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.

Gating strategy

CD45RO Ki-67
(Active primed)

CD45RO Ki-67
(Naïve)

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

FOXP3 expression in Treg subsets.

Frequency of 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).

- CD4+CD25+FOXP3+CD127- 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+CD127) among TB, LTBI, and EC.
- FOXP3 MFI is higher in activated primed Treg of TB patients than LTBI and EC.

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.

- FOXP3+CD4+CD25+Ki-67+ cells are shown in 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.

Change in Treg subsets in TB patients after treatment

Fig. 5 The frequency (left) and FOXP3 intensity (right) of Treg subtypes 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).

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 FOXP3+Ki-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).

Acknowledgements

- Health Centers and Nurses at the TB Clinics
- Study participants

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

PBMC isolated and frozen

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

Treg vs. Effector

• Data acquisition - FACS Cato
• Data analysis – FlowJo & GraphPad Prism

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