Poster # 265

Germinal Center B cells Induce TFH Activation and HIV Replication

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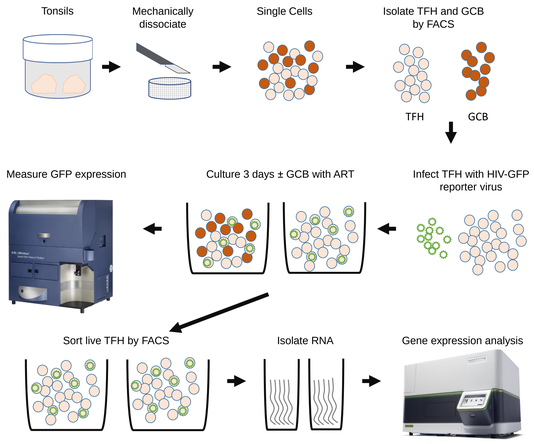
BACKGROUND

T follicular helper cells (TFH) are major sources of HIV replication in people living with HIV infection. T cell activation enhances HIV replication[1], cytokine secretion including IL-2 and TNF, and expression of several receptors including OX40, CD25, CD69 and 4-1BB[2]. In ex vivo infected tonsils, HIV replication is elevated in activated T cells expressing OX40 and reduced levels of CD127[3]. Although much is known of the impact of TFH on germinal center B cell (GCB) activation and function, little is known of the influence of GCB on the activation state of TFH in HIV infection. We hypothesized that GCB induce activation and HIV replication in TFH.

METHODS

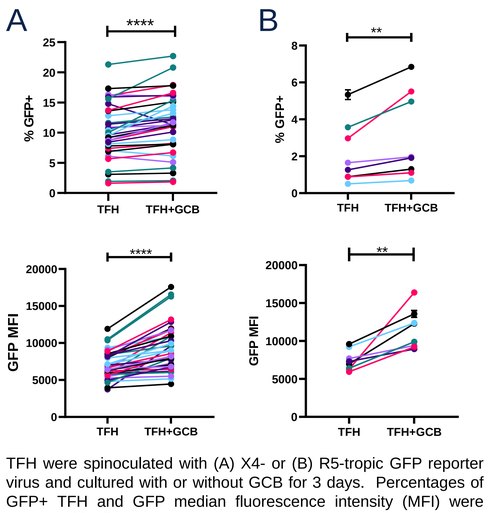
TFH (7-AAD$^-$CD3$^+$CD8$^-$CXCR5^{hi}PD-1^{hi}) and GCB (7-AAD$^-$CD19$^+$IgD$^-$CD38$^+$) were sorted from tonsils of individuals at low risk of HIV infection undergoing routine tonsillectomies. TFH were spinoculated with X4- or R5-tropic GFP reporter virus and cultured for 3 days in the presence or absence of GCB in RPMI media with 15% FBS and saquinavir. Percent GFP+TFH and GFP MFI were measured by flow cytometry. In a subset of experiments, TFH spinoculated with X4-GFP were isolated after being cultured in the absence or presence of GCB. RNA was extracted and individual transcripts for 785 genes were quantified using an nCounter Host Response Panel and analyzed using Rosalind. Fold changes and p-values were calculated and p-value adjustment was performed using the Benjamini-Hochberg method of estimating false discovery rates.

Tonsil model to study HIV replication in TFH



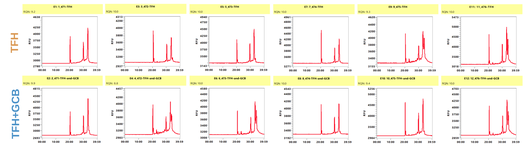
Schematic of experimental design for assessment of HIV replication in TFH and gene expression analysis.

GCB enhance HIV replication in X4 and R5 infected TFH



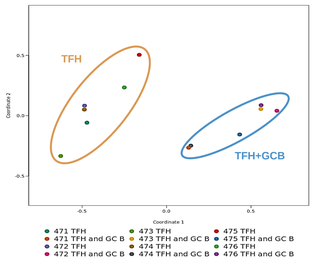
TFH were spinoculated with (A) X4- or (B) R5-tropic GFP reporter virus and cultured with or without GCB for 3 days. Percentages of GFP+ TFH and GFP median fluorescence intensity (MFI) were measured by flow cytometry.

RNA quality



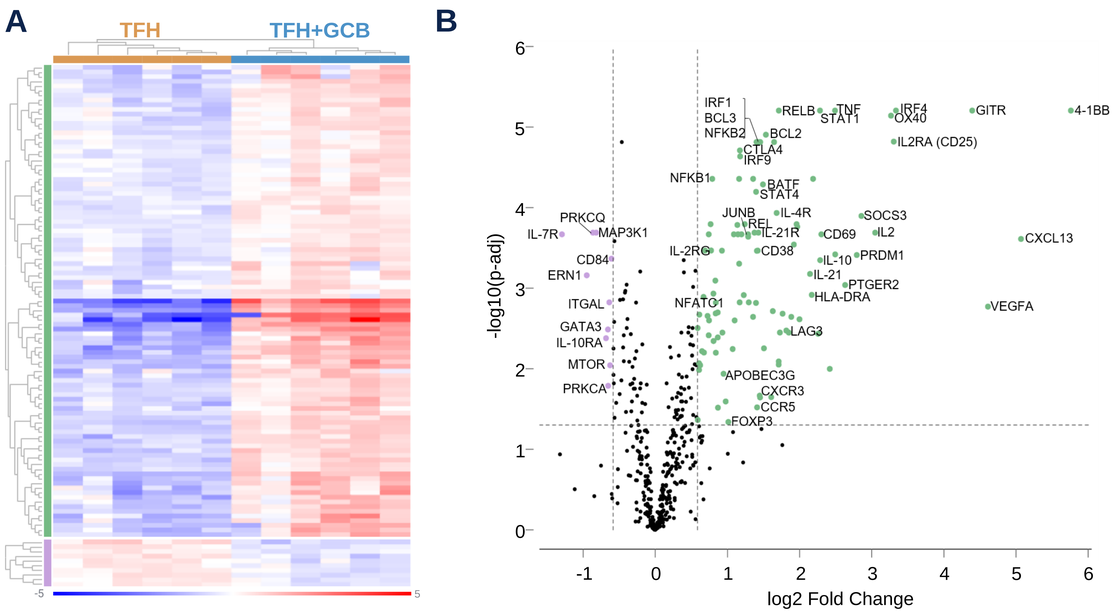
RNA was isolated from sorted TFH using a PicoPure RNA Isolation Kit. RNA quality (RQN) and concentration were evaluated using a Fragment Analyzer.

MDS Plot



RNA gene expression analysis data were plotted on a multidimensional scaling (MDS) plot highlighting clustering of TFH and TFH+GCB conditions.

GCB alter several TFH genes associated with cellular activation and augmented HIV replication



RNA gene expression analysis of the 785 gene Host Response Panel identified 101 TFH genes that were significantly upregulated ≥1.5-fold and 10 genes downregulated ≥1.5-fold in the presence of GCB as shown by the (A) heatmap and (B) volcano plot.

(C) Gene expression profiles of select genes associated with increased HIV replication and/or T cell activation.

CONCLUSIONS

- GCB augment HIV replication in TFH.
- GCB induce expression of several genes associated with T cell activation
- GCB promote a TFH phenotype correlated with increased HIV replication.
- A better understanding of the mechanisms that drive GCB-mediated effects on TFH could suggest new targets for suppression of HIV replication in TFH.

ACKNOWLDEGEMENTS

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REFERENCES

1. McDougal, JS et al. Jimmunol 1985 doi:10.4049/jimmunol.135.5.3151.

2. Reiss, S et al. PLOS One 2017 doi:10.1371/journal.pone.0186998

3. Cavrois, M et al. Cell Reports 2017 doi:10.1016/j.celrep.2017.06.087

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