CARDIAC MORBIDITY IN HIV IS ASSOCIATED WITH CHECKPOINT INHIBITOR LAG-3

 # 683

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Abstract

Background: Cardiovascular disease (CVD) is a major contributor to mortality and morbidity in HIV infection. Elevated expression of certain checkpoint inhibitor (CPI) molecules (e.g. PD-1, TIGIT, LAG3, TIM3) on T cells is associated with dampened immunity. Recent evidence for expression of receptors for CPI at the tissue level with selective enrichment of LAG3 receptors in the heart point to a control mechanism for organ immune homeostasis, (*Rev Immunity 44, 2016*). This study was aimed at defining relationship of CPI and CVD in HIV infection.

Methods: Study participants were recruited in YRG CARE, Chennai, India. ART naive viremic, (Gp1, n=102) and ART experienced aviremic (Gp2, n=172) were compared to healthy volunteers (Gp3, n=64) in a cross-sectional analysis of cardiac function and immunologic markers including CPI on CD4 and CD8 T cells.

Results: In Gp 1 findings of CVD were significant (Mann-Whitney' U test) for lower cardiac ejection time, stroke volume, stroke volume index, cardiac output and small arterial elasticity with higher systemic vascular resistance compared to Gp2 and Gp3 respectively. Large arterial elasticity was lower in comparison to Gp2. For CPI molecules in Gp1, 2 and 3, frequencies of LAG3 (Gp1: 4.9±3.4; Gp2: 2.5±1.5; Gp3: 2.4±1.3) and PD1 (Gp1: 6.2±7.3; Gp2: 1.5±2.5; Gp3: 0.9±1.6) expressing CD4 T cells in Gp1 were higher compared to Gp2 and Gp3 while those of TIGIT (Gp1: 34.2±14.9; Gp2: 33.5±11.8; Gp3: 30.1±7.8) and Tim3 (Gp1: 1.7±2.7; Gp2: 1.3±1; Gp3: 1.3±0.9) did not differ significantly. Frequencies of CD4+ T cells positive for LAG3, PD1, and for dual expression of LAG3 plus PD1 were inversely correlated in Gp1 with cardiac ejection time, cardiac output, cardiac index, stroke volume, stroke volume index and systemic vascular resistance and in Gp 2 with large artery elasticity, and except for PD1, also with small artery elasticity.

Conclusions: In ART naive HIV subjects continuous antigenic stimulation of the immune system results in upregulation of multiple CPI molecules. Our finding of the association of LAG3 and PD1 expressing CD4 cells in cardiac morbidity points to a dominant role of these pathways in regulating cardiac health, given that LAG3 receptors are enriched at the tissue level in the heart. Investigations to understand the immune mechanisms involved could provide insight into potential role of LAG3 immunotherapy (which is in early clinical trials in cancer) in prevention or treatment of cardiac dysfunction in HIV.

Background

- Cardiovascular disease is a major contributor to mortality and morbidity in HIV infection.
- Recent evidence points to expression of receptors for checkpoint inhibitor (CPI) molecules at the tissue level for controlling organ immune homeostasis, with the heart showing selective enriched for LAG 3 receptors[1, 2].
- This cross sectional study was aimed at defining relationship of CPI molecules and CVD in HIV infection. We conducted the study in treatment naïve viremic patients as well as in patients on ART with viral suppression and healthy age-matched controls.

Objectives

- The aim of the study was to analyze the expression of CPI molecules LAG3, PD1, TIGIT and Tim 3 on CD4 T cells in HIV+ subjects who were treatment naïve (group 1) and aviremic on ART (group 2) for their relationship to measures of cardiac function and arterial stiffness as compared to healthy controls (group 3)

Study Participants

- This study was conducted at YRG CARE, a tertiary care center located at Chennai in South India, and enrolled 274 male and female subjects with chronic HIV infection (HIV+) and 64 HIV-uninfected healthy controls (HC) in the age range >18 yr-50 yr
- Among the HIV+, 102 subjects were ART naïve (group 1) and 172 subjects were on ART for >12 months (group 2) with viral suppression (plasma viral load <40 copies/mL).
- 63 healthy controls and were categorized as group 3.
- An additional group of 24 age and sex matched healthy adult volunteers from the US was included for the analysis of checkpoint inhibitors on different immune cell subsets.
- The study was approved by both YRG care and UM Institutional Review Board.

Characteristics of study participants

	Group 1	Group 2	Group 3	P- value* Gp 1 vs 2
Number of participants	102	172	63	-
Gender, M/F	47/55	116/56	22/41	
Age (in years)	36.5 ±5.8	38.6 ±5.9	36.8 ±7.09	0.068
Median Duration of infection in months; (range)	15 (2-42)	72 (39.5-92)	NA	<0.001*
Mean Nadir CD4+ T-cell count, cells/ µL; (range)	NA	312.3 ± 199 (5-1425)	NA	—
Mean CD4+ T-cell count at the time of study entry; cells/ µL) ± SD (range)	421.4 ± 321.6 (21-1897)	743.4 ± 321.6 (154-1863)	NA	<0.001*
Median Duration of ART in months; (range)	—	43 (22-72)	NA	—
Body Mass Index (Kg/m²); Median (range)	22.7 (20.1-26.3)	22 (19- 25.8)	24.6 (22.5- 27.1)	—

Group 1 vs 3 or Group 2 vs 3 not significant except for BMI at P=0.013 and 0.006 respectively

Methods

Sample collection

- All participants had a one time blood draw for collection of plasma and peripheral blood mononuclear cells (PBMC).
- Blood samples were processed within an hour of collection according to guidelines of the ACTG.
- Plasma was stored in aliquots at -80ºC and PBMC were cryopreserved in liquid nitrogen in aliquots of 5 million cells/mL

Measures of cardiac function and arterial stiffness

HDI **PulseWave™ CR-2000**



Cardiac function measures
- Cardiac ejection time
- Pulse rate, stroke volume
- stroke volume index
- cardiac output
- cardiac index
- cardiac ejection time

Arterial measures
- Large artery elasticity index
- Small artery elasticity index
- Systemic vascular resistance

Flow cytometry for analysis of checkpoint inhibitor (CPI) molecules

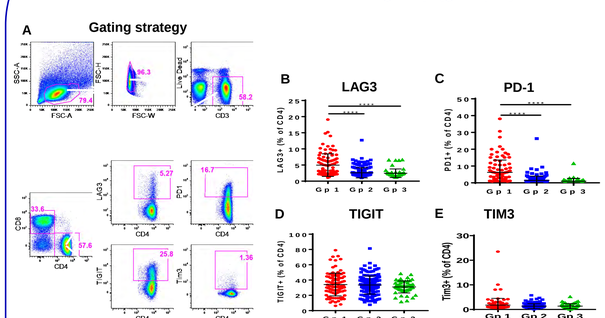
- Cryopreserved PBMC were thawed, rested overnight, and 1x10[6] cells were stained with LIVE/DEAD® Aqua followed by staining for surface markers CD3, CD4, CD8, and the CPI molecules PD1, TIGIT, TIM3, and LAG-3 using the panel 1 shown below, acquired on a Flow cytometer (BD LSRFortessa) and analyzed by FlowJo V10 (Treestar).
- Frequencies of CPI molecules (single or double positive) were analyzed on live (Aqua-) CD3+CD4+ T cells.
- A separate antibody panel (panel 2) containing markers for monocytes (CD14, CD16) and NK cells (CD56) along with HLA-DR and T cell specific markers for CD4 and CD8 T cells was used for analyzing CPI molecules in a cohort of US healthy donors.
- Monocytes were gated as CD45+CD3-CD56-HLA-DR+CD14+ and NK cells as CD45+CD3-CD14-CD56+ populations.

Panels used for flow cytometery staining

	UV	Violet					Red			Green				Blue		
	BUV 395	BUV 496	BV421	BV605	BV650	BV711	BV480	Alexa 647	APC-H7	Alexa 700	PE	ECD	PE-Cy5.5	PE-Cy7	BB515	PerCP-Cy5.5	PerCP
Panel 1 India Cohort	Live Dead	CD3	TIM3	CD27	2B4	EOMES	Tbet	CXCR5	CD4	CD160	PD1	CCR7		LAG3	CD45RO	TIGIT	CD8
Panel 2: US cohort	Live Dead	CD3	TIM3	CD25	HLA-DR	CD38	CD16	CD14	CD4	CD19	PD1	CD56	CD45	LAG3	FOXP3	TIGIT	CD8

Results

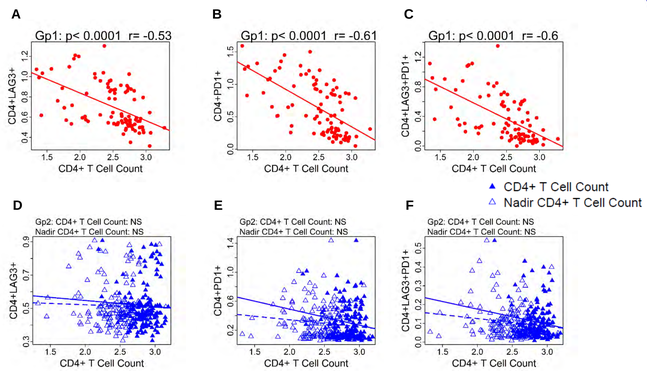
Figure 1: Higher frequencies of LAG3+ and PD1+ CD4 T cells are observed in treatment naïve group (group 1)



Checkpoint inhibitor molecules were analyzed on total CD4 T cells by flow cytometry.
A): Representative flow cytometry showing gating strategy for the analysis of CPI molecules on CD4 T cells.
B-E): Frequencies of CD4 T cells expressing B); LAG-3+, C); PD1+, D); TIGIT, and E); TIM3. Line and error bars within the plot indicate the Mean ± SD. Data compared between groups using Wilcoxon rank-sum test. A p value <0.05 was considered significant. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

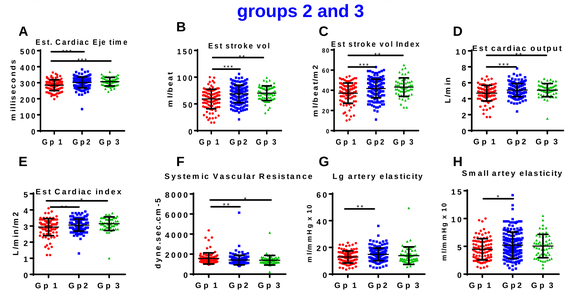
Results (continued)

Figure 2: Absolute CD4 counts correlate with CPI molecule expression on CD4 T cells in treatment naïve individuals (group 1)



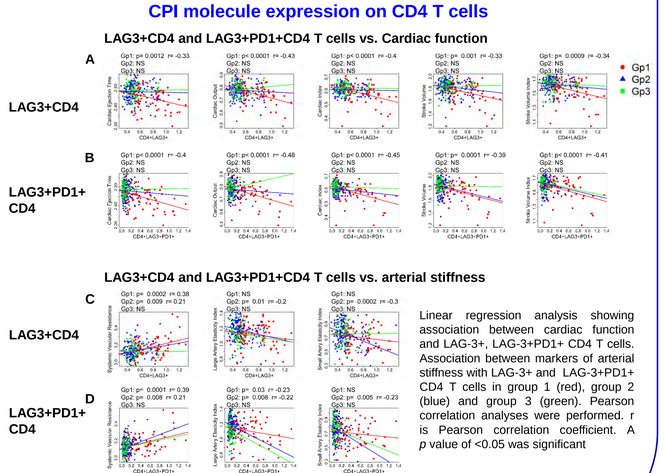
A-C); Linear regression for association between CD4 T cells count at study entry and A); LAG-3+CD4, B); PD1+CD4 and C); LAG-3+PD1+ CD4 T cells in HIV+ treatment naive Gp1. D-F); Linear regression for correlation between CD4 T cells count at study entry (black circles) and nadir CD4 T cells (grey circles) with D); LAG-3+CD4,E); PD1+CD4 and F); LAG-3+PD1+ CD4 T cells in HIV+ ART treated Gp2. For correlation analyses Pearson correlation analyses were performed. r is Pearson correlation coefficient. A *p* value of <0.05 was considered as significant.

Figure 3: Cardiac functions are impaired in group 1 as compared to groups 2 and 3



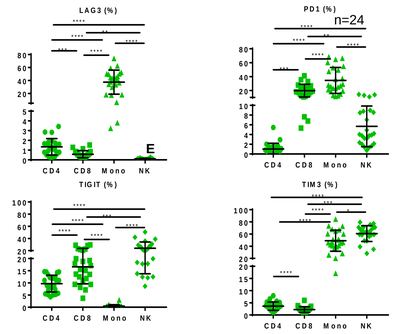
Cardiac function and arterial stiffness related measures were compared between untreated (Gp1), treated (Gp 2) and healthy donors (Gp 3).A-E); Measures of cardiac functions include A); estimated cardiac ejection time, B); estimated stroke volume, C); estimated stroke volume index, D); estimated cardiac output, E); estimated cardiac index. F-H); Measures of arterial stiffness include F); systemic vascular resistance, G); large artery elasticity index and H); small artery elasticity index. Data compared between groups using Wilcoxon rank-sum test. A p value <0.05 was considered significant. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

Figure 4: Markers of cardiac function and arterial stiffness correlate with CPI molecule expression on CD4 T cells



Linear regression analysis showing association between cardiac function and LAG-3+, LAG-3+PD1+ CD4 T cells. Association between markers of arterial stiffness with LAG-3+ and LAG-3+PD1+ CD4 T cells in group 1 (red), group 2 (blue) and group 3 (green). Pearson correlation analyses were performed. r is Pearson correlation coefficient. A *p* value of <0.05 was significant

Figure 5: Distribution of CPI molecules on different immune cells in healthy donors



Checkpoint inhibitor molecules were analyzed on total CD4 T cells by flow cytometry. Dot plots showing frequencies of CD4 T cells, CD8 T cells, monocytes and NK cells expression A); LAG-3, B); PD1, C); TIGIT, D); TIM3. Line and error bars within the plot indicate the Mean ± SD. Data compared between groups using Wilcoxon rank-sum test. A p value <0.05 was considered significant. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Summary

- Cardiac function was decreased with increased vascular resistance in Gp1 compared to Gps 2 and 3 and exhibited an inverse correlation with LAG-3, PD-1 or LAG-3 plus PD1 expressing CD4 T cells.
- Patients in Gp 2, had little evidence of impaired cardiac function but had residual increased vascular resistance involving large and small vessel elasticity which was also inversely correlated with LAG-3+CD4 T cells and was greatest with cells co-expressing LAG-3 and PD1.
- Summary of the association of CPI and cardiac and arterial stiffness measures are shown below

	LAG 3+ (%)		PD1+ (%)		LAG 3+ PD1+ (%)	
	p value	r value	p value	r value	p value	r value
Gp 1 (ART Naïve)						
Cardiac ejection time	0.001	-0.33	0.001	-0.33	<0.0001	-0.4
Cardiac output	<0.0001	-0.38	0.001	-0.33	<0.0001	-0.42
Cardiac index	<0.0001	-0.4	<0.0001	-0.39	<0.0001	-0.45
Stroke volume	0.001	-0.33	0.005	-0.29	<0.0001	-0.39
Stroke volume index	<0.0001	-0.34	0.001	-0.33	<0.0001	-0.41
Systemic vascular-resistance	0.0002	0.38	0.0005	0.36	0.0001	0.39
Large artery elasticity	NS	-	NS	-	0.03	-0.23
Gp 2 (ART treated)						
systemic vascular-resistance	0.009	0.21	0.03	0.17	0.008	-0.21
Large artery elasticity	0.009	-0.21	0.01	-0.21	<0.0001	-0.19
Small artery elasticity	0.001	-0.26	NS	-	0.03	-0.17

Conclusions

- We found a novel association of CD4 T cells expressing LAG-3 alone or LAG-3+PD1 with cardiac dysfunction in treatment naïve HIV+ patients, and with cardiac artery stiffness in both treatment naïve and virally suppressed patients questions to investigate are 1) mechanism of action of LAG-3 affecting the caon ART.
- Key rdiac function and arterial structure, and 2) the underlying basis for synergies of LAG-3 with PD-1 in regulating cardiac function
- This understanding could provide insight into potential role of LAG-3 immunotherapy (which is in early clinical trials in cancer) in prevention or treatment of cardiac dysfunction in HIV+ patients.

References

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Acknowledgements

This work was supported by National Institutes of Health Grant: R 21AI106373 (to S.P.); Indian Council for Medical Research, New Delhi, India grant ECD/NTF/17/2013-14, and by the Laboratory Sciences Core of the Miami Center for AIDS Research (P30AI073961). We are grateful to the study participants and the clinical and laboratory staff at YRG Centre for AIDS Research and Education, VHS, Chennai, India, for facilitation of the study.