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Investigating the roles of diacylglycerol during *Mycobacterium tuberculosis* phagocytosis

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Abstract

Mycobacterium tuberculosis (Mtb) remains a significant global health threat, where an estimated 10 million individuals suffered infection and around 1.4 million people died in 2019 alone. Its success as a pathogen is in part due to its ability to escape cellular immune responses via several mechanisms, among which is the manipulation of lipid metabolic pathways in the host. Previous studies have demonstrated that membrane sphingolipids are implicated in the first step of *Mtb* phagocytosis by different types of phagocytic cells. *Mtb* then prevents phagosome maturation and persists long-term in granuloma structures. Obstructing sphingomyelin and glycosphingolipid biosynthesis has demonstrated that sphingomyelin, not glycosphingolipid, production plays a critical role in phagocytic uptake of *Mtb.* However, the formation of sphingomyelin requires the production of diacylglycerol (DAG) as a byproduct, and the role of DAG produced in this pathway has not been characterized in the context of phagocytosis. There are also other DAG-producing pathways that have yet to be explored in phagocytosis. Our work seeks to utilize a genetic biosensor tool to visualize DAG distribution during phagocytosis by producing RAW264.7 murine macrophage cell lines that stably express a GFP reporter construct fused to the C1 domain from protein kinase C (PKC), deemed C1-GFP. The C1 domain of PKC typically binds DAG in physiological processes, so fusion with GFP will allow visualization experiments involving high-resolution confocal microscopy and live-cell imaging during phagocytosis in wild type and sphingolipid-depleted macrophage cells. This study will shed light on the exact role of DAG during phagocytosis, such as determining whether it is critical at the pathogen contact site, phagocytic cup formation, or cellular entry. Understanding sphingolipid dynamics during *Mtb* infection would allow us to use novel approaches to combat this globally relevant bacterium.