Eomes and T-bet Are Differentially Linked to CD8+ T Cell Exhaustion in HIV Infection

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Poor HIV-specific CD8+ T cell functionality during chronic HIV infection is linked to differential expression of T-bet and Eomes. These characteristics are not reversed despite long-term ART, which partly helps to explain the inability of HIV-specific CD8+ T cells to control viral replication post-ART cessation.

Introduction

- Murine studies have shown that the process of memory formation is highly regulated by the T-box transcription factors T-bet and Eomesodermin (Eomes).
- T-bet regulates the expression of effector functions whereas Eomes is thought to primarily dictate the expression of proteins to maintain a memory CD8+ T cell repertoire.
- Human immunodeficiency virus type 1 (HIV) evades the immune defense and develops into a chronic infection. As a consequence, HIV-specific CD8+ T cells persist throughout the infection and become dysfunctional (exhausted).
- It remains unclear whether a transcriptional link exists between the regulation of CD8+ T cell differentiation and exhaustion in humans.

Aims

To conduct a multi-parametric study to elucidate whether T-bet and Eomes were linked to exhaustion of human viral-specific CD8+ T cells.

Material and methods

- 52 untreated HIV infected individuals, 12 individuals on long-term antiretroviral therapy (ART) and 20 healthy controls were recruited from the HIV clinics in Stockholm, Sweden.
- PBMCs were collected and flow cytometry was used to assess the frequencies of all immunological markers. The samples were acquired on a LSR Fortessa and data analysis was conducted in FlowJo.
- GraphPad Prism and SPICE analysis were conducted to determine differences between groups. All experimental variables between two groups of individuals were analyzed using Mann-Whitney U test and Wilcoxon matched-pairs rank test or between 3 groups by Kruskal-Wallis test. Scatterplots and bars show median and IQR.

Results

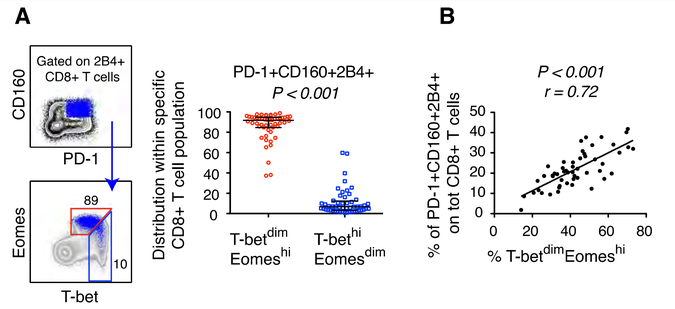


Figure 1. T-bet^{dim}Eomes^{hi} CD8+ T cells express high levels of inhibitory receptors. **(A)** Gating strategy to distinguish the localization of PD-1+CD160+2B4+ CD8+ T cells within the T-bet/Eomes axis. Scatter plot shows the distribution of PD-1+CD160+2B4+ cells between the T-bet^{dim}Eomes^{hi} (red) and T-bet^{hi}Eomes^{dim} (blue) cells. **(B)** Correlation analysis between the frequency of T-bet^{dim} Eomes^{hi} cells and PD-1+CD160+2B4+ CD8+ T cells.

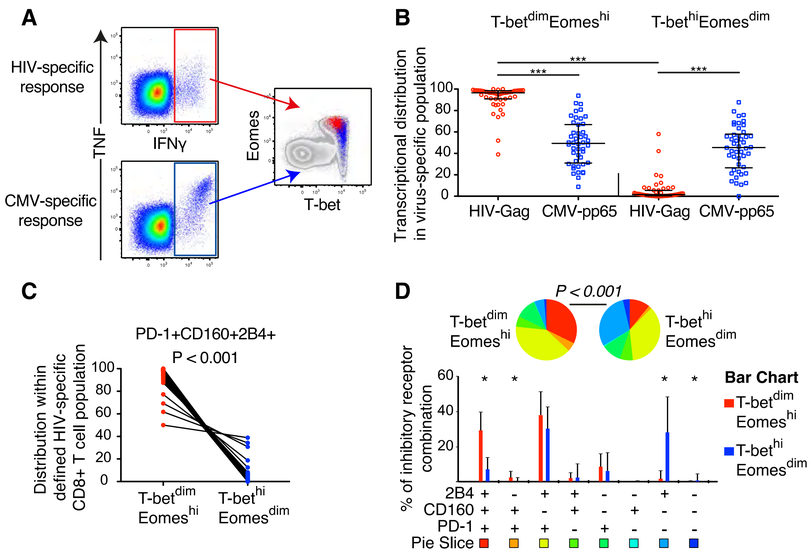


Figure 2. Elevated expression of Eomes is a hallmark of HIV-specific CD8+ T cells with high expression of inhibitory receptors. **(A)** Gating strategy illustrating the localization of HIV-specific (red) and CMV-specific (blue) CD8+ T cells within the T-bet/Eomes axis. **(B)** Distribution within the T-bet^{dim} Eomes^{hi} and T-bet^{hi} Eomes^{dim} population of HIV- and CMV-specific CD8+ T cells. **(C)** Distribution of PD-1+CD160+2B4+ CD8+ T cells within the T-bet^{dim} Eomes^{hi} and T-bet^{hi} Eomes^{dim} populations. **(D)** SPICE analysis of combinations of inhibitory receptors on the T-bet^{dim}Eomes^{hi} (red) and T-bet^{hi} Eomes^{dim} (blue) populations. *** P < 0.001, * P < 0.05.

Conclusions

- Single- and co-expression of inhibitory molecules was associated with a transcriptional profile of T-bet^{dim}Eomes^{hi} in bulk- and HIV-specific CD8+ T cells
- Highly exhausted cells displayed a transitional memory phenotype with poor polyfunctional abilities for both HIV- and CMV-specific CD8+ T cells
- Elevated expression of inhibitory receptors and Eomes was maintained despite long-term ART

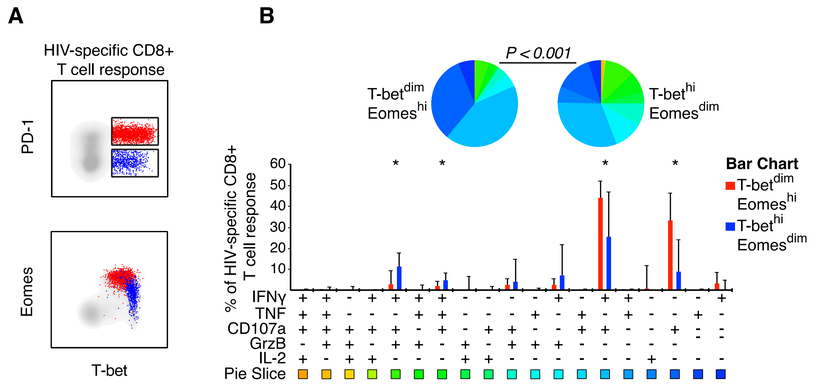


Figure 3. Limited virus-specific functionality is related to co-expression of inhibitory receptors and T-bet^{dim}Eomes^{hi} cells. **(A)** Distribution of PD-1+ (red) and PD-1- (blue) HIV-specific CD8+ T cells within the T-bet/Eomes axis. **(B)** SPICE analysis of all functional combinations between the T-bet^{dim}Eomes^{hi} (red) and T-bet^{hi}Eomes^{dim} (blue) population for HIV-specific CD8+ T cells. * P < 0.05.

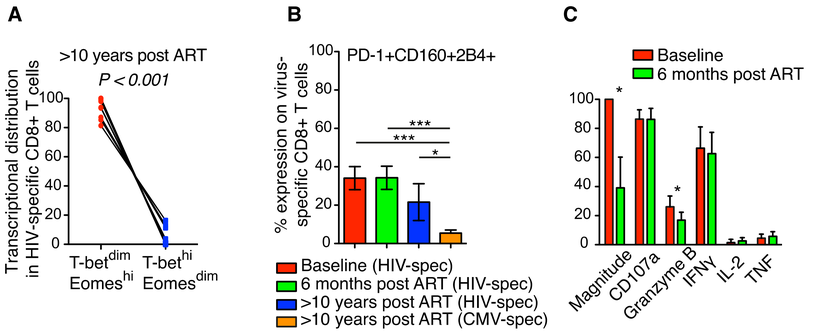


Figure 4. HIV-specific CD8+ T cells possess elevated expression of inhibitory receptors and Eomes despite long-term ART. **(A)** Distribution of HIV-specific CD8+ T cells within the T-bet^{dim}Eomes^{hi} and T-bet^{hi}Eomes^{dim} population after >10 years on ART (n=12). **(B)** Frequency of HIV-specific PD-1+CD160+2B4+ CD8+ T cells at baseline (red), 6 months (green), >10 years on ART (blue) and CMV-specific PD-1+CD160+2B4+ CD8+ T cells >10 years on ART (orange). **(C)** Frequency of each individual function (of total HIV-specific response) before (red) and 6 months after ART (green) (n = 12). *** P < 0.001, * P < 0.05.

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