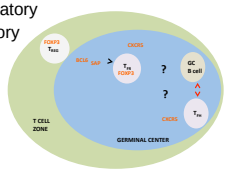
Decreased T_{FR}/T_{FH} ratio in SIV-infected rhesus macaques may contribute to accumulation of T_{FH} cells in chronic infection

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Introduction

T follicular helper cells (T_{FH}) are critical for the development and maintenance of germinal centers (GC) and humoral immune responses (1). T_{FH} accumulate during chronic HIV/SIV infection, possibly as a result of antigenic persistence, and serve as a major site of persistent viral production (2). This SIV/HIV-associated T_{FH} expansion may also reflect lack of regulation by suppressive follicular regulatory CD4$^+$ T cells (T_{FR}). T_{FR} are natural regulatory T-cells (T_{REG}) that migrate into the follicle and, similarly to T_{FH}, up-regulate CXCR5, Bcl6, and PD1 (3).



Here we identified T_{FR} within lymph nodes of rhesus macaques (RM) and confirmed their localization within the GC by immunohistochemistry. Following SIV infection, the T_{FR}/T_{FH} ratio was reduced. Our data suggests that T_{FR} may contribute to the regulation and proliferation of T_{FH} and GC B-cells *in vivo* and that a decreased T_{FR}/T_{FH} ratio in chronic SIV infection may lead to unchecked expansion of both T_{FH} and GC B-cells.

Animals: Ten unvaccinated and SIV-uninfected RM, 13 healthy, SIV-immunized but SIV-uninfected RM, 13 vaccinated and SIV-infected RM. Animals were infected with SIVsmmE660 intra-vaginal challenge at $2.06X10^4$ $TCID_{50}$.

Results

T_{FR} are distinct from T_{FH} and T_{REG} and can be found within lymph nodes of RM.

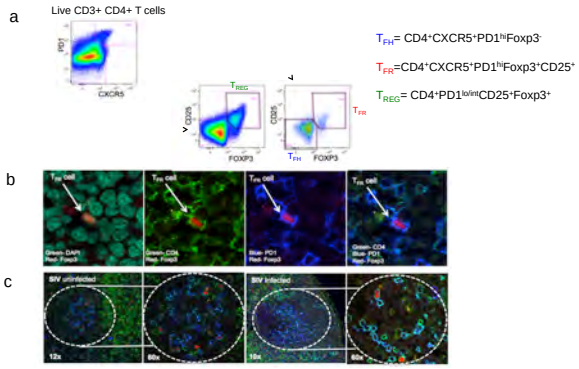


Figure 1 (a) Representative flow cytometry plot of live lymphocytes from lymph node of untreated uninfected RM showing the gating strategy used to define T_{FR}, T_{FH} and T_{REG} cell populations. (b) Representative confocal microscope image showing a single T_{FR} cell(c) Representative image showing T_{FR} cells localized within GCs of uninfected and infected RM.

T_{FR} express markers of both T_{FH} and T_{REG} differentiation.

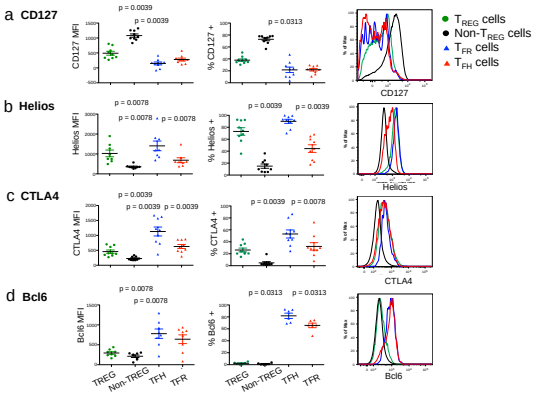


Figure 2. Mean fluorescence intensity, percent positive for expression and representative histograms of markers among T_{REG}, Non-T_{REG}, T_{FR} and T_{FH} cell populations from LN of untreated uninfected RM.

RNA expression patterns confirm that T_{FR} cells share T_{FH} and T_{REG} like phenotype.

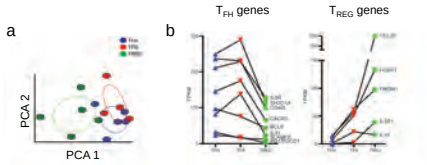


Figure 3 (a) Principal components analysis of RNA transcripts from lymphocytes sorted from 5 uninfected RM. Each circle represents the transcriptome of a sorted population of T_{FH} (blue), T_{REG} (green) of T_{FR} (red) cells from a single animal. (b) Absolute expression in FPKM of key T_{FH} and T_{REG} genes in sorted populations from uninfected RM.

T_{FR} cells decrease as a frequency of T_{FH} cells after SIV infection.

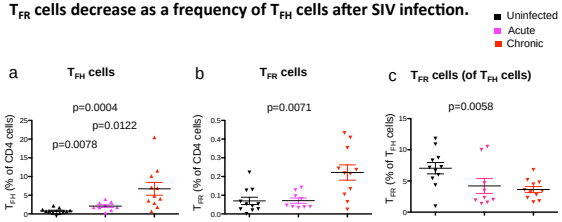


Figure 4. The frequency of (a) T_{FH} and (b) T_{FR} cells within LN of uninfected (black), acutely infected (pink) and chronically infected (red) untreated RM. (c) Frequency of T_{FR} cells (as a percent of T_{FH} cells).

T_{FR} cells frequencies negatively correlate with T_{FH} cell and GC B cell frequencies.

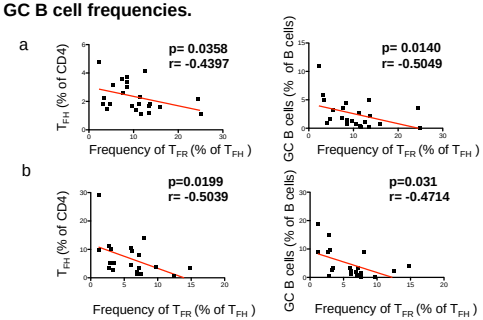


Figure 5.Correlations between the frequencies of T_{FR} (as a frequency of T_{FH}) with the frequencies of T_{FH} and GC B cells within LN of SIV uninfected (a) and SIV infected (b) RM.

Conclusions

The main findings of the current study are the followings:

(i) T_{FR} show a shared or intermediate phenotype as compared to T_{FH} and T_{REG} based on a combination of flow cytometric, histological, and transcriptional analysis by RNA sequencing;

(ii) In healthy, SIV-uninfected RM, the frequencies of T_{FR} are negatively correlated with the levels of both T_{FH} and GC B-cells as well as levels of CD4$^+$ T-cell proliferation;

(iii) following SIV infection, the T_{FR}/T_{FH} ratio was reduced

Collectively these data indicate that while T_{FR} closely resemble T_{FH} in several biological aspects, they are also clearly distinct from this cell subset in terms of both immunophenotype and transcriptional profile.

These results support the hypothesis that these cells play an important immune regulatory role in vivo, and that a relative decline of the T_{FR}/T_{FH} ratio may be involved in establishing a state of chronic immune activation in the B cell areas of lymph nodes during pathogenic HIV and SIV infection.

References

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Acknowledgments: This work was supported by National Institutes of Health Grant U19 AI096187. We would like to thank the animal care and veterinary staff at the Yerkes National Primate Research Center, the Yerkes Genomics Core, Virology Core and the Molecular Pathology Core.