CD8 T cell terminal differentiation and its regulation by DHEA in HIV-TB co-infection

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Background

Tuberculosis (TB) is the first cause of death among HIV+ patients. Decreased frequencies of terminal effector (TTE) CD8 T cells (CD8Tc) may increase the risk of TB reactivation in persons latently infected with *Mtb* (LTB) [1]. We have previously shown that dehydroepinadrosterone (DHEA) increases protective anti-tubercular immune responses in HIV-TB patients [2].

Aim

To explore the anti-tubercular function and effector/memory phenotype of CD8Tc during HIV-TB co-infection and their modulation by DHEA.

Abbreviations

CD8Tc: CD8 T cell; DHEA: dehydroepiandrosterone; HAART: highly active antiretroviral therapy; HD: healthy donor; LTB: latent tuberculosis infection; *Mtb: Mycobacterium tuberculosis;* Sp. r: Spearman´s rank correlation coefficient; TB: tuberculosis; TCM: central memory T cell; TEM: effector memory T cell; TN: neïve T cell; TST: tubrculin skin test; TTE: terminal effector T cell; *: p<0.05; **: p<0.01; ***: p<0.001.

Methods

Table 1. Carachteristics of the patients enrolled.

	HIV	TB	TST	#	Age (years)	female/male	CD4 count (cells/ul)	Viral Load (copies/ul)
HIV-TB	+	+	n.d.	47	38 (31-43)	21/26	93 (37-213)	39867 (3789-160732)
HIV-LTB	+	-	+	11	35 (28-40)	3/8	361 (261-650)	12934 (756-24590)
HIV	+	-	-	12	35 (32-48)	3/9	286 (141-452)	11225 (25-36581)
HD	-	-	n.d.	25	36 (30-46)	16/9	843 (607-1075)	n.d.

a. Median (interquartile range) is depicted.
b. HIV-TB: HIV-1+ patients with active TB within the frist week of anti-TB treatment; HIV-LTB: HIV-1+ patients with latent TB as determined by TST; HIV: HIV-1+ TST- patients; HD: healthy donors.
c. The present study has a cross-sectional design; Patients were evaluated at Hospital J.A. Fernández, Buenos Aires; No patient received DHEA or glucocorticoid treatment; Some HIV-1 infected individuals were on antiretroviral treatment following current guidelines [3]; The Ethics Committee from Fundación Huésped approved the study; Written informed consent was documented from all study subjects.
d. TST: tubrculin skin test; n.d.: not determined.

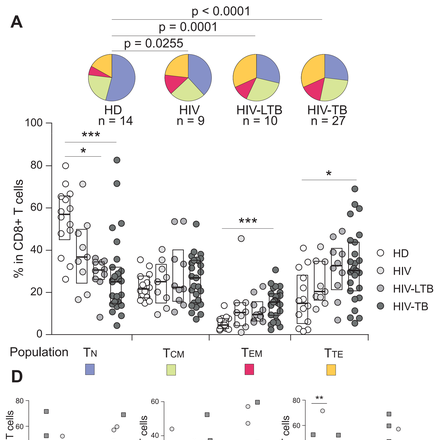


Figure 1. Effector/memory phenotype and transitions in total peripheral CD8 T cells. Recently thawed or freshly isolated PBMC were stained for CD45RA and CD27 expression in CD8 T cells (CD8Tc). **(A)** Cumulative analyses of CD27 and CD45RA expression on CD8Tc from HD, HIV, HIV-LTB and HIV-TB patients and **(B)** representative flow cytometry dot plot examples from an HD (top) and an HIV-TB patient (bottom) are shown. Pie charts summarize the data and each slice corresponds to the mean proportion of each phenotype in CD8Tc. **(C)** % of TTE vs. % of TCM or TEM in HIV-TB (left) and HD (right) subjects. Negative correlations evidence recent effector/memory transitions as described previously [4]. Continuous lines represent linear regression curves and dashed lines represent 95% confidence intervals. **(D)** Cumulative analyses of the expression of CD27 and CD45RA on CD8Tc from HIV-TB subjects stratified according to TB presentation (left), TB burden by smear status (middle) and highly active antiretroviral therapy (HAART, right). **(A, D)** Comparisons of phenotype distribution were performed using the partial permutation test as described [5] and the Kruskal-Wallis test followed by Dunns multiple comparisons post-test. For cumulative analysis, possible phenotypes are shown on the x-axis whereas percentages of distinct T-cell subsets within CD8Tc are shown on the y-axis. Each symbol represents an individual subject. Horizontal lines represent median values and boxes represent interquartile range.

Co-infection with HIV and TB induces a bias to CD8 T cell terminal differentiation (from both TEM and TCM subsets) which is not affected by TB localization or *Mtb* burden but is partially restored after HAART.

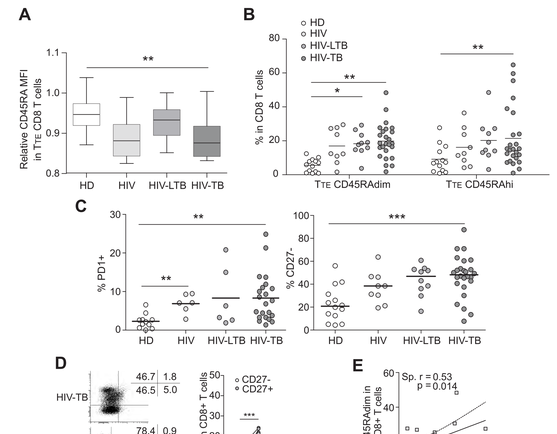


Figure 2. Phenotype of terminally differentiated CD8Tc. Recently thawed or freshly isolated PBMC were stained for the indicated molecules. **(A)** Analysis of the relative mean fluorescence intensity (MFI) of CD45RA in TTE CD8Tc cells as defined by the ratio: [CD45RA MFI on TTE / CD45RA MFI on TEM]. **(B)** % of CD45RAdim TTE and CD45RAhi TTE among CD8Tc, and **(C)** % of PD1+ (left) and CD27neg (right) cells among CD8Tc from HD (n=15), HIV (n=8), HIV-LTB (n=10) and HIV-TB (n=24) subjects. **(D)** Representative dot plots for the expression of CD27 vs. PD1 in total CD8Tc from an HIV-TB and an HD subject (left) and cumulative analysis of PD1 expression in CD27neg and CD27+ CD8Tc from HIV-TB and HD (right). Each line connects the data for an individual patient. Comparisons between the CD27+ and CD27neg populations were performed using the Wilcoxon matched pairs test. **(E)** Correlation analysis between the % of CD45RAdim TTE CD8Tc and the % of PD1 in CD27+/CD8Tc in HIV-TB patients. Continuous lines represent linear regression curves and dashed lines represent 95% confidence intervals. **(A-D)** Comparisons between groups were evaluated by the Kruskal-Wallis test followed by Dunns multiple comparisons post-test. Horizontal lines represent median values, boxes represent interquartile ranges and whiskers represent 95% confidence intervals. Each symbol represents an individual subject.

HIV-TB patients exhibit lower CD45RA expression in TTE CD8Tc and higher PD1 expression restricted to CD27+ CD8Tc, which seem to be associated.

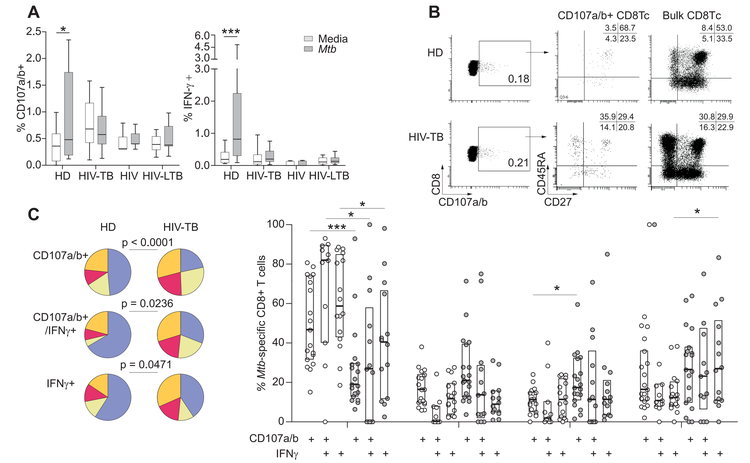
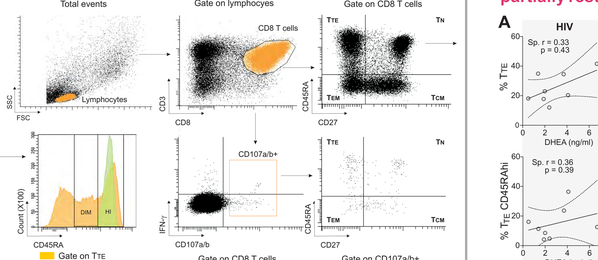


Figure 3. Effector/memory phenotype and transitions in *Mtb*-specific CD8 T cells. (A) Cumulative analyses of *Mtb*-induced (10 μg/ml) CD107a/b and IFN-γ expression in CD8Tc from HD (n=7), HIV (n=7), HIV-LTB (n=7) and HIV-TB (n=10) individuals after 16 h *in-vitro* stimulation. Comparisons between groups were performed using the Kruskal-Wallis test followed by Dunns multiple comparisons post-test. Whiskers represent 95% confidence intervals. **(B)** Representative dot plots of CD107a/b vs. CD8 expression in CD8Tc (left); CD27 vs. CD45RA in CD107a/b+ CD8Tc (middle) and CD27 vs. CD45RA in bulk CD8Tc (right) from an HD (top) and an HIV-TB patient (bottom). **(C)** Cumulative analysis of CD27 and CD45RA expression on *Mtb*-specific CD8Tc from HD and HIV-TB individuals after 3 days *Mtb*-stimulation (10 μg/ml). Comparisons of phenotype distribution were performed using the partial permutation test and the Kruskal-Wallis test followed by Dunns multiple comparisons post-test. Possible phenotypes are shown on the x-axis whereas percentages of distinct T-cell subsets within *Mtb*-specific CD8Tc are shown on the y-axis. Pie charts summarize the data and each slice corresponds to the mean proportion of *Mtb*-specific CD8Tc of each phenotype. **(D)** Correlation analysis between %TTE and % TN, TCM or TEM in CD107a/b+ CD8Tc were evaluated in HIV-TB (white background) and HD (grey background) subjects. Continuous lines represent linear regression curves and dashed lines represent 95% confidence intervals. **(A and C)** Horizontal lines represent median values and boxes represent interquartile range. **(C and D)** Each symbol represents an individual subject.

***Mtb*-specific CD8Tc responses are impaired during HIV infection. *Mtb*-specific CD8Tc in HIV-TB patients are biased to more differentiated stages independently of cell function.**



Gating strategy used for flow cytometry data analysis. Illustration data were derived from one representative HIV-TB subject stimulated with *Mtb*. Initial gating was performed on a dot plot of forward scatter (FSC) versus side scatter (SSC) to select small lymphocytes (top-left panel). Then, gating was performed on a dot plot of FSC-area (FSC-A) versus FSC-height (FSC-H) to remove doublets and dead cells were excluded on the basis of LIVE/DEAD fluorescence (not shown). Subsequently, gate was made on CD3+/CD8+ (CD8 T cells, top-middle). **Identification of effector/memory subsets:** Following gating in CD8 T cells, Naïve (TN) cells were identified as CD45RA+/CD27+, Central Memory (TCM) as CD45RAneg/CD27+, Effector Memory (TEM) as CD45RAneg/CD27neg and Terminal Effector (TTE) cells as CD45RA+/CD27neg events (top-right). For the identification of CD45RAhi and CD45RAdim TTE cells, a threshold was established using CD45RA fluorescence intensity of TN cells for each individual as depicted in the figure (bottom-left). **Identification and phenotype of *Mtb*-specific cells:** Following gating in CD8 T cells, a gate was set for cytokoic degranulating cells (CD107a/b+) and IFN-γ-producing cells (bottom-middle). Then, effector/memory subsets were identified among *Mtb*-specific cells using a gating strategy based on the distribution of CD45RA and CD27 on bulk CD8 T cells that was conserved in the analyses of *Mtb*-specific CD8 T cells for each individual. Figure shows the analysis of effector/memory subsets among CD107a/b+ CD8+ T cells (bottom-right).

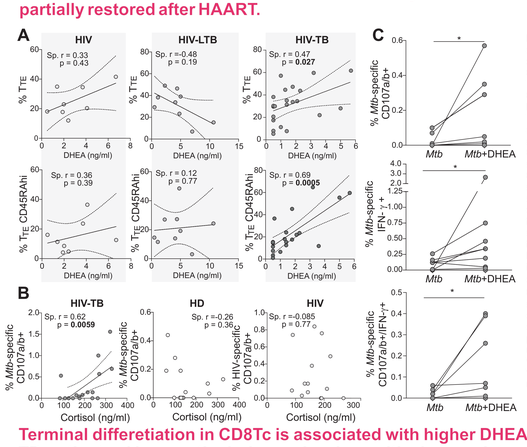


Figure 4. Adrenal hormone levels and their association with CD8 T cell differentiation and function. (A) Correlation analysis between the % of TTE (top) or TTE CD45RAhi (bottom) in CD8Tc and DHEA plasma levels in HIV+ (left), HIV-LTB (middle) and HIV-TB (right) subjects. **(B)** Correlation analysis between cortisol plasma levels and the % of *Mtb*-specific CD107a/b+ CD8Tc in HIV-TB (left) and HD (middle) subjects or the % of HIV-specific CD107a/b+ CD8Tc in chronic HIV+ subjects (right). Continuous lines represent linear regression curves and dashed lines represent 95% confidence intervals. Each symbol represents an individual subject. **(C)** PBMC from HIV-TB patients with CD4 counts higher than 100 cells/μl (n=10) were incubated in the presence or absence of *Mtb* (1 μg/ml) with or without DHEA (10^{-7} M) for 16 h and then the expression of CD107a/b and IFN-γ was evaluated in CD8Tc. Each line connects the data for an individual patient. Comparisons between treatments were performed using the Wilcoxon matched pairs test. **(B and C)** Values were media-subtracted and negative values were set to zero.

Terminal differetiation in CD8Tc is associated with higher DHEA plasma levels in HIV-TB patients. Also, cortisol plasma levels positively correlate with the proportion of cytotoxic *Mtb*-specific CD8Tc while *in-vitro* DHEA treatment increase *Mtb*-specific CD8Tc in HIV-TB patients.

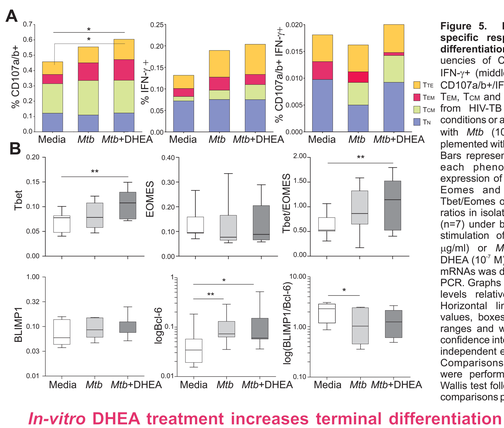


Figure 5. DHEA effect on *Mtb*-specific responses and terminal differentiation in CD8Tc. (A) Frequencies of CD107a/b+ (left, n=17), IFN-γ+ (middle, n=11) or bifunctional CD107a/b+/IFN-γ+ (right, n=13) TN, TEM, TCM and TTE cells among CD8Tc from HIV-TB patients under basal conditions or after stimulation of PBMC with *Mtb* (10 μg/ml) or *Mtb* supplemented with DHEA (10^{-7} M) for 72 h. Bars represent the median value for each phenotype. **(B)** Relative expression of Ttx-21 (Tbet), Blimp-1, Eomes and BCL-6 mRNAs and Tbet/Eomes or Blimp-1/BCL-6 mRNA ratios in isolated CD8+ cells from HD (n=7) under basal conditions or after stimulation of PBMC with *Mtb* (10 μg/ml) or *Mtb* supplemented with DHEA (10^{-7} M) for 72 h. Expression of mRNAs was determined by Real Time PCR. Graphs show mRNA expression levels relative to GAPDH mRNA. Horizontal lines represent median values, boxes represent interquartile ranges and whiskers represent 95% confidence intervals of 8 donors from 3 independent experiments. **(A and B)** Comparisons between treatments were performed using the Kruskal-Wallis test followed by Dunns multiple comparisons post-test.

***In-vitro* DHEA treatment increases terminal differentiation in cytotoxic *Mtb*-specific CD8Tc and favors the expression of transcription factors that drive terminal differentiation in CD8Tc.**

Conclusions

HIV-TB patients showed a bias to more differentiated stages in CD8 T cells, both in bulk and *Mtb*-specific cells. However, the lower levels of CD45RA observed on TTE CD8 T cells could reflect lower cell functionality, based on CD45RA role in enhancing TCR signaling [6].

CD8 T cells from HIV-TB patients also exibited higher expression of PD-1 in CD27+ CD8 T cells and a positive correlation between CD45RAdim proportions in TTE cells and PD-1 expression in CD27+CD8 T cells which is consistent with an arrest of exhausted cells on a less differentiated and poor functional phenotype, as seen for HIV+ patients [7].

Compared to HD, HIV+ patients showed an impaired *Mtb*-specific CD8 T cell response. HIV-TB co-infected patients showed elevated TTE and TEM CD8Tc in *Mtb*-specific IFN-γ+ and CD107a/b+ cells respectively. We also found that *Mtb*-specific CD107a/b+ CD8 T cells were fueled from TCM in HIV-TB, whereas in HD, they originated mainly from TN cells.

Finally, the present study shows that DHEA enhances CD8 T cell responses and terminal differentiation in HIV-TB co-infected individuals, suggesting a protective role for DHEA against mycobacteria in these patients.

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