Chapter 1

Introduction

Tuberculosis (TB) is, perhaps one of the oldest known deadly diseases, caused by the bacillus *Mycobacterium tuberculosis*. Various cultures of the world gave the illness different names: *yaksma* (India), *phthisis* (Greek), *consumption* (Latin), *white plague* (Europe) and *chakyoncay* (Incan). Ancient medical literature pertaining to every civilization had references to this menace. *M. tuberculosis* is believed to kill more people annually than any other single organism on the face of the planet, and tuberculosis-related illnesses are the fourth most deadly type of infectious disease known to man. The disease attacks both man and animals, affects all ages and every organ in the body, and ranges from latent to hyperacute, killing young and old alike, the famous and the unknown. A single airborne droplet from cough or sneeze of a patient can infect others, if inhaled. Surprisingly, only 5% to 10% of these newly contacted people may ever develop symptoms. Another problem with this disease is its undefinitive manifestations which confuse even experienced physicians. Non-pulmonary forms of TB often remain undetected till it is fully blown. These unique features made TB virtually impossible to eradicate and qualified this dreadful disease to be declared a world emergency in the year 1993 by World Health Organization (WHO). As this disease becomes complicated, evasive and ubiquitous, it is important to understand what makes it so uniquely invincible.

1.1 History of Tuberculosis

Ancient Indian scriptures written in Sanskrit sometime between 1500 and 700 BC mention the first known description of tuberculous spondylitis. Conclusive evidences from the paleopathological studies on spinal columns of the Egyptian mummies have confirmed the possible presence of tuberculosis close to 5,000 years back.[1] For ages, nobody has any clue on the pathogenesis of this disease and it remained incurable till 20th century. It was considered more

like a slow death sentence to the very unfortunate patients.

Several hypotheses on pathogenesis of this disease evolved out of visionary thinking and meticulous observations. In 1720, the English doctor Benjamin Marten was the first to state that TB could be caused by "wonderfully minute living creatures." In 1839, Johann Lukas Schönlein labelled the disease "tuberculosis". In 1865, French military doctor Jean-Antoine Villemin demonstrated that TB could be passed from people to cattle and from cattle to rabbits by conducting an experiment in which tuberculous matter from human dead bodies was injected into laboratory rabbits which became infected.[2] Invention of the modern stethoscope by Rene Laennecin 1816, revolutionized diagnosis of TB.

1.1.1 Discovery of the Pathogen

Microscopic observation of pathogenic microorganisms dates back to 17th century. But the observations heavily depended on staining techniques. Mycobacterium remained unidentified till 1882, due its "stain proof" lipid-rich cell wall. The German physician and a Nobel Prize recipient, Robert Koch has identified *Mycobacterium tuberculosis* via acid-fast staining method.[3] In 1881, he began working on a tissue isolated from a deceased ape that had died of tuberculosis, isolating the rod-shaped bacteria by growing it in culture dishes separated from any other germs. He then inoculated healthy guinea pigs with the TB bacteria. The guinea pigs became sick and Koch observed the tuberculosis bacteria growing in them. He then removed some bacteria from the infected guinea pigs and grew them in yet another culture. Finally, he infected a second group of healthy animals with this cultured bacteria. When those animals contracted TB, he could be sure that the same bacteria were responsible. This involved procedure (known as *Koch's Postulates*) soon became the standard method for identification of disease-causing organisms. In 1890, Koch

discovered that a substance released from the TB bacilli caused an allergic reaction in people who have been exposed to TB. This substance, called tuberculin, is being used as a main component in diagnostic test kit for TB.

1.1.2 Tuberculosis: Global Scenario

Despite the advances in chemotherapy and the BCG (Bacillus Calmette-Guerin) vaccine, tuberculosis remains a major global threat. It is the second most prevalent infectious disease, next to HIV, in the world today than at any other time in human history. *M. tuberculosis* has an amazing ability to hide and hibernate by finding a 'safe house' inside our own tissues leading to development of latent form of the disease. But, this seemingly harmless reservoir of infected people can develop active disease at later stages of their life. They may even unknowingly transmit the disease to others, thus making it almost impossible to eradicate this scourge.

According to WHO statistics of 2012, a predicted number of 1.3 million people died with TB and around 8.6 million people newly contracted this disease (includes HIV positives also). The total resource requirements to combat TB and multi-drug-resistant TB (MDR-TB) are estimated to be US$ 4.8 billion each year over 2014-2016. The World Health Organization (WHO) and the Global Fund to Fight AIDS, TB and Malaria estimate that, 118 low and middle income countries are certainly eligible for funding from the Global fund, which totals to US$ 1.6 billion to bridge the funding gap of 2014-2016. Since 1995, 22 million lives were saved and 56 million people were successfully cured and there is a 45% decrease in the TB mortality since 1990. In spite of these achievements 3 million people are estimated to be infected every year and there is an alarming burden of MDR-TB crisis.[4] India stands alone as the highest weigh down country with 2.2 million active TB cases. In another estimate it was found that around

40% of the population contracted this germ and mostly in the latent form.[5]

1.2 Etiology and Pathophysiology

M. tuberculosis primarily invades the host through the pulmonary tract. As we know TB, the contagious microbes pass through airborne particles generated by an infected person's (especially with pulmonary or laryngeal TB) cough or sneeze or even a burst of laughter. It can also be transmitted by unpasteurized milk, as animals can be infected with the bacteria. Children nearly always contract the disease from an infected adult.

M. tuberculosis is an intracellular pathogen that establishes infection in oxygen-rich alveolar macrophages of the lung. The infection renders damaged parts of lung into a dry and cheese like tissue, which soon hardens into a scar tissue. The severity of the attack depends on whether the virulent form of bacterial infection spreads to other parts of the body or not. Tuberculosis infection in the blood, the meninges (membranes around the brain and spinal cord), and the kidneys are the most serious. Children between the ages of 6 and 24 months are the most susceptible to meningitis; it is the chief cause of tuberculosis death among children.

Once the bacteria invade the lungs, the body's immune system sends out white blood cells which build walls of fibers around the bacteria to keep them confined, forming small, hard lumps known as "tubercles." Once the body has formed tubercles to encapsulate the bacteria, the primary infection may be contained and, although the person will always test positive for the TB bacteria, the disease itself may not develop. Later in life, if the walls containing the germs are broken down, the lungs once again become infected. If the immune system is initially unsuccessful in walling off the germs, a full case of TB develops. The new bacilli

grow and multiply and the lung tissue actually dies and becomes soft. Liquid from the tissue is coughed up leaving a cavity in the lung. Cavities may have already formed before a person even notices symptoms such as a cough or fever. Eventually, however, coughing becomes painful and brings up blood with the lung tissue. By this time, the case is well advanced. If large areas of the lungs are damaged, breathing becomes difficult and the body fails to deliver necessary oxygen to tissues. The bacilli may spread to other tissues of the body causing secondary infections and complications, leading to extra-pulmonary tuberculosis. If treated with antibiotics and other drugs, the patient may recover, usually over a period of time.

1.2.1 *M. Tuberculosis* (MTB) Complex

The MTB complex consists of human and animal pathogens that are acid-alcohol fast bacilli. *M. tuberculosis* and seven very closely related mycobacterial species (*M. bovis, M. africanum, M. microti, M. caprae, M. pinnipedii, M. canetti* and *M. mungi*) together comprise MTB complex. MTB found in dogs, cats, pigs[6] and some wild animals infects human and non-human primates.[7] *Mycobacterium bovis* is an agent that causes bovine tuberculosis and infects a wide range of domestic and wild hosts. This strain is used for making BCG vaccine. Studies based on DNA homology have proved that there is a close evolutionary relationship and 95-100% DNA relatedness among the members of complex and suggests that these members belong to the same genus.[8]

M. tuberculosis is capable of affecting almost all parts of the body except hair and nail.[3] Apart from this, Mycobacterium includes more than 50 other species, often collectively referred to as non- tuberculous mycobacteria.

1.2.2 Structural Complexity of *M. Tuberculosis* Cell Wall

M. tuberculosis is a bacillus shielded by a unique, thick lipid-rich cell wall which differs from that of most other bacteria (Fig. 1.1). This forms a diffusion barrier, 100–1000 fold less permeable to hydrophilic molecules than that of *Escherichia coli*.[9] The cellular envelope is composed of a core of three macromolecules covalently linked to each other. They are peptidoglycan (PG), arabinogalactan (AG), and mycolic acids (long fatty acids i.e. C60-C90) and a lipopolysaccharide, lipoarabinomannan (LAM), which is thought to be anchored to the plasma membrane. The cross linked network of peptidoglycan constitutes the arabinogalactan, where few of the muramic acid residues are substituted with complex polysaccharide and in addition it is acylated at its distal end with mycolic acids to the peptidoglycan. The entire complex is abbreviated as the mAGP (mycolylarabinogalactan–peptidoglycan) and is essential for the viability in *M. tuberculosis* and other mycobacteria.[10]

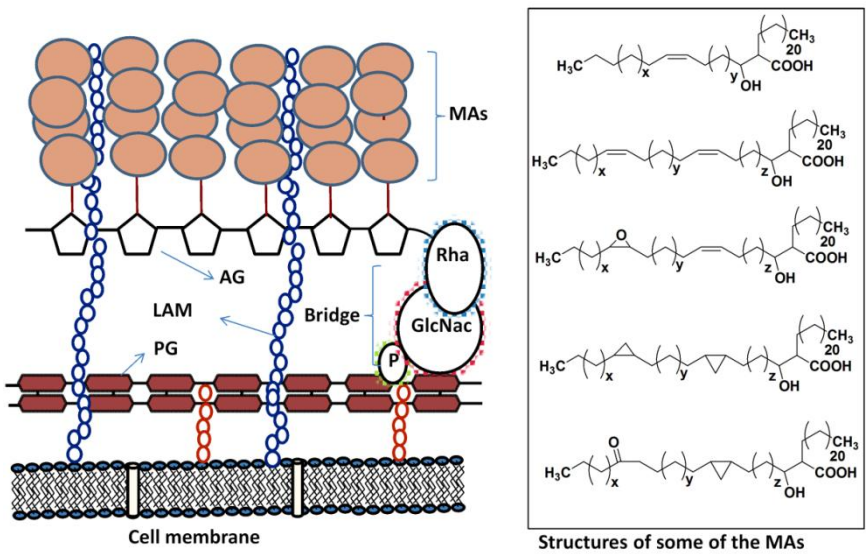

Fig. 1.1 *Cell envelope of M. tuberculosis with structural components.*

Mycolic acids are high molecular weight fatty acids carrying an ά-alkyl-β-hydroxy functionalities and bound typically as bundles of arabinogalactan. They appear primarily as tetramycolylpentaarbinosyl clusters, but also in extractable lipids mainly as trehalose 6, 6'- dimycolate (cord factor). The main part of the branched chain is called ''meromycolic acid'' and the other part ά-branch.[11] The characteristic features of these mycolic acids are that they are of the largest (C_{60}-C_{90}) and they have the largest ά -branch (C_{20}-C_{25}). The long chains are embedded with cyclopropane ring and double bond functionalities, which help maintain its constituency by producing 'kinks' in the molecules. In addition to β -hydroxyl group, it is assumed that the above groups might also contain oxygen functionality and the main carbon backbone may have methyl branches.[12]

The unusually complex mycobacterial cell wall contains many other macromolecules including LAM[13, 14], many extractable lipids, including glycolipids (glycopeptidolipids, GPL[15, 16]; lipooligosachharides, LOS[17, 18]; phenolic glycolipids, PGL [19-22]) and other classes of free lipids (sulpholipids, SL; phthioceroldimycocerosate, PDM).[23-26] LAM and arabinomannan exhibits a wide spectrum of immune regulatory functions including immune suppression, suppression of T-cell activation, inhibition of murine macrophages activation mediated through γ-interferon; cytotoxic oxygen free radical scavenging and protein kinase C activity inhibition. The glycopeptidolipids or phenolic glycolipids which are located either on the cell surface or outside the cell wall also prone to be involved in the generation of pathogenesis.[26] Cell walls containing carbohydrate layers also are indicted in virulence by averting non specific phagocytosis.

Mycolic acids are biosynthesized by Claisen type condensation and reduction of C_{16} fatty acids.[27] The four distinct steps involved in the biosynthesis include -

synthesis of C_{24}-C_{26} straight chain saturated fatty acids to provide C-1 and C-2 of the ά-alkyl chain; synthesis of the backbone of meromycolic acids of C_{40}-C_{60}; modification of meromycolic acids to introduce functional groups other than β-hydroxy and the final condensation step to provide mycolic acids. The role of several enzymes in the biosynthetic processes of mycolic acids were thoroughly studied at the gene level and can be used for development of effective antimycobacterial agents.[28-31]

The outer membrane of the mycobacterial cell wall is an important target for anti-mycobacterial agents, in particular the biosynthesis of cell wall components. The mycobacterial cell wall is very hydrophobic, resulting in an efficient barrier to a range of antimycobacterial agents.[32] Uptake of any drugs through the outer membrane requires the drugs to be lipophilic in nature although there is evidence of the presence of porin channels in the mycobacterial cell envelope through which both nutrients and drugs could diffuse.[33, 34] Currently TB is treated with agents that target mycolic acid biosynthesis including isoniazid (INH), inhibitors of nucleic acid biosynthesis such as rifampicin (RIF) which binds and inhibits mycobacterial DNA-dependent RNA polymerase, and the aminoglycoside antibiotic streptomycin (SM) which targets protein synthesis.[35]

The winning strategy of this humble microbe may now be attributed to its highly evolved chemical armory including unusually waxy cell wall, which could conceal most of the bacterial proteins from getting exposed to the host immune system and also release chemicals to modulate immune responses. The microbe also has developed several suave adaptation tactics to survive in varied environmental conditions including hypoxia, nutrient deprivation, exogenous stress conditions and intraphagosomal environment.[36] Though this lends a very complicated picture to comprehend, fortunately, unrelenting efforts by innumerable people around the world fructified and helped mankind overpower

this menace. The following chapters succinctly present all these invaluable accounts.

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