TFH-SPECIFIC FEATURES ASSOCIATED WITH NEUTRALIZATION IN CHRONIC HIV-1 INFECTION

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BACKGROUND

Follicular helper T −cells (Tfh) are critical for the development of neutralizing antibodies following infection and vaccination. During HIV−1 disease, Tfh accumulate in the germinal centers (GC) of lymphoid tissues[1] possibly due to immune activation. Despite this accumulation, only a small percentage of HIV−infected individuals are able to mount a broadly neutralizing response[2]. The drivers of this discrepancy at tissue level remain to be elucidated. We therefore performed a detailed analysis of the immunophenotype, topology and transcriptional signatures of Tfh in viremic HIV+ donors with and without evidence of serum neutralizing activity

METHODS

LN cell suspensions and matched formalin−fixed, paraffin embedded tissues were analyzed using 30−parameter flow cytometry, quantitative multiplexed confocal imaging and single−cell RNA analysis using the 10x genomics 3´scRNA−seq kit. Neutralization activity of matched serum samples was determined using a single−round−of−infection Env−pseudotyped virus assay. We classified donors into two groups; non-neutralizers had serum ID50 > 40 for ≤1⁄20 pseudoviruses, while neutralizers had serum ID50 > 40 for at least 6⁄20 pseudoviruses (median, range 4-19).

RESULTS

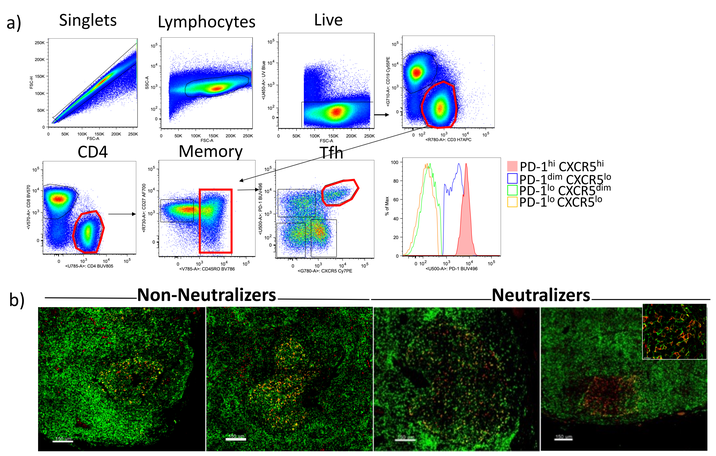


Figure 1. a) Gating Strategy used for Tfh phenotyping in flow cytometry experiments b) Confocal microscopy images of B-cell follicles showing representative examples of CD4 (green), PD-1 (red) distribution in Non-neutralizers (left) and Neutralizers (right). Images were acquired on an TCS SP8 confocal microscope with a 40x objective (NA 1.3). Depicted captions are at 150um

HIV neutralizers harbor higher frequencies of central memory CD4 T-cells, higher frequencies of Tfh, and higher in-situ ratios of follicular CD4 PD-1 compared with non-neutralizers. *(Figures 1, 2, 3)*

Tfh cells with an activated phenotype are enriched in HIV neutralizers consistent with an increased capacity for optimal T-cell help *(Figure 2)*

Neutralizer Tfh cells possess stem-like gene signatures, characterized by a marked upregulation of TCF7 *(Figure 4)*

Flow Cytometry

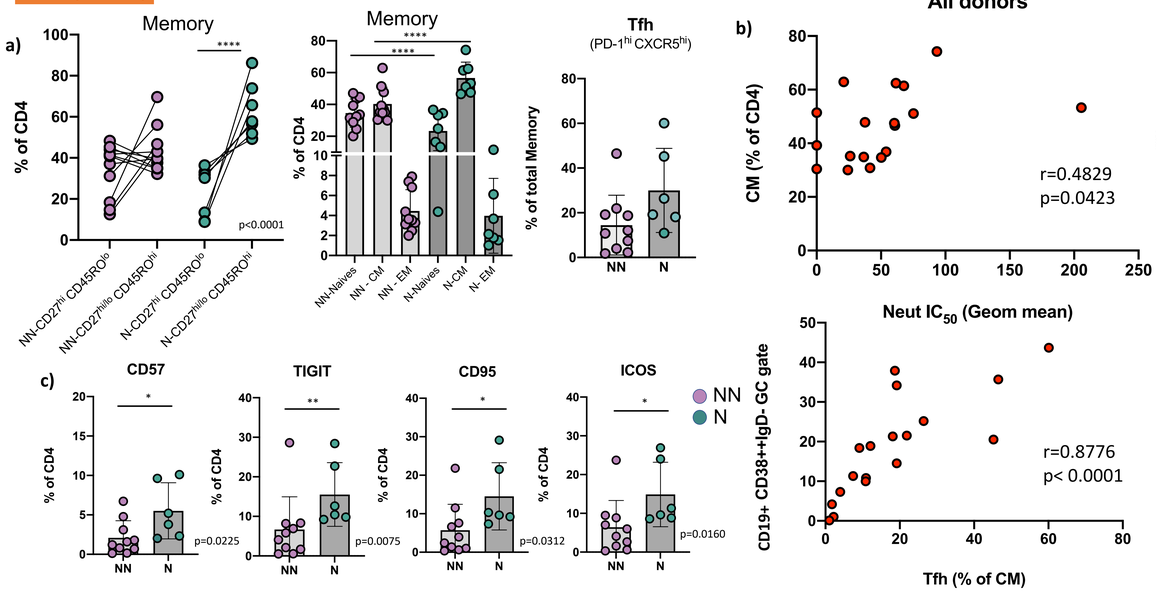


Figure 2. a) Frequencies of CM CD4 T cells and Tfh in Non-neutralizers (n= 11) and Neutralizers (n=7) as measured by flow cytometry. Due to extremely low cells numbers, Tfh analysis for one individual was not possible b) Spearman correlation plots of CM frequencies and Neutralization IC_{50}s as well as CD19+ CD38++IgD- cells and Tfh cells in all participants c) Dot plot depicting the expression of CD57, TIGIT, CD95 and ICOS on Tfh cells in NN vs N as measured by flow cytometry. p values shown calculated using the Mann-Whitney test.

Imaging Analysis - Histocytometry

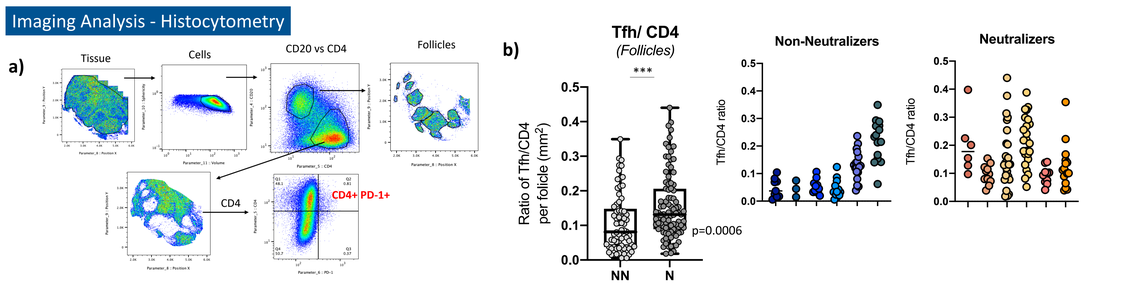


Figure 3 a) Gating for the characterization of Tfh (CD4+ PD-1+ in LN tissue sections using quantitative image analysis (histocytometry) and b) Box plots depicting the ratio of Tfh/CD4 within $CD20^{hi/dim}$ areas in the LN of neutralizers and non-neutralizers as determined by confocal imaging analysis and histocytometry. Inset plots depict measurements in each individual donor by category (each circle is one follicle).

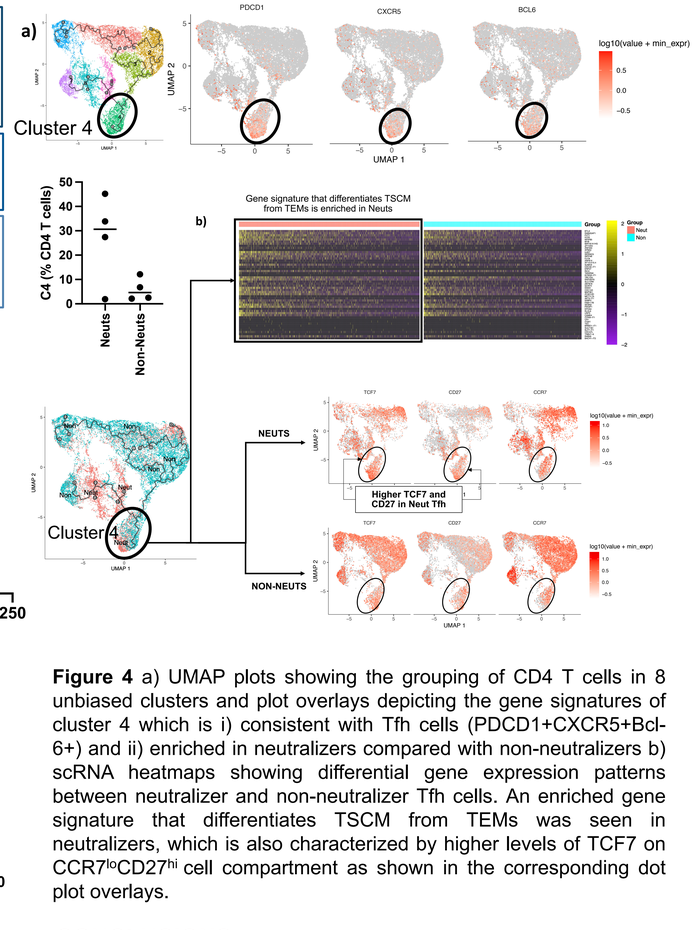


Figure 4 a) UMAP plots showing the grouping of CD4 T cells in 8 unbiased clusters and plot overlays depicting the gene signatures of cluster 4 which is i) consistent with Tfh cells (PDCD1+CXCR5+Bcl-6+) and ii) enriched in neutralizers compared with non-neutralizers b) scRNA heatmaps showing differential gene expression patterns between neutralizer and non-neutralizer Tfh cells. An enriched gene signature that differentiates TSCM from TEMs was seen in neutralizers, which is also characterized by higher levels of TCF7 on $CCR7^{lo}CD27^{hi}$ cell compartment as shown in the corresponding dot plot overlays.

CONCLUSIONS

Our results suggest distinct follicular Tfh accumulation, phenotypic, differentiation and transcriptional signatures in the lymph nodes of untreated, chronically HIV-infected individuals with and without neutralizing antibodies. Long-lived Tfh cells in neutralizers could be resistant to HIV and could provide long-lasting immunity by supporting broadly neutralizing humoral responses.

ADDITIONAL INFORMATION

[1] Perreau M et al, JEM 2012 (210):1 143-156; Lindqvist M et al, JCI 2012(122):9 3271-80; Petrovas C et al, JCI 2012 122(9): 3281-94

[2] Van Gils MJ et al, Virology 2013; 435:46-56

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