Vaccine Induced Follicular CD8 T cells Enhance Control of Pathogenic SIV Infection

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Background

Recent data demonstrated that a significant fraction of latently-infected cells reside in the germinal center (GC) resident T follicular helper cells (Tfh) during HAART of HIV-infected humans/SIV-infected macaques. Therapeutic interventions aimed at achieving a functional cure must target these viral reservoirs. Induction of highly functional anti-viral CD8 T cells that can home to GC (follicular CD8) represents an attractive therapeutic strategy. However, GCs are generally thought to exclude CD8 T cells and very little is known about whether T cell based vaccines induce anti-viral CD8 T cells with the potential to migrate to the GC and influence HIV/SIV replication in Tfh. Here, we addressed this in the context of DNA/MVA vaccination and pathogenic SIV infection in rhesus macaques (RM).

Aberrant Accumulation of PD-1^{hi} CD4 T Cells in Lymphoid Sites of Vaccine Mediated Controllers

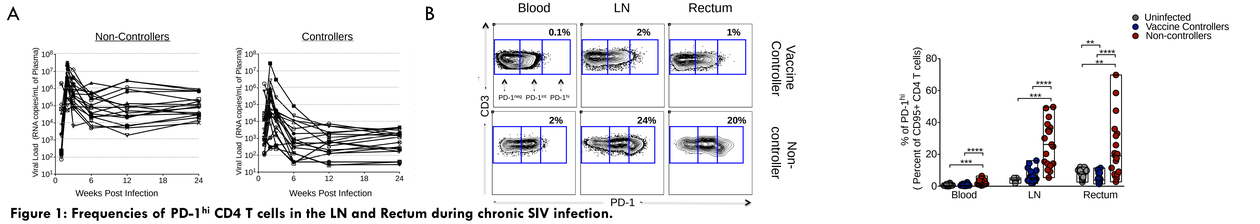

Figure 1: Frequencies of PD-1^{hi} CD4 T cells in the LN and Rectum during chronic SIV infection.
(A) Cohort of SIV infected non-controlling (n=18) and vaccine controlling RM (n=18) defined as RM with viral loads > or < 10^4 RNA copies/mL of plasma, respectively, at set-point. **(B)** Representative plots of PD-1 expression in the blood, LN, and rectum of SIV controller and SIV non-controller RM and flow cytometric analysis of the percentage of PD-1^{hi} CD95+ CD4 T cells in the blood, LN, and rectum of SIV naive (n=16, 7, and 17, respectively) SIV+ controller (n= 20) and SIV+ non-controller (n=20) RM. Scatter Plot shows median. *P < 0.05, **P < 0.01, ***P < 0.001.

SIV+ Tfh Cells Localize to the B cell Follicles and Contribute to Ongoing Viral Production

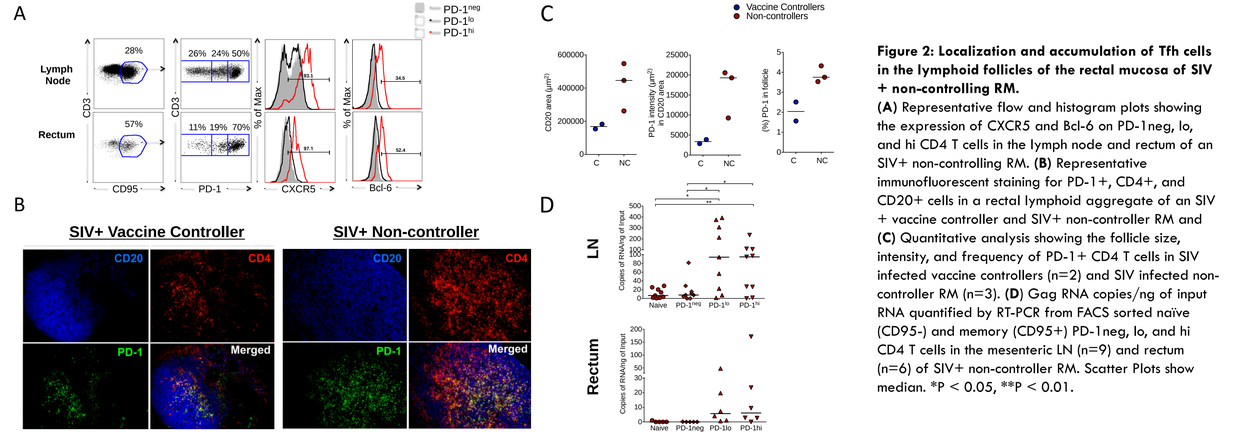

Figure 2: Localization and accumulation of Tfh cells in the lymphoid follicles of the rectal mucosa of SIV + non-controlling RM.
(A) Representative flow and histogram plots showing the expression of CXCR5 and Bcl-6 on PD-1neg, lo, and hi CD4 T cells in the lymph node and rectum of an SIV+ non-controlling RM. **(B)** Representative immunofluorescent staining for PD-1+, CD4+, and CD20+ cells in a rectal lymphoid aggregate of an SIV + vaccine controller and SIV+ non-controller RM and **(C)** Quantitative analysis showing the follicle size, intensity, and frequency of PD-1+ CD4 T cells in SIV infected vaccine controllers (n=2) and SIV infected non-controller RM (n=3). **(D)** Gag RNA copies/ng of input RNA quantified by RT-PCR from FACS sorted naïve (CD95-) and memory (CD95+) PD-1neg, lo, and hi CD4 T cells in the mesenteric LN (n=9) and rectum (n=6) of SIV+ non-controller RM. Scatter Plots show median. *P < 0.05, **P < 0.01.

Increased Co-localization of CD8 T cells with PD-1^{hi} Cells in the Germinal Centers of Vaccinated Viral Controllers

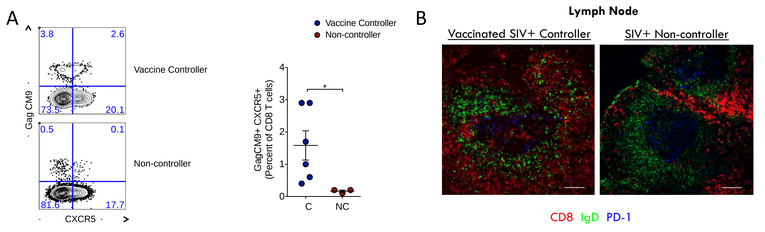

Figure 3. Characteristics of CXCR5+ CD8 T cells in LN of SIV infected vaccinated controlling and non-controlling RM.
(A) Representative plots and percentage of CXCR5+ Gag CM9+ CD8 T cells in LN of SIV+ controller (n=6) and SIV+ non-controller (n=3) RM. **(B)** Representative immunofluorescent staining of PD-1+, CD8+, and IgD+ cells of a mesenteric LN (MLN) section of an SIV+ vaccine controller and SIV+ non-controller RM. Scatter Plots show median. *P < 0.05.

SIV Specific CXCR5+ CD8 T cells Limit Tfh Expansion *In Vitro*

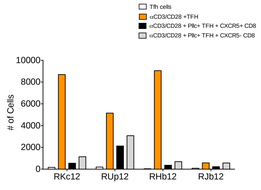

Figure 4. CXCR5+ CD8 T cells have the potential to limit Tfh expansion *in vitro*. Bar graphs representing 4 individual A01+ SIV infected viral controlling RM, in which autologous P11c pulsed CXCR5^{hi} PD-1^{hi} CD4 T cells were co-cultured with autologous CXCR5+ or CXCR5- CD8 T cells for 5 days. Cells were isolated from the LN.

CXCR5+ GagCM9+ CD8 T cells Closely Resemble a Tfh Genetic Profile

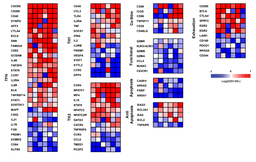

Figure 5. GagCM9+ CXCR5+ CD8 T cells display a Tfh like genetic profile. GagCM9+ CXCR5+ and CXCR5- CD8 T cells were sorted from the mesenteric lymph node (MLN) of vaccinated controller RM. Microarray analysis shows the gene expression represented as a $\log_2$ fold change of CXCR5+ to CXCR5- CD8 T cells.

Conclusions

- PD-1^{hi} CD4 T cells are enriched in sites of preferential SIV replication in an SIV naïve RM.
- Characteristics of these cells during *uncontrolled SIV infection*:
  - PD-1^{hi} CD4 T cells accumulate at lymphoid sites
  - Localize to the B cell follicles of lymphoid aggregates in the rectum and LN and actively support continued viral production
  - PD-1^{hi} cells localize to immune privileged sites such as the GC of lymphoid follicles with limited anti-viral CD8 T cell accessibility
- Characteristics of these cells during *vaccine-mediated control of SIV infection*:
  - No observed expansion of PD-1^{hi} CD4 T cells at lymphoid sites
  - Maintain higher frequencies of follicular SIV specific CD8 T cells
  - CD8 T cells are capable of infiltrating lymphoid sites and targeting infected PD-1^{hi} CD4 T cells
- These data implicate targeted therapy to disrupt follicular sites that may harbor depots of continued viral production

Grant Funding

NIH Grants R01 AI074471, R01 AI071852, and RC2 CA149086 to RRA; Yerkes National Primate Research Center base grant, P51 RR00165; Emory CFAR grant, P30 AI050409