T cell responses to TITAN mRNA vaccine antigens in adults with *Mycobacterium tuberculosis* infection and tuberculosis disease

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Background

1. The TCR Informed TB AntigeN (TITAN) mRNA vaccine is a novel TB vaccine candidate currently in pre-clinical development.

2. Antigen selection for the TITAN vaccine was informed by recent research that identified CFP-10, PE13, PPE18, and Wbbl1 as antigens targeted by T cells expressing conserved CDR3 motifs enriched in individuals who control *Mycobacterium tuberculosis* (*Mtb*) infection.[1]

3. Efficacy of the TITAN vaccine will depend partially upon the ability of TITAN antigens to induce antigen-specific T cells in humans.

Aim: Characterise antigen-specific T cell responses to TITAN antigens in healthy adults and individuals with TB disease.

Methods

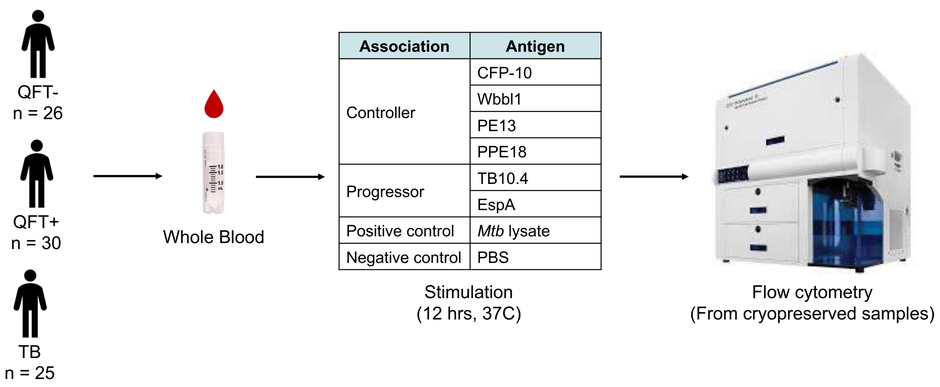


Figure 1. Experimental method. Whole blood was collected from 26 QuantiFERON-TB Gold negative (QFT-) adults, 30 QFT+ adults, and 25 adults with microbiologically-confirmed TB disease. Cells were stimulated with 15-mer peptide pools spanning CFP-10, PE13, PPE18, or WbbL1 and cryopreserved. Cells were then thawed and stained with an optimized antibody panel and frequencies of cytokine-expressing antigen-specific T cells were measured by flow cytometry. EspA and TB10.4 (targeted by T cells associated with progression to TB disease) were used for comparison, *Mtb* lysate was used as a positive control, and PBS was used as a negative control.

Results

Gating strategy for TITAN antigen-specific T cells

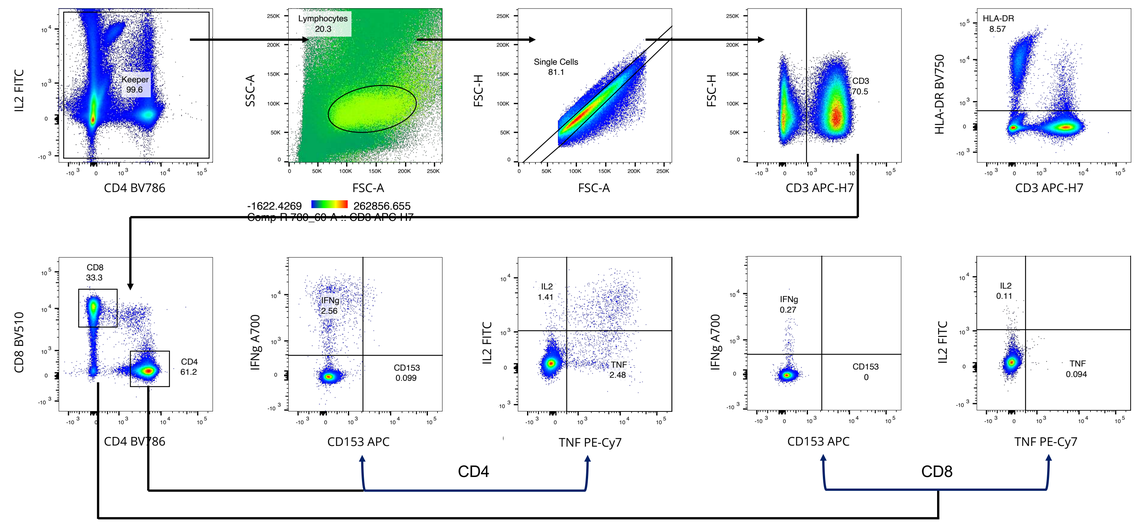


Figure 2. Gating strategy. Cryopreserved whole blood from each participant stimulated with CFP-10, Wbbl1, PE13, PPE18, TB10.4, EspA peptide pools, *Mtb* lysate (positive control), or PBS (unstimulated negative control) were thawed and stained with an optimized antibody panel (CD3-APC-H7, CD4-BV786, CD8-BV510, IFN-γ-AlexaFlour700, TNF-PE-Cy7, IL-2-FITC, HLA-DR-BV750, CD153-APC). Cells were acquired by flow cytometry on a BD FACSymphony Cell Analyzer. Representative plots show response to CFP-10.

Percent of individuals classified as "responders" based on T cell responses

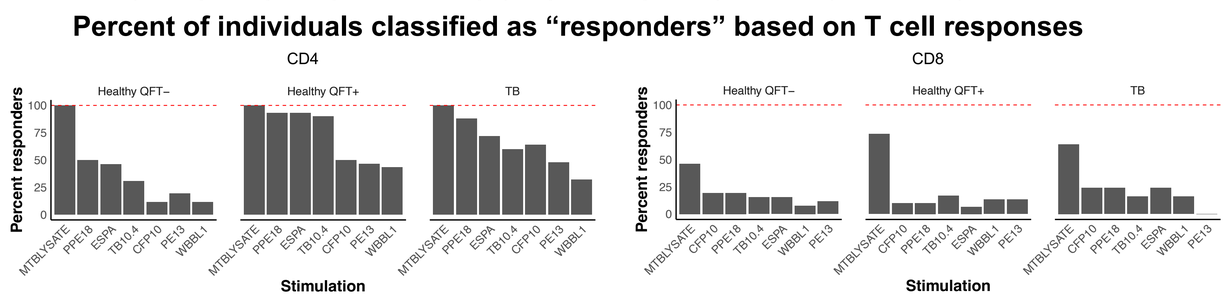
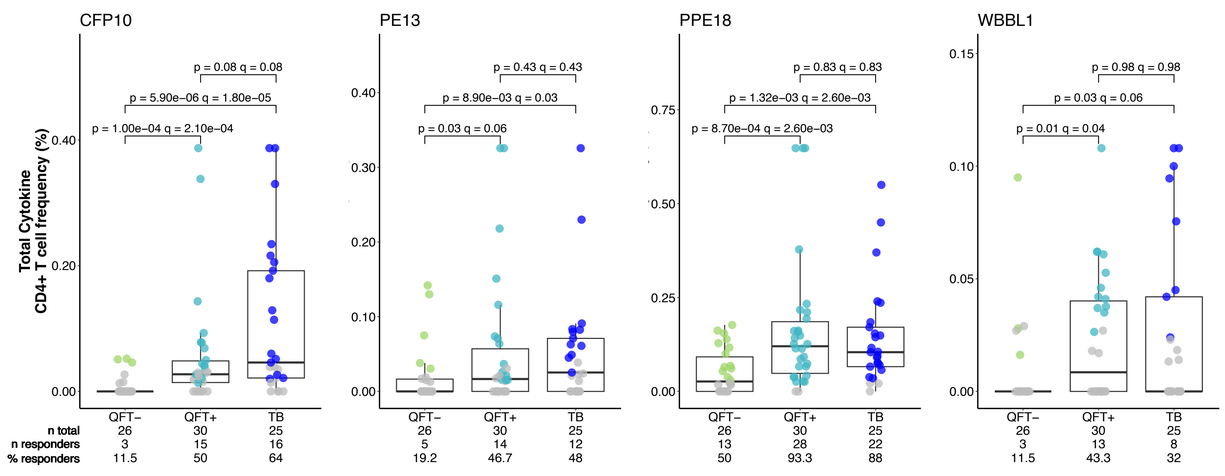


Figure 3. Percent responders. Participants were classified as "responders" or "non-responders" for each antigen based on expression of IL-2, IFN-γ, and / or TNF on CD4 and CD8 T cells. "Responders" are defined as individuals with frequencies of cytokine-positive cells that are significantly higher (Fisher's exact) in the stimulation condition compared to the unstimulated control. The percent of responders ((n responders / total participants per group)*100%) is reported here. The percentage of individuals classified as responders based on CD4 T cell cytokine expression are shown in the left panel. The right panel shows percent responders for CD8 T cells. Stimulation conditions are ordered from highest to lowest percent responders.

Results

Frequencies of CD4 T cells positive for IL-2, IFN-γ, or TNF in response to antigen stimulation



Frequencies of CD8 T cells positive for IL-2, IFN-γ, or TNF in response to antigen stimulation

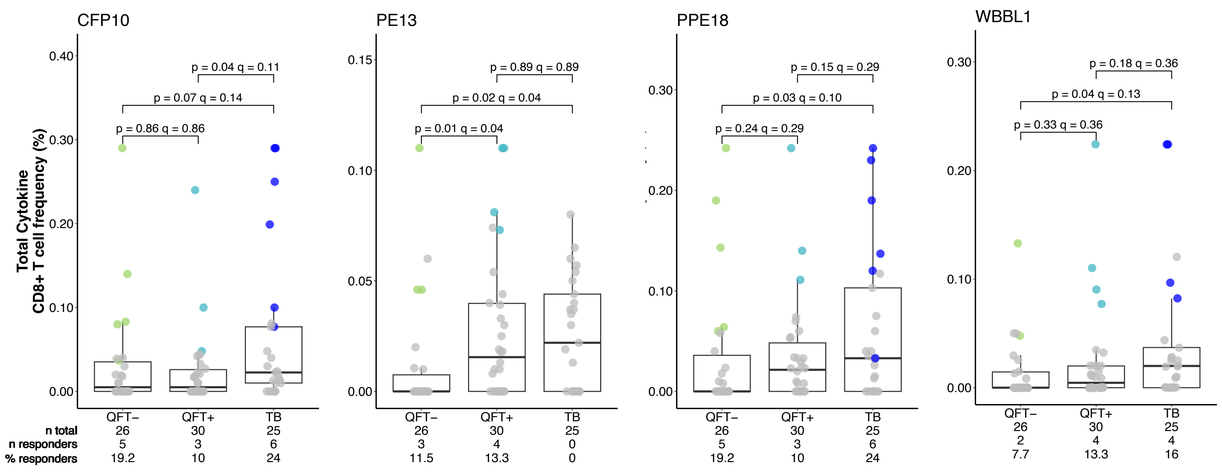
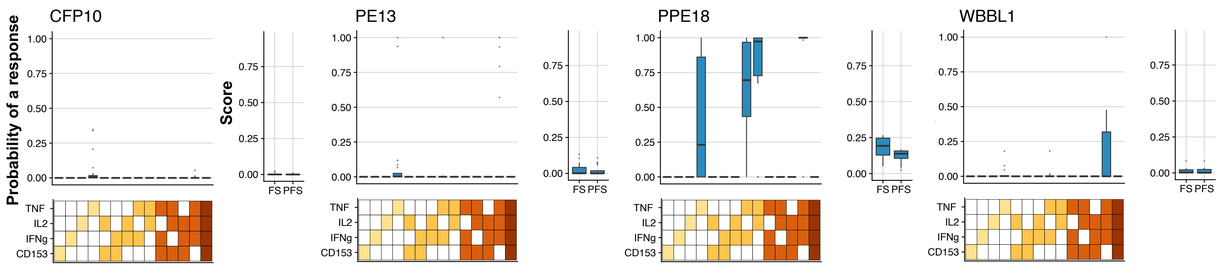
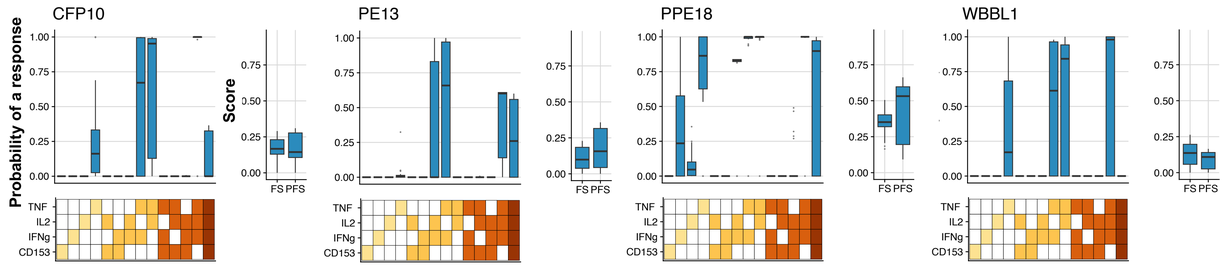


Figure 4. Frequencies of cytokine-expressing CD4 and CD8 T cells. The background-subtracted frequencies of CD4 (left) and CD8 (right) T cells that express IL-2, IFN-γ, and / or TNF following stimulation with each of the TITAN vaccine antigens are reported for QFT- and QFT+ individuals as well as individuals with TB disease. Colorized points represent responders. Gray points represent non-responders. The number and percent of responders for each group is reported below each respective bar plot. Statistical significance was determined by Wilcoxon test. Holm's method was used to correct for multiple comparisons.

COMPASS posterior probability of response for CD4 T cells in QFT- individuals



COMPASS posterior probability of response for CD4 T cells in QFT+ individuals



COMPASS posterior probability of response for CD4 T cells in individuals with TB disease

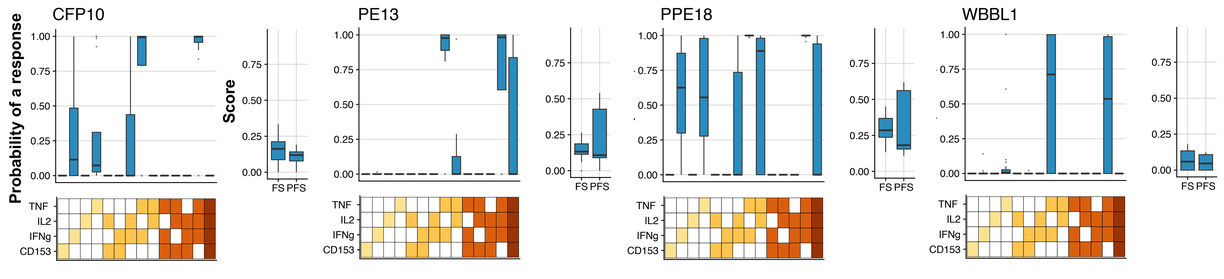


Figure 5. Probability of response for all cytokine combinations. Box and whisker plots showing the median, IQR (boxes) and range (error bars) of posterior probabilities of response, computed by COMPASS analysis, to each TITAN antigen for QFT- and QFT+ individuals as well as individuals with TB disease. These probabilities were then used to compute functionality (FS, single cytokine response) and polyfunctionality (PFS, multiple cytokine response) scores.

Conclusions

1. Individuals with evidence of *Mtb* infection or TB disease are more likely to respond to antigens intended for inclusion in the TITAN mRNA vaccine compared to individuals with no evidence of infection.
   a. CD8 T cell response is present, but less robust than the CD4 T cell response.

2. Frequencies of cytokine-expressing T cells following stimulation with TITAN antigens are higher in those with evidence of *Mtb* infection or TB disease, compared to QFT- participants.

3. TITAN antigen-stimulated CD4 T cells in individuals with evidence of *Mtb* infection or TB disease exhibit both single cytokine expression and polyfunctional phenotypes, suggesting a range of T cell differentiation.

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References: 1. Musvosvi, *et al*. (2023). *Nat Med*. doi: 10.1038/s41591-022-02110-9