RAPID T FOLLICULAR HELPER CELL RECONSTITUTION IN LYMPH NODES AFTER ART INITIATION

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Background and Study Rationale

T follicular helper cells (Tfh), defined as $CD3^+CD4^+PD\text{-}1^+CXCR5^+$, are specialized T cells, residing in the B cell follicles that assist in B cell survival, proliferation, antibody class switching, and differentiation. The loss of Tfh cells, in chronically infected HIV+ individuals, is associated with immune dysfunction. Tfh cells have also been implicated as a persistent reservoir of virus-infected cells in persons treated with antiretrovirals (ART) with undetectable plasma HIV RNA levels. Herein, we studied the dynamics of Tfh cells in lymph nodes (LN) before and shortly after ART initiation.

Study Design & Methods

Twenty two ART-naïve patients with CD4 counts <100cells/µL were enrolled in a prospective study, Inguinal LNs were surgically resected at baseline (pre-ART) and 4-8 weeks post-ART (13 paired samples) and cells were extracted for immunophenotyping by flow cytometry. Mann-Whitney test and Wilcoxon matched pair test were used to compare groups, while Spearman's rank correction coefficient was used to test associations.

Results

22 patients, with a median age of 36 (22-53), 6 females and 16 males, were included and underwent LN biopsies including 13 with paired (pre/post-ART) sampling. Periph eral blood (PB) measurements are shown in Table 1.

Table 1. Pre and post-ART characteristics of study participants. Median values are shown with IQR in parenthesis.

	CD4 T cells (PB)	% CD4 (LN)	CD8 T cells (PB)	% CD8 (LN)	Plasma HIV-RNA
Pre-ART	19 cells/µL (10-28)	6.43 (1.09-12.7)	513 (397-623)	71.9 (61.5-84.2)	214,062 c/mL (103897-525400)
Post-ART	103 cells/µL (84-169)	15.05 (10.5-31)	713 (419-970)	37.8 (18.4-62.9)	71 c/mL (40-274)

Results

Subsets of CD4 T cells (naïve, central and effector memory and Treg) did not differ significantly post-ART in LN. The CD8 percentage in LN decreased significantly (X vs y, P) post-ART. The percent of CD4 T cells in LN increased post-ART although the difference was only significant in paired analysis of the 13 paired sample subgroup (X vs Y, P) (**Figure 1a**). In contrast, the percentage of Tfh cells increased post-ART treatment in both paired and unpaired analysis (**Figure 1b**). This increase of Tfh cells was not associated with baseline or post-ART HIV-RNA or CD4 count recovery (**Figure 1c**).

Figure 1

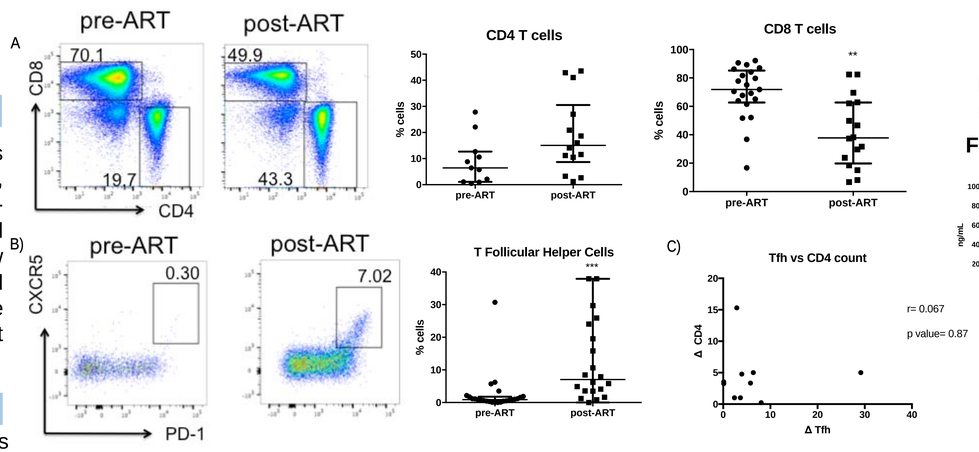


Figure 1. % Tfh in LN increases post-ART and does not correlate to peripheral blood CD4 restoration

A) % CD4 and CD8 of CD3 cells in the lymph node pre-ART and 6-8 weeks post-ART. B) % Tfh cells defined by total $CD3^+CD4^+$ $CXCR5^+PD\text{-}1^+$. C) Spearman's rank correlation coefficient correlating change in CD4 T cells and change in Tfh frequencies

Immune phenotyping reveal no significant changes in expression of CTLA-4 in Tfh (**Figure 2a**). Instead, post-ART Tfh cells had increased levels of CXCR3, a marker associated with higher plasma viremia and Tfh cells that produce higher levels of IL-21 and IFNγ (**Figure 2c**).

Although the proportion of Tfh in LN increases post-ART, these Tfh cells have lower levels of ICOS expression, a co-receptor that enhances the Tfh transcription factor, BCL6 (**Figure 2b)**.

Figure 2

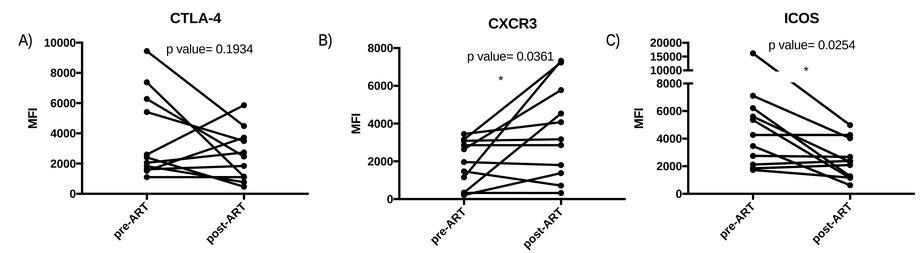


Figure 2. Characterization of Tfh pre and post-ART. MFI of $CD3^+CD4^+$ $CXCR5^+PD\text{-}1^+$ A) CTLA-4 B) CXCR3 C) ICOS

Most inflammatory plasma biomarkers significantly decrease post-ART (**Figure 3)**. Biomarker levels did not correlate with %Tfh in LN, except for IL-12p70 and % of Tfh pre-ART (r= 0.594, p= 0.032).

Figure 3

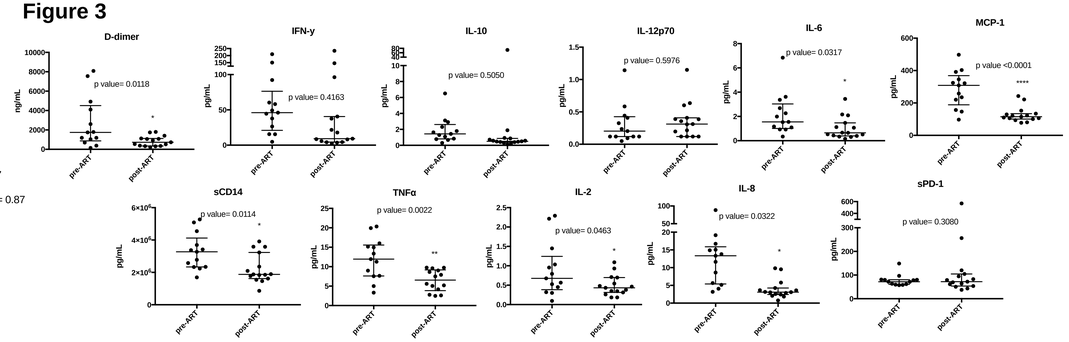


Figure 3. Biomarkers pre and post-ART in plasma matched to LN biopsy dates

Conclusion

- Tfh emergence in lymph nodes of advanced patients occurs early after ART initiation and is independent of plasma viremia, CD4 restoration, or decrease of inflammatory biomarkers in the plasma.
- The mechanisms of this accumulation are unclear and may have implications in the functionality and role of Tfh cells as HIV reservoirs.
- Further studying the localization and phenotype of these Tfh cells in pre and post-ART LNs may help elucidate the effects of ART initiation and HIV-related inflammation on LN dynamics in advanced disease.

References

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