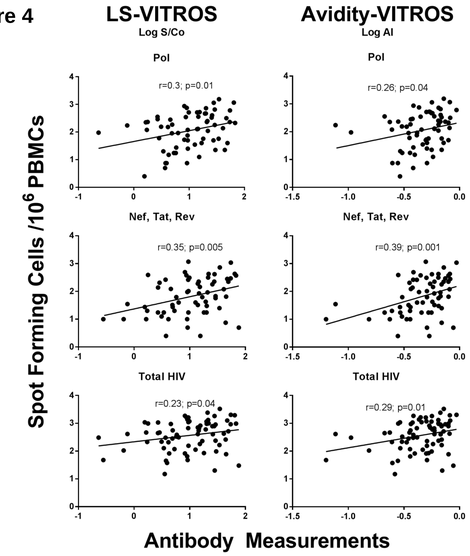
Table 3

		Gag	Pol	Env	Nef/Tat/ Rev	Vpr/Vif/ Vpu	Total HIV	CMV-pp65	EBV BZLF1
HIV DNA CD4	Spearmans r	0.099	0.107	0.087	**0.228**	0.05	0.136	0.124	0.053
	p	0.341	0.301	0.403	**0.026**	0.631	0.189	0.223	0.618
HIV RNA CD4	Spearmans r	-0.087	-0.1	0.006	0.093	0.04	-0.04	0.096	-0.094
	p	0.407	0.343	0.956	0.375	0.704	0.695	0.358	0.368
Cell Free RNA	Spearmans r	0.055	-0.007	0.084	0.039	0.102	0.049	-0.031	0.157
	p	0.59	0.945	0.409	0.705	0.318	0.631	0.758	0.123

Results IV: Antibody levels correlate with ELISPOT responses to Pol, Nef/Tat/Rev and total HIV proteins

Antibody levels for both LS-VITROS and Avidity Index correlated with T cell responses to Pol, Nef/Tat/Rev and Total HIV antigen ELISPOT responses (Figure 4).

Figure 4



Summary/Conclusions

In individuals on long-term ART, HIV-specific Ab to ENV/p24 and T cell responses to Nef/Tat/Rev correlate with each other and with HIV DNA levels but not with CA HIV RNA or residual plasma viremia.

The correlations found between IFNγ T cell responses, Ab and HIV DNA levels suggest that host immune responses may be used to estimate the frequency of HIV-infected cells in patients on suppressive ART.

The total frequency of HIV-infected cells (HIV DNA) may be a better marker of antigen expression that drives immune responses on ART than CA RNA in blood or residual viremia, which reflect activity of only a small fraction of proviruses that can be induced to express antigen.

Positive correlations between HIV immune responses and HIV DNA suggests that the immune system is sensing, but not clearing, infected cells, perhaps because of immune dysfunction. Sensing of infected cells by immune responses suggests that tracking these measures may be a method of assessing the impact of reservoir reducing strategies. Ongoing work to characterize concentrations of antigen-specific Ab responses may provide insights into antigen-expression in the latent reservoir.

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