From India to Canada

PATH 417
Case 3 Week 1
Bacterial Pathogenesis Questions
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The Case

53-year-old Robert K. **immigrated from India** about **a year ago**. **Over the past month** he has had **fevers, chills, night sweats and a chronic productive cough.** He goes to see his family doctor who confirms a fever of 38.5°C. Upon auscultation she also finds **crackles in the right lung** and **decreased breath sounds in the right lower lung** field. She sends Robert for a **chest X-ray** and gives him three sterile containers with instructions to generate **three deep sputum samples** over three mornings. After the samples are examined in the Microbiology Laboratory the Public Health Unit notifies Robert K. to report to the local hospital for further assessment.

Question 1

Encounter: Where do these organisms normally reside, geographically and host wise, and what are the bacterial characteristics that leave them suited to these places of residence? How would our patient have come in contact with these bacteria?

In order to answer this question, we first need to know what's wrong with Robert. In other words, we need to know what type of infection(s) he has, if any, and what are the possible causative agent(s).

What complicates this case is the fact that Robert K. is presenting with 'constitutional symptoms,' which are a group of symptoms affecting many different systems of the body.[1] Examples include weight loss, fatigue, malaise, decreased appetite, fevers, chills, and night sweats. In general, constitutional symptoms are very non-specific and can be due to a number of diseases and conditions. Thus, it is hard to determine what is wrong with Robert.

In addition to the constitutional symptoms noted above, Robert K. is also presenting with a chronic productive cough - a cough lasting longer than 3 weeks (and hence chronic), and one which produces mucus (productive).[2] This symptom is indicative of pulmonary disease, the possibility of which is also affirmed by signs of decreased breath sounds in the right lower lung and crackles heard during inspiration.

This leads me to believe that Robert K. may have Tuberculosis (TB), an airborne disease caused by *Mycobacterium tuberculosis (M. tuberculosis)* (Figure 1).[3] It predominantly affects the lungs, but may also cause disease in the brain, spinal cord, lymph nodes, kidneys, or larynx.[4] Exposure to *M. tuberculosis* may cause latent TB infection, or active TB disease.[5] In latent TB infection, *M. tuberculosis* is dormant inside the human host. It is diagnosed by a positive tuberculin skin test or serum antigen-stimulated IFN-γ release assay. The infected individual is not (yet) ill with the disease and cannot transmit the disease. Latent TB infection may be successfully limited by the host immune response, or it may progress to active TB disease, which presents with symptoms (as is the case with Robert). A person with active TB disease is capable of infecting 10 – 15 other people through close contact over the course of one year. It is usually determined by a positive sputum smear in case of pulmonary or laryngeal disease.

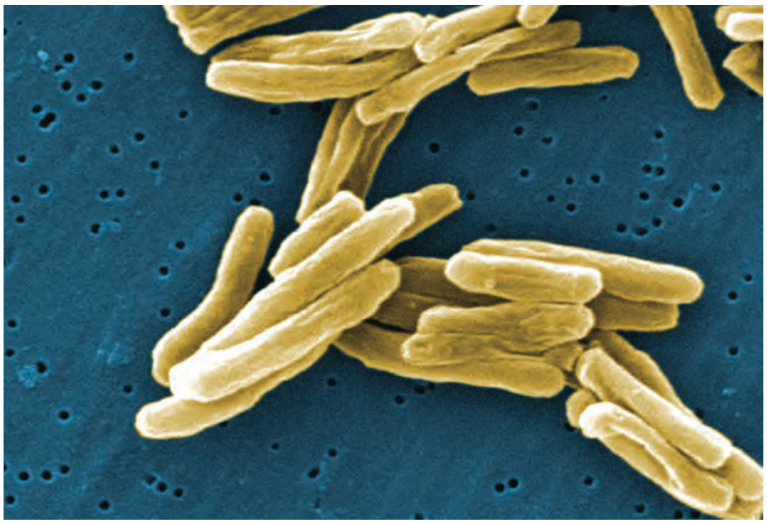


Figure 1: *Mycobacterium tuberculosis,* the causative agent of Tuberculosis

Geographic residence and survival mechanisms

TB has a worldwide distribution (Figure 2). In 2014, 9.6 million people fell ill with TB and 1.5 million died from the disease. Furthermore, over 95% of TB deaths occur in low- and middle-income countries. This is a key piece of information for Robert's case, as he has immigrated from India a year ago. TB is one of the top 10 communicable disease in India, and is the 7th leading cause of death in India.[6] These findings may be attributed to the living conditions in India, which increase the risk of exposure to TB: overcrowding, deterioration in public health systems in developing countries, high prevalence of HIV/AIDS in population.

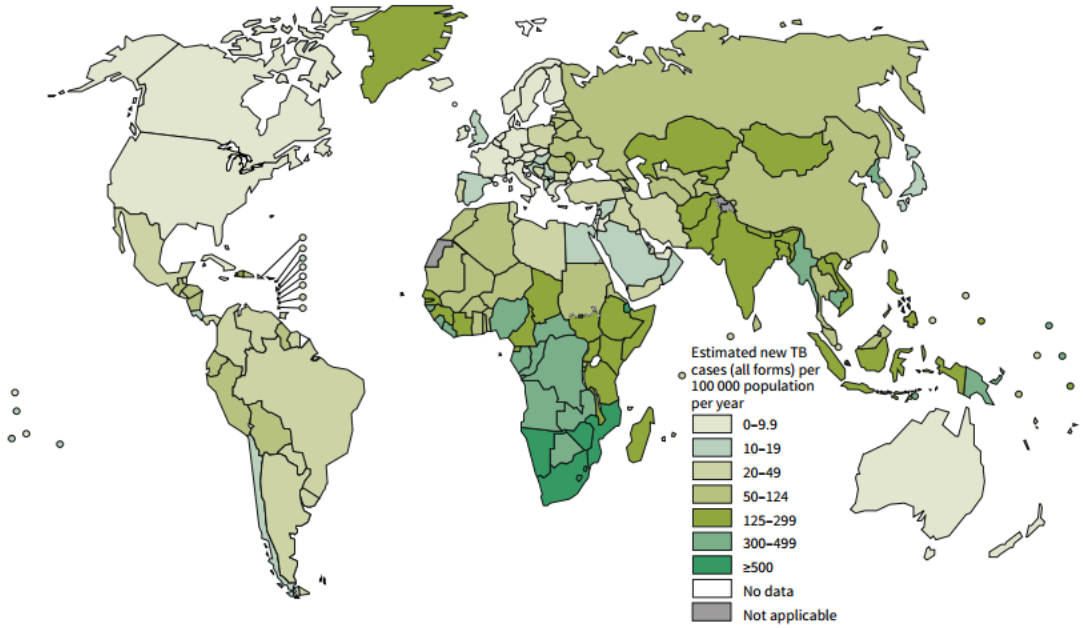


Figure 2: The distribution and estimated TB incidence rates in 2013

The Centre for Disease Control (CDC) lists these other known facts of *M. tuberculosis* and TB:[7]

- TB is among the top 5 causes of death for women aged 15 to 44
- In 2014, an estimated 1 million children became ill with TB and 140, 000 children died of TB
- TB is a leading killer of HIV-positive people: in 2015, 1 in 3 HIV deaths was due to TB
- Globally in 2014, an estimated 480, 000 people developed multidrug-resistant TB which is caused by strains of *M. tuberculosis* that are resistant to isoniazid and rifampicin
- World Health Organization (WHO): the Millennium Development Goal (MDG) target of halting and reversing the TB epidemic by 2015 has been met globally. TB incidence has fallen by an average of 1.5% per year since 2000 and is now 18% lower than the level of 2000
- The TB death rate dropped 47% between 1990 and 2015
- An estimated 43 million lives were saved through TB diagnosis and treatment between 2000 and 2014

M. tuberculosis can survive in the environment:[8]

- months on dry inanimate surfaces
- 8 weeks in cockroach feces
- 19 days in sputum on carpet
- 88 days in sputum on wood
- 4 weeks in moist and dry soil
- more than 74 days in environment if protected from light

The cell wall envelope is the primary bacterial characteristic which enables survival of *M. tuberculosis,* both in the environment and in the human host.[9] The complexity of the cell wall envelope includes long chain α-alkyl β-hydroxy mycolic acids which are responsible for the extremely low permeability of mycobacterial cells. The complexity of the cell wall also confers resistance to disinfectants such as quaternary ammonium compounds, chlorhexidine gluconate, and iodophore which have been reported to be ineffective against *M. tuberculosis*.[10]

Host residence and survival mechanisms

M. tuberculosis is not part of the normal flora of humans. Once a patient comes in contact with *M. tuberculosis,* the bacteria will infect the lungs primarily, but in some cases can also spread to sites such as the kidney, bones and stomach.[11]

These bacteria have a number of virulence factors[a] which enable survival in the host:

(1) lipid and fatty acid metabolism
(2) cell envelope proteins
(3) proteins inhibiting antimicrobial effectors of the host macrophages
(4) protein kinases
(5) proteases
(6) metal-transporter proteins
(7) gene expression regulators

(1) Lipid and Fatty Acid Metabolism

M. tuberculosis has the ability to metabolise lipid and fatty acids using several enzymes belonging to the fatty acid Synthetase (FASII) system.[12] This is a notable virulence factor, as bacteria usually metabolise carbon sources for survival. However, *M. tuberculosis* is able to adapt its metabolism in the host to one that is based on fatty acids instead of carbohydrates.[13]

An important fatty acid in the pathogenicity of *M. tuberculosis* is mycolic acid.[14] Mycolic acids are very long chain α-alkyl β-hydroxy fatty acids which are esterified to both the outer peptidoglycan layer as well as to glycerol and trehalose in the cell wall of *M. tuberculosis*. Mycolic acids display great variability in structure, as they can contain many functional groups, such as methyl groups. These methyl groups may be further modified by conversion to cyclopropane rings, methoxy or keto groups. As such, there are many sub-families of mycolic acids.

The presence of mycolic acids increases the pathogenicity of *M. tuberculosis* in the following ways:[15]

(1) resistance to therapeutic agents due to the structural variability exhibited by mycolic acids
(2) resistance to chemical damage and dehydration
(3) allow bacteria to grow inside host macrophages, without detection by host immune response
(4) allow for interaction with host innate immune receptors

These functions of mycolic acids in increasing the pathogenicity of *M. tuberculosis* can be attributed to their physical structure. This has been shown in previous studies in which modification of the functional groups of mycolic acid lead to attenuation of growth of the bacteria *in vivo*.[16]

[a] Virulence factors: molecules produced by pathogens which increase their ability to cause disease in host, or enable survival in host

In addition to the presence of mycolic acids in the cell wall of *M. tuberculosis,* there are a number of other complex lipids and glycolipids present. The most notable of these lipids is phthiocerol dimycocerosates (PDIM).[17] These lipids have 2 functions: (1) structural and (2) host-cell interactions.

As part of their structural role, these lipids contribute to the low permeability of the cell wall to nutrients and antibacterial drugs. This feature slows down the growth of the bacteria and makes disease caused by pathogenic species difficult to treat.[18]

Some of these lipids are key ligands for host cell receptors, allowing molecular docking between host phagocytes and mycobacteria to allow for cell invasion (see question 2 below).[19] Still other lipids are involved in avoiding lysosomal fusion and acidification, intracellular trafficking and vacuole maturation arrest, modification of host cell signalling affecting the secretion of cytokines necessary for host protection, and in host inflammation processes during tuberculosis infection.

Other virulence genes involved in fatty acid/lipid metabolism include:[20]

(1) Fad33: an acyl-CoA synthase which aids *M. tuberculosis* virulence by supporting tissue-specific replication (Fad33 allows bacterial replication in host liver)
(2) Isocitrate lyases (Icl1 and Icl2): allow *M. tuberculosis* to convert isocitrate to succinate (a fatty acid source for bacterial survival) in the glyoxylate cycle
(3) Phospholipase C-type enzymes: aid in bacterial growth in late phase infection of host macrophages

(2) Cell Envelope Proteins[21]

Cell Wall proteins

Outer membrane proteins (OMPs)

Cell wall proteins include the outer membrane proteins (OMPs), which function in the uptake of small hydrophilic compounds across outer membranes, efflux processes and in energy-dependent uptake of nutrients. OMPs also play a major role in attachment and invasion (see question 2 below) of host cells. An important OMP that has been described in mycobacteria is OmpATb. OmpATb is a porin which has the ability to function in the extreme acidic conditions of host phagosomes, thereby allowing survival of *M. tuberculosis*.

The efflux function of OMPs has also been noted to increase drug resistance of *M. tuberculosis* as it is able to effectively pump out therapeutic agents from the bacterial cell.

Exported receptive protein (Erp)

Erp is a cell wall associated surface protein that has been shown to aid *M. tuberculosis* multiplication in cultured macrophages in mice, suggesting that Erp contributes to virulence of M tuberculosis in humans.

Fibronectin binding protein (Fbp)

Fbp is a complex of 3 proteins (A, B, and C2) which functions to bind to fibronectin, which in turn allows the adhesion of mycobacteria to host mucosal surfaces facilitating entry into host cells (see question 2). It is also involved in the synthesis of cell wall lipids.

Mammalian cell entry (Mce) protein

Mce proteins confer mycobacteria the ability to enter into mammalian cells and survive inside the macrophage and cause a latent TB infection. Another function of Mce proteins is to transport essential compounds required by mycobacteria for survival, such as cholesterol.

Heparin-binding hemagglutinin (HbhA)

HbhA is an adhesin on the cell wall and functions to promote the attachment of mycobacteria to host epithelial cells and fibroblasts.

PstA1 and PhoT

These are proteins involved in the import of inorganic phosphate into mycobacteria during starvation, allowing mycobacteria survival in host phagosomes.

CaeA

A carboxylesterase located on cell surface involved in modulating innate immune control of infection. CaeA confers mycobacteria the ability to continue growing in host macrophages.

KefB

A potassium/proton anti-port which effluxes, or exports, potassium from the cytoplasm of *M. tuberculosis* and uptakes protons from the host phagosome, thereby increasing luminal pH. This prevents phagosomal acidification induced in host macrophages in response to bacterial infection, and thus elimination of the bacteria from host.

Opp-Dpp ABC Transport proteins

These are permeases involved in the uptake of small peptides which modulate intracellular signalling pathways and thereby favor the survival of *M. tuberculosis* inside cells.

CtaC

CtaC is a subunit of cytochrome *c* oxidase and is important for bacterial growth under aerobic conditions.

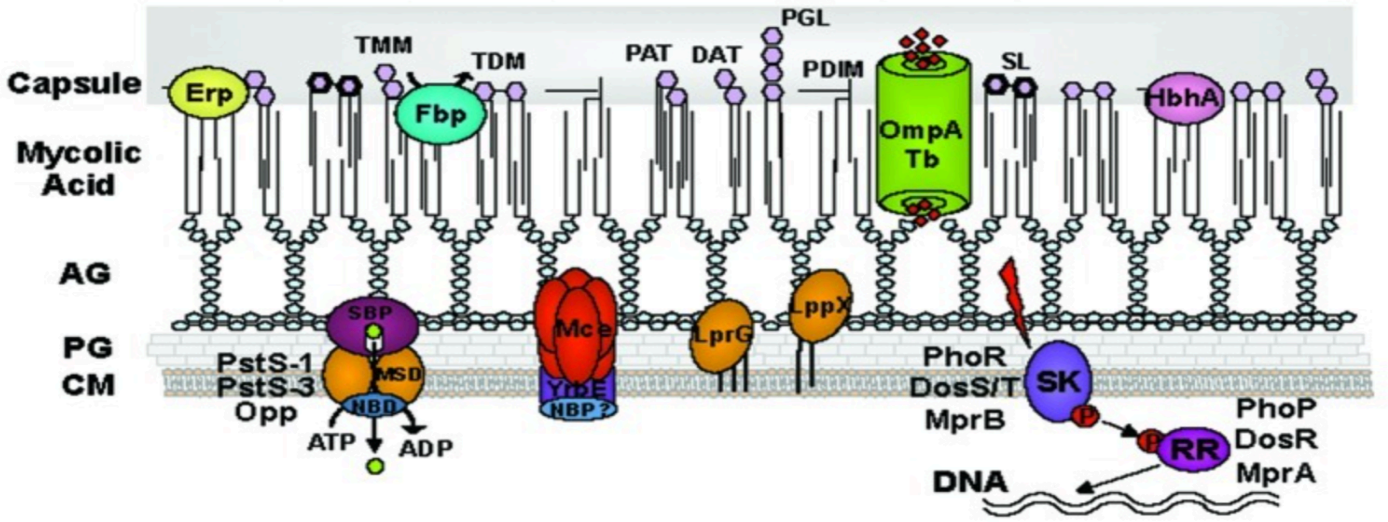


Figure 3: The core of the cell wall is comprised of peptidoglycan covalently bound to a linear galactofuran, which is in turn attached to several strands of a highly branched arabinofuran joined to mycolic acids. These lipids are perpendicularly oriented to the plane of the membrane and provide a special barrier responsible for many of the physiological and disease-inducing aspects of mycobacteria. Within this "core" are the PDIMs, TDM, SLs and PIMs. In turn, phosphatidyl-myoinositol mannosides, lipomannan (LM) and lipoarabinomanan (LAM) are anchored to the plasma membrane and extend to the exterior of the cell wall.

Lipoproteins

Using bioinformatics, it has been predicated that the *M. tuberculosis* genome potentially encodes 48 to 99 lipoproteints representing 1.2 to 2.5% of the proteome. The general functions of lipoproteins in mycobacteria include:

- Transport
- Cell wall metabolism
- Cell adhesion
- Signalling and protein degradation

Secretion systems

Secretion systems are important in bacterial functioning, adaptation, survival, and interaction with host cells. *M. tuberculosis* contains a total of five type seven secretion systems (T7SS) (also known as ESX), which secrete distinct immune modulators during the macrophage infection cycle. For example, ESX-1 secretes effector proteins that are critical for the translocation of *M. tuberculosis* to the cytosol of host cells. The ESX-5 system, induces a caspase-independent cell death (apoptosis) of host cells after translocation has taken place.

M. tuberculosis also has a Type II secretion system by which lipoproteins are exported.

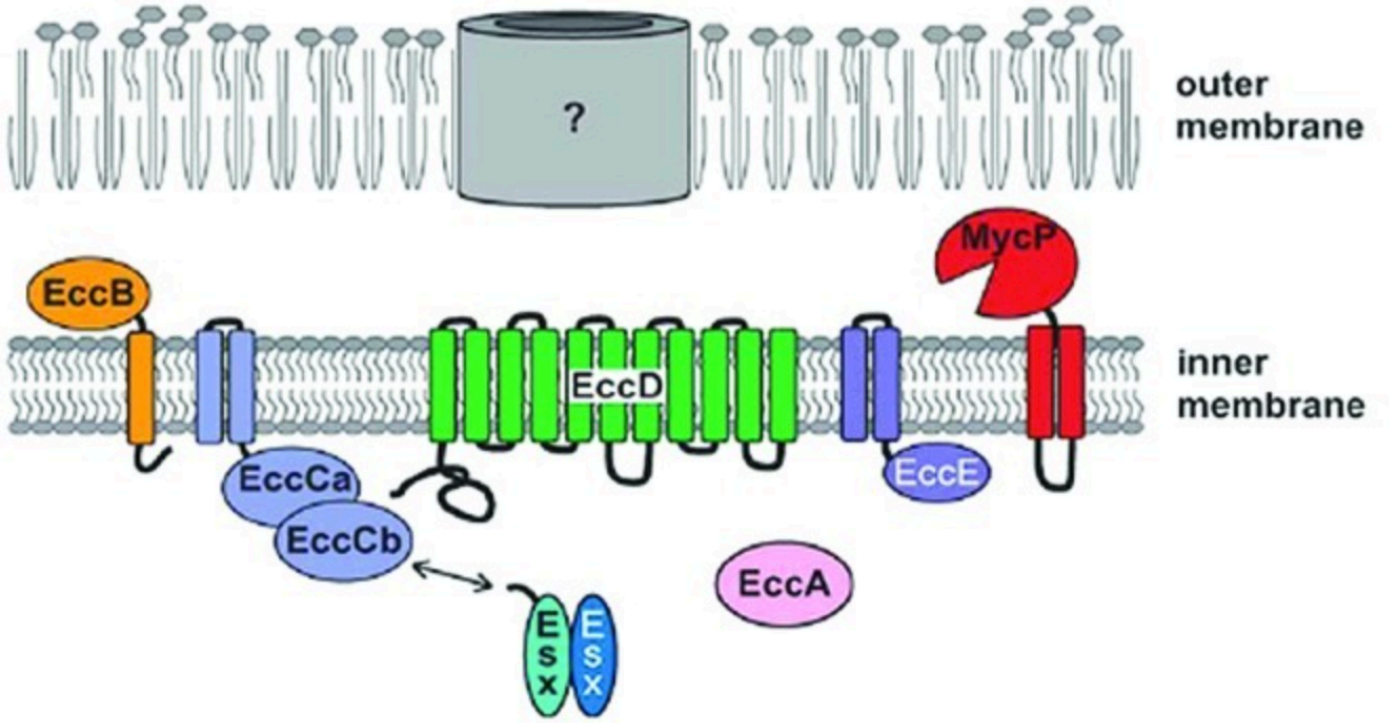


Figure 4: The T7SS (ESX) secretion system in *M. tuberculosis*. *M. tuberculosis* contains 5 of these systems. Four of the five systems have conserved genes (known as ESX conserved components (Ecc)) and also contains genes encoding for proteins (ESX-1 secretion-associated proteins (Esp)).

(3) Proteins inhibiting antimicrobial responses of host macrophages [22]

During infection, host macrophages function in phagocytosis, in recruitment of other immune cells and in presentation of microbial antigens to cells of the adaptive immune system. However, mycobacteria, including *M. tuberculosis* has evolved mechanisms to counteract the macrophage microbicidal ability, thereby increasing their survival in hosts. These mechanisms include ways to avoid oxidative and nitrosative stresses, phagosome arresting and inhibition of apoptosis (Figure 5 A, B, C, respectively.)

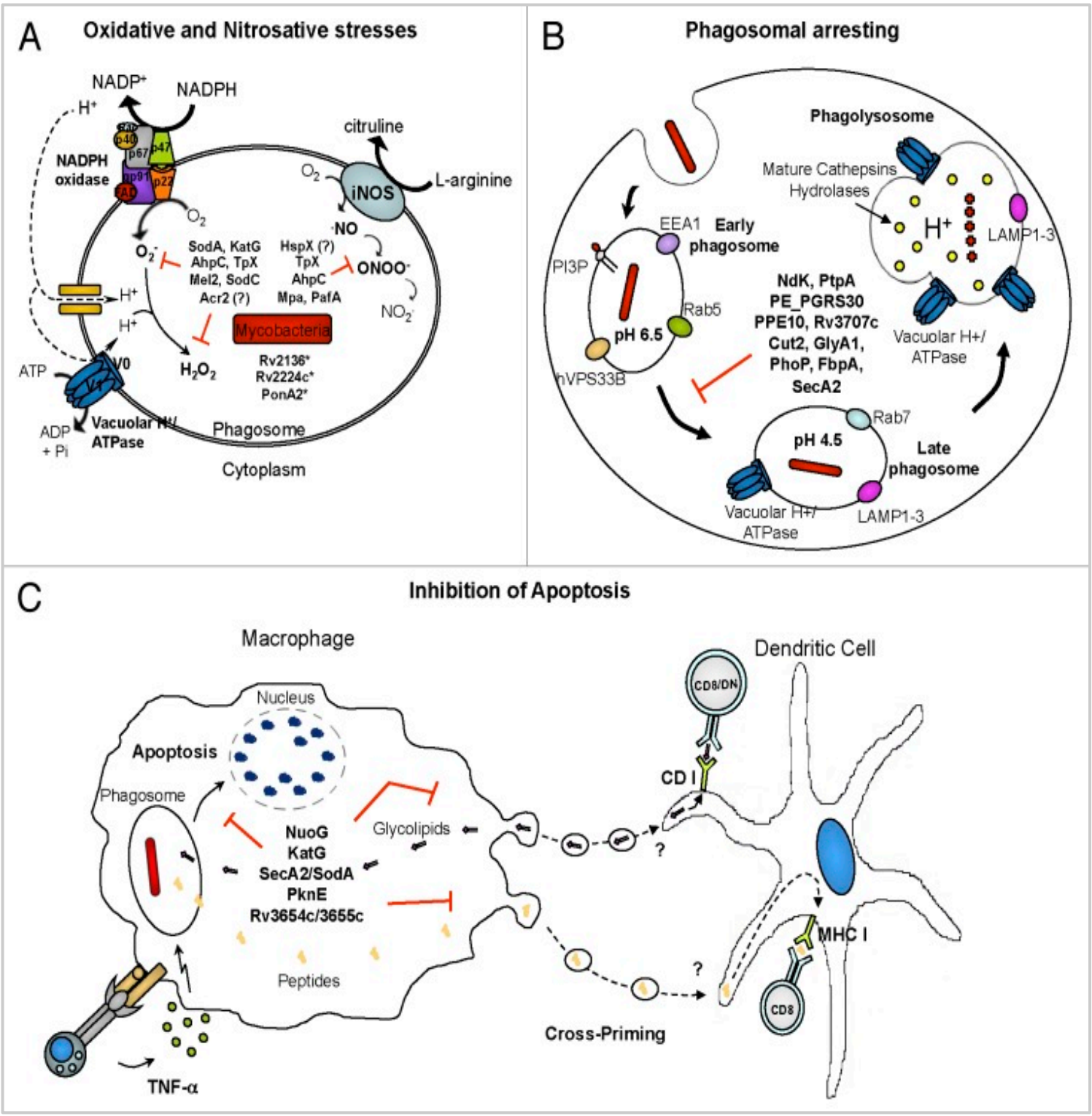


Figure 5: The various defence mechanisms used by *M. tuberculosis* to counteract the microbicidal activities of host macrophages: (A) Oxidative and nitrosative stresses (B) phagosome arresting (C) inhibition of apoptosis

Oxidative and nitrosative stresses

After phagocytosis of bacteria, host cells produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) with have the ability to kill bacteria by inducing damaging to bacterial nucleic acids, proteins, lipids and carbohydrates. However, mycobacteria have a number of defense mechanisms to protect themselves against the damaging effects of these agents, including a highly impermeable cell wall (see above) to act as a barrier against ROS and RNS.

These defence mechanisms are accomplished by many mycobacterial proteins, which are summarized in Figure 5A:

Acr family proteins

The α-crystallin (Acr) family of proteins are produced in response to hypoxia or nitric oxide. Although their exact function is unknown, it has been proposed that this family of proteins has a role in maintenance of long-term viability during latent TB infections and in replication during initial TB infection.

Rv2136c, Rv2224c and PonA2

These proteins increase resistance of mycobacteria to low pH, which is characteristic of phagosomes, and to sodium dodecyl sulphate (detergent), heat shock as well as to ROS and RON. They are believed to have a role in peptidoglycan biosynthesis, and thus contribute to the cell wall structure of mycobacteria.

AhpC (Alcohol hydroperoxide reductase C)

AhpC functions to reduce organic peroxides, and thus mycobacteria are able to evade harmful effects of the respiratory oxidative burst process that takes place in host macrophages.

SodC (superoxide dismutase)

M. tuberculosis has two genes encoding SOD proteins, *sodA* and *sodC,* the transcription of which is upregulated upon macrophage infection. These enzymes function to detoxifiy ROS by converting O2- into molecular oxygen and hydrogen peroxide.

Mel2

This protein plays an important role in resistance of mycobacteria to both ROS and RNS in activated macrophages.

KatG

KatG is an enzyme with catalase-peroxidase activity that degrades H2O2 and organic peroxides in *M. tuberculosis*.
TpX

A thiol peroxidase enzyme that catalyzes the reduction of hydroperoxides and peroxynitrite in *M. tuberculosis* and is thus essential for protecting mycobacteria against the RNS and ROS produced by host cells during TB infection.

Phagosome arresting

Another means by which mycobacteria inhibit antimicrobial responses of macrophages is through phagosome arresting. The proteins involved in this process are summarized in Figure 5B.

Normally, pathogenic microorganisms, such as mycobacteria, are phagocytosed into cellular compartments called phagosomes in which they can be killed or digested by degradative enzymes. A phagosome fuses with lysosomes as part of their maturation process to form phaglysosomes. The acidic environment of phagolysosomes is deadly to pathogens.

However, some intracellular pathogens, including mycobacteria, have the ability to continue to grow inside phagolysosomes or escape into the cytoplasm before the phagosome fuses with the lysosome. Some bacteria can also prevent the phagosome-lysosome fusion by manipulating host macrophages. The proteins involved in phaogsome arresting in *M. tuberculosis* are:

Ndk Protein

A nucleoside diphosphate kinase with ATP and GTP binding and hydrolysis activity which functions to inhibit the recruitment of effectors to phagosome maturation.

PtpA Protein

A low-molecular tyrosine phosphatase which is involved in the inhibition of a host protein (VPS33B) required for regulation of membrane fusion within the endocytic pathway, resulting in arrested phagosome maturation by *M. tuberculosis*.

PE_PGRS30

This protein functions to inhibit phagosome-lysosome fusion by *M. tuberculosis* and to also increase replication of mycobacteria within host macrophages.

Inhibition of apoptosis

Apoptosis is defined as programmed cell death. It is one of the major mechanisms of the host innate immune response in order to eliminate or control the infection through the killing of infected cells. *M. tuberculosis* has developed a number of anti-apoptotic capacities to counteract this host response. An example of one such protein is the NuoG protein (Figure 5C). NuoG is a subunit part of the type I NADH dehydrogenase which functions to inhibit the host extrinsic TNF-α dependent apoptosis pathway; suppress neutrophil apoptosis; and delay the host adaptive immune response. Other proteins involved in inhibition of apoptosis, which are summarized in Figure 5C, include SecA2/SodA, PknE, Rv3654c and Rv3655c.

(4) Protein Kinases [23]

M. tuberculosis genome encodes 11 eukaryotic-like serine-threonine protein kinases through which it is able to regulate its metabolism in response to external stimuli via signal-transduction pathways. They are mainly localized in the cell membrane and cell of *M. tuberculosis*, however some may be found localized in the cytoplasm. A few of the general functions of protein kinases include:

- Modulation of different cellular events such as environmental adaptation, differentiation, and cell division in response to environmental clues
- Determine cell shape, morphology, and cell division
- Provide substrates for enzymes involved in peptidoglycan and mycolic acid biosynthesis
- Regulate cell growth, septum formation, glucose transport in *M. tuberculosis*
- Aid in replication of *M. tuberculosis* inside macrophages

(5) Proteases [24]

Proteases are required for cellular homeostasis as they control proteins involved in transcription, regulation, metabolism and virulence. Microbial pathogens, including *M. tuberculosis,* frequently use extracellular proteases to induce host tissue destruction or to modulate the host immune response through inactivation of host immunoglobulins and complement proteins. They may also function in activating key regulatory proteins or peptides and acquiring nutrients by hydrolyzing host proteins. The genome of *M. tuberculosis* encodes over 100 proteases, including:

Serine proteases

Serine proteases are secreted enzymes which cleave peptide bonds in non-specific host proteins to provide bacteria with readily importable peptides.

ATP-dependent proteases

These proteases require ATP as energy to degrade specific proteins in intracellular environments of the host. http://www.ncbi.nlm.nih.gov/pubmed/17074491

Metalloproteases

These are proteases which use metals, such as zinc, for their catalytic activities. The genome of *M. tuberculosis* encodes 3 zinc-dependent metalloproteases, two of which are described here:

Zmp1

A metalloprotease which functions to cleave host proteins involved in a specialized inflammatory caspase activating protein complex. Without these proteins, the host cannot generate IL-1β which is dependent on the caspase activating protein complex, and thus the host cannot effectively elimination the TB infection.

Rip1

M. tuberculosis requires sigma factors to direct RNA polymerases (RNAP) to specific promoters during cell envelope development. In the absence of external stimuli, these sigma factors are held inactive by trans-membrane anti-sigma factors. In response to external stimuli, these anti-sigma factors are degraded via proteolysis by Rip1 which allows the sigma factors to direct RNAP to appropriate promoters and initiate cell envelope development in *M. tuberculosis*.

Proteasome-associated proteases

In eukaryotes, a proteasome is responsible for the degradation of proteins that are targeted for destruction via ubiquitin tagging. A similar system has been identified in *M. tuberculosis,* which uses two proteasome-associated proteins named Mpa and PafA. These proteins function to protect mycobacteria against RNI (see above). Although the exact mechanism is not known, it is hypothesized that these two proteins may dispose of NO-damaged proteins that are toxic to the bacterial cell through degradation using the bacterial proteasome.

(6) Metal Transporter Proteins [25]

M. tuberculosis requires metals such iron, magnesium, cobalt, copper, manganese and zinc for survival in both the environment or within the host. These metals are part of prosthetic groups or serve as enzyme co-factors. As with any living system, *M. tuberculosis* requires a balance of these metals – too little or too much could be toxic. As

such, *M. tuberculosis* has evolved many strategies to accomplish this balance by importing metal into their cytoplasm, or pumping them out to the extracellular medium.

(7) Gene Expression Regulators [26]

During an active TB infection, the host immune response essentially "walls in" *M. tuberculosis* into granulomatous lesions, which are a collection of immune cells attempting to prevent the spread of the infection. The environment within these lesions is toxic to *M. tuberculosis,* and include low-oxygen tension, nutrient depletion, ROS and RNS, altered pH toxic lipid moieties and agents which are able to perturb the cell wall of *M. tuberculosis*.

Accordingly, the genome of *M. tuberculosis* encodes a variety of gene expression regulators to be able to survive within granulomatous lesions. Alternative sigma factors are an example of a gene expression regulator in *M. tuberculosis*. In response to stress, the level and activity of alternative sigma factors increases, thereby allowing the transcription of a discrete set of genes by bacterial RNAP. These genes function to contribute to stress responses and increase the likelihood of survival of *M. tuberculosis* within granulomatous lesions.

Patient Contact

TB is spread from person to person through the air (Figure 6). When people with active TB cough, sneeze or spit, they propel the TB germs into the air, which leads to the creation of airborne droplets containing bacterial agents.[27] Because of the speed that tubercle bacilli have when they are being coughed up, they get catapulted into the air, and easily reach another person standing one or two meters away.[28] These airborne droplets are small enough in size (1 – 2 μm or less) and are thus able to gain passage into the lower respiratory tract.[29] Due to their small size, these airborne droplets are also called droplet nuclei.[30] Droplets of large sizes are prevented from reaching the lower respiratory tract through the physical barriers of the nasopharnyx and upper respiratory tract. A person needs to inhale only a few of these airborne droplets to become infected.

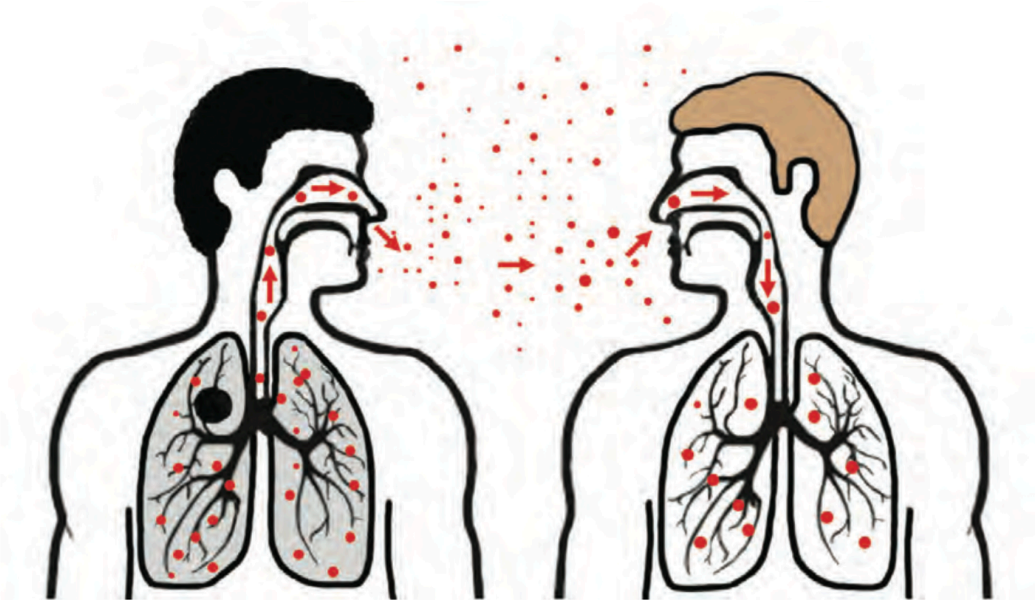


Figure 6: *M. tuberculosis* is spread when a patient with active TB disease sneezes, coughs or spits and releases tiny airborne droplet nuclei containing bacilli which then may be inhaled by another person

Once the airborne droplets reach the lungs, 4 outcomes are possible:[31]

(1) The initial host response may completely and effectively kill all bacilli and the patient has no change of developing TB in the future

(2) The organisms may begin to multiply and grow immediately after infection, causing what is known as primary TB
(3) The host response may effectively control and contain the bacilli, but not effectively eliminate them, in what is known as a latent TB infection; the bacilli become dormant
(4) The latent organisms may be reactivated in the future and cause active TB disease; this occurs in about 5 – 10% of patient with latent TB infection

TB cannot be spread by shaking hands, sharing food or drinks, touching bed linens or toilet seats, sharing toothbrushes or kissing.[32]

(1) TB and air travel [33]

Given that approximately 1/3 of the world's population is infected with TB and with the increasing ease, availability and duration of air travel, with large numbers of people travelling internationally, as in Robert's case, the spread of TB is rampant. In a confined space, such as an airplane, where the air is also re-circulated, the risk of transmitting or contracting TB is extremely high since droplet nuclei are small enough to remain suspended in the air. In Robert's case, he may have gotten infected from an airline passenger or crew member with active TB disease, who did not know they had TB due to the vagueness of symptoms, or did know but chose not to tell authorities and boarded the flight anyway.[b]

[b] The following news article presents a case of one such woman: http://www.rense.com/general79/tbin.htm

Question 2

Entry: how do these bacteria enter into the human host and where do they take up residence? What are the molecular, cellular and/or physiological factors at play in this site specificity and in the initial adherence step?

As mentioned in question 1, *M. tuberculosis* enter the human host through inhalation of airborne droplets containing *M. tuberculosis*.[34] These airborne droplets are small in size (1 – 2 μm or less) and are thus able to pass the physical barriers of the upper respiratory tract (Figure 7A).[35] The airborne droplets then travel into the lower respiratory tract (Figure 7A) where *M. tuberculosis* then takes up residence in the lungs, specifically in the alveoli (Figure 7B and 7C). [36] The bacteria can also spread to other sites via the bloodstream or lymphatics (see question 3).

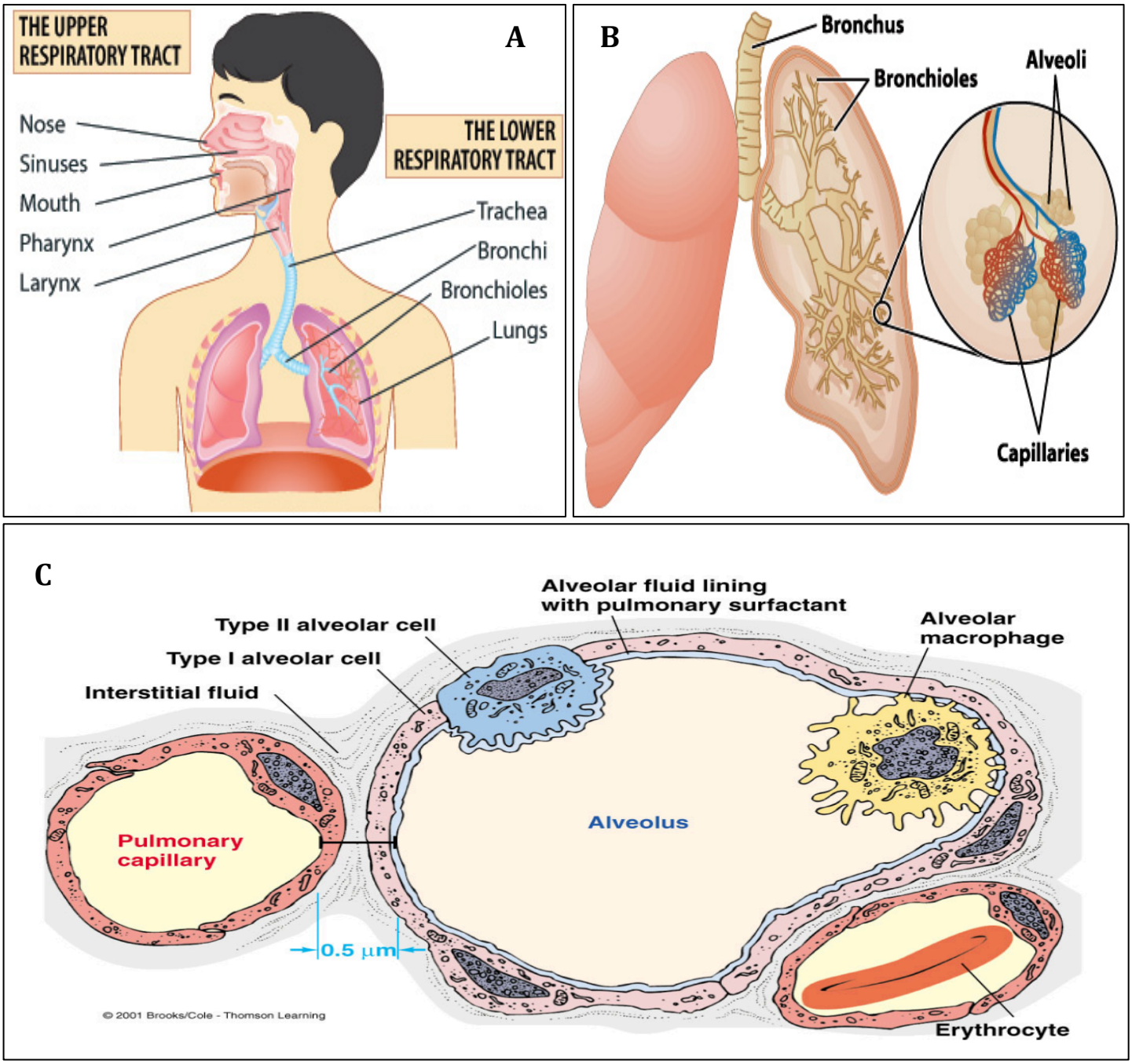


Figure 7: (A) The classification of the respiratory tract (B) Lung anatomy (C) The cells of the lung

Upon entry into the alveoli, the first contact of *M. tuberculosis* is thought to be with resident macrophages, called dust cells (Figure 7C).[37] These cells are located within the alveolar lumen and are able to scavenge for pathogens within the alveoli. In the alveoli, mycobacteria may also encounter dendritic cells, which function in antigen presentation to activate the adaptive immune response and in the dissemination of mycobacteria due to their migratory nature.[38] However, most studies have focused on the interaction of *M. tuberculosis* – macrophage, which will be the focus of this section.

M. tuberculosis – macrophage interaction

Phagocytosis of *M. tuberculosis* is accomplished via cell surface receptors expressed on macrophages. These receptors include complement and mannose receptors, and other cell surface receptor molecules (Figure 8).[39] The expression of these receptors is up-regulated by variety of mediators, including PGE_2 and IL-4. In contrast, the expression of these receptors is down-regulated by IFN-gamma, which therefore decreases the ability of macrophages to bind to mycobacteria.[40]

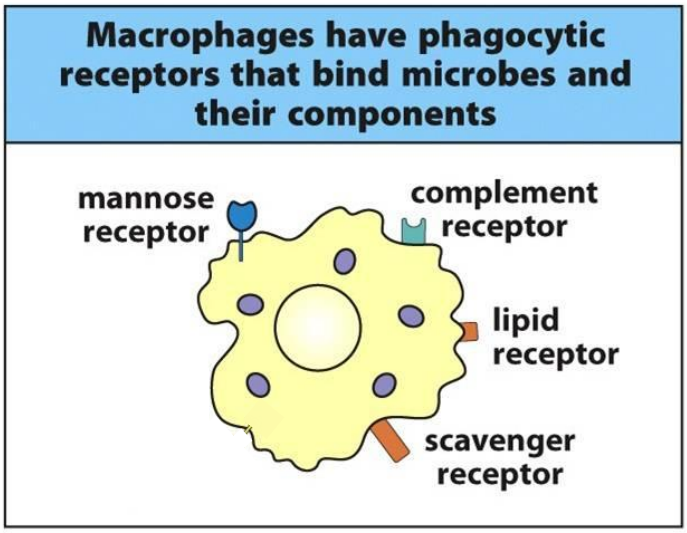


Figure 8: Various receptors expressed on macrophages

Complement receptors

M. tuberculosis is capable of activating the alternative pathway of complement activation, resulting in opsonization with complement proteins C3band C3bi.[41] When coated with these complement proteins, mycobacteria are able to bind to complement receptors 1, 3 and 4 (CR1, CR3, CR4, respectively) and can subsequently be phagocytosed into macrophages which express CR1, CR3, CR4.[42]

M. tuberculosis is also able to bind to CR's without the use of complement proteins through their endogenous capsular polysaccharides located in the capsule of their cell envelopes.[43]

Mannose receptors

Mature macrophages bind and internalize *M. tuberculosis* via mannose receptors located on the cell surface of macrophages. These mannose receptors bind to the *M. tuberculosis* ligand liparabinomannan (LAM) which is located on the bacterial cell wall (see Figure 3). LAM contains terminal mannose residues that interact with mannose receptors.[44]

In addition to phagocytosis of *M. tuberculosis,* mannose receptors can mediate the delivery and presentation of bacterial antigens to T helper cells or to cytotoxic T cells.[45]

Other receptors [46]

(1) CD14

CD14 is a phosphatidylinositol glycan-linked membrane protein which binds to lipopolysaccharides of gram-negative bacteria, and is also able to bind to LAM of *M. tuberculosis*. CD14 has been demonstrated for attachment of mycobacteria to microglia (resident phagocytic cells of the brain) as well as attachment of mycobacteria to alveolar macrophages.

(2) Scavenger receptors

These macrophage receptors are able to bind to a wide variety of ligands, including to LAM and to sulfolipids of *M. tuberculosis*. It is not yet known whether scavenger receptors play a role in simply binding to *M. tuberculosis* and aiding phagocytosis by other immune cells, or if they have a direct role in phagocytosis.

(3) Fcγ receptors

Individuals who have had a past active TB infection will have circulating anti-tuberculosis antibodies (IgG) in their serum. Upon re-infection, the IgG molecules will coat mycobacteria, which are then ingested by macrophages using Fcγ receptors. Once ingested, *M. tuberculosis* enter vesicles which fuse with lysosomes containing degradative enzymes and toxic metabolities which will attempt to eliminate the TB infection.

Figure 9 summarizes the events which take place after macrophages have phagocytosed *M. tuberculosis* using the various receptors described.

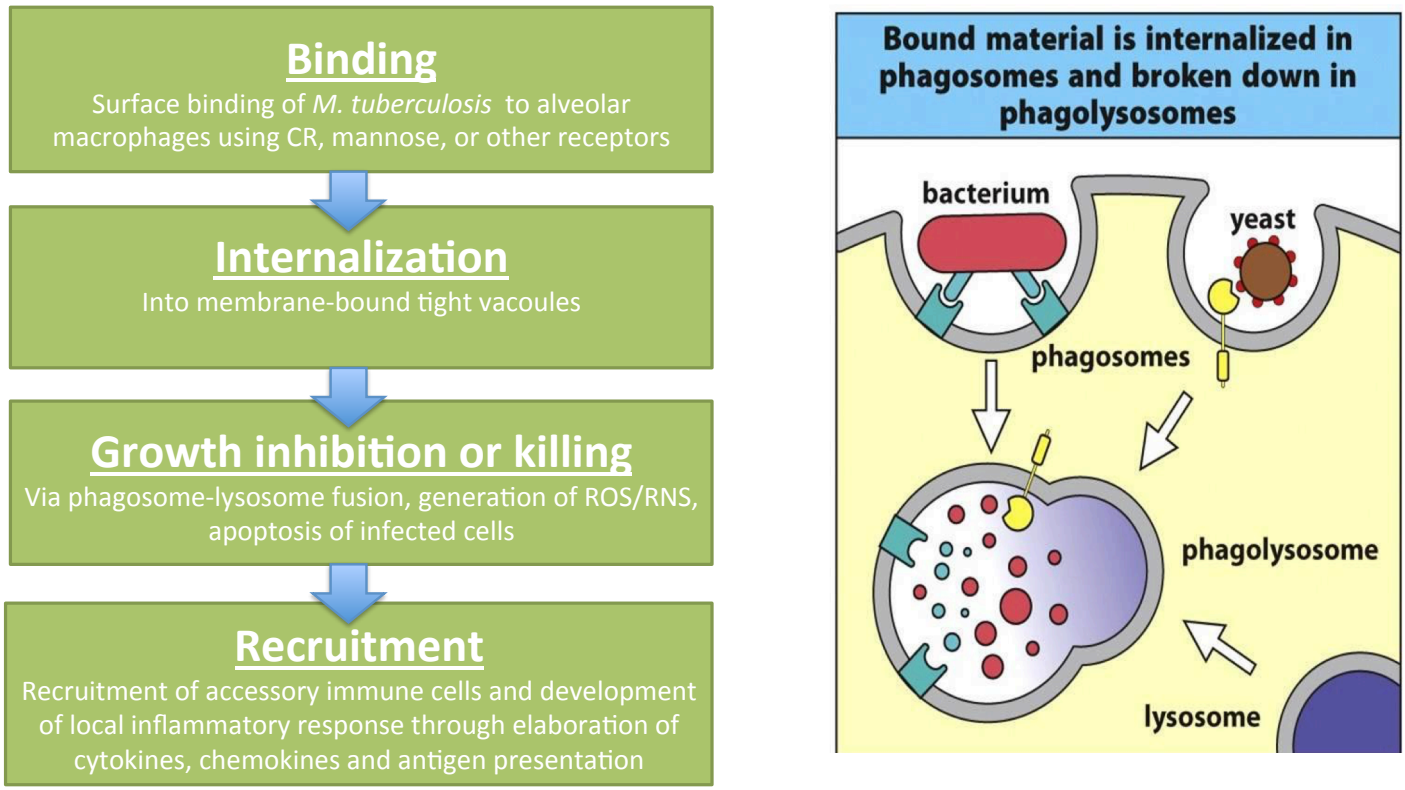


Figure 9: The process of phagocytosis

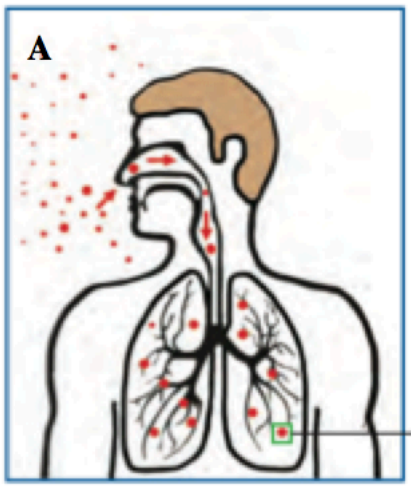
M. tuberculosis displays many diverse ligands on its surface, and as such is able to engage multiple receptors of multiple types simultaneously.[47] Different receptors may be engaged by *M. tuberculosis* depending on the state and differentiation of macrophages. For example, as macrophages mature from monocytes, the expression of CR3 decreases, while that of CR4, mannose receptors and scavenger receptors increases.[48]

More so, the receptor-mediated route of entry of *M. tuberculosis* may affect subsequent events.[49] For example, mannose receptors can deliver LAM to the endocytic compartments containing receptors for antigen presentation to T cells. It is not clear whether other receptors can perform this function.

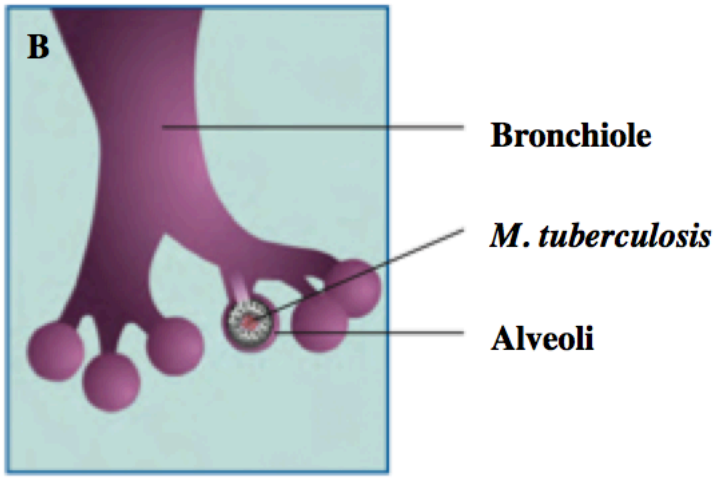
Question 3

Multiplication and Spread: do these organisms remain at the entry site and/or do they spread beyond the initial site; are there, for instance, secondary sites of infection? Do they remain extracellular and/or do they enter into cells and what are the molecular and cellular determinants of these events?

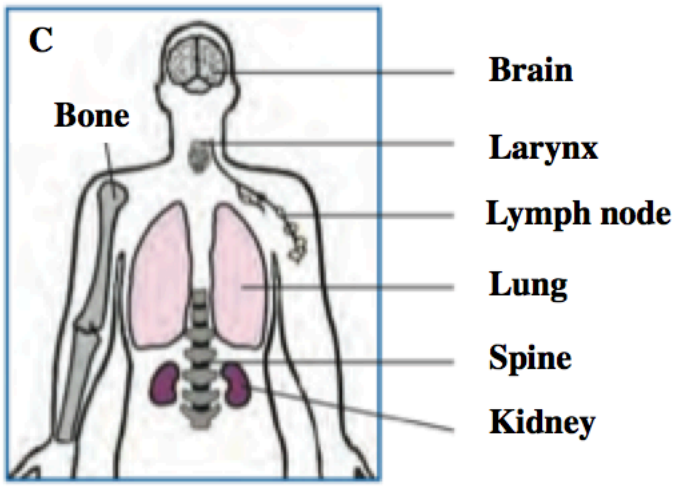
Figure 10 describes the entire process of a TB infection, from entry to multiplication and finally to spread.[50]



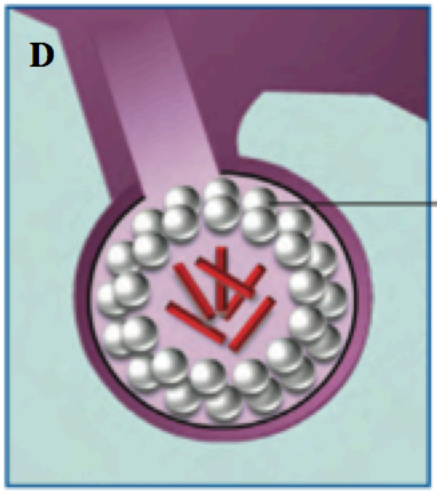
Airborne droplets, or droplet nuclei, containing *M. tuberculosis* are inhaled, enter the lungs, and travel to the alveoli.



Bacteria multiply in the alveoli.

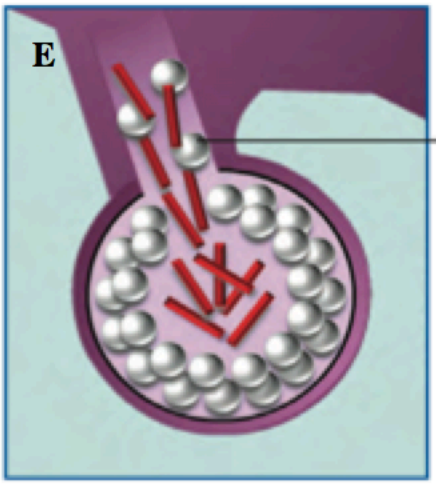


A small number of bacteria may enter the bloodstream and spread throughout the body. The mycobacteria may reach any part of the body, including the brain, larynx, lymph nodes, spine, bone or kidneys.



If the mycobacteria are contained with the lungs, within 2 to 8 weeks, resident macrophages will ingest and surround the mycobacteria (see question 2). These host immune cells form a "wall" around the bacteria, called a granuloma, that functions to contain and control the bacilli as to prevent the progression to active TB disease.

Note: in this figure, the bacilli are in the lungs but this process can occur in different areas of the body outlined in (C)



If the host immune system cannot keep the bacteria under control, the granuloma breaks down and the bacilli begin to multiply and spread through destruction of lung tissue. The bacteria may also enter the pulmonary capillaries, which form an extensive network around alveoli, and cause disseminated TB. In this case, the patient will present with symptoms characteristic of active TB disease (e.g. fever, chills, night sweats, chronic productive cough).

As can be seen in Figure 10, it is evident that *M. tuberculosis* do not remain at the primary site of infection (the lungs) but instead may spread to secondary sites of infections through the lymphatic or blood system.[51]

At all sites of infection, whether primary or secondary, the events outlined in Figure 10 D and E occur. Namely, resident macrophages of the infected site phagocytose mycobacteria and attempt to wall of the infection through the formation of granulomas. If unable to do so, or if the macrophages die and release live bacilli, the mycobacteria will multiply and spread rapidly and cause active TB disease in the infected site.[52]

The risk of a TB infection developing into active TB disease is greater for a person with a weakened immune system. Conditions/situations that weaken the immune system and increase the risk of TB disease include:[53]

- HIV and AIDS
- organ transplants
- a type of lung disease called silicosis
- chronic kidney failure requiring dialysis
- cancer of the head and neck
- having been infected with TB within the past two years
- a chest x-ray showing signs of old TB
- diabetes mellitus (all types)
- being underweight (body mass index < 20)
- being under five years of age when first infected with TB
- cigarette smoking (one pack a day or more)
- treatment with TNF-α inhibitors (e.g., for auto-immune disorders such as rheumatoid arthritis) or glucocorticoids

Clinical presentations of TB disease in various body sites are shown in Figure 11.

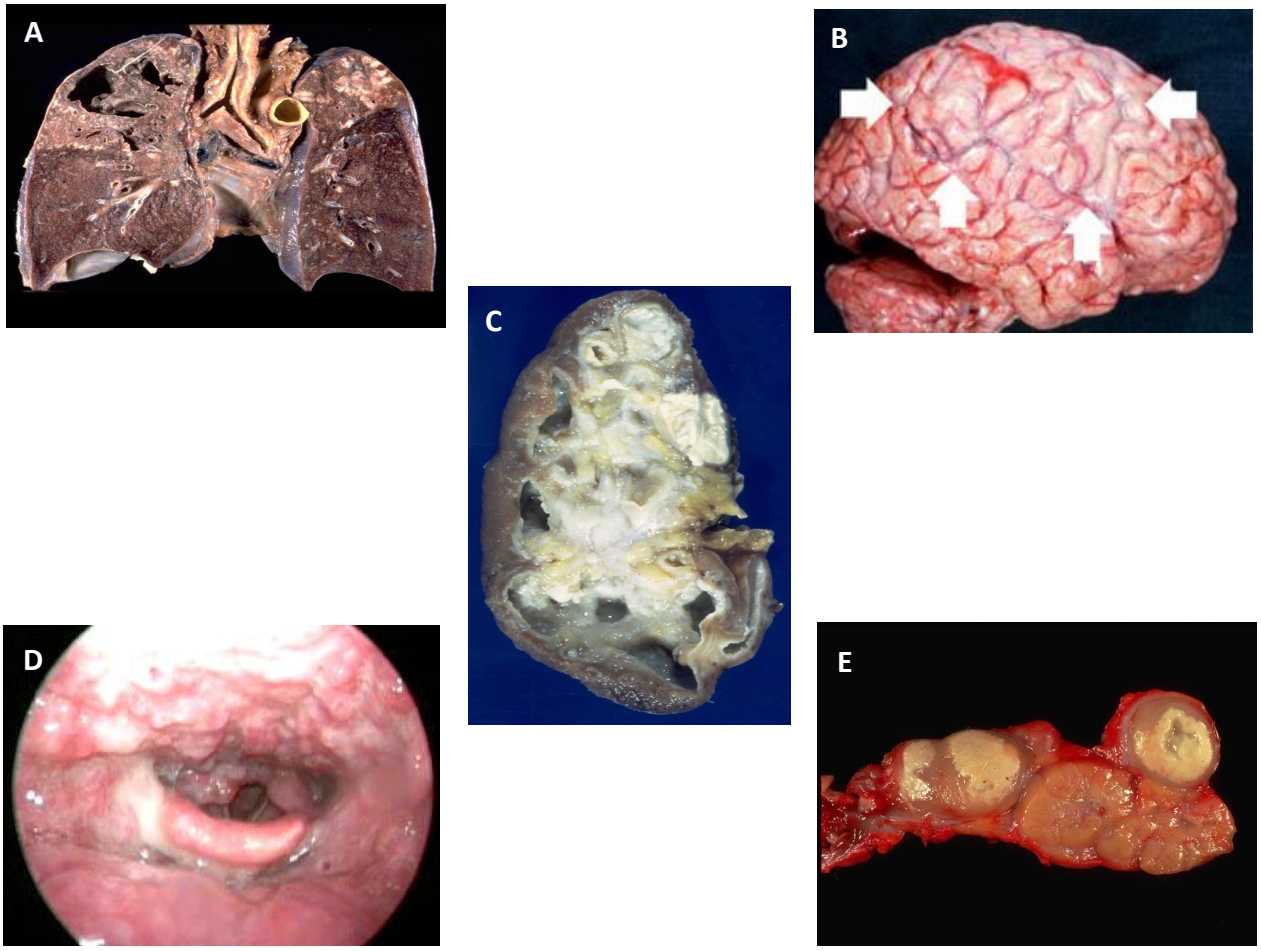


Figure 11: The clinical presentations of active TB disease. (A) Pulmonary TB (B) meningitis (C) Renal TB (D) laryngeal TB (E) TB in lymph nodes

As can be seen in Figure 11A, pulmonary TB presents with diseased tissues that hava cheese-like appearance (caseation) as well as with cavitation (see question 4 below).[54] In the brain (Figure 11B), purulent exudate is shown via the white arrows, which leads to the blockage of brain blood vessels.[55] As with the lungs, the kidneys (Figure 11C), present with caseaous necrosis and granulomas composed of epithelioid cells and immune cells.[56] Laryngeal TB (Figure 11D) presents with caseation, ulceration and fibrosis, all of which lead to stenosis of the larynx and consequently hoarseness of a patient's voice.[57] Lastly, Figure 11E shows granuloma formation and extensive inflammation in TB of the lymph nodes.[58]

Question 4

Bacterial Damage: do the bacteria cause any direct damage to the host (or is the damage fully attributable to the host response, as indicated below) and, if so, what is the nature of the bacterial damage? Can it be linked to any of the signs and symptoms in this case?

Direct Bacterial Damage

During active TB disease, the bacilli break out of the granuloma and become reactivated. Once reactivated, the bacteria destroy lung tissue around the granuloma through the action of bacterial proteases.[59]

Host Immune Response Damage

In an initial infection with TB, the host immune response functions to create a granuloma (Figure 12) around the bacilli. A granuloma is a mass of immune cells, including macrophages, dendritic cells, T cells and epithelioid cells, which functions to "wall off" the infection to prevent its spread.[60] In this case, the infected individual is said to have a latent TB infection.[61]

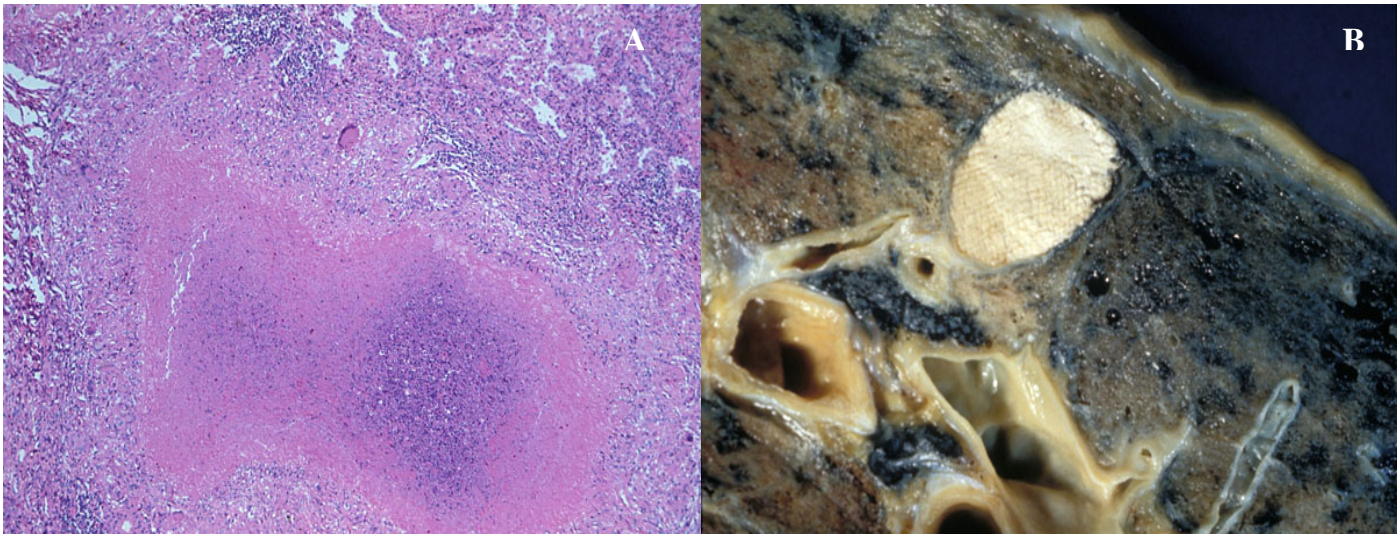


Figure 12: (A) Histologic appearance of a granuloma in the lungs showing an aggregation of cells (B) Gross pathology of a granuloma in the lungs

However, if the host immune response is weakened due to risk factors mentioned above, the granuloma breaks down and the mycobacteria rapidly spread and multiply. In this way, a latent TB infection progress to active TB disease, in which the patient presents with symptoms of TB disease such as fever, chills, night sweats and a chronic productive cough. These symptoms can be attributed to damage incurred by the host immune response during active TB disease.

(1) Symptoms

Robert K. is presenting with a cough because this is the body's normal response to get rid of foreign particles, including pathogens, in the respiratory tract.[62] Furthermore, coughing can also be attributed to the ensuing inflammation in the lungs and airways.[63] The inflammation is caused by immune cells trying to eradicate the infection. Initially, people with pulmonary TB have a dry, persistent cough, which is often worst at night. This dry cough may progress to a productive cough as the destruction of lung tissue worsens and sputum is coughed up.[64] As the disease progresses, sputum of patients with pulmonary TB may present with blood stains as a result of tissue destruction and inflammation.[65]

The cough may be accompanied by a fever and with sweating, which are more pronounced at night.[66] A fever occurs as part of the normal response of the body to get rid of pathogens. Biochemical substances, known as pyrogens, are released either by infected body tissue or by pathogens themselves, and trigger the hypothalamus which then signals the body to retain and generate more heat, leading to a fever.[67] Examples of pyrogens include TNF-α and interleukins.[68] A high body temperature may impair the replication process of *M. tuberculosis,* which is temperature-sensitive, and therefore contribute to the elimination of infection.[69] Chills, much like a fever, cause the core body temperature to rise through rapid muscle contraction and relaxation which produce heat.[70] Chills often are associated with fevers.

As the fever is more pronounced at night, so is the accompanying sweating. Sweating is the body's natural response to decrease body temperatures when they reach beyond a certain threshold level. In this case, sweating is counteracting the effects of the fever on the human body.[71]

Although the exact mechanism of why fever, chills and sweating are more pronounced at night during a TB infection is not known, it is hypothesized that these diurnal patterns in temperature fluctuations occur due to a nocturnal rise in bacterial loads and pyrogenic cytokines. In other words, bacterial loads and the amounts of pyrogenic cytokines increase at night, and the body responds by inducing fevers, chills and sweats at night to fight the infection.[72]

Weight loss, although not reported in this case, is also a common symptom of pulmonary TB because the body uses a big part of its energy reserve to fight the infection in the lungs. This means that energy cannot be stored in the body to gain weight and stay healthy.[73]

(2) Signs

The signs which the family doctor noted, namely crackles in the right lung and decreased breath sounds in the right lower lung, can be attributed to the host response as well.

Firstly, mycobacteria up-regulate the expression of collagenase and gelatinase in peripheral blood monocytes. These two proteins of the extracellular matrix serve to digest collagen and other matrix proteins, leading to caseaous necrosis (death of tissue) in the centre of the granuloma (this is also caused by bacterial proteases).[74] Necrosis is also induced by TNF-α released by activated macrophages, and by killing of infected cells (apoptosis) by cytotoxic T cells.[75]

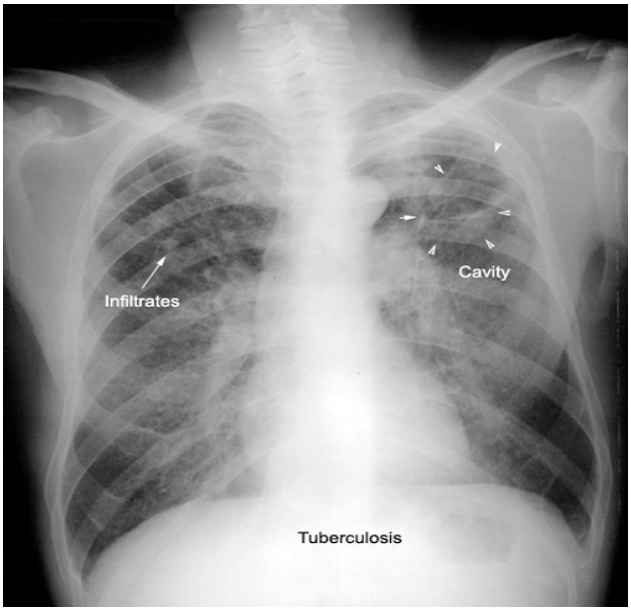


Figure 13: Chest X-ray of TB patient illustrating cavitations

In response to necrosis, deposition of connective tissue will occur and lead to fibrosis, or hardening of the lung tissue. Normally, the lung tissue is very thin to allow for efficient gas exchange. With necrosis and fibrosis, the alveolar wall is thickened, leading to impairment in gas exchange. As gas is unable to diffuse efficiently through thick barriers, crackles during inspiration and decreased breath sounds are noted by the physician. A second effect of fibrosis and necrosis, in addition to impairing gas exchange abilities, is cavitation. Cavitation is the breaking and tearing down of fibrotic and necrotic tissue, leading to formation of pits in the lung tissue, which can be seen on X-rays to diagnose pulmonary TB (Figure 13), and hence why the doctor ordered a chest X-ray for Robert K. Furthermore, these cavitations contain sputum with about 1 million bacilli per millilitre. These sputum samples are also ordered for patients with TB for diagnosis.

Reasons for why the right lung is predominantly affected in TB cases are not well understood. However, anatomical factors may be one reason (Figure 14).[76] In general, the right lung is more prone to infection due to its angular position: the right bronchus as an angle of 25°, whereas the left bronchus has an angle of 45° to accommodate for the heart. As such, the right bronchus appears to be a more direct continuation of the trachea than the left bronchus. When airborne droplets enter the upper respiratory tract, they have a greater probability of going down the right bronchus into the right lung rather than the left side.

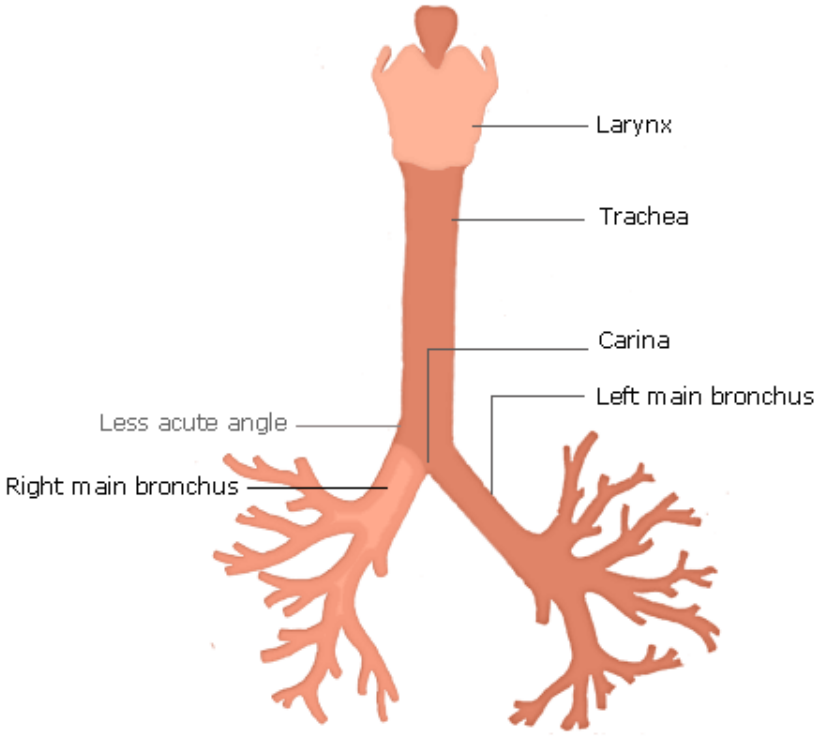


Figure 14: Anatomy of the right and left respiratory tract explains why the right lung is mostly affected in TB

An important point to note is that TB does not frequently affect the lower lung field, as is the case with Robert.[77] Usually, TB affects the apex (top) of the lung due to a higher ventilation: perfusion ratio (V/Q).[78] In other words, this means that the top of the lungs receive more oxygen than the bottom of the lungs. Since *M. tuberculosis* is an obligate aerobe, it establishes an infection in the apex of the lung. In Robert's case, a lower lung field TB infection may indicate immunodeficiency.[79]

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FIGURES

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Figure 6:

http://www.cdc.gov/tb/education/corecurr/pdf/chapter2.pdf

Figure 7:

A: http://cdn.return2health.net/articles/wp-content/uploads/The-upper-respiratory-tract21.jpg
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C: http://mcph.uic.edu/home

Figure 8:

https://classconnection.s3.amazonaws.com/665/flashcards/817665/jpg/macrophages_express_receptors_that_enable_them_to_take_up_microbes_by_phagocytosis-1444E930BC46ECCC005.jpg

Figure 9:

https://classconnection.s3.amazonaws.com/665/flashcards/817665/jpg/macrophages_express_receptors_that_enable_them_to_take_up_microbes_by_phagocytosis-1444E930BC46ECCC005.jpg

Figure 10:

http://www.cdc.gov/tb/education/corecurr/pdf/chapter2.pdf

Figure 11:

A: http://granuloma.homestead.com/files/tb_gross_lung34.jpg
B: http://img.wikinut.com/img/1d-0k5vg-qn3n4_q/jpeg/0/Tuberculous-Meningitis-Infection-In-The-Brain.jpeg
C: http://granuloma.homestead.com/files/tb_gross_extrapulm_kidney57.jpg
D: https://i.ytimg.com/vi/GnwVMlor-v8/maxresdefault.jpg
E: http://granuloma.homestead.com/files/gross_tb_lymphnode3.jpg

Figure 12:

A: http://www.fujita-hu.ac.jp/~tsutsumi/case/case076.htm
B: http://www.fujita-hu.ac.jp/~tsutsumi/case/case076.htm

Figure 13:

http://www.lumen.luc.edu/lumen/MedEd/medicine/pulmonar/cxr/atlas/images/237a1.jpg

Figure 14:

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