CMV Reactivation and Immune Aging in HIV-infected Individuals Virologically Suppressed on ART

#1709

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Introduction

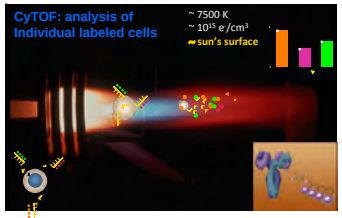
- Patients with well-controlled HIV on antiretroviral therapy are not restored to full health and exhibit features characteristic of the elderly, including:
  - oIncreased rates of heart disease
  - oCognitive impairment
  - oFragility fractures, and frailty
- Persistent immune abnormalities such as T cell and macrophage/monocyte activation and early immune senescence is associated with the these conditions
- We hypothesize that reactivation of chronic herpes viruses (specifically CMV) may drive the persistent immune changes in HIV infection despite good control with ART

Sample and Assay Selection

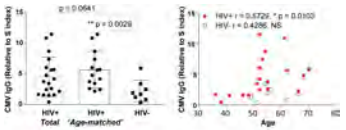
Study Participants- Participants were selected from virologically-suppressed HIV-infected (n = 20) and sociodemographically-matched HIV-uninfected individuals enrolled in the Stanford HIV Aging Cohort (SHAC).

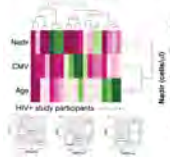
Assays– Plasma was assessed for CMV IgG antibody titer, and flow cytometric analysis on peripheral blood mononuclear cells allowed phenotyping of total immune subsets (bulk) and CMV-responsive T cells, identified by IFN$_\gamma$ production following CMV peptide pool stimulation.

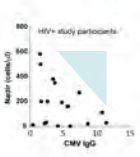
A subset of HLA-A2+ were analyzed for CMV-tetramer cells and more extensive phenotypic and function markers by Cytometry by Time-Of-Flight (CyTOF).



CMV IgG Titers







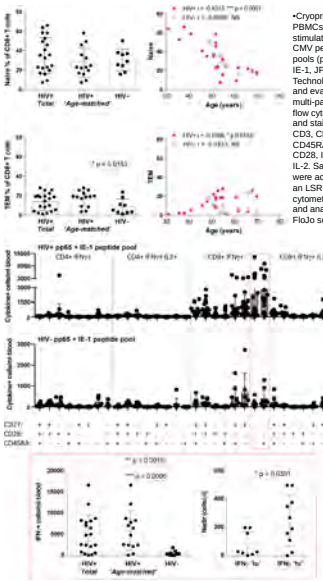
- Plasma was assayed for CMV IgG titers (Gold Standard Diagnostics)
- HIV+ individuals had higher plasma CMV IgG levels, significantly increased with age
  - oA notable absence was HIV+ individuals with high nadir and high CMV IgG

Patient Characteristics

Table 1: Patient Characteristics (of CMV+ participants)

Characteristic	All HIV+ (n=19)	Age matched HIV+ (n=14)	HIV- (n=8)
Median Age - Years (IQR)	52 (46-61)	54.5 (52-63)	55.5 (51.75-65)
Median Years of HIV Diagnosis (IQR)	18.8 (10.5-22.7)	21.1 (14.4-23.7)	NA
Median Current CD4 (cells/μL) (IQR)	633 (440-885)	516 (433-768)	
Median Nadir CD4 (cells/μL) (IQR)	160 (23-350)	135 (22.25-218)	
Median Years on Effective Treatment (IQR)	5.3 (2.1-6.8)	5.5 (4.2-8.8)	

CMV peptide pool stimulation



- Cryopreserved PBMCs were stimulated with CMV peptide pools (pp65 and IE-1, JPT Technologies) and evaluated by multi-parameter flow cytometry and stained for CD3, CD4, CD8, CD45RA, CD27, CD28, IFNg, IL-2. Samples were acquired on an LSR-II flow cytometer (BD) and analyzed by FloJo software.

CMV specific CD8+ T cell analysis by CyTOF

Table 2: Phenotypic/functional markers assessed with CyTOF

Type	Subgroup	Markers
Phenotypic (surface markers)	Subsets	CD45RO/RA, CD62L, CCR7
	Activation	CD38, HLA-DR, CD69, CD25
	Proliferation	CD127 (IL-7R), CD57
	Exhaustion	CD27 (loss), CD28 (loss)
	Co-stimulation	CD40L, CTLA-4
Functional	Cytokines	IFNγ, IL2, GMCSF, MIP1b, TNFα, IL-4, IL-10, IL-13, IL-17A, IL-17F
	Cytotoxicity	CD107 (degranulation), GranzymeB Perforin
	Antigen specific	Tetramers

MHCI-A2-restricted CMV epitopes for this study:

Specificity	Peptide Sequence
IE-1$_{316-324}$	VLEETSVML
IE-1$_{272-280}$	FMDILTTCVET
pp65$_{492-500}$	NLVPMVATV
pp65$_{185-193}$	QIMVGARLTV
pp65$_{320-328}$	LMNGQQIFL

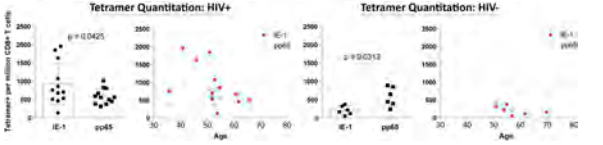
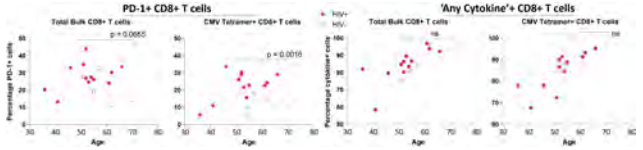


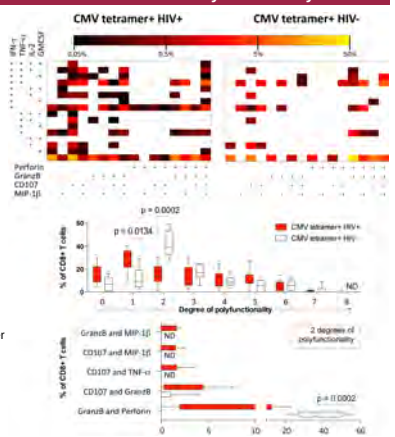
Table 3: Comparison of Bulk CD8+ T cells to IE-1 and pp65 specific cells, median (IQR)

Analysis	Population	Age and HIV status				
		36-46 HIV+	51-55 HIV-	51-55 HIV+	57-70 HIV-	61-66 HIV+
% naïve CD45RA+ CCR7+ (of parent gate)	Bulk CD8+ T cells	25.4 (23.7-49.5)	14.8 (14.4-22.8)	15.4 (9.93-26.5)	4.19 (3.08-5.48)	7.32 (5.68-16.4)
	IE-1 tetramer+	***73.3 (71.4-80.0)***	33.3	***67.6 (58.3-73.3)***	32.5 (25.0-40.0)	***60.3 (44.8-62.5)***
	pp65 tetramer+	23.1	26.2 (25.0-27.3)	22.4 (18.8-29.9)	10.8 (10.4-11.2)	23.2 (15.5-40.0)
% TEM CD45RA+ CCR7- (of parent gate)	Bulk CD8+ T cells	18.9 (13.7-30.0)	22.2 (5.88-23.3)	14.4 (9.4-19.4)	15.1 (10.7-22.3)	8.12 (5.23-25.6)
	IE-1 tetramer+	20.0 (18.6-28.6)	40.0	16.9 (10.2-29.3)	26.7 (20.0-33.3)	12.5 (10.3-36.2)
	pp65 tetramer+	42.3	31.3 (18.2-44.4)	51.4 (28.5-65.5)	34.8 (31.2-38.3)	33.3 (25.7-52.4)
% IFNγ (of parent gate)	Bulk CD8+ T cells	***44.2 (13.5-47.2)***	21.1 (18.5-28.2)	***41.4 (34.2-46.3)***	28.6 (14.5-35.2)	***56.3 (50.7-72.8)***
	IE-1 tetramer+	***21.4 (0-27.9)***	6.67	***14.3 (13.5-20.9)***	24.2 (8.33-40.0)	41.4 (34.5-47.1)
	pp65 tetramer+	***57.7***	11.5 (9.09-13.9)	***34.7 (30.0-42.6)***	11.8 (2.08-21.5)	***42.9 (40.5-63.8)***
% TNFα (of parent gate)	Bulk CD8+ T cells	22.7 (5.56-26.0)	12.2 (10.0-13.3)	21.8 (16.2-25.3)	12.9 (6.78-31.0)	28.4 (27.6-47.7)
	IE-1 tetramer+	0.0 (0.0-17.4)	13.3	11.4 (5.35-15.1)	25.9 (25.0-26.7)	13.8 (13.8-36.5)
	pp65 tetramer+	19.2	6.95 (0-13.9)	18.5 (15.3-23.3)	11.0 (4.17-17.8)	20.0 (19.0-44.9)
% Granzyme B (of parent gate)	Bulk CD8+ T cells	45.9 (33.8-50.0)	56.9 (44.7-58.6)	48.8 (42.8-59.9)	***80.9 (62.3-90.1)***	64.3 (29.2-68.1)
	IE-1 tetramer+	14.3 (4.0-25.6)	***33.3***	14.7 (11.1-26.5)	***65.8 (58.3-73.3)***	35.6 (15.5-51.7)
	pp65 tetramer+	76.9	72.1 (63.6-80.6)	76.3 (73.8-85.0)	***96.1 (95.8-96.3)***	78.3 (60.0-88.1)
% Perforin (of parent gate)	Bulk CD8+ T cells	***22.9 (17.5-24.1)***	47.3 (32.1-54.4)	***30.6 (25.6-40.1)***	66.7 (39.3-84.8)	***18.7 (13.9-33.1)***
	IE-1 tetramer+	***9.30 (4.0-14.3)***	26.7	***17.1 (10.7-19.0)***	61.7 (50.0-73.3)	***19.0 (10.6-25.9)***
	pp65 tetramer+	57.7	74.9 (63.6-86.1)	69.9 (64.9-76.6)	96.5 (95.8-97.2)	60.0 (50.7-76.2)

Bulk versus CMV specific comparison



Combinatorial Cytokine Analysis



Cryopreserved PBMCs were stimulated with PMA/Ionomycin and evaluated by CyTOF for CMV tetramers and antibodies (Table 2). Comparison between bulk CD8+ T cell response and CMV specific (IE-1 and p65) CD8+ T cells for phenotype and cytokine production (Table 3), as well as assessment of exhaustion marker PD-1 and combinatorial cytokine functionality

Conclusions

- HIV+ had higher plasma CMV IgG levels, significantly increased with age
  - oWith no HIV+ individuals: high nadir and high CMV IgG suggesting least depleted CD4 pool leads to best viral control
- HIV+ had significantly more CMV reactive TEM CD8+ T cells- best with nadir high
- CD8+ T cell functional and phenotypic changes in HIV+ individuals heightened in CMV responsive cells, with interesting antigen specific effects

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