Poster # 00172

TFR Reduce HIV-1 Infected TFH *in vitro* in an IL-2 Dependent Manner

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BACKGROUND

Follicular CD4+ T cells (TFH) are highly permissive to HIV-1 infection and a major reservoir of HIV-1 in lymphoid tissues (1). Follicular regulatory CD4+ T cells (TFR) limit TFH numbers and function in vitro and in vivo (2-3). We hypothesized that TFR inhibit HIV-1 replication in TFH.

METHODS

TFH (CD3+CD8-CXCR5+CD25-) isolated from tonsils of individuals at low risk for HIV were spinoculated with the GFP reporter virus NLENG1, labeled with the proliferation dye VPD450, and co-cultured 1:1 with autologous TFR (CD3+CD8-CXCR5+CD25+) or TFH (control) for 5 days in Advanced R-10 media and 10 IU/ml IL-2. Percent GFP+VPD450+ TFH were determined by flow cytometry. Live VPD450+ TFH were isolated on a cell sorter, and total and integrated HIV DNA were quantified using QPCR. Cell counts were measured using counting beads. In some experiments, 0, 10, 30, or 100 IU/ml of IL-2, or blocking antibodies to TGF-β, CTLA-4, CD39, or IL-10 at 10 µg/ml were added. IL-2 supernatant concentrations were measured by ELISA. Statistical analyses were performed with non-parametric Wilcoxon matched-pairs test using GraphPad Prism 8.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$, and ns = not significant.

RESULTS

Figure 1. Methods schematic (A) representative FACS (B) and flow gating (C)

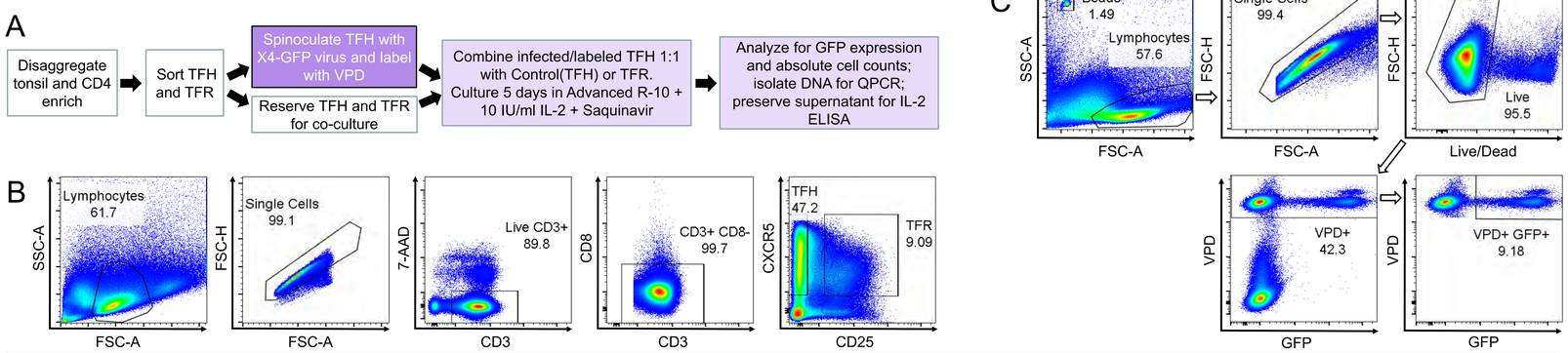


Figure 2. TFR suppress HIV replication in TFH

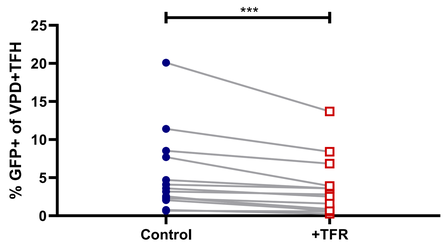


Figure 3. TFR reduce integrated (A) and total (B) HIV DNA in TFH

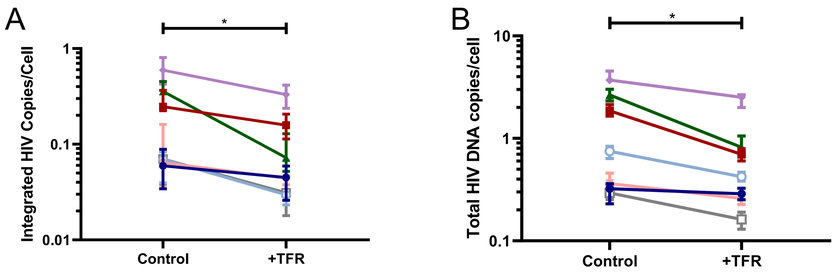


Figure 4. TGF-β, CD39, CTLA-4 or IL-10 blockade does not reverse TFR mediated reduction of GFP+ TFH

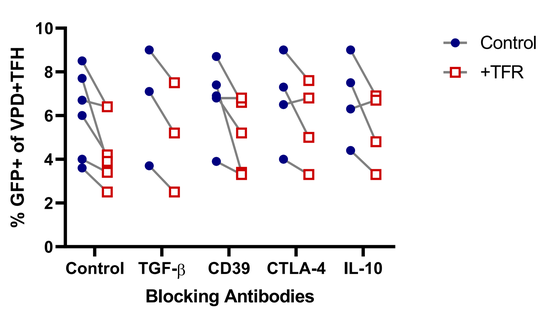


Figure 5. IL-2 enhances % GFP+ TFH (A) and viability (B)

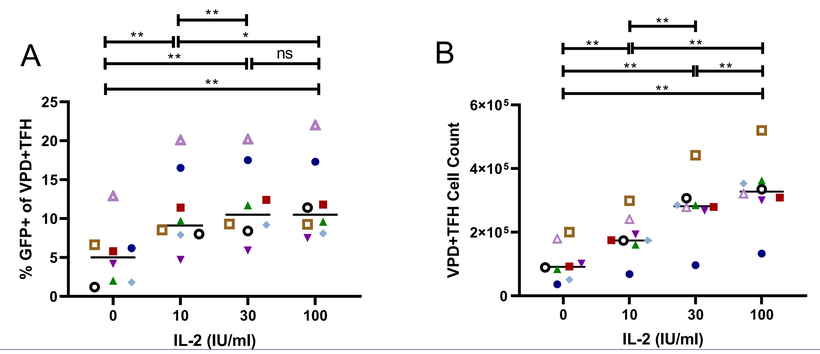


Figure 6. TFR mediated reduction of GFP+ TFH (A) and TFH viability (B) occur only at low doses of IL-2

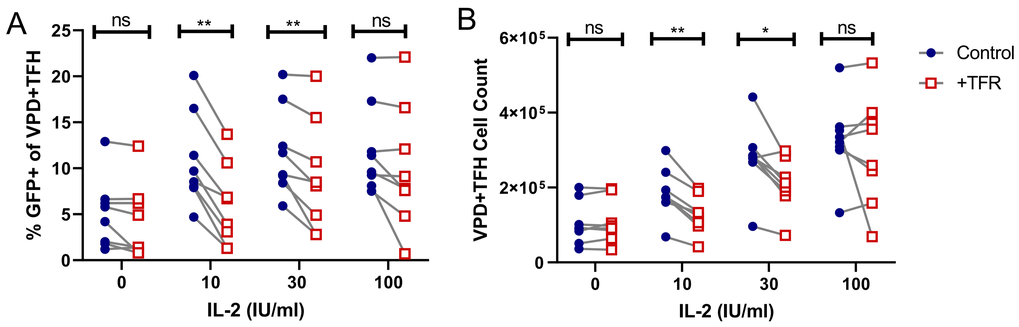
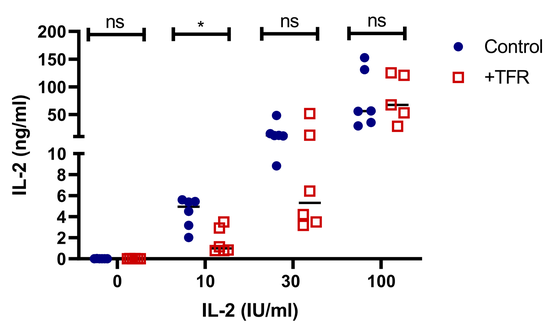


Figure 7. TFR decrease IL-2 supernatant concentrations only at low doses of IL-2



CONCLUSIONS

IL-2 promoted TFH viability and HIV-1 associated GFP expression in vitro. TFR reduced HIV infected and productively replicating TFH in low IL-2 conditions by limiting IL-2 concentrations. Consumption of IL-2 in B cell follicles may be one mechanism by which TFR reduce HIV-expressing TFH in vivo.

ACKNOWLDEGEMENTS

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