Regulatory T-cell activity but not conventional HIV-specific T-cell responses are associated with protection from HIV-1 infection.

Laura Pattacini[1], Jared M. Baeten[2,3,4], Katherine K. Thomas[2], Tayler R. Fluharty[1], Pamela M. Murnane[2,3], Deborah Donnell[2,5], Elizabeth Bukusi[2,6,7], Allan Ronald[8], Nelly Mugo[2,9], Jairam R. Lingappa[2,4,10], Connie Celum[2,3,4], M. Juliana McElrath[1,2,4], Jennifer M. Lund[*,1,2], for the Partners PrEP Study Team

[1] Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, U.S.A.
[2] Department of Global Health, University of Washington, Seattle, WA 98195, U.S.A.
[3] Department of Epidemiology, University of Washington, Seattle, WA 98195, U.S.A.
[4] Department of Medicine, University of Washington, Seattle, WA 98195, U.S.A.
[5] Statistical Center for HIV-1/AIDS Research and Prevention, FHCRC, Seattle, WA 98109, U.S.A.
[6] Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya
[7] Department of Obstetrics and Gynecology, University of Washington, Seattle, WA 98195, U.S.A.
[8] Department of Medical Microbiology, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
[9] Centre for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya
[10] Department of Pediatrics, University of Washington, Seattle, WA 98195, U.S.A.

FRED HUTCH
CURES START HERE™

Background: HIV-1-exposed seronegative (HESN) individuals provide a relevant model to identify correlates of protection from HIV-1 infection. Currently, there are two main non-exclusive mechanisms to explain the reason for protection from HIV-1 infection in HESN: a status of immune quiescence and the presence of HIV-1-specific immune responses.

Methods: Within a prospective cohort among African male and female HESN who were at high risk of HIV infection as a result of having an HIV infected heterosexual partner, we conducted a nested case-control study to assess immune responses among 179 subjects, including 30 who acquired HIV (cases) and 149 who remained HIV uninfected (controls). Peripheral blood mononuclear cells were collected prospectively, prior to HIV acquisition. All samples were obtained from individuals participating in the Partners PrEP Study (ClinicalTrials.gov number NCT00557245).

Results: We detected a significantly higher percentage of Tregs in controls, with the frequency of Tregs among CD3+ T-cells being 3.6%, as compared to cases, with 3.1% Tregs (p=0.04). No statistically significant differences in Treg activation were observed between controls and cases, as measured by CD39, CTLA-4, and ICOS expression.

As expected, we observed that Treg frequency inversely correlated with CD4+ and CD8+ T-cell activation as measured by co-expression of CD38 and HLA-DR (both p<0.0001). We next examined the association between Treg frequency and T-cell maturation and found Treg frequency to be directly correlated with CD4+ central memory T-cells (p<0.0001) and inversely with CD4+ effector memory T-cells (p=0.02).

When using intracellular cytokine staining to compare frequency, magnitude and breadth of HIV-specific T-cell responses we found no significant difference between controls and cases.

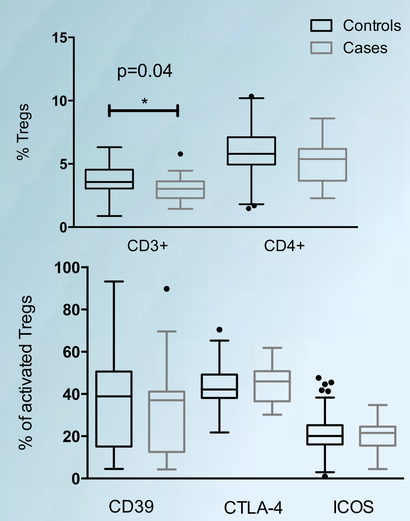
Conclusion: Our findings support the hypothesis that low immune activation assists in protection from HIV-1 infection.

Characteristics of cases and controls.

Characteristic	N (%) or median (IQR)	
	Cases (N=30)	Controls (N=149)
Female	14 (47)	51 (34)
Age, years	29 (25, 36)	29 (24, 36)
Number of sex acts, prior month	6 (3, 9)	4 (3, 8)
Any unprotected sex, prior month	14 (48)	72 (49)
Assigned active PrEP (vs. placebo)	14 (16)	73 (76)
Baseline CD4+ T-cell count of HIV-1 infected partner, cells/μL	466 (390, 676)	550 (391, 664)
Baseline plasma HIV-1 RNA concentration of HIV-1 infected partner, log_{10} copies/mL	4.4 (3.9, 4.7)	4.5 (3.8, 4.9)
Partnership characteristics		
Risk score (Kahle et al., 2013)	6 (4, 7)	6 (5, 7)

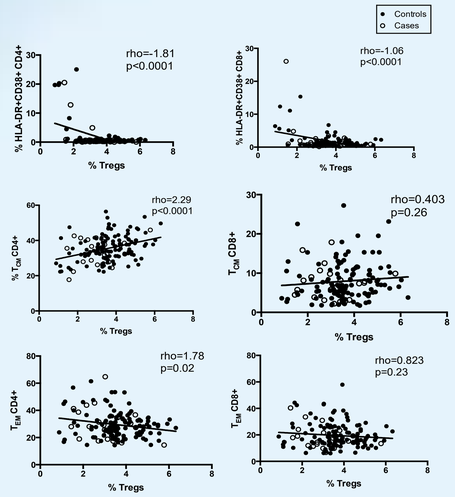
- Controls were determined at enrollment to be at particularly elevated risk of HIV acquisition (5 or higher based on a risk score optimized for serodiscordant couples). Samples tested from controls were at 12 months into the study.
- Samples tested from the cases were the last available PMBC before the participant became infected and were collected a median of 183 days (interquartile range 28-578) before HIV infection.

Regulatory T cell (Treg) frequency is higher in controls as compared to cases



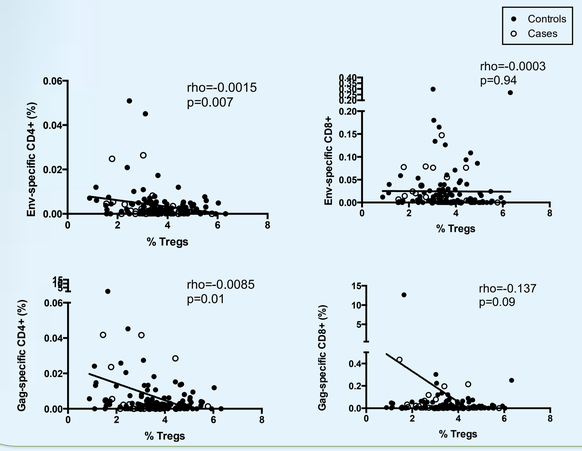
Frequency of Tregs, identified as CD3+ CD4+ CD25hi CD127dim FoxP3+ cells, were quantified by ex-vivo detection of specific markers by flow cytometry.

Treg frequency correlates negatively with T cell activation and positively with the frequency of central memory (T_{CM}) CD4+ T cells



Correlation plot and linear regression lines showing the associations between Treg frequency and T-cell activation frequency (top); Treg and Central Memory T-cell (T_{CM}) frequencies (center) Treg and Effector Memory T-cells (T_{EM}) frequencies (bottom). CD4+ T-cells are shown on the left part of the figure and CD8+ T-cell on the right.

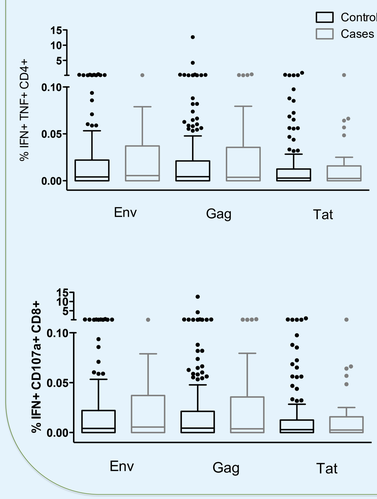
Treg frequency correlates negatively with HIV-specific CD4+, but not CD8+ T cell responses



Correlation plot and linear regression lines showing the associations between Treg frequency and magnitude of HIV-specific responses to Env (top) or Gag (bottom), with CD4+ T-cells shown on the left and CD8+ T-cells on the right.

HIV-1-specific T-cell responses are comparable in cases and controls

Cell type	Cell Markers	Peptide pool	Frequency* Cases	Frequency* Controls	OR (95% CI)**	p-value
CD8+	IFN-g⁺CD107a	Gag	5 (17.2) [n=29]	10 (6.8) [n=146]	2.8 (0.7-10.0)	0.16
		Env	3 (11.1) [n=27]	15 (10.6) [n=142]	1.1 (0.2-4.2)	>0.99
		Tat	2 (7.7) [n=26]	7 (5.3) [n=131]	1.5 (0.1-8.5)	0.91
		Any	5 (17.2) [n=29]	18 (12.2) [n=147]	1.5 (0.4-4.7)	0.64
CD4+	IFN-g⁺TNF-a	Gag	1 (3.4) [n=29]	5 (3.4) [n=146]	1.0 (0.0-9.5)	>0.99
		Env	0 (0.0) [n=27]	4 (2.8) [n=142]	1.0 (0.0-5.9)	0.99
		Tat	0 (0.0) [n=26]	3 (2.3) [n=131]	1.3 (0.0-8.8)	>0.99
		Any	1 (3.4) [n=29]	8 (5.4) [n=147]	0.6 (0.0-5.0)	>0.99



- HIV-specific immune responses were detected by intracellular cytokine staining using global potential T-cell epitope 11mer peptide pools for Gag, Env, and Tat, with positive responses defined as dual production of IFN–γ and CD107a for CD8+ T-cell responses, IFN-γ and TNF-α for CD4+ T-cell responses, and IFN-γ and CD107a for NK responses. Logistic regression was used to assess if the frequency and magnitude of immune responses were associated with protection from HIV acquisition.

Conclusions

- **Increased Treg frequency correlates with protection from HIV-1 infection.**
- **HIV-1 specific T-cell responses in blood of HESN do not protect from HIV-1 acquisition.**
- **Tregs favor the formation of central over effector memory CD4+ T-cells.**

Funding : NIAID/NIH 1R01AI096968 and Bill and Melinda Gates Foundation (Grant ID# 47674)