Longitudinal Dynamics on Follicular CD4+ T cells in Acute SIV Infection

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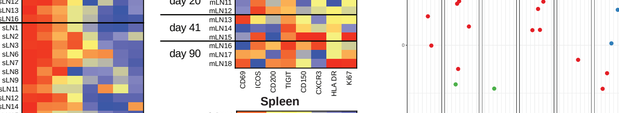
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BACKGROUND

- Follicular T helper CD4+ (Tfh) cells play a critical role in germinal center (GC) formation and B cell maturation.
- GCs in lymph nodes (LN), particularly within Tfh cells, are sites for preferential SIV infection and replication.
- Changes in Tfh phenotype and functions in early acute SIV infection may be a major determinant in the development effective antibody-mediated control of SIV infection.

STUDY DESIGN

Eighteen rhesus macaques were intravenously (i.v.) infected with SIVmac251 and underwent staggered necropsy (in sets of 3 animals per time point) during acute and chronic infection (Roberts, PLoSPath 2016).

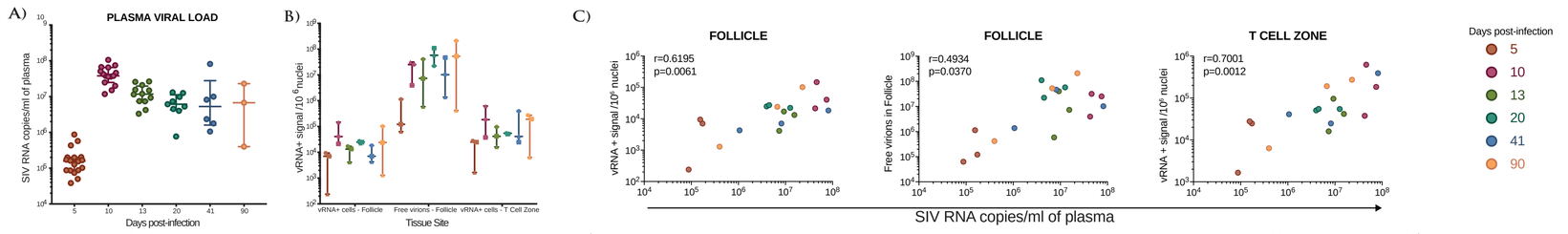


1. IMMUNOPHENOTYPING: SLN, MLN, SPLEEN

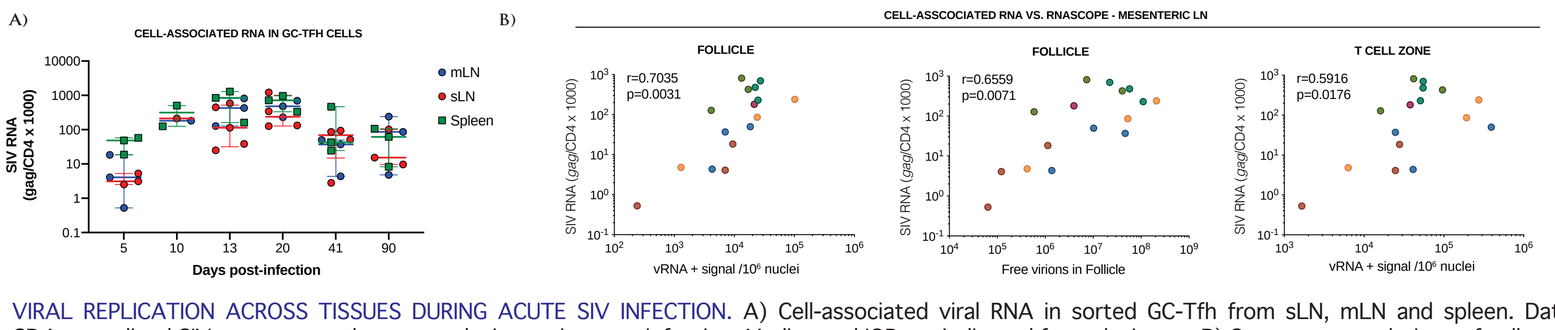
LINEAGE MARKERS	MEMORY SUBSETS	GC-TFH PHENOTYPE
CD3	CCR7	PD-1
CD4	CD28	CXCR5
CD8	CD95	CD69
CD14		CD200
CD16	ACTIVATION MARKERS	TIGIT
CD20	Ki67	ICOS
	HLA-DR	CD150

FREQUENCY AND PHENOTYPE DYNAMICS OF GC-TFH CELLS ACROSS TISSUES IN ACUTE SIV INFECTION. A) Gating strategy to identify GC-Tfh cells. B) and C) Changes in frequency of GC-Tfh cells in surface LN (sLN), mesenteric LN (mLN) and spleen. Lines and whiskers indicate median and IQR, respectively. D) Heatmap summary of phenotypic characteristics of GC-Tfh cells across tissues after SIV infection. E) Principal component analyses based on phenotypic variations of Tfh cells.

2. VIRAL QUANTIFICATION IN BLOOD AND TISSUES



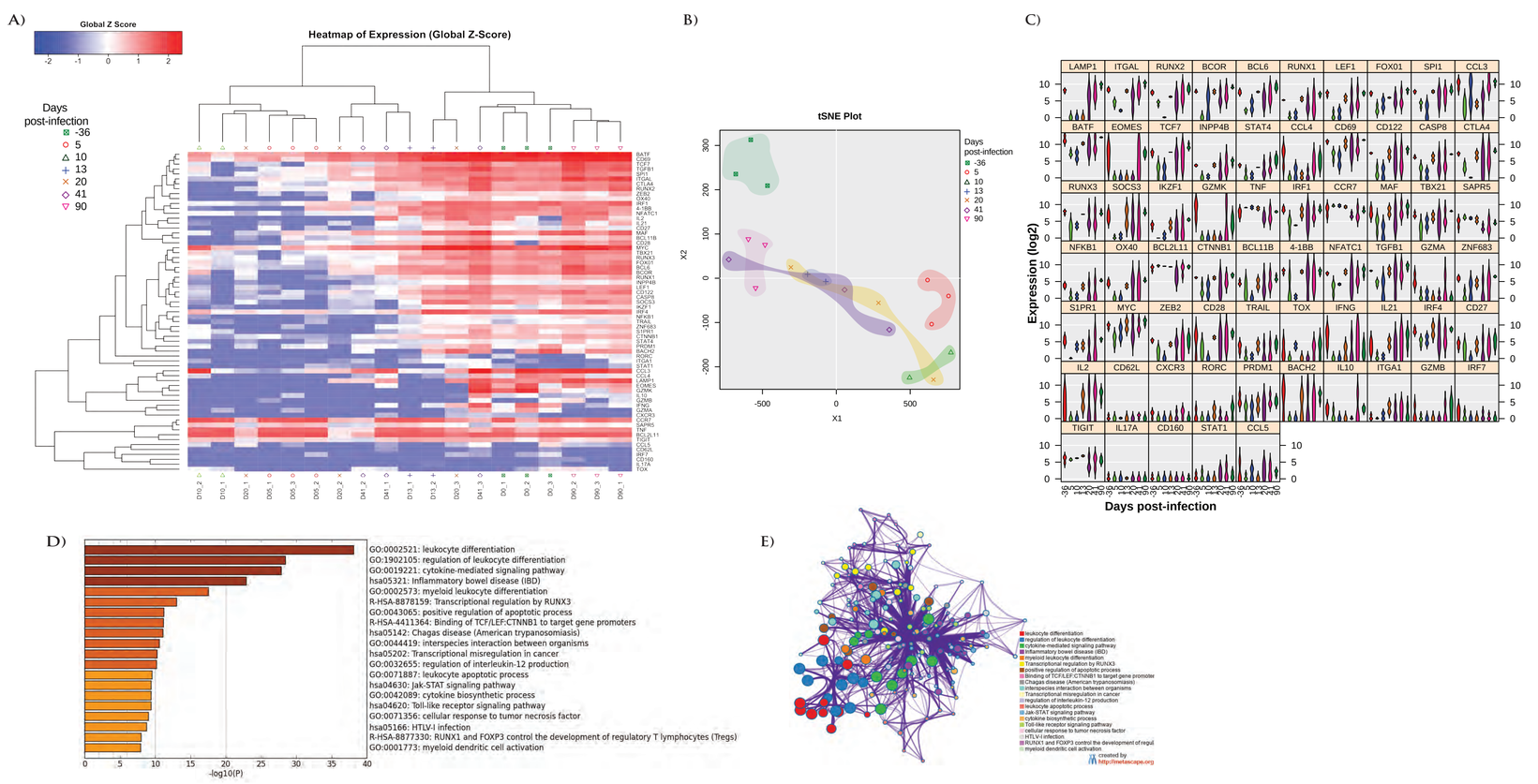
VIRAL LOAD DYNAMICS IN PLASMA AND MESENTERIC LN. A) SIV RNA copies per ml of plasma were quantified using standardized PCR methods. B) Virus positive (vRNA+) cells per million were measured using RNAScope in situ hibridization in paraffin embeded mesenteric LN samples. Lines and whiskers indicate median and IQR, respectively. C) Spearman correlations of plasma viremia and vRNA+ cells or free virions in mLN.



VIRAL REPLICATION ACROSS TISSUES DURING ACUTE SIV INFECTION. A) Cell-associated viral RNA in sorted GC-Tfh from sLN, mLN and spleen. Data show CD4-normalized SIV *gag* copy numbers at each time point post-infection. Median and IQR are indicated foreach tissue. B) Spearman correlations of cell associated RNA in mLN and RNAScope in different areas of the mLN.

3. TRANSCRIPTIONAL PROFILING AND RESPONSIVENESS OF GC-TFH CELLS

- Whole LNMC from LN were cultured for 3 hours with PMA/ionomycin or medium alone at 37 °C and 5% CO_2. For pre-infection time point, cells from a sLN were used as there were no mLN available. All post- infection data was generated from mLN.
- GC-Tfh were FACS sorted at 100 cells per sample, and gene expression was measured by high throughput TaqMan based assays using Fludigm platform.
- Pathway enrichment analyses were performed using Metascape.



LOSS OF GC-TFH RESPONSIVENESS AFTER EARLY SIV INFECTION FROM MESENTERIC LN. A) Heatmap and cluster analyses of gene expression after PMA/ionomycin stimulation (D0 label corresponds to day -36/pre-infection time point). B) tSNE analyses identifying clusters of samples based on gene expression. Colored clusters indicate a specific time point. C) Violin plots showing the dynamic variation in activation induced gene expression. The most significant gene shown in the top left (ANOVA test, $p<0.05$). D) Heatmap of enriched pathways colored by p-values. E) Network of enriched pathways colored by cluster, where nodes that share the same cluster are typically close to each other. Size of the nodes correspond to the p-values indicated on D.

MAIN FINDINGS

- The frequency of Tfh cells decreased at day 10 post-infection (p.i.) and partially rebounded after 20 days in all tissues.
- PCA showed similar phenotypic profiles in Tfh from mLN and sLN. In contrast, Tfh isolated from the spleen clustered separately after 10 days p.i., and this separation was driven mainly by CXCR3 expression.
- Although plasma viremia (pVL) peaked day 10 p.i., vRNA in mLNs was detectable as early as day 5 p.i. within follicles and the T cell zone.
- While pVL decreased after 20 days, tissue vRNA was increased until 90 days p.i. but was not preferentially found within the follicles during this early period.
- pVL correlated with free virions and vRNA+ cells in both follicles and T cell zone within the mLN.
- Very early following infection, transcriptional profiling of Tfh-related genes showed profound modulation of cytokine production and inflammatory pathways.
- Tfh responsiveness to stimulation decreased as early as day 5 p.i. This functional ability was partially recovered after 20 days p.i. irrespective of the increasing vRNA found the tissue.
- tSNE analyses showed independent clustering pre- and post-infection, and Tfh cells from day 90 p.i. had the most similar profile to pre-infection suggesting a partial recovery in responsiveness in later stages of infection.

CONCLUSION

SIV infection has a profound effect in Tfh frequencies, phenotypic and genetic profiles across tissues since acute infection. This effect suggests a temporal decrease in Tfh ability to provide B cell help during early stages of infection associated with high levels of viremia in blood and tissues, that may directly impact or delay the early induction of SIV-specific antibody production.

FUNDING

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