Ex-vivo Characterization of Regulatory T Cells in Pulmonary Tuberculosis Patients, Latently Infected Persons, and Healthy Endemic Controls.

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Introduction

Regulatory T cells (Treg) are an essential arm of adaptive immunity not only in tolerance and autoimmunity but also in infectious diseases. In Tuberculosis (TB), it has been suggested that the frequency of Tregs is higher in the blood of TB patients when compared to healthy controls (HC) or latent TB infection (LTBI) and contributes to suppression of the Th1 immune response in patients. However, the discovery that FOXP3, the hallmark marker of Tregs is not exclusive to Tregs and the lack of specific markers for Tregs, have made it a challenge to fully understand the role of Tregs in TB. More recently, the activation marker Ki-67 and memory/primed T cell marker (CD45RO) were shown to delineate regulatory T cells into functional subsets of effector like (activated primed) Treg and naïve Treg. In this study, we used a combination of six markers to identify and characterize regulatory T cells in TB patients (active disease and cure), LTBI persons, and healthy controls in a TB endemic area.

Materials and Methods

Study sites

- Health centers in Addis Ababa
- AHRI

Study participants:

TB patients (TB, N=13):
Newly diagnosed, smear positive.

Latent infection (LTBI, N=8):
QuantiFeron Positive

Healthy endemic controls
(EC, N=9): QuantiFeron negative

All participants:

- Age 18 to 65 years old
- No previous history of TB

Workflow

Blood collected in Heparin Vacutainer tube

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PBMC isolated and frozen

↓

Intracellular Flowcytometry (CD4,CD8,CD25,CD127, CD45RO,FOXP3,Ki-67)

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- Data acquisition - FACSCanto
- Data analysis – FlowJo & GraphPad Prism

Results and Conclusions

Gating strategy

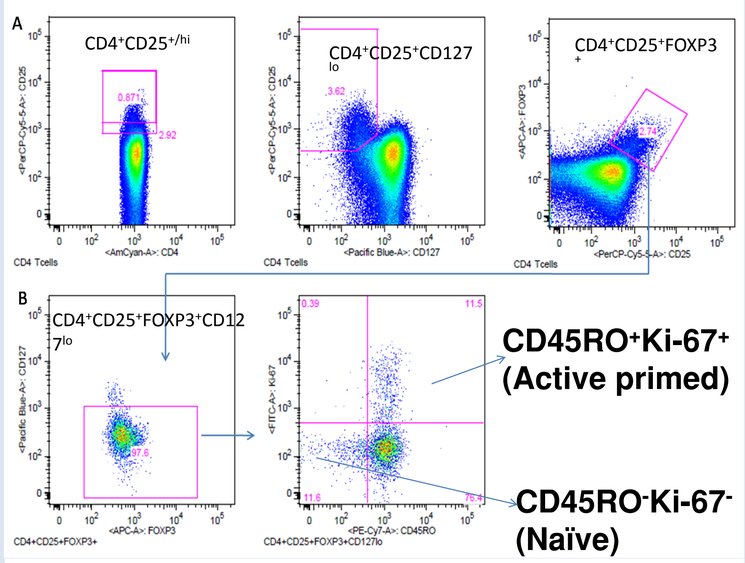

Fig. 1 Gating strategy includes: Lymphocyte gate → Live cells → CD4 T cells → Treg populations.

Treg gates

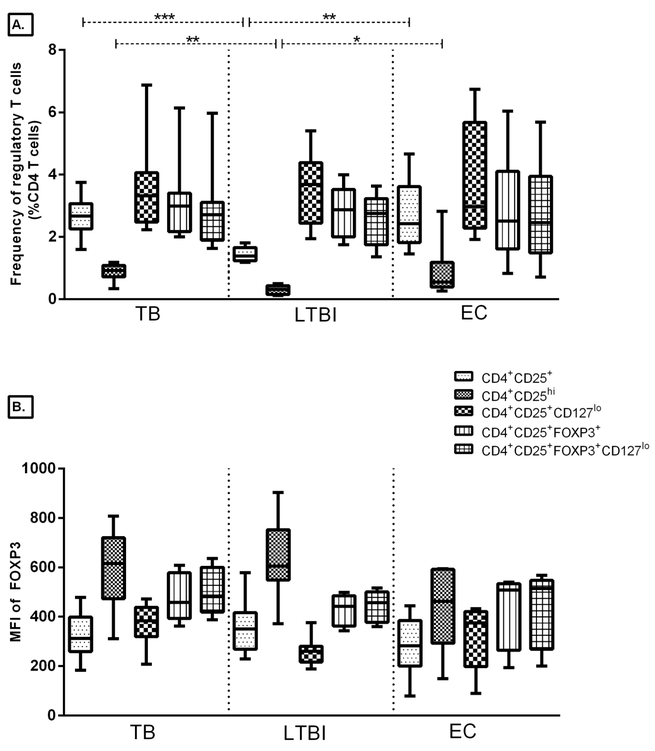

Fig. 2 Treg gates. Box-plots represent IQR with Min to Max values, line at median. Significant results of Kruskal-Wallis test are indicated by dashed lines.

➢CD4+CD25hi cells have the highest intensity of FOXP3; and their frequency is higher in TB patients than LTBI persons.

➢No difference in the frequency of Treg (defined by CD4+CD25+FOXP3+CD127lo) among TB, LTBI, and EC.

Change in Treg subsets in TB patients after treatment

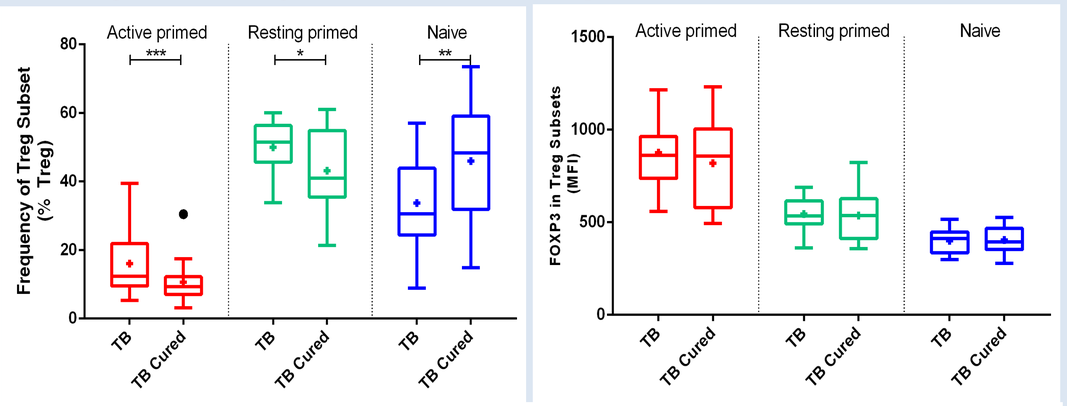

Fig. 5 The frequency (left) and FOXP3 intensity (right) of Treg subsets are shown as Active Primed (Red), Resting Primed (Green), or Naïve Treg (Blue) in TB patients before and after treatment. Box plots show median and inter-quartile range. P value of Wilcoxon signed rank test are indicated as * (*P<0.05), ** (P<0.01*), and *** (*P<0.001*).

➢ Activated primed subset of Treg (CD45RO+Ki-67+) are higher in TB disease and decline in frequency after treatment.

➢Naïve subset of Treg are lower in active TB disease and increase after treatment.

➢ No change in the intensity of FOXP3 in Treg subsets after treatment.

FOXP3 expression in Treg subsets.

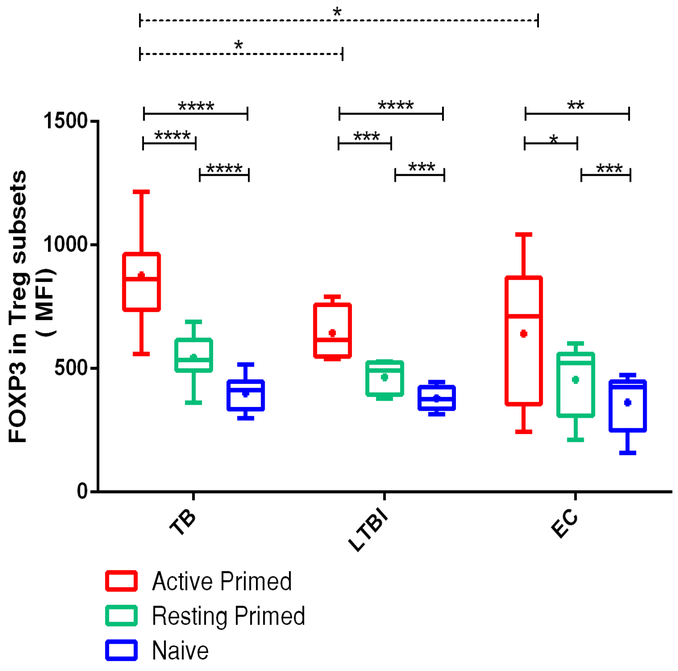

Fig. 3 Results of one way ANOVA (dashed lines) and repeated measures ANOVA (solid lines) are indicated by (*P<0.05*), **(*P<0.01*),*** (*P<0.001*), and **** (*P<0.0001*).

➢FOXP3 MFI is higher in activated primed Treg of TB patients than LTBI and EC.

Frequency of Treg subsets.

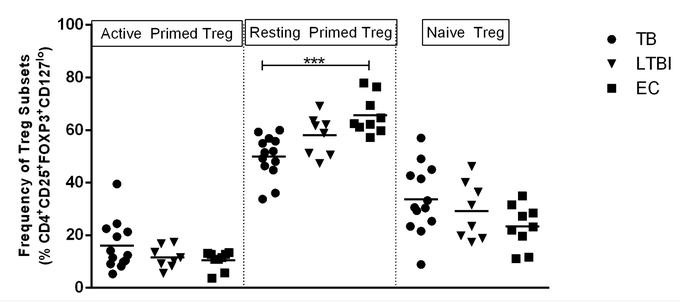

Fig. 4 The frequency of Treg subsets among study groups, Line at Median.

➢No difference in the frequency of Active Primed and Naïve Treg subsets among TB, LTBI, and EC.

➢ Resting Primed Treg subset higher in EC than TB patients.

Conclusions & Discussion

❖ **Association of Treg frequency with TB varies depending on the phenotypic markers used.**

❖ Ki-67 and CD45RO enable identification of functional subsets of Treg where there is a dynamic change in TB disease and cure.

❖**Activated primed Treg (highly suppressive) are higher In active TB disease and decline after treatment.**

❖ No difference in frequency of FOXP3+Treg among TB, LTBI, and EC.

Limitations:

- Small sample size
- Resting primed Treg population include FOXP3loKi-67- non-regulatory and pro-inflammatory cytokine producing T cells.

Recommendations for Future Studies:

- Consider confounding factors common in TB endemic areas (nutritional status, co-infection with helminth, and exposure to Non-Tuberculous Mycobacteria).

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- Study participants

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