MEDICAL MICROBIOLOGY

UNIT III : Tuberculosis

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Tuberculosis

- Tuberculosis (TB) is caused by bacteria (*Mycobacterium tuberculosis*) that most often affect the lungs.
- Tuberculosis is curable and preventable.
- TB is spread from person to person through the air.
- When people with lung TB cough, sneeze or spit, they propel the TB germs into the air. A person needs to inhale only a few of these germs to become infected.
- About one-quarter of the world's population has a TB infection, which means people have been infected by TB bacteria but are not (yet) ill with the disease and cannot transmit i

- Mycobacterium-
- slender rods, show branching ,filamentous forms resembling fungal mycelia.
- Fungus like bacterium
- In human being tuberculosis caused by *Mycobacterium tuberculosis* and *Mycobacterium bovis.*
- ***Mycobacterium tuberculosis***

Etiology: Morphology

Straight or slightly curved rod about 3x0.3µm.

Occur singly or in pair or as small clumps.

M.Bovis is short rod.

Gram positive but not in true sense

Acid fast.: once stained with Zeel Neelson carbol fuchsine resist decolourization with 20 %H_2SO_4.

General characters of the genus

- Slender rods
- Resist staining

→but once stained, resist decolorization by dilute mineral acids

→ hence called **ACID FAST BACILLI** (AFB)

- Aerobic, Non-motile, Non-sporing, Non-capsulated.
- Growth generally slow
- Genus includes
  - Obligate parasites
  - Opportunist pathogens
  - Saprophytes

Mycobacterium tuberculosis complex

- Includes Human and Bovine mycobacterium
- M .Africanism Tropical Africa
- M. microti do not cause human infections but in small mammals

M. bovis

- Primarily infection among the cattle
- M.bovis infects Tonsils, Cervical nodes, can produce Scrofula.
- Enter through Intestines – infects the Ileocecal region.

What are atypical Mycobacterium

- Infects birds, cold blooded animals worm blooded animals
- Present in environment
- Opportunistic pathogens
- Others – Saprophytic bacteria

-M butryicum present in butter

-M. phlei

-M smegmatis – present in Smegma

Mycobacterium tuberculosis (MTB)

- **Ziehl – Neelsen stain** – Once stained by Carbol fuchsin, resist decolorization by 20% Sulphuric acide and absolute alcohol.
→Acid & Alcohol Fast (AFB)
- **Fluorescent dyes** like Auramine O or Rhodamine also stain and the decolorization is resisted.

- Reason for Acid & Alcohol fastness –
  - Presence of unsaponifiable wax Mycolic acid
  - Semi permeable membrane around the cell
  - Property of cell wall and related to integrity of the cell wall
- Staining may be uniform or granular

MTB : Cultural characters

- **Slow-growing:** generation time of 12 to 18 hours (c.f. 20-30 minutes for *Escherichia coli*).
- Colonies appear after 2 weeks or at 6-8 weeks
- **Hydrophobic** with a high lipid content in the cell wall.
- Because the cells are hydrophobic and tend to clump together, they are impermeable to the usual stains, e.g. **Gram's stain.**

- MTB - Obligate aerobes.
- MTB grows more luxuriantly than M. bovis.
- Addition of 0.5% Glycerol supports growth of human strains.
- No effect or inhibitory effect on bovine strains.

MTB : Culture media

- **Solid media** –
  - Egg containing
    - Lowenstein-Jensen Medium
    - Petragnini medium
    - Dorset
  - Blood containing –
    - Tarshi's
  - Serum containing –
    - Loeffler's serum slope
  - Potato containing –
    - Pawlowsky's
- **Liquid media** –
  - Dubos'
  - Middlebrooks
  - Proskauer & Beck's
  - Sula's
  - Sauton's

1.Lowenstein Jensen medium –LJ medium .Commonly employed.

It contain egg, mineral salt and malachite green as selective agent which inhibit other bacteria.

M. Tuberculosis form dry ,rough, raised irregular colonies with wrinkled surface are creamy white and become buff coloured on further incubation,hard ,tenaceous

M.bovis form flat smooth, moist, white and soft colonies.

Liquid media- not used for routine cultivation but used for sensitivity testing ,preparation of antigen and vaccine.

Growth begins at the bottom and creeps up sides and form surface pellicle, serpentine cord

Virulent strains form long serpentine cord in broth

Resistance

- Not specifically resistant to heat: destroyed by 60° C x 20 min.
- In sputum: can survive 20-30 hrs
- Relatively resistant to disinfectants. Survives exposure to
  - 5 % Phenol
  - 15 % Sulphuric acid
  - 3 % Nitric acid
  - 5 % Oxalic acid
  - 4 % NaOH

Biochemical reactions

- **Niacin test** – Human MTB produces niacin when grown in egg medium.
- **Aryl Sulphatase test** – Enzyme Aryl sulphatase formed by only atypical mycobacteria.
- **Neutral red test** – Virulent strains of tubercle bacilli bind neutral red in alkaline solution while avirulent strains can not.
- **Catalase–paroxidase test** – Most atypical mycobacteria are strongly catalase positive while MTB is weakly positive.

- MTB is strongly peroxidase positive while atypical mycobacteria are negative.

- **Nitrate reduction test** – Positive in MTB and negative in M. bovis

Antigenic properties:

1. **Lipid**: cell wall is rich in long chain fatty acid called as mycolic acid and contain phosphatide, wax

Mycolic acid along with peptidoglycan is responsible for formation of **granuloma**.-lesion

2. **Protein** : tuberculo protein, responsible for tuberculin reaction , allergy test, an induce **delayed hypersensitivity in the host**.
3. **Polysaccharides**: Give group specificity , can induce immediate hypersensitivity.

Tuberculosis:

Source:

Source of infection is **open cases of tuberculosis**.

Mode of infection :**direct inhalation** of aerosolize bacilli, droplet nuclei of expectorated sputum.

Coughing ,sneezing and speaking release as many as 3000 infectious nuclei per cough.

Spread occur most often among households other close and prolonged contact of open cases(10,000 bacilli/ml of sputum).

Tubercle bacilli

Upper respiratory tract –in lung ingested by alveolar macrophages.

Several factor determine out come – virulence, I.D., host resistance etc

Toxin not produced , basis of virulence may be ability to resist intracellular killing and survive in macrophages.

Various components of cell are responsible for pathogenesis.

Cell mediated immunity is the only specific immune mechanism.

Activated CD4+ helper T cell releasing **cytokines** such gamma interferon, interleukin 1,and 2 and others exert different biological effects.

Th1 dependent cytokines activate macrophages

Formation in infected tissues of a characteristic lesion called tubercle is essential feature.

Tubercle is **avascular granuloma** .It contain a central zone containing giant cells with or without **caseation,cavitation** necrosis.Peripheral zone is made up of lymphocytes and fibroblasts. soft cheese , blood capilaries-bleeding,

Tuberculosis lesions are pr wo types.

Exudative and productive

Exudative	Productive
Acute inflamatory reaction.	Lesion is cellular. Tubercles,caseation .necrosis,
Accumulation of **edema** fluid, neutrophiles,lymphocytes.	
Large number of virulent bacilli	

Depending upon time of infection and type of response tuberculosis is of two types

Primary tuberculosis:

1. Is initial infection by tubercle bacilli in a host. In endemic countries like India usually occur in young children.
2. The bacilli engulfed by aleveolar macrophages multiply and give rise to a **tuberculous pneumonea**, in lower lobe or lower part of the upper lobe of the lungs(Ghon focus).
3. It is primary complex. Occur about 3-8 wk from about time of infection.
4. There is development of tuberculin hypersensitivity. Lesions calcified and heals spontaneously in 2-6 months.

Post primary tuberculosis-secondary-

It is reactivation of latent infection ,endogenous infection.

Affect upper lobe of the lungs.

Lesions/lesios- undergo necros death of cell, tissue destruction necrolysed ,slough off and cavitation.

The necrotic material break out in airways and lead to expectoration of bacteria containing sputum.

Epidemiology : Tuberculosis is an ancient disease.

1. Every year 8-8 million cases of tuberculosis appear.
2. India is one of the worst affected country .
3. Half a million people die due to

Laboratory diagnosis:

1. Microscopy
2. Isolation
3. Demonstration of hypersensitivity to tuberculoprotein.
4. Molecular biology techniques

Specimen-Sputum care

Decontamination and concentration of the sputum.

Petroff's method: homogenization sample

Sputum +4%NaOH—frequent shaking at 37oC 20 min- clear-centrifuge and neutralize by addition of 0.1N HCl. Sample can be used for microscopy ,culture , animal inoculation.

Microscopy

Acid fast staining –**Ziehl Neelson technique**

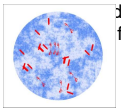
Prepare smear, dry and heat fix.

Flooded with ZNCF ,steam for 5-7 min.

Wash decolourize with **20% sulphuric acid** till no more colour comes out, followed by **decolourization with 95%** ethanol. 2 min, wash and counter stain with **malachite green or methylene blue**.

Under oil immersion d fast bacilli observe bright red fast green in colour .

Smear is Graded –



Number of AFB	Fields	Report
No AFB	In 100 immersion fields	Negative
1-9	In 100 immersion fields	Exact No
10-99	In 100 immersion fields	1+
1-10	Per-field examination	2+
>10	Per-field examination	3+

Source-RNTCP Lab manual 2009. RNTCP: Revised National Tuberculosis Control Program, AFB: Acid-fast bacilli

Culturing

Two bottles of LJ medium inoculated with pre treated sputum sample

Incubation at 37 0C examine after 4 days for rapid growing **Saprophytes.**

Examined twice weekly there after.

Negative report—if growth absent after 8-10 wks.

If growth present –Acid fast staining is performed

Biochemical test :niacin positive acid fast bacilli indicate *M.tuberculosis.*

Antibiotic sensitivity test :As antibiotic resistance is common problem , it gives guide for treatment.

Symptoms

1. Breathing difficulty. dyspnoea
2. Chest pain.
3. Cough (usually with mucus)
4. Coughing up-sputum with blood.
5. Excessive sweating, particularly at night.
6. Fatigue-tiredness
7. Fever.
8. **Weight loss.**
9.
10.

Immunodiagnosis;

Mantoux test**:** Demonstration of hypersensitivity to tuberculon protein s standard test for immunodiagnosis.

It is also called as **tuberculin test**

0.1 ml tuberculoprotein –PPD injected on fore arm with tuberculein syringe. And examine after 48 -72 hr.

If there is swelling ,redening test is positive 10 mm- Positive- indicate infection or immunization with BCG.

If diameter of lesion is 5mm and less test negative

Prophylaxis

Immunoprophylaxis-BCG –Bacillus Calmette Geurien vaccine.

Live attenuated vaccine

M.bovis strain attenuated in glycerine bile potato medim 239 times over a period of **13 years.**

Immunity last for **10-15 yr.**

Administered to babies by intra dermal injection on deltoid. Immediately after birth.

Treatment: DOT

Antituberculous drugs are of two types

1.Bactericidal an bacteriostatic

Bactericidal :Rifampicin-R and Pyrazinamide(Z) called sterilizing drugs

Isoniazide (H),streptomycein(S)

Bacteriostatic:Ethambutol(E)

Reigim / course is of 6-7 months.

1.HRZE- given 3 times a wk-2months

2.HR -given 3 times a wk for 4-5 months.