Poster #0197

Lymph Node Treg Subsets are Expanded in Some HIV+ People on Suppressive ART

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BACKGROUND

- Tregs including follicular regulatory T cells (TFR) are expanded in lymph nodes of untreated HIV+ people.
- TFR impair TFH responses and humoral immunity in untreated disease.
- Little is known about the impact of antiretroviral therapy (ART) on Treg and TFR populations in secondary lymphoid organs.

METHODS

Study subjects: Inguinal lymph nodes (LNs) were obtained from untreated HIV seropositive (HIV+ART-), treated HIV seropositive (HIV+ART+) and HIV seronegative (HIV-) individuals after informed consent in accordance with the IRBs from the University of Colorado, the University of Hawaii at Manoa and the University of Arizona.

Staining: Formalin fixed paraffin embedded LNs were stained and analyzed for HIV RNA+ cells using *in situ* hybridization (Advanced Cell Diagnostics' (ACD) branched DNA technique) and Tregs using immunofluorescent staining for FoxP3, CD8 and CD20 (Abcam). Stained sections were imaged with a Leica Aperio scanner at 40X. Sections were quantified for both HIV RNA+ cells and Tregs (FoxP3+CD8-) cells within follicular regions (F) (defined by CD20 staining) and extrafollicular regions (EF). Total and follicular areas were determined using Imagescope (Leica) to calculate frequencies. Mann-Whitney tests and Spearmen coefficients (r) were determined using Prism 7.01 (GraphPad).

Contact Information

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Table 1: Subject Characteristics.

Subject ID	Age	Sex	CD4 T cell count (cell x10^6/L)	Viral Load (RNA log_{10} copies/mL)	Years on Treatment
HIV Seropositive – Untreated (HIV+ART-)					
LN12	47	M	391	3.86	na
LN20	50	M	215	3.64	na
LN106	40	M	706	5.61	na
LN80	42	M	628	3.94	na
LN113	65	M	895	1.7	na
LN137	41	M	319	5.09	na
LN7	42	M	318	4.07	na
LN25	34	M	553	4.36	na
HIV Seropositive – Treated (HIV+ART+)					
HI5332	48	M	1039	<1.3	25
HI5334	63	M	1153	<1.3	18
HI5335	59	M	462	<1.3	7
HI5410	61	M	223	<1.3	20
HI5411	54	M	860	Not Detected	29
HI5412	66	M	538	Not Detected	21
HIV Seronegative (HIV-)					
HI5333	37	M			
HI5336	53	M			
HI5409	49	M			
HI5413	53	M			
LN96	34	M			
LN34	36	M			

Figure 1: Tregs and TFR were identified in LN sections from HIV+ART- (A), HIV+ART+ (B) and HIV- (C) by staining for FoxP3 (white), CD8 (red), DAPI (blue) and CD20 (not shown). Tregs were defined as FoxP3+CD8- cells (arrows) and TFR were Tregs located within B cell follicles (F) as defined by CD20 staining (not shown) used to draw pink lines to define extrafollicular (EF) and follicular (F) regions.

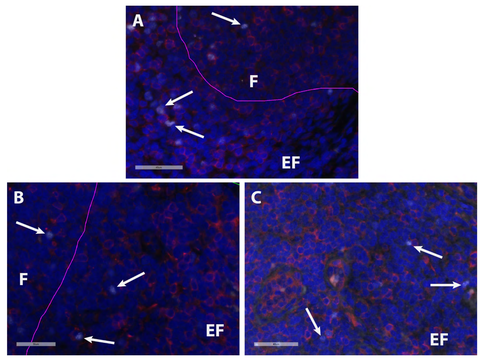
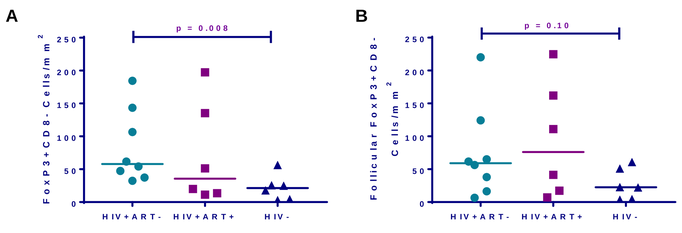


Figure 2: Frequencies of Tregs (A) were significantly higher in LNs from HIV+ART- when compared to LNs from HIV-. Frequencies of TFR (B) tended to be higher in HIV+ART- compared to HIV-. HIV+ART+ had higher Tregs and TFR compared to HIV- subjects but differences were not statistically significant. Further they demonstrated a dichotomy with three HIV+ART+ similar to HIV- and three HIV+ART+ similar to HIV+ART-.



RESULTS

Figure 3: HIV RNA+ cells (gold, arrow) were readily detected in LN of all HIV+ART+ as shown in representative images both in follicular regions (A) and extrafollicular regions (B). CD20 staining (not shown) was used to define follicles (F) and extrafollicular regions (EF).

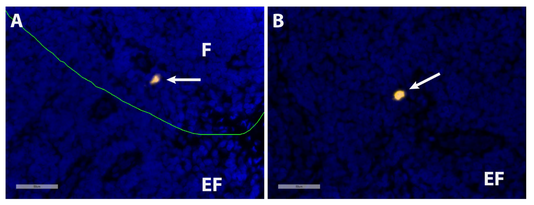


Figure 4: HIV RNA+ cells were significantly less frequent in LN from HIV+ART+ (median 0.28 cells/mm^2) compared to HIV+ART- (median 1.04 cells/mm^2).

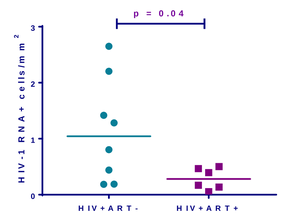


Figure 5: HIV-1 RNA+ cells in the LN from HIV+ART+ were significantly more frequent in the follicle (median 0.40 cells/mm^2) than in the extrafollicular region (median 0.23 cells/mm^2, p = 0.03).

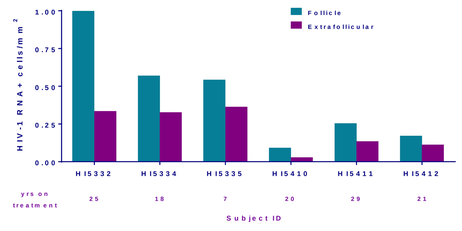
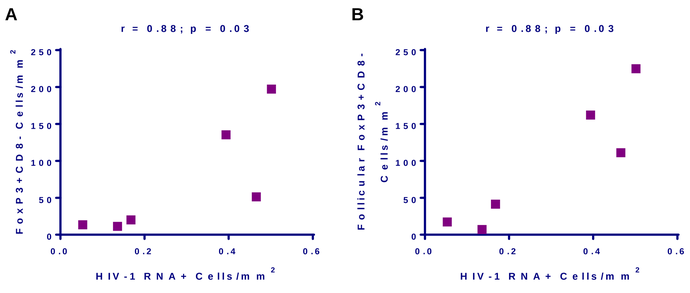


Figure 6: Frequencies of Tregs and TFR express a positive correlation with frequencies of HIV-1 RNA+ cells in LNs from HIV+ART+.



SUMMARY

- Frequencies of Tregs and TFR are increased in LNs from HIV+ART- and tended to be higher in HIV+ART+.
- Three HIV+ART+ had frequencies of Tregs and TFR similar to HIV+ART-, while three had frequencies similar to LNs from HIV-.
- HIV RNA+ cells were detected using RNAscope in LNs from HIV+ART+ but at frequencies much less than LNs from HIV+ART-.
- LNs from HIV+ART+ have higher frequencies of HIV RNA+ cells in the follicle compared to the extrafollicular region as has previously been reported in HIV+ART- (1, 2).
- Frequencies of Tregs and TFR in LNs from HIV+ART+ correlate with frequencies of HIV RNA+ cells.

CONCLUSIONS

Persistent expansion of Tregs including TFR is seen in LN of some ART-treated individuals with plasma virus suppression and is related to tissue HIV RNA expression. Expansions in TFR may contribute to impairments in humoral immunity seen in some ART-treated individuals.

References

1. Folkvord, et. al, AIDS Res Hum Retroviruses, 2005, 21:363-370.
2. Kohler, et. al, J Immunol, 2016, 196(6):2711-2722.