Outline

- Macrophages ingestion & processing of TB bacilli
- Phagosome & Phagolysosome formation
- Bacterial & Host Factors influencing phagosome & lysosomes formation
- Role of macrophages & Monocytes
- Role of T-cells & Humoral Immunity in TB infection
- Role of Th1 and Th2 and cytokines
- Granuloma Formation
- Factors favouring spread of TB
- Mantoux / Skin Test
Importance of Immunity

- Not all persons TB infection leads to clinical disease
- Low immunity associated with high risk of severe TB infection
- Vaccination prevents disseminated TB eg meningitis
- Mantoux positive person has low risk of acquiring TB than negative person
Infection results from Droplet nuclei (1-5 micron) inhalation

Cilia expels most of bacilli; only <10% reach alveoli

Macrophages phagocytosis (phagosomes)
(Dendritic cells less important in Resp Tract)

Bind via surface receptors (incl. complement receptors, mannose receptor, immunoglobulin GFc receptor, and type A scavenger receptors.

Phagosome bind lysosomes- ----> phagolysozymes
Innate immunity – How does Macrophages recognise foreign against self antigens?

‘Pattern Recognition Receptors’
(Toll-Like Receptors)
Fate of TB infection

- Macrophages kill bacteria (majority)-no residual signs/symptoms
- Rapid multiplication and spread
- Local infection, Granuloma formation, healing and calcification
- Latent infection & reactivation
Survival of TB in Macrophages

- Depends on:
  - (1) Virulence of Tb bacteria
  - (2) Host Factors
    - genetics
    - Macrophages ability to response/phagocytose
    - Cytokines activity
    - T-cell response (CD4&CD8)
    - Balance of Th1 and Th2 and cytokines
    - B-cell response
Survival of TB in Macrophages

- **Bacteria Factors:**
  - Bacterial Cell Wall (glycolipid *lipoarabinomannan* (LAM));
    - inhibits the intracellular increase of Ca$^{2+}$.
    - Thus the Ca$^{2+}$/calmodulin pathway (leading to *phagosome-lysosome* fusion) is impaired,
    - and the bacilli may survive within the *phagosomes*.
  - If the bacilli are successful in arresting phagosome maturation, then replication begins and the *macrophage eventually ruptures* and releases its bacillary contents.
Mycobacterial Lipids and Proteins

- MycoTB Lipids are important for its recognition by APCs (dendritic cells) via its Toll-Like Receptors (TLRs).

- *M. tuberculosis* possesses various protein antigens. Some are present in the cytoplasm and cell wall; others are secreted.

- Secreted antigens are more important in eliciting a T lymphocyte response.

- Among the antigens that may play a protective role are the 30-kDa (or 85B) and ESAT-6 antigens.

- Protective immunity is probably the result of reactivity to many different mycobacterial antigens.
Virulence of Tubercle Bacilli

- Several genes thought to confer virulence to *M. tuberculosis* have been identified.
  - The *katG* gene encodes for catalase/peroxidase enzymes that protect against oxidative stress;
  - *rpoV* is the main sigma factor initiating transcription of several genes.
  - *erp* gene, encoding a protein required for multiplication, also contributes to virulence

- Defects in these genes result in loss of virulence.

- The *Beijing/W genotype family* have been identified in outbreak conditions in a variety of settings worldwide and have been associated with higher mortality rates and occasionally with multidrug resistance.
Innate Resistance to Infection

- **Genetics:** Host genetic factors play a key role in innate non-immune resistance to infection with *M. Tuberculosis* & the development of disease.

- The existence of this resistance, which is polygenic in nature, is suggested by the differing degrees of susceptibility to tuberculosis in different populations. Example;

  - In mice, a gene called *Nramp1* (natural resistance–associated macrophage protein 1) plays a regulatory role in resistance/susceptibility to mycobacteria.

  - The human homologue **NRAMP1**, which maps to chromosome 2q, may play a role in determining susceptibility to tuberculosis, as is suggested by a study among West Africans.
Innate Resistance to Infection

- Polymorphisms in multiple genes, such as those encoding for:
  - Histocompatibility leukocyte antigen (HLA),
  - interferon (IFN-),
  - T cell growth factor (TGF-),
  - interleukin (IL) 10,
  - mannose-binding protein,
  - IFN- receptor,
  - Toll-like receptor (TLR) 2,
  - vitamin D receptor, &
  - IL-1, have been associated with susceptibility to tuberculosis.
Host Response

- 1st Phagosome-lysosomes fusion \rightarrow \text{prevents survival of bacteria} \rightarrow, ultimately killing the macrophage.

- **Cells lysis** - release of a variety of chemo-attractant (e.g., complement components, bacterial molecules, and cytokines)

- **Recruitment** of additional immature monocyte-derived macrophages, including dendritic cells, which migrate to the draining lymph nodes and present mycobacterial antigens to T lymphocytes.

- At this point, the development of CMI and humoral immunity begins. These initial stages of infection are usually asymptomatic.
Host Response

- About 2–4 weeks after infection,

  (1) a Macrophage-activating CMI response &

  (2) Tissue-Damaging response.

- The *macrophage-activating response*
  - is a T cell–mediated (CMI) phenomenon
  - resulting in the activation of macrophages that are capable of killing and digesting tubercle bacilli.
The Macrophage-Activating Response

- **CMI is critical at this early stage.**

- In the majority of infected individuals, local macrophages are activated when bacillary antigens processed by macrophages stimulate T lymphocytes to release a variety of lymphokines.

- Activated Macrophages aggregate around the lesion's center and effectively neutralize tubercle bacilli without causing further tissue destruction.

- **Caseous Necrosis:** resembles soft cheese—a phenomenon that may also be observed in other conditions, such as neoplasms.

- **Healed lesion**---(1) viable bacilli may remain dormant within macrophages or in the necrotic material for many years or (2) may later undergo calcification.
The tissue-damaging Response

Delayed-type hypersensitivity (DTH) reaction to various bacillary antigens;

- it destroys unactivated macrophages that contain multiplying bacilli

- also causes caseous necrosis of the involved tissues. Although both of these responses can inhibit mycobacterial growth, it is the balance between the two that determines the form of tuberculosis that will develop subsequently.
In a minority of cases, the macrophage-activating response is weak, and mycobacterial growth can be inhibited only by intensified DTH reactions, which lead to lung tissue destruction.

i.e., lesion progresses ----> surrounding tissue is progressively damaged.
The Delayed-Type Hypersensitivity Reaction

- Liquefaction of the center of the lesion (caseous material) expand, destroy & invade bronchial walls & blood vessels→ cavities form→ bacilli multiply→ erode into bronchial airway→ discharge caseous necrosis+ large bacilli→ coughed up→ infect others,
Cavity-TB Bronchopneumonia
Granuloma Formation

- T cell
- B cell
- Neutrophil
- NK cell
- Epithelioid macrophage
- Apoptotic infected epithelioid macrophage
- Macrophage
- Apoptotic infected macrophage
- Necrotic infected macrophage
- Giant cell
- Foam cell
- Mycobacterium tuberculosis
Granuloma Formation

- **Formed upon development of Specific Immunity**

- Accumulation of large numbers of activated macrophages at the site of the primary lesion, granulomatous lesions (+/- tubercles) are formed.

- **Cells in Granuloma:**
  - Lymphocytes
  - Activated Macrophages
  - Epithelioid & Giant Cells (derived from Macrophages)

- Limit bacterial Growth within M (low oxygen & pH)

- Produce central necrosis (some TB bacilli survive)

- Heal to **calcification**
Extra-pulmonary Spread

- Lowered Immunity (HIV, poor nutrition, cancer, elderly)
- Children /Adults without strong immunity
- Transplant/Chemotherapy/Steroid treatment
Extra-pulmonary Spread

- In the early stages of infection, bacilli are usually transported by macrophages to regional lymph nodes, from which they gain access to the bloodstream and disseminate widely throughout the body—large weight-bearing joints (spine, hip, knee & sacroiliac).

- The resulting lesions may undergo the same evolution as those in the lungs, although most tend to heal.

- In young children with poor natural immunity, hematogenous dissemination may result in fatal miliary tuberculosis or tuberculous meningitis.
Role of Macrophages and Monocytes

- Involved in Antigen Presentation (CMI).
- Activated /infected Macrophages- process antigen & present for T-cell recognition;
- Produce cytokines – enhance CMI, produce clinical effects such as fever, wt loss & sweating and mediate pathology (granuloma).
Role of Macrophages and Monocytes

- Example:
  - Activated Monocytes and macrophages produce nitric oxide, which leads to antimycobacterial activity and increases synthesis of cytokines such as tumor necrosis factor (TNF-) and IL-1, which in turn regulate release of reactive nitrogen intermediates.

- In addition, macrophages can undergo apoptosis—a defensive mechanism to prevent release of cytokines and bacilli via their sequestration in the apoptotic cell.
Role of Macrophages

- **Overview of macrophage-lymphocyte interactions in tuberculosis.**
  - (Th1) and (NK cells) secrete interferon gamma,
  - INF-gamma activates alveolar macrophages to produce a variety of substances, including reactive oxygen and nitrogen species (inhibit & kill TB)
  - INF-gamma suppress Th2 function; hence, decrease IL4, IL5
  - Macrophages secrete interleukin-12 (IL-12)-positive feedback loop to amplify this pathway.

Role of Humoral Immunity

- Role (although evidence is accumulating on the existence of LAM antibodies, which may prevent dissemination of infection in children).

- As compared to CMI which confers partial protection against *M. tuberculosis*,
Role of T Lymphocytes

- T-cells recognise antigen presented (by alveolar macrophages, monocytes, and dendritic cell) in association with MHC II via specific TCR $\rightarrow$ activation of T-cells $\rightarrow$ production of cytokines $\rightarrow$ mediate CD8 or humoral responses.

- This is critical step in CMI leading to activation of CD8 cells and B-cells.

- Qualitative and quantitative defects of CD4+ T cells explain the inability of HIV-infected individuals to contain mycobacterial proliferation.
Role of T Lymphocytes

- Activated CD4+ T lymphocytes can differentiate into cytokine-producing $T_H^1$ or $T_H^2$ cells.

- $T_H^1$ cells produce IFN— an activator of macrophages and monocytes—and IL-2 (more important in TB infection)

- $T_H^2$ cells produce IL-4, IL-5, IL-10, and IL-13 and also may promote humoral immunity (less important).
Role of T lymphocytes

**Good TB control**
- **T<sub>H1</sub> response**
  - IFN-γ
  - IL-2
- Activate Macrophages & Monocytes
- Protective immunity

**Poor TB control**
- **T<sub>H2</sub> response**
  - IL-4
  - IL-5
- Antibody production, IgE, Eosinophil, Lepromatous leprosy

**IL-12**

- Activate Macrophages & Monocytes
- Protective immunity
- Impaired immunity
Role of T Lymphocytes

- CD8 cell lyses infected cells and produce INF&TNF
- NK cells –co-regulates CD8 activity
- Enhance mycobacterium killing
Figure 34-7

Regulation of the immune system, emphasizing a pivotal role of the helper T cells. MHC, major histocompatibility complex.
Skin Test Reactivity

- Coincident with the appearance of immunity, DTH to *M. tuberculosis* develops.

- This reactivity is the basis of the TST, which is used primarily for the detection of *M. tuberculosis* infection in persons without symptoms.

- The cellular mechanisms responsible for TST reactivity are related mainly to previously sensitized CD4+ T lymphocytes, which are attracted to the skin-test site.
Skin Test Reactivity (Mantoux Test)

- Elicits CMI /DTH reaction
- Measured area of red induration
- Cut-off for positive 5mm-10mm size; PNG >10mm
References


- www.uptodate.com