The role of triacylglycerols and repurposing DGAT1 inhibitors for the treatment of *Mycobacterium tuberculosis*

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**ABSTRACT**

Latent tuberculosis poses a significant threat to global health through the incubation of undiagnosed infections within the community, and through its tolerance to antibiotics. This Special Features article explores the mechanisms by which the dormant *Mycobacterium tuberculosis* pathogen can store energy in the form of lipid inclusion bodies and triacylglycerols, which may be key in the development of novel therapeutics to treat TB.

Tuberculosis (TB) is an infection of global significance, caused by *Mycobacterium tuberculosis*, and is second only to Covid-19 in the number of deaths caused annually by a single infectious agent. Most cases begin with the inhalation of the bacterium, which leads to a primary infection of the lungs, although TB can also cause systemic disease. *M. tuberculosis* is remarkable in its ability to survive long periods of dormancy, during which the infection is referred to as latent TB. Several physiological and metabolic changes occur between the host and the pathogen in latent TB which lead to the formation of granulomas. The granuloma, is a hallmark of TB, typically preventing bacterial proliferation, however, it does not allow the host to completely eradicate the infection.

Briefly, when the bacterium is inhaled, it is phagocytosed by alveolar macrophages. Further immune cells are recruited to the site, while phagosomal maturation is interrupted by the bacillus, which prevents acidification of the phagosomal compartment. Macrophages constitute a large part of the granuloma, undergoing changes at the site of infection, some becoming lipid loaded foamy macrophages, others fusing to form multi-nucleated giant cells (Ramakrishnan, 2012). Further to these changes, mature macrophages in the TB granuloma can develop into epithelioid cells with an inter-linked arrangement, providing structure (Ramakrishnan, 2012). Additional immune cells are also recruited to the periphery of the granuloma, forming a diversely populated capsule with a central cavity of *M. tuberculosis* bacilli and necrotic infected macrophages, surrounded by both epithelioid and foamy macrophages, neutrophils, giant and dendritic cells, and an outer border of T- and B-cells (Fig. 1) (Ramakrishnan, 2012).

Granuloma formation, which restricts the growth and division of the pathogen, contributes to the clinical complication of antibiotic tolerance and resistance. Under the hypoxic conditions of the granuloma, the metabolic and phenotypic changes that occur in *M. tuberculosis* (Wayne and Hayes, 1996) render antibiotics, such as rifampicin, isoniazid, ethambutol and pyrazinamide, which target actively replicating bacteria, ineffective.

Dormant *M. tuberculosis* bacilli are faced with the challenge of fueling the maintenance of essential cell processes in this nutrient poor environment. While encased in the granuloma, *M. tuberculosis* are able produce ATP, however the cellular levels of ATP are considerably lower than actively replicating *M. tuberculosis*. This depletion of cellular energy results in a reduction of biochemical activity within the cell which is largely responsible for the reduction in antibiotic efficiency, as the biosynthetic pathways which are targeted by antibiotics are downregulated.

Both prokaryotic and eukaryotic organisms use fatty acids as an important energy source, as they yield a higher amount of energy than other molecules, such as carbohydrates and can easily be stored within the cell (Serafin et al., 2018). *M. tuberculosis* is no different; in fact, during dormancy, the role of fatty acids for energy storage is particularly significant. Fatty acids are known to stimulate oxygen uptake in *M. tuberculosis* and can be utilised for the release of energy and carbohydrate synthesis.

Lipids such as fatty acids can be broken down through the tricarboxylic acid cycle (TCA), in which the oxidation of acetyl-CoA results in the formation of adenosine triphosphate (ATP) and carbon dioxide. Additionally, the glyoxylate shunt is a pathway through which acetyl-CoA is converted to a dicarboxylic acid, succinate, which is involved in the synthesis of carbohydrates. These two cycles are sufficient when run in parallel for fatty acids to be used as the main carbon source, which helps cells endure hypoxic environments.

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Moreover, for efficient storage, fatty acids are accumulated as triacylglycerols (TAGs) in the adipose tissue of most mammals and in aggregated lipids within bacterial cells, known as intracellular lipid inclusion (ILI) bodies (Serafim et al., 2018). The amount of lipid inclusion bodies in the cytoplasm of *M. tuberculosis* increases during cell dormancy, and accumulation of TAG can be induced under hypoxic environments and nitrogen deprivation, suggesting a key role in *M. tuberculosis* survival within the host (Sirakova et al., 2006; Santucci et al., 2019). A protein identified in *Mycobacterium bovis* BCG, BCG_1721, has bi-functional activity for both the accumulation and hydrolysis of TAG and is important for the growth of cells following resuscitation (Low et al., 2010). In addition to intracellular TAG, there is strong evidence that both diacylglycerol (DAG) and TAG are present in the outer membrane of mycobacterial species. The export of TAG from the cytoplasm to the outer membrane by an efflux pump (Rv1410) and lipoprotein LprG has been observed, which supports the theory that TAG provides an undefined function to the complex *M. tuberculosis* cell wall (Martinot et al., 2016; Viljoen et al., 2016).

The synthesis of diacylglycerol (DAG) is the first committed step in mycobacterial TAG synthesis. The PlsB/PlsC acyltransferases sequentially esterify glycerol-3-phosphate resulting in phosphatidic acid, which is dephosphorylated by a phosphatidic acid phosphatase and used in the synthesis of DAG and TAG. Fifteen genes have been identified in *M. tuberculosis* as encoding putative triacylglycerol synthase (Tgs) enzymes which are responsible for acylating DAG using acyl-CoAs (Fig. 2, Table 1) (Daniel et al., 2004). When expressed in *Escherichia coli*, the tgs activity of the cell lysate increased for all expressed proteins compared to wild type *E. coli*, and the four Tgs proteins with highest tgs activity were designated Tgs1 (Rv3130c), Tgs2 (Rv3734c), Tgs3 (Rv3234c) and Tgs4 (Rv3088) (Daniel et al., 2004).

*M. tuberculosis* has a group of 48 genes known as the DosR dormancy regulon, a regulatory system which is activated under conditions of hypoxia. Tgs1 is a powerfully inducible gene located within the DosR region and is upregulated in *M. tuberculosis* clinical isolates (Chauhan and Tyagi, 2009). The involvement of Tgs1 in virulence and the rapid formation of necrotising granulomas has been demonstrated in zebrafish, and the deletion of Tgs1 is known to cause a dramatic (but not complete) reduction in TAGs (Sirakova et al., 2006; Daniel et al., 2011). Tgs1 has directly been linked to the accumulation of TAG in ILIs and...
Conclusion

Latent TB poses a significant threat to global health through the incubation of undiagnosed infections within the community, and through its tolerance to antibiotics. Tackling the mechanisms by which the dormant pathogen can store energy may be key in the development of novel therapeutics to treat TB. A two-pronged approach of inhibiting de novo TAG synthesis in *M. tuberculosis* and preventing the uptake of host TAG is essential and could dramatically reduce the ability of *M. tuberculosis* to persist within the granuloma. Fortunately, the secretion of niacin by the bacterium prevents one route of host TAG synthesis, via the inhibition of DGAT2. Looking to the future of TB drug discovery, known inhibitors of DGAT1 should be screened against *M. tuberculosis* to establish whether any existing drugs could be exploited as antimycobacterial agents.

Ethics Statement

No ethical issues to report.

CRediT authorship contribution statement

Alice R. Moorey: Conceptualization, Writing – original draft, Writing – review & editing. Gurdyal S. Besra: Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gurdyal Singh Besra reports financial support was provided by Medical Research Council. Gurdyal Singh Besra reports a relationship with Microbiology Society that includes: travel reimbursement.

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Conflicts of interest

As Gurdyal S. Besra, a co-author on this paper, is an Editor of this journal, he was fully blinded to the review process and this paper was independently handled by Neil Gow (Editor-in-Chief).

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