Assessing the Ability of Auxotrophic Mycobacterium tuberculosis Strains to Trigger Trained Innate Immunity in Mouse Macrophages

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Tuberculosis (TB), caused by Mycobacterium tuberculosis, is once again the world's deadliest infectious disease, after being replaced by COVID-19 for three years. The only approved vaccine for TB, the Bacillus Calmette-Guérin (BCG) vaccine, shows limited efficacy in adults, but promising alternatives are auxotrophic strains of M. tuberculosis; they are avirulent, nonlethal, and BSL-2 approved. However, the ability of auxotrophs of M. tuberculosis to trigger trained innate immunity (TII), a key feature of BCG vaccination, has not, until this study, been researched. TII is a heightening of innate immune cell inflammatory response following heterologous stimuli after training by a primary stimulus. I assessed the ability of auxotrophic M. tuberculosis strains mc27901, mc27902, and mc28755 as well as the fast-growing M. smegmatis mc2155 to trigger TII in wildtype and Stat3-/- RAW 264.7 mouse macrophages. Cells were trained with mycobacterial strains or BCG for 24 hours, rested for six days, and restimulated with lipopolysaccharide or lipoteichoic acid. Cytokine expression (IL-6, IL-10, TNF-?) was analyzed via RT-qPCR following TRIzol RNA extraction. I found mc27902 trained WT macrophages to elicit ~3 times greater IL-6 upregulation compared to BCG training with no IL-10 upregulation, indicating mc27902 can trigger TII with a greater pro-inflammatory cytokine response. Additionally, Stat3-/- macrophages displayed less IL-6 and IL-10 upregulation but similar TNF-? responses compared to WT. Surprisingly, STAT3 loss increased mc27902-trained IL-6 and IL-10 upregulation, but had the opposite effect in BCG, suggesting different TII induction mechanisms. These results underscore mc27902's potential to be a valid and superior BCG replacement.

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