

Name: Itumeleng Boshielo

Student number: 1144115

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Abstract

Tuberculosis (TB) is a chronic disease caused by *Mycobacterium tuberculosis* (Mtb). Despite the implementation of multifaceted TB prevention and control efforts, a significant number of people still die from TB. Consistent with this, an uptick in TB-related mortality was recently noted, which has been ascribed to the negative effects of Coronavirus disease-2019 (COVID-19) on TB programs. The complex life cycle of Mtb is largely due to the use of immune evasion mechanisms to establish initial infection, remain dormant in the host, and reactivate pathogenicity under favourable circumstances. The prolonged TB treatment regimen is necessitated by the slow response of bacterial populations to standard TB chemotherapy, a phenomenon that may be caused by persistent, drug-tolerant bacteria. Scientific literature has provided evidence for these types of bacterial populations in the form of Differentially Culturable Tubercle Bacilli (DCTB). It has been demonstrated that DCTB represent drug tolerant bacteria that appear to be cleared at slower rate than organisms detected by routine culture methods. However, it remains unclear if DCTB populations elicit different immune responses when compared to their conventionally culturable counterparts.

Herein, we address this question by optimizing a laboratory model for the generation of DCTB *in vitro* and test the capacity of clinical isolates of Mtb from Lineage 2 (Beijing) and Lineage 4 (LAM) to adopt the DCTB state. Using the Most probable number (MPN) assay, in the presence of culture filtrate (CF) as a source of growth factors to resuscitate DCTB, and colony forming units, the amount of DCTB in our model was quantified. As demonstrated by the limited growth on agar plates and increased growth in liquid media supplemented with CF from an axenic culture of Mtb, our findings demonstrated that carbon starvation was able to generate DCTB from clinical Mtb strains. After generating these populations, we stimulated whole blood with DCTB and conventionally culturable populations and report on the stimulation of a select set of cytokines (IFN- γ , IL-4, IL-5, IL-6, IL-12p70 and TNF- α) using a Bead Array Multiplex Immunoassay. In comparison to H37Rv-DCTB and LAM-DCTB, Beijing-DCTB induced significantly reduced levels of IL-5 and TNF- α . When comparing cytokine production between culturable and DCTB populations, within a single strain, we noted that LAM-DCTB was delayed in the production of IFN- γ whilst Beijing-DCTB was not able to induce production of this cytokine when compared to conventionally culturable counterparts. These data suggest that shifting to a non-replicating DCTB state does indeed affect the ability of clinical isolates to induce immune responses.

Based on these observations, we next set out to determine if DCTB affects immune responses during treatment of Mtb infected individuals. In prior work, using a prospective observational cohort, we demonstrated a substantive heterogeneity in clearance of DCTB in individuals with drug susceptible TB. We were able to classify these response patterns into three broad groups including (I) participants who were able to clear DCTB within the first two weeks of treatment (treatment-responsive); (II) those with delayed ability to clear these organisms (delayed-responsive) and (III) a group of individuals where DCTB did not change substantively during treatment (non-responders). Given these stark differences in treatment response patterns, we hypothesized that the immune responses associated with these patterns would be substantively different. In the second component of this work, we set out identify immune biomarkers that predict an effective response of DCTB to TB treatment. To quantify cytokines, chemokines and growth factors in plasma from these groups, we used a 65-plex Luminex assay, with a broad selection of targets. Statistically significant differences between these groups were analysed using the Kruskal-Wallis test with Dunn's multiple comparisons, with $p < 0.05$ was considered as statistically significant. When compared to patients who had TB and HIV co-infection, the number of cytokines that may possibly be used to report on the effectiveness of TB treatment was significantly higher in Mtb-only infected patients. This suggests that HIV infection significantly reduces the number of cytokines that can be used to report on TB treatment response. The ROC analysis of I-TAC, G-CSF and VEGF-A showed that these cytokines have a significant discriminatory power to distinguish treatment-responsive and non-responsive patients from HCs using DCTB as the measure of treatment response. No unifying cytokine signature that predicted DCTB response in all groups was identified. Together, our results indicate that some inflammatory markers are elevated in individuals with TB that rapidly clear bacteria during treatment. Given that these responses are based on DCTB, which represent drug tolerant populations, these select cytokines may be useful in evaluating the effectiveness of novel shorter TB treatment regimens.