Lecture 2 – TB immune evasion and immunopathology

Summary:

- Mycobacterium tuberculosis is one of the world most prevalent infections
  - 33% of the world is infected (2 billion), 10% die from TB
  - Pre-disposes to other infections, most TB in sub-Sahara Africa is cause by HIV infection
  - Most of the TB in Australia is from immigration, PNG has the highest incidence in the world
  - 3 – 20% of cases are antibiotic resistant strains, with 10% untreatably so
- Mtb is successful because
  - Has a privileged niche – generally naïve, few commensals with few alarms (PAMPs-PRRs)
  - Multiplies in a protect macrophage – mainly infects macrophages, but can use other cells
    - Disseminates via macrophages – kills macrophages → bronchi → next person
  - Induces "walling off" – protects the infection via hypoxia (unsuitable for immune/any cells)

<u>Avoiding initial detection</u>

- Mtb compromises infectivity to reach lower in the lungs (commensal free niche)
  - Large cough droplets → URTI → commensals + TLRS → killed
  - Smaller cough droplets → LRTI → few commensals/TLRs/macrophages → Mtb uptake
    - Simultaneously harder to access, but better outcomes for Mtb
- Mtb recruits the quiescent macrophages to infect them
  - Mtb is covered in a PDIM mask → harder for PRRs to access PAMPs
  - There is slight interaction of the surface lipid recruiter (PGL) with the macrophage → secretion of CCL2 from the macrophage to the macrophage to recruit it
- **Phthiocerol dimycocerosate (PDIM)** = surface lipid mask
  - Expressed by <u>all</u> clinical isolates → essential for virulence/infection (confirmed with animal)
  - It is metabolically costly (worthwhile to avoid detection) and lost during in vitro passage
- **Phenolic glycolipid (PGL)** = surface lipid "recruiter"
  - Not found in all clinical isolates/strains
  - It is found in areas of low endemnicity to aid transmission (lower levels in high TB areas)

<u>Use and Abuse of the Macrophage</u>

- Pathway
  - Bacteria are endocytosed → phagosome → lysosome fusion → phagolysosome → killed
    - Mtb makes kinase **PknG**, phosphorylates proteins to prevent the fusion event
    - Some fusion can occur → lysis of Mtb
  - Mtb replicates in the phagosome but needs to escape
    - Makes ESX1 (secretion pathway) to secrete ESAT6 (major pathogenic loci + protein)
      - ESAT6 needs ESX1 to facilitate movement
    - Permeablises the phagosome/phagolysosome (if survival) → cytosol → escape Mac
- **PknG = protein kinase G** = inhibits the formation of phagolysosome
  - Similar protein to eukaryotic protein kinases
  - Phosphorylates host proteins to prevent phagosome-lysosome fusion
- **ESX = (espA gene)** = specialised secretion system for ESAT-6
  - Essential for virulence, NOTE: deleted in BCG (bovus TB) = vaccine → highly attenuated
- **ESAT-6** = essential for virulence and important for recruiting and killing macrophages
  - Required for permeabilisation of the phagosome + escape to the macrophage cytoplasm
  - 6kDa early secretory antigenic target → potent T cell antigen
- Other functions of ESX and ESAT-6
  - ESAT6 is secreted from dead/dying macrophages → acts on epithelial cells → upregulated MMP9 → recruits more macrophages to the site → granuloma formation + caseation
    - Mtb may exploit the formation of granulomas
- Mtb has two strategies to recruit macrophages
  - Upregulate CCL2 → attract macrophages = increasing Mtb niche
    - Early on = quiet, keeps noise low = keeps the infection a secret
  - Increase expression of MMP9 on epithelial cells for more macrophages = later one + louder

<u>Mtb blocks/influences the adaptive immune response</u>

- If there was Mtb in the phagolysosome alive long enough to make some ESX/ESAT-6 → permeabilises the phagolysosome → releases the debris of the dead Mtb (i.e. DNA) → triggers cytosolic TLRs → STING → TBK1 → IRF3 → Type I IFN production

    - Low levels of Type I IFN → helps the adaptive immune response
    - Chronic levels of type I IFN → inhibits T cell/adaptive cell activity
- NOTE: gamma IFN is completely different + made by adaptive cells
    - IFNg acts on macrophages to downregulate the signalling pathways and tryptophan levels
        - Aims to active the macrophage to a bactericidal phenotype
    - BUT, Mtb makes its own tryptophan and thus is refractory/resistant to this change
- Mtb induces lytic cell death more than apoptosis
    - In the granuloma there are epithelial cells and dead/dying infected macrophages
    - To induce propagation Mtb induces lytic cell death (necroptosis/pyropotis), unsure how
        - Membrane permeabilises → releases the contents of the cell, and thus any alive Mtb can escape prior to degradation by proteases → eventually casemates → bronchus → transmission of the organism
    - Apoptosis does occur, isn't the biggest type of cell death occurring, but the contents of a cell is disintegrated prior to cell permeabilisation → no Mtb escapes alive

Inflammatory cytokines + TNF

- **Neutrophils** are attracted to the necrotic centre → (theoretically) kill Mtb BUT also release lots of IL-1 → contribute to pyroptotic death → assists the transmission/release of Mtb
- **IL-1(a and b)** from other cells can induce host cell activation (inflammation → collateral damage of tissues) + thus killing BUT, it induced lytic cell death
- **TNF**
    - There is a clear role of TNF in TB given no TNF or no signalling via TNF → severe TB disease
        - Patients with RA on anti-TNF + latent TB → resurgence once TNF removed
- TNF + TNF-R1
    - Activate NF-kB → activate macrophage → bactericidal pathway → Mtb killed
    - Activates death inducing signalling complex → apoptosis of macrophages
        - Also kills the organism
        - KO of TNF–induced apoptosis (via caspase 8 KO) and necroptosis (via MLKL KO) → increase TB load (+ no role of necroptosis) = apoptosis important
    - Activates necroptosis of macrophages → lytic death = BAD
        - But KO of MLKL (essential for necroptosis) = no change in TB load
        - Therefore, this is NOT contributing to TB disease/pathogenesis
- TNF effect on adaptive immunity
    - KO of TNF–induced apoptosis (via caspase 8 KO) and necroptosis (via MLKL KO) → reduced number of active adaptive cells
    - Thus, TNF is promoting the presentation of antigens to the immune system and driving the adaptive immune response
        - Indispensable for the control of Mtb

Outcomes of Mtb infection

- Unusual for patients to move straight to overt disease, most people can control infection → Immune control/Latency = there is Mtb turn over but this is control = no overt disease
    - This is known due to Quantiferron assays for TNF levels
- Conversion to overt/active disease occurs with immunosuppression (e.g. anti-TNF biologics, HIV, steroids, environmental stress [malnutrition, immigration]) → cure with Antibiotics

Summary

- Mtb has adapted with host → does a lot to go unnoticed (access privileged niche = no commensals = naïve macrophages = less TLRs AND lipid layers to hide)
- Once infected → increases niche/macrophage infiltration (1st quiet CCL2; later MMP9 via ESAT6)
- Aims to prevent Mtb killing pathways = inhibits phagosome-lysosome fusion (PknG) + inhibits apoptosis (unsure how) + promotes lytic death/necroptosis → escapes and infect