DYNAMICS OF HIV-SPECIFIC T-CELLS ON DURABLE ART DIFFER BY ANTIGEN RECOGNIZED & BY SEX

0303

Eva M. Stevenson[1], **Adam R. Ward[1,2]**, Allison S. Thomas[3], Thomas R. Dilling[1], John K. Bui[1], John W. Mellors[4], Rajesh T. Gandhi[5], Deborah K. McMahon[4], Joseph J. Eron[6], Ronald J. Bosch[7], Christina M. Lalama[4], Joshua C. Cyktor[4], and R. Brad Jones[1,2] for the A5321 Team

[1]Weill Cornell Medicine, New York, NY, USA, [2]George Washington University, Washington, DC, USA, [3]Boston University School of Medicine, Boston, MA, USA, [4]University of Pittsburgh, Pittsburgh, PA, USA, [5]Massachusetts General Hospital, Boston, MA, USA, [6]University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, [7]Harvard T.H. Chan School of Public Health, Boston, MA, USA

BACKGROUND

- HIV-specific CD8$^+$ T-cell responses decay in the early stages of suppressive antiretroviral therapy (ART), likely due to a drastic reduction in HIV antigen expression, with an approximate half-life of 39 weeks after initial viral suppression is achieved[1]
- HIV-specific CD8$^+$ and CD4$^+$ T-cell responses have been found even after up to 4 years on suppressive ART[2]
- **Few studies have assessed the longitudinal dynamics and durability of HIV-specific T-cell responses on long-term ART, or their relationship with persistent HIV reservoirs**
  - A recent study found responses were highly stable over time, but no association was observed with the size of the replication-competent reservoir by QVOA[3]; however, defective proviruses produce HIV antigens[4,5], and HIV cell-associated DNA (CA-DNA) may thus provide a more accurate proxy of antigenic burden
- We previously demonstrated that HIV-specific T-cell responses are readily detectable in 96 participants from the ACTG A5321 cohort with a median of 7 years on suppressive ART[6]
  - Magnitudes of responses to a pool of the early HIV gene products Nef/Tat/Rev, but not other gene products or CMV, correlated with CA-DNA levels (**Figure 1**), providing initial evidence for ongoing antigen expression and T-cell recognition *in vivo*

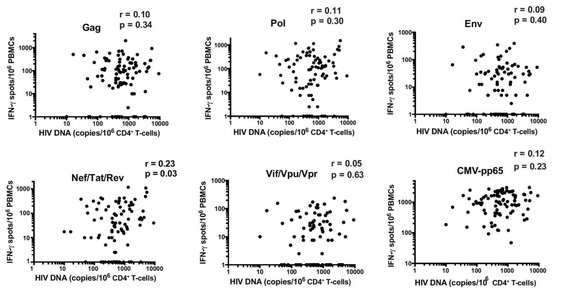


Figure 1. Correlations between IFN-γ-producing T-cell responses with HIV DNA levels. Responses were measured by ELISPOT assay on PBMCs collected at A5321 study entry (median 7 years on ART). Each dot represents a single individual. Spearman's r and *p*-values are given for each peptide pool.

- ***Based on our previous results, we therefore HYPOTHESIZED that decay of Nef/Tat/Rev-specific T-cell responses would inversely correlate with frequencies of HIV-infected cells by CA-DNA***

METHODS

- The AIDS Clinical Trials Group (ACTG) HIV Reservoirs Cohort Study (A5321) enrolled participants with documented sustained viral suppression for a range of 4-15 years at study entry, who initiated ART during chronic infection in other ACTG trials
  - Twice yearly blood draws were performed for virologic, immunologic, and pharmacologic assays
  - PBMC samples collected at *24 weeks* and *168 weeks* post-A5321 entry were obtained from the ACTG for n=49 participants (**Table 1**) randomly selected from the original n=96 participants we previously performed IFN-γ ELISPOT measurements on (i.e. *week 0*, median 7 years on ART)
    - IFN-γ ELISPOT assays were performed to measure responses to pools of overlapping 15-mer peptides spanning the HIV gene products Gag, Env, Pol, Nef, Nef/Tat/Rev (combined pool), as well as CMVpp65, on batched week 24 and week 168 samples
    - Longitudinal relationships between T-cell responses by gene product and by sex were assessed in linear mixed-effects models; median half-lives were calculated by gene product overall and stratified by sex; relationships between participant-specific slopes of change in T-cell responses and virologic and clinical measures were assessed by Spearman correlations, including partial correlations adjusting for potential confounders

In individuals on stable long-term ART, the rates of decay of HIV-specific T-cell responses targeting the early gene products Nef, Tat, and Rev - over a 3 year period - were inversely correlated with frequencies of infected cells (HIV DNA). This suggests that ongoing expression and T-cell recognition of these antigens occurs in treated individuals. Sex-based differences were observed, with female participants demonstrating higher magnitudes of responses against all HIV gene products longitudinally.

RESULTS

Table 1. Demographic, virologic, and clinical characteristics of longitudinal sub-study participants

Continuous Variables	Median	Range		Categorical Variables	n	%
		Lower	Upper			
Age at A5321 entry	48	23	74	Sex		
Years on ART at A5321 entry	6.6	4.2	14.8	Female	11	22.45%
HIV CA-DNA at A5321 entry (cps/10^6 CD4+ T-cells)	515.7	5.2	5494.0	Male	38	77.55%
HIV CA-RNA at A5321 entry (cps/10^6 CD4+ T-cells)	24.2	13.6	898.9	Race/Ethnicity		
HIV plasma RNA via iSCA at A5321 entry (cps/mL)	0.4	0.4	8.8	American Indian/Alaskan Native	1	2.04%
Pre-ART %CD38+HLA-DR+ on CD4+ cells	15.90%	4.90%	66.10%	Black (non-Hispanic)	5	10.20%
Pre-ART %CD38+HLA-DR+ on CD8+ cells	47.30%	17.30%	83.30%	Hispanic (regardless of Race)	16	32.65%
%PD-1+ CD4+ cells at A5321 entry	36.75%	1.20%	83.10%	White (non-Hispanic)	27	55.10%
%PD-1+ CD8+ cells at A5321 entry	35.40%	0.70%	84.90%			
Pre-ART plasma HIV-1 RNA (log$_{10}$cps/mL)	4.6	2.3	5.9			
Pre-ART CD4+ T-cell count (cells/mm^3)	287.5	15.5	708.5			

Table 2. Linear mixed-effects model results for differences in HIV-specific T-cell responses by gene product and time

Comparison	Mean Difference	95% LCL	95% UCL	*p*-Value[1]
Comparisons between timepoints by gene product				
Gag				
0 vs. 24	-185.2	-411.8	41.4	0.128
0 vs. 168	-176.7	-434.5	81.2	0.230
24 vs. 168	8.5	-251.2	268.2	0.996
Env				
0 vs. 24	-46.4	-89.6	-3.1	**0.033**
0 vs. 168	-62.8	-200.1	74.5	0.511
24 vs. 168	-16.5	-179.9	147.0	0.967
Pol				
0 vs. 24	-22.0	-154.1	110.1	0.913
0 vs. 168	25.7	-130.7	182.0	0.916
24 vs. 168	47.7	-155.3	250.6	0.834
Nef/Tat/Rev				
0 vs. 24	24.7	-75.4	124.7	0.820
0 vs. 168	-23.8	-171.3	123.8	0.919
24 vs. 168	-48.4	-238.7	141.8	0.808

Comparison	Mean Difference	95% LCL	95% UCL	*p*-Value[1]
Comparisons between gene products longitudinally				
Gag vs. Env	267.7	131.3	404.2	**<0.001**
Gag vs. Pol	97.9	-55.8	251.6	0.354
Gag vs. Nef/Tat/Rev	167.1	20.7	313.4	**0.018**
Env vs. Pol	-169.9	-274.2	-65.5	**<0.001**
Env vs. Nef/Tat/Rev	-100.7	-193.7	-7.7	**0.028**
Pol vs. Nef/Tat/Rev	69.2	-47.9	186.2	0.422

[1]Adjusted for multiple comparisons using single-step adjustment method

All HIV-specific responses by gene product

Bars - Median (Q1, Q3)

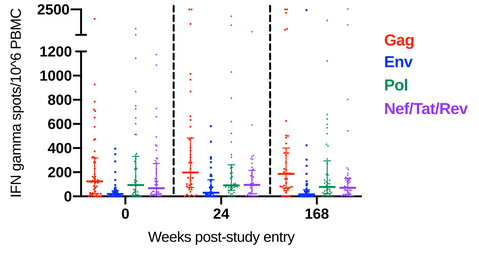
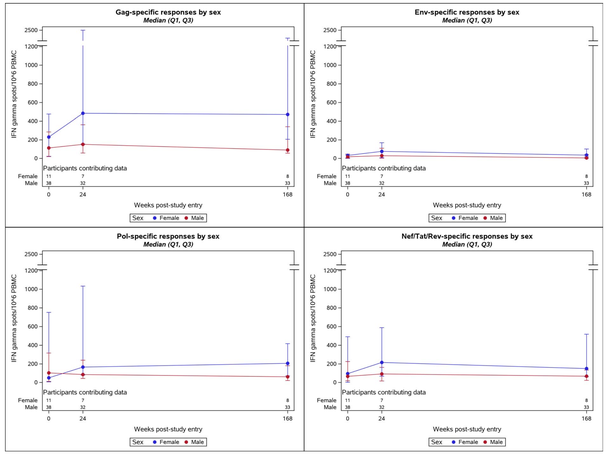


Figure 2. All HIV-specific T-cell responses by gene product.
↑ Responses against all gene products remain detectable over the entire study period, starting at a median 7 years on ART

Figure 3. HIV-specific T-cell responses longitudinally by participant sex and gene product.
→ Responses exhibit long half-lives overall, with differences by sex: Gag 32.4yrs (F 75.1yrs, M 3.7yrs); Env 3.5yrs (F 2.5yrs, M 1.1yrs); Pol 12.1yrs (F no decay, M 6.1yrs); Nef/Tat/Rev 6.9yrs (F 5.3yrs, M 6.1yrs)



RESULTS CONTINUED

Table 3. Linear mixed-effects model results for female vs. male participant differences

Response	Beta[1]	95% LCL	95% UCL	*p*-Value	Adjusted Beta[2]	95% LCL	95% UCL	*p*-Value
Gag	2.55	1.37	4.73	**0.004**	2.01	1.01	4.01	**0.048**
Env	1.76	1.15	2.69	**0.010**	1.64	1.05	2.55	**0.030**
Pol	3.38	1.63	7.00	**0.002**	3.40	1.84	6.30	**<0.001**
Nef/Tat/Rev	4.74	3.00	7.49	**<0.001**	3.14	1.86	5.31	**<0.001**
CMV	0.83	0.48	1.41	0.476	n/a	n/a	n/a	n/a

[1]Unadjusted estimates modeling sex=M as the reference value
[2]Controlling for Pre-ART plasma HIV-1 RNA (log$_{10}$cps/mL) and Pre-ART CD4+ T-cell count (cells/mm^3)

↑ Female vs. male participants display higher magnitudes of responses longitudinally for all HIV gene products, but not for CMV, before and after controlling for pre-ART HIV RNA and CD4$^+$ T-cell count (**Table 3**)

Table 4. Spearman correlations between slopes of change in T-cell responses with virologic and clinical measures

Variable		*Gag* 24-168 weeks	*Env* 24-168 weeks	*Pol* 24-168 weeks	*Nef/Tat/Rev* 24-168 weeks	*CMV* 24-168 weeks	*Nef*[2] 24-168 weeks
HIV CA-DNA at A5321 entry (cps/10^6 CD4+ T-cells)	Spearman r	0.15	0.24	0.17	**0.36**	0.00	**0.508**
	p-value	0.398	0.158	0.339	**0.034**	0.983	**0.001**
	n	35	35	35	35	33	37
	Adjusted r[1]	0.11	0.27	0.15	0.34	-	**0.496**
	p-value	0.531	0.127	0.412	0.056	-	**0.003**
HIV CA-RNA at A5321 entry (cps/10^6 CD4+ T-cells)	Spearman r	0.28	0.14	0.10	0.23	0.07	**0.455**
	p-value	0.121	0.424	0.595	0.203	0.694	**0.006**
	n	33	33	33	33	31	35
	Adjusted r[1]	0.29	0.18	0.16	0.24	-	**0.405**
	p-value	0.113	0.325	0.404	0.196	-	**0.019**
HIV plasma RNA via iSCA at A5321 entry (cps/mL)	Spearman r	-0.31	-0.23	-0.06	-0.20	0.02	0.158
	p-value	0.071	0.191	0.743	0.265	0.905	0.358
	n	34	34	34	34	32	36
	Adjusted r[1]	-0.45	-0.26	-0.09	-0.31	-	-0.104
	p-value	0.009	0.153	0.621	0.081	-	0.560
Pre-ART %CD38+HLA-DR+ on CD4+ cells	Spearman r	0.05	0.14	0.02	0.24	0.03	**0.558**
	p-value	0.804	0.475	0.936	0.193	0.890	**0.001**
	n	30	30	30	30	28	32
	Adjusted r[1]	0.14	0.29	0.12	0.29	-	**0.436**
	p-value	0.467	0.129	0.534	0.132	-	**0.016**
Pre-ART %CD38+HLA-DR+ on CD8+ cells	Spearman r	0.19	0.07	0.25	0.23	0.34	**0.526**
	p-value	0.326	0.703	0.187	0.219	0.077	**0.002**
	n	30	30	30	30	28	32
	Adjusted r[1]	0.27	0.26	0.36	0.24	-	**0.366**
	p-value	0.161	0.187	0.062	0.215	-	**0.047**
Age at A5321 entry	Spearman r	-0.06	-0.13	-0.01	0.00	0.27	-0.173
	p-value	0.737	0.450	0.953	0.985	0.132	0.305
	n	35	35	35	35	33	37
	Adjusted r[1]	-0.10	-0.12	-0.07	-0.03	-	-0.179
	p-value	0.569	0.500	0.714	0.856	-	0.304
%PD-1+ CD4+ cells at A5321 entry	Spearman r	0.30	0.32	-0.15	-0.15	-0.16	0.008
	p-value	0.095	0.076	0.405	0.403	0.388	0.966
	n	32	32	32	32	30	33
	Adjusted r[1]	0.29	0.34	-0.16	-0.22	-	-0.093
	p-value	0.119	0.069	0.392	0.234	-	0.618
%PD-1+ CD8+ cells at A5321 entry	Spearman r	0.26	0.31	-0.19	-0.16	-0.16	0.069
	p-value	0.146	0.089	0.295	0.378	0.397	0.703
	n	32	32	32	32	30	33
	Adjusted r[1]	0.26	0.32	-0.19	-0.23	-	0.087
	p-value	0.170	0.084	0.305	0.230	-	0.642
Pre-ART plasma HIV-1 RNA (log$_{10}$cps/mL)	Spearman r	0.07	-0.03	-0.02	0.08	0.17	**0.472**
	p-value	0.678	0.862	0.900	0.659	0.340	**0.003**
	n	35	35	35	35	33	37
Pre-ART CD4+ T-cell count (cells/mm^3)	Spearman r	-0.16	0.06	-0.17	-0.14	**-0.42**	-0.291
	p-value	0.368	0.718	0.337	0.407	**0.016**	0.080
	n	35	35	35	35	33	37

[1]Controlling for Pre-ART plasma HIV-1 RNA (log$_{10}$cps/mL) and Pre-ART CD4+ T-cell count (cells/mm^3)
[2]Measured separately

← Magnitudes of HIV-specific T-cell responses differ longitudinally by antigen recognized, but generally not between timepoints for each gene product (**Table 2**)

↑ Higher levels of CA-DNA at study entry were associated with lesser decay of Nef/Tat/Rev-specific responses from weeks 24 to 168; correlations were not observed between: i) CA-DNA and slopes of change in T-cell responses to other gene products, nor ii) between the slopes of any HIV-specific T-cell response and CA-RNA, plasma HIV-RNA, %CD38$^+$HLA-DR$^+$ T-cells, age, or PD-1 expression (**Table 4**)

↑ Separately, we measured responses to Nef and observed correlations between slopes of change from weeks 24 to 168 with: CA-DNA, CA-RNA, and %CD38$^+$HLA-DR$^+$ CD4$^+$ and CD8$^+$ T-cells (**Table 4**)

CONCLUSIONS

- HIV-specific T-cell responses were stable, demonstrating long half-lives, with sex-specific differences
  - Female participants had higher magnitudes of HIV-specific T-cell responses longitudinally vs. male participants
    - These results may help explain findings that females have lower residual viremia in this cohort overall[7] and in our longitudinal sub-sample of n=49 participants
- Higher CA-DNA at study entry correlated with slower rates of decay in Nef/Tat/Rev-specific T-cell responses on long-term ART, and Nef responses separately, consistent with maintenance of these responses by ongoing antigen recognition on stable long-term ART

[1]Casazza et al. 2001, J. Virol., [2]Appay et al. 2002, AIDS, [3]Xu et al. 2019, Mol Ther Methods Clin Dev., [4]Imamichi et al. 2016, PNAS, [5]Imamichi et al. 2020, PNAS, [6]Thomas et al. 2017, PLoS Pathog., [7]Cyktor et al. 2019, CROI

ACKNOWLEDGEMENTS

- ACTG A5321 cohort study participants
- This work was supported by an Administrative Supplement to the NIH funded Martin Delaney 'I4C' Collaboratory - UM1-AI126603 (Barouch). It was also supported in part by the Martin Delaney 'BELIEVE' Collaboratory - UM1-AI26617 (Jones & Nixon).