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Title: Different polyfunctional characteristics of RD1-specific CD4+ T-cells in active TB disease and LTBI

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Body: Introduction: CD4+ T-cells and their cytokines are crucial for protection against M. tuberculosis (Mtb). Analyses of the cytokines coexpressed by polyfunctional T-cells can help in discriminating different tuberculosis (TB) stages. Aims: To evaluate by flow cytometry the functional status and phenotype of Mtb-specific CD4+ T-cells in TB subjects at different stages. Methods: We enrolled 25 TB patients before and after therapy (active/past TB) and 39 latent TB infection (LTBI), classified as recent/remote infection and LTBI post-prophylaxis. We evaluated the CD4+ intracellular cytokines production (IFNγ, TNFα, IL2) and memory/effector status after in vitro whole blood stimulation with RD1 antigens. Results Magnitude of CD4+ T-cells is higher in active TB compared to the other groups, although the differences are not significant. Double IFNγ+TNFα+ CD4+ T-cells are significantly higher in active TB than in past TB (p=0.03) and in LTBI (p=0.002), whereas triple IFNγ+TNFα+IL2+ are significantly associated to LTBI post-prophylaxis compared to active TB (p=0.02). The proportion of total IFNγ CD4+ T-cells increases whereas the proportion of total IL-2 CD4+ T-cells decreases in active TB compared to LTBI (p=0.02). Effector memory CD4 T-cells are significantly higher in active TB than in LTBI (p<0.01), whereas central memory cells are higher in LTBI than active TB (p=0.03). Conclusions: Double IFNγ+TNFα+ CD4 T-cells are associated to active TB disease whereas triple polyfunctional cells are associated to infection containment. These results may be helpful for better characterizing TB immune responses and generating tools for TB stages identification.