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

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Results

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 all functional combinations between the T-betEomes (red) and T-betEomes (blue) population for HIV-specific CD8+ T cells. * P < 0.05.

Conclusions

- Single- and co-expression of inhibitory molecules was associated with a transcriptional profile of T-betdimEomes 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 1. T-betEomes 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-betEomes (red) and T-betEomes (blue) cells. (B) Correlation analysis between the frequency of T-betEomes 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-betEomes and T-betEomes population of HIV- and CMV-specific CD8+ T cells. (C) Distribution of PD-1+CD160+2B4+ CD8+ T cells within the T-betEomes and T-betEomes populations. (D) SPICE analysis of combinations of inhibitory receptors on the T-betEomes (red) and T-betEomes (blue) populations. *** P < 0.001, * P < 0.05.

Figure 3. Limited virus-specific functionality is related to co-expression of inhibitory receptors and T-betEomes 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-betEomes (red) and T-betEomes (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-betEomes and T-betEomes 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.

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.

Figure 4. HIV-specific CD8+ T cell exhaustion in human restimmunized individuals. (A) Distribution of HIV-specific PD-1hi2B4hiEomeshi cells within the T-betEomes axis. (B) SPICE analysis of all functional combinations between the T-betEomes (red) and T-betEomes (blue) population for HIV-specific CD8+ T cells. * P < 0.05.