264. Characterization of Pre-Existing HIV-Specific CD4 T Cells in Uninfected Individuals

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Background

- The magnitude of CD4 T cell responses to a given HIV/SIV immunogen can be highly variable in humans and NHPs, but determinants poorly known
- Class II tetramers for common pathogen epitopes in seronegative donors identify cross-reactive CD4 T cells; >50% of these CD4 T cells have a memory phenotype (*Su/Davis, Immunity 2013*)
- HIV-specific lines derived from naïve and memory subsets of seronegative donors (after stimulation with PHA/high IL-2/allogeneic PBMCs) frequently target epitopes that are immunodominant in HIV+ subjects *(Campion/Sallusto JEM 2014)*
- These two studies show frequent cross reactivity between the viral sequences investigated and bacterial sequences from the commensal flora

Hypotheses

- A pre-immune repertoire of HIV cross-reactive CD4 T cells can be identified in a subpopulation of infected donors
- This cross-reactive subset contains functional differentiated memory CD4 T cells
- This pre-existing subset may influence antigen-specific responses upon vaccination or infection

Objectives

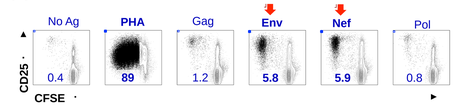
- Define the prevalence and specificity of HIV-cross reactive CD4 T cell responses in uninfected donors
- Determine whether HIV cross-reactive CD4 T cells in uninfected donors target epitopes that are also frequently recognized in infected subjects
- Determine the differentiation profile and functional characteristics of these CD4 T cells compared to HIV-specific Thelper responses in HIV-infected individuals

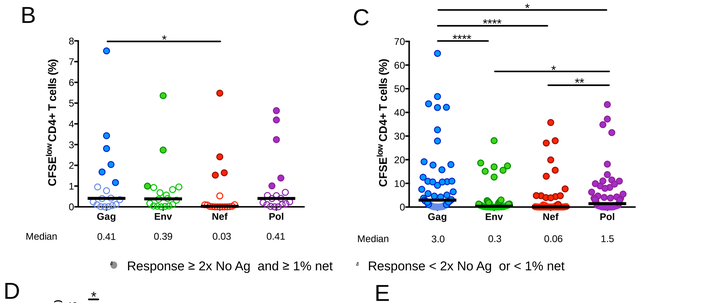
Study population

	UNINFECTED DONORS (UD)	HIV-INFECTED INDIVIDUALS (HI)
Subjects studied (n)	18	53
Gender (n male/ n female)	14/4	49/4
[male % - female %]	78% - 22%	92% - 8%
Age (median years)	47	47
[range]	[22-70]	[19-70]
CD4 T cell count (median cell/mm^3)		501
[range]		[123-1110]
Viral load (median vRNA copies/mL)		<40
[range]		<40 - >1,000,000

Proliferative responses to HIV antigens can be detected in some uninfected donors

Figure 1. CFSE proliferative responses to HIV peptide pools. **A)** example and **C**) summary data on uninfected (UD) subjects; **C**) summary results on HIV-seropositive (HI) participants. **DE**) Comparison of the HIV antigens immuodominance patterns in UD and HI subjects.



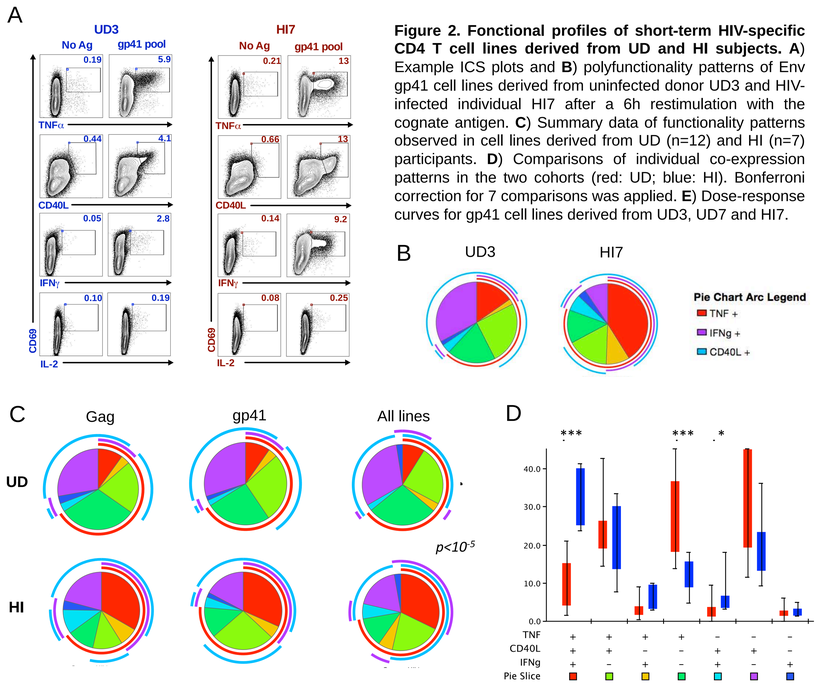


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Contingency Table	Uninfected Donors	HIV+ Individuals
Gag immunodominance	4 (22%)	30 (57%)
Non-Gag immunodominance	14 (78%)	23 (43%)
Total	N=18	N=53

Fisher's exact test: p=0.0146

HIV-specific CD4 T cell lines derived from uninfected donors and infected individuals present distinct functionality patterns



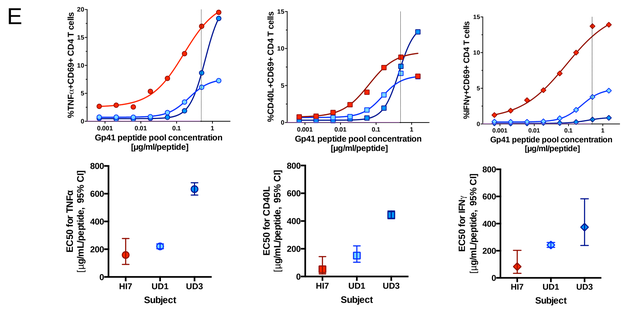


Figure 2. Fonctional profiles of short-term HIV-specific CD4 T cell lines derived from UD and HI subjects. A) Example ICS plots and **B**) polyfunctionality patterns of Env gp41 cell lines derived from uninfected donor UD3 and HIV-infected individual HI7 after a 6h restimulation with the cognate antigen. **C**) Summary data of functionality patterns observed in cell lines derived from UD (n=12) and HI (n=7) participants. **D**) Comparisons of individual co-expression patterns in the two cohorts (red: UD; blue: HI). Bonferroni correction for 7 comparisons was applied. **E**) Dose-response curves for gp41 cell lines derived from UD3, UD7 and HI7.

Uninfected donors share some immunodominant gp41 epitopes with HIV-infected individuals

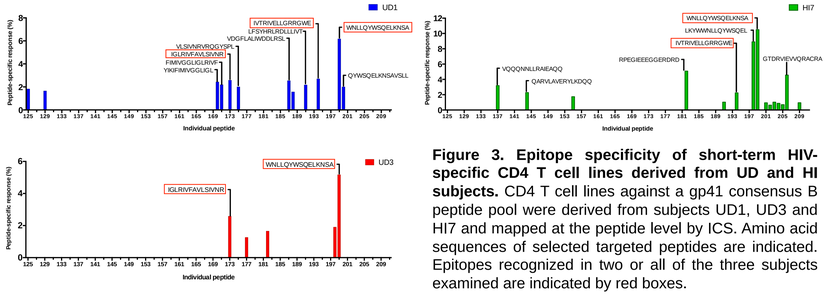


Figure 3. Epitope specificity of short-term HIV-specific CD4 T cell lines derived from UD and HI subjects. CD4 T cell lines against a gp41 consensus B peptide pool were derived from subjects UD1, UD3 and HI7 and mapped at the peptide level by ICS. Amino acid sequences of selected targeted peptides are indicated. Epitopes recognized in two or all of the three subjects examined are indicated by red boxes.

Detection of HIV cross-reactive CD4 T cells in uninfected donors by activation-induced marker assays

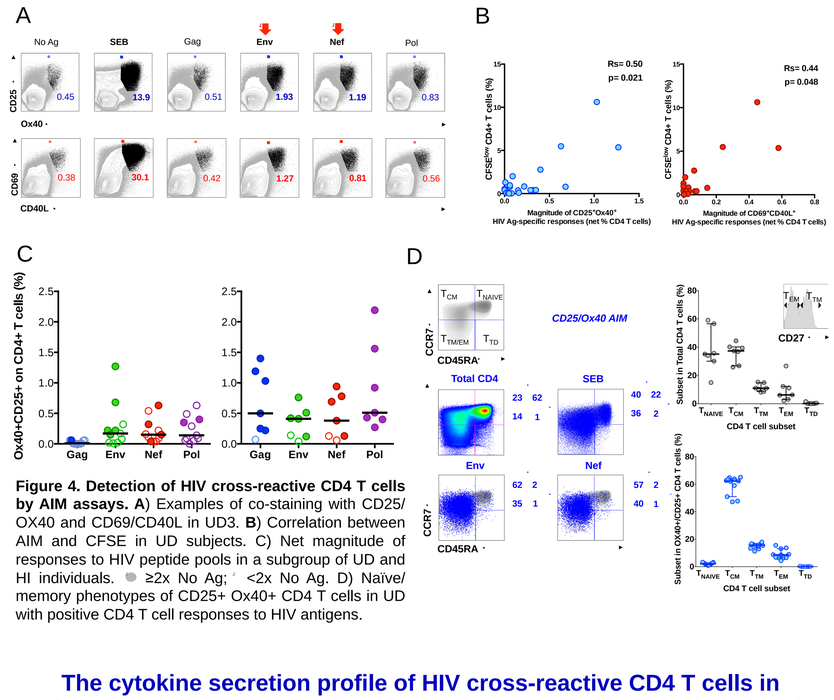


Figure 4. Detection of HIV cross-reactive CD4 T cells by AIM assays. A) Examples of co-staining with CD25/ OX40 and CD69/CD40L in UD3. **B**) Correlation between AIM and CFSE in UD subjects. C) Net magnitude of responses to HIV peptide pools in a subgroup of UD and HI individuals. ● ≥2x No Ag; ○ <2x No Ag. D) Naïve/ memory phenotypes of CD25+ Ox40+ CD4 T cells in UD with positive CD4 T cell responses to HIV antigens.

The cytokine secretion profile of HIV cross-reactive CD4 T cells in uninfected donors differs from the pattern observed in HIV+ individuals

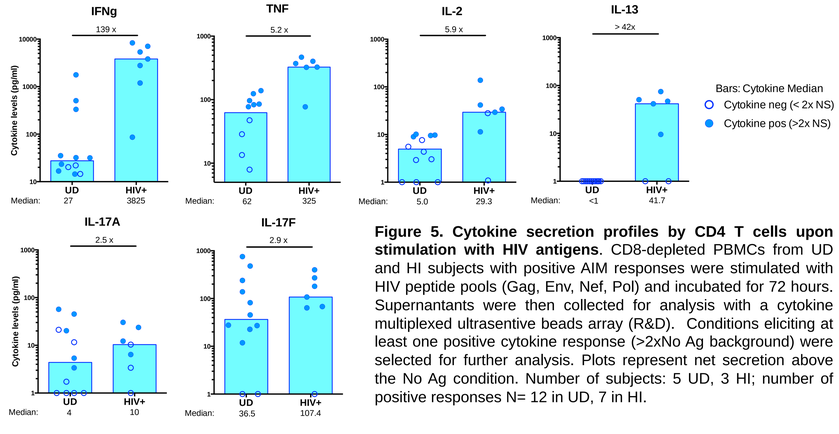


Figure 5. Cytokine secretion profiles by CD4 T cells upon stimulation with HIV antigens. CD8-depleted PBMCs from UD and HI subjects with positive AIM responses were stimulated with HIV peptide pools (Gag, Env, Nef, Pol) and incubated for 72 hours. Supernantants were then collected for analysis with a cytokine multiplexed ultrasentive beads array (R&D). Conditions eliciting at least one positive cytokine response (>2xNo Ag background) were selected for further analysis. Plots represent net secretion above the No Ag condition. Number of subjects: 5 UD, 3 HI; number of positive responses N= 12 in UD, 7 in HI.

Conclusions

- Cross-reactive CD4 T cells against HIV-antigens are frequent in uninfected donors, including against Env epitopes
- They can be detected by assays that show higher sensitivy than previously used techniques
- Also in contrast to previous studies, the large majority of thes cross-reactive CD4s are memory cells (-> they have already undergone lineage commitment)
- Functions compared to HIV+: proliferative capacity frequently detected, limited cytokine secretion, relative preservation of IL-17A/F production
- *Can these responses influence CD4 T cell responses to subsquent HIV vaccine administration or HIV infection?*

References

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