Immune Checkpoint Signatures Associated With Impaired Cognition in People Living With HIV

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SUMMARY: The immune checkpoints (ICs) interactions have been associated with HIV comorbidities in people with HIV (PWH), yet the link to HIV associated cognitive impairment remains unclear. Here we studied 100 PWH on suppressive ART and persons without HIV (PWoH) and conducted extensive cognitive assessment and cellular immunophenotyping. We observed higher PD-1+ TIGIT+ CD4 T cells frequencies correlated with worse both verbal memory and fine motor performance in PWH. PD-1^{High} CD4+ T cells *in vitro* increased PD-L1 expression on neuroblastoma cells and this was associated with an increase in TNF-α production that is not reversed by PD-L1 blockade.

BACKGROUND

- HIV associated brain injury still persists among people with HIV (PWH) despite virally suppressive by antiretroviral therapy (ART).
- Evidence support the important role of immune checkpoint receptors (PD-1, TIGIT, TIM-3, LAG-3) and their cognate ligands (eg: PD-L1, PVR) in immune dysfunction in PWH even on suppressive ART and links to co-morbidities.
- Immune checkpoint blockade (ICB) has been shown to restore immune function in oncology and their relevance in the setting of HIV are ongoing.

Whether IC pathways are linked to neurocognitive deficit in people living with HIV on suppressive ART, remains unclear.

METHODS

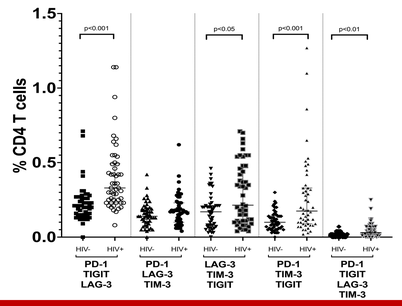
- Cross sectional study of virally-suppressed PWH (n=50) on stable ART and people without HIV (PWoH) (n=50) recruited in Metro Manila, Philippines from 2018-2019. Neuropsychological (NP) performance tests were conducted in 5 cognitive domains: Attention/Working Memory, Motor, Information Processing Speed, Language, Visuospatial, and Memory
- We assessed expression of ICs and their cognate ligands on T cells and monocytes in peripheral blood using multiparametric flow cytometry
- We assessed for association with ICs and ligand expression with NP test.
- *In vitro* co-cultures models of IC-expressing CD4 T cells and neurons were developed to assess IC ligand expression and release of cytokines.

RESULTS

Table 1. Study participants characteristics.

	People without HIV (N=50)			People with HIV (N=50)			
	Median	IQR		Median	IQR		p value
Age	30	28	33.25	33	28.75	35	0.05
Sex (Male)	49 (96.1%)	--	--	48 (96.0%)	--	--	1
Education (Years)	15	14	17	15.5	14	16.25	0.85
Recent CD4+ Count, cells/uL	--	--	--	503	344.25	641.75	--
Nadir CD4+ Count	--	--	--	124	26.75	299.75	--
Antiretroviral Regimen							
3TC/TDF/EFV	--	--	--	34 (68%)	--	--	--
3TC/AZT+EFV	--	--	--	7 (14%)	--	--	--
3TC/AZT+NVP	--	--	--	4 (8%)	--	--	--
3TC/AZT+TDF+LPVir	--	--	--	3 (6%)	--	--	--
3TC/AZT+LPVir	--	--	--	1 (2%)	--	--	--
3TC+TDF+LPVir	--	--	--	1 (2%)	--	--	--

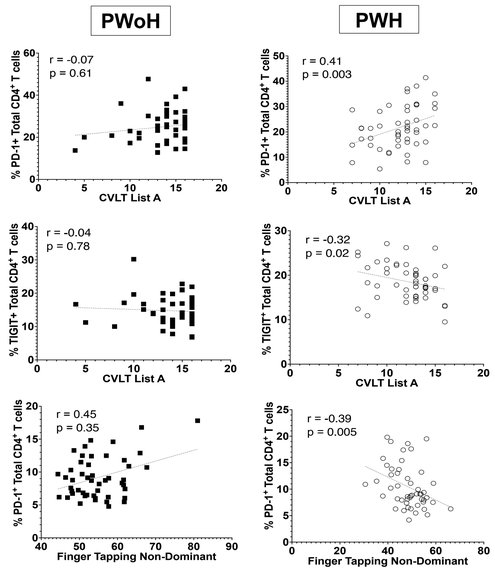
Figure 1. Comparison of IC receptor-bearing T cell in PWH (HIV+) and PWoH (HIV-) and associations with NP test scores.



Graphs show an expansion of multiple ICs CD4⁺ T cells in PWH compared to PWoH. n=50 per group. No differences were observed in cognate ligand expression (data not shown). p<0.05.

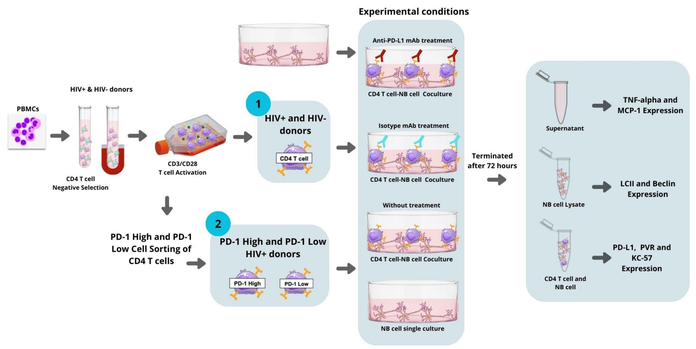
RESULTS

Figure 2. Associations of IC receptor-bearing on CD4 T cells and Neurocognitive Performance (NP) test scores in PWH and PWoH.



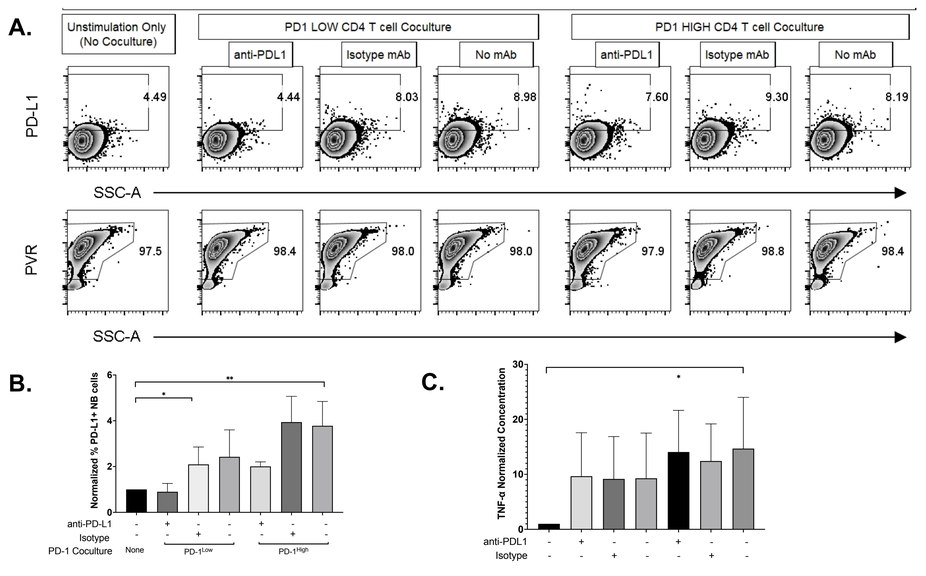
Plots show significant associations between the frequencies (%) of IC receptors on CD4⁺ T cells and NP score a in PWH and not in PWoH. n=50 per group; p<0.05. No association in both groups with IC on CD8 T cells.

Figure 3. Experimental Overview: *In vitro* co-culture system of T cells and neuroblastoma (NB) cell line with treatment of anti-PD-L1 mAb in two designs: **[1]** CD4⁺ T cells from PWH and PWoH and **[2]** PD-1^{High} and PD-1^{Low} CD4⁺ T cells from PWH



RESULTS

Figure 4. Changes of PD-L1 expression and TNF-α production of PD-1^{High} or PD1^{Low} CD4+ T cells in coculture with NB cells in the presence or absence of PD-1 blockade



Representative flow cytometry plots of **A.)** PD-L1+ and PVR+ NB cells co-cultured with PD-1^{High} vs PD-1^{Low} CD4+ T cells in the presence of anti-PD-L1 mAb or isotype. **B.)** Graph of PD-L1+ NB cell frequencies in co-culture with either PD-1^{High} CD4⁺ T or PD-1^{Low} CD4⁺ T cells **C.)** TNF-α production PD-1^{High} CD4+ T cells in co-culture studies with anti-PD-L1 mAb (n=3).

SUMMARY

- Expansion of PD-1+ TIGIT+ LAG-3+ TIM-3+ on both CD4⁺ in PWH compared to PWoH.
- Higher PD-1+ and TIGIT+ CD4⁺ T cells frequencies correlated with worse both verbal memory (CVLT) and fine motor performance (Finger tapping) in PWH and not in PWoH.
- Given that neuronal cells have been known to express PD-L1 and PVR, a known TIGIT ligand, we further confirm the expression of PDL-1 and PVR on neuroblastoma cells and assessed the interaction of IC-expressing CD4 T cells with neuroblastoma cells.
- We observed that PD-1^{High} CD4⁺ T cells *in vitro* increased PD-L1 expression on neuroblastoma cells and this was associated with an increase in TNF-a production.
- PD-L1 blockade reduced neuronal expression of PD-L1 but was insufficient to reverse the inflammatory response.

CONCLUSIONS

• We have identified important immune checkpoint signatures (IC) that uniquely correlated in PWH with worse neuropsychological performance and this was not observed in PWoH.
• IC could be potentially used as biomarkers for HIV-associated brain injury.
• Presence of PD-1^{High} CD4+ T cells *in vitro* induced expansion of PD-L1 on NB cells with concomitant increase in TNF-α production. This was not reversed with PD-L1 blockade. Overall, our findings suggest that immune checkpoint signature links with neurocognitive deficit in virally suppressed PWH on ART and that PD-1^{High} CD4+ T cells could influence neuronal inflammation elucidating the role of exhausted T cells in neuroHIV immunopathology.

ADDITIONAL KEY INFORMATION

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