CD38⁺CXCR3⁺ Tfh cells serve as active HIV reservoir in the total Tfh cell population

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Abstract

Background. T follicular helper cells (Tfhs) are a phenotypically heterogeneous cell population generally defined by the expression of CXCR5 and PD-1. Tfhs serve as a major reservoir for HIV transcription and production in both viremic and long-term ART treated subjects. In the presents study, we have dissected the phenotypic and functional heterogeneity of Tfhs and the role of the different Tfhs populations in serving as HIV reservoir and their relationship with HIV-specific B cell responses.

Methods. Lymph nodes (LN) biopsies were obtained from 17 HIV uninfected, 27 viremic untreated and 24 aviremic ART treated subjects. Definition of B and T cell populations and cytokines was performed by mass cytometry using a panel of 40 metal-conjugated antibodies. To dissect the heterogeneity of Tfh cells, we performed self-organizing map (FlowSOM) and consensus clustering. Cell-associated HIV-RNA was assessed in Tfh cells sorted on the basis of CD38 and CXCR3 expression ($CD38^+CXCR3^+$, $CD38^+CXCR3^-$, $CD38^-CXCR3^+$ and $CD38^-CXCR3^-$).

Results. Unsupervised clustering identified 20 different populations of Tfh cells within $CXCR5^{high}PD\text{-}1^{high}$ Tfh cells. $CD38^+CXCR3^+$ Tfhs significantly increased in viremics (41.18%) as compared to ART treated (17.8%) and HIV uninfected (6.9%) subjects ($p< 0.0001$). Frequencies of $CD38^+CXCR3^+$ Tfh cells positively correlated with the percentage of total germinal center B cells ($r = 0.58$, $P < 0.0001$) and GC $gp140^+$ B cells ($p<0.0003$). $CD38^+CXCR3^+$ Tfhs expressed higher levels of expression of BCL-6, ICOS, CD57, CD40L, CCR5, HLA-DR, T-bet as compared to the $CD38^+CXCR3^-$, $CD38^-CXCR3^+$ and the $CD38^-CXCR3^-$ and of production of the Tfh signature cytokine IL-21 (averages of 33%) as compared to the other three Tfh cell populations ($p<0.0002$). Of note, only the percentage of $CD38^+CXCR3^+$ Tfhs correlated with viremia ($r = 0.5$, $P = 0.01$) in untreated subjects. More importantly, $CD38^+CXCR3^+$ Tfhs were greatly enriched in cell-associated HIV-RNA as compared to $CD38^+CXCR3^-$ (average 3.3 fold), $CD38^-CXCR3^+$ (average 6.24 fold) and $CD38^-CXCR3^-$ (average 8.24 fold) Tfh cell populations ($p<0.05$).

Conclusions. $CD38^+CXCR3^+$ Tfhs correspond to a population of phenotypically and functionally active Tfh cells. The higher levels of expression of CCR5 may render these cells more susceptible to HIV infection and it explains why $CD38^+CXCR3^+$ Tfh cells serve as the major active HIV reservoir within the total Tfh cell population.

Table I : Study cohort: Clinical data

	Values	
ART	ART Treated (n= 27)	Naive (n=24)
Age (years), mean (SD)	48.5 (6.7)	36 (8.4)
Male/female ratio	19/8	19/5
Duration of HIV Infection (years)	11.6 (3-23.27)	2.24 (0.1-13)
Viral Load (copies/ml)	<20	5.54E+05 (8.30E+02-1.00E+07)
CD4 count (cells/ul) [interquartile range (IQR)]	683 (490-928)	543.5 (486.5-642.5)

Results

Figure 1: High dimensional analysis of Tfh cells in HIV infected and uninfected individuals

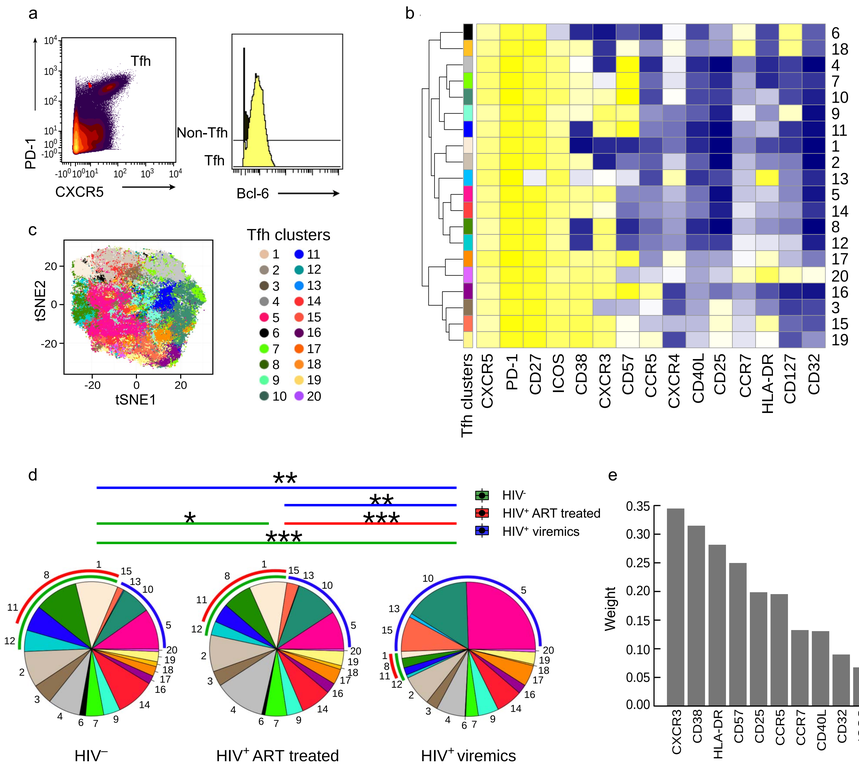


Figure 2: $CD38^+CXCR3^+$ Tfh cells are increased in viremics and represent a distinct Tfh population

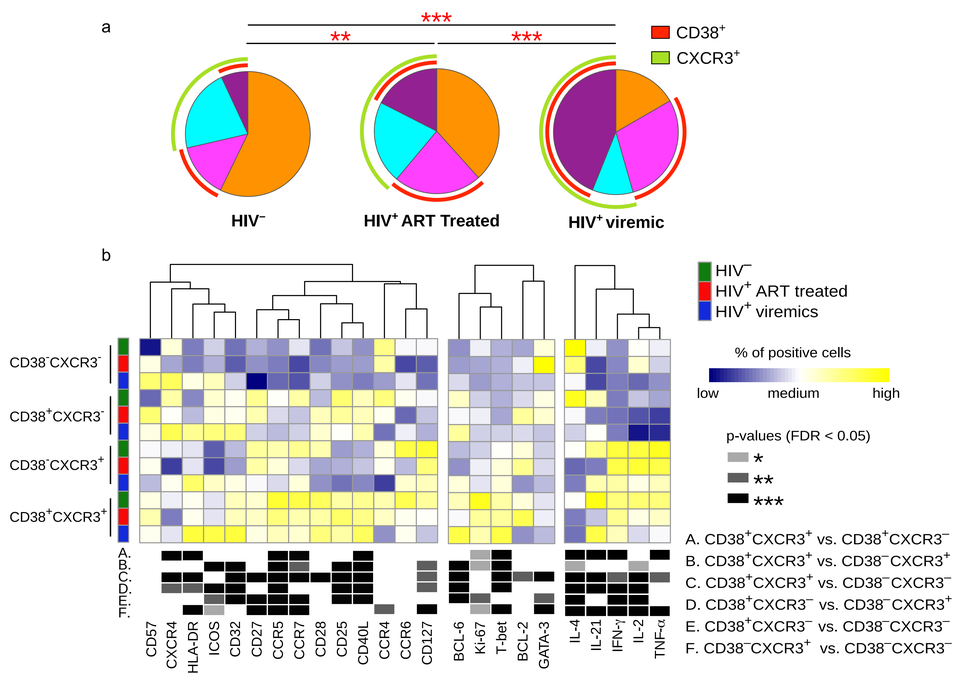


Figure 3. Functional analyses of Tfhs on the basis of $CD38^+$ and $CXCR3^+$expression

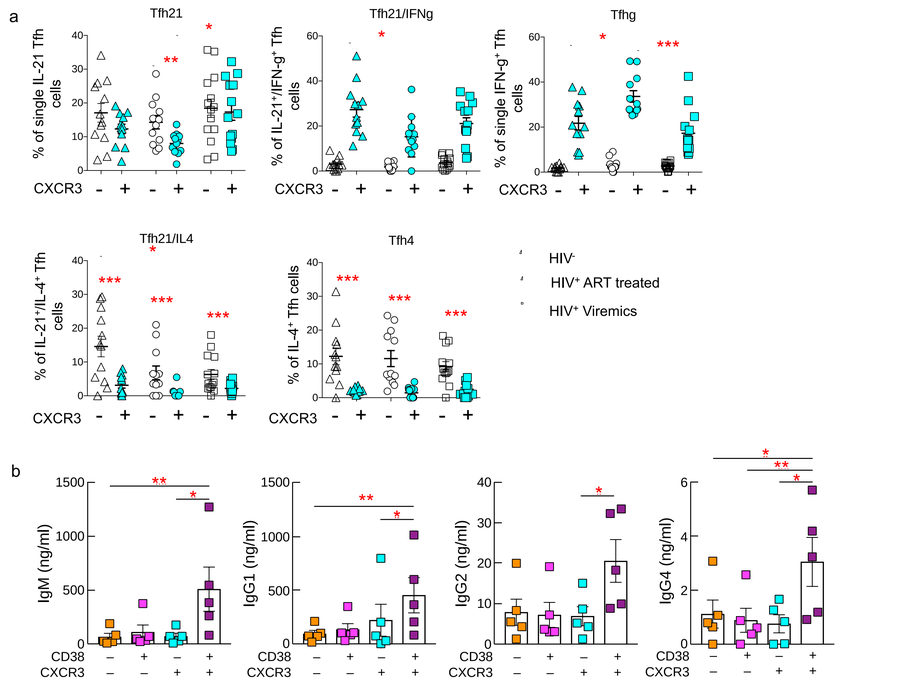


Figure 3: Frequencies of gp140 specific GC B cells in lymph nodes correlate with the percentage of $CD38^+CXCR3^+$ Tfhs

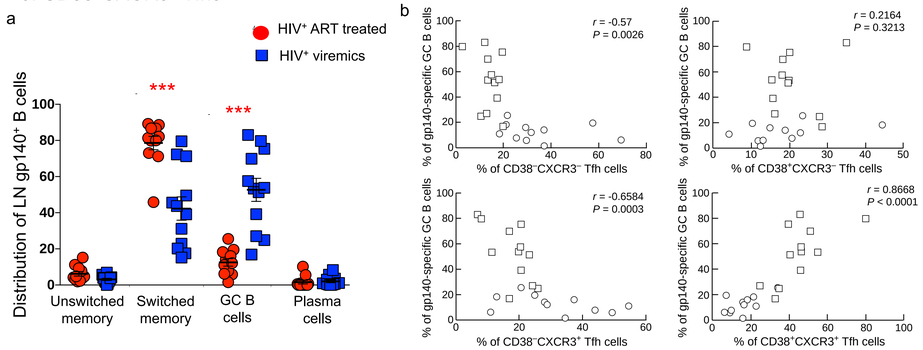


Figure 4. $CD38^+CXCR3^+$ Tfh cells enrich in cell associated HIV-RNA

