234 - Persistent Dysregulation of HIV-Specific T Follicular Helper Cells during ART

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Background

- Germinal center T follicular helper cells (GC Tfh) provide B cell help for antibody responses[1]
- GC Tfh cells and circulating Tfh (cTfh) are impaired during HIV infection, contributing to inefficient humoral response to HIV[2]
- Impairment of these cells is not normalized by antiretroviral therapy (ART)[3]
- Increasing interest for humoral responses in HIV cure strategies
- Most previous studies of Tfh/cTfh limited to bulk population
- Current methods to study antigen (Ag) -specific T cells are limited[4]
- Mechanisms of impairment are unknown

- **General modulation that affects T cells of different specificities?**
- **Antigen-specific modulation that specifically affects HIV-specific cells?**

Study population

CHARACTERISTIC	HIV+ on ART (n=27)
Viral load	<40 copies/ml
Time on treatment (years)	4.3 [1-25]
CD4 count	630 [101-1091]
CD4:CD8 ratio	1.12 [0.13-3.60]
Age (years)	48 [19-70]
Gender (M/F)	27/0
CMV (positive/negative)	23/4
HBV (resolved infection/vaccinated/not immune or unknown)	12/6/9

Fig 1. Antigen-specific CD4+ T cells can be identified by surface upregulation of CD69/CD40L

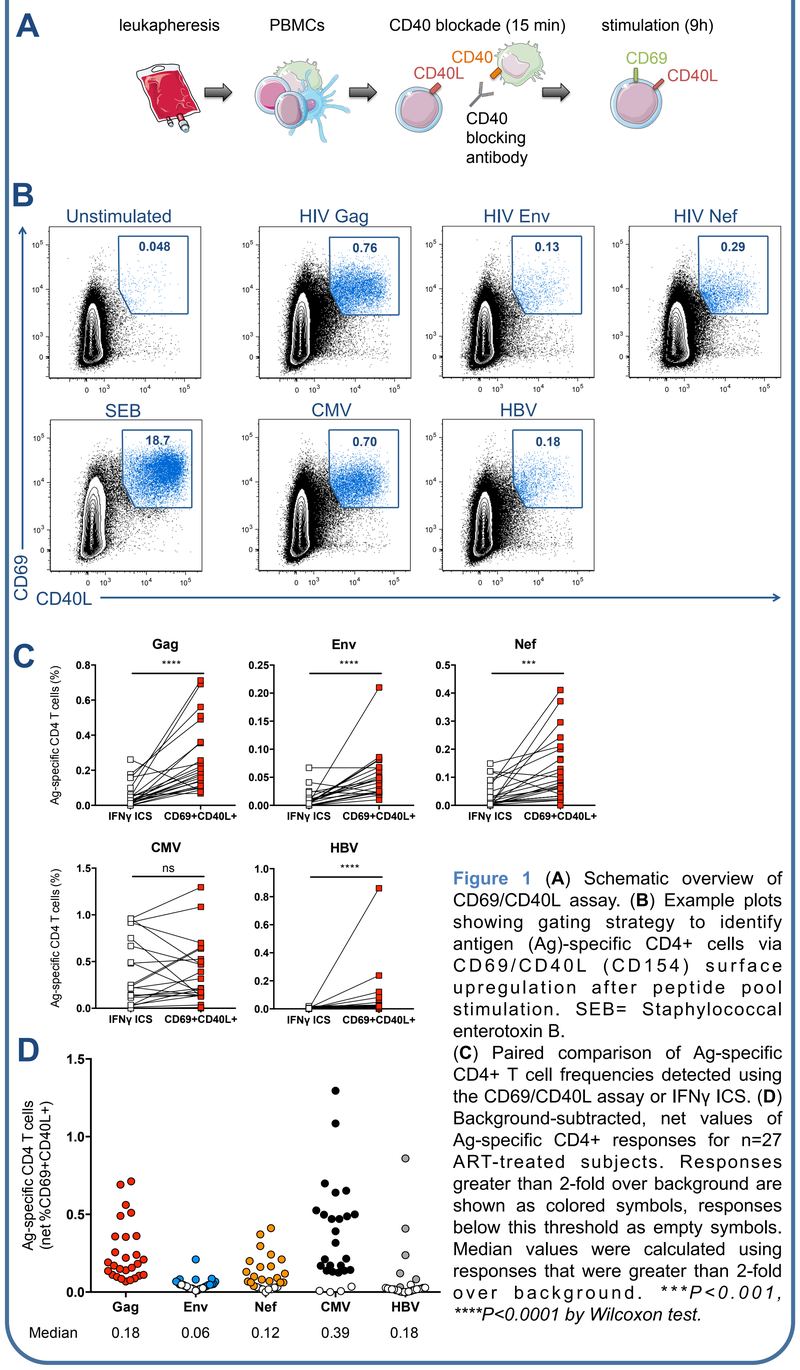


Figure 1 (**A**) Schematic overview of CD69/CD40L assay. (**B**) Example plots showing gating strategy to identify antigen (Ag)-specific CD4+ cells via CD69/CD40L (CD154) surface upregulation after peptide pool stimulation. SEB= Staphylococcal enterotoxin B.
(**C**) Paired comparison of Ag-specific CD4+ T cell frequencies detected using the CD69/CD40L assay or IFNγ ICS. (**D**) Background-subtracted, net values of Ag-specific CD4+ responses for n=27 ART-treated subjects. Responses greater than 2-fold over background are shown as colored symbols, responses below this threshold as empty symbols. Median values were calculated using responses that were greater than 2-fold over background. ****P<0.001, ****P<0.0001 by Wilcoxon test.*

Fig 2. HIV-specific CD4+ T cells show markers of circulating follicular helper T cells

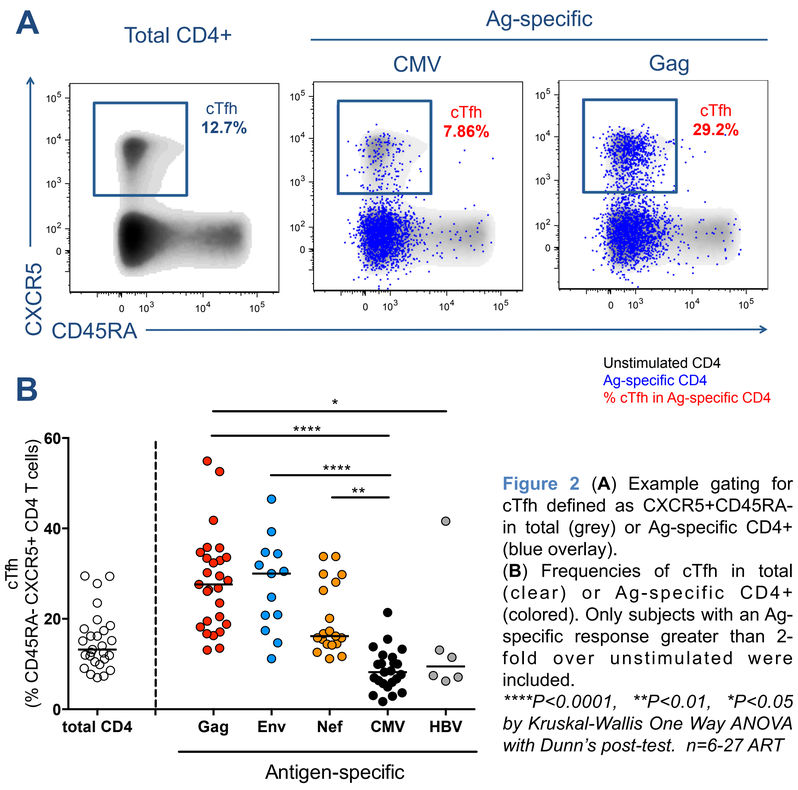


Figure 2 (**A**) Example gating for cTfh defined as CXCR5+CD45RA- in total (grey) or Ag-specific CD4+ (blue overlay).
(**B**) Frequencies of cTfh in total (clear) or Ag-specific CD4+ (colored). Only subjects with an Ag-specific response greater than 2-fold over unstimulated were included.
*****P<0.0001, **P<0.01, *P<0.05 by Kruskal-Wallis One Way ANOVA with Dunn's post-test. n=6-27 ART*

Fig 3. Gag-specific cTfh cells are predominantly Th1-polarized

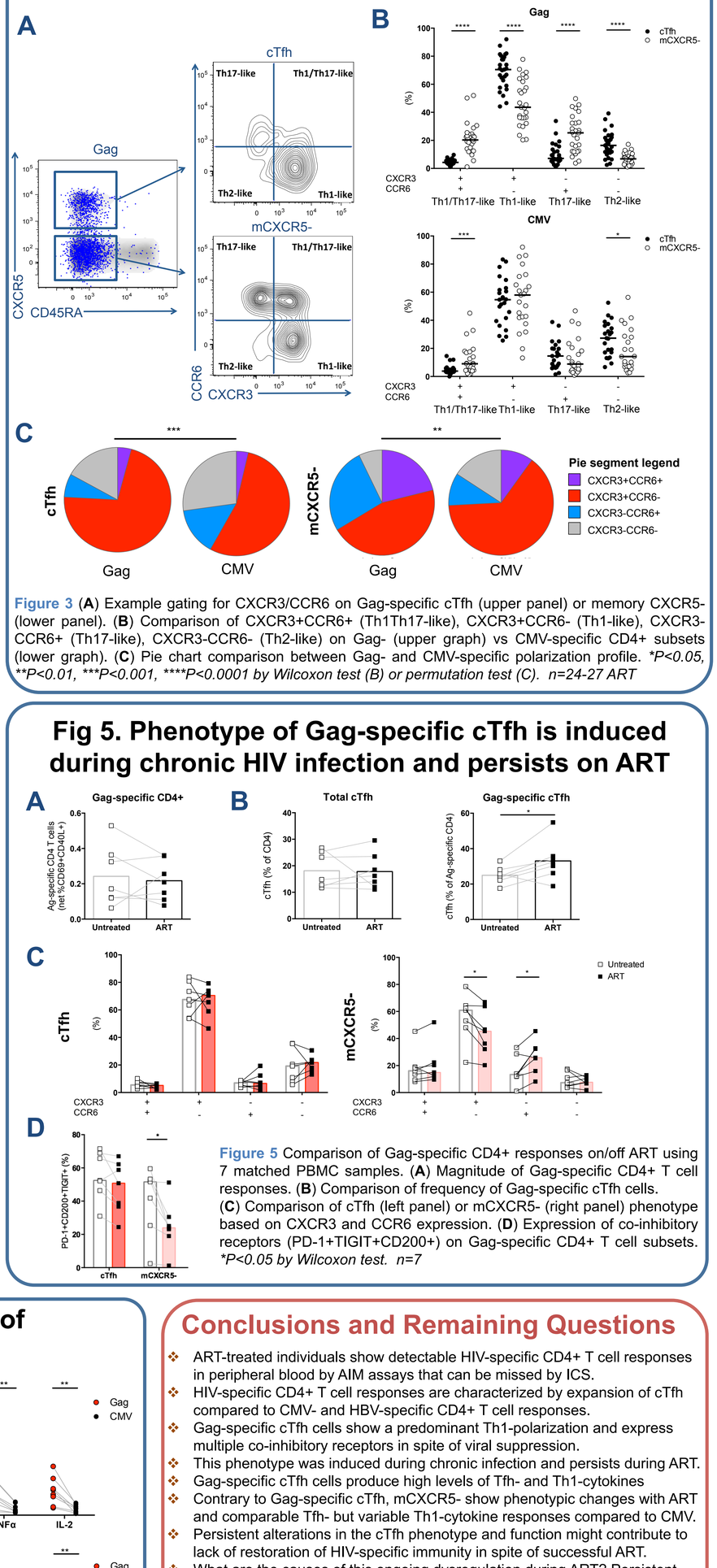


Figure 3 (**A**) Example gating for CXCR3/CCR6 on Gag-specific cTfh (upper panel) or memory CXCR5- (lower panel). (**B**) Comparison of CXCR3+CCR6+ (Th1Th17-like), CXCR3+CCR6- (Th1-like), CXCR3-CCR6+ (Th17-like), CXCR3-CCR6- (Th2-like) on Gag- (upper graph) vs CMV-specific CD4+ subsets (lower graph). (**C**) Pie chart comparison between Gag- and CMV-specific polarization profile. **P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 by Wilcoxon test (B) or permutation test (C). n=24-27 ART*

Fig 4. Gag-specific cTfh cells express multiple co-inhibitory receptors

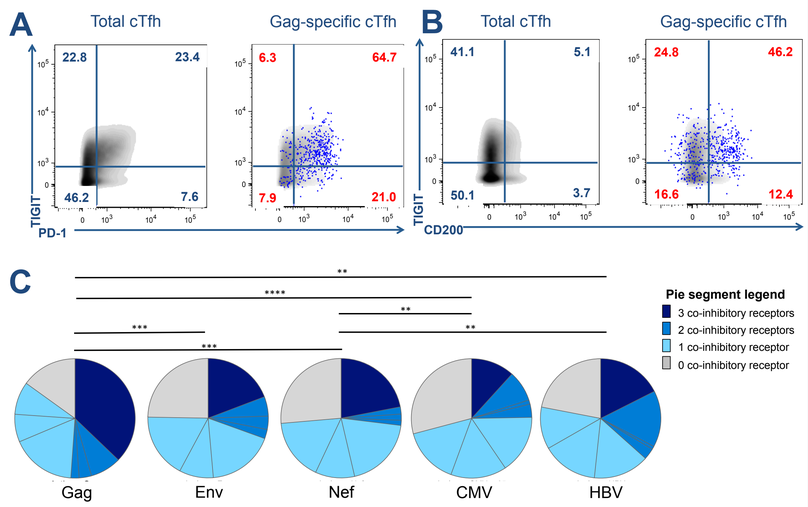


Figure 4 (**AB**) Example gating of TIGIT/PD-1 (A) or TIGIT/CD200 (B) expression on total cTfh (grey) or Gag-specific cTfh (blue overlay). (**C**) Comparison of co-inhibitory receptor coexpression on Ag-specific cTfh cells. Only subjects with an Ag-specific response greater than 2-fold over unstimulated were included. ***P<0.01, ***P<0.001, ****P<0.0001 by Permutation test. n=6-27 ART*

Fig 5. Phenotype of Gag-specific cTfh is induced during chronic HIV infection and persists on ART

Figure 5 Comparison of Gag-specific CD4+ responses on/off ART using 7 matched PBMC samples. (**A**) Magnitude of Gag-specific CD4+ T cell responses. (**B**) Comparison of frequency of Gag-specific cTfh cells.
(**C**) Comparison of cTfh (left panel) or mCXCR5- (right panel) phenotype based on CXCR3 and CCR6 expression. (**D**) Expression of co-inhibitory receptors (PD-1+TIGIT+CD200+) on Gag-specific CD4+ T cell subsets. **P<0.05 by Wilcoxon test. n=7*

Fig 6. Gag-specific cTfh produce high levels of Tfh- and Th1-cytokines

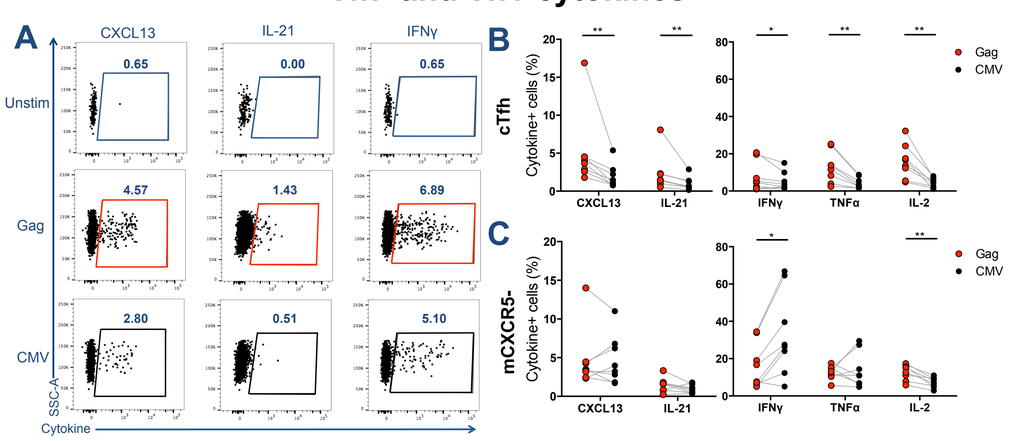


Figure 6 (**A**) Example plots showing expression of CXCL13, IL-21 or IFNγ in CD69+CD40L+ cTfh cells. (**BC**) Frequency of cytokine+ cells for antigen-specific cTfh (B) or memory CXCR5- cells (C). **P<0.05, **P<0.01 by Wilcoxon test. n=8*

Conclusions and Remaining Questions

- ART-treated individuals show detectable HIV-specific CD4+ T cell responses in peripheral blood by AIM assays that can be missed by ICS.
- HIV-specific CD4+ T cell responses are characterized by expansion of cTfh compared to CMV- and HBV-specific CD4+ T cell responses.
- Gag-specific cTfh cells show a predominant Th1-polarization and express multiple co-inhibitory receptors in spite of viral suppression.
- This phenotype was induced during chronic infection and persists during ART.
- Gag-specific cTfh cells produce high levels of Tfh- and Th1-cytokines
- Contrary to Gag-specific cTfh, mCXCR5- show phenotypic changes with ART and comparable Tfh- but variable Th1-cytokine responses compared to CMV.
- Persistent alterations in the cTfh phenotype and function might contribute to lack of restoration of HIV-specific immunity in spite of successful ART.
- What are the causes of this ongoing dysregulation during ART? Persistent antigen? Hardwired alterations that are not reversible by ART?

References

1 – Crotty et al., Annual Review of Immunology, 2013
2 – Miles et al., Frontiers in Immunology, 2016
3 – Cubas et al., J of Immunology, 2015
4 – Reiss/Baxter et al., PlosOne, 2017

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