Evolution of HIV-specific CD8+ T cell responses in hyperacute HIV infection

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Summary

To determine the relationship between onset of viremia and T cell immunity in a chronic human viral infection, we performed twice-weekly plasma HIV RNA screening of high-risk uninfected women as part of a comprehensive prevention and empowerment program. Twelve hyperacute infections were identified, with a median initial viral load of $1.8*10^3$ RNA copies/ml. Onset of viremia induced vigorous CD8+ T cell activation from a naïve precursor pool, peaking as high as 77%, with minimal bystander activation. These briskly proliferating cells exhibited potent HIV-specific cytotoxicity as measured by degranulation in response to infected cells, but underwent rapid apoptosis and failed to upregulate the IL-7 receptor involved in T cell survival. The rapidity to peak CD8+ T cell activation and absolute magnitude of activation were both inversely correlated with set point viremia, indicating that the initial dynamics of HIV-specific CD8+ T cells following onset of viremia modulate subsequent viral control.

Study setup and sample collection

Phase I: Surveillance

- Twice weekly HIV RNA PCR testing via finger stick blood draws
- Quarterly blood and mucosal sampling of the female genital track

Phase II: Acute Infection

Sample collection schedule in weeks

Blood draws: 0,1,2,3,4,5,6..8 → 12..24..36..48 → 100……152

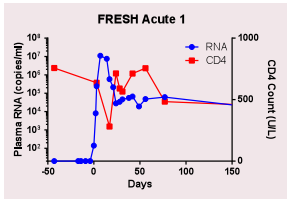


Figure 1: Dynamics of absolute CD4 count and plasma viral loads during early HIV infection

Experimental setup

Longitudinal analysis of HIV induced CD8+ T cell responses

Recently activated (CD38+HLA DR+) CD8+ T cells

Recently stimulated cycling (BCL2low, Ki67+) CD8+ T cells

HIV-specific CD8+ T cells (Elispot, ICS and Tetramer staining)

Results

Massive activation of CD8+ T cells during acute HIV infection

- Massive activation of CD8+ T cells during hyperacute HIV infection **(fig. 2a,b)**
- Greater time to peak activation positively correlate with higher viral load set point. **(fig. 2c)**
- The frequency of activated (CD38+HLA DR+) CD8+ T cells at peak activation inversely correlate with HIV viral set point. **(fig . 2d)**

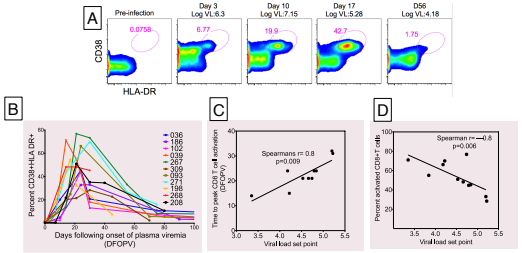


Figure 2: (a) FACS plots show CD8+ cells gated on CD38 and HLA-DR double positive populations. **(b)** Dynamics of activated (CD38+HLA-DR+) CD8+ cells for 11 donors. **(c)** Correlation between time (in days) to peak activation and viral load setpoint. **(d)** Correlation between frequency of activated CD8+ cells at peak activation and viral load set point.

CD8+ T cell proliferation and apoptosis following onset of plasma viremia

Massive proliferation of proapoptotic CD8+ T cells following during hyperacute HIV infection

The frequency of proliferating ($BCL2_{low}$ $Ki67_{high}$) CD8+ T cells at peak activation inversely correlated with HIV viral set point

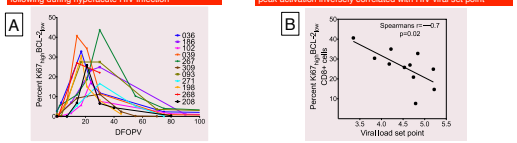


Figure 3: (a) Dynamics of proliferating/proapoptotic (CD38+HLA-DR+) CD8+ cells for 11 donors. **(b)** Correlation between frequency of proliferating CD8+ cells at peak activation and viral load set point.

Evolution of HIV-specific CD8+ T cell responses during acute HIV infection

HIV-specific CD8+ T cell responses are detectable within 1 to 3 days following onset of plasma viremia

HIV-specific (tetramer+) CD8+ T cells have a diminished capacity to secrete IFN-γ during peak activation

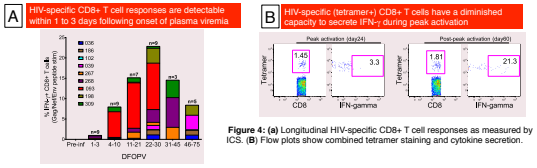


Figure 4: (a) Longitudinal HIV-specific CD8+ T cell responses as measured by ICS. **(B)** Flow plots show combined tetramer staining and cytokine secretion.

Acute HIV induces strong HIV-specific CD8+ T cells with degranulation potential

HIV-specific CD8+ T cells mobilized CD107a upon stimulation with HIV infected CD4+ T cells

CD107 responses are significantly greater than IFN-γ, TNF-a, and IL-2 responses

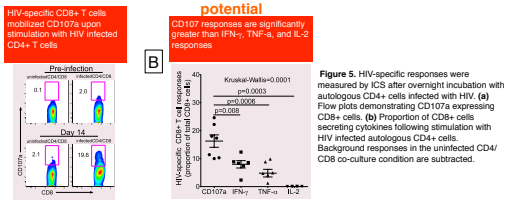
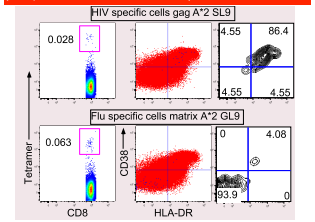


Figure 5. HIV-specific responses were measured by ICS after overnight incubation with autologous CD4+ cells infected with HIV. **(a)** Flow plots demonstrating CD107a expressing CD8+ cells. **(b)** Proportion of CD8+ cells secreting cytokines following stimulation with HIV infected autologous CD4+ cells. Background responses in the uninfected CD4/CD8 co-culture condition are subtracted.

Limited CD8+ T cells bystander activation during acute HIV infection

Figure 6: Intra-donor activation (CD8+ CD38+ HLA-DR+ cells) profiles of HIV-specific (A*0201 SL9) and influenza virus specific (A*0201 GL9 matrix protein) in a donor with detectable tetramer responses for influenza and HIV



Apoptosis, not exhaustion causes rapid loss of HIV-specific CD8+ T cells during hyperacute HIV infection

- No significant increase in PD-1 during hyperacute HIV infection until after contraction of responses. **(Fig 7a).**
- Activated cells express decreased levels of BCL-2, high levels of CD95, a potent inducer of apoptosis and fail to upregulate CD127, which is important for cell survival **(Fig 7b).**
- Tetramer staining show that HIV-specific cells were selectively more susceptible to apoptosis compared to bystander (CMV-specific) CD8+ T cell responses **(Fig 7c).**

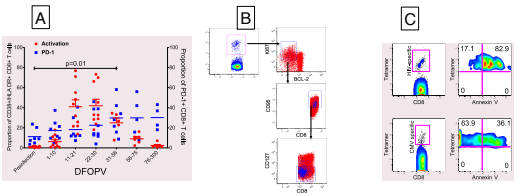


Figure 7. (a) Longitudinal analysis of CD38/HLA-DR dual expression and PD-1 expression on CD8+ cells after HIV infection. **(b)** Phenotypic analysis of HIV specific tetramer+ CD8+ cells. Data representative of one of the five donors tested are shown. **(c)** *Ex vivo* expression of annexin V by HIV tetramer+ and CMV tetramer+ cells are shown in upper right quadrants of the flow plots.

Conclusions

- Acute HIV infection stimulates a vigorous CTL response
- Magnitude of the initial CTL response is larger than previously thought
- A large fraction of HIV induced activated CD8+ T cells are HIV-specific
- Initial response fails to upregulete critical molecules for long-term survival
- The timing and strength of the initial CTL response impact early HIV replication

Acknowledgments

We would like to thank the FRESH participants, as well as the FRESH and HPP laboratory staff. This work was supported by the Bill and Melinda Gates Foundation, the Witten Family Foundation, Dan and Marjie Sullivan, the Mark and Lisa Schwartz Foundation, the International AIDS Vaccine Initiative (IAVI) (UKZNRSA1001), the NIAID (R37AI067073), an NIH funded Center for AIDS Research (P30 AI060354), which is supported by the following NIH Co-Funding and Participating Institutes and Centers:IAID, NCI, NICHD, NHLBI, NIDA, NIMH, NIA, FIC, and OAR, and the Howard Hughes medical Institute

Presented at CROI 2015 – Seattle, Washington, USA