Medical Bacteriology - lecture 13

*Mycobacterium*

*Actinomycetes*
Mycobacterium tuberculosis

- Large, very weakly gram positive rods, **Obligate aerobes**, related to Actinomycetes, non spore forming, non motile
- **very slow growing**- slow generation time (12-24 hours).
- Acid fast bacteria (Ziehl-Neelsen stain)
- facultative intracellular parasite, usually of macrophages
- rich in lipids - Mycolic acids in (**Mycobacteria, Nocardia**)
- Egg yolk agar and Lowenstein- Jensen agar (Selective media)
- 1N NaOH.
- More resistant to chemical agents than other bacteria
- Many non pathogenic mycobacteria are parts of human normal flora
- **Medically important:**
  - **M. tuberculosis** is cause agent of tuberculosis in humans.
  - **Tuberculosis (TB)** is the leading cause of death in the world. Most people with TB infection have a positive reaction to the **tuberculin skin test** (purified protein derivative). **Bacille Calmette-Guerin** (BCG-vaccine).
  - **M. bovis** is the agent of TB in cows and rarely in humans (Both cows & humans can serve as reservoirs)- Humans can infected by the consumption of unpasteurized milk. This route of transmission can lead to the development of **extrapulmonary TB.**
  - **M. leprae**, the causative agent of **leprosy**
Stages of the Tuberculosis Disease

• **Incubation period**: 4-6 weeks.
• **Source of infection**: Tuberculous patients
• **Route of infection**: Respiratory- Inhalation of droplet nuclei- Ingestion of contaminated milk.
• **The disease manifests with low fever, night sweating, significant weight loss, fatigue and weakness.**

- **Disease progression depends on**
  - Strain of MTB
  - Prior exposure
  - Vaccination
  - Infectious dose
  - Immune status of the host

• **Stage 1: Droplet nuclei (Primary nodule) (tubercle)**: inhaled. One droplet nuclei contains no more than 3 bacilli. Droplet nuclei are so small that they can remain air-borne for extended periods of time.

• **Stage 2: (Tissue necrosis)** Begins 7-21 days after initial infection. MTB multiplies within macrophages until the macrophages burst.

• **Stage 3: (Consolidation)** the individual becomes tuberculin-positive. The host developing a cell mediated immune response. An antibody will not control of a MTB infection because MTB is intracellular and if extracellular, it is resistant to complement killing due to the high lipid concentration in its cell wall. at this stage that **tubercle formation** begins. The center of the tubercle is characterized by semi-solid or "cheesy" necrosis". MTB cannot multiply within these tubercles because of the low pH. MTB can, however, persist within these tubercles for extended periods. **(more contagious).**

• **Stage 4: (Calcification)** MTB uses macrophages to replicate, and the tubercle grows. The growing tubercle may invade a bronchus. If this happens, MTB infection can spread to other parts of the lung.
Tuberculosis begins when droplet nuclei reach the alveoli. When a person inhales air that contains droplets, most of the larger droplets become lodged in the upper respiratory tract (the nose and throat), where infection is unlikely to develop. However, the smaller droplet nuclei may reach the small air sacs of the lung (the alveoli), where infection begins.

**Pathogenesis**

- Inhaled aerosols
  - Engulfed by alveolar macrophages
  - Bacilli replicate
  - Macrophages die
  - Infected macrophages migrate
  - Develop Ghon’s focus
  - Cell mediated immune response
  - Stops cycle of destruction and spread

- Viable but non replicating bacilli present in macrophages

**Evidence of Infection with M. tuberculosis**
- Chest x-ray / positive skin test
Cell Wall Structure

- It is unique among procaryotes, and it is a major determinant of virulence for the bacterium

- The cell wall complex contains
  - Peptidoglycan
  - complex lipids (consists of three major components, mycolic acids, cord factor and wax-D)

- Mycolic acids: found in cell walls of Mycobacterium, Corynebacterium and Nocardia- a significant determinant of virulence- prevent attack of the mycobacteria by cationic proteins, lysozyme and oxygen radicals in the phagocytes.

- Cord Factor: is toxic to host cells and inhibit PMN migration- most abundant in virulent strains of MTB.

- Wax-D: is the major component of Complete Freund's adjuvant (CFA). (a solution of water-in-oil emulsion used as an immunopotentaitor (booster). containing heat-killed mycobacteria or mycobacterial cell wall components, is an effective means of active cellular and humoral antibody response. It is known to stimulate production of tumor necrosis factor, which is thought to kill the T cells responsible for the autoimmune destruction)

- The high concentration of lipids in the cell wall of M. tuberculosis properties:
  - Impermeability to stains and dyes
  - Resistance to many antibiotics
  - Resistance to killing by acidic and alkaline compounds
  - Resistance to osmotic lysis by complement.
  - Resistance to lethal oxidations and survival inside of macrophages
Mycobacterium leprae

- Gram positive- acid fast bacilli- non motile- aerobic
- Causes leprosy
- Mostly found in warm tropical countries
- Strict human pathogens
- Obligate intracellular parasite- Cannot be cultivated in-vitro (Not grown in non-living bacteriologic media).
- Eg. Foot pads of mice
  - Armadillos
- Characteristic lesions are grown in laboratory animals.

**Clinical features:**
- Incubation period is months to years.
- Route of infection is through nasal mucus secretion

*Mycobacterium leprae* taken from a leprosy skin lesion.
### MYCOBACTERIA ASSOCIATED WITH HUMAN DISEASE

<table>
<thead>
<tr>
<th>Mycobacterium</th>
<th>Environmental contaminant</th>
<th>Reservoir</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em></td>
<td>No</td>
<td>Human</td>
</tr>
<tr>
<td><em>M. bovis</em></td>
<td>No</td>
<td>Human, cattle</td>
</tr>
<tr>
<td><em>M. leprae</em></td>
<td>No</td>
<td>Human</td>
</tr>
<tr>
<td><em>M. kansasii</em></td>
<td>Rarely</td>
<td>Water, cattle</td>
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<td><em>M. marinum</em></td>
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<tr>
<td><em>M. scrofulaceum</em></td>
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</tr>
<tr>
<td><em>M. avium intracellulare</em></td>
<td>Possibly</td>
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<td><em>M. ulcerans</em></td>
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<tr>
<td><em>M. fortuitum</em></td>
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</tr>
<tr>
<td><em>M. chelonae</em></td>
<td>Yes</td>
<td>Soil, water, animals</td>
</tr>
</tbody>
</table>
Actinomycetes

- Gram positive bacilli, facultative or strictly anaerobic- aerobic
- Branching filaments
- Actinomyces are morphologically similar to Nocardia except that they Actinomyces are not acid-fast
- **Grow slowly** in culture (up to two weeks or more) and cause infections that are slow to develop and tend to be chronic, abscesses, dental caries
- Normal flora of the upper respiratory, gastrointestinal and female genital tracts
- Low virulence potential, causing opportunistic disease following disruption of mucosal barriers by trauma, surgery or infection
- *Actinomyces bovis* (cows)
- Aerobic actinomycetes whose cell walls lack mycolic acid: Streptomyces species
Nocardia

- Weakly gram positive bacilli, branching long filamentous cells
- Acid fast
- Common found in soil, aquatic environment, humans (oral flora) and animals
- Sterolies
- Transmission (inhalation, skin) Most nocardia infections are acquired by inhalation of the bacteria or through traumatic introduction
- Treatment (long term antibiotics therapy)

- *Nocardia madurae*
- Madura foot

Fig. 6. *Nocardia* spp. Filamentous forms are partially acid fast with a branched beaded appearance on Fite or modified Kinyoun stain (A, 1,000× original magnification). The branching filamentous nature is nicely highlighted with GMS (B, 1,000× original magnification).
Review Questions

• What is the major phenotypic characteristic of mycobacterium? (5 points)

• Mycabacteria contain three medically important species, write them and write its diseases?

• What is the human Tuberculosis stages, which is more Contagious, which one can be seen apparently under X-rays, What site of the body for TB disease?

• What is the components of the rich lipid layer on the Mycobacterial cell wall, what is the major virulence determinants of the lipid components. What is the beneficial effect the Lipid layer?

• What is the types of *Mycobacterium leprae* diseases, *M. leprae* cannot be cultured in laboratory, because it cannot survive outside of mammalian cells. So, how it can be diagnosed?

• Give two examples of Branching (Filamintos) bacteria?