TFH cells Functional Profile Drives Abnormal B Cell Maturation in HIV infection

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Abstract

Background. The mechanisms underlying abnormalities in B cell maturation and antibody response in HIV-1 infection have been only partially elucidated. The large majority of the studies have been performed in blood B cells while limited information is available on B cell populations in lymph nodes and on the association between the functional profile of T follicular helper (Tfh) cells and the maturation of B cell populations in lymph nodes.

Methods. In the present study, we have investigated the phenotype and function of T follicular helper cells (Tfh) and B cells in lymph nodes of healthy and HIV-1 infected individuals using cutting-edge technologies such as mass cytometry and single cell gene expression. Lymph node biopsies were obtained from 21 HIV-1 infected viremic individuals naive to antiretroviral therapy and 18 healthy HIV negative individuals undergoing surgery for vascular pathologies or herniorrhaphy. Lymph node mononuclear cells (LNMC) were stimulated with phorbol-12-myristate-13-acetate (PMA) and ionomycin for 6 hours and stained with a unique panel of 32 markers primarily defining memory CD4 T cell and B cell populations including antibodies measuring differentiation, trafficking receptors and function.

Results. In HIV-1 infected individuals, we demonstrate a significant increase (2-3 fold) in the Tfh cells defined by the CXCR3+T-bet+ phenotype and IFN-γ production along with a significant decrease (2-3 fold) in Tfh cells expressing CCR4, CCR6 and producing IL-4. The CXCR3, T-bet and IFN-γ signatures of Tfh cells in HIV infection were strongly associated with the appearance of memory B cells expressing CXCR3 and T-bet, which accounted for 30% of memory B cells. CXCR3+ B cells had decreased expression of CXCR4 and CXCR5 and antibody production by CXCR3+ cells was different both quantitatively, e.g. higher producer cells, and qualitatively, *e.g.* lower levels of hyper somatic mutations, as compared to CXCR3- B cells. We then identified IFN-γ as the causative factor inducing the differentiation of the CXCR3+T-bet+ memory B cells and as a strong suppressive factor of antibody production.

Conclusions. The identification of Th1-like Tfh cells and IFN-γ as the main mechanisms affecting B cell maturation and antibody production in HIV infection will provide novel insights into strategies to develop optimal antibody responses in HIV infection and following vaccination.

Study group at enrollment

Subject ID	Age	Sex	Duration of HIV infection (years)	CD4 Cell Count at enrollment (cells/ul)	Viral Load at Enrollment (copies/ml)	Treatment Status
#RB021	34	M	6.1	517	3,000	Naive
#RB017	34	M	0.2	602	32,000	Naive
#RB018	48	M	2	475	26,000	Naive
#MP031	32	F	0.2	819	90,000	Naive
#MP034	24	M	0.3	549	5,000	Naive
#MP037	31	M	0.1	174	10,000,000	Naive
#MP049	21	F	15	813	21	Naive
#MP038	38	M	0.1	550	1,500,000	Naive
#MP046	45	M	0.1	704	20,000	Naive
#MP056	35	F	13	670	830	Naive
#RB019	NA	F	NA	634	73,400	Naive
#SA005	38	M	8	651	5,186	Naive
#SA035	37	M	7	1095	<20	Naive
#AVIB 1003	50	F	NA	720	214,622	Naive
#AVIB 1005	55	M	NA	859	16,164	Naive
#AVIB 1006	33	M	NA	691	141,628	Naive
#AVIB 1027	30	F	NA	867	60,828	Naive
#AVIB 1028	32	M	NA	535	25,760	Naive
#AVIB 1042	33	M	NA	470	121,000	Naive
#AVIB 1043	34	M	NA	442	246,000	Naive
#CAN 2132	26	M	NA	487	57,690	Naive

Results

Figure 1: Phenotype and function of Tfh cells

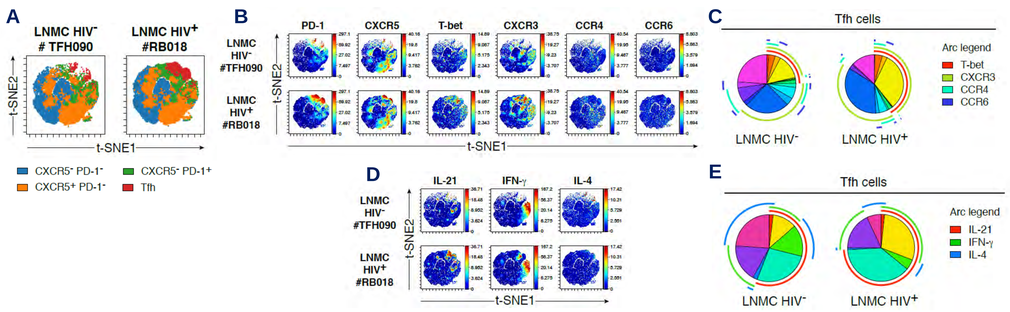


Figure 2: Gene expression profile of Tfh cells at single cell level in HIV-negative and HIV infected individuals by Fluidigm Biomark

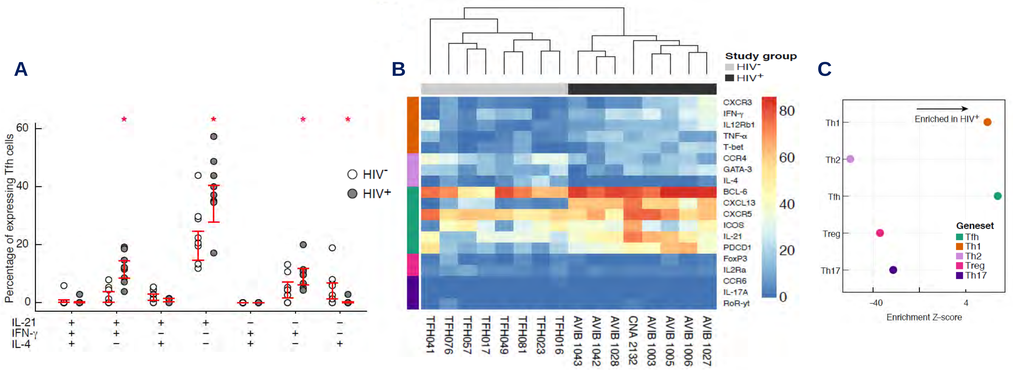


Figure 3: Phenotype and function of germinal center B cells

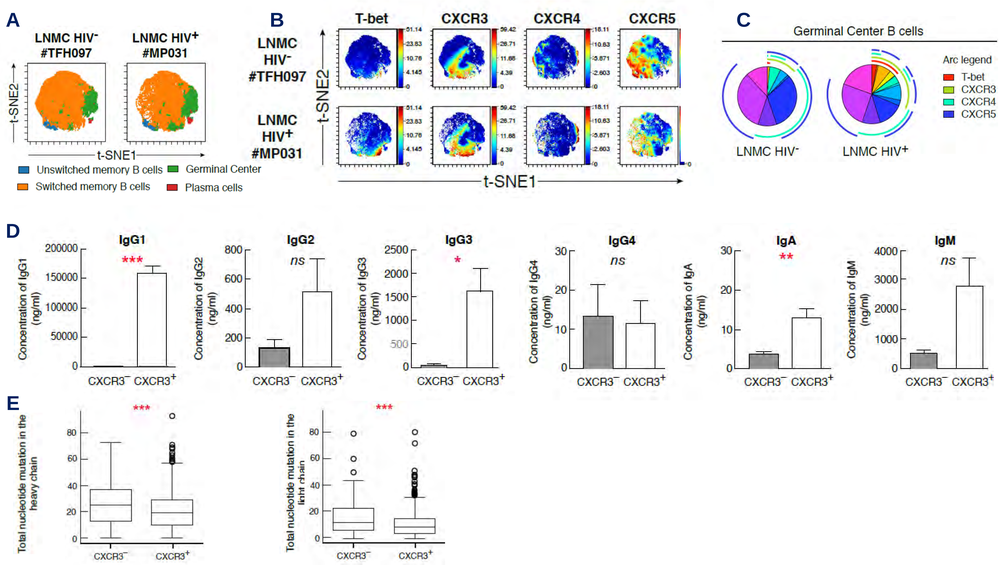


Figure 4: Correlations between phenotypic and functional Tfh markers and B cell phenotype

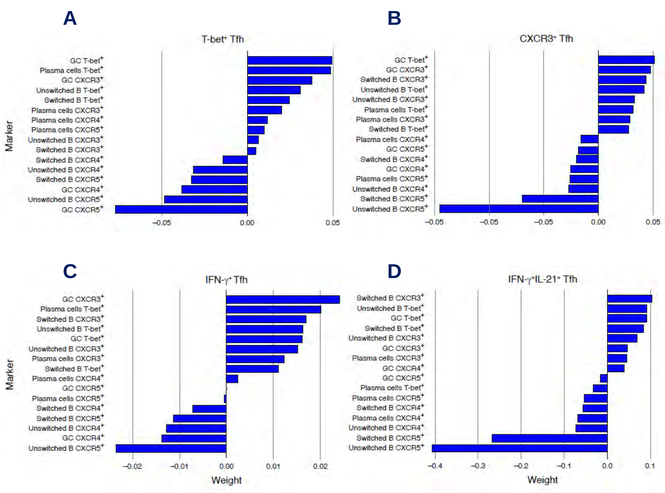
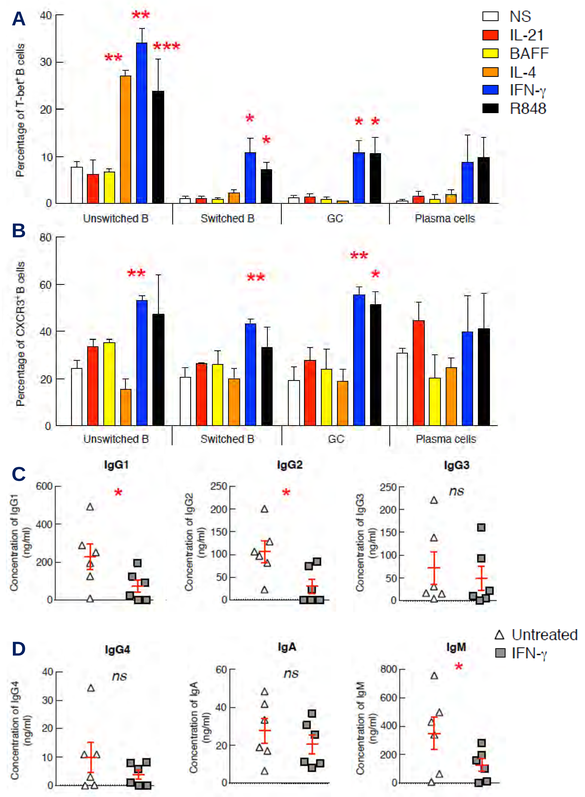


Figure 5: Effects of IFN-γ treatment



Conclusions

In the present study, we demonstrated that the identification of Th1-like Tfh cells and IFN-γ as the main mechanisms affecting B cell maturation and antibody production in HIV infection will provide novel insights into strategies to develop optimal antibody responses in HIV infection and following vaccination.