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Mycobacterium Tuberculosis

- Mycobacteria features;
  1. Aerobic
  2. Acid-fast bacilli → it is the only bacteria that is acid fast
     → it means the organism ability to retain the stain (carbol-fuchsin) although it is treated with ethanol hydrochloric acid mixture
  3. has **high lipid content** in their cell wall → make them acid fast
  4. They are neither gram positive nor gram negative
  5. They poorly stained with gram stain
- Major types;
  1. Mycobacterium tuberculosis → cause **tuberculosis**
  2. Mycobacterium leprae → cause of **leprosy**
  3. Atypical Mycobacteria
     .. Mycobacterium avium-intracellulare complex
     .. Mycobacterium kansasii
     → Cause tuberculosis-like disease

Mycobacterium Tuberculosis;

- Causes more deaths than any other microorganism
- Each year, 1.7 million people die of TB and 9 million die of TB
- Important properties;
  1. **Grows slowly**
     ∴ Culture specimen must be held for 6-8 weeks
  2. **Cultured in bacteriologic media**
     → Media used is Lowenstein – Jensen medium that contain;
     a) Nutrients → egg yolk
     b) Dyes → malachite green
        inhibit normal flora found in the sputum specimen
  3. **Aerobic**
     ∴ Causes disease in highly oxygenated tissues
  4. **They have the acid fast property**
     → Due to the long chain FA found in their cell wall → mycolic acid
     → They are resistant to acids and alkalis
     ∴ NaOH is used to destroy unwanted bacteria, human cells , mucus but not the organism
  5. **Characterized by the** cord factor
     → Responsible for the virulence of the organism
  6. **Contain several proteins that combine with waxes**
     → Elicit the delayed hypersensitivity
     → They are the antigens found in the PPD test
  7. **Resistant to dehydration**
     ∴ Survive in dry sputum and cause them easily to be transmitted by aerosol

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8. **Some strains are resistant to antimycobacterial drugs (INH) and other strains are resistant to multiple drugs**
   → due to chromosomal mutations;
   a) Mutations in the gene encoding the catalase pyridoxase
   b) Mutations in the gene encoding mycolic acid synthesis
9. **Have no plasmid**

- Transmission and epidemiology;
  - Transmitted by **respiratory aerosols** produced by;
    1. Coughing of **smear positive** people → sputum has detectable bacilli
    2. Coughing of **smear negative** people → 20 % of case
  - The initial site of infection → LUNGS
  - *M. tuberculosis* found in **reticuloendothelial cells** (macrophages)
  - **Humans** are the natural reservoir for the organism
  - **Mycobacterium bovis;**
    1. cause tuberculosis
    2. Mostly in developing countries
    3. Found in not pasteurized cow's milk → cause GI tuberculosis
  - Most cases of TB result from the reactivation of the organism in elderly people and malnourished men
  - Occur mostly in socioeconomically disadvantage people → poor housing – poor nutrition
    ∴ Most common in;
    1. Native Americans
    2. African Americans
    3. Eskimos

- Pathogenesis
  - It **does not** produce exotoxins and it does not contain endotoxins
  - Infect **macrophages** and other reticuloendothelial cells
  - Survive and multiplies within **phagosomes**
  - Produced exported repetitive protein → prevent phagosome from fusing with lysosome and thus prevent the organism from degradation by lysosome enzymes
  - Produce two types of lesions;
    1. Exudative lesions → consist of acute inflammatory response occur in the intial site of infection
    2. Granulomatous lesion →consist of central area of Langhans giant cells (epithelioid)
       → contain the tubercle bacilii surrounded by epithelioid cells
       Tubercle = granuloma surrounded by fibrous tissue and has centaral part that undergoes caseation necrosis
  - **Primary** lesions occur in lower lobes of lungs
  - Exudative lesion + draining lymph node = Ghon complex
  - **Reactivation** lobes occur in
    1. Apexes of the lungs
    2. Well oxygenated areas (brain, kidneys & bone)

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3. Immnocompromised patients

- **Mechanisms of organism spread;**
  1. Tubercle erode into bronchus → empty it's caseous content → spreads it to
     .. other part of lung
     .. GI if it is swallowed
     .. To other person if it is expectorated
  2. Dissemination via blood to parts of the body
     May occur at;
     a) **Early stage** → if the cell-mediated immunity fails to contain the infection
     b) **Late stage** → if the person become immunocompromised

- Immunity and hypersensitivity
  - After recovery from primary infection , resistance is mediated by **cell immunity** → Th-1 helper T-cells + macrophage
  - **Antibodies** are formed
    → play no role in resistance and not used for diagnosis
  - **High risk people;**
    1. Patient deficient in cellular immunity
    2. Mutation in gamma interferon receptor gene
    3. Mutations in Nramp gene
  - Prior infection detected by **tuberculin skin test**
    → **PPD** is used as an antigen
    → evaluated by measuring the diameter of **induration**
    1. 15 mm or more induration is + in persons with no risk factors
    2. 10 mm or more induration is + in person with high risk factor
    3. 5 mm or more induration is + in;
       .. Person who has deficient cell-mediated immunity
       .. Person who was in close contact with person with TB

    → + test indicate previous infection but not necessarily an active disease
    → Immunization with BCG can result in + test
    → People with PPD reaction more than 15 mm are assumed that are infected even if they take BCG
    → The test may result in – test in patient who is expected to be +
  - Gene called *Nramp* → give **natural resistance** to TB
    → Nramp protein found in the membrane of phagosome

- Clinical features;

1. **Pulmonary tuberculosis**
   - Fever
   - Fatigue
   - Night sweats
   - Weight loss
   - Cough and Hemoptysis

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- Scrofula → cervical lymphadenitis
- Erythma nodosum → tender nodules at the extensor surfaces of tibia and ulna

2. The disseminated forms of TB;
   a) **Military TB**
   b) **Tuberculous meningitis**
   c) **Tuberculous osteomyelitis**
   d) **Gastrointestinal TB**
      - Abdominal pain
      - Diarrhea
      - Fever
      - Weight loss
      - Intestinal obstruction
      - Caused due;

        .. Swallowing of M.tuberulosis after being coughed

        .. Mycobacterium bovis when it is ingested from not pasteurized milk

   e) **Oropharyngeal TB**
      - Painless ulcer
      - Local adenopathy
   f) **Renal TB**
      - Dysuria
      - Hematuria
      - Flank pain
      - WBCs in urine

→ Remicade drug used in treating rheumatoid arthritis may cause the reactivation of TB

- **Laboratory diagnosis;**

1. **Acid-fast staining of sputum**

   .. stains used are;

   a) Kinyoun stain
   b) Zeihl-Neelsen stain
   c) Auramine stain → for fluorescence microscope

   .. Process;

   Specimen is treated with NaOH and centrifugation → cultured on special media;

   a) Lowenstein-Jensen agar
   b) BACTEC medium → contain radioactive metabolites
   c) Liquid medium

      → After growth of microorganism .. organism then biochemically identified

2. Nucleic acid amplification test

   → detect presence of M.tuberculosis directly

   → highly specific but the sensitivities varies

3. Susceptibility test → because of drug resistance

   → it takes long time

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4. Molecular test → detect mutations that may cause the TB
5. **Luciferase assay** → detect drug resistant organisms
   .. Luciferase → enzyme isolated from fireflies that produce light in the presence of ATP
6. PPD skin test

7. **Interferon gamma release assay IGRA**
   .. Has two versions;
   a) Quantiferon TB
   b) T-spot. TB
      → the blood cells from patient are exposed to antigen of M.tuberculosis
      → gamma interferon released from cells is measured
   .. Specific for TB
   .. Not influenced by BCG vaccine

- **Treatment and resistant**
  - Multidrug therapy is used to prevent the emergence of drug-resistance
  - Therapy lasts for months but the sputum become noninfectious within 2-3 weeks
  - The **goals** of therapy
    1. Reach intracellular organisms
    2. Destroy the caseous material
    3. Kill the organism since it is slow growing
    4. Inactivate persisters within lesions
  - The failure to complete the full course of therapy is a major problem that cause the survival of TB
    → **DOT** →direct observed therapy → the health care workers observe patient taking the medications
  - **XDR** (extensively drug resistance) strains → resistant to INH, rifampin, fluoroquinolone and one additional drug

- **Prevention**
  - BCG vaccine;
    1. Live attenuated M.bovis
    2. Prevent the appearance of the disease
    3. Effectiveness ranges 0 – 70 %
    4. Used in areas where the incidence of the disease is high
    5. Should not be given to immunocompromized patients
       → it might cause disseminated diease
  - Better housing
  - Good nutrition
  - Use of masks when dealing with patients
  - Contact tracing
  - PPD skin test
  - Pasteurization of milk and destruction of infected cattle

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Atypical mycobacteria

- Spread in the **environment** unlike M.tuberculosis
- Called **mycobacteria other that tuberculosis MOTTS**
- Classified into 4 groups
  1. Group I; photochromogens
     → grow slowly
     → produce yellow-orange pigment colony when exposed to light
  2. Group II; scotochromogens
     → grows slowly
     → produce the pigment in the dark
  3. Group III; nonchromogens
     → grow slowly
     → produce little or no pigment in the absence or present of light
  4. Group IV;
     → grows rapidlv