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Antiretroviral Therapy Fails to Restore Mycobacterium Tuberculosis-specific Th1 and Th17 CD4 Responses

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Introduction

- HIV-1 infection is the biggest risk factor for the development of active tuberculosis (TB).
- Antiretroviral therapy (ART) substantially reduces the TB risk in HIV+ve individuals, however TB incidence remains greater than for those who are HIV-ve and is greatest during the first year of therapy.[1,2]
- Both the Th_1 and the recently-described Th_{17} Mtb-specific CD4 T cell response are believed to play an important role in anti-Mtb immunity.[3]
- **We hypothesized that this lack of protection results from the failure of key components of the Mtb-specific Th_1 and Th_{17} CD4 T cell responses to restore in individuals on ART.**

Materials and methods

- 115 subjects were recruited in a TB-endemic setting in Bloemfontein, South Africa.
- The Mtb-specific T cell responses of HIV+ve (n=71) subjects were evaluated before initiation of ART, and at 6 and 12 months thereafter.



- HIV-ve (n=44) subjects were recruited as a comparative group.
- Expression on CD4 T cells of IFN γ, TNF α, IL-2 and IL-17 in response to Mtb antigens (PPD, ESAT-6/CFP-10 (EC) and α-crystallin (Rv2031c)) were measured using a whole-blood flow cytometry assay.[4]

Results

1. ART results in increases in CD4 count, CD4:CD8 ratio and BMI after 6 and 12 months of therapy.

Characteristic	HIV-ve	HIV+ve
	n= 44	n= 71
Gender Female - n (%)	20 (45)	46 (65)
Male - n (%)	24 (55)	25 (35)
Age (years)[a]	24 (20 – 32)	36 (31 – 45)
CD4 count (cells/uL) [a]	879 (686 – 1054)	201 (155 – 279)
CD4:CD8 Ratio	1.54 (1.04 – 1.98)	0.21 (0.14 – 0.28)
Viral load (RNA copies/ml) [a]	N/A	77839 (24736 – 266629)
BMI (kg/m^2) [a]	24.3 (22.7 – 27.8)	24.1 (22.4 – 27.5)
Prev TB - n (%)	0 (0)	6 (8)
BCG scar - n (%)	20 (45)	47 (66)

Table 1. Baseline characteristics of all individuals included in the analysis.
[a] Data expressed as median (Interquartile range)

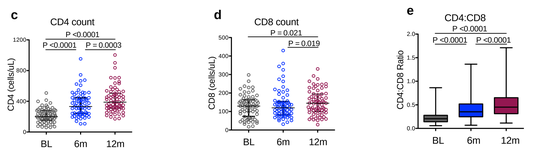


Figure 1. Changes in HIV-1 viral load, CD4 and CD8 T cell counts and BMI in HIV+ve individuals on ART. a) Viral suppression was achieved in all individuals included in this analysis. b) Changes in body mass index (BMI). c) Changes in CD4 T cell counts. d) Changes in CD8 T cell counts e) Changes in CD4:CD8 ratios. All P values generated using Wilcoxon matched pair analyses.

2. There was no significant change in the *total percentage* of HIV +ve IFNγ responders or in the *frequency* of IFNγ+ CD4 T cells responding to EC, PPD or Rv2031c on ART.

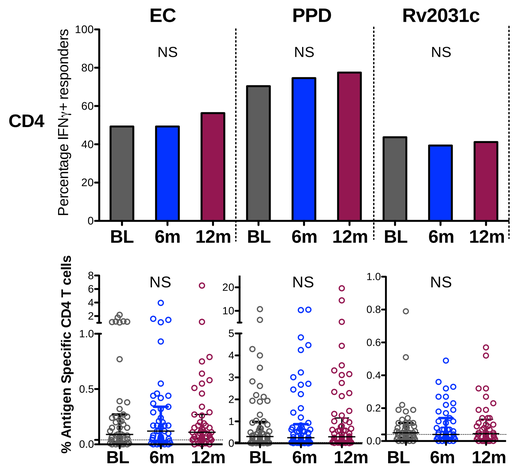


Figure 2. The impact of ART on the percentage of positive responders and frequency of Mtb-specific IFNγ+ CD4 T cells. BL = baseline, 6m = 6 months, 12m = 12 months. N = 71. Significant P values <0.05 are shown for Fisher's Exact tests (upper panel) and using Wilcoxon matched pair analyses (lower panel).

3. Analysis of the *absolute number of Mtb-specific cytokine-expressing CD4 T cells per mL of whole blood* shows significant immune reconstitution of Th_1 responses and limited reconstitution of Th_{17} responses on ART.

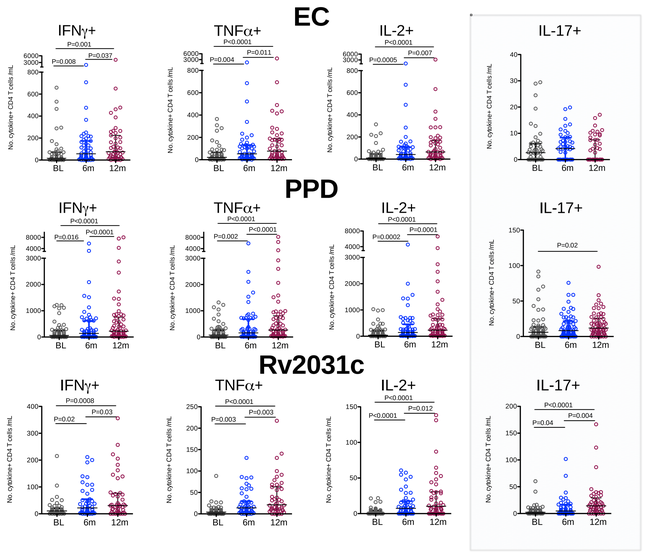


Figure 3. The effect of ART on absolute numbers of CD4 T cells expressing each cytokine measured in response to each antigen. N = 61. Values are expressed as cells/mL whole blood and are compared between each time point. P values are indicated for Wilcoxon matched pair analyses.

4. Preferential restoration of IL-2 and TNF α expressing CD4 T cells and lack of restoration of IL-17+ expressing CD4 T cells.

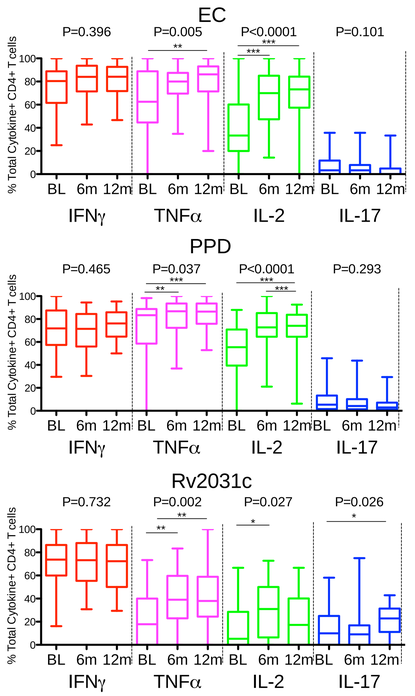


Figure 4. The proportions of IFN γ +, TNF α +, IL-2+ and IL-17+ CD4 T cells among total cytokine positive CD4 T cells over 12 months of ART. Changes in proportions are evaluated across the 3 time points. P values across the top of each panel indicate Kruskal-Wallis tests across each cytokine+ group. Significant differences were confirmed between time points using Mann-Whitney tests. * P <0.05, ** P <0.005, *** P <0.0005

5. ART restores *polyfunctionality* of Th_1 CD4 T cells responding to all Mtb-antigens tested.

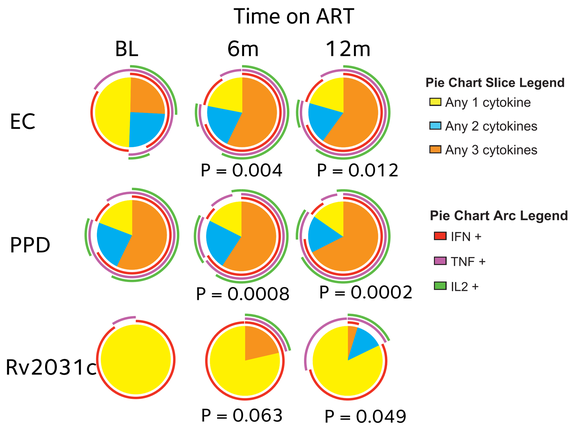


Figure 5. Changes in polyfunctionality of CD4 T cell responses on ART. Pie charts show the fraction of cells expressing 1, 2, or 3 cytokines. The arcs surrounding the pie charts indicate the proportion of the responses contributed by the single cytokines. P values indicate comparisons to baseline and are calculated using a partial permutation analysis (Roederer et al. 2011)[5]

6. Despite immune reconstitution, the *total percentage* of Mtb-specific IFNγ responders and the *frequency* of Mtb-specific IFNγ+ CD4 T cells remained suppressed after 12 months of ART when compared to HIV-ve subjects.

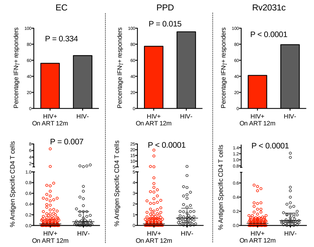


Figure 6. A comparison of the total percentage of IFNγ responders (upper panel) and frequency of IFNγ+ CD4 T cell responses (lower panel) between 71 subjects on ART for12 months and 44 HIV-ve subjects. P values are indicated for Fisher's Exact and Mann-Whitney tests.

7. Despite 12 months of ART, the *absolute number* of all Mtb-specific cytokine-expressing CD4 T cells remained suppressed when compared to HIV-ve subjects.

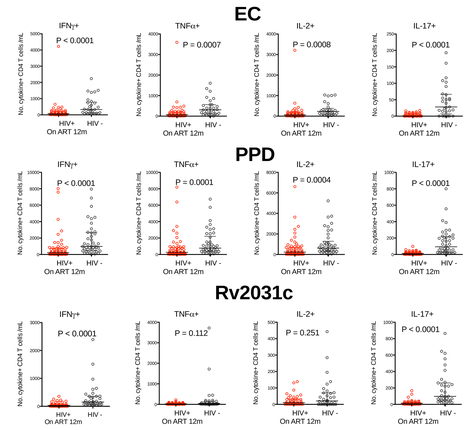


Figure 7. A comparison of absolute numbers of cytokine-expressing CD4 T cells/mL whole blood between 71 subjects on ART for12 months and 44 HIV-ve subjects. P values are indicated for Mann-Whitney tests.

Conclusions

- 12 months of ART only partially restores the magnitude of Mtb-specific CD4 T cell responses.
- Reconstitution is greatest for Mtb-specific Th_1 CD4 responses and is *diminished or absent* for Mtb-specific Th_{17} CD4 responses.
- ART is associated with an increase in polyfunctionality of Mtb-specific CD4 T cell responses.
- This lack of sufficient immune reconstitution is likely to contribute to the observed elevated TB risk on ART, particularly during the first year of therapy.

References

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