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The Role of Cell Wall in *Mycobacterium tuberculosis* Resistance: Summary Review

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Abstract: **Tuberculosis (TB) is one of the oldest diseases known to affect humans and a major cause of death worldwide. TB is caused by bacteria of *Mycobacterium tuberculosis (Mtb)* and characterised by coughing of blood, wasting, spinal deformity, bone defects and deaths. Treatment of TB requires administration of various types of drugs for six months. However, most *Mtb* strains have developed resistance against many types of these drugs. Drugs resistance is focused on many mechanisms such as modification of drug metabolism pathways, bacterial cell wall, inactivation of drug by enzyme, activation of efflux pumps, and modified drug target among others. The cell wall of *Mtb* confers robust protection against any drugs or immune mediators and allows bacterial survival in very harsh condition. The plasma membrane separates the cytosol from the waxy coat which is made up of a granular layer composed of proteins leading to the periplasmic layer where peptidoglycan (PG) is connected to the Mycolic acid chains by arabinogalactan (AG). PG and AG together forms the insoluble cell wall skeleton. GL is Glycolipid, TMM is trehalose monomycolate, PL is Phospholipid and TDM is trehalose 6,6-dimycolate. *MmpL*, most successful drug target, are involved in the transport of various substrate especially lipids across cell membrane and represent one of the most important virulence factors of Mtb. The objective of this summary review is to describe the role of cell wall in *Mtb* resistance.**

Keywords: **Tuberculosis; *Mycobacterium*; Mycolic acid; *MmpL*; resistance.**

1. TUBERCULOSIS

Tuberculosis (TB) is also known as the white plague [1] and the Pott's disease or spinal tuberculosis [2] is characterised by coughing of blood, wasting, spinal deformity, bone defects and deaths. The disease, which is caused by a bacteria, *Mycobacterium tuberculosis (Mtb)*, is known to be one of the most deadly infection in the world and it was the main cause of death in Europe in the late 19th and early 20th century. Although there has been a decline in the number of deaths due to improvement in the health care system, there is an imminent epidemic threat fuelled by the emergence of drug resistant strains of the bacteria and the increasing population of immunocompromised patients [3]. Furthermore HIV Positive infected person has a 50% chance of developing reactivation of TB and those with immunosuppression are more likely to develop active disease after exposure [2].

2. PATHOGENESIS

Humans become infected by inhaling droplets containing the infectious agent that would settle down in the alveolar site where it can be engulfed by macrophages or dendritic cells or alveolar epithelial type II pneumocytes. In healthy individuals these bacteria can be eliminated by macrophages during phagosome-lysosome fusion where the engulfed bacteria is exposed to very harsh conditions such as low pH, contact with free radicals and oxygen species and toxic peptides and enzymes. However in the immunocompromised or vulnerable patient the bacteria can escape the immune system and establishes itself in the lung where it can proliferate and stay in a latent form [2].

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3. THE ORIGIN

The origin of *Mtb* is still being elucidated but it is hypothesised that the bacteria of the genus *Mycobacterium* that was living in the soil might have evolved to invade animals. With the introduction of domestication 10,000 and 25,000 years ago most probably has allowed transmission of the bacteria from livestock to human [2]. *M. bovis*, which is the causative agent of tuberculosis type disease in cattle is believed to be the ancestor of *Mtb*. The disease symptoms such as coughing blood and pain was described in Assyrian clay tablets from seventh century BC. The sudden increase in European population enhanced the spread of the disease in the 16th and 17th century and the epidemic peaked in the 19th century. In 1865 Jean-Antoine Villemin managed to infect laboratory rabbits using infected cadaver and 17 years later Robert Koch reported that the disease was caused by a bacteria. Ultimately the contradictions between Pidoux and colleagues in maintaining the argument that the disease was caused by poor sanitation, poverty, overworking and starvation rather than bacterial infection was cleared in the late 19th and early 20th century when the work of Edward Trudeau showed that the disease is caused by a bacteria, which is transmitted only in certain conditions such as poor sanitation, overcrowding and starvation [2].

4. TREATMENT AND CHALLENGES

Around 8.7 million cases of TB and 1.4 million deaths associated with *Mtb* infection were reported in 2011. Treatment requires administration of various types of drugs for six months and also many drug resistant strains have been reported. Treatment recommended by the World Health Organisation include daily rifampin, isoniazid, pyrazinamide and ethambutol for 2 months, followed by 4 months of daily isoniazid and rifampin. Strict adherence is required but the duration and intensity of treatment may contribute to failures. Also 3.5% of multi drug resistant (MDR) strains (resistant to Isoniazid and Rifampin) were reported in 2011 and emergence of extensively drug resistant (XDR) strains (MDR resistant to quinolone and any of the second-line drugs requires urgent development of new therapeutic agents to limit the spread of TB [4]. A limited number of strains isolated in South Africa, India and Italy are resistant to all drugs and are referred as the Total Drug resistant (TDR) group [5]. Various mechanisms of drug resistance such as modification of drug metabolism pathways, bacterial cell wall, inactivation of drug by enzyme, activation of efflux pumps, and modified drug target among others have been identified and described.

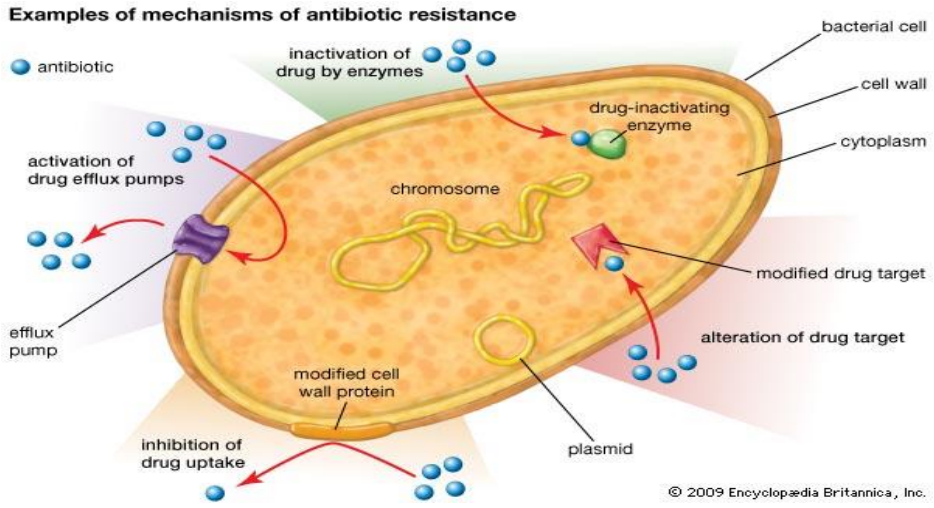

Figure 1. The various mechanisms involved in drug resistance [6]

5. MYCOBACTERIAL CELL WALL

The cell wall of *Mtb* confers robust protection against any drugs or immune mediators and allows bacterial survival in very harsh condition. It is composed of plasma membrane, an outer membrane (mycomembrane), and an outer layer known as the waxy coat [7] as shown in figure 2.

The cell wall contains three components including peptidoglycan, mycolic acid and arabinogalactan that are covalently linked and together they are referred to as mAGP [8]. Mycolic acids (MA), which are 2-alkyl, 3-hydroxy long-chain fatty acids are essential components of the waxy coat conferring resistance to various antibiotics and it is made up of 60-90

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fatty acid chains in *Mtb* [9]. (MA), Arabinogalactan (AG) and Peptidoglycan (PG) complex (MAPc), (another term for mAGP) is composed of cross-linked peptidoglycan that covalently linked to (AG) chains by phosphoryl-N-acetylglucosaminosyl-rhamnosyl linkage units. AG is esterified to various derivatives of MA. (PG) is insoluble and is made up of alternating units of N-acetylglucosamine (GlcNAc) and muramic acid residues, which are also modified by tetrapeptide (L-alanyl-D-isoglutaminyl-meso-diaminopimelyl-D-alanine) side chains. In the synthesis of cell wall Pol-P, which is formed by dephosphorylation of decaprenyl phosphate, is essential and is the rate limiting factor [10].

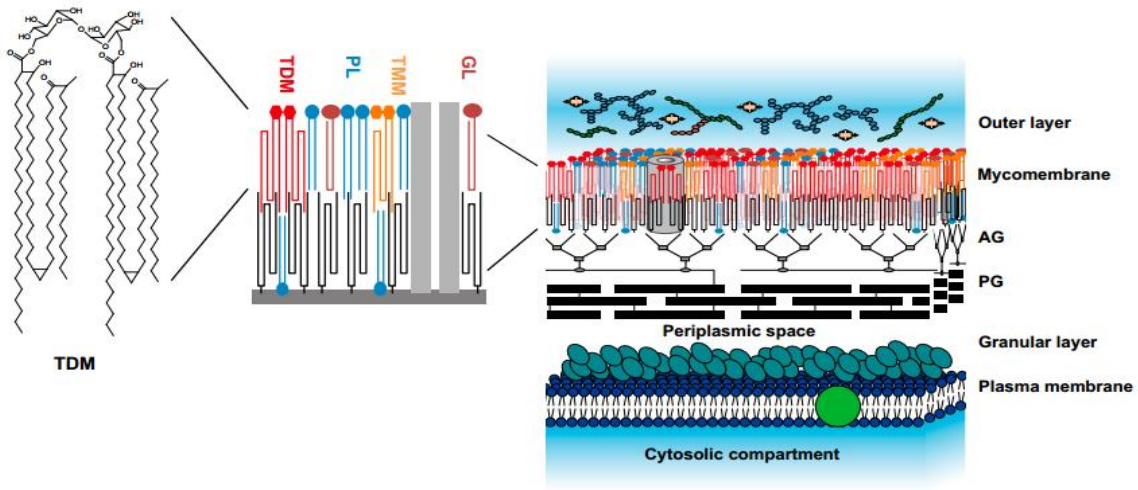

Figure 2. A model of the cell wall of *Mycobacterium tuberculosis* [9]. The plasma membrane separates the cytosol from the waxy coat which is made up of a granular layer composed of proteins leading to the periplasmic layer where peptidoglycan (PG) is connected to the Mycolic acid chains by arabinogalactan (AG). PG and AG together forms the insoluble cell wall skeleton. GL is Glycolipid, TMM is trehalose monomycolate, PL is Phospholipid and TDM is trehalose 6,6-dimycolate.

6. ROLE OF MYCOLIC ACID

(MA) synthesis is a vital step in the development of the waxy coat that protects *Mtb* from any attack. Biosynthesis requires two sets of synthases termed Fatty Acid Synthase I and II (FAS1and FASII). The first group of enzymes (FAS-I) synthesize fatty acids C_{16}-C_{18} and C_{24}-C_{26} while the second group (FAS-II) is responsible for adding multiple malonate units onto the C_{16}–C_{18} to generate the long-chain meromycolic acid (C_{48}–C_{64}) [11]. Further reactions add *cis* or *trans* double bonds, *cis* or *trans* cyclopropane rings and polar groups such as methyl ethers, ketones, esters or epoxides to form the functional groups. .Elongation of the side chain followed by Claisen condensation reaction of C23–C27 by the acyl-AMP ligase FadD32 and polyketide synthase PKs 13 form the alpha and beta keto acyl derivatives, which are further reduced by the enzyme reductase CmrA to form MA derivatives [12]. Finally Mycolic acid is transferred as trehalose monomycolate (TMM) to cell wall where it is incorporated in the waxy coat as trehalose 6,6-dimycolate (TDM) and mycolyl arabinogalactan by the mycolyltransferases of the antigen 85 complex (FbpA, FbpB and FbpC) [13] However the mechanism of transfer to cell wall is still unknown but lipids such as free mycolic acid of the waxy layer are not only essential for the formation of biofilms but also help in immunomodulation [14]. Since mycolic acid contributes to virulence of *Mtb* more researches are being focused in elucidating the mechanism involved in the transport of mycolic acid to the cell wall and outer surface. Knowledge of the mechanism involved in the transfer of Mycolic acid and its incorporation to the various components of the cell wall will enhance the development of new drugs against *Mtb* infection.

7. THE PROTEIN LAYER

The protein layer found within the periplasmic space consists of efflux pumps that transport various compounds across the cell envelope. These efflux pumps have been classified in five major groups including ATP-binding cassette (ABC), resistance nodulation division (RND), major facilitator super-family (MFS), multidrug and toxic-compound extrusion (MATE) and small multidrug resistance (SMR). In the RND family of membrane proteins there is a special group of proteins called Mycobacterial membrane protein large (*MmpL*), which is an inner membrane protein family [5]. In *Mtb*,

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13 actinobacteria-specific inner membrane proteins were found, it belongs to the (RND) family of transmembrane transporters [15].

8. MYCOBACTERIAL MEMBRANE PROTEIN LARGE

MmpL, which consist of two non-transmembrane - loops and 12 transmembrane domains [16], are involved in the transport of various substrate especially lipids across cell membrane and represent one of the most important virulence factors of *Mtb*. Therefore they have been the most successful drug target [15]. It is known that mutation in *MmpL 4* and *7* can affect virulence while *MmpL* 5 and *MmpL*10 are required for survival of *MtB*. *MmpL* 8 and *MmpL 11* mutants are less pathogenic compared to wild type. *M. smegmatis*, which is well known for its use as a non-pathogenic model representing *MtB*. *MmpL4a and MmpL4b* (TmtpB and TmtpC) deletions in *M. smegmatis* is known to be associated with altered colony morphology with reduced motility and biofilm formation. They are also characterised by the absence of glycopeptidolipids (GPLs) on their surfaces [14]. Furthermore the effect of *MmpL 11* deletion was found to be associated with impaired biofilm formation, which was restored by *MmpL* gene from *Mtb*. The *MmpL 11* gene, which shares the same locus as *MmpL3* is found in a much conserved area and the *MmpL11* protein in *M. smegmatis* shares 69% identity with that of *Mtb*, which would explain a similar function. In the absence of the *MmpL11* protein, monomeromycolyl diacylglycerol (MMDAG) and mycolate ester wax are synthesised but not transported to the surface. In addition to that there was an accumulation of the mycolic acid precursor molecule mycolyl phospholipid (MycPL). Similarly *MmpL 3* helps in transport of trehalose monomycolate (TMM) while *MmpL* 11 assists in the transport of (MMDAG) and mycolate ester wax. Both proteins are phylogenically related with 26% amino acid identity in *M smegmatis* [14]. In addition to that they are both implicated in the transport of heme. One of the protein (Rv0203) seems to be playing a key role in recruiting heme and transporting heme to the intracellular space with the help from *MmpL 3* and *MmpL11* [17].

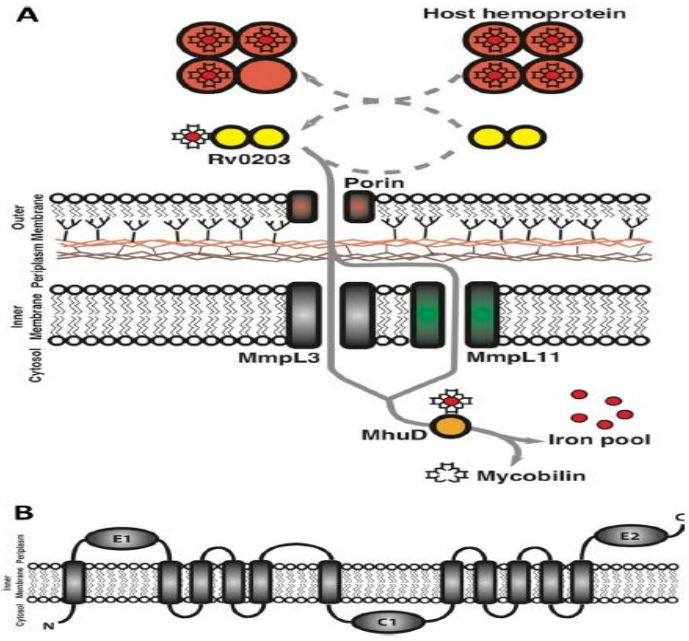

Figure 3. A model of the involvement of *MmpL3* and *MmpL11* in the acquisition of iron from heme molecule [17].

9. THE STRUCTURE AND FUNCTION OF *MmpL3/11*

The *MmpL3* and *MmpL11* genes are found in very close genetic content and also in an area where most genes code for transmembrane proteins while other *MmpL* genes are found in a cluster where most genes are associated the biosynthesis of cell wall-associated glycolipids such as phthiocerol dimycocerosate (PDIM), sulfolipids, polyacylated trehalose, glycopeptidolipids, and lipooligosaccharides [16]. However they are not found anywhere near any gene associated with the synthesis of Mycolic acid. *MmpL3/11/13* have three soluble domains D1, D2 and D3 while the rest of *MmpL* proteins have only two soluble domains D1 and D2. The structure of D1 and D2 domains similar to those of the RND transporter porter domains except that they are shorter (Approximately 150 residues versus 300 residues) [15], which would indicate that both domains are located in the periplasmic region. Compared to RND periplasmic domain that contain one docking

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subdomain and two porter subdomains, *MmpL3/11* seem to have only one porter subdomain βαββαβ motif flanked by α helices (α1 and α4) [18]. Using purified D1 and D2 domains weak or no interaction was observed between the two. Interaction was dependant on the α1 helix and the D1-D2 heterodimers are not stabilised by β sheets. Unlike D2, D1 domain is involved in binding heme. In contrast domain 3, which is found in the cytoplasm, is predicted to be alpha helical and largely unstructured. Since mycolic acid and its derivatives are synthesised in cytoplasm, their transfer to cell wall might involve interaction with the D3 domain, which is located in the cytoplasm.

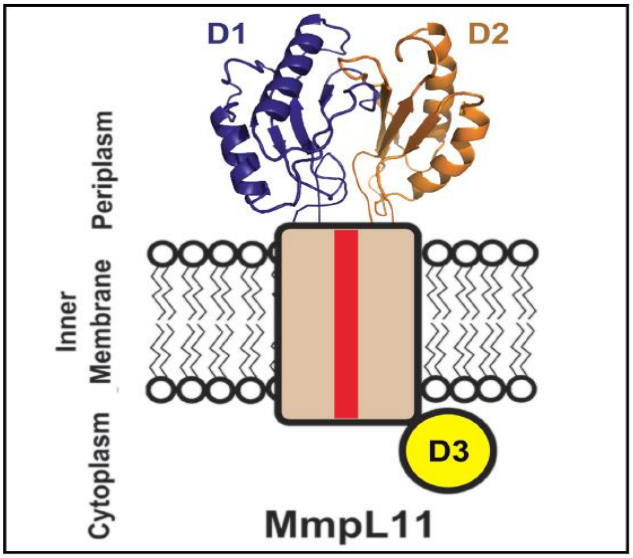

Figure 4. A model of *MmpL11*. It is predicted that the D3 subunit lies in the cytoplasmic while D1 and D2 domains are in the periplasmic region [15].

10. PROTEIN INTERACTION WITH *MmpL*

It is known that many *MmpLs* are involved in the transport of various compounds but the exact mechanism involved is still unknown. It has been suggested that the proton motive force (PMF) of the transmembrane electrochemical proton gradient assist in the transport of substrate across the cytoplasmic membrane [19]. Similar to *MmpL8*, which is required to transport sulfolipid-1 (SL-1), *MmpL7* involved in the transport of phthiocerol dimycocerosate (PDIM) to the cell surface [20]. Using the two hybrid test, in 2005 Jain and Cox managed to show interactions between *MmpL7* domain2 and PpsE, which is a key component required in the extension of fatty acids straight chain to phthiocerol, a precursor of mycocerosic acid (Mas) [20]. Therefore protein interaction such as that between *MmpL7* and PpsE is required for phthiocerol dimycocerosate (PDIM) biosynthesis and transportation across the cell wall. Furthermore it has been proposed that *MmpL* is involved in the formation of scaffold in order to facilitate transport of lipids and Mycobacterial membrane protein small (*MmpS*) are proteins that have been found to help in the formation of membrane-associated scaffolding required for linking lipid biosynthesis and transport system [21]. In addition to the building of the scaffold, flipping of the mycolic acid intermediate and *MmpL* complex may also help in transfer. Interaction of *MmpL* and *MmpS* is also required for other processes such as drug resistance, heme uptake (Protein Rv0203-*MmpL3* and *MmpL11* interaction), and export of siderophore (*MmPL4-MmpS4* and *MmpL5-MmpS5* interactions) [5]. Similar mechanism involving interactions between cytoplasmic proteins and intracytoplasmic domains of *MmpL 3/11* are believed to assist in the transfer of Mycolic acid from cytoplasm to cell wall and outer surface of *Mtb* and identification of the interacting protein would provide useful information not only to elucidate the mechanism involved but also provide a potential target for future anti mycobacterial drug development.

11. CONCLUSION

This paper summarized various mechanisms of drug resistance, bacterial cell wall was one of these mechanisms. Further research is recommended to identify the proteins that interact with *MmpL* transporter from *Mycobacterium Tuberculosis*. Future studies are required for developing candidates for vaccine development and therapeutic strategies against *Mycobacterium tuberculosis*.

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