Robert's Case
Bacterial Pathogenesis

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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 38.5C. 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 3 deep sputum samples over 3 mornings. After the samples are examined in the laboratory, the Public Health Unit notifies Robert K. to report to the local hospital for further assessment.

INTRODUCTION

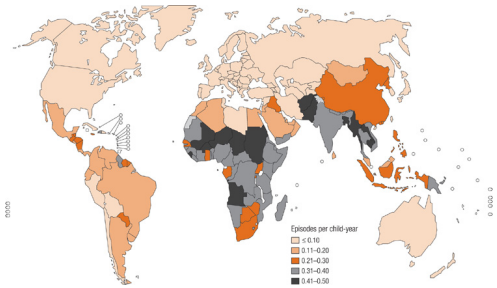
Robert is expressing constitutional symptoms, which affect different areas of the body. These include night sweats, fevers, and chills. However, given his geographic location and symptoms I have narrowed it down to the two most probable bacteria that could have infected Robert K:

1. *Streptococcus pneumoniae*
2. *Mycobacterium tuberculosis*

Question 1

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?

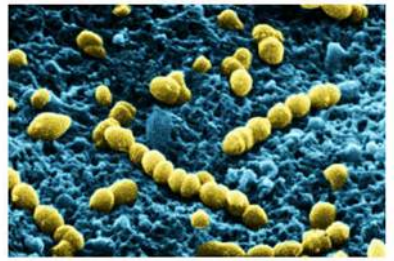
Encounter: *S. Pneumoniae*



A gram positive, non-motile cocci bacteria.

- Often arranged in pairs (diplo-coccus) or short chains.
- Lung infection characterized by cough, fever and difficulty breathing.
- Primarily affects children under 5 years old and adults over 50 years old.
- Highest incidence is in India, China, and multiple African countries.

<u>Two major types:</u>



i. **Community-acquired pneumonia (CAP)**

- develops with people limited contact with healthcare
- Most common cause of CAP (30-50% of pneumonia c
- Leading cause of illness and death worldwide

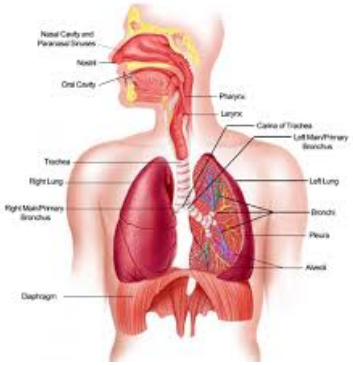
Ii. **Hospital-acquired pneumonia (HAP)**

- Develops with in a patient at a hospital at least 48-72 hours
- Most common cause of death among nosocomial infections.

Encounter: *S. Pneumoniae*

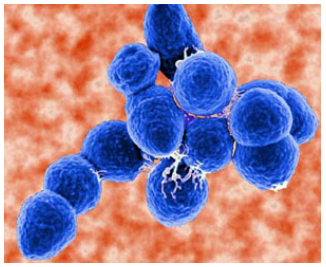
Img. NaturalNews

- *S. Pneumoniae* is most often spread via airborne droplets
- A result of an infected person coughing, sneezing or person-person oral contact.
- Bacteria enter lung via inhalation and reside in the upper respiratory tract.
- Nasopharynx is main residential location of bacteria.
- Colonization is regulated by mucosal immune response.
- A poor mucosal immunity can lead to the bacteria colonizing sterile parts of the airway, leading to infection.
- *S. pneumoniae* is susceptible to cold, dry and hot temperatures. not able to survive outside a human host for long periods.



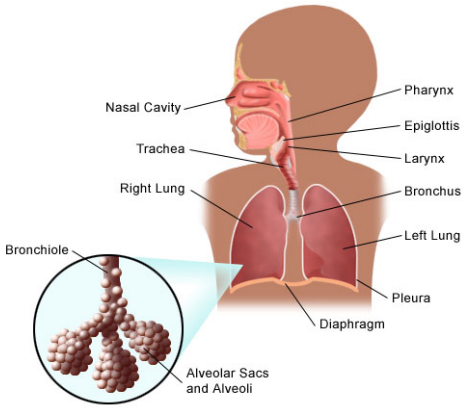
Img.Organs of the Body

Encounter: *S. Pneumoniae*



Bacterial Characteristics

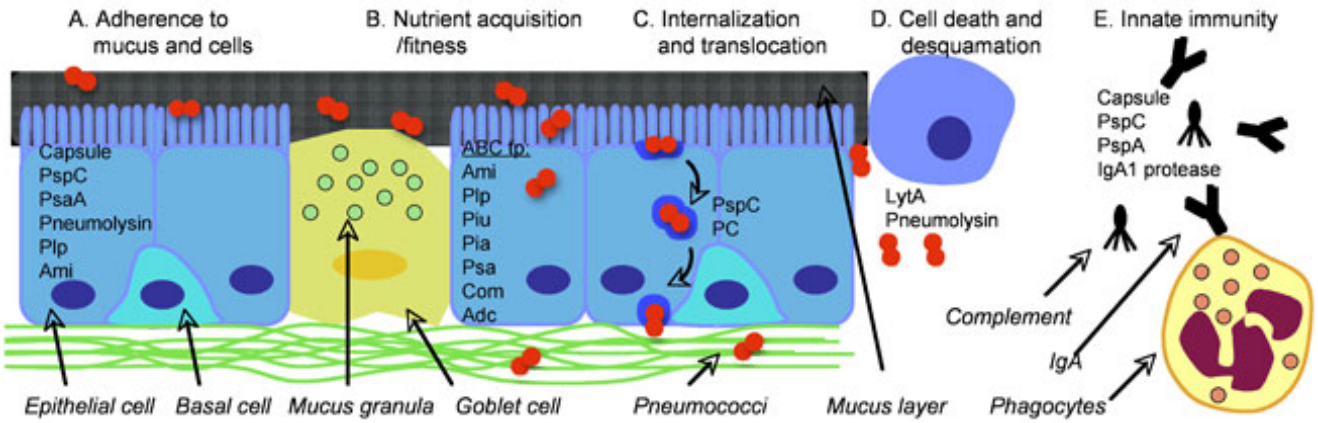
- *S. pneumoniae* is able to make a biofilm that allows the bacteria to adhere to the mucosal surfaces of the mucosal lining of the respiratory system.
  - requires CiaR/H two component system which regulates genes required for biofilm formation.
- Capsule interferes with phagocytosis by macrophages and neutrophils by preventing binding of complement to cell surface.
- Pilli allows attachment to epithelial cells and colonization of upper respiratory tract.
- Pneumococcal serine-rich repeat protein (PsrP) and pyruvate oxidase both help bacterial adhesion.
- IgA proteases degrade the endogenous IgA antibodies



Encounter: *S. Pneumoniae*

Bacterial Characteristics

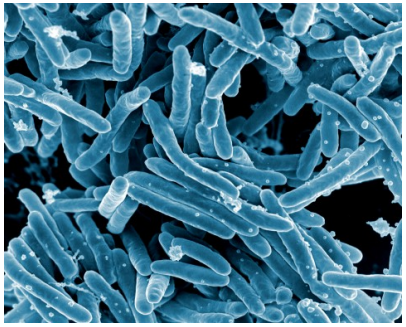
- Choline binding proteins inhibit complement mediated opsonisation
- Cell wall phosphorylcholine acts as an adhesin and docking site for choline binding proteins.
- The colonization by *S.pneumoniae* requires CiaR/H two component system which regulates genes required for biofilm formation.
- Pneumococcal serine-rich repeat protein (PsrP) and pyruvate oxidase both positively help the bacteria in adhesion.



Img. Anderson Hakson Lab

Mycobacterium Tuberculosis

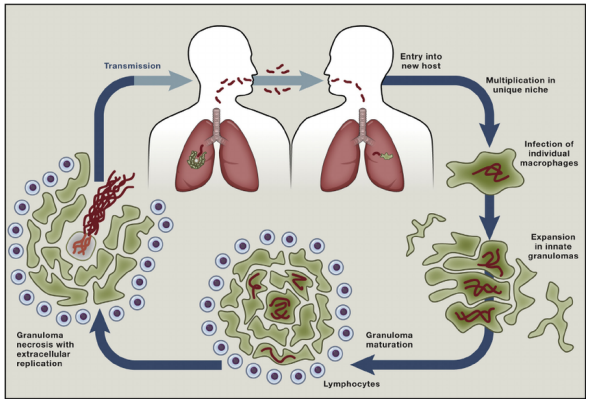
Encounter: MTB



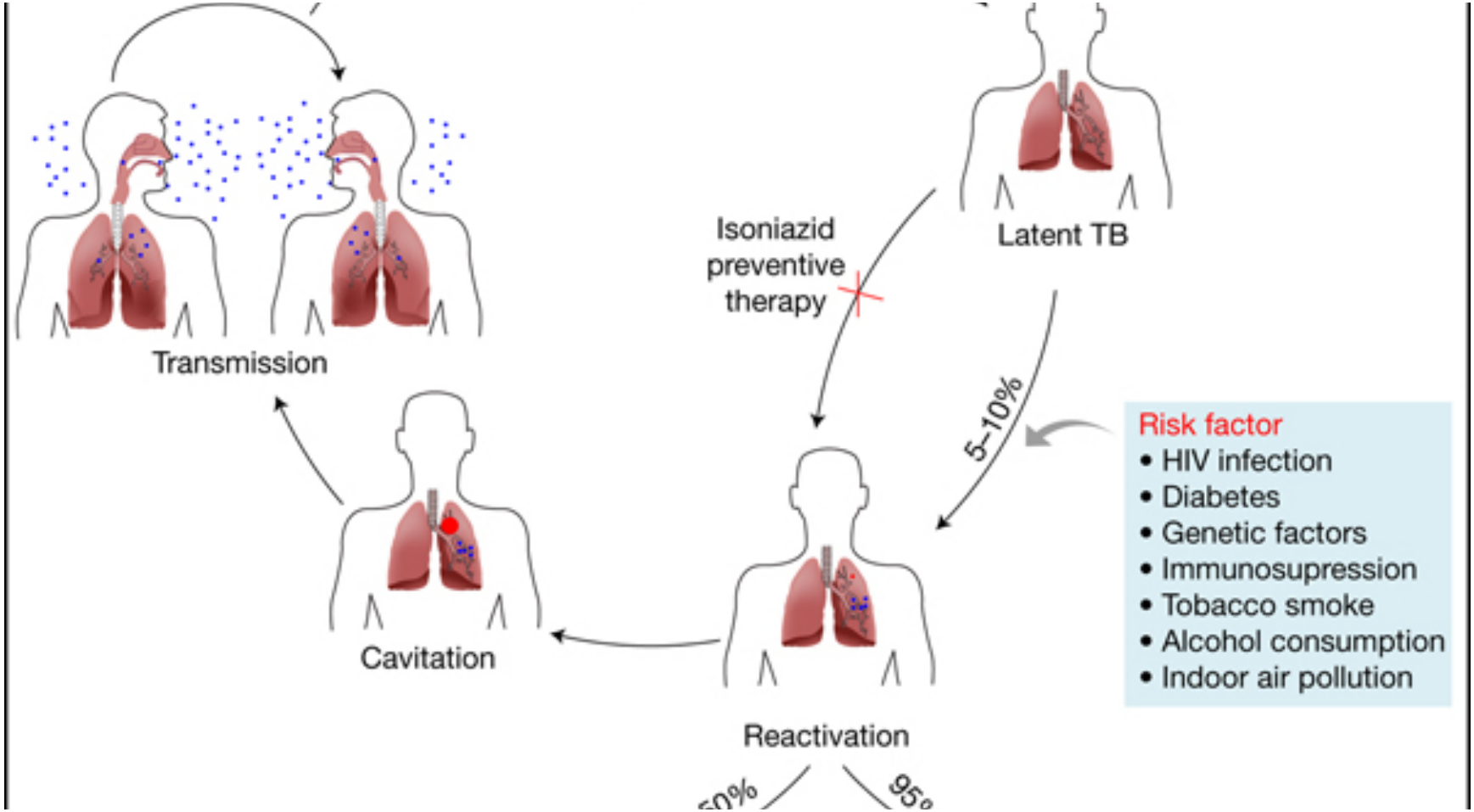
- Bacteria that predominately affects the lungs but can also spread to lymph nodes, larynx and brain.
- Symptoms include: chronic coughing, chest pain, fatigue, night sweats, fever.
- <u>Two forms:</u>

i. **Active**: can be spread to others and individual exhibits symptoms.

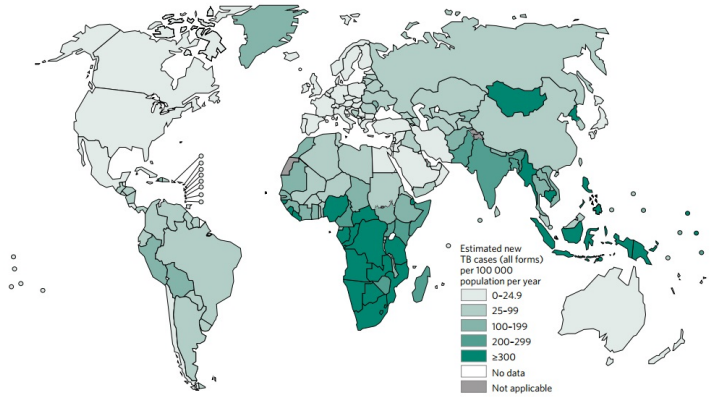
Ii. **Latent**: cannot be spread and carriers do not exhibit symptoms. Multiple triggers can cause the activation of MTB including having a compromised immune system such as patients with HIV.



MTB Lifecycle



Encounter: MTB

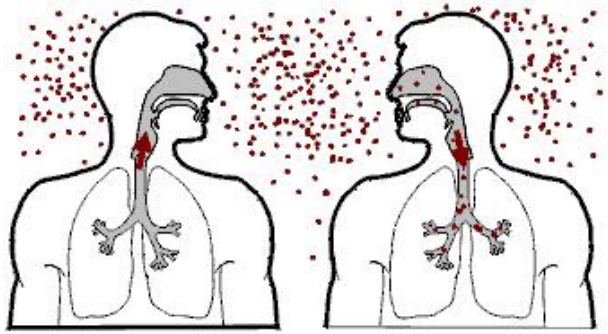


Geographical Distribution:

MTB can be found worldwide, however, some countries are at higher prevalence.

- Most common in developing countries: India, Pakistan, Indonesia, China, South Africa and the Soviet Union being among the top, according to the World Health Organization.
- Humans are the only known reservoir and it is not part of the normal flora.
- Second most common cause of death, with 10.4 million people worldwide infected and with 1.8 million deaths annually (WHO).
- Majority are asymptomatic carriers with the latent form of MTB, meaning they cannot spread the disease to others. However, they can have a positive TB skin test.
- India has 2.2 million cases of MTB out of 9 million cases worldwide.
- MTB kills one person every two minutes in India.

Encounter: MTB



Transmission:

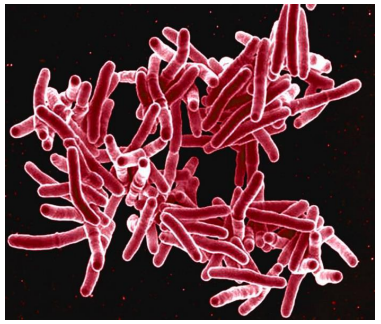
- MTB enters the host via aerosol droplets released from actively infected individuals that sneeze, cough or have person-person oral contact.
  - Droplets can remain suspended in air for hours.
  - Only those with the active form of TB are contagious.
- Other modes of transmission: **percutaneous transmission, venereal transmission**.
- Environmental factors that increase chance of transmission include:

➢ poor ventilation and small enclosed spaces.

MTB can survive outside the host for weeks to months depending on the type of environment. In a dry environment it can last survive for months, and if protected from sunlight.

Healthcare providers, HIV patients and people in residential facilities (nursing homes, prisons) are most at risk.

Encounter: MTB



- The bacteria passes into the respiratory system when inhaled and begins colonization.
- MTB enters the alveoli where it comes in contact with resident lung macrophages or alveolar epithelial type II pneumocytes.
- MTB can evade phagocytosis by their cell wall that contains mycolic acids, affecting the permeability of the cell surface
- Also prevents attack from lysozyme, cationic proteins and complement deposits in serum, acting like a proective shield against the reactive oxygen species released by macrophages
- Mycolic acids also allow protection against certain antibiotics and other antibacterial agents

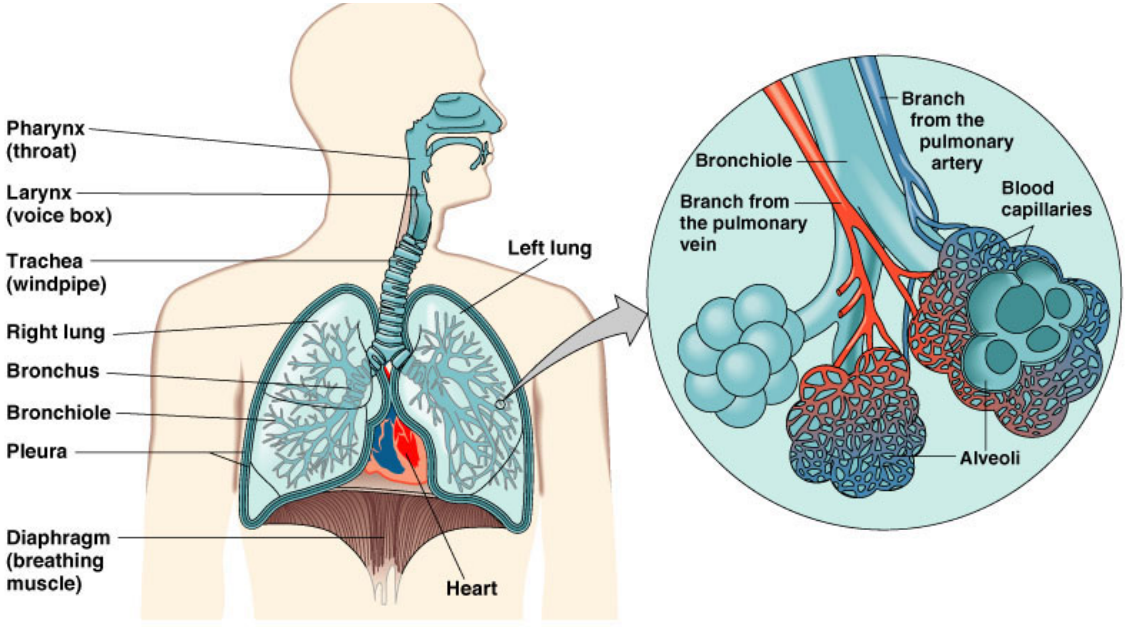
Question 2

How do these bacteria enter into the human host and where do they take up residence.

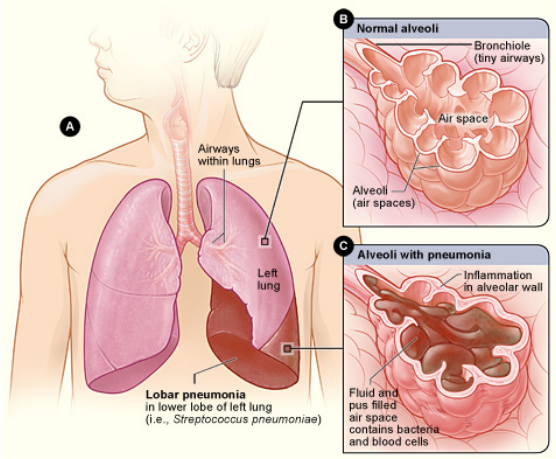
What are the **molecular, cellular and/or physiological factors at play** in this site specificity and in the **initial adherence step**.

Respiratory Tract

- The nose and mouth lead to the pharynx then trachea which branches into two bronchi.
- These further divide into bronchioles.
- Which in turn divide into alveoli, where gas exchange occurs.
- Once inhalation of the droplets occur, they can reach the alveoli and infection ensues.



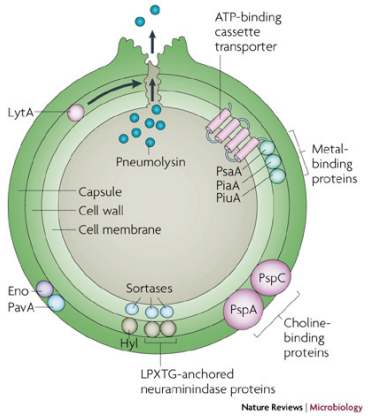
Entry: *S. Pneumoniae*



<u>Entry + Residence</u>

- *S. pneumoniae* is a resident of the normal respiratory flora.
  - Can act as a secondary invader (ie. post-virus).
- Enters via the nose and mouth through droplet nuclei expelled from infected patients sneezing, coughing, speaking or through person-person oral contact.
- Most bacteria get trapped in the upper respiratory tract. However, if there is no damage in the respiratory epithelium, the bacteria can make their way down the tract to the alveoli.
- Once in the alveoli, *S.pneumoniae* will adhere to the respiratory epithelial cells.

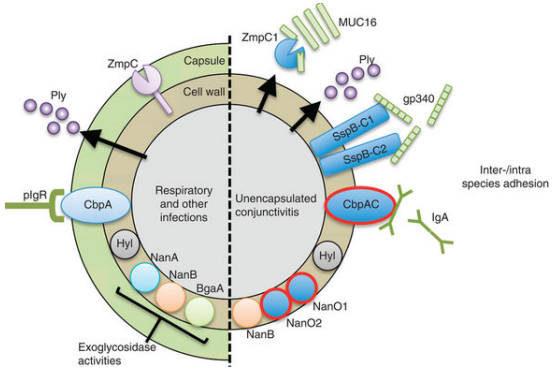
Entry: *S. Pneumoniae*



Molecular, Cellular and Physiological factors:

- Host-bacterial interactions are mediated by the *S.pneumoniae* enzymes: Neuraminidase (NanA), B glucosidase (BgaA) and B-N-glucosaminidase.
- These facilitate the attachment to epithelial cells
  - Cleaving terminal sugars from host glycolipids in the human lung epithelium.
- **GlcNAc receptors** on host cell surface are exposed and can be used for the bacterial adherence.
- **Polysaccharide capsule**: Anti-phagocytic. Plays a key role in virulence by preventing complement fixation and thus preventing opsonization, resulting in phagocytosis.
- **Pneumnococcal surface adhesin (PsaA)**: Cell wall surface protein mediating adherence to host cells. Increased production of interleukin 1 and tumor necrosis factor (TNF) to better facilitate entry into host cell by also upregulating host's PAFr (platelet activating factor).

Entry: *S. Pneumoniae*



Molecular, Cellular and Physiological factors:

- **Choline Binding protein (CbpA**): A major adhesin that binds to complement C3 and polymeric Ig receptor on nasopharyngeal mucosa which promotes uptake of bacteria into cells.
  - Uses CbpA to act as PAF (platelet activating factor) and bind to host PAFR allowing entrace to vacuole through endocytosis allowing bacteria to infect the respiratory epithelium.
  - Other adhesins: CbpG, CbpD.
- **Phosphocholine (ChoP)**: Cell wall component, is essential for adherence and bacterial entry into host cell. Acts as a adhesin station for CbpA.
- **IgA1 protease**: Secreted by S.pneumoniae, enhances adhesion by increasing proximity of ChoP to PAFr.
- **Pili (PI-1, PI-2)**: Play a role in adhesion to epithelial cells. They are hair-like structures extending from S.pneumoniae.
- **Pneumolysin**: An intracellular protein that results in the lysis of host cells and activates complement. May also inhibite cytokine secretion by host cells that create an immune response against the bacteria.

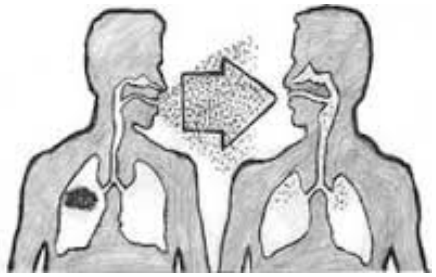
Summary of virulence factors of S.Pneumoniae

Table 1 | **Pneumococcal virulence factors and their main role in colonization and disease**

Pneumococcal virulence factors and disease	Main role in colonization
Upper-airway colonization	
Capsule	Prevents entrapment in the nasal mucus, thereby allowing access to epithelial surfaces. Also inhibits effective opsonophagocytosis.
ChoP	Binds to rPAF on the epithelial surface of the human nasopharynx.
CbpA (also known as PspC)	Binds to human secretory component on a polymeric Ig receptor during the first stage of translocation across the epithelium.
NanA, BgaA and StrH	Act sequentially to cleave terminal sugars from human glycoconjugates, which might reveal receptors for adherence.
Hyl	Breaks down hyaluronan-containing extracellular matrix components.
PavA	Binds to fibronectin.
Eno	Binds to plasminogen.
Competition in upper airway	
Bacteriocin (pneumocin)	Small antimicrobial peptide that targets members of the same species.
Respiratory-tract infection and pneumonia	
Ply	Cytolytic toxin that also activates complement. An important determinant of virulence in *in vivo* models of disease. Wide range of effects on host immune components at sub-lytic concentrations.
PspA	Prevents binding of C3 onto pneumococcal surface. Also binds lactoferrin.
LytA	Digests the cell wall, which results in the release of Ply.
PsaA	Component of the ABC transport system, which is involved in resistance to oxidative stress.
PiaA and PiuA	Component of the ABC transport system.
NanA and NanB	Aid colonization by revealing receptors for adherence, modifying the surfaces of competing bacteria that are within the same niche and/or modifying the function of host clearance glycoproteins.
IgA	Cleaves human IgA1.

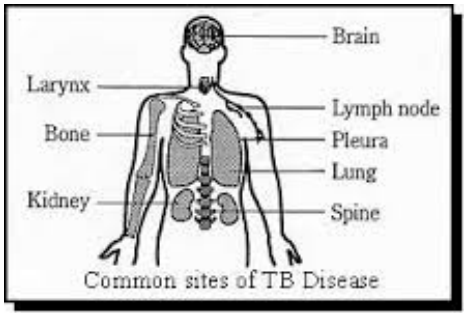
Img. Kadigolu

Entry: MTB



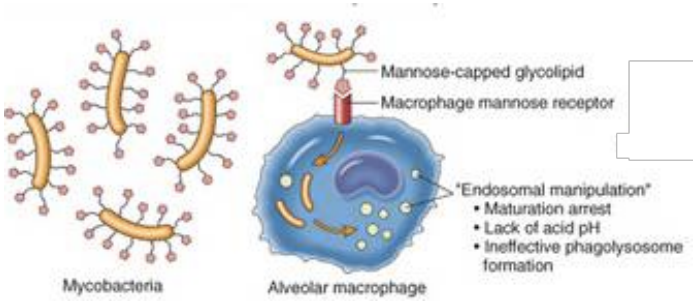
Entry + Residence:

- MTB enters the host through respiratory system, through inhalation of infectious aerosol droplets expelled by infected individuals. The droplets enter the nose and mouth and make their way down the trachea and into the alveoli.



- Droplet nuclei reach the alveoli and the bacteria encounter resident macrophages or pneumocytes and are phagocytosed, inducing an immune response, and lyse out of the cells to spread to other locations.
- The main site of infection in the lungs are the superior lobes and apex of lung.
- MTB can also occur outside the lung: central nervous system, lymphatic and genitourinary systems or in the bones and joints.
- Spreading of MTB from the primary site of infection can occur through the blood to other locations in the body.

Entry: MTB



Molecular, Cellular and Physiological factors:

Mannose Receptors

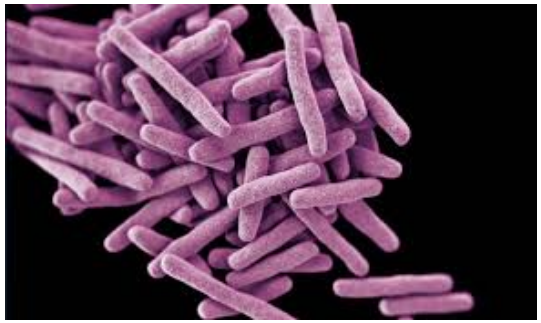
- Mannose receptors on alveolar macrophages are monomeric transmembrane proteins and mediates production of reactive oxygen species.
- Mannose receptors bind to liparabinomannan (LAM) on MTB.
- Mannose receptors can also mediate delivery of LAM to endocytic compartments that contain CD1b
  - Facilitates presentation of mycolic acid and lipoglycan antigens to CD4-CD8- or CD8+ T cells, causing an immune reaction.

Complement receptors: MTB is opsonized with complement proteins.

Fc-gamma receptors: Bind to IgG (anti-TB antibodies) upon reinfection of TB for phagocytosis. These IgG are present in individuals who have already be infected with MTB.

Scavenger receptors: bing to many types of ligands (ie. sulfolipids) on MTB.

Entry: MTB



Bacterial Characteristics and Adherence:

- **Surfactant protein A**: Glycoprotein found of alveolar surfaces that enhance binding and uptake of bacterium.
  - Up-regulates mannos receptor.
- **Pili**: Used in initial colonization of host.
- **Fibronectin-binding proteins (FbpA):** aids in mucosal tissue colonization
- **Mycolic acids:** on cell wall are used for adherence in host cell.
- **Mammalian cell entry**: promote changes in host cell plasma membrane and assist with entry.
- **TACO** (tryptophan aspartate coat protein): MTB recruits TACO to phagosomes to prevent its delivery to lysosomes and get degraded.
- **LAM:** A cell wall glycolipid, protects MTB from host respiratory burst and depresses IFN gamma.
- **Antigen 85 complex**: Proteins secreted by MTB that binds extracellular protein fibronectin and aids in bacterial protection against immune system.
- **Oxidative stress proteins**: Enzymes such as peroxidases, utilized by bacteria to counteract oxidative stresses.

Question 3

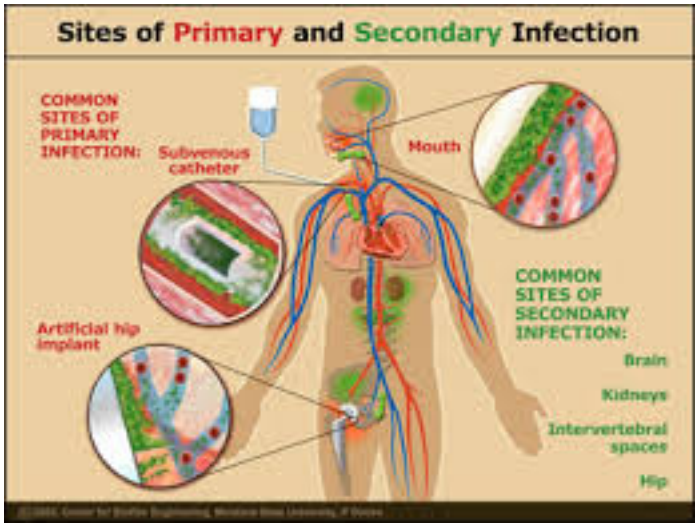
Do these organisms remain at the entry site and/or do they spread beyond the initial site

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

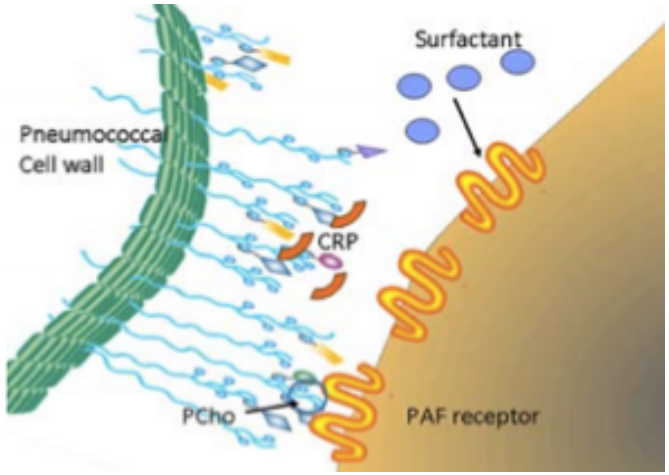
S.Pneumoniae

- *S.pneumoniae* can infect the lung but also has secondary sites of infection.
- Secondary infection: an infection that occurs during or after treatment for another infection. It may be caused by a treatment or by changes in immune system.
- *S.pneumoniae* can act as further invade following a disturbance in the mucosal flora or post-viral infection.



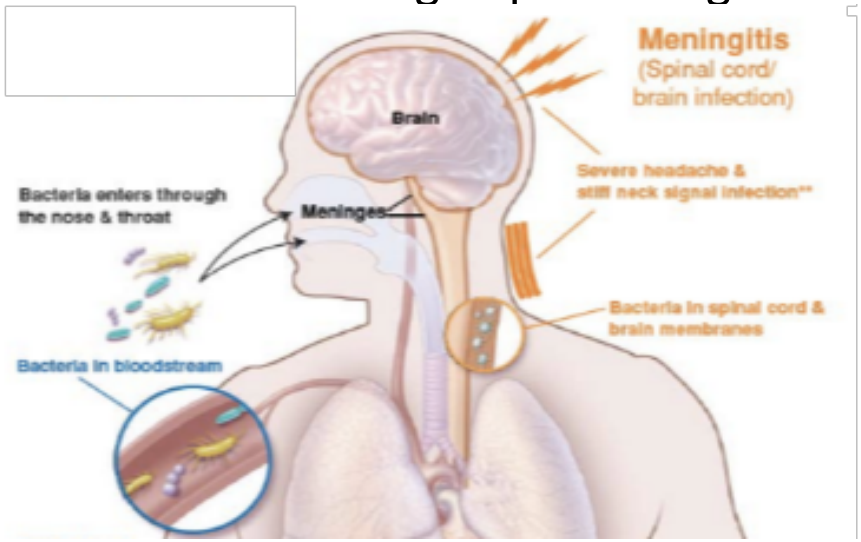
Multiplication and Spread: *S.Pneumoniae and Bacteremia*

- This bacteria can spread and cause a number of secondary infections including bacteremia, and meningitis.
- Bacteremia can happen when *S.pneumoniae* enters the blood, which is normally a sterile environment.
  - The bacteria is able to survive in the blood due to its capsule, that is anti-phagocytic. In addition, the bacteria expresses surface proteins such as PspA that interferes with complement-mediated bacterial clearance.
  - PAFr interactions with ChoP can allow the bacteria to traverse the lung epithelium and the endothelium of the blood-brain barrier.
  - Can also lead to septicimia (blood poisoning).
  - Now in the blood, the bacteria can spread to other regions in the body.



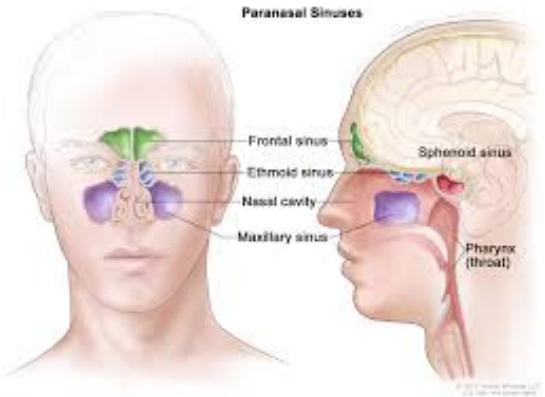
S.Pneumoniae and Meningitis

- Meningitis is an inflammation of the lining around the brain and spinal cord (meninges) with the most severe cases caused by S.pneumoniae.
- Pneumonococcal meningitis occurs when the bacteria that invaded the bloodstream move across to infect the meninges. The meninges are filled with a liquid called cerebrospinal fluid (CSF) where the S.pneumoniae can multiply and release substances that cause swelling and damage.
- *S.pneumoniae* inteferes with the inter-epithelial tight junctions to get across the blood-brain barrier.
  - Does so via tPA mediated binding of plasminogen to the bacterial surface.



Other secondary sites of infection

- There are many other secondary sites of infection of *S.pneumoniae*.
- Some of these include: paranasal sinusitis, meningitis, osteomyeletis, septic arthritis, endocaritis, an



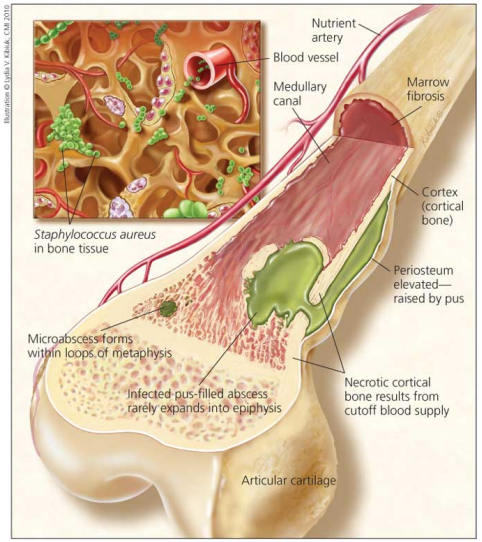
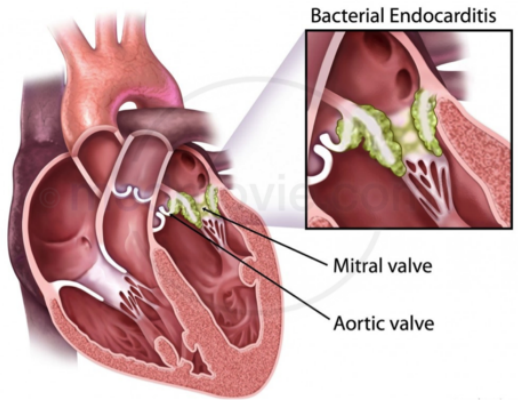


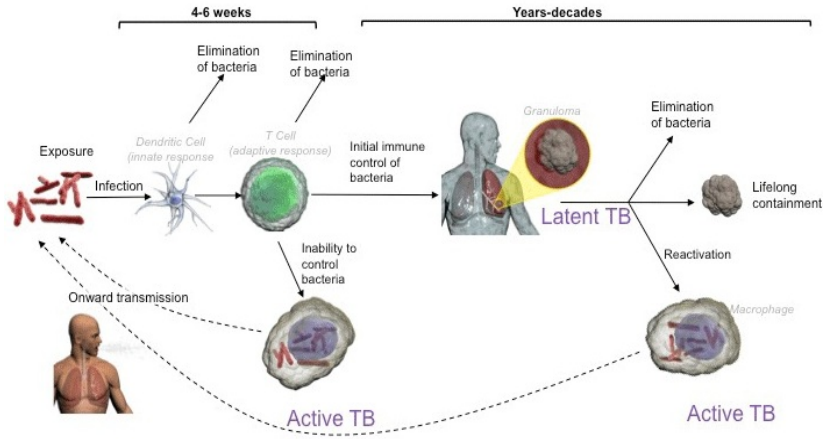
Figure 1 – *This diagram shows hematogenous osteomyelitis of a tubular bone in a child.*



Multiplication and Spread: MTB

Two forms of MTB: Active or Latent.

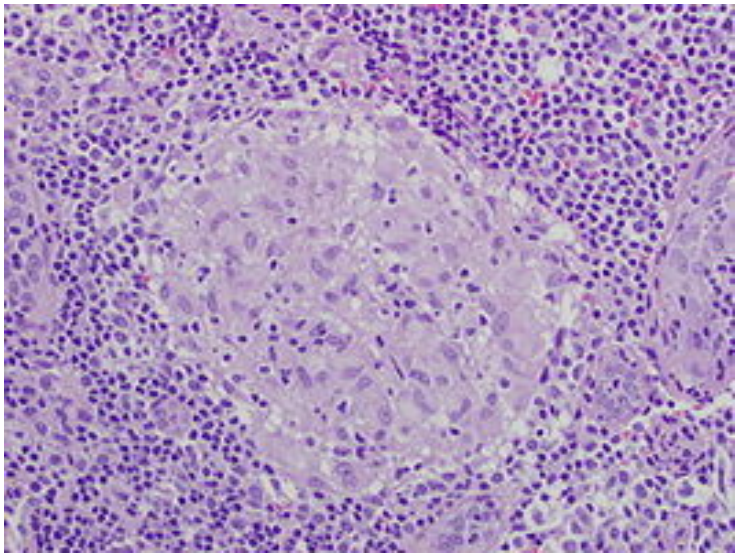
- **Latent Tuberculosis** (LMTB):
  - The majority of those infected are asymptomatic and the bacteria remain in the macrophages resulting in the formation of granulomas.
  - Onset can last from days to years
  - Possible causes of reactivation: Immunosuppression (chemotherapy), malnutrition and systemic diseases.



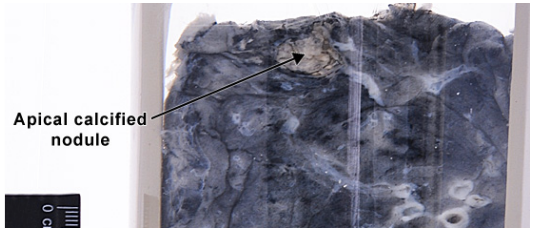
Multiplication and Spread: MTB

Granulomas are masses of immune cells (ie. macrophages) which prevent the spread of infection.

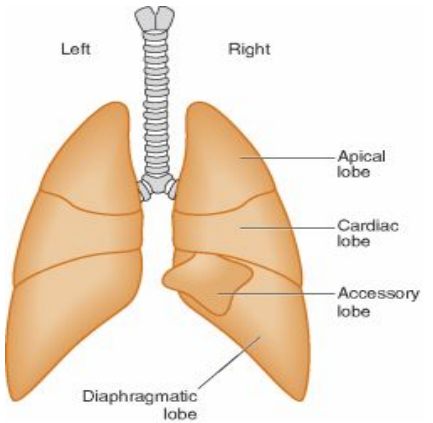
- The breakdown of granulomas may allow bacteria to multiply extra-cellularly.
- Mass increase may cause rupturing in the fibrotic wall and bacterial spreading via vessels or airway.



Multiplication and Spread: MTB

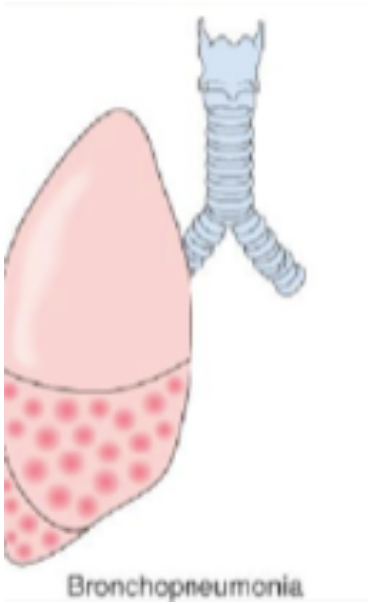


- Secondary pulmonary tuberculosis primarily occurs as a result of re-activation of latent MTB (90% of cases).
- The initial lesion is the apical nodule which consists of a solid nodular mass. Left untreated, lesions evolve with pleural fibrosis leading to secondary progressive tuberculosis.
- This includes: Apical caviatary fibrocaseous tuberculosis and miliary tuberculosis.



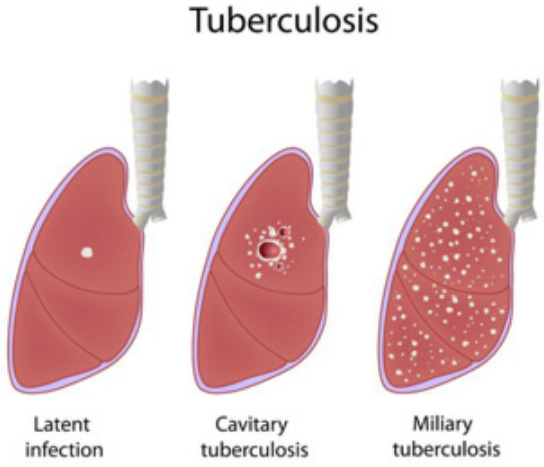
Tuberculous Bronchopneumonia

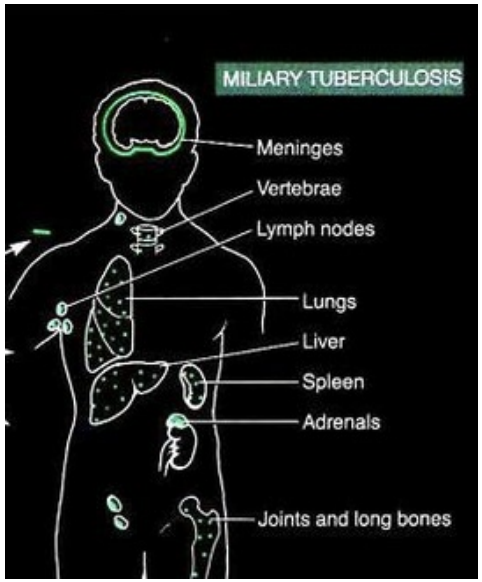
- Caused by the spread of bacteria from primary focus in the lung via the bronchi.
- This results in the inflammation of the walls of the bronchioles.
- The bacteria adhere to the bronchi walls and elicit an immune response with cytokines and chemokines such as IL6. Macrophages in the site phagocytose the bacteria and they multiply and lyse the leukocyte.



Miliary Tuberculosis

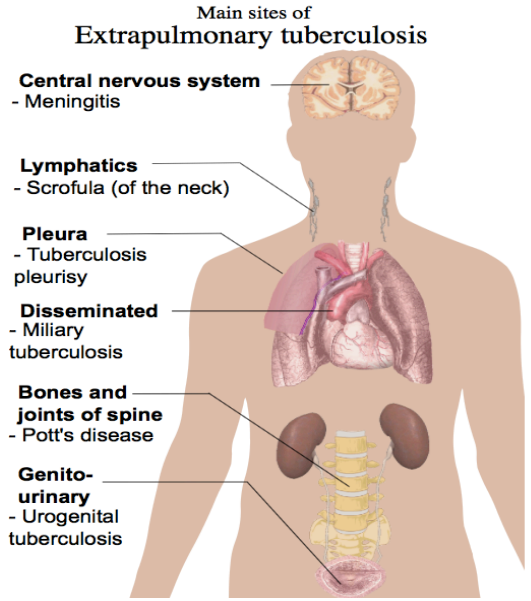
- Occurs when a tuberculous lesion erodes into a blood vessel disseminating millions of tubercle bacilli into the bloodstream and throughout the body.
- Spreading occurs via lymphatic ducts and blood.
- Symptoms include fever, chills weakness, and malaise driven by the chemokines and cytokines released by infected host cells and immune cells.





Other Secondary Sites of Infection MTB

- There are also extra-pulmonary sites of infection for tuberculosis.
- Examples include skeletal tuberculosis, CNS tuberculosis and abdominal tuberculosis.
- These can be further subdivided such as in CNS tuberculosis which includes meningitis, intracranial tuberculomas and spinal tuberculous arachnoidis.



Question 4

Do the bacteria cause any direct damage to the host or is the damage fully attributable to the host response, as indicated. 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?

S.Pneumonia and MTB

Lung Dysfunction will occur in both:

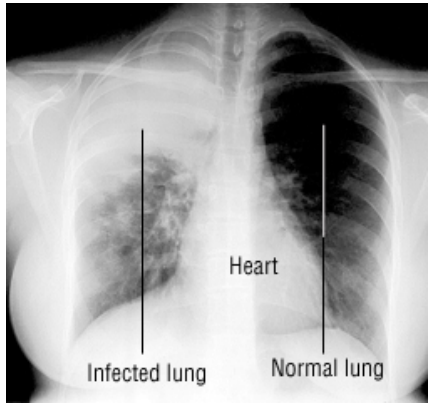
- Decreased oxygen and carbon dioxide exchange
- Increased inflammatory response
  - Release of pro-inflammatory cytokines such as IL-6, that can cause damage to surrounding tissues
- Airway blockage
  - Rapid bacterial growth
  - Cell death

Bacterial Damage: S. Pneumoniae

Link with Signs and Symptoms:

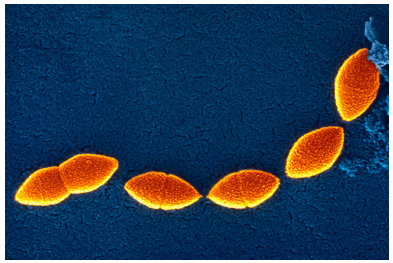
- **Chronic cough**: irritation and damage to ciliated epithelial cells of respiratory system associated with pneumolysin.
  - Accumulation of immune cells in inflammed lungs and increased production of mucous.
- **Fevers, chills and night sweats:** reflect the inflammatory response, with the release of cytokines and chemokines such as IL-6 and TNF.
- **Crackles and decreased breathing in the lower right lung:** fmay indicate host cell lysis and apoptosis as well as accumulation of fluids and dead cells.

Bacterial Damage: S.pneumoniae



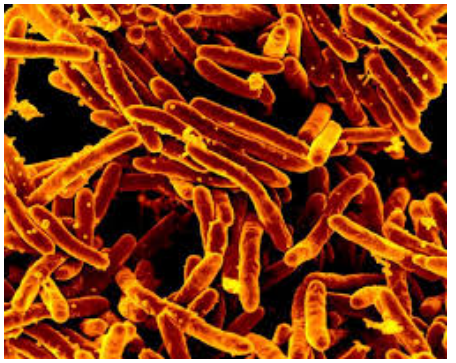
- Activation of immune inflammatory response
  - Complement system = Opsonization for phagocytosis
  - Neutrophils, macrophages, T cells, etc.
  - Release of cytokines (IL-6, TNF, IL10) triggering fever and night sweats.
- The inflammatory response itself can damage the host epithelial cells, and immune cells through the release of different chemokines, cytokines and induced cell death.
- Bacterial lysis causes the release of S.pneumoniae antigens which further increase the inflammatory response.
- The bacteria produces pneumolysin and hydrogen peroxide which can greatly damage and kill host cells.
- The bacterial pili trigger a greater TNF response.

Bacterial Damage: S.Pneumoniae



- **Capsule** induces a heightened immunoglobulin response which recruits more immune cells.
- Inflammatory mediators further increase local inflammation, fluid accumulation and cell death.
- *S.pneumoniae* also secretes **exotoxin**s:
  - Pneumolysin causes lysis of host cells and activates complement cascades.
  - Pneumolysin acts via cholesterol and can have a greater effect as it is a critical componenent in cell walls.
  - Hydrogen peroxide induces apoptosis in host cells.
- In addition, S.pneumoniae produces nitric oxide which can lead to septic shock.
- In turn, this disrupts the alveolar epithelium and increases the fluid accumulation in the alveolar space.

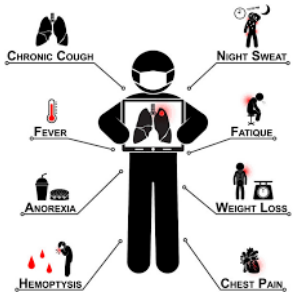
Bacterial Damage: MTB



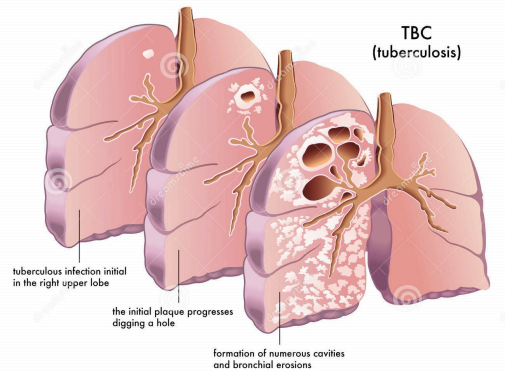
Link with Signs and Symptoms:

- The signs and symptoms can be linked to the damage that MTB emits on the host.
- **Fever, chills and night sweats**: associated with immune inflammatory response of cytokines, chemokines, immune cells, etc. With IL-6 inducing a fever. Increase in heat is as a result of the body trying to kill off heat-sensitive bacteria, impairing their replication.
- **Chronic Productive Cough**: damage to the host lung tissue and build up of dead cells. In addition, further production of mucus is increased to trap the bacteria. The chronic cough leads to constant inflammation in the lungs and further damage, that can result in a cough with blood.
- **Crackles and decreased breathing sounds**: Build up of fluid, granulomas and damage to lung epithelia.
    - Killing of lung parenchymal cells involved in oxygen uptake leads to a reduction in oxygen levels.
    - Formation of tuberculosis nodules leads to obstructions in the lungs, making breathing more difficult.
    - Necrosis (death of a lot of cells in tissue) can lead to fibrosis and hardening of lung tissue resulting in reduction of gas exchange in the alveoli.

Bacterial Damage: MTB

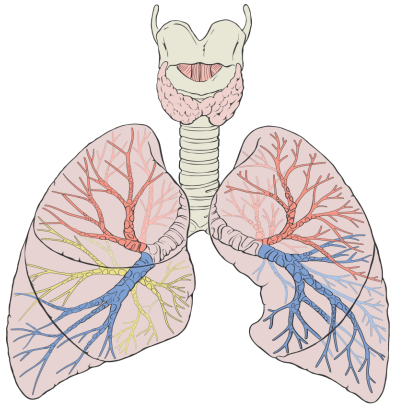


- Tuberculosis nodules have low pH and anoxic environment resulting in cesation necrosis in that the regional tissue is destroyed to a semi-solid consistency.
- The caseous centers of the nodules can liquefy, causing fibrotic wall rupturing leading to further damage.
- Bacterial proteases that are released (IgA1) help bacterial binding and cell lysis once the bacteria exit the cell.
- Furthermore, tuberculosis necrotixing toxn (TNT) released can induce apoptosis in macrophages and lead to bacteria escaping.
- In addition, the reactive oxygen species formed by macrophages and neutrophils in attempts to kill the bacteria can also damage the host cells.
- Cytokines produced by host cells in response to the invasion such as IL1, TNF, IL6 recruit more immune cells and further augmenting the inflammatory responses .



Summary

Robert K. could have contracted either S.pneumoniae or M.tuberculosis. It is important that he takes the appropriate measures and tests in order to restore his health and not infect others. Both these bacteria are highly contagious and easily spread with different virulence factors that increase their pathogenesis and thus Robert undergoes correct treatment.



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- Kadigolu Pneumoniae Paper http://go.galegroup.com.ezproxy.library.ubc.ca/ps/i.do?p=HRCA&u=ubcolumbia&id=GALE|A190890746&v=2.1&it=r&sid=summon&authCount=1

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