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The Cell Wall of Mycobacteria

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ABSTRACT *The cell wall of mycobacteria deserves special mention due to its unique structure among prokaryotes and a major determinant of virulence. It is composed of peptidoglycan and more than 60% of the cell wall has complex lipids. Mycolic acids are unique α-branched lipids found in other genera like Nocardia and Corynebacteria. The unique cell wall is responsible for impermeability to stains and dyes, resistance to many antibiotics, killing by acidic and alkaline compounds, resistance to osmotic lysis by complement deposition and survival inside macrophages.*

Introduction

Mycobacteria are nonmotile, nonspore-forming, and aerobic or microaerophilic straight or slightly curved bacilli. There are nearly 200 species grouped into 'slowly growing' and 'rapidly growing' mycobacteria. They belong to the family Mycobacteriaceae, order Actinomycetales, and are related to other mycolic acid-containing genera. They are acid and alcohol-fast, contain mycolic acids containing 60-90 carbon atoms. The unique structure of their cell wall is a major determinant of virulence.

Structure of the mycobacterial cell wall

Mycobacteria have a complex outer envelope composed of several distinct layers. The innermost layer is the plasma membrane, which has an asymmetric bilayer structure. Proteins, phosphatidylinositol mannosides and lipoarabinomannan are inserted into it. Next lies the peptidoglycan (PG) layer, which determines the shape of the cell and is similar to peptidoglycans of gram positive bacteria. It contains repeating disaccharide units of N-acetyl-glucosamine-(β1-4)-N-glycolylmuramic acid, cross-linked via L-alanyl-D-isoglutaminyl-meso-diaminopimelyl-D-alanine, except in M. leprae, where the L-alanine is replaced by glycine [1]. Some muramic acid residues are linked to a branched-chain polysaccharide arabinogalactan via phosphodiester bonds. Arabinogalactan molecules are esterified to high-molecular-weight mycolic acids. The outer surface of the mycobacterium is formed by intercalation of medium-chain (mycoserates) and short-chain (acylglycerols) lipids, glycolipids and peptidoglycolipids into the mycolic acids. Porins and transport proteins are found throughout the various layers.

Researchers have recently found that mycobacterial PG contains both N-glycolyl and N-acetyl modifications [2]. The actual percentage of glycolylation in the muramic acid residues of mycobacteria is still debatable. Approximately 25% of cross-links in the PG of M smegmatis (and other mycobacteria) are unique in that the bonds are between two diamino-pimelic acid (DAP) molecules (DAP-DAP) while the remaining cross-links are the typical DAP-alanine. [3]

The arabinogalactan possesses a branched structure and consists mainly of 1-5 linked D-arabinofuranose units and 1-4 linked D-galactopyranose units, the two sugars in approximate molecular proportions of 5 to 2 [4]. The arabinogalactan is consistently shorter in M leprae than in M tuberculosis (13 versus 23 residues, respectively). More arabinogalactan molecules are present per peptidoglycan molecule in the M leprae cell wall (3.0 AG molecules/ 10 repeating units of PG) than in M tuberculosis (1.3 AG molecules/ 10 repeating units of PG). Thus, the cell wall of M leprae has a higher degree of mycolylation per 10 repeating units of PG (20.8 for M leprae compared to 16.4 for M tuberculosis) [5].

The outer surfaces of mycobacteria have a high content of lipids. This contributes to their tendency to form clumps and cords and ability to survive for a long period of time. Lipoarabinomannan (LAM) is related to the O-antigenic lipopolysaccharides of other bacteria [6]. LAM is anchored in the cell membrane and extends through the cell wall to the surface of the mycobacterium. The membrane anchor is a diacylglycerol moiety which contains tuberculostearic acid and palmitic acid in M tuberculosis. The termini of LAM molecules may be branched hexa-arabinosides or linear tetra-arabinosides.

In slow-growing, pathogenic mycobacteria like M tuberculosis and M leprae, the LAMs are capped at the terminal β-Ara residue with mannose residues and are referred as ManLAMs. The fast-growing mycobacteria like M smegmatis and M fortuitum have phosphatidylinositol-capped LAMs known as PILAMs. In M chelonae LAMs are devoid of caps and are referred to as AraLAMs [7].

The genus Mycobacterium is characterised by the presence of mycolic acids. The mycobacterial mycolic acids are α-branched, β-hydroxy fatty acids with 60-90 carbon atoms in the primary chain. Mycolic acids have been described as "monstrous mycobacterial molecules" discovered by R J Anderson during his classical systematic investigation of the chemistry of the lipids of mycobacteria [8]. There are three principal categories, (i) the corynemycolic acids, ranging from C_{28} to C_{40}, found mainly in Corynebacteria, (ii) the nocardomycolic acids, ranging from C_{40} to C_{60}, found in Nocardia and (iii) the mycobacterial mycolic acids, ranging from C_{60} to C_{90} [9]. Studies using cryo-electron microscopy have shown that the inner half of the outer membrane of mycobacteria is consistent with the mycolic acids occurring in a folded configuration based on a Langmuir monolayer packing. Mycolic acids are categorised according to oxygen-containing modifications found in the primary chain. The methyl branches or methylene groups of the cyclopropane rings of the long mycolic acid chains are

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introduced by C-methylation. Each species of Mycobacterium synthesizes a unique set of mycolic acids, which has been exploited for identifying them [10].

Trehalose dimycolate (TDM), also known as cord factor, is responsible for systemic toxicity and cytokine induction. It is a 6-6' dimycolate of trehalose shown by Noll et al in 1956 [11]. It is a unique glycolipid with a trehalose and two molecules of mycolic acids. Its molecular structure varies within mycobacterial species [12] and its antigenic structure profoundly influences host immune response.

The outer surface of mycobacteria contains a heterogenous group of biologically and immunologically active medium- and short-chain lipids, analogous to the O-antigens of gram-negative enteric bacteria. They can be peptidoglycolipids, which contain mycoserosic acid, sugars and aminoacids, or phenol-phthiocerol glycosides. The C-mycosides are structurally well-defined hydrophobic glycopeptidolipids found in several mycobacterial species [13]. They are responsible for determining serotype, colony morphology and virulence.The C-mycosides are the serologically active typing antigens of the Mycobacterium avium-intracellulare complex. Mycosides lacking amino acids are derivatives of phenol-phthiocerol and have been isolated and characterised from M kansasii, M bovis, M marinum and M leprae. Phenolic glycolipid I (PGL-I) is found only in M leprae and is synthesised and shed in large amounts. It is extremely lipophilic due to its phthiocerol dimycocerosyl component and evokes a high content of IgM antibodies [14]. The immune response to the trisaccharide component of PGL-I is used in immunodiagnostic tests for M leprae. PGL-I downregulates the inflammatory immune response and inhibits dendritic cell maturation and activation, thus resulting in the persistence of disease.

Biological activity of the mycobacterial cell wall

The mycobacterial cell wall has a number of biological activities. Freund's adjuvant (heat-killed mycobacterial cells in a water-in-oil emulsion containing the antigen in the water phase) is well known to immunologists. The murein layer as well as long-chain mycolic acids are required for the adjuvant action. A water-soluble fragment of the peptidoglycan called muramyl dipeptide (MDP) (N-acetylmuramyl-L-alanyl-D-isoglutamine) also acts as an adjuvant. MDP has the advantages over heat-killed mycobacteria in that it can be chemically synthesized and is commercially available. Injection of intact mycobacterial cells into mice can produce immunity to heterologous infections [15]. Bacille Calmette Guerin (BCG) produces a stimulatory effect on the phagocytic activity of the reticuloendothelial system and increases resistance of mice to Salmonella enteritidis. The pretreatment of mice with Freund's adjuvant shortens the delay in the production of interferon appearance after viral infection. The N-glycolyl-muramic acid and N-acetyl muramic acid (NAM) could tighten the PG sacculus by providing more opportunities for hydrogen bonding and increase resistance to β-lactams and lysozyme [16].

LAM contributes to inhibition of interferon-γ-mediated activation of macrophages, induction of cytokine production and release by macrophages, scavenging of reactive oxygen intermediates and suppression of T-cell proliferation. The arabinose units are targets of circulating antibodies and their degree of mannosylation influences the biological activities of LAM. The key ligand in dendritic cell (DC)-specific intracellular adhesion molecule-3 grabbing non-integrin (SIGN) has been identified as LAM. This influences bacterial persistence and host immunity [17]. Mycolic acids prevent the attack of mycobacteria by cationic proteins, lysozyme and oxygen radicals in the phagocytic granule. They also protect extracellular mycobacteria from complement deposition in serum. Trehalose dimycolate (TDM) causes structural disintegration of the mitochondrial membrane and a decrease in mitochondrial respiration and phosphorylation. It has an ability to induce a wide range of chemokines (MCP-1, MIP-1alpha, IL-8) and cytokines (e.g., IL-12, IFN-gamma, TNF-alpha, IL-4, IL-6, IL-10) [18].

The peculiar structure of the cell wall also contributes to resistance against antibiotics. Mycobacteria show an intrinsic resistance to many antibiotics and chemotherapeutic agents. Hydrophilic agents cross the cell wall slowly as the mycobacterial porin is inefficient in allowing the permeation of solutes and exists in low concentration. Lipophilic agents may be slowed down by the lipid bilayer which is of low fluidity and abnormal thickness [19]. The M chelonae cell wall is 30 times less permeable to hydrophilic molecules than E coli [20] while M smegmatis was found to be 20 times less permeable than E coli [21]. The permeation ability of a lipophilic molecule is inversely related to the fluidity of the cell wall, which decreases as the length of fatty acids in the mycolic acid layer increases [22]. Lipophilic drugs, such as the fluoroquinolones or rifamycins, pass more easily through the lipid-rich cell wall and thus are more active [23].

Conclusions

The cell wall of mycobacteria is strikingly different from that of other gram positive bacteria. This unique structure contributes to their virulence and prolonged, antibiotic resistant infections are caused by these organisms. A deeper understanding of the structure and biological functions will help us to tackle the heavy burden of mycobacterial infections in our country.

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