Proportion of SIV-Infected Memory T Helper Subsets Correlates to Size of Population

Stephen Lai[1], Joseph C. Mudd[1], Jason M. Brenchley[1]

[1]Barrier Immunity Section, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases

National Institute of Allergy and Infectious Diseases

BACKGROUND

- Th17 cells are important for the maintenance of mucosal epithelial integrity
- There is a preferential loss of Th17 cells throughout the course of HIV/SIV infection
- Loss of Th17 cells weakens the mucosal epithelial barrier, leading to microbial translocation, chronic inflammation, and disease progression
- The mechanisms underlying preferential Th17 cell loss are unclear
- Some groups have shown that Th17 cells are preferentially infected in vitro (*Ancuta et al*. 2017)
- Many other IL-17 producing cell types that do not express viral entry receptors, such as ILC-3s, or IL-17 producing CD8s (Tc17s) are also preferentially lost

EXPERIMENTAL HYPOTHESIS

If the depletion of Th17 cells is due to preferential infection, then we expect to see more viral DNA present in Th17 cells as compared to other Th subsets.

We would also expect that if Th17 cells were preferentially infected, then CCR6+ Th17 and Th17-like cells would comprise a greater proportion of the total Th Memory infected pool

METHODS

- Lymphocytes from the peripheral blood, spleen, and mesenteric lymph nodes (MLN) of SIV+ ARV-naïve and ARV-treated Asian macaques were stimulated for 6 hours with PMA and ionomycin in the presence of Brefeldin A.
- Lymphocytes were stained with antibodies for specific functional and phenotypic markers
- Functionally-defined T Helper Memory Subsets were isolated by Fluorescent Activated Cell Sorting (FACS).
- Viral DNA in each subset was assessed using quantitative PCR

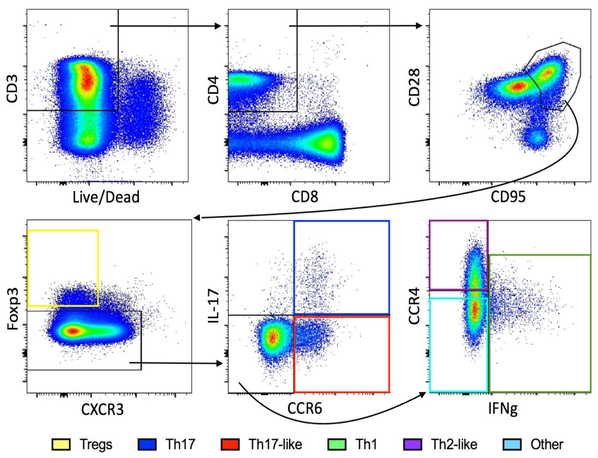


Figure 1. Gating strategy used for the isolation of functionally-defined T Helper Memory cells. From singlet lymphocytes, Live, CD4 Memory T cells were sorted into Foxp3+ T Regulatory cells (Tregs), IL-17 producing CCR6+ Th17 cells, CCR6+ cells Th17-like cells, IFNg-producing Th1 cells, CCR4+ Th2-like cells, and all the other cells which did not express the phenotypic and functional markers we have defined. We call this group "Other."

RESULTS

The "Other" Subset is the Largest Proportion of CD4 Memory cells in PBMC, Spleen, and MLN of ARV-naïve and ARV-treated macaques

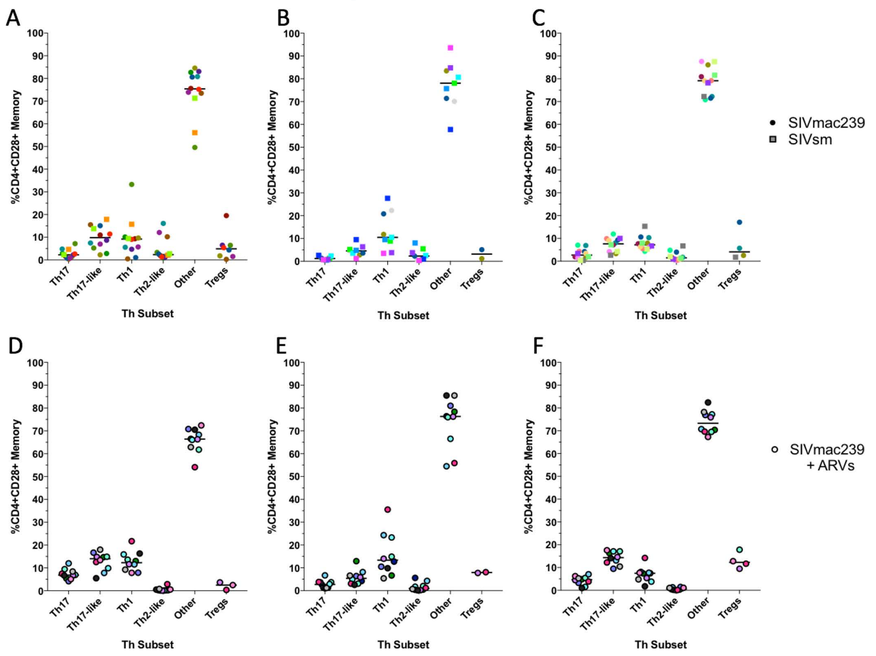


Figure 2. Size of each CD4 Memory Subset as a percentage of CD4+CD28+ Memory cells for A) ARV-naïve PBMC, B) ARV-naïve Spleen, and C) ARV-naïve MLN, as well as D) ARV-treated PBMC, E) ARV-treated Spleen, F) ARV-treated MLN.

Relatively Equal Levels of SIV infection in CD4 Memory Subsets

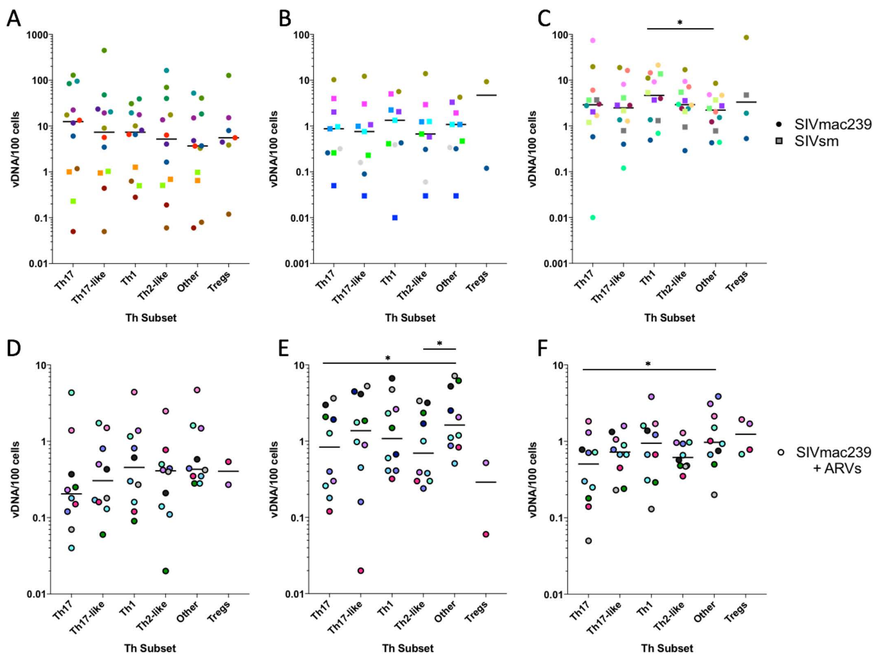
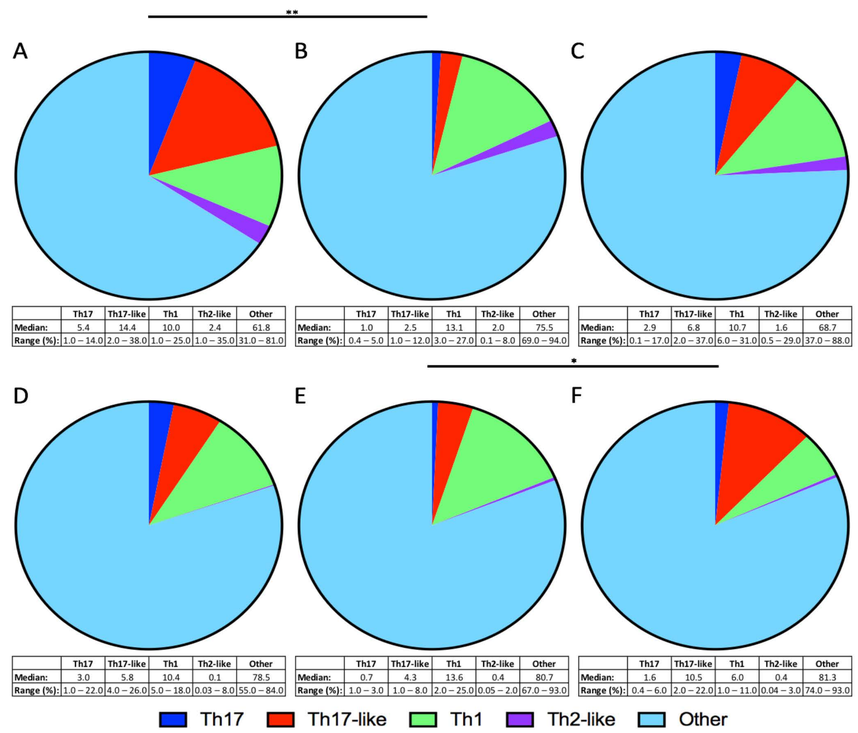


Figure 3. There are relatively equal levels of SIV DNA detected in the CD4 Memory Subsets of A) ARV-naïve PBMC, B) ARV-naïve Spleen, and C) ARV-naïve MLN, as well as D) ARV-treated PBMC, E) ARV-treated Spleen, F) ARV-treated MLN.

T Helper Subset Contribution to Total Infected Pool



	Th17	Th17-like	Th1	Th2-like	Other
Median:	5.4	14.4	10.0	2.4	61.8
Range (%):	1.0 – 14.0	2.0 – 38.0	1.0 – 25.0	1.0 – 35.0	31.0 – 81.0

	Th17	Th17-like	Th1	Th2-like	Other
Median:	1.0	2.5	13.1	2.0	75.5
Range (%):	0.4 – 5.0	1.0 – 12.0	3.0 – 27.0	0.1 – 8.0	69.0 – 94.0

	Th17	Th17-like	Th1	Th2-like	Other
Median:	2.9	6.8	10.7	1.6	68.7
Range (%):	0.1 – 17.0	2.0 – 37.0	6.0 – 31.0	0.5 – 29.0	37.0 – 88.0

	Th17	Th17-like	Th1	Th2-like	Other
Median:	3.0	5.8	10.4	0.1	78.5
Range (%):	1.0 – 22.0	4.0 – 26.0	5.0 – 18.0	0.03 – 8.0	55.0 – 84.0

	Th17	Th17-like	Th1	Th2-like	Other
Median:	0.7	4.3	13.6	0.4	80.7
Range (%):	1.0 – 3.0	1.0 – 8.0	2.0 – 25.0	0.05 – 2.0	67.0 – 93.0

	Th17	Th17-like	Th1	Th2-like	Other
Median:	1.6	10.5	6.0	0.4	81.3
Range (%):	0.4 – 6.0	2.0 – 22.0	1.0 – 11.0	0.04 – 3.0	74.0 – 93.0

Figure 4. T Helper Subset contribution to the total infected pool for A) ARV-naïve PBMC, B) ARV-naïve Spleen, and C) ARV-naïve MLN, as well as D) ARV-treated PBMC, E) ARV-treated Spleen, F) ARV-treated MLN.

The Contribution to the Total Infected Memory Pool of Each Subset is Correlated to the Size Each Population

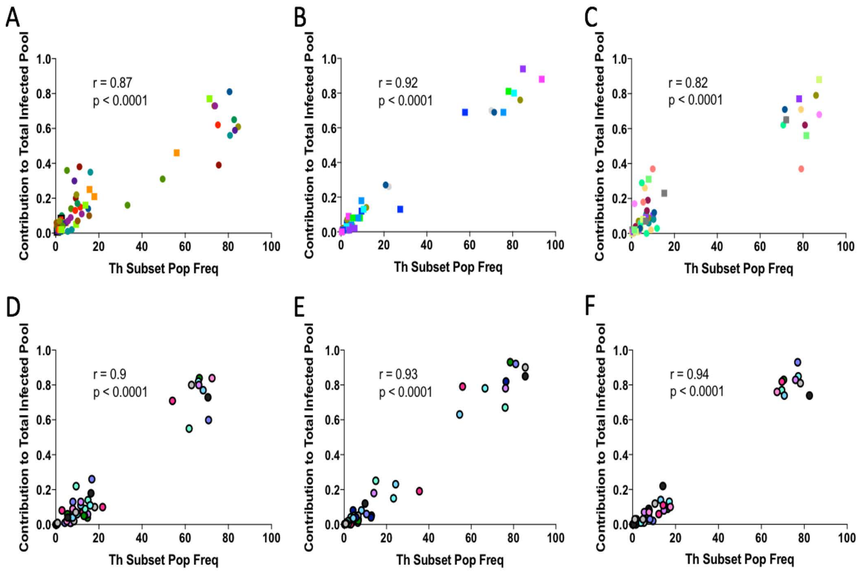


Figure 5. T Helper Memory Subset contribution to the total infected pool is significantly correlated to the size of each subset population. This is true for A) ARV-naïve PBMC, B) ARV-naïve Spleen, and C) ARV-naïve MLN, as well as D) ARV-treated PBMC, E) ARV-treated Spleen, F) ARV-treated MLN.

CONCLUSION

•Th17 cells are infected at equal levels as other T Helper Memory cell subsets
•The degree each subset is infected is strongly correlated to the size of the subset, making preferential infection by SIV unlikely to be the cause for the depletion of Th17 cells

ACKNOWLEDGEMENTS

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