Tuberculosis

Dr. Shehab Ahmed Lafi

- This group of bacteria have rigid cell wall rich with mycolic acid which is water resistant waxy material,so they are known as Mycobacteria.

Characters of acid fast bacteria

- **Mycobacteria are acid fast bacteria , they resist decolorization with acid due to their waxy cell wall .**
- **This group of bacteria is weakly stained with gram stain as weak gram positive bacteria .**

- **We need to use strong dye which is Carbolfuchsin with heating below boiling to enhance dye entrance to the waxy layer of the cell wall.**

classification

- **Mycobacteria are of many types:**
- **A- TB complex bacilli :**
- **Mycobacterium tuberculosis , *Mycobacterium bovis***

- **B- Lepromatous Mycobacteria :*Mycobacteria lepri***

- **C- Avian complex (Atypical mycobacterium)**
- ***Mycobacterium avium***
-
- **D-Opportunistic Mycobacteria :**
- ***Mycobacterium ulcerans* , *Mycobacterium balani***

- **E- non pathogenic Mycobacteria :*Mycobacterium smegmatis***

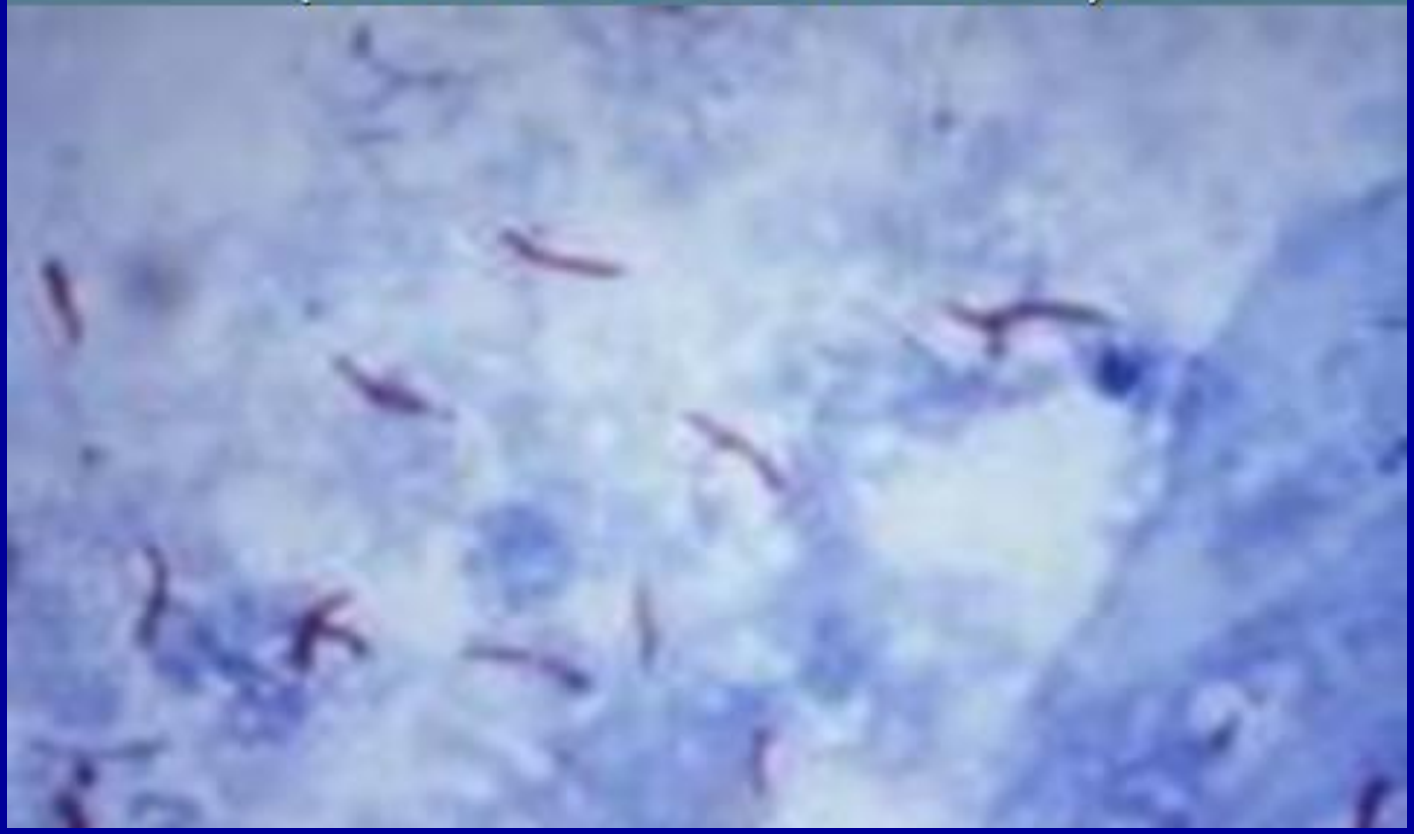
- **The most common and important mycobacterial species affecting humans is**
- ***Mycobacterium tuberculosis*** **(Mtb), which causes 'tuberculosis'.**
- ***M. bovis,*** **which causes tuberculosis in cattle and causes zoonotic infect to human via ingestion of milk and meat of infected animal.**
- ***M. leprae*** **is the causative agent of leprosy**
- **There are several uncommon species which cause opportunistic infection in the immunocompromised individals and collectively named 'atypical' mycobacteria as *Mycobacterium avium* which causes a TB-like disease especially prevalent in AIDS patients.**

- **General Characteristics**

- Mycobacteria are strictly aerobic, nonsporing intracellular bacilli. They are resistant to decolorization by mineral acid or alcohol after staining with carbol fuchsin, hence called ‘acid-fast bacilli’. The later forms a strong complex with the mycolic acid content of the cell wall, therefore the organism is only decolorized with 20% sulfuric acid and alcohol. This is the basis of the **Ziel-Neelsen**

- Staining method for *Mycobacterium tuberculosis* is the Ziehl- Neelsen stain. When this method is used, the M.TB. smear is fixed, stained with carbol-fuchsin (a pink dye), and decolorized with acid-alcohol. The smear is counterstained with methylene-blue or certain other dyes. Acid-fast bacilli appear pink .

Mycobacterium tuberculosis (Ziehl-Neeslsen stain)



Mycobacterium tuberculosis. Acid-fast stain.

Cultural characters

- **Medically important Mycobacterium species grow slowly. Their generation (doubling) time ranges from a few hours to >2 days, compared to 40–60 minutes for most other bacteria.**
- **Colonies may take 2–3 weeks to develop from the time of inoculation of culture media.**
- **Mycobacteria do not grow on ordinary laboratory media but grow well on Lowenstein-Jensen (LJ) medium, containing egg, asparagine, glycerol and malachite green. The latter inhibits contaminants.**
- **Colonial morphologies on LJ agar slopes are used in identification of species**

- ***Mycobacterium tuberculosis*** **grow also on other semi synthetic media like Dorset egg agar which is similar to LJ medium.**

Other media are Oleic acid medium and Middlebrook also enable Mycobacterium growth ,

Fluid medium like Dubos medium consists of Tween 80 as fatty material .

- **Colonies on Lowenstien Jensen LJ. medium are rough dry irregular they are white first become yellow or buff later. *Mycobacterium tuberculosis* colonies are well described as buff , tuff and rough take 2-3 weeks to develop at 37 C.**

Colonies of *Mycobacterium tuberculosis* on Lowenstein-Jensen medium.

- **Resistance to physical and chemical agents , mycobacterium tuberculosis are sensitive to sunlight , it may be killed within 2 hours. Temperature 60 C kills them within 20 min . 5% Phenol solution kills them within 24 hours.**

Antigenic structure

- **The contents of the cell wall imposed in antigenicity of *Mycobacterium tuberculosis***
- **Mycolic Acid is thought to be a significant determinant of virulence in Mycobacterium tuberculosis . Probably, it prevents attack of the mycobacteria lysozyme and oxygen radicals in the phagocytic granule.**
- **-They also protect extracellular mycobacteria from complement in serum.**

Cord Factor is responsible for the serpentine cording (chains of cells form distinctive serpentine cord). Cord factor is an inhibitor to PMN migration. **Cord factor** is most abundantly produced in virulent strains of M.TB. .

- **Wax-D** in the cell envelope is the major component of Freund's complete adjuvant (CFA).

Bacterial protein is involved with delayed type of hypersensitivity,

(The concept of Tuberculin test)

- In summary, the high concentration of lipids in the cell wall of Mycobacterium tuberculosis has been associated with these properties of the bacterium:

- • Impermeability to stains and dyes
  • Resistance to many antibiotics
  • Resistance to killing by acidic and alkaline compounds
  • Resistance to osmotic lysis via complement deposition
  • Resistance to lethal oxidations and survival inside of macrophages

Pathigenesis

Tuberculosis (TB) is a slow-progressing chronic disease of human and animals caused mainly by *M. tuberculosis,* less commonly by *M. bovis* and by atypical Mycobacteria in immunocopromised patients, These bacteria enter the body primarily through respiratory or alimentary tracts, or by contact, and form ‘tubercles’ in the lung or other parts of the body.

- TB infection means that M.TB. is in the body but the immune system is keeping the bacteria under control. The immune system does this by producing macrophages that surround the tubercle bacilli. The cells form a hard shell that keeps the bacilli contained and under control. Most people with TB infection have a positive reaction to the tuberculin skin test.

- People who have TB infection but not TB disease are NOT infectious, i.e., they cannot spread the infection to other people. These people usually have a normal chest x-ray.
- TB infection is not considered a case of TB. Major similarities and differences between TB infection and TB disease are shown below

TB Infection

- M.TB. present
- Tuberculin skin test positive
- Chest X-ray normal
- Sputum smears and cultures are negative
- No symptoms
- Not infectious
- Not defined as a case of TB

TB disease in lungs

- M.TB. Present
- Tuberculin skin test positive
- Chest X-ray usually reveals lesion
- Sputum smears and cultures positive
- Symptoms such as cough, fever, weight loss
- Often infectious before treatment
- Defined as a case of TB

Predisposing factors for TB infection include

- :

- • Close contact with large populations of people, i.e., schools, nursing homes, dormitories, prisons, etc.
  • Poor nutrition
  • iv drug use
  • Alcoholism
- HIV infection is the predisposing factor for M.TB. infection.

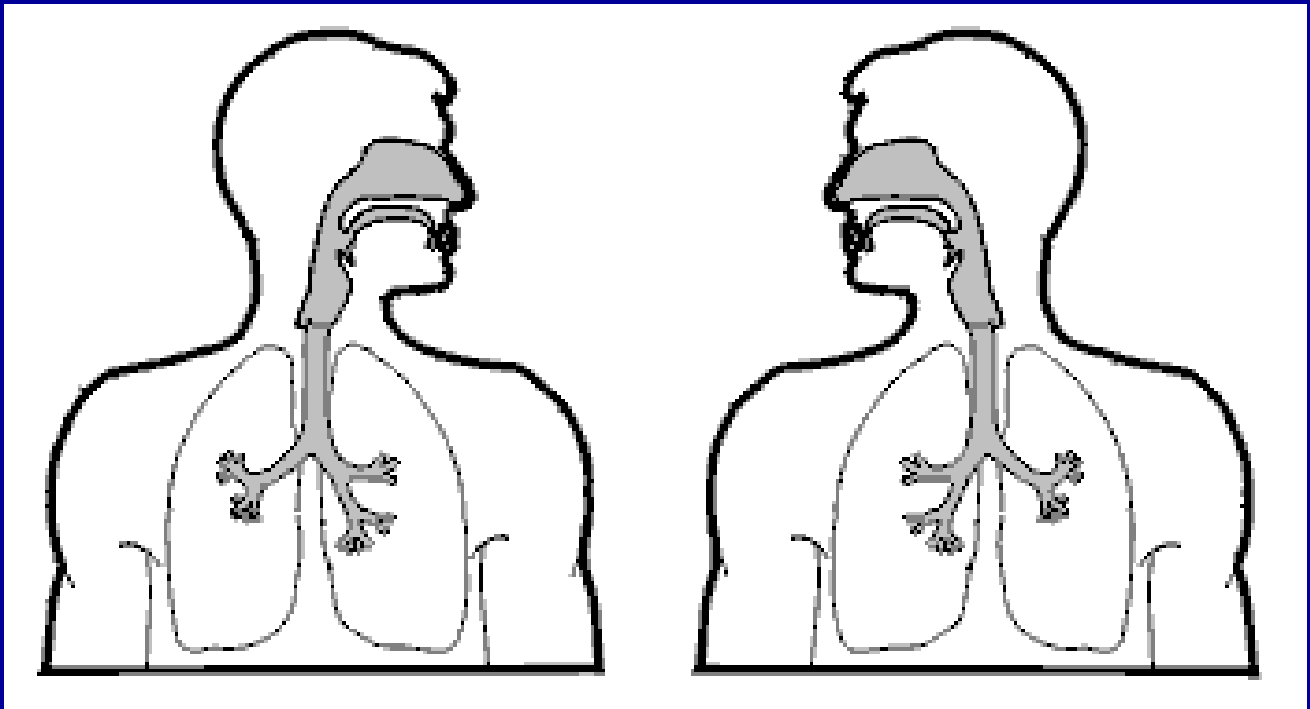
Predisposing factors for TB infection include

- Strain of M.TB.
- Prior exposure
- Vaccination
- Infectious dose
- Immune status of the host

Stages of the Disease

- Droplet nuclei are inhaled. Droplet nuclei are generated by during talking coughing and sneezing. Coughing generates about 3000 droplet nuclei. Talking for 5 minutes generates 3000 droplet nuclei . Sneezing generates the most droplet nuclei by far, which can spread to individuals up to 10 feet away.

Tuberculosis begins when droplet nuclei reach the alveoli. When a person Inhales air that contains droplets . The smaller droplet nuclei may reach the small air sacs of the lung (the alveoli), where infection begins.



Spread of droplet nuclei from one individual to another. After droplet nuclei are inhaled, bacteria are nonspecifically taken up by alveolar macrophages. However, the macrophages are not activated and are unable to destroy the intracellular organisms.

- Begins 7-21 days after initial infection. M.TB. multiplies virtually unrestricted within inactivated macrophages until the macrophages burst. Other macrophages also phagocytose M.TB., but they are also inactivated and hence can not destroy M.TB.

- At this stage lymphocytes begin to infiltrate. The lymphocytes, specifically T-cells, recognize processed and presented M.TB. antigen This results in T-cell activation and the liberation of cytokines including gamma interferon (IFN).

- In this stage that the individual becomes tuberculin-positive.
- Activated macrophages and T-cells also secrete cytokines that may also play a role in the development of immune pathology, including Interleukin 1 (IL-l), tumor necrosis factor (TNF), and gamma IFN.

- It is also at this stage that tubercle formation begins. The center of the tubercle is characterized by "caseation necrosis" meaning semi-solid or "cheesy" consistency. M.TB. cannot multiply within these tubercles because of the low pH and anoxic environment. M.TB. can, however, persist within these tubercles for extended periods.

- The growing tubercle may invade a bronchus. If this happens, M.TB. infection can spread to other parts of the lung. Similarly the tubercle may invade an artery or other blood supply line.

- **The hematogenous spread of M.TB. may result in extrapulmonary tuberculosis otherwise known as milliary tuberculosis.** The name "milliary" is derived from the fact that metastasizing tubercles are about the same size as a millet seed . Huge number of millet like lesions are distributed in viscera , liver , spleen , brain and even bone . Any site could be infected her.

- The secondary lesions caused by milliary TB can occur at almost any anatomical location, but usually involve the genitourinary system, bones, joints, lymph nodes, and peritoneum. **These lesions are of two types:**

- 1. **Exudative lesions** result from the accumulation of PMN's around M.TB. Here the bacteria replicate with virtually no resistance. This situation gives rise to the formation of a "soft tubercle".

- 2. **Productive or granulomatous lesions** occur when the host becomes hypersensitive to tuberculoproteins. This situation gives rise to the formation of a "hard tubercle

- The caseous centers of the tubercles liquify. This liquid is very conducive to M.TB. growth and hence the organism begins to rapidly multiply extracellularly. After time, the large antigen load causes the walls of nearby bronchi to become necrotic and rupture.

- After the first encounter with the organism **(primary infection),** the patient may remain asymptomatic or develop primary pneumonia, meningitis, osteomylitis, disseminated (miliary) tuberculosis or a range of other manifestations. In immunocompetent hosts, the primary infection remains asymptomatic for a long time (years) until the organism finds the opportunity (e.g. immunocompromised status) to reactivate. **Reactivation** of old infection or acquisition of new infection and development of disease is called **'post-primary' tuberculosis**

- Pulmonary tuberculosis is the most common manifestation and lesions usually occur in the upper lobes of the lung.
- In most cases TB progresses and requires treatment. Patients often produce blood-containing sputum (hemoptysis) and suffer persistent high temperature, night sweats, loss of appetite and loss of weight. If untreated, patients may die.

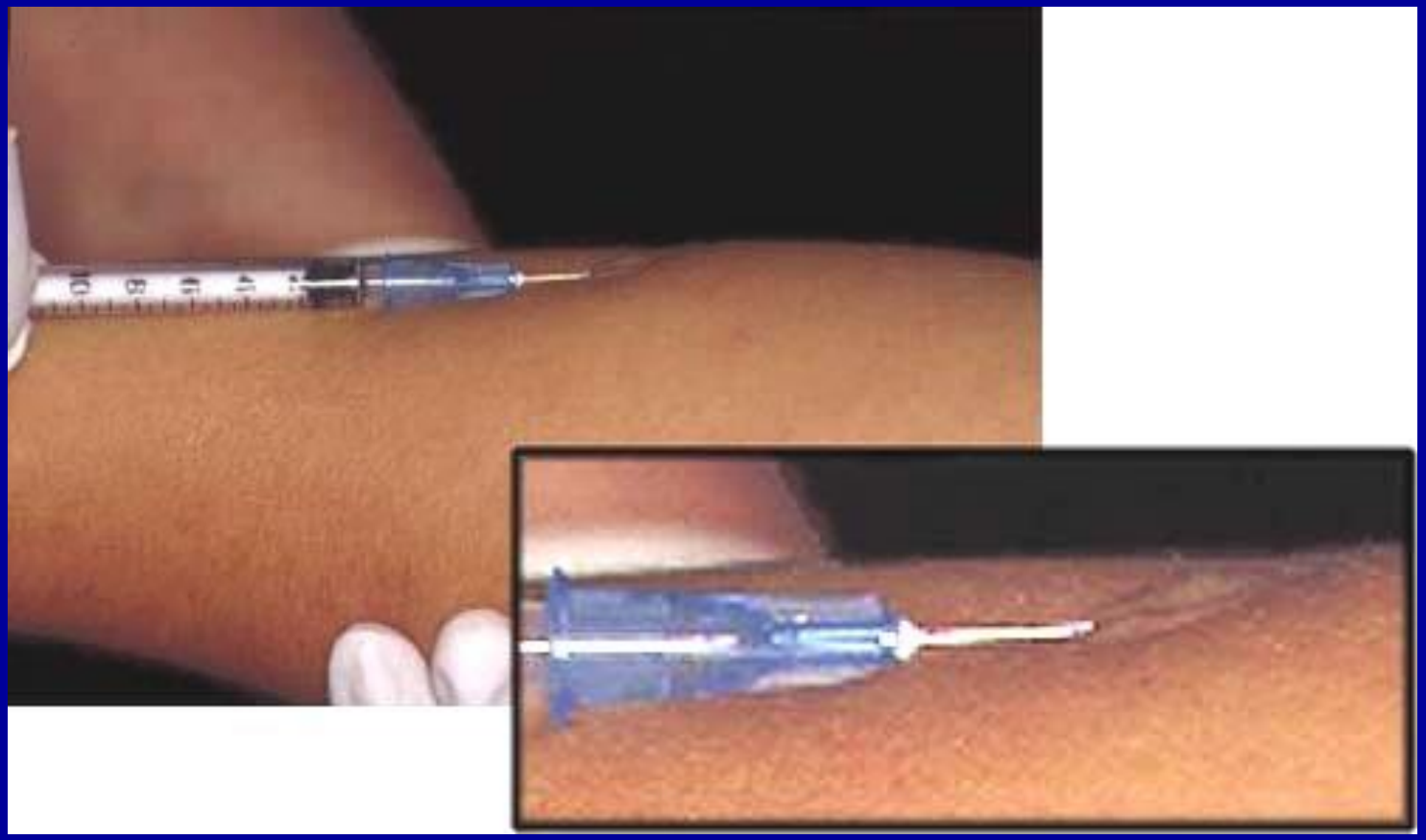
Lab Diagnosis

- **Specimen depends on the site of infection :**
- **Sputum , fresh early morning sputum is recommended for the diagnosis of pulmonary TB.**
- **Gastric wash and laryngeal swab are indicated for pulmonary TB detection in children .**
- **Urine is most likely indicated in urinary TB.**
- **Cerebrospinal fluid CSF in case of neural TB.**
- **Pleural fluid and pleural biopsy in exudative and peracute TB.**
- **Blood for miliary TB.**
- **Synovial fluid for Joint TB.**
- **Peritoneal fluid and feces in digestive TB.**

- In cases of pulmonary tuberculosis, **early morning sputum** is most appropriate for microbiological examination. Microscopy can be performed using conventional **ZN** or **auramine staining**. The latter, examined under a fluorescent microscope, is a sensitive but not very specific method of detection. Suspected positive sputum is then stained with ZN (examined under light microscope) and cultured on LJ slopes. Broth cultures may speed up isolation and molecular techniques (e.g. PCR) may accelerate diagnosis.

Skin test (Tuberculin Test)

- Skin Testing is performed as the tuberculin or Mantoux test. PPD (purified protein derivative) is employed as the test antigen in the Mantoux test. PPD is prepared from culture of M.TB., specifically Old Tuberculin (OT). 5 TU (tuberculin units), of PPD, in a O.1 ml volume is intracutaneously injected in the forearm. The test is read within 48-72 hours



Administering the Mantoux test.

Interpretation of tuberculin test

The test is considered positive if the diameter of the resulting lesion is 10 mm or greater. The lesion is characterized by erythema (redness) and swelling and induration (raised and hard).

- False positive tests usually manifest themselves as lesser reactions. These lesser reactions could indicate prior exposure or infection with other Mycobacteria or vaccination with BCG. However, in places were the vaccine is not used, lesser reactions should be regarded as highly suspicious.

- NegativeTuberclin:
- Actual negative: it is real negative result due to absence of infection.
- False Negative: It was due to immnocompromization factors like long cortisone therapy , Hiv infection, radiotherapy

Treatment

- Because administration of a single drug often leads to the development of a bacterial population resistant to that drug, effective regimens for the treatment of TB must contain multiple drugs to which the organisms are susceptible. When two or more drugs are used simultaneously, each helps prevent the emergence of tubercle bacilli resistant to the others.

prevention

- A vaccine against M.TB. is available. It is called BCG (Bacillus of Calmette and Guerin), after the two Frenchmen that developed it. BCG consists of a live attenuated strain derived from *Mycobacterium bovis*. This strain of *Mycobacterium* has remained avirulent for over 60 years.

Mycobacterium leprae

- It is weak acid fast bacillus , strict human pathogen found intracellular (inside endothelial and giant cells) as single bacilli and bundles

they can not grow on cell free medium , clinical specimens are inoculated into the foot pad of mice , mild granulomatous lesions are developed with limited multiplication of bacilli .

This provides a mean for testing antileprosy drugs bacilli are showing high growth in cooler parts

Pathogenesis

- Leprosy bacilli are obligate human pathogens, the most important routes of infection are skin and nasal mucosa it needs close and prolonged contact
- The incubation period is 5-8 years and the lesions involve the cooler parts of the body like tip of the nose and ear , eyes and testicles . the disease occur in two forms :
- Lepromatous form
- Tubrculoid form

Lepromatous type

- It is progressive and malignant course with nodular skin lesions and abundant AFB in the lesion.(High bacteriological index , it means 100 bacterial cells / oil immersion lens field).
- Lepromin test is negative due to deficient cell mediated immunity leontiasis is seen on the face of patient .

Tuberculoid type

- The course id benign and non progressive with macular skin lesions . sever nerve involvement is clear her .
- .

Diagnosis

1- Acid fast stain for smear or biopsy

2- Skin test Lpromin test

3- PCR test

Treatment : with anti leprosy drugs