Tuberculosis- Part. 7

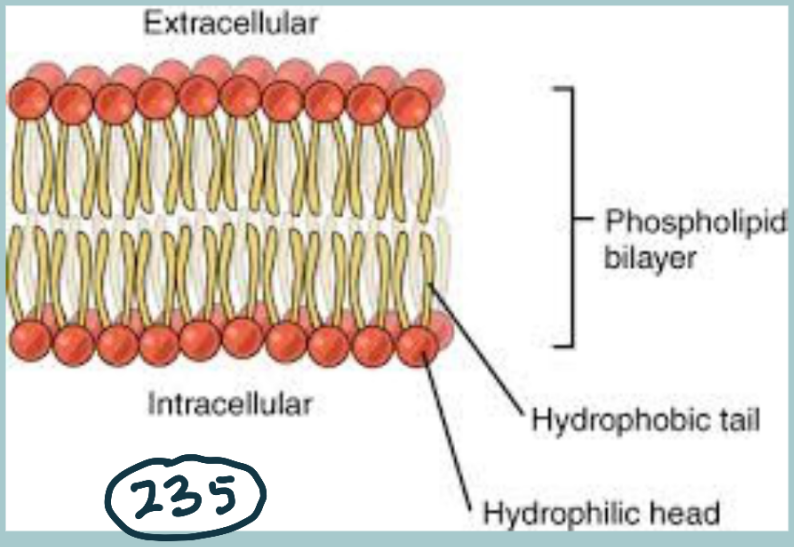
Structure of Mycobacterial Cell envelope and drugs acting on it

12.5. Cell envelope

All bacteria have an *envelope* which is defined as the *plasma or cell membrane* and all the layers external to it. Apart from *plasma membrane* a bacterial cell has a cell wall and at least one *additional layer* (*capsule* or slimy layer). The cell envelope of bacteria therefore has plasma membrane, cell wall and a capsule. The structure of cell envelope in gram positive and gram negative bacteria is different. Gram negative bacteria has cell wall sandwiched between two membranes- inner and outer (inner is plasma membrane), it's cell wall is thin, and it has lipopolysaccharide in the outer membrane which is an endotoxin. The configuration of cell envelope of mycobacteria which are gram positive organisms is also slightly different. It has mycolic acid in the cell wall which other bacteria do not have. Let us unpeel the different layers, inside out. It has plasma membrane, periplasmic space, layer of peptidoglycon, layer of arabinogalacton and mycolic acid which together form the cell wall and outer free lipids and the outermost capsule.

Layer 1: *Plasma membrane* (235)is common to all living cells. It surrounds the cytoplasm of cell. It is the most important component of the cell envelope and all living cells, bacteria or human, are equipped with it.

It is made of two layers of phosphate- lipid (fatty acid) within which proteins float. It has a phosphate head



which is water loving (or polar) and fatty acid tail which is water hating (non polar). The two layers are arranged in such a way the heads face out and tails are tucked inside facing each other. So, what gives a membrane its shape? It is the way phospholipids will arrange themselves. The hydrophilic heads love water, so they want to face the outside or inside of the cell. The hydrophobic tails want to be away from the water, so they will interact with each other. So, the tails snuggle into each other and away from aqueous regions. The heads are on the surface in contact with the aqueous environment. Since the cell is surrounded by water environment and it contains water inside it is essential that water loving heads face outside and inside. The tail part provides the permeability barrier- the

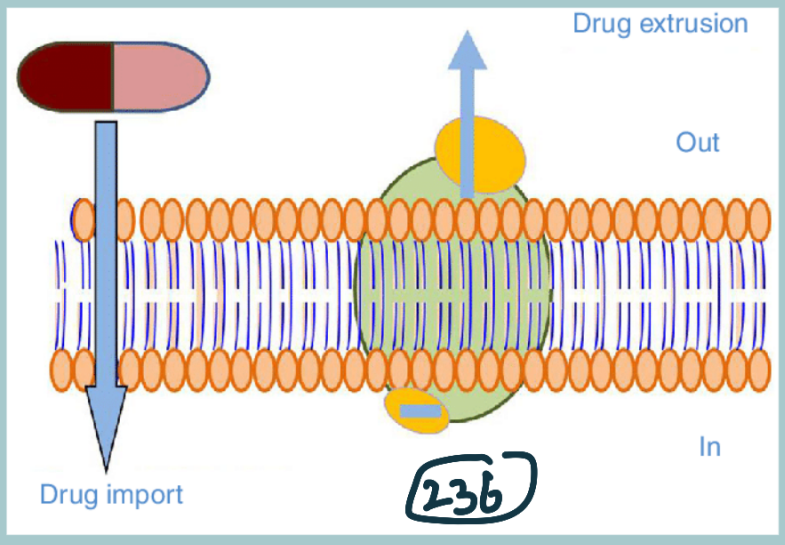
two tails of the two layers face each other- and selectively permits certain substances- *small molecules, non charged particles or fat soluble substances* to pass through and others are prevented from moving across. If you are fat soluble you get free pass. If you are water soluble you are allowed inside through special protein channels or transport proteins. It is not a solid structure but a constantly moving fluid. It is like fluid with proteins, carbohydrates and lipids inside moving freely. It displays lateral movement or fluidity. When you pick it with a blunt pin it makes a dent and flows around it. This is advantageous when baby cells are growing. This allows the membrane to grow without losing its elasticity or expand without breaking. If there is a cut it can easily rejoin. The membrane is less fluid in Mycobacteria so that it is less flexible than in other bacteria. This is due to the fact that the bilayer is densely packed with fatty acids.

It is responsible for permeability of cell envelope controlling movement of substances in and out, synthesis of cell wall components by ribosome which is attached to the plasma membrane (the components synthesised by plasma membrane bound ribosome become part of cell wall or capsule or secreted outside).

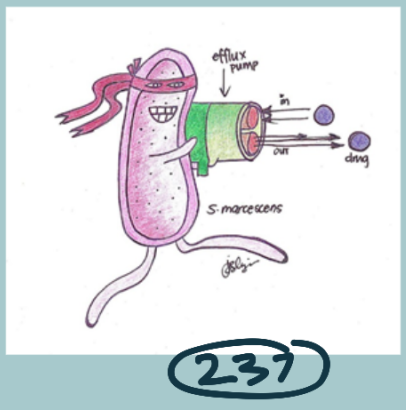
There is also an *efflux pump (236)* (efflux is expel out)

in the

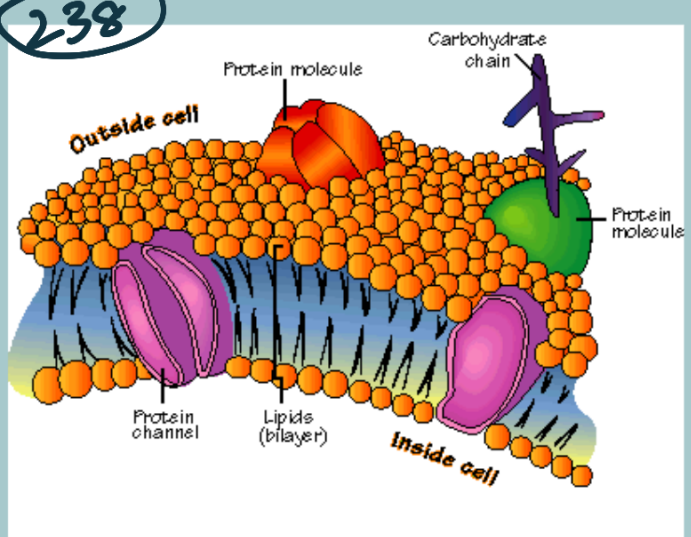
plasma membrane which pushes unwanted or undesirable substances like antibiotic outside.



Part of the resistance in aminoglycosides is due to the action of efflux pump (237)which expels the antibiotic from the cytoplasm.



There are certain areas in the cell membrane where the phospholipids are more densely packed and there is lot of cholesterol in between the fatty acids. Bacterial PL membrane does not have cholesterol, instead it has hopanoids similar to cholesterol. In this special area where fatty acids are densely packed and there is lot of hopanoids. There are special proteins which float along the membrane in these fatty acid and cholesterol dense areas like "butter on buttermilk". These proteins are called *associated proteins (238)*which are temporarily or permanently attached to plasma

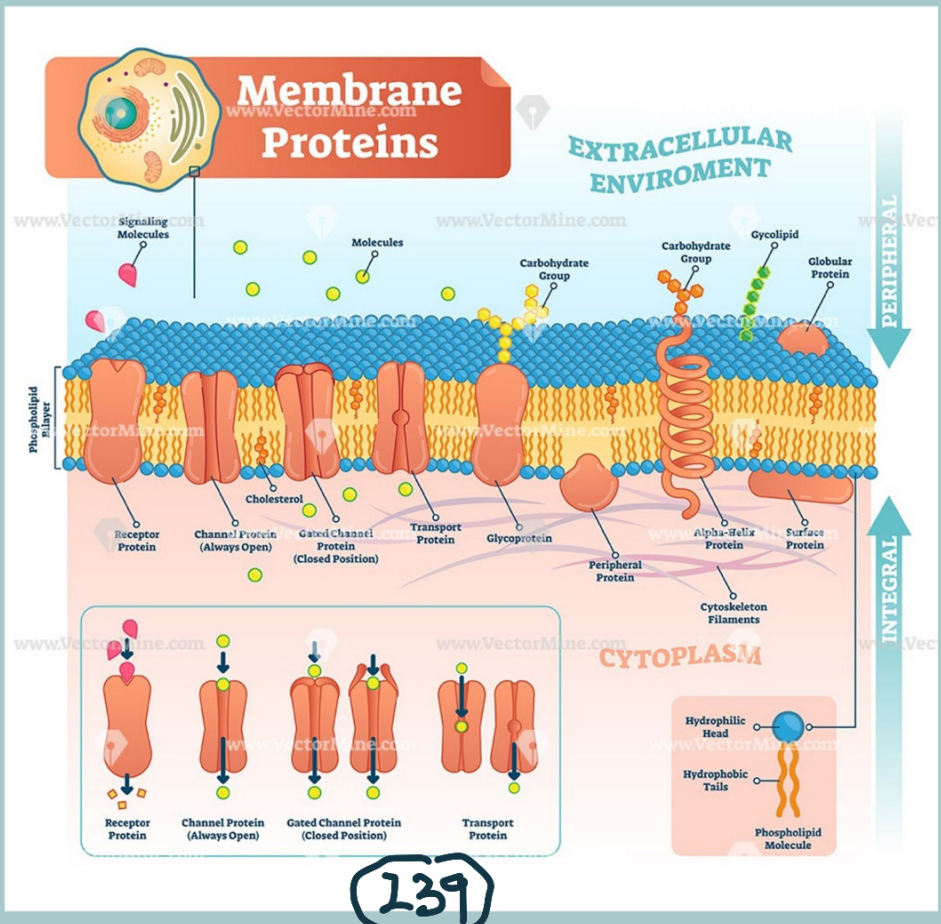


membrane.

Each of these proteins has a specific function, and that function often requires them to extend beyond the membrane. Some membrane proteins sit on the surface. Some proteins pass through and through or some may be attached to outside or inside the membrane. Those that pass throughout the membrane are called *integral proteins or transmembrane proteins*. These proteins have an extracellular and intracellular part or domain. Those proteins which are found entirely outside or inside are called *peripheral proteins(*20 to 30%)- loosely attached to membrane by noncovalent bonding. (Covalent bond is strong) So it can be easily removed. Integral proteins are tightly connected, cannot be removed without disrupting the membrane. These proteins are called *receptors*. Others are anchored in the membrane and have pieces (called domains) on one or both sides of it.

Proteins with extracellular domains (which means they're outside the cell) are usually involved with cell-to-cell communications or interactions. Proteins that reside mainly within a membrane usually form channels or pores to allow molecules to cross the membrane. Proteins with intracellular domains (which means they're inside the cell) have the widest range of function.

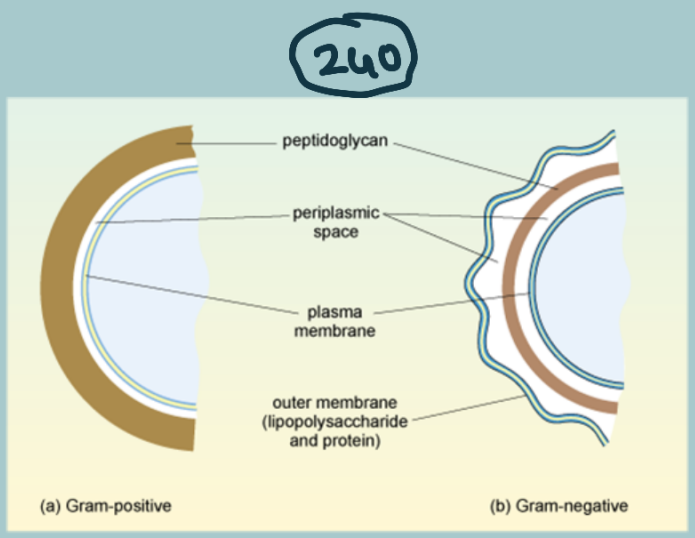
These membrane proteins (238,239) can serve a variety of key functions:



- Enzymes – These proteins have extracellular part which binds to ligand which stimulates the intracellular part and activates enzyme which brings about the change inside the cell.
- Transport – large molecules or charged molecules or water soluble molecules cannot pass through plasma membrane by passive diffusion. They are facilitated to diffuse across by transport proteins. Example is sodium ion or glucose.
- Recognition – May function as markers for cellular identification - Pathogen Associated Molecular Pattern (PAMP) on the surface of pathogen identifies the pathogen like an ID badge.
- Transduction – They allow molecules to diffuse across and initiate action.

Layer 2:

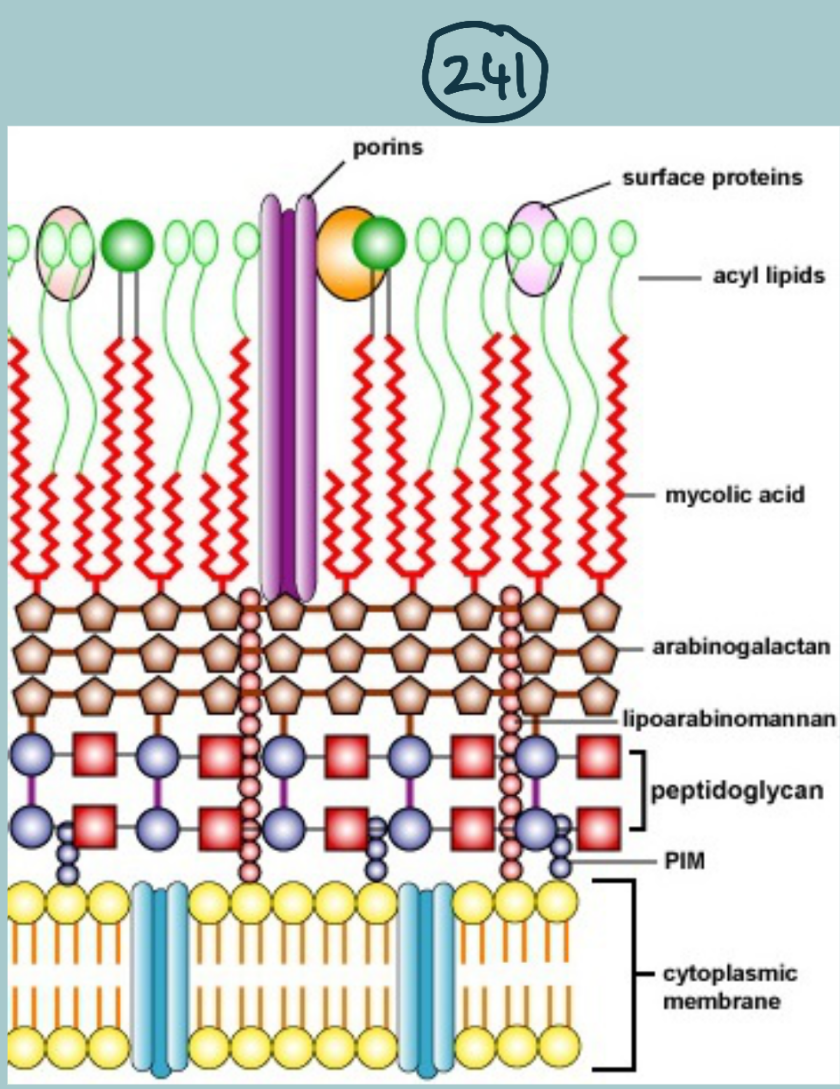
This is not exactly a layer but a space outside the plasma membrane called periplasmic space (240). Found in this space are binding proteins for various substances, enzymes which degrade substances (like



protease) and antibiotic detoxifying enzymes (β-lactamases, alkyl sulfodehydrases, and aminoglycoside phosphorylating enzymes).

Layer 3:

Cell wall (241). It is made of three distinct layers- *peptidoglycon, arabinogalactan and mycolic acid.*

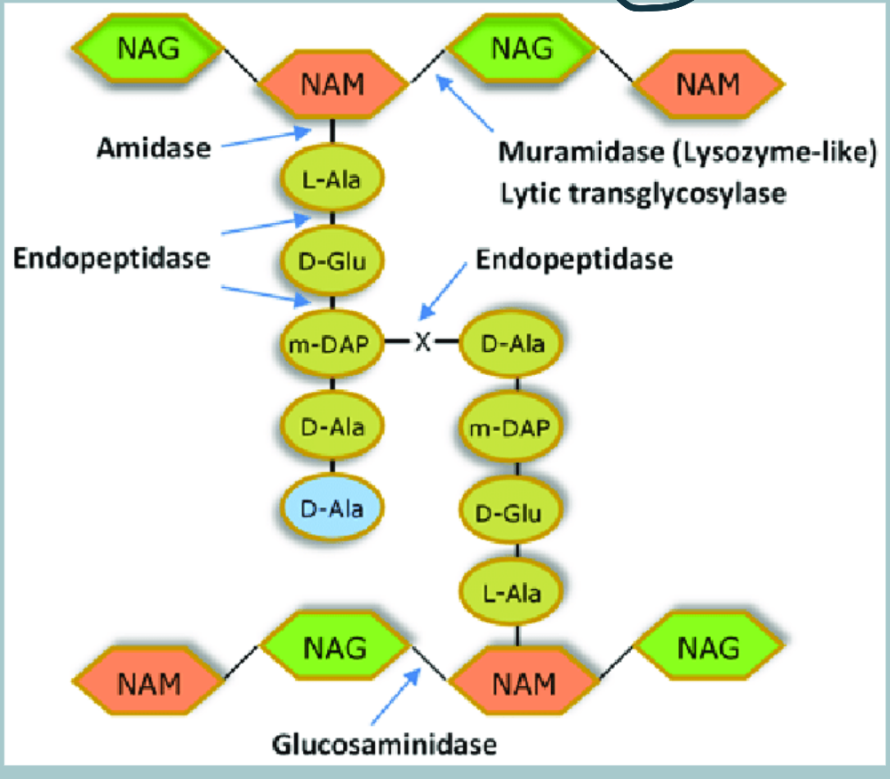


The cell wall is not present in human cell. This is an advantage because drugs which act

on cell wall do not have an effect on human host cells.

Cell wall gives cell shape, rigidity, prevents cell death by osmotic lysis, protects cell from toxic substances and contributes to pathogenesis.

The first of the three layers is *peptidoglycon(242).* It is made of two sugars (glycon= sugar) - NAM and NAG -linked to each other side by side to form a strand and arranged in layers around the phospholipid membrane like onion skin. Sprouting out of the sugars is a short peptide (aminoacids) chain. The sugars are cross linked to each other by covalent bonds between peptides. Cross linking results in one dense interconnected network. There is more cross linking in gram positive. There are several enzymes involved in the cross linking- enzyme which binds NAM and NAG, enzyme which bonds peptides, enzymes which bond NAM with peptide. Drugs acting on any of these enzymes will prevent the formation of peptidoglycon



thereby preventing formation of cell wall.

In gram positive organisms the PG is several layers thick but in gram negative it is two to three layers thick. In mycobacterium tuberculosis the thickness is in between. Even though it is multilayered it is a single molecule.

Peptidoglycon is produced in cytoplasm at the plasma membrane in periplasmic space and moves out as it is produced . It is strong but elastic. It can stretch and contract to osmotic pressure. It has rigidity of backbone and flexibility of cross-links. It acts like covering of foot ball. It gives rigidity and prevents inner bladder from expanding beyond it. Cell wall protects the cell against osmotic pressure.if the cell is in hypotonic solution water diffuses into cell. The cell swells. Without peptidoglycon wall the pressure on the membrane will become too much and so it will burst the cell. In hypersonic solution water flows out, cell shrivel up and dies. Peptidoglycon layer is therefore very important component of cell envelope. It is porous. Even large proteins are allowed. Only extremely large proteins are not.

Next to peptidoglycon is the *arabinogalacton (241)* layer. It is made of sugars, arabinose and galactose. The

main part is galactose with arabinose as side chain. It is linked to mycolic acid at one end and peptidoglycon at the other.

This layer, therefore, lies in between outer mycolic acid and inner peptidoglycon and therefore it tethers mycolic acid to peptidoglycon. Along with peptidoglycon and mycolic acid, it forms maAGPG complex giving the shape, rigidity and other characteristics of cell wall.

The drug etambutol prevents the formation of both arabinose and galactose thereby preventing the formation of mycolic acid and killing the cell.

The next layer is *mycolic acid (241)*. It is an essential component of mycobacterial cell wall and is special. About 60% of its cell dry weight is lipid. Mycolic acid which is made of long chain fatty acid is dense and it provides virulence, permeability barrier, resistance to chemical damage and dehydration, limits the effectiveness of watersoluble antibiotics, and is important for growth and survival. Mycolic acids also allow the bacterium to grow inside macrophages, effectively hiding it from the host immune system. Mycolic acids are produced in cytoplasm by a series of fatty acid synthase enzyme and shuttled by transporter protein outside the cell and attached to arabinogalactan

and linked to peptidoglycon. Any drug acting on these enzymes(fatty acid synthase) prevents the formation of MA thereby killing the cell. The ma-AG-PG complex protects the tubercle bacillus from antibiotics and host's immune system.

Layer 4:

The layer outside of mycolic acid has free lipids which include glycolipids including phenolic glycolipid. This layer is loosely attached and it can be wrenched off without much difficulty.

Layer 5:

The capsule consists of polysaccharides and proteins with very little lipids.

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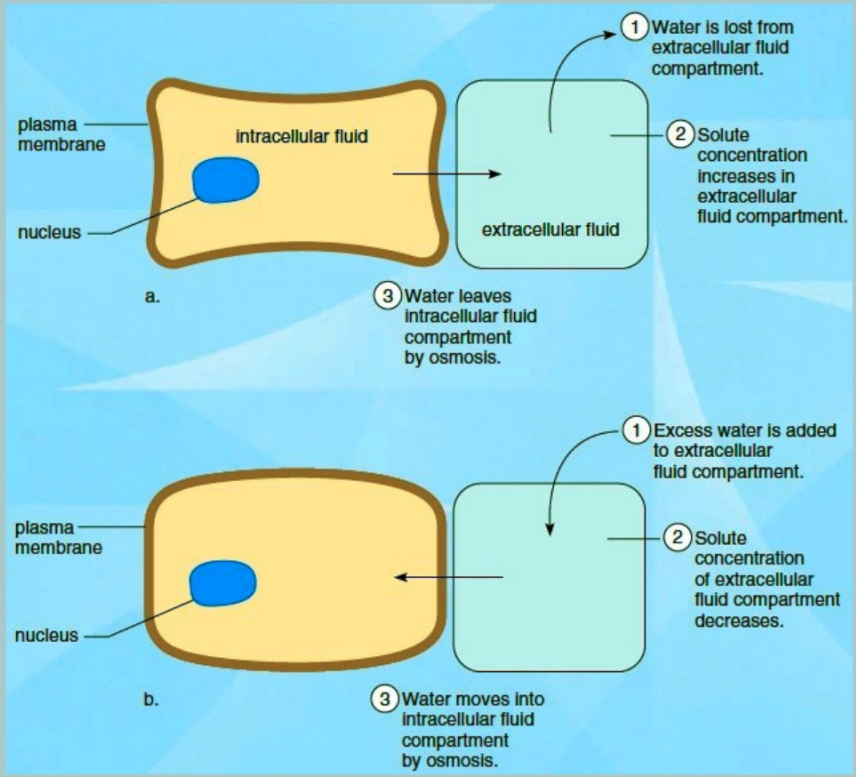
Drugs acting on cell wall

synthesis do not act on already formed cell wall. They act on actively multiplying cell, not on resting cell. Why? Actively multiplying cell is forming baby cells. When a cell divides two



baby cells are produced. The bacterial baby cell has only cell membrane and no cell wall. As the baby develops, the osmotic pressure inside cell goes up and at the same time the cell wall is synthsised so that the cell prevents the accumulation of water inside cell and resulting in osmotic burst (243, 244) The osmolarity inside the cell is always higher than that outside. The drugs can act to inhibit cell wall synthesis, therefire, only when the bacteria are actively multiplying and producing baby cells. The drugs can then prevent the formation of cell wall on baby cells so that they will not survive.

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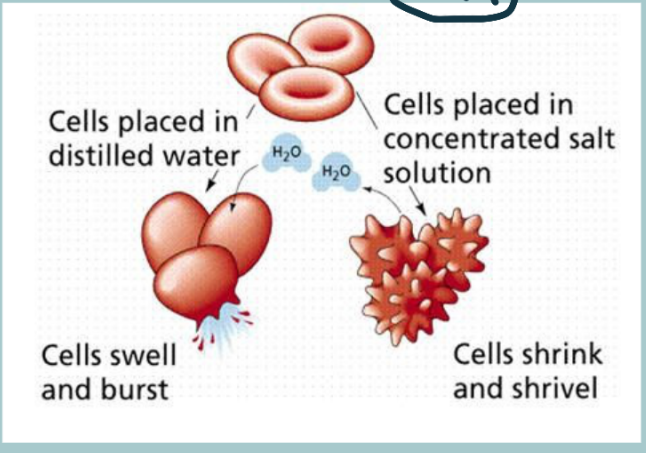

There are several Pathogen Associated Molecular Pattern (receptor) on the cell wall like LAM which are easily recognized by macrophages (every pathogen has unique PAMP receptors on their surface which are recognized by receptors on the surface of macrophages. Hence the receptor on macrophage sees these PAMP they say, " Here you are , my dear M.tuberculosis". They can recognise they are foreigners, they are pathogens, and which pathogens). The bacilli often cover these PAMP with a layer of fat so that they are not recognized by macrophages.

Mycobacteria are impermeable to several substances. This is mainly because of its thick wall containing plenty of lipids. This also affects the permeability to nutrients. This is the reason why mycobacteria are slow growing, the generation time of M.tuberculosis is an average 20 hours whereas for some of the

other bacteria it is a matter of a few minutes.

There is also a very efficient efflux pump which pumps out all substances including antibiotics which are not unwanted by the bacillus.

The two structures of M.tuberculosis which are targeted are mycolic acid, and peptidoglycon. There are several enzymes involved in the production of these two and therefore are good targets for drugs.

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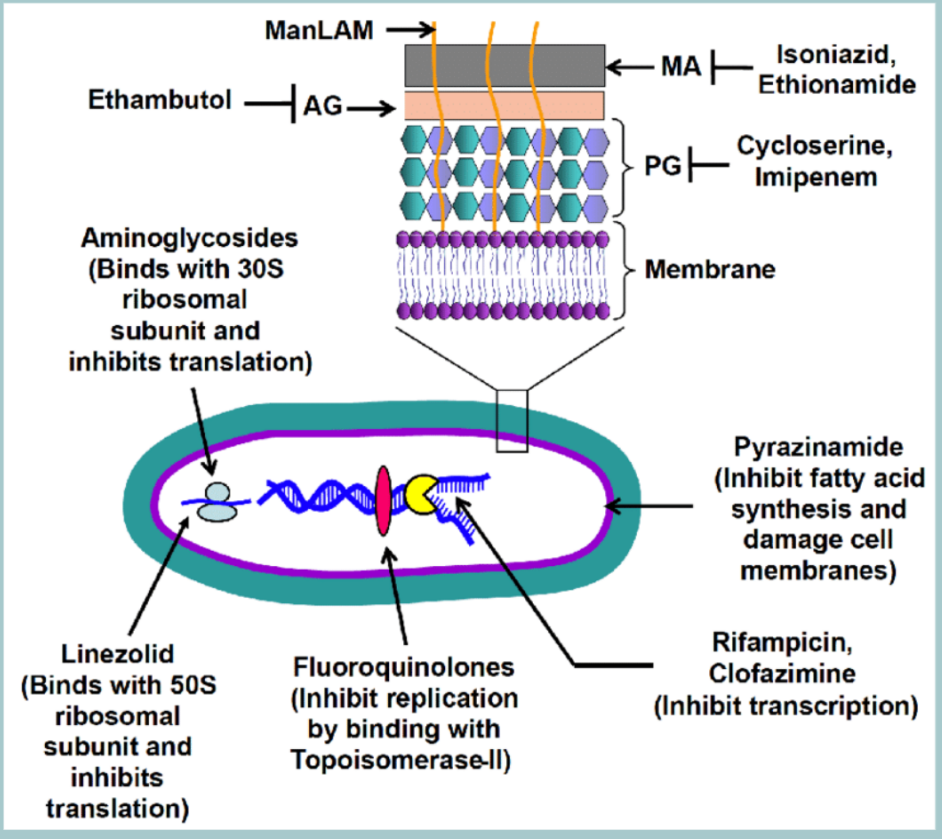
Drugs with action on cell wall (245)



Isoniazid:
It is a prodrug which is converted to active drug by KatG (catalase peroxidase) of M.tuberculosis. This active drug inhibits two enzymes- InhA and KasA which are essential for mycolic acid synthesis. Resistance is due to mutation of KatG and KasA and overexpression of InhA genes. KatG mutation results in high resistance and the other two, low resistance.

Ethionamide and prothionamide:

Ethionamide is a prodrug which is activated by the enzyme ethA , a mono-oxygenase in *Mycobacterium tuberculosis*, and then binds NAD+ to form an adduct (compound) which inhibits InhA in the same way as isoniazid. The mechanism of action is thought to be through disruption of mycolic acid.

Isoxyl or thiocarlide or tiocarlide:

It is a thiourea drug used in the treatment of tuberculosis, inhibiting synthesis of oleic acid and tuberculostearic acid thereby inhibiting formation of mycolic acid. Invented by a Belgian scientist it is proved to be useful in the treatment of MDR tuberculosis.

Thioacetazone:

It is a weak antitubercular drug and it kills bacteria by inhibiting mycolic acid synthesis.

Pyrazinamide:

Pyrazinamide is converted to active form, pyrazinoic acid by pyrazinamidase of M.tuberculosis. The net effect is that more pyrazinoic acid accumulates inside the bacillus at acid pH than at neutral pH. Pyrazinoic

acid was thought to inhibit the enzyme fatty acid synthase (FAS) I, which is required by the bacterium to synthesise fatty acids. This produces fatty acids which are added into long chain by fatty acid synthase 2.

It has also been suggested that the accumulation of pyrazinoic acid disrupts membrane potential and interferes with energy production, necessary for survival of M. tuberculosis at an acidic site of infection. Pyrazinoic acid has also been shown to bind to the ribosomal protein and inhibit trans-translation. This may explain the ability of the drug to kill dormant mycobacteria.

Delamanid:

It acts by inhibiting the synthesis of mycobacterial cell wall components, methoxy mycolic acid and ketomycolic acid. Delamanid is a pro-drug which gets activated by the enzyme nitroreductase. A reactive intermediate metabolite, formed is considered to play a vital role in the inhibition of mycolic acid production.

Pretomanid:

It has two actions. One is against nonreplicating bacteria in anaerobic condition. It is prodrug and is activated by the nitroreductase of the bacteria to an intermediate derivative which produces nitric oxide that kills the bacteria. The second action is on actively multiplying bacteria in aerobic condition in which it kills by inhibiting cell wall synthesis. The exact mechanism of this is not clear.

Ethambutol

Ethambutol is bacteriostatic against actively growing TB bacilli. It works by obstructing the formation of cell wall. Mycolic acids attach to the arabinose residues of arabinogalactan and form mycolyl-arabinogalactan-peptidoglycan complex in the cell wall. It disrupts arabinogalactan synthesis by inhibiting the enzyme arabinosyl transferase. Disruption of the arabinogalactan synthesis inhibits the formation of this complex and leads to increased permeability of the cell wall.

Cycloserine

Inhibits the enzymes responsible for formation of peptide side chain (aminoacid alanine) of peptidoglycon thereby preventing the cell wall synthesis.