

Tubercle Bacilli in Spinal Tuberculosis - Morphology, Cell Wall Features, Behaviour and Drugs

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Abstract

Tubercle bacilli are tiny thin rod-shaped, non-spore-forming, non-motile obligate aerobic bacteria measuring 3 µm in length and 0.5 µm in width without pili for adherence and without producing adhesion molecule, and are acid-fast bacilli (AFB) with thick waxy cell wall having poorly developed porins, being present in the planktonic form. Tubercle bacilli are very slowly replicating (12 hours of generation time) only in presence of oxygen. Tuberculosis is a product of war between the host phagocytes and M tuberculosis in tissue. Phagocyte and M tuberculosis have very different cell wall composition, but both have very similar chemical weapons in them for fight and defense. Mycobacterium produces various mycolic acid compounds to form waxy cell wall and to defend it not to be digested by phagocytes, and not to be killed by the antituberculous drugs. Mycobacterium forms granuloma (tubercle) which is the specific reticuloendothelial tissue reaction to the specific type of irritants in normal person. It does not produce chondrolytic enzymes to destroy cartilage and disk, and does not adhere to any biomaterials.

Thick waxy coat of Mycobacterium impedes the entry of nutrient through the poorly developed and scarce porins, and thus limits growth rate, but it also protects the bacilli from host defenses and antibiotics.

Keywords: Tuberculosis; Cell; Mycobacteria

Introduction

Pulmonary tuberculosis starts with the delivery of M tuberculosis through the cough-produced droplet nuclei, sized 5 µm, and containing 5 M tuberculosis. Also tubercle bacillus is delivered to gut by drinking the unpasteurized cow milk, and ingesting the bacilli contained sputum. Once the bacilli which get into artery or Batson's venous plexus will be delivered to sinusoid in bone. These facts indicate that tubercle bacilli inseminate in the tissues and organs by passive transmission. And intracellular existence (inside macrophage) is also passive through the phagocytosis. That is, tubercle bacillus cannot actively get into the cell by its own motility [1-8].

Following exposure to M tuberculosis, about 10% of individuals develop active pulmonary tuberculosis, while the majority do not. It would be interesting to disclose the factors why some develop active disease after exposure and why the others do not. In humans, there are two populations of resident macrophages in the lung; alveolar and interstitial. Alveolar macrophages are the first line defenders against inhaled microbes that enter the lung. These macrophages are extremely active and highly phagocytic cells that kill with great efficiency. Interstitial macrophages are found in the stroma of the lung and are smaller and less phagocytic than alveolar macrophages. It is important to keep in mind the route of entry here and the need to protect the alveolar spaces where gas exchange occurs. Alveolar macrophages can inhibit the proliferation of Mycobacterium by producing NO (nitric oxide) and related reactive nitrogen intermediate (RNI) which have been reported to possess antimycobacterial activity. The balance of stimulatory and inhibitory cytokines for NO production may play a critical role in the defense mechanism against M tuberculosis considering that NO production is up-regulated or down-regulated by the cytokines [2,4,7,9].

Mast cells and neutrophils also generate reactive nitrogen intermediates that serve as potent cytotoxic agents.

Tuberculosis has no lethal dose (LD₅₀; Numbers of organisms required to kill 50% of the hosts), and fortunately has only infection dose (ID₅₀; Numbers of organisms required to 50% of the population to show sign of infection).

According to the studies conducted in tuberculosis prevalent countries before prechemotherapy era, it was shown that untreated tuberculosis was often fatal. About one third of the patients died within one year after diagnosis, and one-half died within 5 years. The five year mortality rate among the sputum smear-positive patients was 65%. Of the survivors at five years up to 60% had undergone spontaneous remission, while remainders were still excreting the contagious bacilli [7,10].

Patients infected with M tuberculosis have a 50% risk of reactivation in the first 2 years and then a 5% life time risk. Patients with "high five" HIV will have a 5+5% risk of reactivation per year.

In the chemotherapy era, patients had a very high chance of cure with effective, timely and proper chemotherapy. However, improper chemotherapy while reducing mortality rates might also result in large numbers of chronic infectious cases, often with drug resistant bacilli [9].

Once in the past, tuberculosis was known as "consumption", the emaciation disease. The known cause of the loss of body weight in tuberculosis patient is the Mycobacterium produced tumor necrosis factor (cachectin, a variant of mycosides) [8,11].

The effect of hyperalimentation on cachectin release (TNF) in the tuberculosis patients has never been studied.

Tubercle bacilli are obligate aerobes, and often facultative aerobes. Mycobacterial behavioural characteristics in pulmonary tuberculosis are not different from osteoarticular tuberculosis. However, the bacillary activities at the two organ sites (lesions) are somewhat different because

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M tuberculosis inseminates first the organ of high oxygen, the upper lung lobe through cough droplets, and metaphysis by hematogenous spread from primary lesion in case of bone involvement. As a local factors of bone, intraosseous vascular anatomy and bacterial chemiadsorptive characteristics to cartilage can be listed for bacterial lodgement; metaphysis where there is a change from high flow arterioles

to low-flow venous sinusoid. Poorly developed RES or lack of RES cells (lack of tissue macrophage) in the sinusoid is listed as local anatomic defect (locus minoris resistance).

Host has several beneficial positions such as emergency mobilization system (cellular forces supported by enhanced immune system), while M tuberculosis cannot increase its population numbers in short period of time because of its slow division (generation time, 12 hours). Thus, the host cells can form the fortified lesion (granuloma), constructed by host phagocytic cells aggregation. The mycobacterium inseminated area slowly grows, cascades, liquefies and cavitates. This slow erosive infection occurs at the site of the macrophage and T-cells battle against tubercle bacilli locally to wall off the bacilli. That is, following insemination of tubercle bacilli in the healthy bone tissue, the initial response is seen as the reticuloendothelial deposits. This is characterized by 1. Accumulation of polymorphonuclear leucocytes which are rapidly replaced by 2. Macrophages and monocytes, and the high phagocytic members of RES. Tubercle bacilli are phagocytosed and broken down. Their cell wall lipid is dispersed throughout the cytoplasm of the mononuclears. Thus, transforming those cells into epithelioid cells. Epithelioid cells are the characteristic feature of the tuberculous reaction.

Some of macrophages succeed in phagocytosing and breaking up the inseminated bacilli. These macrophages then run toward a local lymphnode, and present part of the bacilli to T helper cells (CD₄). The sensitized T-cells then multiply and enter the circulation in search of M tuberculosis. When the T-cells encounter their antigenic target, they release lymphokines that serve to attract macrophages and activate them when they arrive. Those activated macrophages can now destroy the bacilli.

During this stage, macrophages' attack actually results in local destruction and necrosis of the inseminated tissue (bone). The necrosed tissue looks like "a granular creamy cheese" and is called "caseous necrosis". This soft caseous center is surrounded by macrophages, multinucleated giant cells, fibroblasts, and collagen deposits, and it frequently calcified.

Within this granuloma, the bacilli are kept at bay, but remain "viable". In bone sometimes later, the bacilli may grow again perhaps due to a depressed host resistance (latent tuberculosis). However in the immunocompromised patients granuloma cannot be formed [5].

Tuberculous Mycobacteriology

M tuberculosis is a tiny thin obligate aerobic, acid fast and Gram positive pathogen. It is metabolically catalase- and phenylase-positive. It has no adhesion pili or fimbriae in cell wall surface. Its population in the infected osteoarticular lesion is small in numbers (paucibacillary), and bacilli present in planktonic form even after the repeated replication, and never produce glycocalyx adhesion molecule. Thus, it does not cause biomaterial centered infection.

The slow mycobacterial growth is not the by-product of "Starvation" response. Because, even in the rich-nutrient environment mycobacterial growth is same because of poorly developed porins in the cell wall. However, in case of other bacteria, bacterial growth rate slows when nutrient declines. Daughter cells become smaller and begin to experience what is termed as "Starvation response". My interpretation on the tiny thin size of M tuberculosis is closely related with the chronic "starvation" due to poor nutritional supply through the scarce porins in the thick waxy cell wall.

Bacteria convert unsaturated fatty acids to cyclopropane during starvation and acid stress conditions under which membranes require

stiffening. Cyclopropane conversion is an important factor in the pathogenesis of mycobacterium tuberculosis.

M tuberculosis is very well-clad in armor (the thick slippery waxy cell wall), and can stand well the poor nutrient milieu (famine) and oxygen deficiency (becomes anaerobic). But its small size is not the result of true starvation.

Mycobacterial growth is inhibited within the necrotic environment by low oxygen tension and low PH. Its growth takes up to 6-8 weeks for the visible colony. The colonies that form lump together due to their hydrophobic lipid nature, resulting in clumped colonies.

Tubercle bacillus has ability to shift down into one or both of the two non-replicating stages; microaerophilic and anaerobic persistence. Anaerobic persistence is responsible for the mycobacterial ability to lie dormant in the host for long period of time with the capacity to revive and activate disease at a later time.

Oxygen is one of the most important chemical requirements for bacillary growth. Oxygen has two roles against the bacilli; aerobes and anaerobes. In some bacteria metabolically oxygen is essentially needed for growth (aerobes), though it can be facultative. Many bacteria do not require oxygen only for growth (anaerobes) but also actually die in the presence of oxygen (obligate anaerobes). Thus, oxygen has a "dark side" during normal bacterial respiration.

Structure and Chemical Composition of Mycobacterial Cell Wall and its Characteristics

Primary structure or backbone of the bacterial wall is "peptidoglycan", a structure composed of repeating molecules of the sugars N-acetyl glycosamine (NAG) and N-acetyl muramic acid (NAM) which together with small peptide chains from the meshwork of the cell wall. There are several enzymes including transglycosylase, transpeptidase, polymerase, and hydrolase that work together to construct a peptidoglycan backbone. M tuberculosis builds up the exceptionally complex cell envelope by synthesizing a waxy lipid called mycolic acids (called unusual membrane lipids) and its compounds by combining with other compounds such as phenolic glycolipids (Figure 1).

Mycolic acids in mycobacterial cell envelopes are extremely diverse and include some of the longest-chain acids, known up to 90 carbons, and are linked to arabinogalactan (a polymer of arabinose and galactose), built in the peptidoglycan, which does not exist in mammalian cells (Figure 2).

The layer just outside the bacterial cytoplasmic membrane is the peptidoglycan layer or cell wall which is composed of replicating disaccharides with 4 amino acids in a side chain extending from each disaccharide. The amino acid chains of the peptidoglycan covalently bind to other amino acids from neighbouring chains. This results in stable cross-linked structures.

In summary complex mycobacterial cell wall includes a peptidoglycan layer linked to a chain of galactose polymer (galactan) and arabinose polymer (arabinan). Arabinan forms esterlinks to mycolic acids which form an outer bilayer with phenolic glycolipids. Outside the outer bilayer is a capsule of loosely associated phospholipids and phenolic glycolipids. Mycolic acids in the cell envelope occupies as much as 60% of cell wall, and provide the basis for acid-fast staining in which cells retains the dye carbolfuchsin. Mycolic acid contains a hydroxyl acid back bone with two hydrocarbon chains – one comparable in length to typical membrane lipids (about 20 carbons), the other about threefold longer. The long chain includes ketones, methoxyl groups, and cyclopropane rings. Hundreds of different forms are known. The

phenolic glycolipids include a phenol group, also linked to sugar chains (Figure 2).

Cell wall is the protective crater structure of the bacterial cell. Peptidoglycan is constructed inside the cell and transported to the outside, and forms a meshwork formation for the cell wall, and is a target for antibiotics.

There are one class of lipid that only acid-fast organisms have, and that is involved in cell wall thickness, virulence and protective cellular and immune activities (adjuvant) (Figure 1).

According to Cladwin and Tratter [2], “mycolic acid” (a large fatty acid) combining with other chemicals forms other compounds, known as mycoside, cord factor, sulfatide, and wax D (Figure 1).

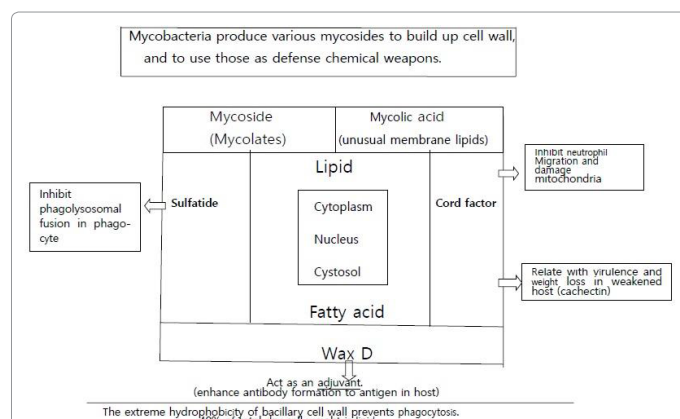


Figure 1: Cell wall chemical structure of *M tuberculosis*. This sketch does not represent the layering of cell wall. The role of the mycolic acid (membrane lipid) and its compounds are illustrated. True envelope structure will be illustrated separately in Figure 2.

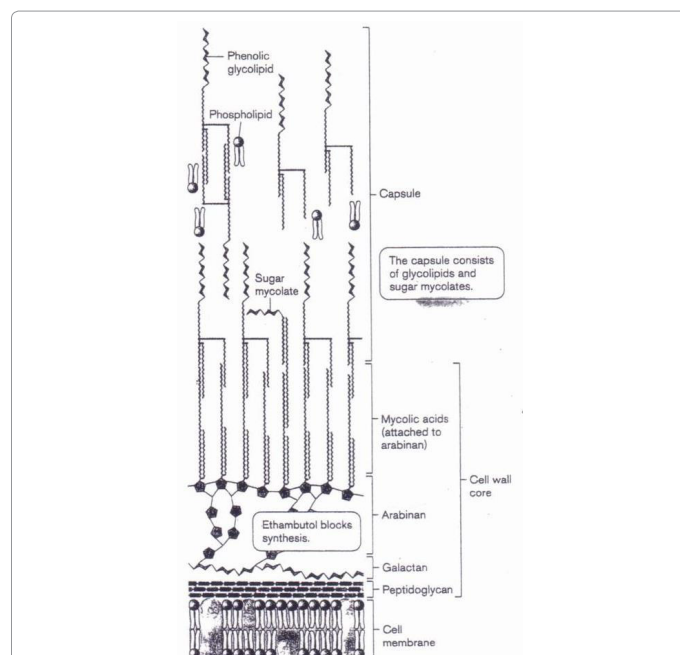


Figure 2: Mycobacterial envelope structures.

A complex cell wall includes a peptidoglycan layer linked to a chain of galactose polymer (arabinan). Arabinan forms ester links to mycolic acids, which form an outer bilayer with phenolic glycolipids. Outside the outer bilayer is a capsule of loosely associated phospholipids and phenolic glycolipids [1,3].

a. Mycoside (glycolipid) is formed by the bounded mycolic acid to a carbohydrate.

b. Cord factor (trehalose) is a combined compound; mycoside which is formed by 2 united mycolic acid and disaccharide. This factor is found only in the virulent strain. It also releases TNF or cachectin which relates with weight loss and cachexia in the tuberculosis patient.

c. Sulfatides are the mycosides which resemble the cord factor, and formed by sulfate attached mycoside and disaccharide. The facultative intracellular nature of mycobacterium tuberculosis during early infection may be partly attributed to the sulfatides which inhibits phagolysosomal fusion.

d. Wax D is a complicated mycoside which does adjuvant role; Enhances antibody formation to antigen, and activates the protective cellular immune system.

Fatty acid in the mycoside cell wall structure that stiffen the membrane are increased under stress condition such as starvation and acidity. The extreme hydrophobicity of the phenol derivatives of mycobacterial cell wall generates a waxy surface that prevents macrophage's phagocytosis.

The lipid wall protects the mycobacterium from the host defense. Mycobacterial behaviour becomes somewhat different due to the percentile differences of the cell wall chemical composition of each bacilli. Waxy cell wall protects itself against antiseptics, disinfectant, and antibiotics.

Mycobacterial waxy cell coat impedes the entry of nutrient through scarce and poorly developed porins and thus limits grow rate, but also protect the bacterium from the host defense and antibiotics. For this reason the cure of tuberculosis requires an exceptionally long course of antibiotic therapy. Arabinogalactan biosynthesis can be inhibited by ethambutol.

Cytocidal Chemicals Produced by Phagocytes and *M Tuberculosis*

Both the host phagocytes and mycobacteria can produce chemical weapons (missiles) to disarm (to dysfunction the cytolytic enzymes or to detoxify the cytotoxic substances) the opponent. Both sides ironically carry the very similar chemical weapons. Because all the cells (host and pathogens) have similar internal structure and metabolic activity for their survival.

Once when the “tit-for-tat” war starts between phagocytes and pathogens, the first step of war starts at the cellular level which is augmented by the chemical weapons (enzymatic crossfiring).

Oxygen is benefit to aerobes that can use it as a terminal electron acceptor to extract energy from nutrients, while oxygen is toxic to all cells that do not have enzymes capable of efficiently destroying the reactive oxygen species, for example, anaerobes.

In the phagocytic killing pathways against bacteria there are two; oxygen-dependent and oxygen-independent ones. Oxygen-dependent mechanisms (non-enzymatic) are probably activated through the Toll-like receptors, and kill through the production of various oxygen radicals. NADPH oxidase, myeloperoxidase, and nitric oxide synthetase in the phagosome membranes are extremely important. NADPH oxidase yields superoxide ion (O_2^-), hydrogen peroxide and ultimately hydroxyl radicals (OH^\bullet) and ions (OH^-).

Oxygen independent mechanism (enzymatic) include the enzyme like lysozyme to destroy the cell wall compounds such as lactoferrin to

sequester iron away from the microbe and defensins, small cationic antimicrobial peptides.

Reactive nitrogen intermediates, generated by the phagocytes serve as potent cytotoxic agents. Nitric oxide (NO) is synthesized by NO synthetase. Further oxidation of NO by oxygen yields nitrite (NO₂) and nitrate (NO₃) ions. All of these reactive oxygen species attack bacterial membranes and proteins.

These mechanisms cause the large increase in oxygen consumption noted during the phagocytosis, called the “oxidative burst”. The reactive chemical species formed during “oxidative burst” do little to harm the phagocyte because the burst is limited to the phagosome and because the various reactive oxygen species such as superoxide are very short-lived. Although these phagocytes are very good at clearing infectious agents, many bacteria have developed ways to outsmart the aspect of innate immunity.

The enzymes consist of cytolytic (digestive enzyme, the lysosomal enzymes) and free oxygen radical-detoxifying enzymes (catalase, peroxidase, superoxide dismutase). Host phagocytes and mycobacteria produce the oxygen for their needs in the tissue; for own respiration and additionally to use it as the defense chemical weapons. Excessively produced (oxygen burst) remnant free oxygen radicals if not detoxified work as cytotoxic substance which damage their own cells and the surrounding tissues. To detoxify the excess free oxygen radicals, the cells again use the catalase, peroxidase, and superoxide dismutase, and neutralize their surroundings (Figure 3).

For example, both aerobes and facultative anaerobes produce an enzymes called “superoxide dismutase (SOD)” which work for

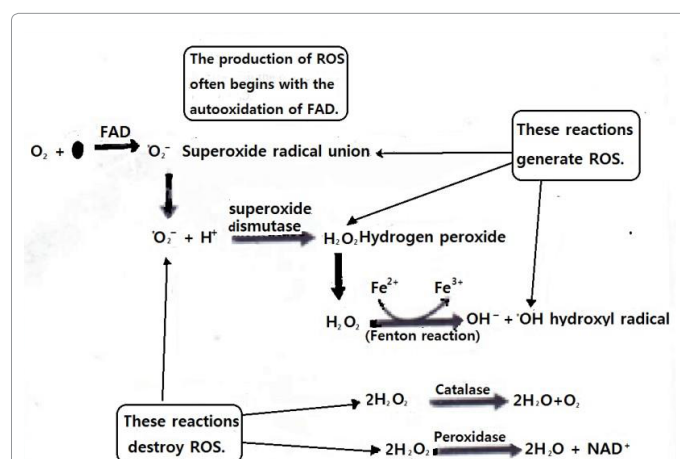
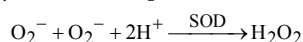


Figure 3: Generation and destruction of reactive oxygen species (ROS).

The autooxidation of flavin adenine dinucleotide (FAD) and the Fenton reaction occur spontaneously to produce superoxide and hydroxyl radicals, respectively. The other reactions require enzymes. FAD is a cofactor for a number of enzymes (for example, NADH dehydrogenase 2). Catalase and peroxidase do not produce ROS but detoxify hydrogen peroxide.

Nitrogen intermediates serve as potent cytotoxic agents. Nitric oxide (NO) is synthesized by NO synthetase. Further oxidation of NO by oxygen yields nitrite (NO₂) and nitrate (NO₃) ions. All of these reactive oxygen species attack bacterial membrane and proteins. These mechanisms cause the large increase in oxygen consumption noted during phagocytosis, called the “oxidative burst”. Myeloperoxidase present only in neutrophils converts hydrogen peroxide and chloride ions to hypochlorous acid (HOCl).

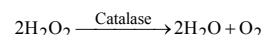
- The “oxidative burst”, a large increase in oxygen consumption during phagocytosis, results in the production of superoxide ions, nitric oxide, and other reactive oxygen species.

Unfortunately SOD produce the cytotoxic chemical, “hydrogen peroxide (H₂O₂)”, and it contains the peroxide anion O₂²⁻ which is just as toxic to the bacteria as free radical oxygen; this toxic peroxide anion is the active component of antimicrobial agents such as hydrogen peroxide

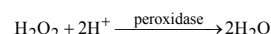
(H₂O₂) and benzole peroxide.

To avoid this toxic anion, bacilli have developed two enzymes to neutralize; Two secreted enzymes are catalase and peroxidase.

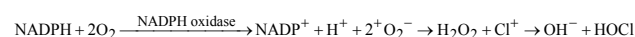
- Catalase causes hydrogen peroxide to water and oxygen.



- Peroxidase causes H₂O₂, not to water plus molecular oxygen, but rather to just water.



Myeloperoxidase present only in neutrophils converts hydrogen peroxide and chloride ions to hypochlorous acid (HOCl)



Oxygen is a benefit to aerobe which uses oxygen as a terminal electron acceptor to extract energy from nutrients. However, oxygen is toxic to all cells that do not have enzymes capable of efficiently destroying the reactive oxygen species (ROS).

Some aerobes can survive in oxygen, but do not have the ability to use oxygen.

Any organism that possesses NADH dehydrogenase 2, - aerobe or anaerobe -, will in the presence of oxygen inadvertently autooxidize the FAD (flavin adenine dinucleotide) cofactor within the enzyme, and produce dangerous amounts of superoxide radicals (O₂⁻). Superoxide will degrade to H₂O₂, another reactive molecule.

Iron, present as a cofactor in several enzymes, can then catalyze a reaction with H₂O₂ to produce the highly toxic hydroxyl radical (OH·). All of those molecules seriously damage DNA, RNA, proteins and lipids. Consequently, oxygen is actually an extreme environment in which survival requires special talent.

Macrophage uses oxygen derivatives such as hydrogen peroxide (H₂O₂) and hypochlorite ions for digestive phase. All oxygen derivatives work by destroying the pathogens’s plasma membrane. In case of too large pathogen cells to be ingested, toxic compounds from lysosome will be released onto pathogen outside of macrophage and thus also damage the surrounding tissue.

Phagocytes against M Tuberculosis

Phagocytes recognize alien cells and particles; For phagocytosis to proceed, neutrophils and macrophages *must* first recognize the surface of a particle as foreign, and is selective for particles recognized as foreign to the body (Figure 4).

As first step of phagocytosis, phagocyte’s pseudopod adheres to the surface of M tuberculosis, and gradually wraps the mycobacterium entirely. The wrapped mycobacterium by pseudopod, brought inside the phagocyte, is called phagosome. The fused phagosomes with lysosomes enable the destruction of the pathogen cells. However, several microorganisms have evolved ways to defeat this process. Mycobacterium inhibits phagolysosomal fusion by the mycobacteria-produced sulfatide.

When a phagocyte surface interacts with the surface of another body cell, the phagocyte becomes temporarily paralysed (inept at

pseudopod formation). Paralysis allows the phagocyte to evaluate whether the other cell is friend or foe, self or nonself. Self-recognition involves glycoproteins located on the white blood cell membrane binding inhibitory glycoproteins present on all host cell membranes. The inhibitory glycoprotein on human cells is called CD₄₇ (Figure 4). Because invading bacteria lack these inhibitory surface molecules, they can readily be engulfed. Mycobacterium is easily recognized and engulfed by phagocytosis. On the other hand mycobacteria to protect itself from phagocyte possess polysaccharide capsules that are too slippery for pseudopod to grab. This is where innate immunity and adaptive immunity join cellular forces. Adaptive immunity produces anticapsular antibodies that aid the innate immune mechanism of phagocytosis through a process; known opsonization (The process of antibodies binding to the capsule is called opsonization). The Fc portion of the antibodies binds to reception on the macrophage surface [1,3].

Virulence of Tubercle Bacilli

Bacillary pathogenicity has long been an issue. Mycobacterium is a problematic pathogen for the host defense because of its unique cell wall. Among the human pathogens, virulence is the potential to cause disease or virulence refers to just how harmful a given pathogen is to a host. In certain circumstances, organisms become pathogenic, but some mildly virulent.

Two criteria are defined in assessing the bacterial virulence; Lethal dose 50% (LD₅₀) and infection dose 50% (ID₅₀). Pathogens having the lower LD₅₀ and ID₅₀ values are the most virulent.

Mycobacterial virulence belongs only to infection dose, because it grows very slowly. We authors put emphasis more on the infection and infection establishment rates, and also the reactivation rates.

These factors clinically closely related each other in virulence;

1. Epidemiology; study of the factors determining the frequency and distribution of the disease.
2. Pathogenesis; study of how disease develops.
3. Host defense.

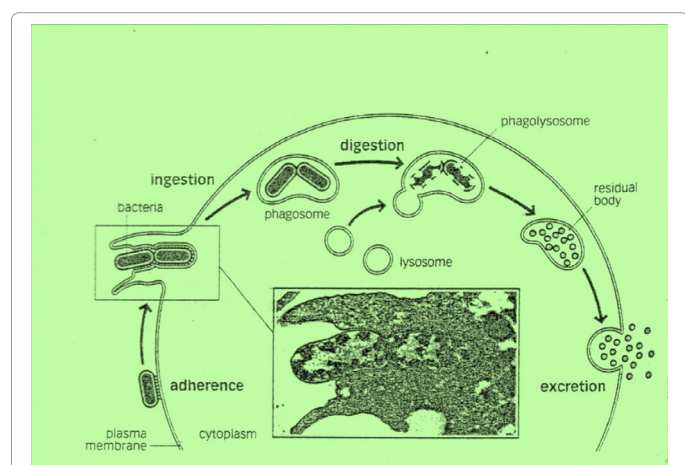


Figure 4: Four of the five phases of phagocytosis: adherence, ingestion, digestion, and excretion.

As first step of phagocytosis, phagocyte's pseudopod adhere to the surface of M tuberculosis, and gradually wrap the mycobacterium entirely. The wrapped mycobacterium by pseudopod, brought inside the phagocyte, is called phagosome. The fused phagosomes with lysosomes enable the destruction of the pathogen cells. However, several microorganisms have evolved ways to defeat this process. Mycobacterium inhibits phagolysosomal fusion by the mycobacteria-produced sulfatide.

Cord factor out of several known mycolic acid compounds in the mycobacterial cell wall was found only in the virulent strain.

What does a Mycobacterium Make Pathogenic?

It is not easy for pathogens to produce disease in human, Virulence factors (successful pathogen) are required for the organism to persist in the host, to cause disease, and to escape on host defense so that the infection can continue. This has long been an issue.

Pathogen *must* be able to accomplish the followings:

Virulence depends on genetic factor.

1. Potential pathogen *must* be able to adhere, penetrate, and persist in host cells ("get-in and stay-in rule"), but tubercle bacilli cannot adhere and penetrate.

Also pathogen *must* be able to avoid, evade, or compromise the host defense mechanisms.

2. Pathogen *must* damage the host tissue or organ, and permit the spread of the infection.

3. Pathogen *must* be able to exist from one host and infect another host (transmission of disease).

Surviving Environment of Bacterial Pathogen

There are two external environments. External to host and either on or in host;

1. Opportunistic and 2. Primary. Tubercle bacilli is the primary one.

Behavior of tissue Inseminated Tubercle Bacilli Against Phagocytes

Both host phagocyte and mycobacterium survive independently as a separate entity, though mycobacterium possibly can survive inside the macrophage which serves as host. Interestingly both have very similar intracellular morphology and functions. But both have great structural differences in the cell wall (Figures 1 and 2).

Tubercle bacilli are small in numbers in the lesion, but are better armed than the phagocytes.

It behaves differently according to extra- and intra-cellular environmental changes such as PH and oxygen tension without its own structural changes.

Tubercle bacilli are resistant to killing by macrophages, and can be found alive within the macrophages (intracellular mycobacteria). This resistance against the host defense can lead to long-term (chronic) infection and the formation of granuloma. Host mobilizes all the cellular (phagocytes) forces producing chemical weapons (chemokines and lysosomal enzymes including leukocidin) and immune system to fight against the mycobacterium.

Phagocytosis is selective for the particles recognized as foreign to the body. In the phagocytes' killing processes of mycobacterium, the first step is the phagosome formation through the process of entire wrapping of the bacilli by phagocyte-pseudopod which is followed by succeeding engulfment into the phagocyte. Phagosome in the phagocyte cytoplasm fuses with lysosome by virtue of their similar membrane structure. This fusion allows the lysosomal enzymes to come into contact with the mycobacterium and destroy it. However, in tuberculosis the phagolysosomal fusion process is hindered or inhibited by the mycobacterium-produced sulfatide. Thus, some of the engulfed mycobacteria eventually escape from phagosome into the cytoplasm of

macrophage where the bacilli will increase in numbers, and the bacilli outside from the phagocytes spread to the lymphnode where again they will enter the blood and distribute throughout the body.

In addition to phagolysosomal enzymes, macrophage uses oxygen derivatives such as hydrogen peroxide (H_2O_2), hypochloride ion in digestion phase. All oxygen derivatives work by destroying the pathogen's plasma membrane.

Mycobacterial Survival in the Tissue

Mycobacteria survive inside the granulomas which are bodies made-up of host defense cells such as neutrophils, macrophages, T-cells, B-cells, dendrite cells, fibroblast and matrix component. Granuloma forms as an activated macrophages aggregate which grows into gigantic cells. Question is how the mycobacteria survive at initial contact with cells that are programmed to phagocytose and kill them! To fight against this host phagocytic process, mycobacterium produces sulfatides, cord factors and wax D which are the main cell wall components. Cord factor inhibits neutrophil migration and damage mitochondria, and also relate with virulence and weight loss (cachectin production).

Intracellular M tuberculosis

Intracellular pathogens that grow in eukaryotic cytoplasm can be a serious problem for the host. An example is M tuberculosis which enters the host cell not only through phagocytosis but also enters host cell in ways that bypass the endosome formation, or if mycobacteria do enter via an endosome those can escape from that compartment. These pathogens block normal host cell clearance pathways. To circumvent this problem, eukaryotic host cells (not just phagocytes) have taken a cell function normally used to degrade damaged organ cells (called autophagy; eating of one's own flesh) and have adapted it to clear themselves or intracellular pathogens. That is, intracellular mycobacterium can be sequestered from the cytoplasm (via an autophagosome) and being killed following fusion with a lysosome by autophagy. During the process of autophagy, the cell constructs a double membrane around the organism or damaged organelles. This structure, called the autophagosome, sequesters the microbe from the nutrient-rich cytosol. Lysosome then fuses with the autophagosome, depositing degradative enzymes that digest the organism. Although the phagocytes are very good at clearing infectious agents, many bacteria have developed ways to outsmart this aspect of innate immunity. Ever adapting intracellular microbes, however, have formed ways to suppress autophagy and survive.

An example is M tuberculosis.

Tissue and Organ Damage by Mycobacterium

Bacterial pathogen can produce digestive enzymes and/or toxins which damage the tissue. Some of the infection-associated host tissue damage is due to an overcompensating host defense, when the host defense reactions designed to fight the infection are too severe.

Pathogen uses multiple methods to survive and thrive, while the host defense (immune system) becomes involved immediately in an attempt to kill or expel the pathogens. Consequently the infected host can protect its own tissue and organ from damage; Host defense for invading pathogen is the key to protect the host body unharmed. Body mobilizes two immune systems to expel or kill pathogen: innate and adaptive immune systems.

Some pathogens utilize the defending host cells as a place to hide from the host defense. Only the outcome can define the success or failure of host defense.

Mycobacterial Response to Implant

Various implants are already proven to be inert to mycobacterium, because mycobacterium doesn't produce adhesion molecules known as biofilm (glycocalyx). Thus, biomaterial for reconstruction of the destroyed bone and/or joint by tuberculosis can be safely used, and also it doesn't interfere the effectiveness of chemotherapy.

However, the use of biomaterial for the infected lesions of the HIV positive patient is obviously inappropriate, because those patients are susceptible to a variety of other opportunistic infections, particularly the pyogenic bacterial infections.

Evolution of Tubercle Bacilli

Tubercle bacilli itself evolve to adapt and survive in the environment where they are by developing the drug resistance simply through increased production of sulfatides and cord factor.

Mycobacterial Response to Chemotherapy

It is most important to remind all the treating physicians a fact that the thick waxy envelope of tubercle bacilli excludes many antibiotics, and offers exceptional protection from host defense, enabling bacilli to colonize their hosts over long periods. The mycobacterial envelope also retards uptake of nutrients. As a result, bacilli grow extremely slowly, and it is a challenge to culture to the laboratory. Therefore, 9-12 months of the combined chemotherapy is needed.

The treating physician should make accurate diagnosis of osteoarticular tuberculosis at an earliest stage, and then start blasting his or her multidrug shotgun chemotherapy at all potential targets and continue over 9-12 months; Fire early and hit everything. Thus, consequently tuberculosis can be aborted medically without sequelae and avoid development of complicated osteoarticular tuberculosis which leads to surgical management.

Chemotherapy usually consists of a triple therapy involving isoniazid (INH), pyrazinamide (PZA) and rifampicin (RFP). These three drugs are taken once a day for two months, followed by a regimen of INH and RFP for nine more months. If the tubercle bacilli strain is drug resistant, the initial regimen should include ethambutol.

It is good news that new drugs, known as pyronins which can replace rifampicin become available. Because pyronins also target the RNA polymerase (RNAP) as rifampicin did, when mycobacterium becomes resistant to rifampicin.

It could be concluded that tubercle bacilli in osteoarticular tuberculosis can be easily suppressed or eradicated by the orchestrated management between patients and physicians regardless of bacillary chemosensitivity and/or chemo-resistance. Thus, it can be said that osteoarticular tuberculosis including spinal tuberculosis is a treatable medical condition.

Effect of antituberculous agents depends on which physiological stage tubercle bacilli are; replicating and non-replicating stages. That is, at the given time it reflects the recognized

mode of action of those antituberculous agents (Table 3).

Mechanisms of Action of Antituberculous Agents

1. Isoniazid (INH) is bacteriostatic and narrow spectrum antibiotic. It becomes active only in presence of an enzyme, Kat G, produced by M tuberculosis. It inhibits the synthesis of mycolic acid.

2. Streptomycin acts through its binding to the 30s ribosomal

Growth rate	Distribution	Susceptibility
Rapid growing	Cavities	Isoniazid, Rifampin, Streptomycin
Slow growing May have intermittent spurts of activity	Solid caseous lesion	Rifampin *(Pyronins)
Slow growing in acid environment	Inside macrophages	Pyrazinamide
Semidormant bacilli	Any tissue	Pyrazinamide
Dormant bacilli	Solid caseous lesions and inside macrophages	Not killed by any drug

Table 3: Bacterial susceptibility on drugs depending on the tuberculous lesions.

subunit which affects polypeptide synthesis, and ultimately leads to inhibition of translation. Streptomycin is bacteriocidal and broad-spectrum.

3. Pyrazinamide is converted to bacteriocidal pyrazinoic acid inside the bacterial cell by pyrazinamidase/nicotinamidase. Pyrazinamide appears to kill a population of semidormant tubercle bacilli that are not affected by other antituberculous drugs.

4. Ethambutol is bacteriostatic. Single ethambutol is actually not very effective. Ethambutol in concert with isoniazid inhibits the incorporation of mycolic acid (arabinogalactan biosynthesis) into the growing bacterial cell wall. Combination of isoniazid, ethambutol and rifampicin is now the treatment of choice for tuberculosis, and the administration of these combinations of drugs will also lower the potential for the development of resistance.

5. Rifampicin is bacteriostatic and broad spectrum antibiotic, and targets the RNA polymerase.

6. Rapid growing tubercle bacilli in the cavities are sensitive to INH, rifampicin and streptomycin.

7. Slow growing bacilli with intermittent spurts of activity in solid caseous lesion are sensitive to rifampicin. If the bacilli are resistant to rifampicin, new drug, targeting the RNA polymerase, called “pyronins” can replace rifampicin.

8. Intracellular (in macrophage) slow growing mycobacteria in acid environment are sensitive to pyrazinamide.

9. Semidormant bacilli in any tissue are sensitive to pyrazinamide’

10. Dormant bacilli in solid caseous lesion and inside the macrophages do not respond any drug. Only the revived bacilli from dormant state can respond to chemotherapeutic agents.

Conflict of Interest

No benefit in any form has been or will be received from a commercial party, related directly or indirectly to the subject of this manuscript.

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