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Mini-Review

Triacylglycerol: nourishing molecule in endurance of *Mycobacterium tuberculosis*

PRATAP C MALI[1] and LAXMAN S MEENA[2]*

[1]*Department of Zoology, University of Rajasthan, Jaipur 322 219, India*

[2]*CSIR–Institute of Genomics and Integrative Biology, New Delhi 110 007, India*

**Corresponding author (Email, Meena@igib.res.in, Laxmansm72@yahoo.com)*

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The ability of *Mycobacterium tuberculosis* (*M. tuberculosis*) to accumulate lipid-rich molecules as an energy source obtained from host cell debris remains interesting. Additionally, the potential of *M. tuberculosis* to survive under different stress conditions leading to its dormant state in pathogenesis remains elusive. The exact mechanism by which these lipid bodies generated in *M. tuberculosis* infection and utilized by bacilli inside infected macrophage for its survival is still not understood. In this, during bacillary infection, many metabolic pathways are involved that influence the survival of *M. tuberculosis* for their own support. However, the exact energy source derived from infecting host cells remain elusive. Therefore, this study highlights several alternative energy sources in the form of triacylglycerol (TAG) and fatty acids, i.e. oleic acids accumulation, which are essential in dormancy-like state under *M. tuberculosis* infection. The prominent stage in tuberculosis (TB) infection is re-establishment of *M. tuberculosis* under stress conditions and deployment of a confined strategy to utilize these biomolecules for its persistence survival. So, growing in our understanding of these pathways will help us in accelerating therapies, which could reduce TB prevalence world widely.

Keywords. Foamy macrophages; LipY; *Mycobacterium tuberculosis*; TAG; Tgs

Abbreviations: FM, foamy macrophages; TAG, triacylglycerol; TB, tuberculosis; Tgs, triacylglycerol synthase

1. Introduction

Tuberculosis (TB) remains the most deadly disease caused by the pathogen *M. tuberculosis* (Meena and Rajni 2010; Meena 2015). Additionally, emergence of latent TB into drug resistance forms like multi-drug resistant (MDR), extremely drug resistant (XDR), along with the immune-compromised state, make it highly infectious (Ahmad *et al.* 2016). Besides, the tactical organization of *M. tuberculosis*'s complex virulence factors and the survival capacity of the bacilli also limit our knowledge of TB pathology (Mukhopadhyay *et al.* 2012; Meena and Meena 2015). It is estimated that approximately 6.1 million TB cases arise annually, of which approximately 55% of the cases are co-infection with HIV, thus increasing TB burden worldwide (Kumari and Meena 2014; WHO 2016). The weakened host immunity under *M. tuberculosis* infection improves its survival succession rate for a prolonged time, which further leads to repetitive active infection. In TB pathogenesis, the pathogen mostly affects the major alveolar macrophage via infectious aerosol and modulates generalized bactericidal action of the host to prevent its own destruction (Neil and William 1998; Sasindran and Torrelles 2011). Moreover, the continual virulent effect of *M. tuberculosis* and its ability to survive within host cells for decades impedes our understanding of TB biology (Meena and Rajni 2010; Gengenbacher and Kaufmann 2012). So, the mycobacterium possessing multiple strategies and its consumption of energy sources in the dormancy-like stage maximize its pathological impact on the infected host cell (Rajni *et al.* 2011; Meena and Meena 2016). It was established that biosynthesis and intracellular accumulation of triacylglycerol (TAG) (Reed *et al.* 2007) act as a major source of energy at different stages of the infection, which remain to be understood. A variety of fatty acids can be esterified at the glycerol backbone originated by TAG dissolution, which serves as energy source by the production of acetyl-coA (Walker *et al.* 1970). However, in previous studies it was shown that *M. tuberculosis* accumulates a large amount of TAG and fatty acid, which serves as a carbon energy source reservoir during the dormancy stage and allow its persistency (Daniel *et al.* 2011; Monu and Meena 2016). Interestingly, the genes responsible for TAG synthesis/

accumulation under different stress condition leading to *M. tuberculosis* dormancy-like state remain largely unknown.

Current studies have shown that the triacylglycerol synthase (tgs) genes possesses the accumulating capability that brings *M. tuberculosis* into the dormancy-like state (Sirakova *et al.* 2006). However, with respect to these tgs accumulation genes, mycobacterium survival rate being equally affected by other growth conditions like acidic, stress and hypoxia (oxygen depletion), etc., is also very common (Sirakova *et al.* 2006; Deb *et al.* 2009). As reported by a study, exclusion of tgs1 leads to the complete loss of TAG accumulation (Daniel *et al.* 2004; Sirakova *et al.* 2006; Deb *et al.* 2009). During the time course of this study, the bacilli consisted of distinguished genes to accumulate TAG and fatty acid, which allowed it to go into the dormant stage of infection (Russell 2003). In one study, it was shown that the mutant/disruption of triacylglycerol synthase (tgs) gene of *M. tuberculosis* exhibited decline in TAG accumulation (Garay et al. 2014).

2. Synergy between energy sources and *M. tuberculosis's* dormant stage

One of the major problems in TB control is the lack in knowledge of about survival of *M. tuberculosis* for long decades in the dormancy-like stage (Meena and Rajni 2010). Metabolism of fatty acid is a vital feature for maintaining the dormant state of *M. tuberculosis*. The synergism between tricarboxylic acid cycle (TCA) and glyoxalate cycle maintain the vital use of acetyl coA, a common substrate produced by catabolism of host fatty acids. In favourable conditions, *M. tuberculosis* generates energy through TCA cycle, and it uses glyoxalate cycle in harsh situations (Gengenbacher and Kaufmann 2012). However, the consumption of fatty acids is thought to be a major source of energy among biomolecules which is required for the persistence phase in *M. tuberculosis* infection (Meena and Kolattukudy 2013). A highly likely possibility is the fatty acids stored in host cell's inclusion bodies as an energy reserve in the form of TAG. Previous studies demonstrated that, TAG found in the inclusion bodies were obtained from the infected host (Garton *et al.* 2002) and that reflects the favourable condition for dormant bacilli. Besides *M. tuberculosis* infection, in other microbial infections, these synthesized lipid-rich vesicles are actively utilized by microorganisms and deploy carbon source for their living (Murphy 2001). Further studies on TAG shows there is substrates specificity that (Monu and Meena 2016) further acts as reservoirs energy sources, which are still bare in *M. tuberculosis*. These are generated from host's cell/tissue, and their further degradation is thought to be a source of *M. tuberculosis* survival in dormant stage. Fifteen members

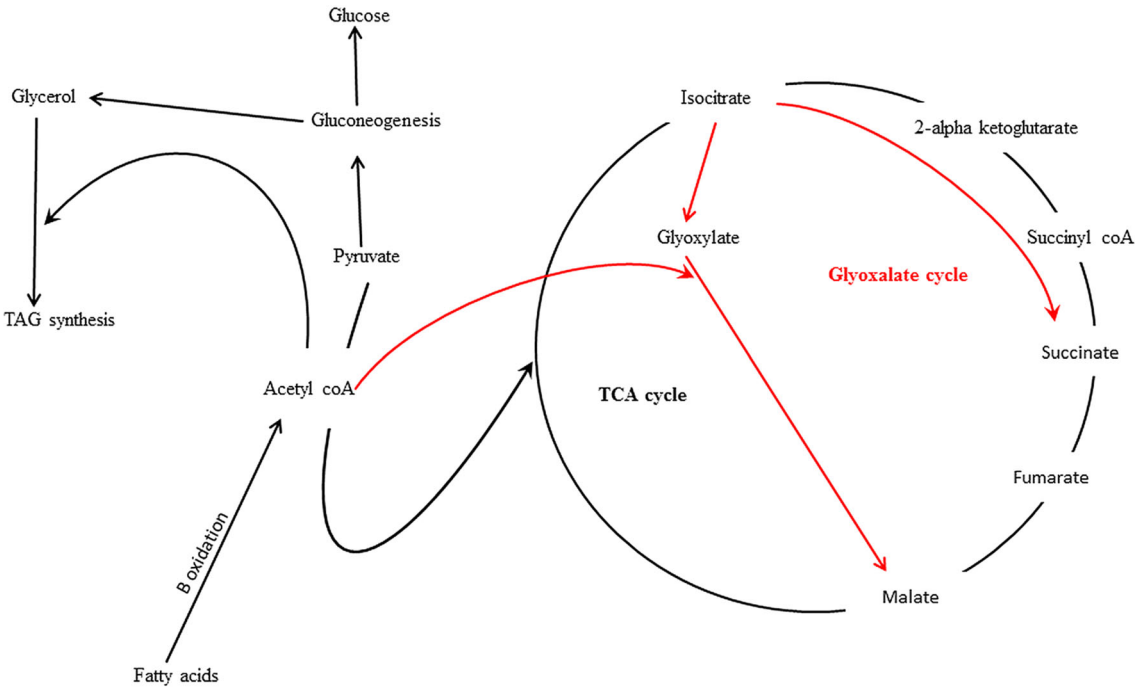

Figure 1. Involved metabolic pathways of Mtb during infection. Acetyl coA formed by the β-oxidation of fatty acids. Bacilli metabolizes C2 units by TCA cycle and intermediate glyoxalate pathway. Generation of pyruvate and gluconeogenesis replenishes the other glycolytic substrate. C3 units and acetyl coA required for the synthesis of TAG which is used in dormancy. Here bifurcation of carbon flux between TCA and glyoxalate cycle maintain the dormancy stage of Mtb.

of a class of diacylglycerol acyltransferase genes were identified and reported as tgs on the basis of *Acinetobacter calcoaceticus* (*A. Calcoaceticus*) gene homology (Kalscheuer and Steinbuchel 2003; Daniel *et al.* 2004); 24 members of various lipases/esterases and 7 members of cutinase-like protein (CULP) are found in *M. tuberculosis*. CULPs of Mtb does not degrade cutin; rather they work as lipases, esterase and phospholipases to serve diverse physiological functions which are used in storage and degradation of host fatty acids (Rastogi *et al.* 2016). In view of this evidence, it has also shown that, when mycobacterium cells are supplemented with exogenous fatty acids, these lipids bodies are formed (Deb *et al.* 2009).

However, bacilli have to be considered as anaerobic regulative bacteria that generate succinate molecules by consumption of two molecules of acetyl-coA through glyoxylate shunt mechanism (Monu and Meena 2016). The continual generation of energy source occurred when both tricarboxylic acid (TCA) cycle and glyoxylate shunt ran simultaneously (Mckinney *et al.* 2000). The balance between TCA cycle and glyoxylate cycle intermediates forms homeostasis in storing fatty acids during the latent phase (Kornberg 1965). In *M. tuberculosis*, fatty acid consumption depends on the bifurcation of carbon flux between the TCA and glyoxalate cycles (Murima *et al.* 2016). These studies can demonstrate that *M. tuberculosis* contained isocitrate lyase (ICL) genes which may be required to switch its role from isocitrate dehydrogenease (ICD) which is involved in TCA cycle (Garnak and Reeves 1979) in relation with its diet dependency from TAG to lipid source in mycobacterium's dormancy-like stage (Bishai 2000). Similarly, it was postulated that the other gene of *M. tuberculosis,* Rv3130c, is potentially involved in tgs induction during hypoxic conditions (Daniel *et al.* 2004) (figure 1).

In our perception, *M. tuberculosis* enters into the persistence stage within the host cell and starts solely on the lipid-rich host cell's debris in evolving granulomas and induction of tgs-associated genes aid to control energy supplement. It is more obvious that in this new environment, a glyoxylate shunt may supply the necessary precursors required for assembly of mycobacterial cell envelope as well as an alternative energy source in the dormancy-like state (Meena

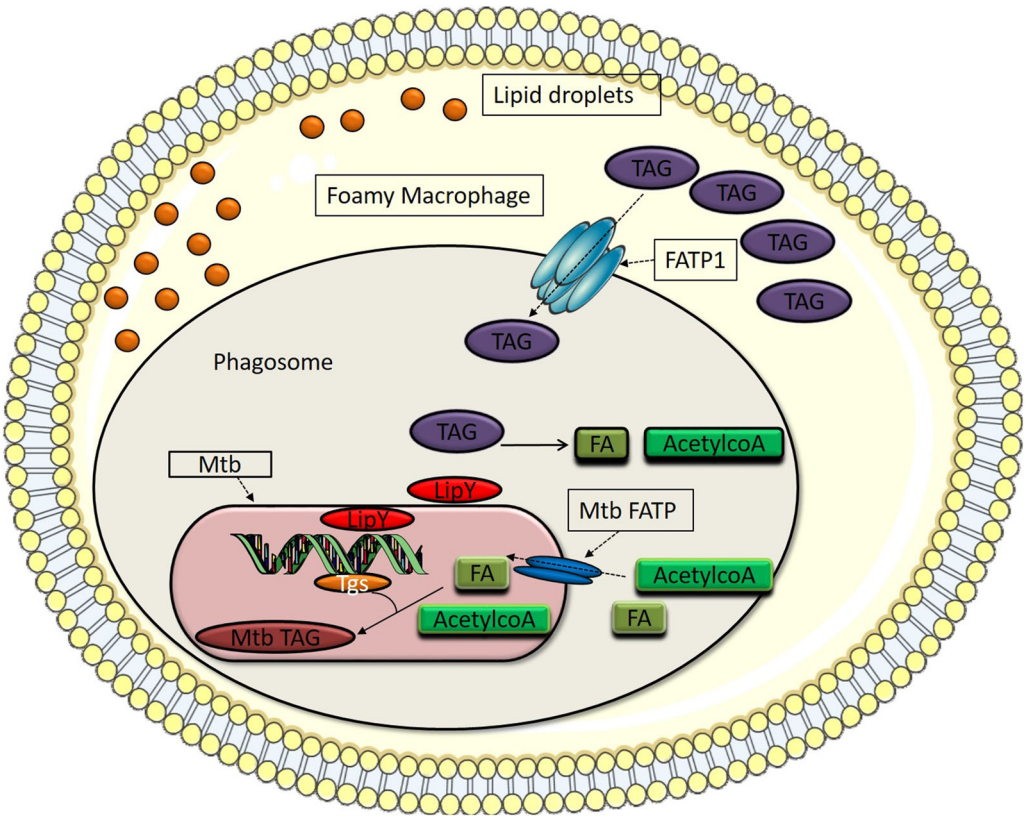

Figure 2. Graphical representation of TAG synthesis in *M. tuberculosis* with the help of tgs enzyme. Survival of *M. tuberculosis* within host macrophages is ensure by the utilization of its two important genes tgs and LipY. Host fatty acids are stored in the form of TAG within inclusion bodies in macrophages. During dormancy period LipY genes is activated which initiate the transport of host TAG with the help of its membrane transporter fatp1 (fatty acid transporter protein 1) in to the phagosome and catalyze them into fatty acids and acetyl-coA. Host fatty acids and acetyl-coA again transport within bacilli through mycobacterium transporter of fatty acid Fatp where *M. tuberculosis* enzyme tgs synthesize and convert them into bacilli TAG which is used by bacterium as energy source during its dormant stage.

et al. 2013). However, it is still an uncovered phenomenon to understand the exact mechanism involved in *M. tuberculosis* infection and how it TAG and fatty acids resting inside the macrophage are utilized.

M. tuberculosis is known to be obligate aerobes, and it has also been well known that mycobacterium encounters hypoxic environments in acute disease as well as in latent infection (Flynn and Chan 2001). Lip genes might be important for proper exploitation of host TAG during dormancy and upon renaissance after dormancy stage. These genes are responsible for the breakdown of host TAG and release fatty acids, and they also carry out the transportation of fatty acids inside the bacilli (Deb *et al.* 2006). Lip Y (Rv3097c) of mycobacterium contains the PE domain, which is a signal sequence for transportation of this protein to the surface of the bacilli by the ESX-5 transport system where it hydrolyses the host TAG (Mishra *et al.* 2008). It was established that, under oxygen depletion/tension, the bacillus terminates its own growth and enters into a non-replicating or dormant stage and retains the resistance to all conventional anti-TB drugs (Wayne and Hayes 1996). Our strategy was based on the hypothesis that bacilli that were disrupted for their enzymes including nitric reductase (Tan *et al.* 2010) and ICL implicated in the metabolic adaptation during the dormancy-like state. Hence, we proposed this hypothesis including fatty acids and tgs genes involvement in food supplement, which could be more significant for *M. tuberculosis* survival under the dormant stage (figure 2).

3. Foamy macrophages and dormant *M. tuberculosis*

Generally, the storage form of fatty acid is TAG in the adipose tissue of mammals, seed oils of plants and inclusion bodies in prokaryotes so as to serve as energy sources during dormancy or hibernation (Daniel *et al.* 2004). In the same way *M. tuberculosis* also needs a rich energy reservoir to sustain in the latent phase for its growth and survival, and so this nutrient-rich environment is provided by foamy macrophages (FMs) (Russell 2007). During initial infection, host immunity responds to bacilli by slowing the replication process and creating the basis for the pathogen to enter the dormant phase and become resistant to antibiotics (Gomez and Mckinney 2004). In mature granuloma structure, which is a distinctive feature of TB, many macrophages are packed with a large number of lipid-free vacuoles, and these macrophages occupied with lipid-containing bodies are termed as foamy macrophages (Cardona *et al.* 2000). These cells are enclosed by a layer of lymphocytes, and later a tight coat of fibroblasts encompasses the assembly (Saunders and Cooper 2000). The term foamy describes the lipid-loaded nature of these cells. It has been described earlier that only virulent strains of *M. tuberculosis* can induce the formation of FMs, and oxygenated mycolic acid plays a key role in induction. Normal macrophages after converting into FMs lose the microbicidal activity (Peyron *et al.* 2008). TAG accumulation is the critical event for the *M. tuberculosis* dormant stage, which depicts the importance of the *M. tuberculosis* enzyme triacylglycerol synthase 1 (tgs1). Tgs1 acts as the major contributor in TAG synthesis in the pathogen; as shown in a study, omission of tgs1 leads to the complete loss of TAG accumulation (Daniel *et al.* 2004; Sirakova *et al.* 2006; Deb *et al.* 2009). It has been revealed that TAG accumulated by *M. tuberculosis* is derived by using host fatty acids and the composition is much similar to host TAG (Garton *et al.* 2008).

4. Summary

The TAG accumulation capability of these genes indicated that not all tgs mutants showed significant effect under unfavourable growth conditions in *M. tuberculosis* infection. Despite many studies being carried out, the major challenge is still the understanding of how mycobacterium consumes these biomolecules and induction of their respective genes under hypoxic conditions which adapt the bacilli to the dormant-stage infection. FMs are the altered macrophage cells which are involved in the survival and growth of *M. tuberculosis* during dormancy. FMs are characterized by the activity of tgs1, hypoxic conditions, and accumulation of TAG. The conversion of macrophage into FMs inside the granuloma by the bacilli is an interesting way to deal with the stressful condition inside the host and to survive long term. Among many unresolved questions, one more important is through which mechanism in *M. tuberculosis* survive and what the energy sources are on which it survives during dormancy-like stage.

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