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 HIV/AIDS-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 ($T_{FR}$) that migrate into the follicle and, similarly to $T_{FH}$, up-regulate CXCXR5, 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 $T_{FR}$ 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$^4$ TCID50.

Results

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

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

Figure 1. 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 $T_{FH}$ cells localized within GCs of uninfected and infected RM.

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

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

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 $T_{FR}$ (blue), $T_{REG}$ (green), $T_{FH}$ (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.

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

Figure 5. Correlations between the frequencies of $T_{FH}$ (as a frequency of $T_{FR}$) 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) $T_{FH}$ show a shared or intermediate phenotype 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 $T_{FH}$ are negatively correlated with the levels of both $T_{FR}$ and GC B-cells as well as levels of CD4$^+$ T cell proliferation.

(iii) Following SIV infection, the $T_{FH}/T_{FH}$ ratio was reduced.

Collectively these data indicate that while $T_{FH}$ 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 $T_{FH}/T_{FH}$ 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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