Persistence of Activated PD-1⁺ *Mtb*-specific CD4⁺ T Cells is Associated with Coronary Artery Disease During and After Tuberculosis Treatment

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BACKGROUND

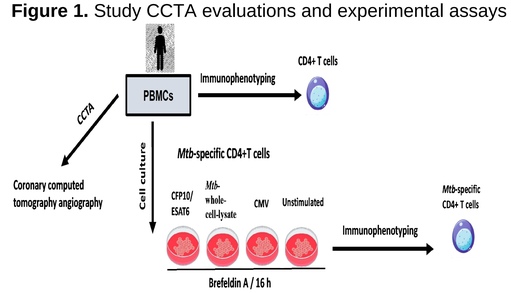
- Individuals who recovered from tuberculosis (TB) disease (post-TB) remain at increased risk of cardiovascular events and mortality. However, the underlying mechanisms of cardiovascular disease in post-TB remain unknown
- *Mycobacterium tuberculosis* (*Mtb*)-specific CD4⁺ T cells have an essential role in TB control and pathogenesis. The profile of *Mtb*-specific CD4⁺ T cells and its association with atherosclerotic disease has not been studied. Herein, we aimed to characterize the immune profile of *Mtb*-specific CD4⁺ T cells in post-TB and explore their relationship with coronary artery disease (CAD)
- Hypothesis: Individuals post-TB have alterations in their *Mtb*-specific CD4⁺ T cells that can contribute to CAD pathogenesis

METHODS

- **Study design:** Cross-sectional study of individuals 40 to 70 years of age in Lima, Peru
- Participants underwent a coronary computed tomography angiography (CCTA) and provided blood for PBMC isolation. See **Figure 1** below.
- **Study groups:**

❑ **LTBI:** defined by a positive QuantiFERON-TB test
❑ **TB-on-treatment**: individuals with active TB on isoniazid/rifampin consolidation treatment
❑ **Post-TB**: clinically-cured individuals within one year of TB treatment completion

Figure 1. Study CCTA evaluations and experimental assays



RESULTS

Characteristics of the study population*

Characteristics	LTBI (n=41)	TB on treatment (n=24)	Post TB treatment (n=11)
Age in year	53 (47–59)	58 (46–64)	49 (45–63)
Male sex	46%	50%	45%
Body mass index**	27.1 (25.8–29)	25.4 (23.7–28.0)	25.0 (23.9–29.0)
Hypertension	17%	8%	9%
Diabetes mellitus	26%	25%	22%
Current tobacco use	10%	9%	0%
Hemoglobin A1c	5.7 (5.5–7.1)	5.7 (5.5–8.4)	5.6 (5.4–7.2)
Total cholesterol	198 (176–224)	181 (141–202)	196 (174–221)
HDL cholesterol**	42.0(37–47)	52.5 (44–61.7)	59.0 (45–71)
LDL cholesterol	114 (99–135)	109.7 (80–121)	119 (80–141)
ASCVD PCE score	5.8 (1.7–10)	4.2 (1.3–9.5)	2.8 (2.1–7.9)

* Data presented as median with interquartile range in parenthesis or as percentages (%)
** p<0.05

TB-on-treatment and post-TB groups exhibited higher percentage of *Mtb*-specific CD4⁺ T cells after *Mtb*-lysate stimulation, compared to LTBI

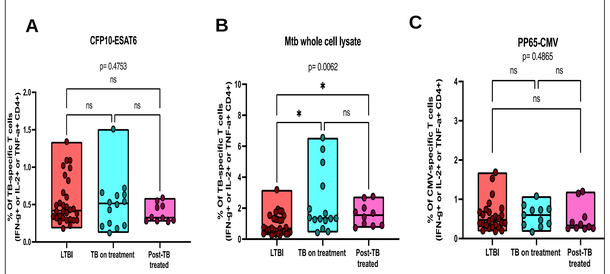


Figure 2. *Mtb*-specific CD4⁺ T cells in LTBI, TB-on-treatment, and post-TB. Percentage of *Mtb*-specific CD4⁺ T cells (IFN-γ+ or TNF-α+ or IL-2+) from LTBI, TB-on-treatment and post-TB in response to **(A)** CFP-10/ESAT6 pool peptides, **(B)** *Mtb* whole cell lysate, and **(C)** pp65-CMV peptides as a control. *P* values are from Kruskal-Wallis and Dunn's multiple tests. Significant differences are indicated at the top of the figure (*p<0.05)

Mtb-specific CD4⁺ T cells in TB-on-treatment and post-TB groups exhibited an activated and exhausting phenotype

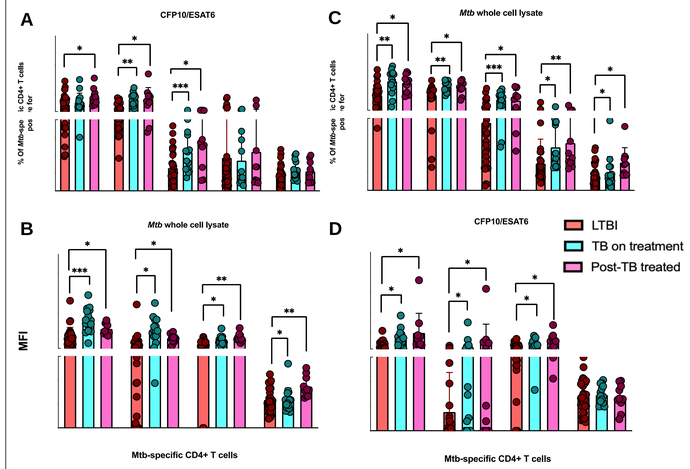


Figure 3. TB-on-treatment and post-TB groups exhibited overexpression of HLA-DR, CD38 and PD1 markers on *Mtb*-specific CD4+ T cells. (A) Percentage and **(B)** MFI of immune markers on *Mtb*-specific CD4+ T cells in response to *CFP10/ESAT6*. **(C)** Percentage and **(D)** MFI of immune markers on *Mtb*-specific CD4+ T cells in response to *Mtb* lysate. Significant differences are indicated at the top of the figure (*p<0.05) (**p<0.01) (***p<0.0001). *P* values are from Kruskal-Wallis and Dunn's multiple tests. Significant differences are indicated at the top of the figure (*p<0.05)

TB-on-treatment group exhibited a higher percentage of *Mtb*-specific CD4⁺ T cells positive to ≥2 cytokines upon *Mtb*-lysate stimulation, compared LTBI and post-TB groups

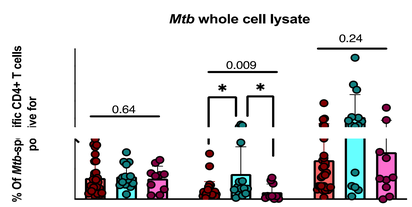


Figure 4. Polyfunctional capacity of *Mtb*-specific CD4⁺ T cell from LTBI, TB-on-treatment, and post-TB groups in response to *Mtb* lysate. Percentage of *Mtb*-specific CD4⁺ T cells positive for one, two and three cytokines in response to *Mtb* whole cell lysate. *P* values are from Kruskal-Wallis and Dunn's multiple tests. Significant differences are indicated at the top of the figure (*p<0.05). The black lines on the top of the figures correspond to the Kruskal-Wallis test among LTBI, TB-on-treatment, and post-TB groups

Expression of PD1 on *Mtb*-specific CD4⁺ T cells from TB-on-treatment and post-TB individuals positively correlated with CAD-RADS score

- Median fluorescence intensity (MFI) of PD1 on *Mtb*-specific CD4⁺ T cells from TB-on-treatment and post-TB groups positively correlated with CAD-RADS score (Spearman rho=+0.502; p=0.017)

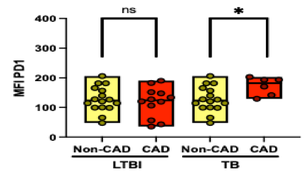


Figure 5. MFI of PD1 on *Mtb*-specific CD4+ T cells in individuals with and without CAD within the LTBI and TB groups. MFI of PD1 on *Mtb*-specific CD4+ T cells in LTBI and TB groups with CAD and non-CAD in response to CFP10/ESAT6. The TB group included TB-on-treatment and post-TB individuals. Significant differences are indicated at the top of the figure (*p<0.05). *P* values are from Mann-Whitney tests. Significant differences are indicated at the top of the figure (*p<0.05). NS: not significant differences

CONCLUSIONS

- Despite clinical TB cure, individuals post-TB exhibited signs of persistent immune activation and exhaustion of their *Mtb*-specific CD4⁺ T cell population
- PD1 expression on *Mtb*-specific CD4+ T cells was associated with coronary artery disease during and after TB treatment
- Whether alterations in *Mtb*-specific CD4⁺ T cell responses contribute to pathogenesis and/or could be used as biomarkers of increased cardiovascular risk during and after TB treatment will be explored in future studies

ACKNOWLEDGEMENTS

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