Targeting *M. tuberculosis* counteractome to potentiate BCG-induced trained immunity

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1. Introduction

Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis (TB), remains a global health crisis. The pathogenicity of MTB involves its ability to adapt, survive and replicate within the human host cells. Recent studies have identified mycobacterial genes (termed 'counteractome') that enable MTB to survive CD4 T-cell mediated immunity. Understanding and targeting these survival mechanisms may advance therapeutic interventions that may augment vaccine efficacy. Bacillus Calmette Guerin (BCG) is the only approved TB vaccine. While efficient in children, this vaccine results in poor protection in adults. Recently, the BCG vaccine was shown to function through the induction of 'trained immunity' as it acts as a driver of a memory-like innate immune response. However, the protective dynamics of BCG-mediated immunity and mechanisms utilized by MTB to subvert killing effects of 'trained immunity', remain poorly understood. Therefore, the aim of this project is to determine protective correlates of BCG-induced trained immunity, and to identify, validate and target genes utilized by MTB to counteract the BCG-induced immunity.

2. Method to characterize protective correlates of trained immunity in BCG- trained PBMC's

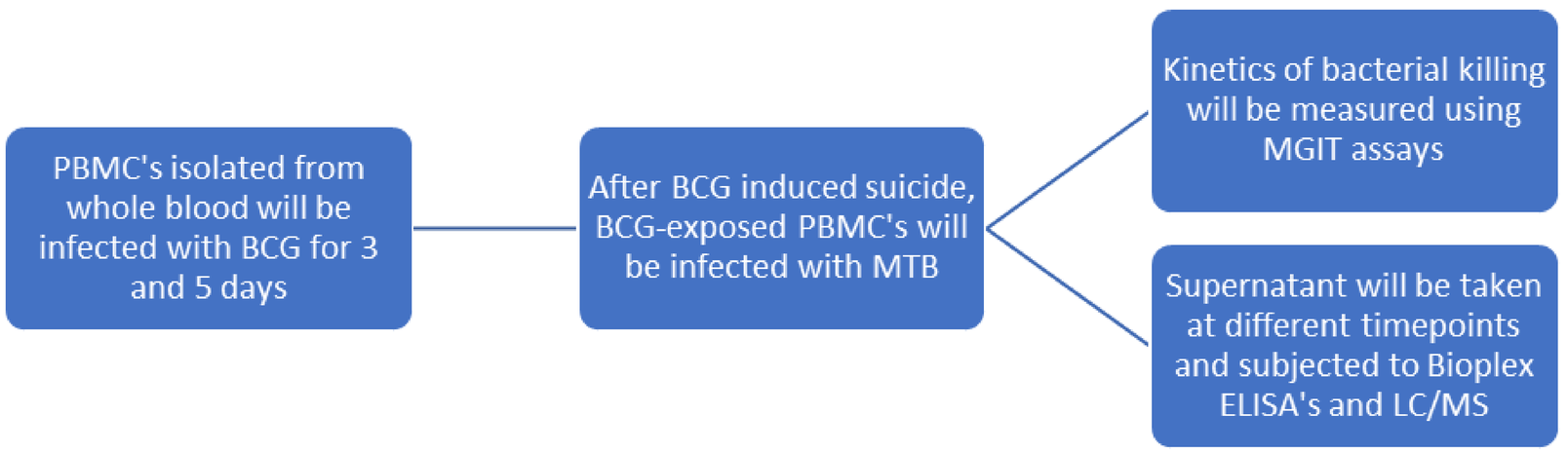

Fig #1 Diagram illustrating the process to be used to characterize protective correlates of trained immunity in BCG- trained PBMC's. PBMC's isolated from whole blood will be infected with an adapted BCG strain for 3 and 5 days. This BCG strain is adapted such that under certain conditions, it will commit suicide. Thus, after 3 and 5 days, the BCG will be induced to commit suicide after which the BCG-exposed PBMC's will then be infected with MTB. The kinetics of MTB killing of the BCG-exposed PBMC's will then be measured using MGIT assays. Furthermore, the supernatant of this mix will be taken at different timepoints and subjected LC/MS as well as cytokine and chemokine analysis using Bioplex.

3. Method to identify genes required to specifically survive in BCG-trained monocytes

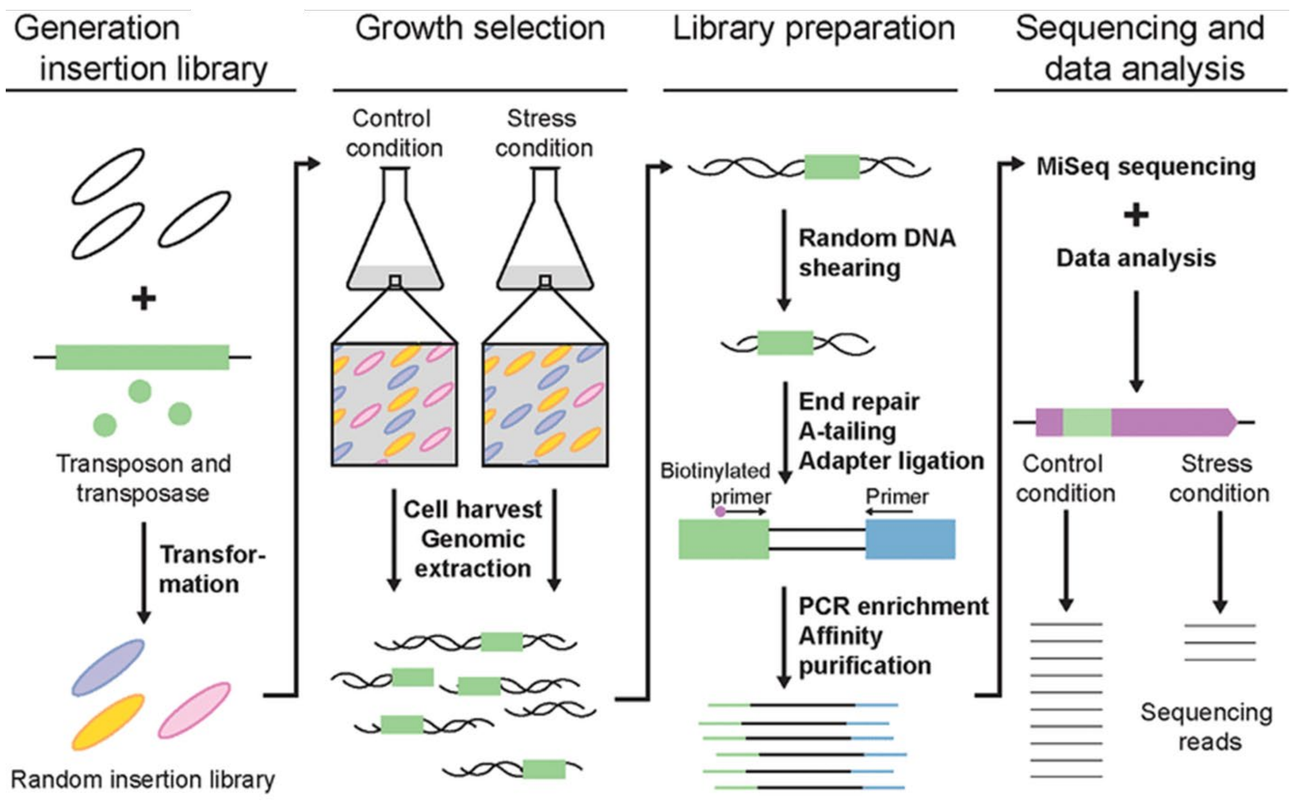

Fig #2 Diagram showing the process of Tn-Seq used to determine genes required survive in BCG-trained monocytes. The BCG trained monocytes will be infected with an input MTB library that has been mutagenized with a transposon and on ~day 5 post-infection, the cells will be lysed. This lysate containing surviving MTB (output) will be plated on agar. Whole genome sequencing analyses of the input and output MTB library will identify the MTB mutants that survived in trained monocytes, revealing genes (counteractome) that are essential to survive this condition. Adapted from Calero et al 2017.

4. Results

- Tn-Seq library of MTB has been successfully created. Moving forward, we will be performing the growth selection phase shown is Fig #2.
- Removal of lysed red blood cells from previously frozen whole blood has been optimised using the Hemovoid™ kit.

5. Conclusion

We have optimized methods to identify BCG protective correlates in previously frozen whole blood allowing proteomic signatures to be determined using mass spectrometry.

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