The breadth of expandable central memory CD8+ T cells (Tcm) inversely correlates with residual viral loads in HIV elite controllers

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Introduction
Previous studies have shown that elite controllers with minimal effector T cell responses harbor a highly functional, broadly directed central memory (Tcm) population. Here, we investigated the in vivo relevance of this cell population by investigating whether the breadth of expandable Tcm is associated with the magnitude of residual viremia in individuals achieving durable suppression of HIV infection. HIV-specific memory CD8+ T cells were expanded using autologous epitopic and variant peptides. Viral load was measured by an ultrasensitive single-copy PCR assay. Controls showed a greater increase in the overall breadth of Gag responses compared to untreated progressors (p=0.02) as well as treated progressors (p=0.03). Nef and Env memory cells expanded poorly for all groups and their breadth was indistinguishable between the groups (Nef: Kruskal-Wallis=0.003; Env: Kruskal-Wallis=0.05). More importantly, we show that the breadth of Gag-specific Tcm responses is inversely correlated with residual viral load (p=0.05, p=0.02). Together these data reveal a direct link between the abundance of Gag-specific Tcm and prolonged maintenance of low-level viremia. Our studies highlight a CD8+ T cell feature that would be desirable in a vaccine induced T cell response.

Materials and methods
- **Study subjects:** HIV-infected individuals were recruited from outpatient clinics at the Massachusetts General Hospital and affiliated Boston area hospitals.
- A total of 20 elite controllers, 15 untreated chronic progressors and 16 treated chronic progressors were studied.
- **Cultured IFN-γ ELispot:** Peptide-stimulated and unstimulated cells were cultured at 37°C, 5% CO2 for 12 days in RPMI media. ELispot assays were performed using a final concentration of 100ng/ml (20ng in 200µl volume) of overlapping peptides (OLPs) averaged 16 amino acids in length and overlapped by 10 amino acids) spanning the entire HIV gag, nef and env proteins.
- **Virus infection assay:** virally infected CD4+ T cells were incubated in the presence or absence of expanded CD8+ T cells at an adjusted 1:1 ratio to target cells. The number of input CD8+ T cells for each cell culture was adjusted based on the frequency of IFNγ secreting cells. p24 antigen quantification was done by ELISA (PerkinElmer, Boston, MA). Log inhibition values were calculated by subtracting log_{10} p24 values with CD8+ T cells from log_{10} p24 values without CD8+ T cells at day 7.
- **Intracellular cytokine staining and tetramer staining:**
- HIV RNA determination by single copy assay: PCR amplification and sequencing of the HIV gag region was performed for each sample. For each specimen, three replicate reactions were performed for HIV quantification. The number of HIV copies was derived from the calculated number of copies per million of the starting plasma sample.

Experimental design

**Ex-vivo ELISPOT**
Enrichment-phenotyping of tetramer+ cells

Peptide stimulation for 12 days
Rest overnight

Cultured ELispot
CM breadth

Tetramer staining

Phenotypic and functional assays

![Diagram](image_url)

Expandable memory CD8+ T cell responses in elite controllers are predominantly directed against Gag

**Figure 1**

The breadth of HIV-specific memory CD8+ T cells have an inverse correlation with residual plasma viral load in HIV elite controllers

**Figure 3**

Elite controllers maintain a large pool of type-specific expandable memory responses

**Figure 4**

![Diagram](image_url)

Conclusions
- **Elite controllers with minimal effector T cell responses harbor a highly functional broadly directed central memory (Tcm) population.
- The breadth of Gag-specific Tcm responses is inversely correlated with residual viral load.
- Maintenance of larger pool of HIV-specific Tcm for a wide range of viral variants is another feature associated with sustained virus suppression.
- Overall, immune mediated viral suppression in chronic HIV infection is an active and ongoing process.

Literature cited


Acknowledgments
We thank Dr. Thams N‟Mungo and Filippus Pichocki for their input in this study. The cohort was funded by the International HIV Cure Collaborative and the MIT HIV Cure Collaborative.org; BHF and Malala Guses Foundation, the AIDS Healthcare Foundation and the Harvard University Center for AIDS Research (CFAR) and NIH funded project (U01 AI003154).

Presented at AIDS 2014 – Melbourne, Australia