UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

Mycobacterium Tuberculosis- MANIPULATOR OF PROTECTIVE IMMUNITY

ANIL A CHUTURGOON (PhD)
Professor and Head: Discipline of Medical Biochemistry
University of KwaZulu-Natal
Durban, South Africa

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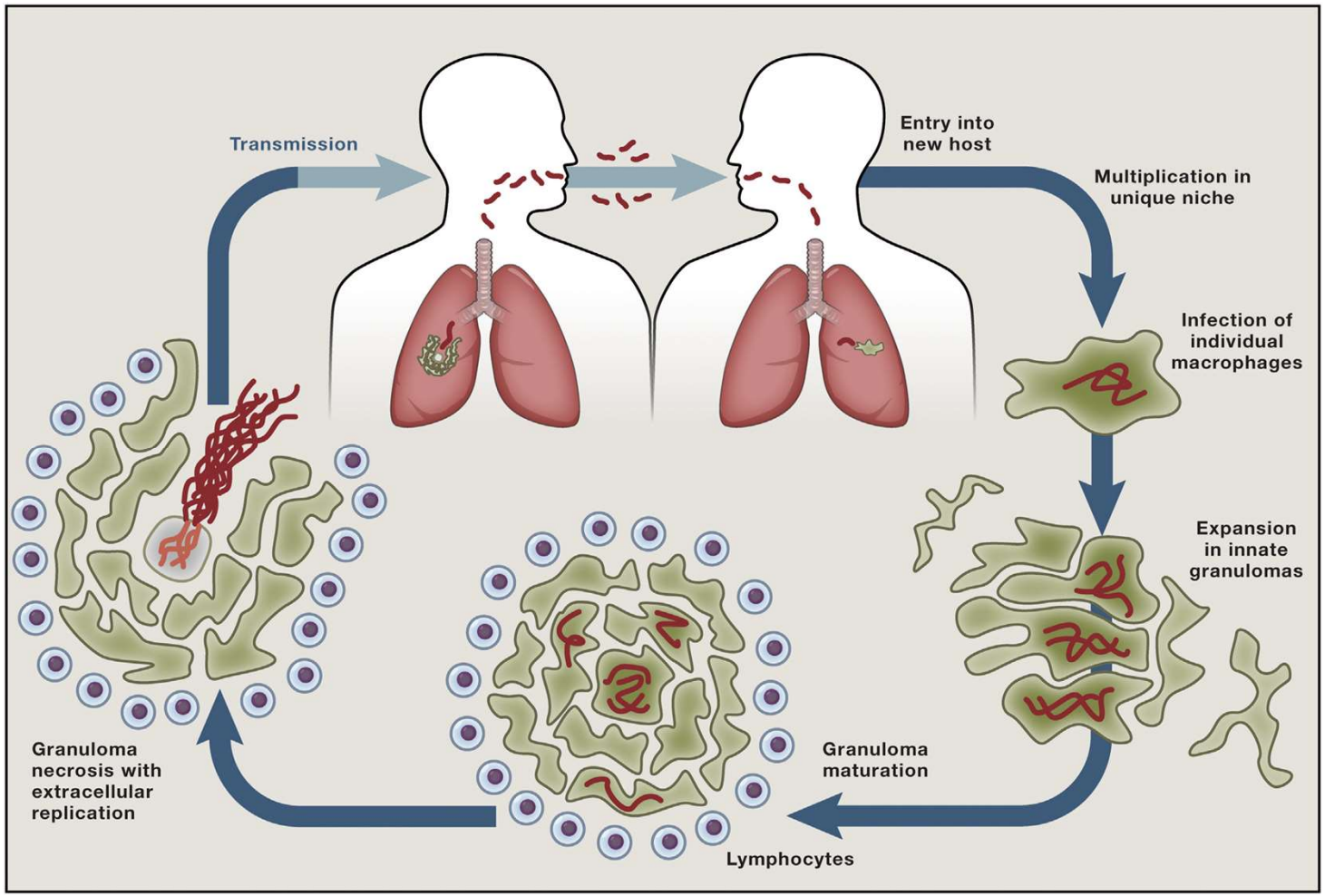
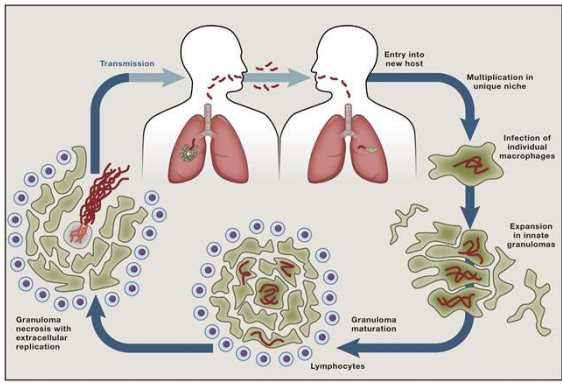
Mycobacterium Tuberculosis

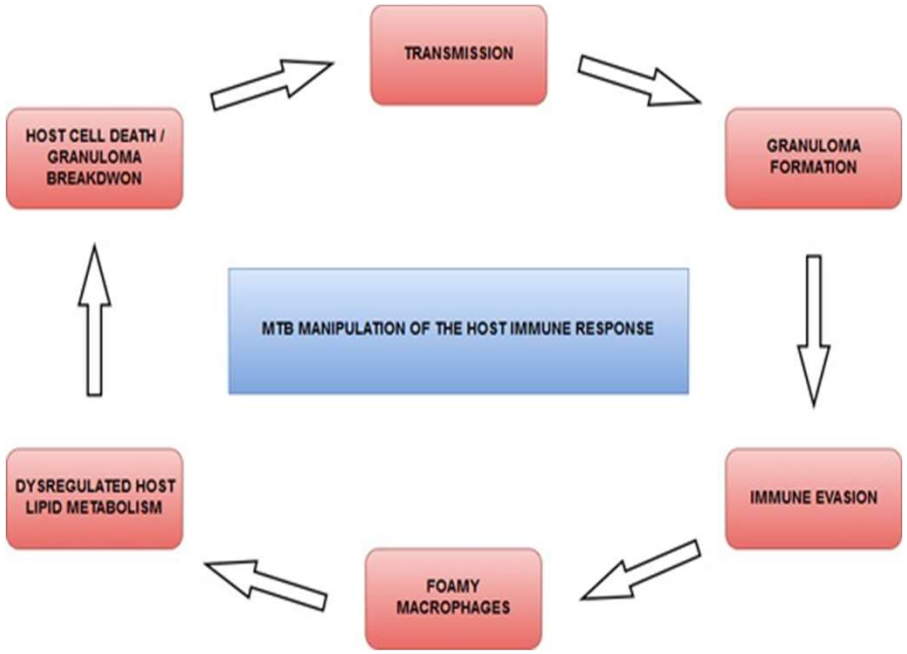


Mycobacterium Tuberculosis

- An infectious respiratory disease (most successful pathogen in human history-70 000 year)
- CDC and WHO (2017):
  - 10 million people became infected worldwide
  - approximately 1.5 million TB related deaths
- Due to lack of an effective vaccine, prolonged treatment periods, and adverse effects of toxic drugs- an understanding of the host and bacterial role in the immune response is crucial
- Particularly important with respect to drug resistant MTB strains
- **A huge threat to global health**

Mycobacterium Tuberculosis



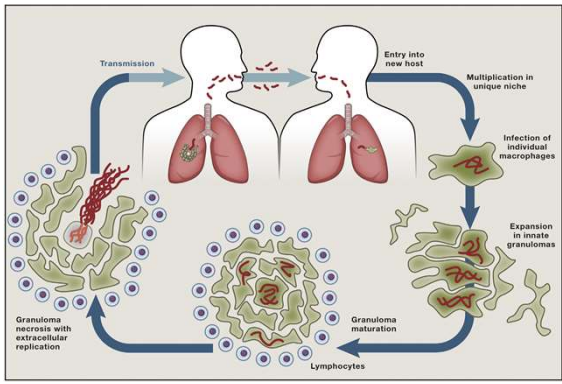


Korb VC, Moodley D, Chuturgoon AA (2016) Int J Mol Sci. doi: 10.3390/ijms17030131

Mycobacterium Tuberculosis

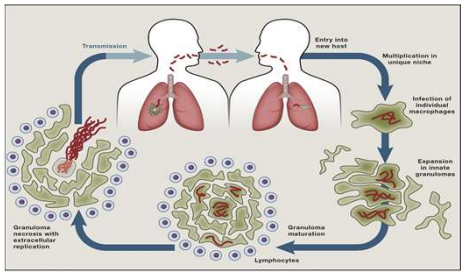
- Classified as a granulomatous inflammatory condition
- Effector cells accumulate at the site of the infection to form a tubercle
- Contains infection, suppresses bacterial replication and dissemination- resulting in subclinical disease
- The granuloma also shields the bacteria from the immune system- provides a niche for bacterial survival
- Latency is considered the hallmark of protective immunity (attributed to the CD4 T-Cell responses)
- Primary active disease/reactivation is considered a failure of CD4 T-cell immunity
- Rather than the absence of immune activity, active TB infection displays areas of intense immune infiltrate

Mycobacterium Tuberculosis



- A contrasting school of thought ascribes active disease progression to MTB-mediated dysregulation of the immune response into a pathological productive infection

DISEASE PATHOGENESIS



- Once bacilli are inhaled, the innate immune response is initiated
- MTB in alveolar space recognise (PRRs)- TLRs on alveolar and interstitial macrophages as well as local DCs- engulfed
- These APCs – present Ag as well as in association with MHC-II – to CD4 T-helper cells
- Up to acquired immunity, macrophages remain permissive intracellular MTB stores
- Period of exponential MTB replication
- Primed T-cells recognise and activate macrophages to eliminate the bacteria through secretion of IFN-ɣ and TNF-α - only controls bacterial replication- but not eradication
- These pro-inflammatory cascades culminate in remodelling of the infection site into a granuloma
- Results in chronic infection – slow/nonreplicating bacteria and progressive pathology

Macrophages

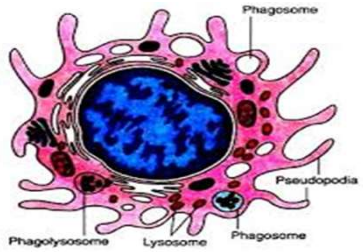


Fig. 6.35 : Typical morphology of a macrophage with lysosomes, pseudopodia, phagosome and phago-lysosome

- Play a dual role in MTB infection
- MTB targets and replicates within modified phagosomes of macrophages
- MTB employs several strategies to evade clearance (innate/adaptive)
- Yet macrophages predominantly kill MTB
- Initially respond with a vigorous pro-inflammatory and anti-microbial response via TLR signalling
- Acute TLR-2 ligation enhances both innate and adaptive immune function – contains infection
- Prolonged TLR-2 signalling – down regulates immune responses through – Foxp3-Treg cells, increased IL-10 secretion, reduced sensitivity to IFN-ɣ, inhibition of MHC-II expression, together with Ag processing and presentation (evades T-cell recognition

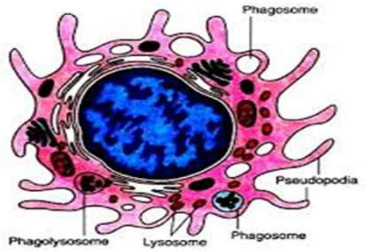
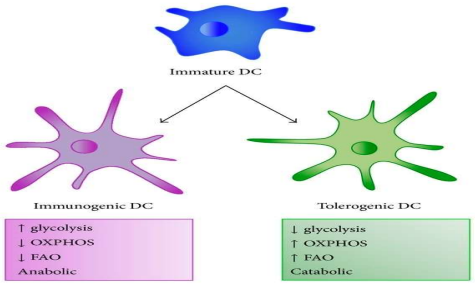


Fig. 6.35 : Typical morphology of a macrophage with lysosomes, pseudopodia, phagosome and phago-lysosome

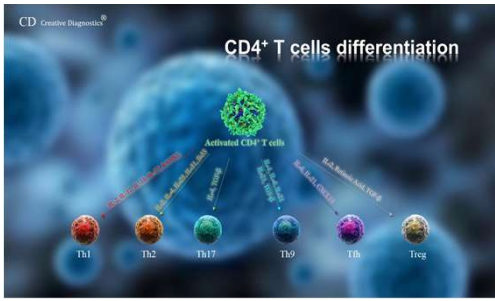
- During chronic phase infection- T-cell immune surveillance is focussed on macrophages:
- MTB turns a liability, viz., its cell wall abundant with PRR ligands, into a mechanism for avoiding recognition by T-cells and inhibition of effector T-cell responses- persistence of bacilli
- Infected alveolar macrophages enter the lung interstitium and establishes a site of infection
- Leads to a localised pro-inflammatory cascade (TNF-α, IL-1,-6 and -12) plus inflammatory chemokines (CCL2, CXCL10)
- The chemokines recruit neutrophils, NKCs, CD4, CD8 and B cells
- Results in a cascade of chemokines and cytokines that amplify cellular recruitment and remodelling of infection site into a granuloma

Dendritic Cells

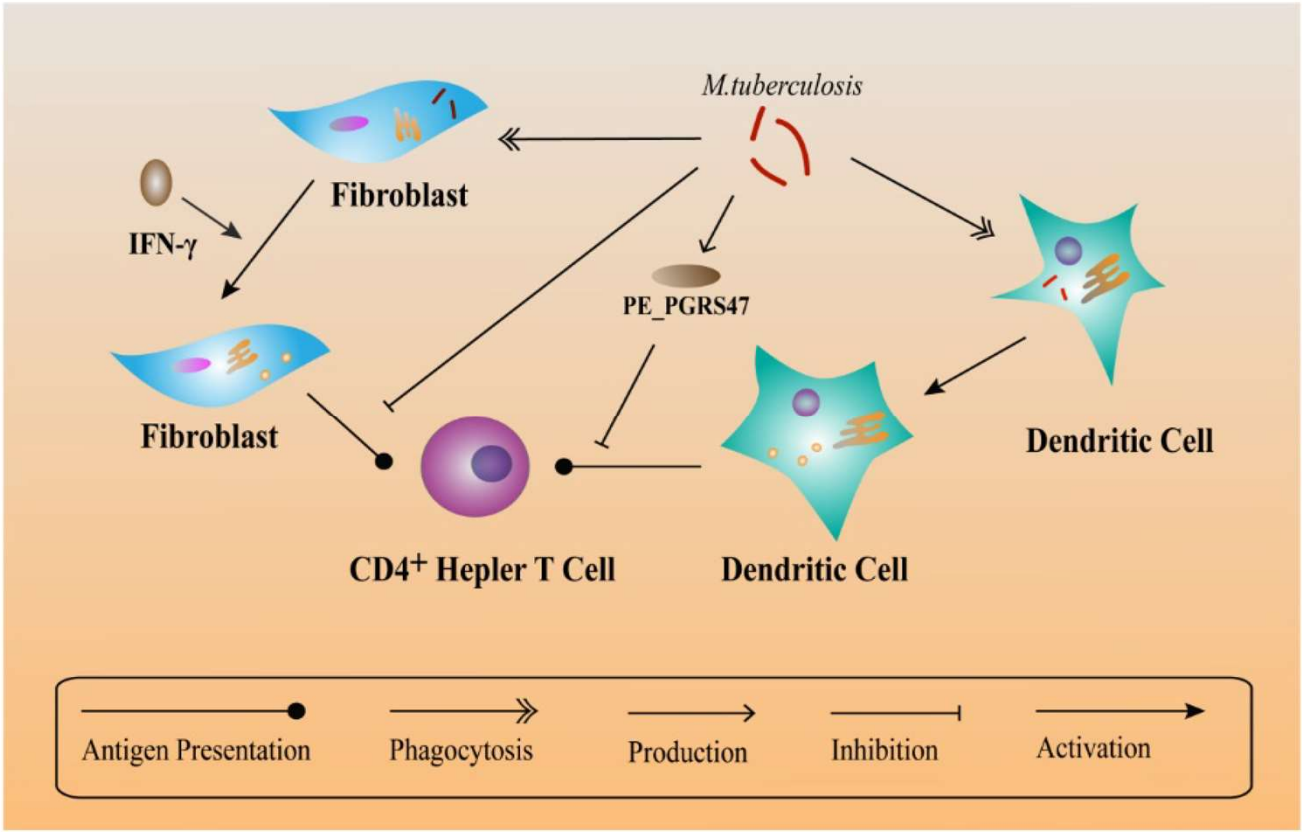


- Switches from innate immune response to acquired specific immunity
- Once MTB is phagocytosed, the DCs produce IL-12, upregulates MHC-I and II and CD-1 associated Ags
- Migrate to the the proximal lymph nodes and prime naïve T-cells
- However, it was shown that MTB inhibits their migration through the CCL-19-CCR7 gradient
- This lag permits exponential bacterial replication
- Further, several MTB factors downregulate DC function (cell envelope associated Ser hydrolase, Hip-1 impairs ROS generation etc)
- Ultimately results in increased expression of SOCS-1, inhibition of: NF-kβ signalling and IL-12 secretion (prevents T-cell priming)

CD4 T-cells

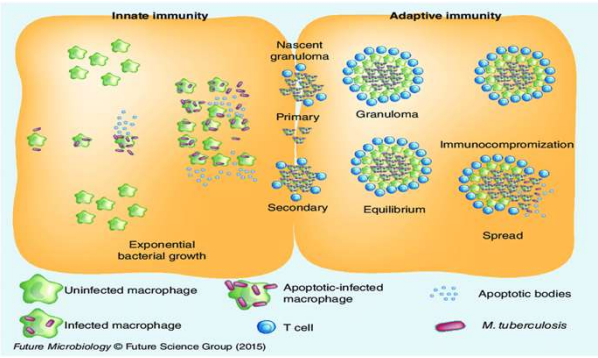


- Controls primary infection provides ongoing immune surveillance of reservoir of persistent bacilli in the granuloma (reactivation originates)
- Selective depletion of CD4 T-cells by HIV significantly increases TB reactivation rates to 5-10% per life year

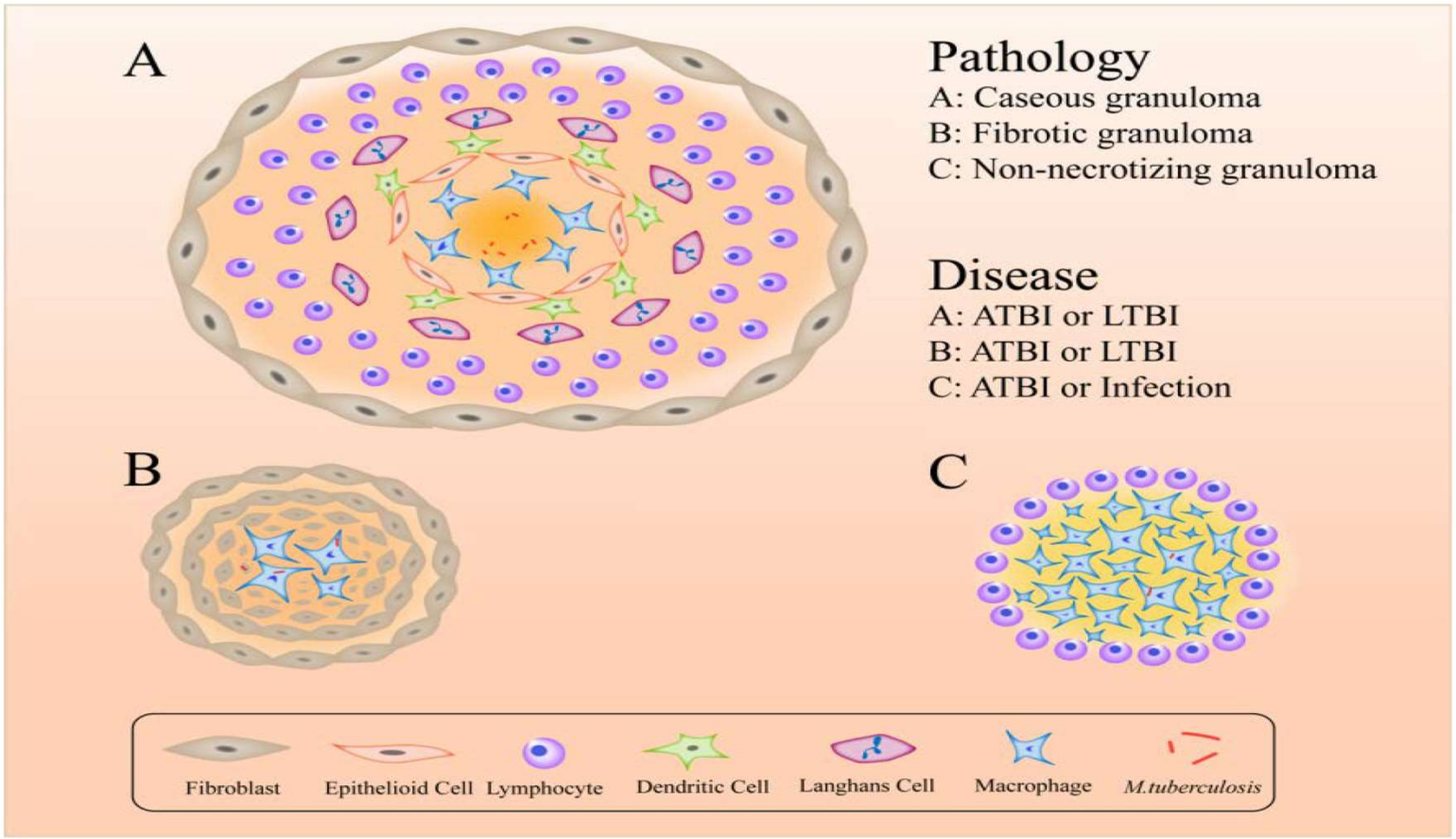


Relationship between MHC class II and M. tuberculosis evasion. As non-professional antigen-presenting cells, fibroblasts also present antigen derived from the processing of heat-killed MTB in addition to presenting peptides and isolated proteins. But MTB-infected fibroblasts are unable to present antigen from the bacteria. Peptide-loaded MHC class II molecules activate T cells to generate an immune response against MTB and this action occurs in the plasma membrane. However, PE_PGRS47 expressed by MTB can inhibit the presentation of MHC class II antigen.

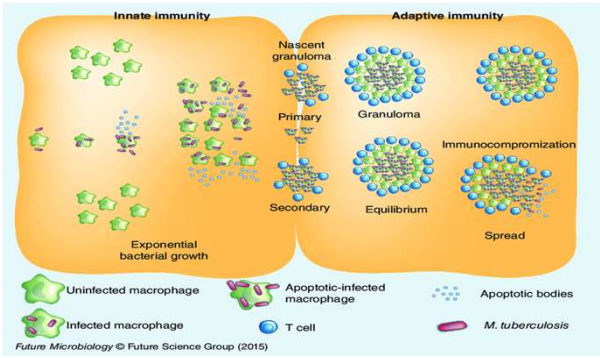
GRANULOMA



- Physically contain the bacilli to prevent dissemination and provides an environment of optimum and localised immune communication
- Bacilli growth is inhibited, partly by macrophage bactericidal mechanisms (creates an oxygen and nutrient deprived environment)
- Has several morphological forms- solid (dense aggregates of infected and uninfected macrophages and lymphocytes), neutrophilic (granulocytic infiltrates/central core of suppuration), and caseous (enlarged necrosis and liquefaction of dead cells-progress to cavities)
- Solid granuloma – containment phase or silent stage of infection (stalemate period when bacilli load is constant and infection enters latency)
- As it goes from solid non-progressive to active cavitary lesions- blood supply decreased- hypoxic environment

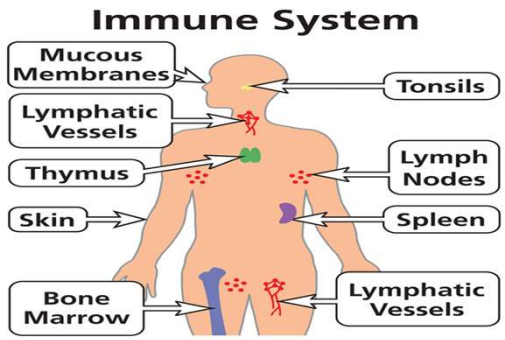


Active (ATB1) TB, latent TB infection (LTB1) or infectious granulomas that provide a shelter for MTB



- ROS and NO – inhibited by hypoxia- macrophages cannot kill bacteria- and hence uncontrolled bacterial replication and granuloma breakdown and dissemination
- MTB proteins and lipids have an established granulomatous effect
- Allows for continued MTB persistence in the core while separated from the bactericidal activity of lymphocytes

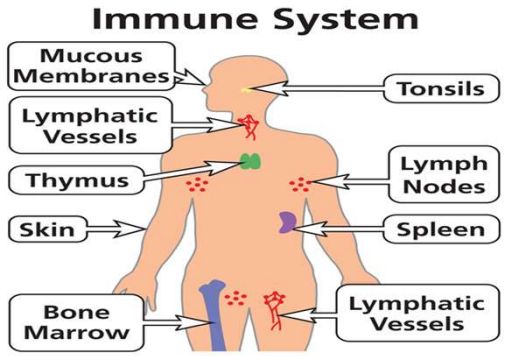
IMMUNE EVASION STRATEGIES



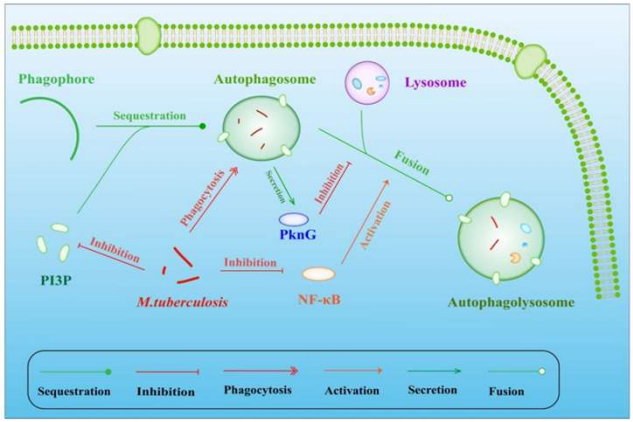
- MTB survives and replicates in macrophages (chronic persistent infection)
- Arrests phagolysosome biogenesis:
  - restricts unfavourable intracellular environment
  - Prevents host effector mechanisms
  - Shields MTB from antigen processing pathways
- Phagosome maturation occurs via acidification (pH=5.0 or less) of the lumen
- Acidic environment:
  - Inhibits bacterial activity
  - Optimises activity of hydrolytic proteases
  - Ensures correct vesicular trafficking/lysosome fusion events
  - Degradation of pathogen into components for Ag processing and presentation
  - Activation of cell-mediated immune response

IMMUNE EVASION STRATEGIES

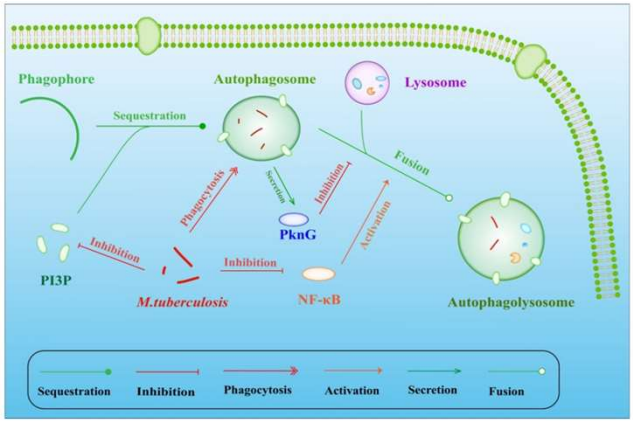
1. Maturation inhibition of phagolysosomes
2. Foamy Macrophages (FM)
3. MTB membrane vesicles
4. Chronic infection and transmission
5. MTB host lipid metabolism
6. Hypoxia and lipid metabolism
7. Host cell death



MTB inhibits maturation of Phagolysosomes

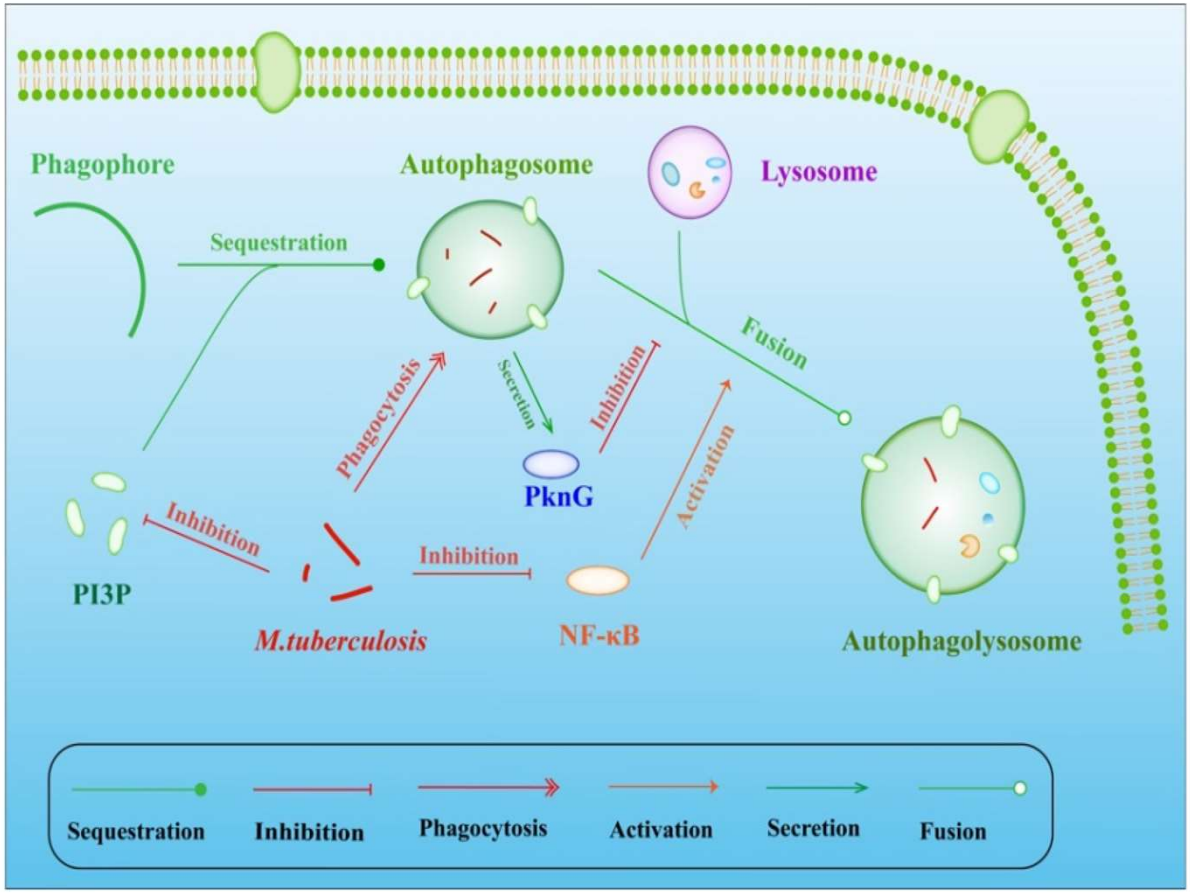


- Acidification occurs via recruitment of V-ATPases
- Pump H^+ across the phagosome membrane
- MTB-containing phagosomes acidify to a maximum of pH6.4 (failure to fuse with lysosomes)
- MTB excludes V-ATPase from phagosome membrane (prevents acidification)
- Mediated by MTB protein tyrosine phosphate (PtpA)
- PtpA binds to H (su) of macrophage V-ATPase and inhibits trafficking of vesicles to phagosome



- PKnG – similar to eukaryotic kinases:
  - Enhances MTB metabolism
  - Growth rate
  - Virulence and drug resistance
  - Inhibits maturation of lysosomes and increases infectivity
  - Prevents fusion of phagosomes and lysosomes
  - Inhibition of fusion is an important mechanism of inhibiting maturation of phagosomes/lysosomes in macrophages
- NF-kβ regulates the release of lysosomal enzymes into phagosomes
- Regulates killing of pathogens
- PI3P synthesis is decreased with increased hydrolysis- suppresses fusion and provides an escape channel for MTB

MOU1



MOU2

Figure 1. MTB evasion by inhibiting fusion of lysosomes with phagosomes. (Zhai et al., 2019, IJMS).

Slide 20

MOU1 Microsoft Office User, 5/27/2019

MOU2 While the secretion of PknG by phagocytic MTB directly inhibits the fusion of phagosomes with lysosomes, the suppression of NF-B also decreases this fusion. As an important component on the phagosome surface, lower biosynthesis and higher hydrolysis of PI3P also suppresses the fusion, providing an escape channel for MTB
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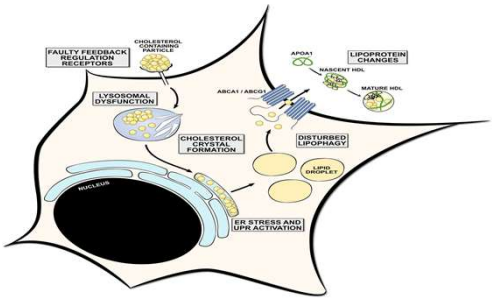
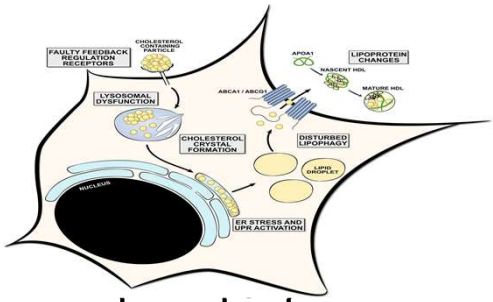
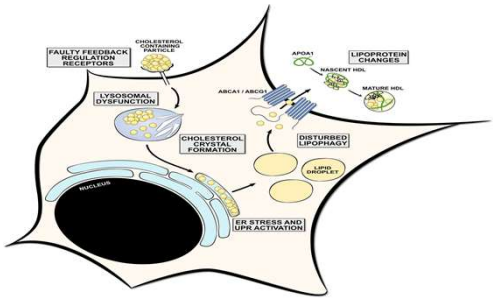
The Foamy Macrophage (FM)



- MTB evades the immune response to allow persistent infection, but simultaneously promotes sufficient immunopathology to ensure its transmission
- Current thinking suggests that MTB dysregulation of host lipid synthesis and lipid accumulation is pivotal in transition from latent to active infection
- The FM (found in pathologies with chronic pro-inflammatory stimulus) is key in sustaining persistent bacterial infection and driver of pathology, leading to cavitation and transmission
- Macrophages – converted into foam cells by an imbalance in the influx and efflux of LDLs (phospholipids, TAGs and cholesterol)
- Following breakdown of LDLs- majority of phospholipids and TAGs are metabolised

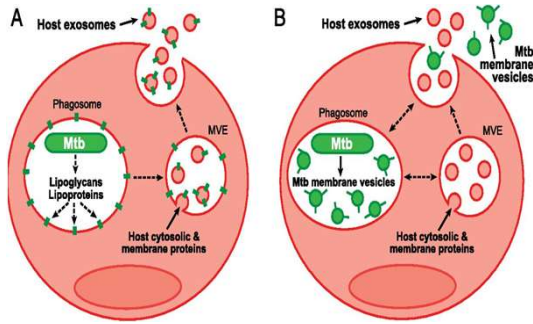


- Cholesterol is transported into the cytosol - either esterified and sequestered into lipid bodies within the ER or is pumped out via ATP-binding cassette transporters
- Evolution from macrophage to FM – through accumulation and progressive transformation of lipid bodies
- FM is in itself a pro-inflammatory cell
- Lipid bodies play a role in inflammatory response in granulomas
- Appear in necrotic lesions
- Increased and continual secretion of TNF-α - strong inducer of necrosis
- Have reduced: ability to mediate phagocytosis and anti-mycobacterial mechanisms; antigen processing capacity; suppress effector T-cells



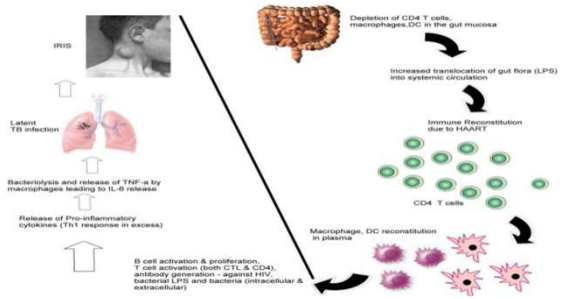
- Produce TGF-β – induces apoptosis of immune effector T-cells
- Produce high levels of iNOS (suppress T-cells in murine TB infection)
- The FM play a role in immune modulation and sheltering of MTB
- FMs can be generated from phagocytosis of dying cells
- Dying cell membranes contain arachidonic acid (AA) precursors and enzymes
- AA- mediated metabolic pathways are implicated in inflammatory response to MTB – either promotes or suppresses inflammation through prostaglandins (PGs), etc

MTB membrane vesicles



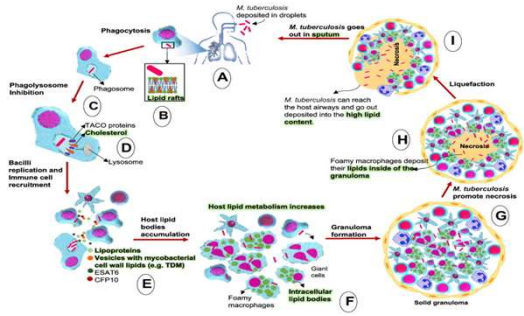
- MTB cell wall components are shed into vesicles within the phagosome
- Vesicles are actively trafficked out and accumulate in multilamellar bodies in micellar structures
- Vesicles are released by exocytosis
- These exosomes are engulfed by bystander cells, including uninfected APCs
- This infers MTB's, beyond the infected macrophage, influences the infected environment

Chronic infection and transmission

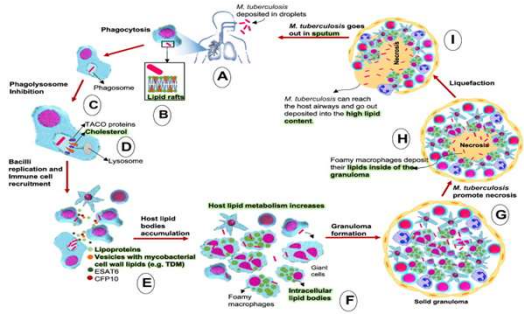


- HIV co-infection is strongest known risk factor for primary and delayed progression from infection to active disease
- Excessive bacterial replication results in necrosis, caseation, liquidation of granuloma and cavitation into bronchi, releasing bacilli
- Necessitates development of a productive cough, resulting in aerosol transmission of MTB
- FM generation is induced by oxygenated mycolic acids and hydroxyl mycolic acids
- Mycolic acids are abundant in MTB- and are virulence factors in chronic infection through hosts lipid accumulation and FM generation

MTB-Host Lipid Metabolism

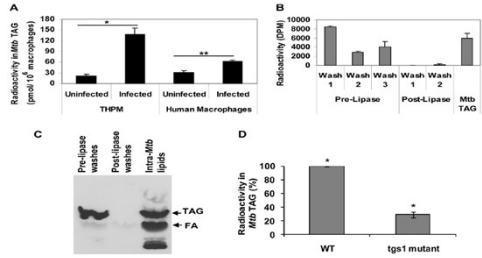


- Intracytoplasmic lipid inclusions have been identified in bacilli isolated from positive sputum
- MTB-containing phagosomes migrate toward the lipid droplets and undergo fusion
- Lipid bodies are released with thinning of the MTB cell wall
- The major component lipids are host TAGs (used for MTB lipid metabolism)
- MTB in FMs, with accumulated lipid inclusions, become phenotypically resistant to Rifampicin and isoniazid (frontline TB drugs)
- Lipids are the major source of energy for latent bacilli and a source of building materials for cell wall during dormancy
- Thus MTB manipulates host cell metabolism, inducing generation of FMs and lipid droplets – for its nutritional and structural requirements



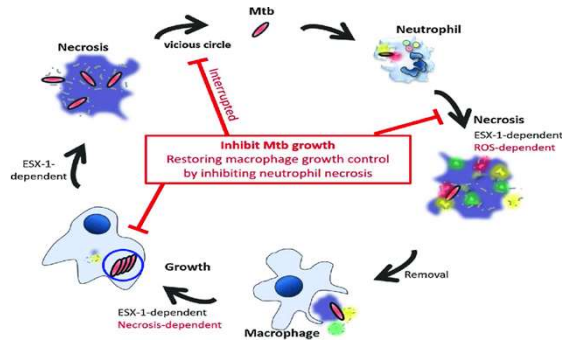
- In chronically infected lung tissue, fatty acids/cholesterol are indicated as source of carbon and energy for MTB metabolism
- IFN-ɣ macrophage activation causes maturation of the MTB phagosome and limits available carbon
- Nutrient restriction is a specific IFN-ɣ mediated defense mechanism
- MTB utilisation of TAGs and cholesterol circumvents the host response

Hypoxia and Lipid metabolism



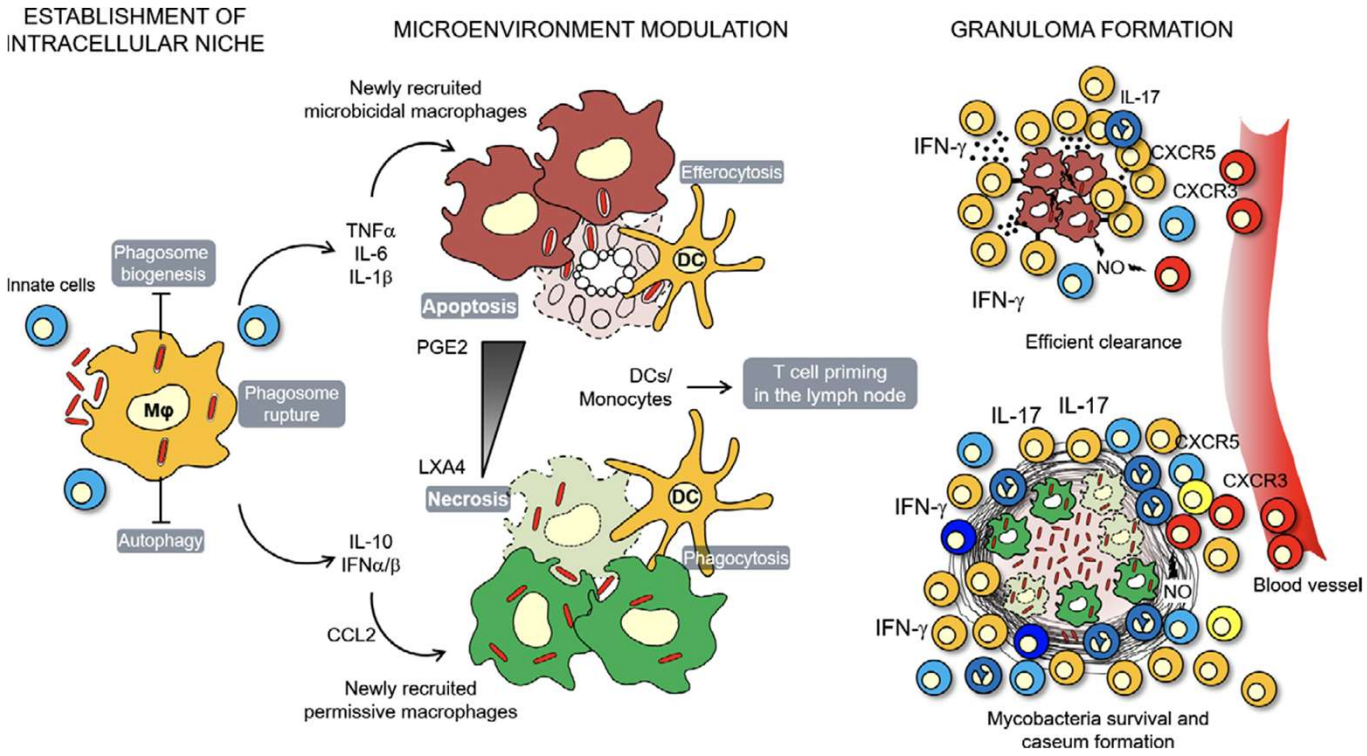
- Chromatin immunoprecipitation and sequencing during normoxia, hypoxia and re-aeration – reveal interconnections between the hypoxic response, lipid catabolism and anabolism and the production of cell wall lipids
- Free mycolic acids increased (form FM)- but decreased on re-aeration
- Change in oxygen status altered about 30% of MTB genes
- In granuloma core, hypoxia induces a non-replicative state- characterised by phenotypic resistance to anti-TB medication, as well as detection by acid fast staining, with a shift to TAGs and cholesterol as nutrient sources
- From PET-CT scans – areas immediately surrounding cavities within lungs of pulmonary TB patients showed marked hypoxia
- Associated with increase in MMP-1 and -9 expression (MMPs breakdown extracellular matrix)
- Hypoxia initiates latent MTB persistence and facilitates events leading to cavitation

Host Cell Death



- Virulent MTB survive in macrophages by preventing apoptosis, phagosome maturation and Ag processing (remains metabolically active and replicates)
- Once high intracellular bacterial load is reached, micro-disruptions in plasma membrane; necrosis occurs – and bacilli disseminated (necrosis promotes inflammation and disease progression)
- Necrosis of the FM in the granuloma leads to accumulation of lipid debris
- Thus MTB subversion of the macrophage response brings about late stage damage in the chronic phase of infection required for transmission and completion of MTB life cycle

Mycobacteria modulate the microenvironment to subvert infected-cell death pathways and recruit permissive macrophages



Progression of the immune response to M. tuberculosis infection.

MOU4 Progression of the immune response to M. tuberculosis infection. Early interaction with phagocytes through PRRs results in the phagocytosis of the bacteria. Virulent M. tuberculosis interferes with intracellular vesicle trafficking and escapes the phagosome to prevent early elimination and establish an intracellular niche. At the same macrophages, and other inflammatory cells in the site of infection, produce inflammatory mediators that can impact the cell death modality of the infected-cell and induce the recruitment of other inflammatory cells. High levels of IFN-αβ induce the recruitment of CCR2-expressing monocytes with limited ability to control bacterial growth. As infected cells dies newly recruited macrophages and monocytes efferocytose or phagocytose the bacteria and a granulomatous reaction begins to form. After the onset of acquired immunity, T cells begin to accumulate and a granuloma is formed. The efficient interaction between T cells and macrophages together with a balanced production of cytokines and chemokines results in a non-damaging inflammatory environment and control of infection. On the other hand, phagocytosis of the bacteria by permissive macrophages with a weak capacity to interact with T cells results in progression of infection. As the granuloma coalesces, the inflammatory environment generated may promote the constant accumulation of T cells contributing to immunopathological consequences to the host.

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CONCLUSION

- Despite centuries of research – relationship between MTB and host immune response remains enigmatic
- Current investigations are starting to elucidate mechanisms employed by MTB in the manipulation of protective immunity from infection to transmission
- Creates novel therapeutic opportunities and to reassess experimental designs and research goals


THANK
YOU!