Life Science & BT Pre PhD Course Work 2022

Paper I- Advance Theory

Chhatrapati Shahu Ji Maharaj University Kanpur

Lecture 3:12.9.22

Survival Strategies of *Mycobacteria*

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LEARNING OBJECTIVES

- Understand basic characteristics of Mycobacteria
    - Understand Pathogenesis
    - 3 main stages
- Describe Major Mechanisms of TB virulence
    - During 3 stages
- Understand Interplay of immune response
    - Immune response at 3 stages
    - Immune-pathology

Tuberculosis

- “The World Health Organization (WHO) estimates that since 2015, tuberculosis has surpassed human immunodeficiency virus infection and acquired immunodeficiency syndrome (HIV/AIDS) as the leading cause of death from an infectious disease worldwide,
- Almost one third of the world's population (2.5 billion people) is infected with *M. tuberculosis.* Approximately 95% of TB cases occur in the developing world. The highest numbers of cases are in Asia, Africa, and the eastern Mediterranean region”.

Tuberculosis (Mycobacterium tuberculosis)
Robert M. Kliegman MD, in Nelson Textbook of Pediatrics, 2020

Microbiology

- Tuberculosis: caused by everal species of the*Mycobacterium* *tuberculosis complex* (MTBC): *M. tuberculosis,M. africanum, M. bovis etc*
- Humans are the only reservoir for *Mycobacterium tuberculosis.*
- The organism is an acid-fast, aerobic bacillus with a high cell wall content of high-molecular-weight lipids.
- Visible growth takes 3 to 8 weeks on solid media.
- An estimated 10,000 organisms/mL are required for sputum smear positivity.
- Incomplete necrosis produces cheesy, acellular material (i.e., caseous necrosis). :Pulmonary cavities contain huge numbers of organisms.
- Several Mycobacteria are antibiotic resistance. Multidrug-resistant tuberculosis (MDR-TB) indicates resistance to both isoniazid and rifampin.
- Extensively drug-resistant tuberculosis (XDR-TB) indicates resistance to isoniazid, rifampin, a fluoroquinolone, and a second-line injectable drug.
- Transmission of XDR-TB is of immense concern.

https://www.sciencedirect.com/topics/medicine-and-dentistry/mycobacterium-tuberculosis

Taxonomy

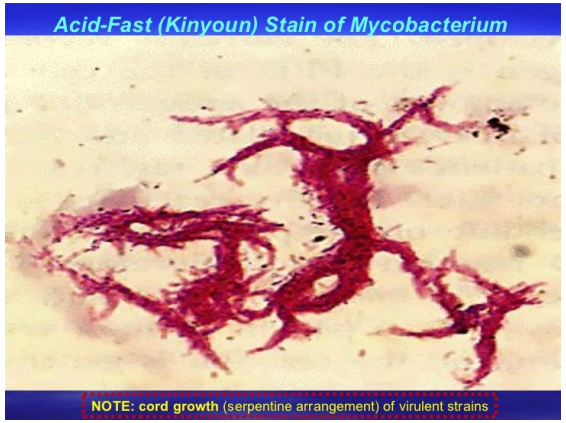
- Order – Actinomycetales
- Family – Mycobacteriacea
- Genus – Mycobacterium
  - Over 130 know described species
  - Most are non-pathogenic (soil/water organisms)
- Usually grouped in 2 divisions
  - Typical Mycobacteria (MTb complex)
  - Atypical Mycobacteria
    - MOTT – slow growers other than tuberculosis
    - Rapid growers

Mycobacterium Tuberculosis Complex

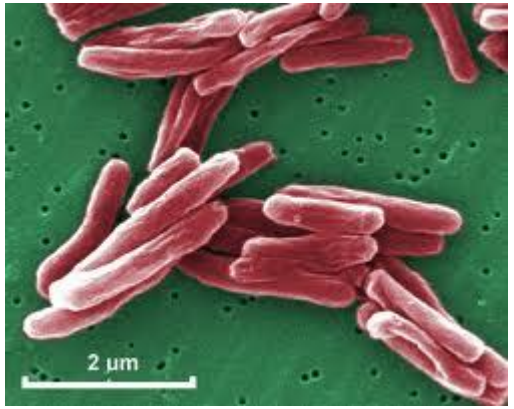
- *Mycobacterium tuberculosis*
- *Mycobacterium bovis (BCG)*
- *Mycbacterium africanum*
- *Mycobacterium microti*
- *Mycobacterium canetii*
- *Mycobacterium caprae*
- *Mycobacterium pinnipedii*
- *Mycobacterium mungi*

- These can all cause Tuberculosis in Humans and animals
- 85% of TB in humans is Mtb
- All members have a distinct host preference
- All are obligate pathogens
- Show 99.5% sequence similarity wthin the group
- Distinguished by rare fixed molecular differences (SNPs, deletions,

General Characteristics of *Mycobacterium*

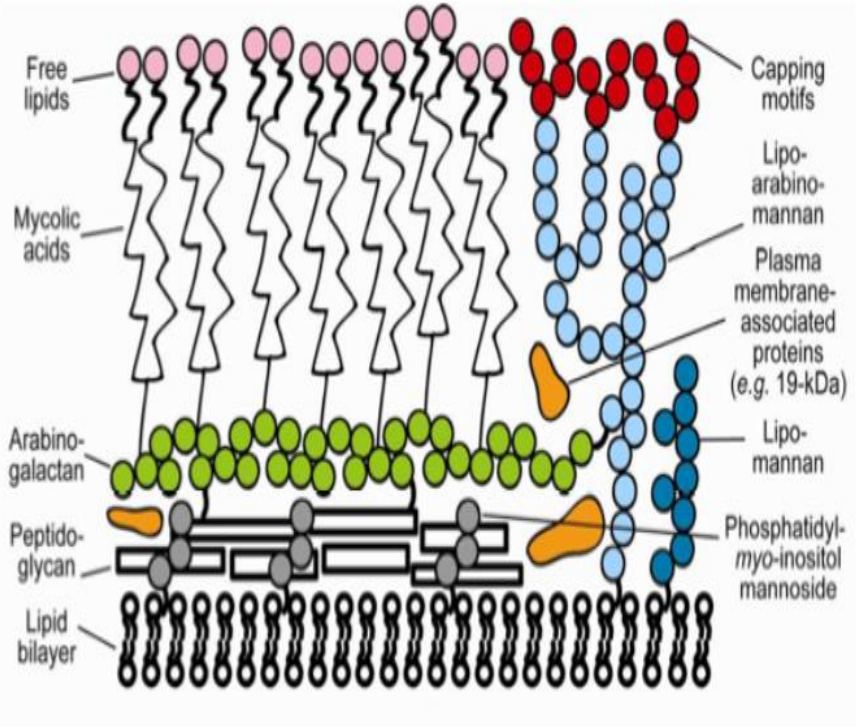


- **Gram Positive (wont actually stain)**
- **Slightly curved rod-shaped bacilli, aerobic, non-motile**
- **Can show filimentous branching like fungus “myco”**
- **Thick lipid rich cell wall**
- **Can remain dormant, non spore forming**
- **Multiplies slowly (18-24 hour generation time)**
- **Acid Fast – resists stain decolorization with acid/alcohol**



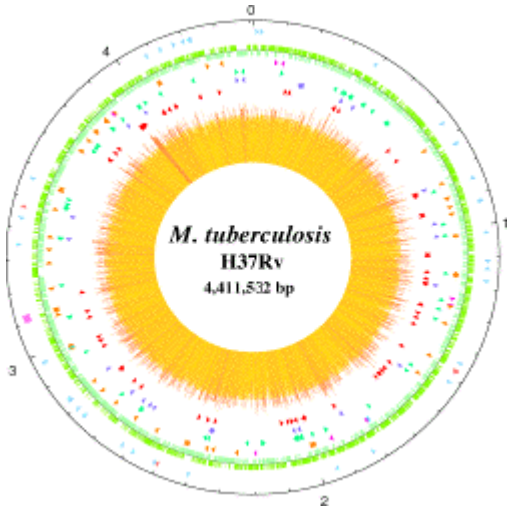
Mycobacterium Cell Wall Structure

- The cell wall of Mycobacteria is unique and responsible for much of its virulence
- Covered with a thick-waxy mix of lipids and polysaccharides
- Lipids
  - Lipopolysaccharides – (Lipoarabinomannan LAM)
- Mycolic acids- characteristic of Mycobacteria
  - comprises over 50% of the dry weight of the cell wall, responsible for acid fastness
  - Mycoloic acids are immunostimulatory (Fruends adjuvant)
- Plasma membrane associated proteins
  - form PPD (protein purified derrivative) – stimulates DTH/T cell response and elicit antibody response
- Cord Factor- (trehalose)
  - Present in virulent strains
  - Inhibit migration of leukocytes
  - Cause chronic granulomas



Mtb Genome

- Over 4 million bp
- DNA has high GC content
- Complete genome sequence of lab strain H37R in 1998
- Much of the genome remains uncharacterized
- Majority of work done in comparison of attentuated to pathogenic strains: M.bovis BCG vs M.tbt
  - Genes in RD1 region are responsible for pathogenicity
- REGION OF DELETION RD1
- 9 .5 Kb
- 9 open reading frames
- None with known function
- antigenic proteins: CFP-10/ TB7.7/ESAT-6
- Deletion of RDI from explains majority of attenuation of M.bovis BCG which is missing this region
  - Region appears to harbor virulence factors



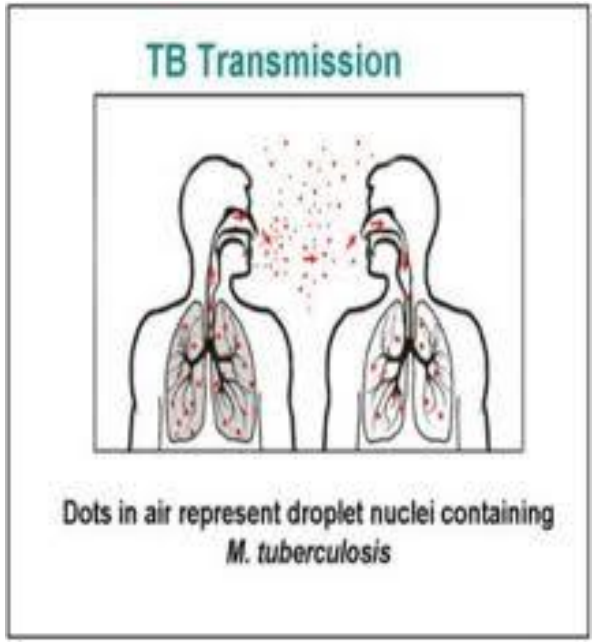
From Cole *et al. 1998 Nature*

Transmission

- Mtb is spread person to person via aerosolized
- droplet nuclei

- Infection is via inhalation of infected droplets

- Expelled when an infectious person coughs,
- sneezes, speaks



Risk Factors/ Epidemiological links

- Socioeconomic status(poverty, homelessnesss, substance abuse
- Crowding (congregate settings: prisons, shelters)
- Immune supression
- Age
- Immune supressive drugs
- Health Care Workers
- Immigrants
- Overall health/immune system status
- Alchoholism
- Smoking
- Diabetes
- TB within the last 2 years (recent infection)
- HIV Coinfection
- Strain Virulence ?
- Genetic Predisposition ? Risk Factors/ Epidemiological
- links
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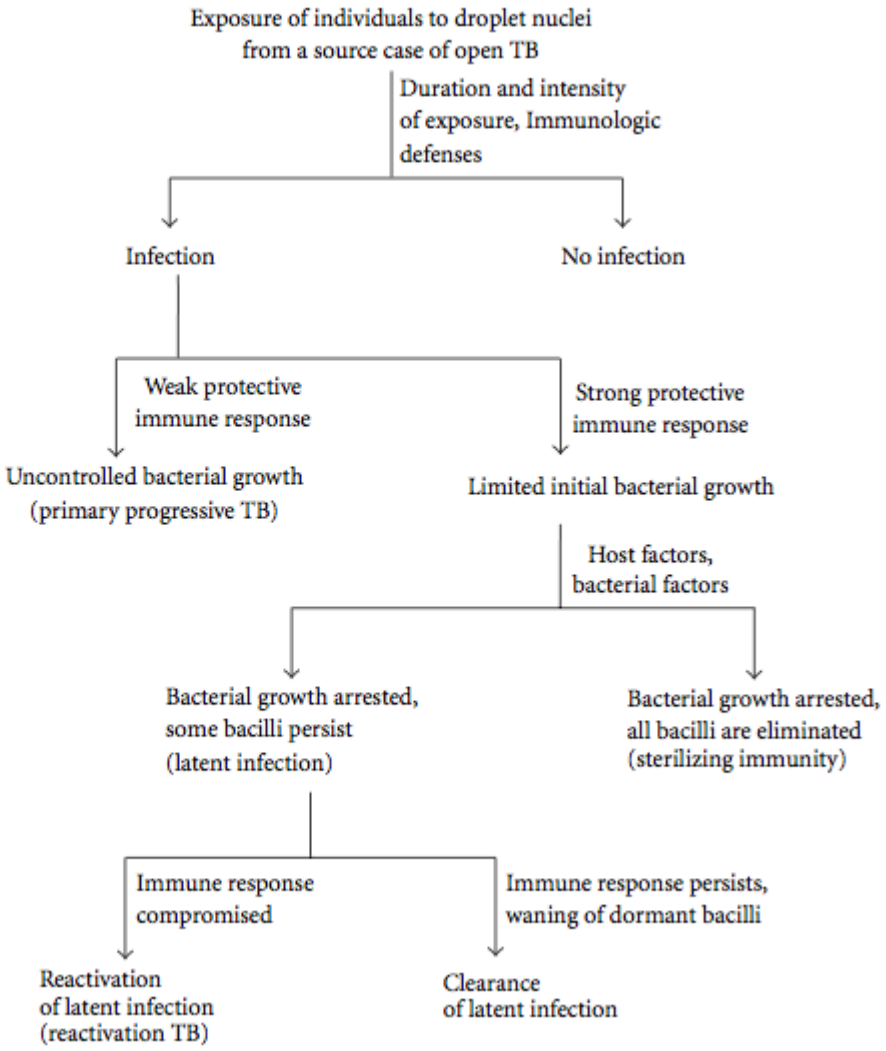
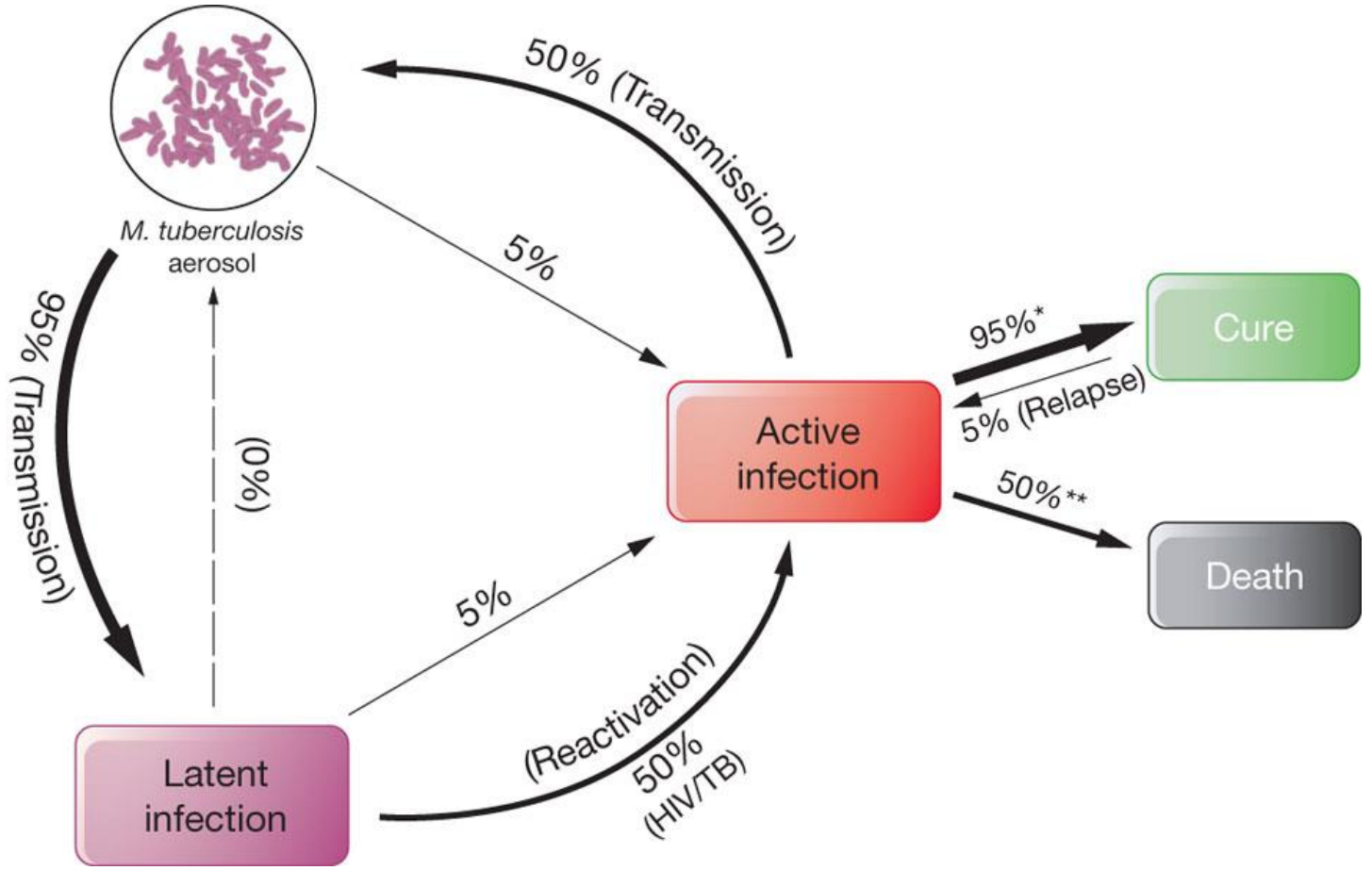
Disease Progression



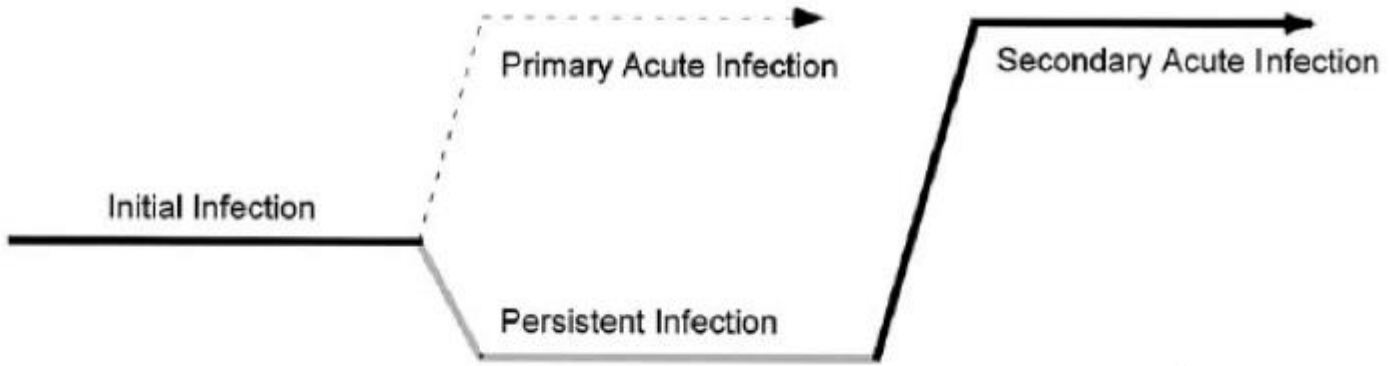
Disease Progression

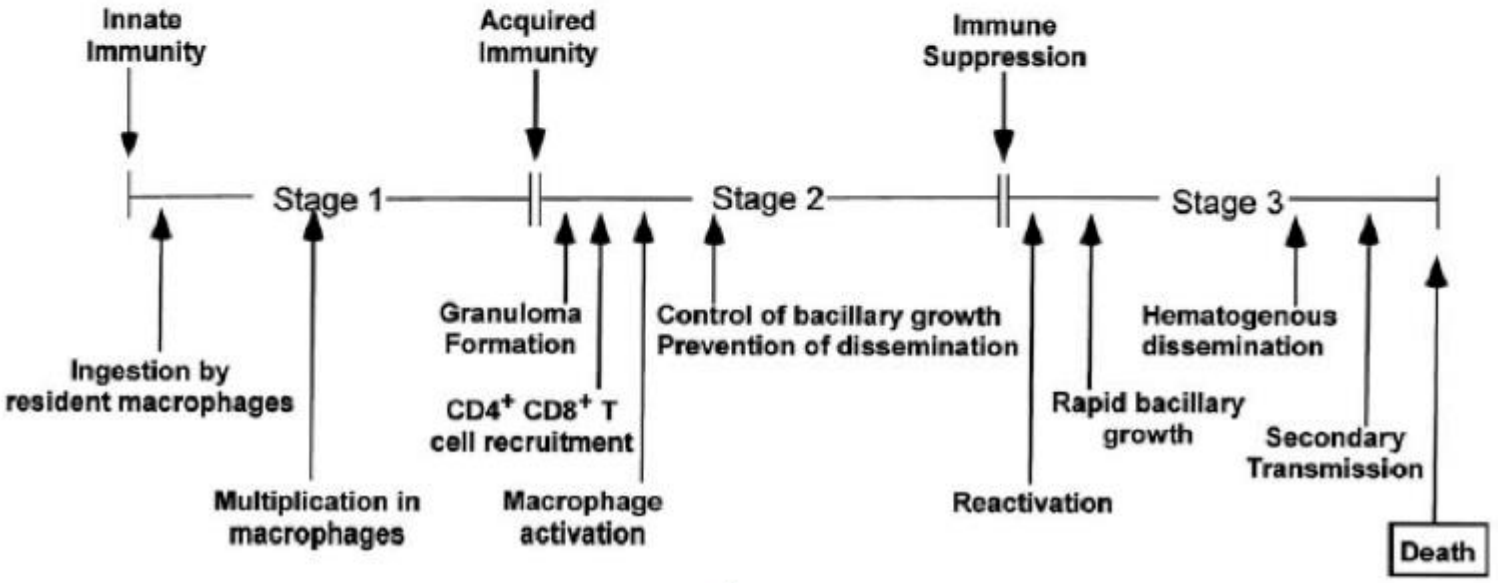


A Koul *et al. Nature* **469**, 483-490 (2011) doi:10.1038/nature09657

Tuberculosis pathogenesis

- Disease course has 3 defined stages (based on timing after exposure):
  - Stage 1: Invasion, pre-cellular immune response
  - Stage 2: Persistence, post cellular response
  - Stage 3: Reactivation / Secondary acute infection
- *M. tuberculosis* is able to evade and modulate components of both the innate and adaptive immune responses, allowing it to persist and but also facilitate its dissemination.





Ahmad S. Pathogenesis, immunology, and diagnosis of latent Mycobacterium tuberculosis infection. Clin Dev Immunol. 2011;2011:814943. doi: 10.1155/2011/814943.

PATHOGENESIS- STAGE 1-3

- Stage 1: Invasion
  - Bacteria get into cell- receptor mediated
  - Bacteria avoid macrophage activation/maturation- hide in phagocytes an multiply
  - Innate immune response is engaged; Inflammatory cell recruitment
  - Eventually adaptive response is engaged
- Stage 2: Persistence
  - Cellular immune response is engaged
  - Leads to formation of granuloma
  - Bacteria can become inert - latency
- Stage 3: Reactivation/secondary activation
  - Inflammatory response balance disruption
  - Tissue Destruction – indirect effect of immune response
-

STAGE 1: INVASION

- Mtb is a facultative intracellular pathogen – requires replicates in macrophages
- Ingested by alveolar macrophages
  - receptor mediated phagocytosis
- Mtb utilizes a wide range of cellular receptors (phagocytic cells)
  - Phagocytosis of either opsonized or non-opsonized bacteria
  - Not all of the receptors for opsonized bacteria are known, but include complement receptors and FcγReceptor
- Receptors include C-type lectin receptors (CLR's), nod-like Receptors (NLR's), scavenger receptors, complement receptors, Toll-like receptors (TLR)
- Ability to infect macrophages is a key pathogenic determinant for bacterial spread

MMR and DC-SIGN

- Major phagocytic cell uptake receptors:These receptors recognize mycobacterial lipoglycans-**ManLAM**
- Man LAM is considered to be a virulence factor as LAM is mannosylated in pathgenic Mtb and arabinose capped (AraLAM) in non-pathogenic environmental mycobacteria
  - Mannose receptor is used to uptake virulent bacteria
- Binding of Mtb thru these specific receptors is thought to influence phagosome maturation and cytokine signaling
- Interaction of the mannose receptor with ManLAM is a key step in limiting phagosome-lysosomal fusion
- The phogocytosis of Mtb via the mannose receptor is associated with an anti-inflammatory response
  - ManLam inhibits mannose-receptor dependant IL-12 production
- Also has direct effects in limiting phagosome-lysosome fusion
- This promotes survival of Mtb in the macrophage

TLR Signaling

- Mtb does not use TLRs to directly gain entry, but triggers these receptors to initiate immune cascades important in TB pathogenesis
- TLRs are expressed on macrophages and DC's among others:Pattern recognition receptors recognizing pathogen associated molecular patterns
- Signalling by TLR is mediated by MyD88 and results in pro-inflammatory cascade signalling via NFkB and MAP-kinase activation
- Direct: Leads to secretion of **TNF-a, IL-1, IL-12, and production of Nitrous Oxide (NO)**
- Indirect: Cross-talk between TLRs and other receptors such as mannose receptor coordinate efficient IFN-γ secretion

- Man-LAM/Mannose receptor and DC-SIGN binding initiates and anti-inflammatory/suppressive response
- TLR-binding by Mtb products initiates a inflammatory/protective response
- *** Balance is critical to pathogenicity****
- Interaction of Mtb bits with TLR's is beneficial to the host
  - proinflammatory response
    - Recruitment of immune cells
    - Engages the adaptive immune response
- Mtb has evolved strategies to circumvent this response which is beneficial for the pathogen

TLR manipulation/ Altered antigen presentation

- The 19kDa lipoprotein of *M. tuberculosis* is an agonist of TLR-2
- TLR's have natural agonist to provide a negative feedback mechanism to control excessive inflammatory response
- Binding of TLR-2 by this Mtb lipoprotein:
  - Modulates innate immunity and antigen presentation
  - Inhibits MHC-II expression and antigen processing by macrophages
    - This limits presentation to CD4 leading to insufficient activation of effector T cell
      - Promotes bacterial invasion/sustained growth

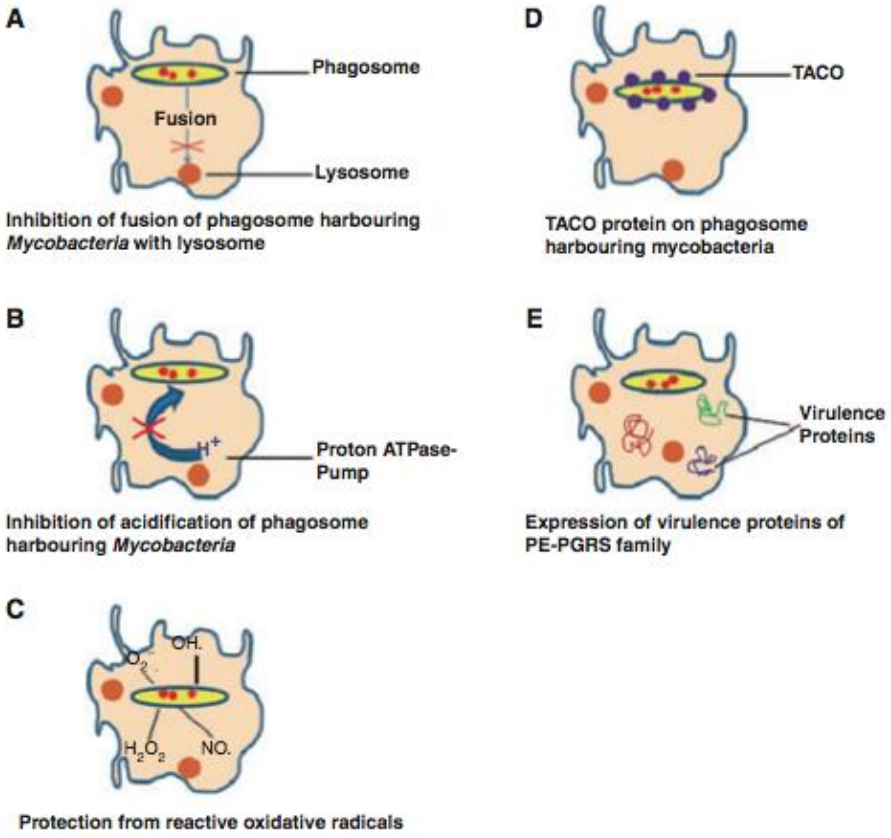
Stage 2: Macrophage maturation arrest

- Following Internalization of the bacteria the macrophage is either:
- **Activated**: host will clear infection or contain it (CMI initiated)
- **If Unactivated:** bacteria survive and replicate in the macrophage
  - Continued replication recruits other immune cells, leads to tissue damage, granuloma formation
- A key to the pathogenesis of Mtb is that the bacteria exploit the macrophage and rely on it for replication by preventing its activation
- There are several ways in which Mtb manipulates the macrophage to avoid killing and create a favorable environment for replication

Inhibition of Phagosomal maturation

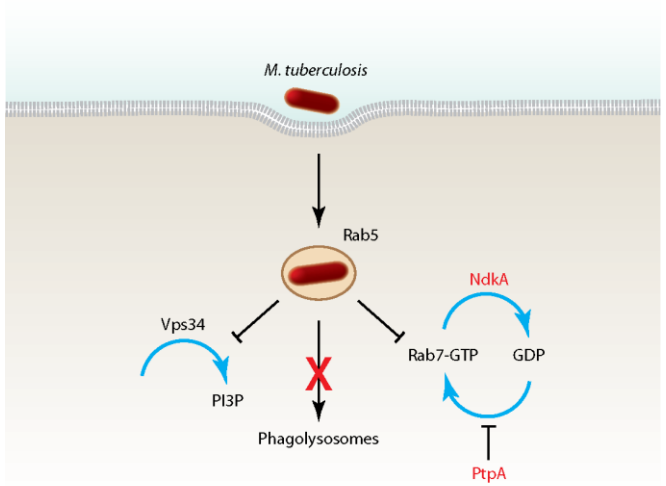
- Impaired fusion of phagosome with lysosome
- Inhibition of phagosome acidification
- Inefficient recruitment of proton-ATPase pump results in a lack of acidification of the phagosome
- Modified maturation of phagosome
- Over expresson of Rab5 on the phagosomes harboring bacilli causes maturation arrest at early endosomal stage

Mtb and macrophages



Bacterial proteins and bacterial lipids play a direct role in the inhibition of phagosome-lysosome fusion

- **Altered Fusion: Phagosomes containing Mtb do not fuse with lysosomes**
  - allows Mtb to acquire nutrients from recycled endosomes while avoiding the acidic /degrading lysosome
- Mannose cap (Man-LAM) appears to be essential to inhibit phagolysosomal fusion
- TDM (cord factor) aslso shows a similar effect as Man-LAM
- **Arrest phagosome maturation:** bacteria continue to grow in an early endosome–like compartment
  - inhibiting phosphatidylinositol 3-phosphate (PI3P) generation on the phagosome and impairing the recruitment of active, GTP-bound Rab7 while retaining Rab5
  - Several Mtb products are thought to be responsible for the inhibition in maturation, including LAM, trehalose dimycolate and sulpholipids, as well as the phosphatase SapM and the kinase PknG
- **Prevention of endosomal acidification:**
  - Mtb produce ammonia which affect movement of lysosomes,
  - destroys intralysosomal enzymes via alkalization
  - There is also no v-ATPase on the mycobacterial vacuole, preventing acidification keeping pH balanced
- Phagosome maturation requires conversion of Rab5 to active, GTP-bound Rab7 and the production of phospatidylinositol-3 phosphate (PI3P) on the phagosomal membrane-Both of these mechanisms are impaired in Mtb infected phagosomes



Philips JA, Ernst JD. 2012.
Annu. Rev. Pathol. Mech. Dis. 7:353–84

Intracellular pathogen

- The ability of Mtb to arrest phagosomal maturation gives the bacteria a protected intracellular niche in which they replicate
- The host innate response is engaged, but not sufficient alone to clear the infection
- Importantly, some ingested bacteria fail to prevent phagosome maturation, and they are delivered to the lysosome, where their replication is curtailed. This activates the cellular immune response
- Eventually the cellular response is engaged, but only after a few weeks following initial infection

Mtb and cellular response

- Th1 mediated response is critical.
- Production of IFN-γ by primarily CD4+ T cells leads to macrophage activation of resting/arrested cells leading to death of Mtb infected cells
- IFN-γ also recruits new macrophages
- γδ Tcells, Th17, NK, Treg,and CD8 subsets also contribute
- the cellular immune system (CD4) is critical for Mtb immunity is that HIV positive patients have increased risk of TB reactivation as they lose CD4 T cells

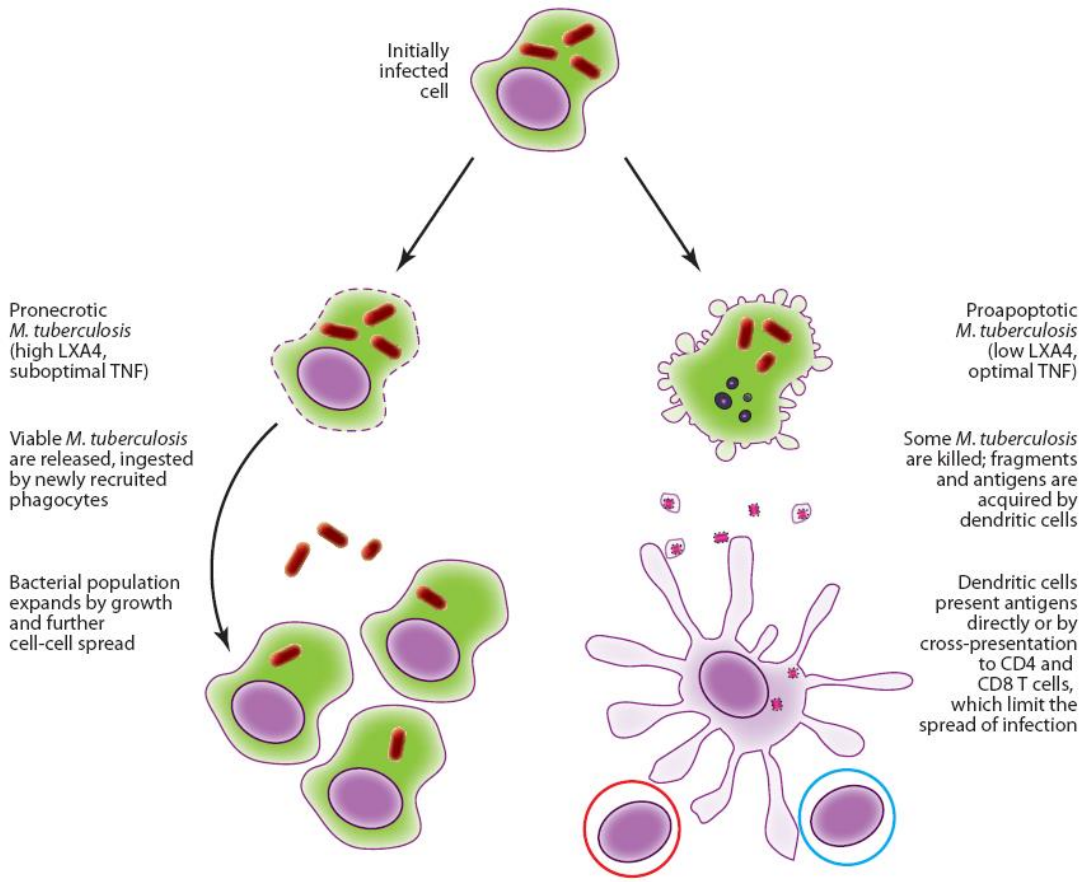
CMI and M.tb

- Following engagement of the cellular immune response, Interferon (IFN)-γ can overcome the early endosome–like arrest of *M. tuberculosis*, promoting delivery of bacteria to autolysosomes, where growth is curtailed
- Mtb has genes for Multiple eukaryotic –like effector to modify/mimic host signaling pathways
  - 11 eukaryotic Ser/Thr Protein kinases (PKn a to PKn L)
  - 2 Serine/Thr phosphatases (PtpA and PtpB)
- Mtb fights back yet again
  - Resists oxidative stress
  - Subverts the cell death pathways: Necrosis vs apoptosis

Resistance to oxidative stress

- Upon activation signals from IFN-γ, the phagosome arrest is overcome and the mycobacterial phagosome fuses with the lysosome
- This exposes the bacterium to the proton-rich lysosome, where hydrolases, reactive nitrogen intermediates (RNI) and reactive oxygen intermediates (ROI) operate most effectively to kill bacteria
- Some Mtb gene products work to counteract production of these intermediates
- Mtb also uses a membrane-bound serine protease encoded by the *Rv3671c* locus
  - helps maintain pH by potentially excluding external protons

- Even if the former mechanisms fail, and cell death ensues, Mtb hijacks the cell death pathway that is favorable
- Manipulation of cell death pathways is a virulence component of Mtb
- Cell can die by 2 major mechanisms: Apoptosis or Necrosis
  - Apoptosis favors the host
    - Cell is degraded neatly, no spilling of guts
    - Assists in downmodulation of the inflammatory response
    - Apoptosis promotes cross-presentation of Mtb antigens to engage the cellular immune response
  - Necrosis is favorable to the pathogen
    - cell guts are spilled out
    - The inflammatory response remains engaged, further recruitment of immune cells
    - Bacteria are freed and go on to infect other macrophages recruited to the site



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•Comparative studies of avirulent H37RVa and virulent H37RV showed:

- H37RV induced production of Lipoxin A4-LXA4 not seen in attenuated strain
- Excessive production of LXA4 is detrimental

•

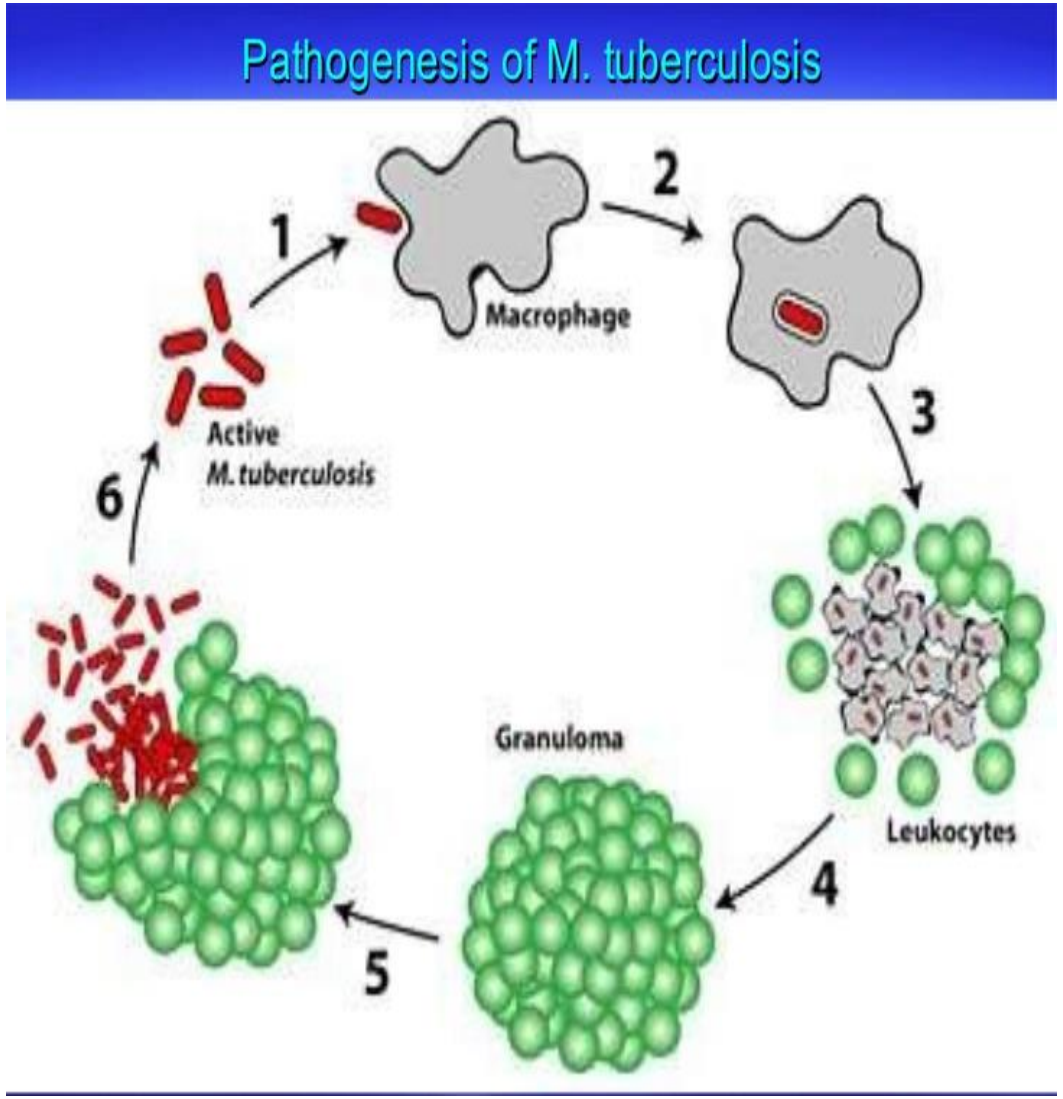
The bacterial genes responsible for manipulation of these factors is not resolved

Necrosis allows bacterial escape and spread and also continues to engage the inflammatory response recruiting more immune cells to the site of infection

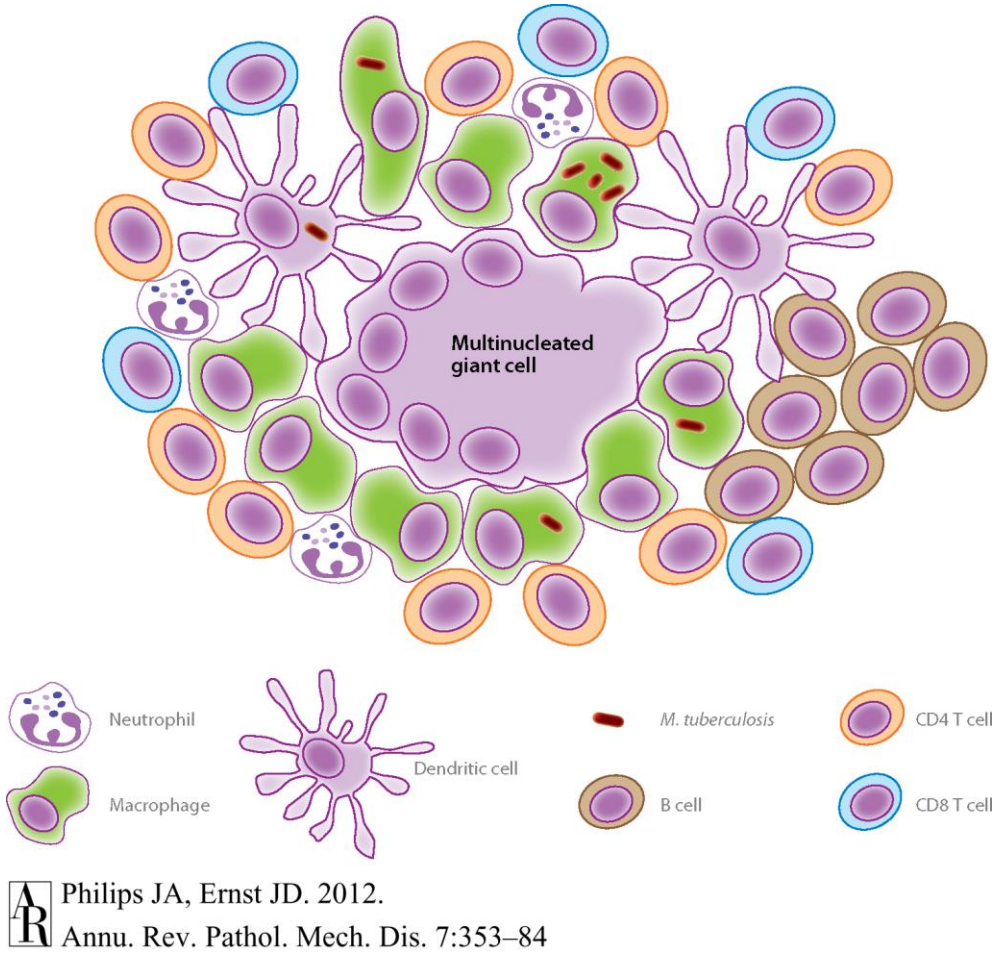
This then leads to the next phase of infection, Granuloma formation

Stage 2: Persistence
Granuloma formation and bacterial latency

- Classical histopathological lesion of M.tb infection
- Host induced to prevent the spread/dissemination of bacteria
- It is a organized aggregate of immune cells that surround a foci of infected tissues
- Formation is initiated upon infection of the macrophage
- Infected macrophages produce a strong pro-inflammatory response
  - TNF-a is key
- These signals recruit more immune cells to the site!
- Macrophages alone are sufficient for the initiation of the granuloma forming multinucleated giant cells MGC's
  - MGC's are associated with virulent mycobacteria
  - Glycolipids such as PIMS, lipomannan (LM), and TDM (trehalose) promote MGC formation
- The fact that mycobacterial products participate in granuloma formation indicate that the granuloma is important to bacterial virulence
-



- Eventually T cells are recruited to the site of infection
  - Their contribution is critical to the granuloma maintenance and disease progression following the acute phase of infection
- Other immune cells also join in
  - Responding to inflammatory signals of infected macrophages



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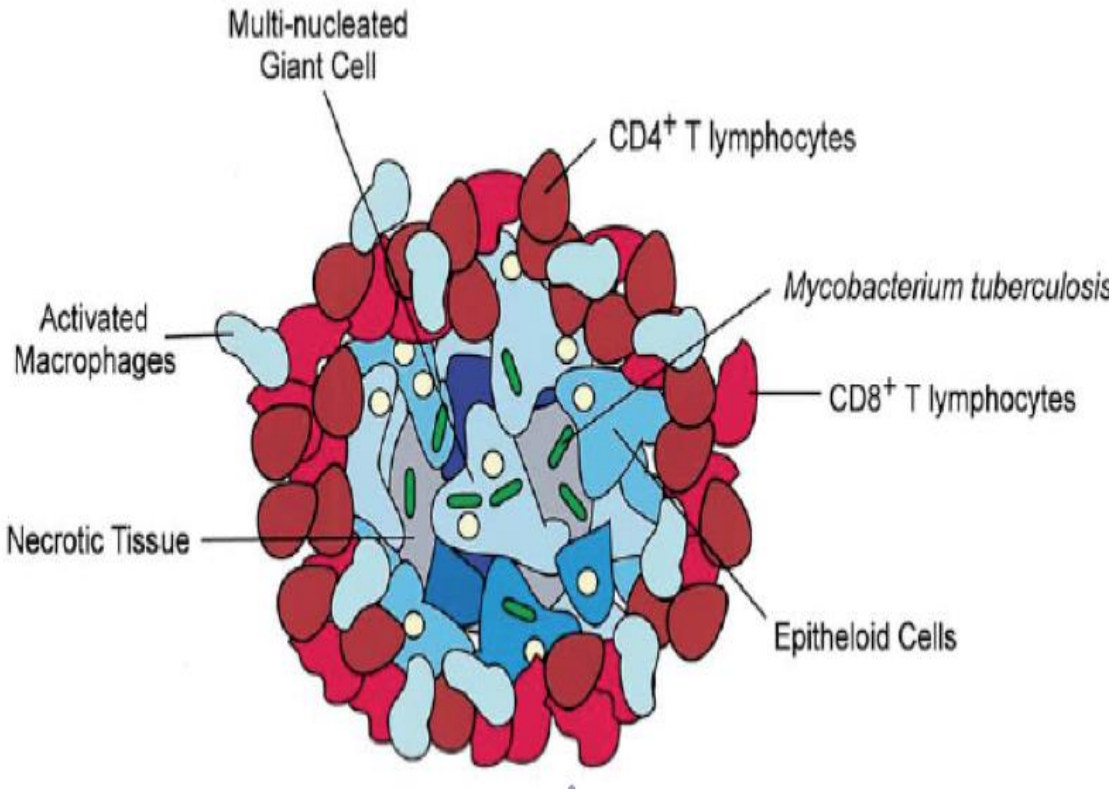
Caseous Centre
Macrophage rich
Giant cells (MGC's)
Majority of Mtb resides in this region

Next layer is epitheliod cells
Outer layer consists of T cells,
External layer of fibroblasts enclose the structure



Host

Site for optimal interactions between pathogens, antigen presenting cells, and T cells

Allows for immune control of infection within the microenvironment

Importantly though, the host response contains bacterial growth but does not eradicate the bacteria

Bacteria

Early granuloma formation recruits more macrophages

that engulf released bacteria

Benefits of late stage granuloma to the bacteria are not clear

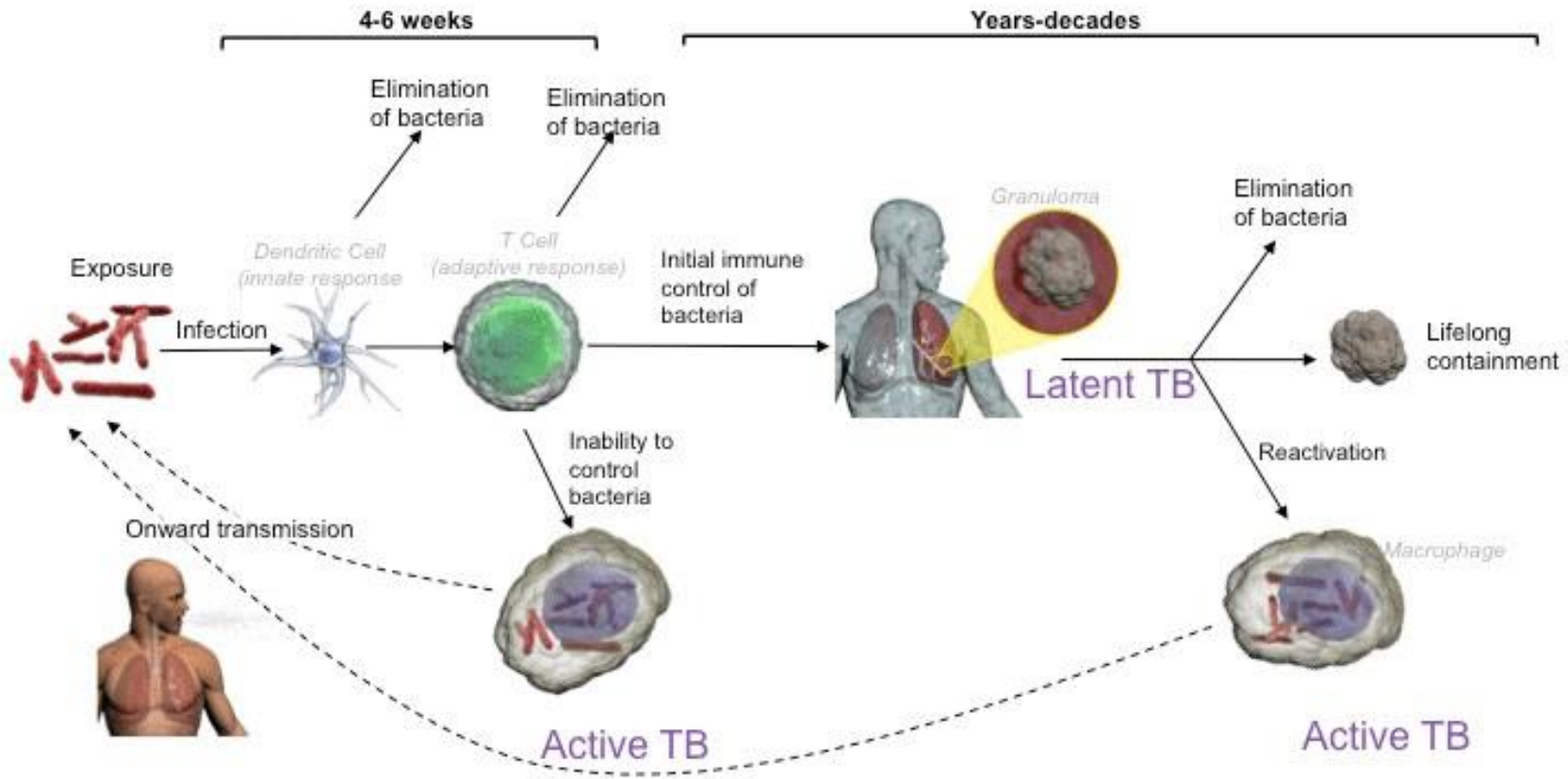
Environment induces a latent state

Latency

- Mtb is able to persist as an intracellular pathogen due to ability to alter phagosomal maturation
- Mtb has capacity for anaerobic existence, similar to spore formers
- Advantage to bacteria
  - Subvert the immune system

- Mtb can survive within a granuloma for decades
- Centre of the granuloma is hypoxic
  - Little nutrient availability , low oxygenation, low pH
- This environment induces a shift in bacterial metabolism in response
  - There are latency associated genes (LATs)
  - Dormancy regulon?
- Dormancy allows bacteria to survive yet escape the activated immune system
- The actively growing bacterial population is killed by the immune response, but latent (nonreplicating) bacteria persist

Natural history of TB infection



Stage 3: Reactivation Loss of immune control and bacterial dissemination

- Occurs in 5-10% of patients with LTBI
- Reasons unknown
- Likely due to a disruption in the balance of the host immune response
- Caused by immune suppression of some sort
- HIV is the most potent risk factor for Mtb reactivation from latency
- The events that lead to reactivation of a latent Mtb infection contained within the granuloma to active disease is not understood
- Recently, discovery of resuscitation promoting factors (RPF's) produced by Mtb have been recognized as critical components for bacterial revival
- Bacteria begin to replicate again and lead to activation of the immune response that leads to most pathology
- Induce TNF-a production
- Tissue destruction allows for bacterial dissemination and transmission

Bacterial dissemination

- Leads to disruption of the granuloma- Mtb can spread
  - Though to be due to collapse of the caseous centre (cheese like in biopsy: necrotic centre)
  - Recent evidence to indicate that neutrophils may carry bacteria to the airway in late-stage inflammation
- Granulomas: Productive lesion has 3 zones (central, mid, peripheral) middle is caseous, outer is fibrous
- Macrophage lytic process causes tissue damage- caseous necrosis

- Eventually T cells are recruited to the site of infection
  - Their contribution is critical to the granuloma maintenance and disease progression following the acute phase of infection
- Other immune cells also join in
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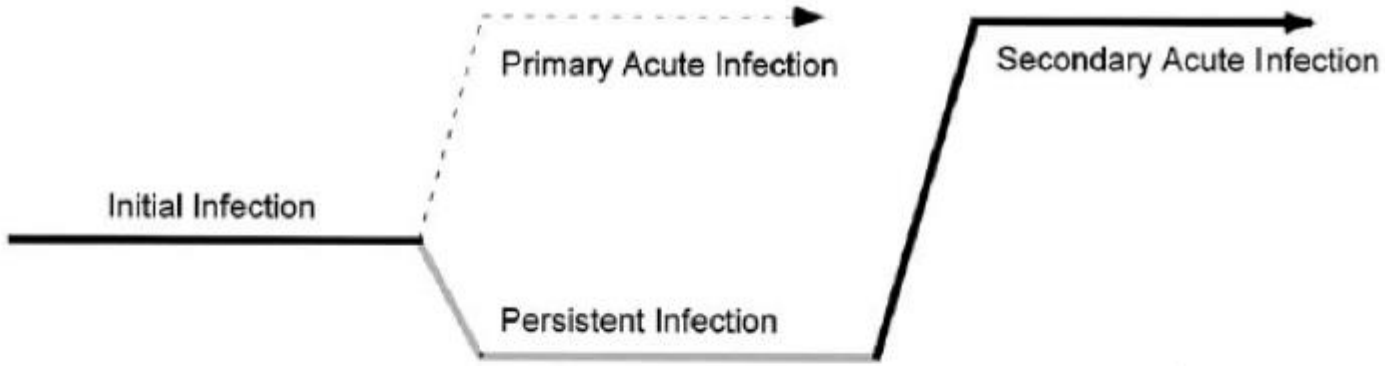
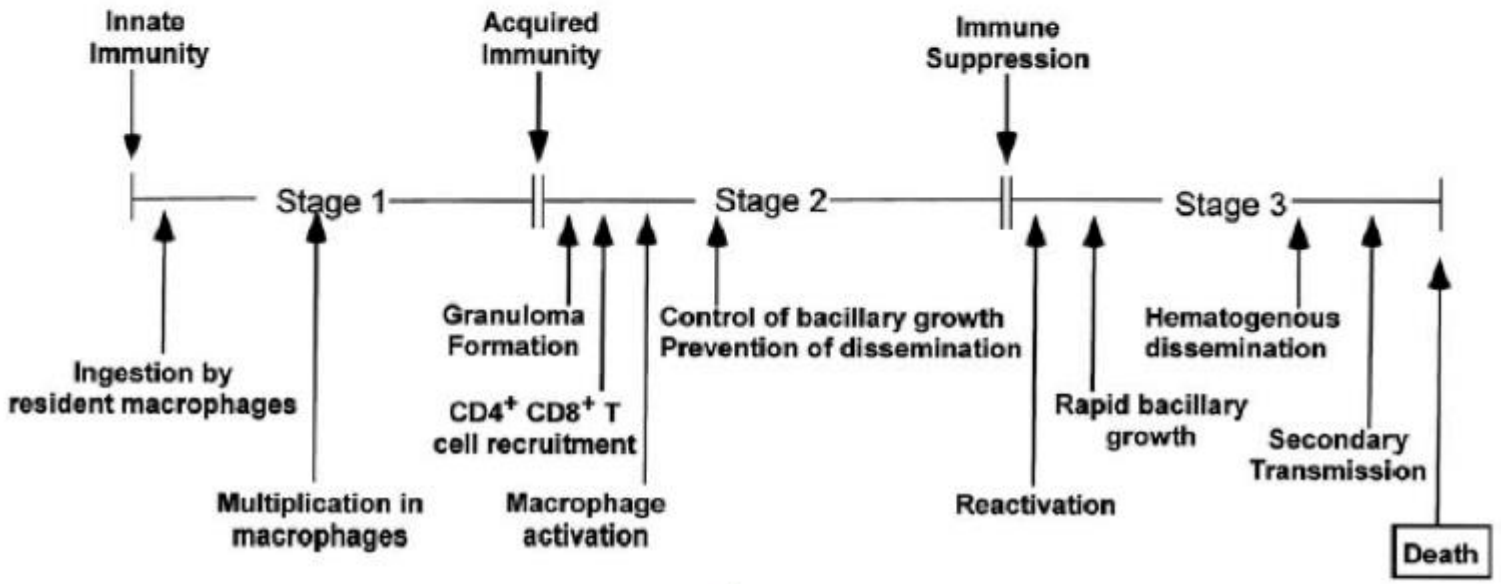


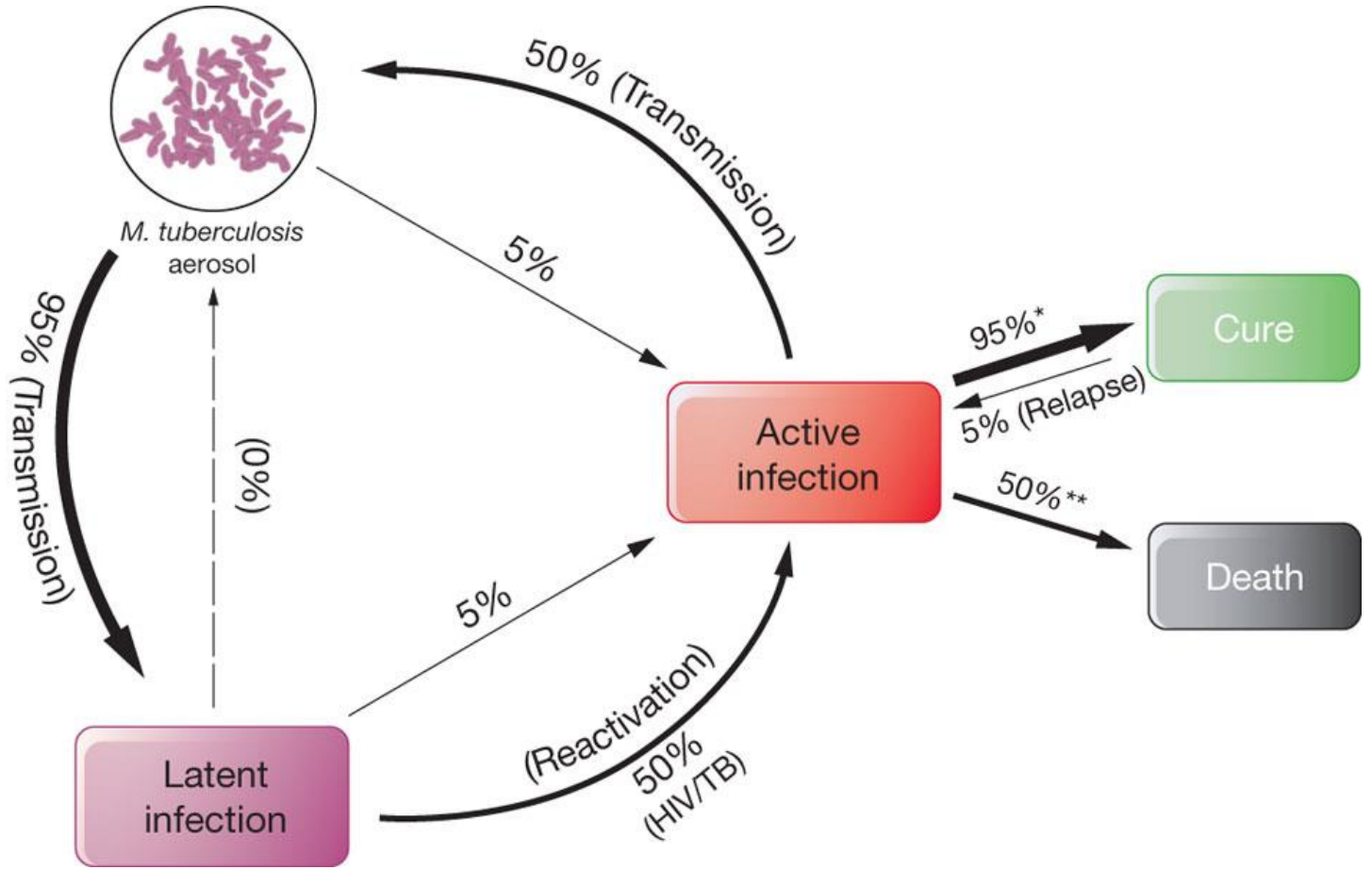


Ahmad S. Pathogenesis, immunology, and diagnosis of latent Mycobacterium tuberculosis infection. Clin Dev Immunol. 2011;2011:814943. doi: 10.1155/2011/814943.

Strategies for Survival- Summary

- Host Response
  - Innate Immunity
  - Adaptive/Cellular Immunity
  - formation of granuloma
- Virulence Tactics
  - Invasion
  - Blockade of macrophage maturation
  - TLR subversion / Modulation of antigen presentation
  - Subversion of oxidative stress
  - Necrosis vs Apoptosis
  - Latency

A Koul *et al. Nature* **469**, 483-490 (2011) doi:10.1038/nature09657



Diagnosis

•Culture is the gold standard.

•Liquid broth cultures require 1 to 3 weeks to detect organisms.

•Nucleic acid amplification tests have sensitivities and specificities that approach culture.

• Three sputum specimens increase sensitivity.

•A radiograph showing a patchy or nodular infiltrate in the lung apices is highly suggestive, especially if the infiltrate is cavitary.

•Pulmonary tuberculosis can occur in persons with normal chest radiographs.

https://www.sciencedirect.com/topics/medicine-and-dentistry/mycobacterium-tuberculosis

Therapy

Multidrug regimens, typically starting with at least four active drugs, are recommended.

At least 6 months of therapy is usually required.

Isoniazid (INH) is the cornerstone of therapy; rifampin, the second major antituberculous agent, causes many drug-drug interactions; pyrazinamide is essential for 6-month regimens.

Adjustment of doses of antiretroviral agents during treatment of tuberculosis is shown in Tables 38-3 and 38-5. The time to initiate antiretroviral therapy during tuberculosis treatment is shown in Table 38-2.

Directly observed therapy is crucial.

Susceptibility testing is important to guide therapy.

With appropriate chemotherapy, patients become noninfectious within 2 weeks.

Treatment of XDR-TB is usually associated with poor outcomes.

Bedaquiline is a recently approved drug for MDR-TB.

https://www.sciencedirect.com/topics/medicine-and-dentistry/mycobacterium-tuberculosis

Vaccine

- *Mycobacterium bovis* bacillus Calmette-Guérin (BCG), vaccine remains the only licensed vaccine for the prevention of TB (Danish 1331 sub-strain).
- It is traditionally given to newborns, and in that population, it has a protective effect.
- However, this is not sustained, and the general consensus is now that the vaccine provides little protection in adult individuals
- A tuberculin skin test is usually carried out before administering BCG. A reactive tuberculin skin test is a contraindication to BCG due to the risk of severe local inflammation and scarring

https://en.wikipedia.org/wiki/BCG_vaccine

Reading Material

- Green ER, Mecsas J. Bacterial Secretion Systems: An Overview. Microbiol Spectr. 2016 Feb;4(1):10.1128/microbiolspec.VMBF-0012-2015. doi: 10.1128/microbiolspec.VMBF-0012-2015. PMID: 26999395; PMCID: PMC4804464.
- Prescott Microbiology Book by Christopher J. Woolverton, Joanne Willey, and Linda Sherwood
- https://www.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.001193?crawler=true
- https://en.wikipedia.org/wiki/Bacterial_secretion_system
- https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Boundless)/*14%3A_Pathogenicity/14.4%3A_Damaging_Host_Cells*

- **THANK YOU**