PD-1 IS AN INSENSITIVE MARKER FOR HIV/SIV RNA+ LYMPH NODE TFH IN UNTREATED INFECTION

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BACKGROUND

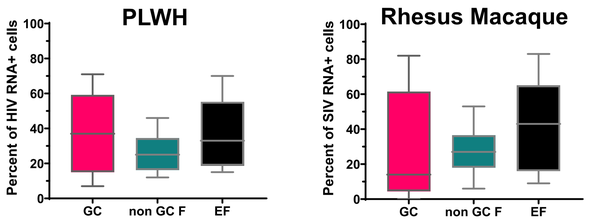
- Follicular T helper cells (TFH) are the major viral RNA expressing (vRNA+) cells in secondary lymphoid tissues during chronic HIV/SIV infection prior to AIDS/SAIDS.
- TFH are variably defined based on location in germinal centers (GC), and/or expression of the canonical transcription factor BCL6 and multiple cell surface markers including PD-1.
- In some in vitro systems, HIV downregulates PD-1, but whether PD-1 is expressed on vRNA+ TFH in vivo is unknown.

Participant Characteristics

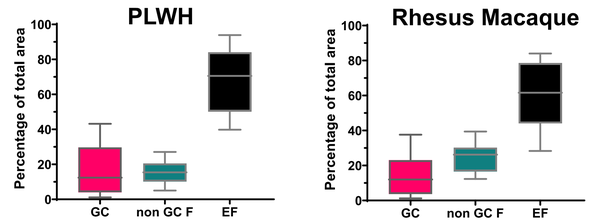
ID	Plasma HIV/SIV RNA (log_{10}Copies mL)	CD4+ T Cell Count (cells/mm3)	Sex	# vRNA+ cells
Human Inguinal LNs				
121	4.26	720	M	59
115	4.21	712	M	52
136	5.24	300	M	395
106	5.61	706	M	58
116	3.26	271	F	63
117	4.35	1117	F	55
120	5.88	327	F	111
129	3.06	547	F	34
Rhesus Macaque LNs				
R02076	6.76	315	F	227
R03141	6.00	324	M	92
Rh2515	4.22	na	F	17
Rh2516	5.55	na	F	68
Rh2520	7.58	na	F	352
R03103	5.23	317	M	74
Rh2123	5.62	na	M	34
Rh2525	6.75	473	F	92
Rh2587	5.42	633	F	105

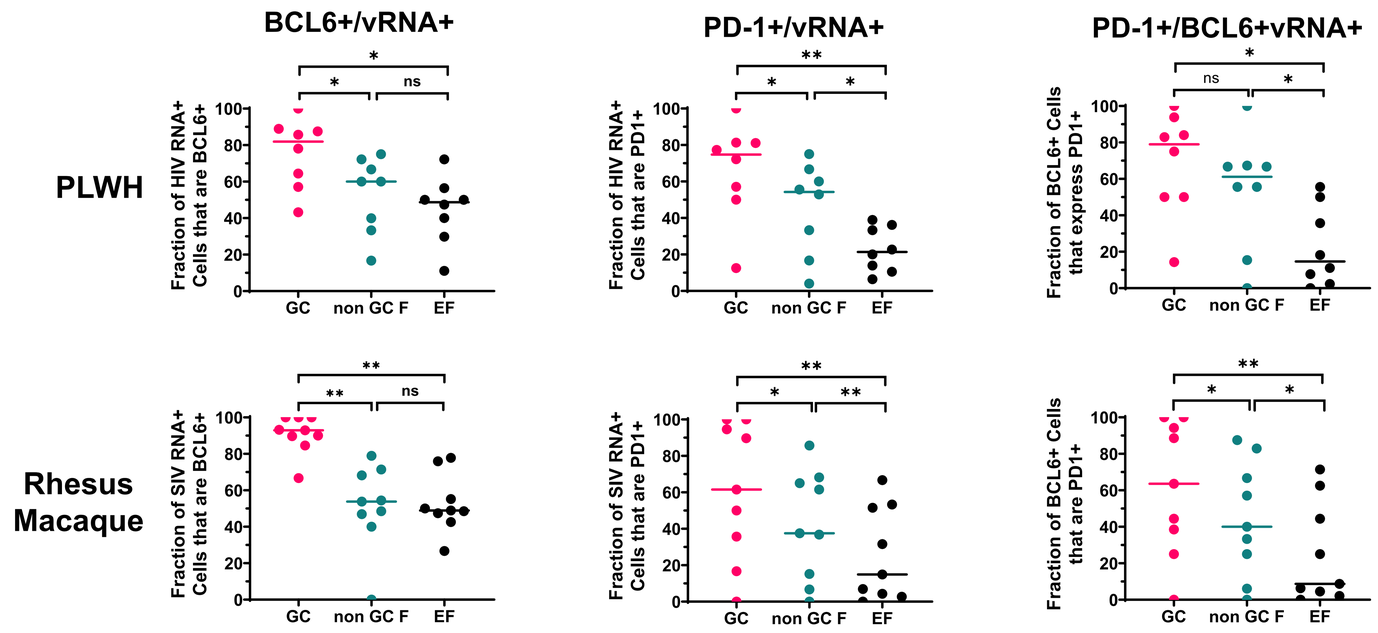
Identification of TFH by PD-1 expression likely underestimates TFH contribution to viral burden.

Fraction of vRNA+ Cells



Fraction of Tissue Area





SUMMARY

- The majority of vRNA+ cells are disproportionately concentrated in the follicular region including GC.
- BCL6 is expressed on most GC vRNA+ cells but only approximately on half of non GC F and EF vRNA+ cells.
- PD-1 expression is highest on GC vRNA+ cells, medium on non GC F and lowest in EF vRNA+ cells.
- PD-1 was not found on 21% of vRNA+ GC TFH in PLWH and 36% in RM, when defining TFH as BCL6+ vRNA+ cells in GC.

Conclusions

- PD-1 is not expressed on a significant fraction of TFH, defined as BCL6+ vRNA+ cells in GC.
- Decreased PD-1 expression on BCL6+ vRNA+ cells outside of the GC is consistent with a model of downregulation of PD-1 on vRNA+ TFH infected in the GC as they migrate into non GC F and EF regions.
- Alternatively, BCL6+ vRNA+ cells in non GC F and EF regions that lack PD-1 may be pre-TFH or activated CD4+ T cells.

Acknowledgements

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Contact Information

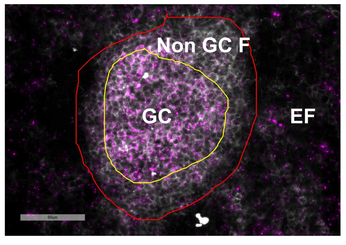
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METHODS

Study Participants: Lymph nodes (LN), frozen in OCT from 8 untreated people living with HIV-1 (PLWH) and 9 Rhesus Macaques with mac239SIV were included in this analysis.

Immunostaining: Frozen LN tissue was sectioned (10µm) and fixed in paraformaldehyde. In situ hybridization for vRNA and BCL6 mRNA was performed using multiplexed *in situ* hybridization (Advanced Cell Diagnostics) followed by immunohistochemical staining for PD-1 and CD20. Slides were imaged with a Leica Aperio scanner at 40X, vRNA+ cells identified and phenotypes determined. CD20+ areas defined follicles (F), clusters of BCL6 within the F defined germinal centers (GC) and CD20- areas defined extrafollicular regions (EF). Non GC F was defined as F excluding GC. vRNA+ phenotypes were defined as BCL6+ only, PD-1+ only, BCL6+/PD-1+, and neither. Nonparametric statistical analyses were performed using Prism 9.5.0 (GraphPad). (*p<0.05; **p<0.01)

LN regions used in this analysis defined by CD20 (white) and BCL6 staining (**magenta**). Bar = 80 µm.



Images of vRNA+ cells (gold) with BCL6 (magenta), PD-1 (aqua) and DAPI (blue). Bar = 20 µm.

