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Review

Guanosine triphosphatases as novel therapeutic targets in tuberculosis

Rajni, Laxman S. Meena *

Institute of Genomics and Integrative Biology, Mall Road, Delhi 110007, India

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ABSTRACT

Tuberculosis (TB) is an infectious disease caused by the aerobic microbe *Mycobacterium tuberculosis* $H_{37}Rv$. Despite the availability of the Bacille Calmette–Guérin (BCG) vaccine and directly observed treatment, short-course (DOTS), TB is a leading cause of death and affects a third of the world's population. The most important factor associated with disease severity is the development of antibiotic-resistant strains, including multidrug-resistant (MDR)-TB and extensively drug-resistant (XDR)-TB. In order to understand disease pathogenesis, it is necessary to delineate the specific features of *M. tuberculosis* that enable it to evade the host defense system and contribute to its virulence. Here, we have reviewed the various characteristics, such as cell wall components, virulence genes, and the role of small guanosine triphosphatases (GTPases) in the pathogenesis of TB. GTPases are known to play a crucial role in the survival and pathogenesis of various pathogens. The key role of these proteins involves interference in phagosome maturation arrest, enabling pathogens to survive by escaping from lysozymes and toxic free radicals. This observation provides a new avenue for the development of anti-TB drugs.

1. Introduction

Tuberculosis (TB) is a leading cause of mortality and affects one third of the world's population. This is a common and most deadly infectious disease, which spreads through the air when an affected person coughs, sneezes, or spits. It mainly attacks the lungs, but can also affect the central nervous system, lymphatic system, circulatory system, genitourinary system, gastrointestinal system, bones, joints, and skin.[1] A number of treatment and preventive strategies have been implemented over the last 50 years, with the Bacille Calmette–Guérin (BCG) vaccine being the most widely used against TB. Some antibiotics, such as rifampin and isoniazid, are also employed in the treatment of TB. However, spontaneous mutation, incomplete and inadequate treatment, poor administrative control, and irregular distribution of drugs have led to the development of multidrug-resistant (MDR)-TB, progressing to extensively drug-resistant (XDR)-TB.[2]

Mycobacterium tuberculosis $H_{37}Rv$ is a unique acid-fast Gram-positive bacterium; it neither contains a phospholipid outer membrane nor retains dye due to the high lipid and mycolic acid content of its cell wall. The *M. tuberculosis* $H_{37}Rv$ cell wall contains large amounts of glycolipid and is especially rich in mycolic acid, peptidoglycan, lipoarabinomannan (LAM), phosphatidylinositol mannosides (PIM), phthiocerol dimycocerosate, cord factor, sulfolipids, and wax D (Figure 1).[3–7]

Several approaches executed from time to time have identified a number of genes conferring survival and persistence within the host. It manipulates host defense pathways resulting in inhibition of apoptosis of infected host cells. These virulence genes include *nuoG*, *erp*, phospholipases c, and *fadE28*.[8–14]

In the past few years, extensive work has been done to understand the role of guanosine triphosphatases (GTPases) in the growth and development of bacteria. GTPases are also known as molecular switch proteins.[15] These proteins specifically bind and hydrolyze GTP, which in turn activates or inactivates the GTPase in a cyclic manner (Figure 2).[15] GTPases are highly conserved and function through RNA or ribosome binding. G1, G2, G3, and G4 motifs are responsible for specific interactions with the guanine nucleotide and effector proteins. Genome sequencing projects have revealed a core of 11 universally conserved GTPases, referred to as elongation factors G and Tu (EF-G and EF-Tu), initiation factor 2 (IF2), LepA, Era, Obg, ThdF/TrmE, Ffh, FtsY, EngA, and YchF.[15] These 11 GTPases are vital for bacterial life, since they regulate the cell cycle and distribution of DNA to daughter cells.[16–19]

Eukaryotic Rab GTPases are important regulators of different steps of the pathways leading to phagosome maturation arrest. Rab GTPases, in particular Rab5 and Rab7, regulate rate-limiting steps leading to maintenance and self-preservation of Mycobacterium within host macrophages (Figure 3).[20] Apart from these, Rab14 has also been identified as an important factor in maintaining the phagosome maturation block.[21] Another GTPase, FtsZ, has been characterized as an assembling GTPase, which functions in prokaryotic cell division. FtsZ antagonists disrupt its assembling activity and cause lethality to a variety of bacteria.[22]

* Corresponding author. Tel.: +91 11 27666156; fax: +91 11 27667471.
E-mail addresses: meena@igib.res.in, Laxmansm72@yahoo.com (L.S. Meena).

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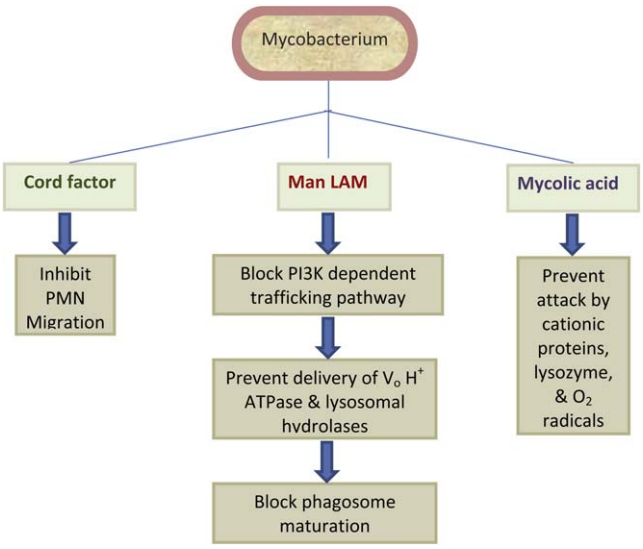

Figure 1. Significance of unique cell wall components of *Mycobacterium tuberculosis* against the host immune system.

These studies show a crucial role of GTPases in the survival of Mycobacterium within the host macrophage.

2. Tuberculosis as the major problem we are facing today

TB remains a global puzzle in spite of effective chemotherapy, the BCG vaccine, and the directly observed treatment, short-course (DOTS) strategy for its treatment. TB is a leading cause of mortality and morbidity and affects a third of the world's current population.[2] Other *Mycobacterium* species such as *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canetti*, and *Mycobacterium microti* also cause TB, but are relatively less common. Latent infection is the most common and significant stage, which if left untreated leads to death in more than half of the patients.[2] The BCG vaccine is one of the world's most widely used vaccines, despite showing variable effectiveness in different clinical trials. Treatment of TB employs anti-TB drugs to kill the bacteria, such as rifampin and isoniazid. A long time duration (around 6–12 months) is required to entirely eradicate Mycobacterium from the body.[23] There are two stages in the infection process: latent TB and active TB, which demand different

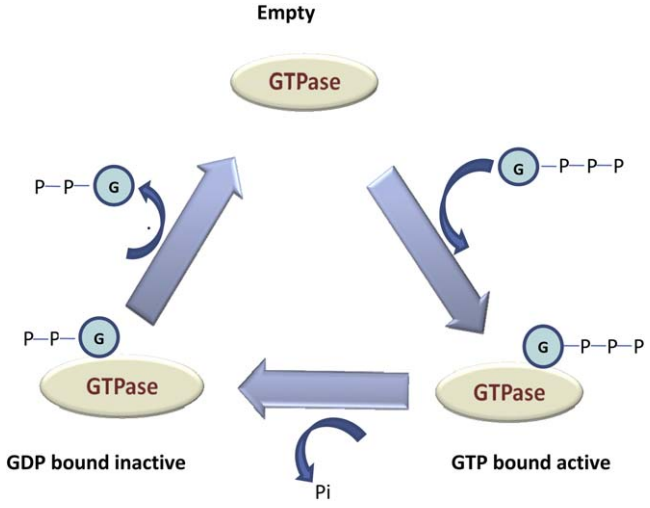

Figure 2. GTPase cycle depicting GTP binding proteins as molecular switch proteins cycling between the active GTP-bound and inactive GDP-bound state.

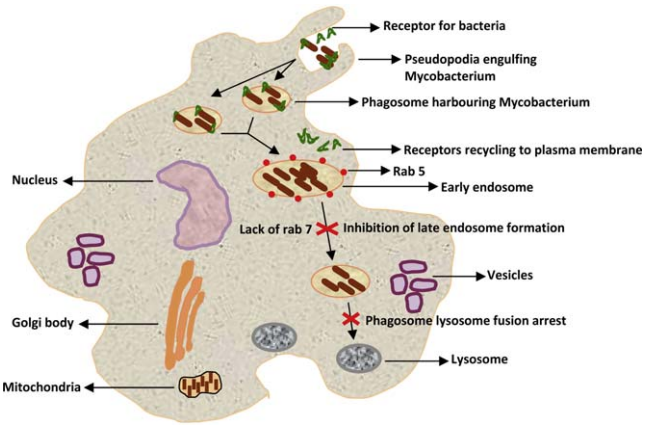

Figure 3. Role of Rab GTPases in phagosome maturation arrest: recruitment of Rab5 on the phagosome results in the formation of early endosome, but lack of Rab7 blocks its maturation into late endosome, ultimately leading to the inhibition of phagosome lysosome fusion.

treatments. Latent TB is treated using a single drug, while active TB is best treated using a combination of several drugs, to reduce the development of antibiotic-resistant bacteria.[24]

Spontaneous mutations in *M. tuberculosis* $H_{37}Rv$, incomplete and inadequate treatment,[25,26] poor administrative control on purchase, irregular distribution of the drugs, and improper quality control and bioavailability tests have led to the development of MDR-TB.[27] MDR-TB is resistant to rifampicin and isoniazid.[27] The DOTS strategy is a key factor involved in TB control.[28] This strategy is based upon clinical trials done in the 1970 s by the TB Research Center, Chennai, India. Mismanagement and ignorance in relation to treatment have led to the emergence of a new form of drug-resistant TB known as XDR-TB.[29] Based on the meeting of the World Health Organization (WHO) XDR-TB task force, XDR-TB has been defined as TB caused by *M. tuberculosis* $H_{37}Rv$ resistant to at least rifampicin and isoniazid among the first-line anti-TB drugs (MDR-TB), as well as resistance to any fluoroquinolones, i.e., ofloxacin, ciprofloxacin, and levofloxacin, and at least one of three second-line anti-TB drugs, i.e., amikacin, kanamycin, and capreomycin.[30] The increasing antibiotic resistance of *M. tuberculosis* $H_{37}Rv$ demands an urgent solution.

3. Mycobacterium tuberculosis—natural warriors

3.1. Unique cell wall structure

The cell wall structure of *M. tuberculosis* $H_{37}Rv$ has become cynosure in the field of research, as it is unique among prokaryotes and is a major determinant of virulence for this bacterium. *M. tuberculosis* $H_{37}Rv$ contains large amounts of glycolipid and is especially rich in mycolic acid, making up approximately 60% of the cell wall. The orientation of mycolic acids is perpendicular to the plane of the membrane, creating a special lipid barrier, which serves as the major factor contributing to the virulence of *M. tuberculosis* $H_{37}Rv$.[3] In addition to mycolic acid, other glycolipids include LAM, PIM, phthiocerol dimycocerosate, cord factor/dimycolyltrehalose, sulfolipids, and wax D.[4] Mycolic acids prevent an attack on Mycobacterium by cationic proteins, lysozymes, and the oxygen radicals in the phagocytic granule. Cord factor is toxic to mammalian cells and is also an inhibitor of polymorphonuclear neutrophil (PMN) migration, an important effector mechanism in the defense against external antigenic agents; cord factor is produced abundantly in virulent strains of *M. tuberculosis* $H_{37}Rv$. Wax D in the cell envelope is the major component of Freund's complete adjuvant (CFA).