- Actinomycetales ⇒ include two bacteria of high medical importance (Mycobacterium / Nocardia)
- (Mycobacterium, Nocardia) ⇒ the only acid-fast bacteria (neither gram (+) nor gram (-))
- HOWEVER, Nocardia asteroids causes Nocardiosis ⇒ described as partially acid fast bacilli (Branching bacilli)
- Family of Mycobacteria ⇒ Mycobacteriaceae
- Genus ⇒ Mycobacteria

Mycobacteria

By: Assis. Prof. Nader Alaridah

Background

⇒ Acid-fast bacilli
⇒ Discovered by Koch (Noble Prize)

- The mycobacteria are rod-shaped, aerobic bacteria that do not form spores.
- (1) *Mycobacterium tuberculosis complex* (MTC) a genetically related group of Mycobacterium species that can cause tuberculosis in humans.

(السلّ، التدرّن)

⇒ 11 million people develop tuberculosis each year with 1.5 million death annually ⇒ highest mortality from a single infectious agent

- (2) *Mycobacterium leprae* causes leprosy.

(Hansen's disease) ⇒ decreased incidence compared to tuberculosis

- *Mycobacterium avium-intracellulare* (M avium complex, or MAC) and other (3) nontuberculous (NTM) mycobacteria frequently infect patients with AIDS, are opportunistic pathogens in other immunocompromised persons, and occasionally cause disease in patients with normal immune systems.

(MOTs) ⇐ Mycobacteria other than Tuberculosis

Mycobacterium Tuberculosis (Mtb)

- It was not until the 19th century, when Robert Koch utilized s new staining method (ZN stain) and applied it to sputum from patients discovering the causal agent of the disease Tuberculosis (TB); Mtb or Koch bacillus. ⇒ Robert Koch

- Tuberculosis , consumption(consume patients ,weight loss), white plaque (extreme pallor seen among patients). * Zaman → top differential diagnosis * + Phthisis is another name of TP + hypoxia

- The family mycobacterium tuberculosis complex(MTC) can cause Tuberculosis (TB) in humans and other livings. (11 member)

- It includes M. tuberculosis (Mtb), Mycobacterium africanum, Mycobacterium bovis, Mycobacterium microti, Mycobacterium caprae, Mycobacterium pinnipedii, Mycobacterium suricatte, Mycobacterium mungi, Mycobacterium dassie, Mycobacterium oryx and Mycobacterium canetti. → the principal member

Morphology

⇒ Acid fast bacilli
⇒ Non-motile
⇒ Non-spore formers
⇒ Uncapsulated
⇒ Obligate aerobes
⇒ Facultitive intracellular

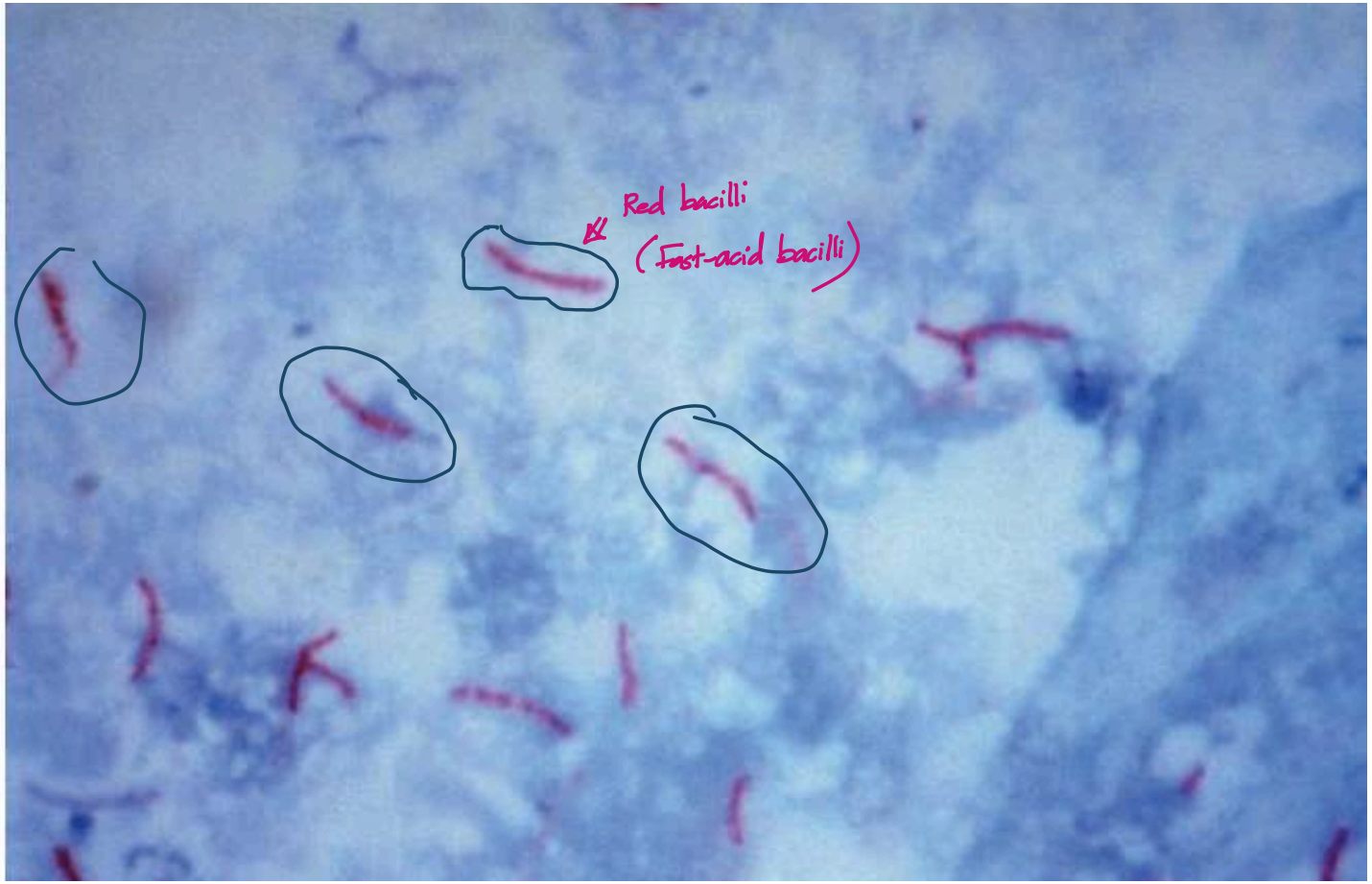
- In tissue, tubercle bacilli are thin, straight rods measuring about 0.3 ~ 3 µ m.
- True tubercle bacilli are characterized by “acid fastness”—that is, 95% ethyl alcohol containing 3% hydrochloric acid (acid-alcohol) quickly decolorizes all bacteria except the mycobacteria.
- Mycobacteria are obligate aerobes and derive energy from the oxidation of many simple carbon compounds.

- Facultitive intracellular ⇒ main targets are circulating monocytes or resident microphages especially in pulmonary TB ⇒ the most common type (80-90)% ≠ TP
- The growth rate is much slower than that of most bacteria. The doubling time of tubercle bacilli is about 18 hours. ⇒ slow doubling time unlike E. coli (minutes)
- Mycobacteria tend to be more resistant to chemical agents than other bacteria because of the hydrophobic nature of the cell surface and their clumped growth,

- This is a high power micrograph of a sputum sample from a patient. These Red bacilli identified as fast-acid bacilli through Ziehl-Neelsen staining. In this method, the sample is stained with carbol fuchsin, a red dye, and then heated. Heating is necessary because mycobacteria, the only prokaryotes with a waxy lipid-rich cell wall containing mycolic acid, have cell walls that are impermeable to most dyes. The heat help to penetrate the cell wall. After staining, the sample is decolorized with strong acid-alcohol solution. While this solution would typically remove the stain from most bacteria, acid fast bacteria bacilli retain the red dye due to their unique cell wall structure. A counter stain, such as methylene blue, is then applied, but the acid fast bacilli remain red. This means that they are neither gram (+) nor (-) although if stained using gram staining might appear weakly positive

- Among bacteria, only Mycobacteria and partially acid-fast Nocardia exhibit this acid fast property

- Among parasites, only Cryptosporidium, Cyclospora and Isospora do (under a modified acid-fast stain.



Mtb Culture

⇒ final / most sensitive mean of diagnosis
⇒ Smear microscopy is a quick and less sensitive procedure

- The media for primary culture of mycobacteria should include a nonselective medium and a selective medium.

⇒ takes weeks the (patient may cannot wait that long)
⇒ even if the result is negative, it doesn't confirm true negative
⇒ to see any growth (3-4 week), to discard the plate as negative (8 weeks)

- Semisynthetic agar media— These media (eg, Middlebrook 7H10 and 7H11) contain defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol .

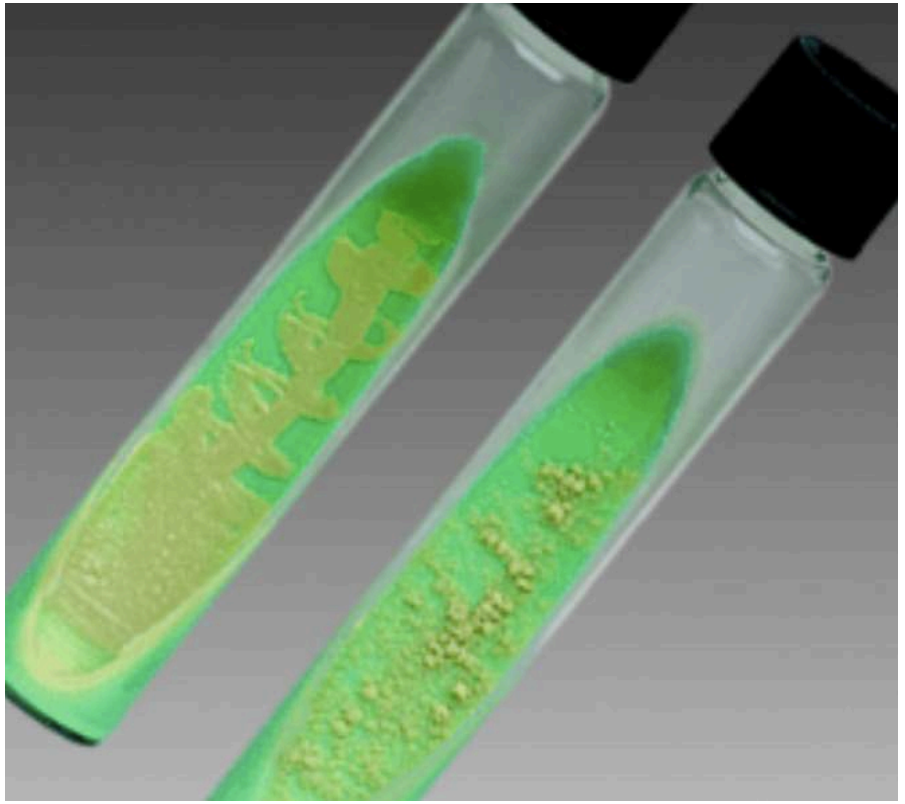
- Inspissated egg media— These media (eg, Löwenstein- Jensen) contain defined salts, glycerol, and complex organic substances (eg, fresh eggs or egg yolks, potato flour, and other ingredients in various combinations.

the one in UJH :)

- Broth media— (eg, Middlebrook 7H9 and 7H12) support the proliferation of small inoculate.

Mtb Colonies

If growth occurs, colonies appear as dry, rough wrinkled colonies. This is a magnified image shows these distinctive colonies.



These plates are known as Löwenstein-Jensen medium and contain malachite green dye which inhibits the growth of normal flora in the sputum samples.

Mtb Cell wall ⇒ Mycobacteria is the only prokaryotes with waxy lipid-rich cell wall ⇒ acid-fast bacilli

- The mycobacterial cell wall is a complex structure that is required for cell growth, resistance to antibiotics and virulence.

- It consists of an inner layer and an outer layer that surrounds the plasma membrane. The inner compartment is composed of three distinct macromolecules — peptidoglycans (PG), arabinogalactans (AG) and mycolic acids (MA) — covalently linked together to form a complex known as the MA-AG-PG complex.

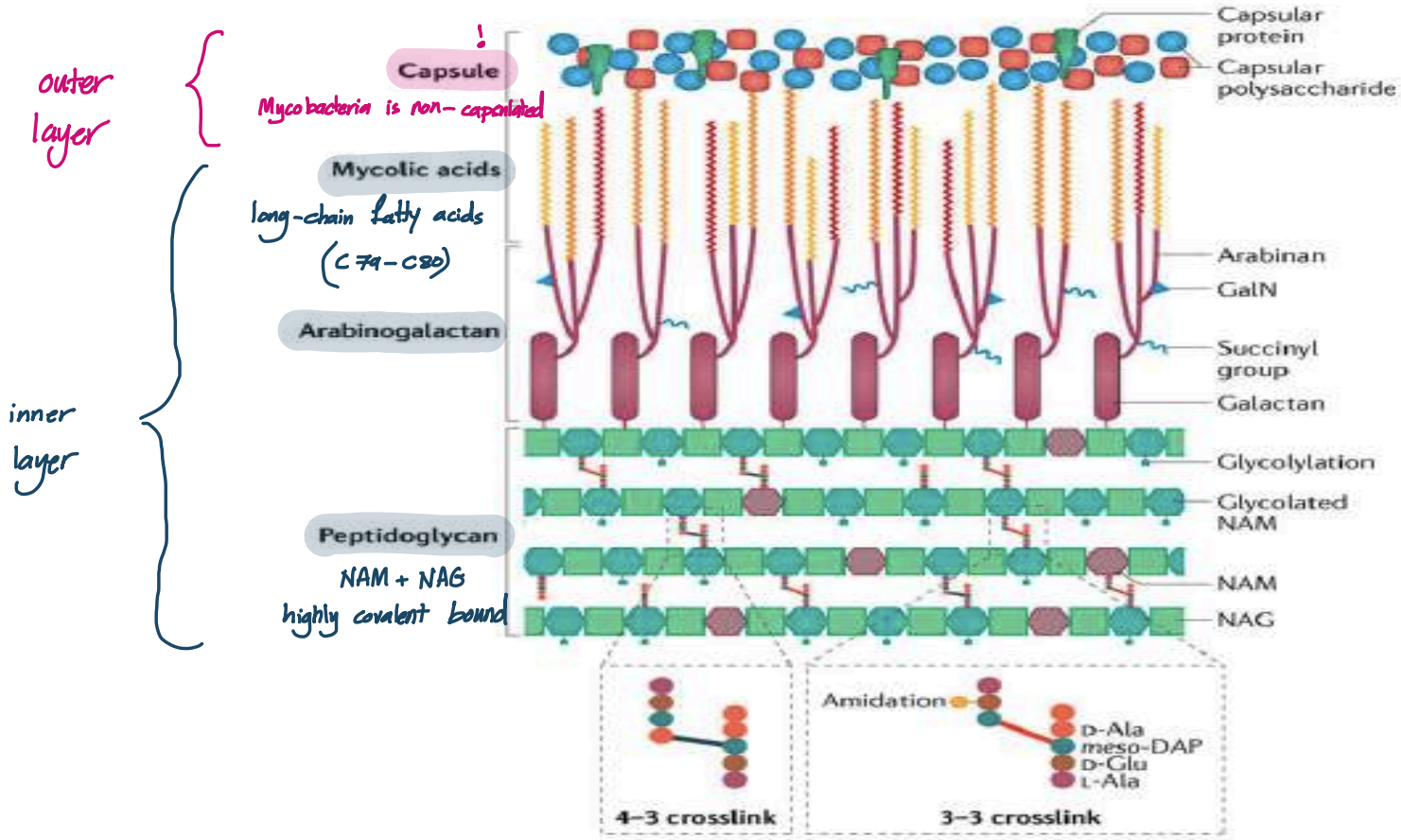
- The peptidoglycan layer surrounds the plasma membrane and comprises long polymers of the repeating disaccharide N-acetyl glucosamine–N-acetyl muramic acid (NAG–NAM) that are linked via peptide bridges.

- Most of the arabinan is ligated with long-carbon-chain mycolic acids, which form the characteristic thick waxy lipid coat of mycobacteria and are major contributors to the impermeability of the cell wall and to virulence.

- Mycolic acids (long-chain fatty acids C78–C90), waxes, and phosphatides , can be found in Mtb cell wall and make up 50% of the dry weight of the mycobacterial cell envelope.

- These mycolic acids are esterified to glycerol and trehalose where trehalose can contain one or two molecules of mycolic acids forming trehalose dimycolates (TDM) (Cord Factor) and trehalose monomycolates (TMM).

Outer layer is composed of lipids, proteins and carbohydrates formed a capsule-like structure rich in virulence factors such as lipoarabinomannan, lipomannan, sulfolipids which inhibit phagolysosome fusion enabling mycobacteria to target macrophages, Trehalosedimycolate which promotes serpentine-like (حلزوني) when cultured in broth and Type VII secretion system (T7SS)



Epidemiology

active TB and show symtomes (pulmonary, extrapulmonary)

- Two TB-related conditions exist; latent TB infection (LTBI) and TB disease. If not treated properly, TB disease can be fatal. People who have latent TB infection do not feel sick, do not have any symptoms, and cannot spread TB to others ⇒ not contagions
  ⇒ reactivation may occur if the patient has high risk of reactivation (immunocompramized / elderly) ⇒ preventive treatment ⇒ Isoniazide for 9 month
- About one third of the worlds population is infected with TB bacteria (TB latency) dormantly
- However, only small proportion of those infected will become sick with TB.
- TB remains a leading cause of infectious diseases morbidity and mortality. In 2015, an estimated 10.4 million new TB cases were seen world wide.
- TB is considered an airborne infectious disease although M. tuberculosis complex organisms can be spread through un-pasteurised milk, direct inoculation and other means.

Estimated TB incidence rates, 2020

- Each year, there are 10-12 million new cases of TB worldwide
  In Jordan, the incidence has decreased to 15 cases per 100,000
  increased migration waves may increase the cases.

- TB is strongly associated with socioeconomic stigma and is more prevalent among individuals suffering from malnutrition, living in poor housing conditions with inadequate ventilation, or those with possible genatic predisposition.

- In South Africa, TB cases reaches thousands per 100,000 people.

- The combination of HIV and TB is fatal as the primary defense against TB is cell-immunity.

Tuberculosis TB

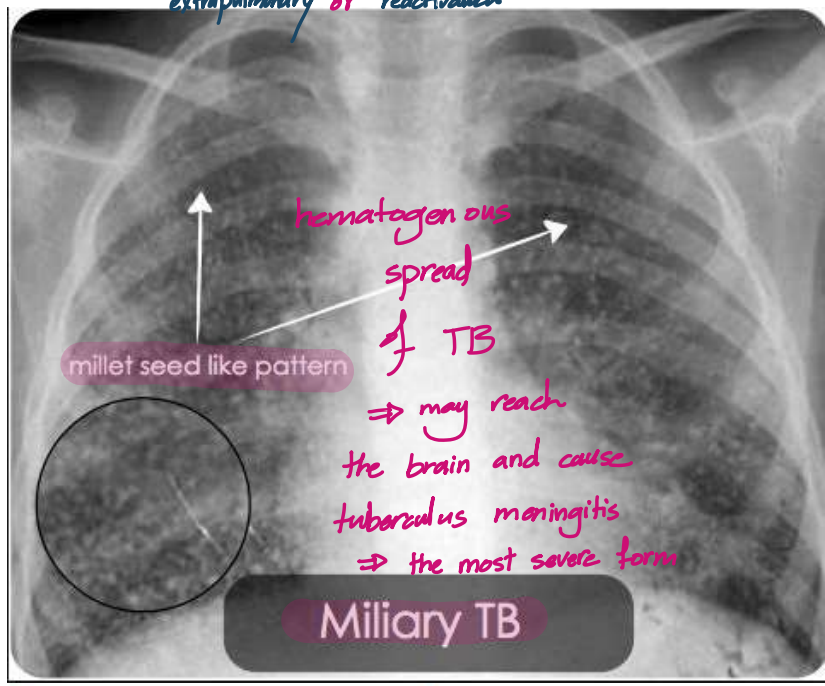
* direct erosion from the granuloma to adjacent areas
* hematogenous spred
* lymphatic spred ⇒ extra pulmonary

Active → pulmonary , Active → pulmonary → extrapulmonary, Active → extrapulmonary or reactivation

- (80-90)% of the cases are pulmonary TB

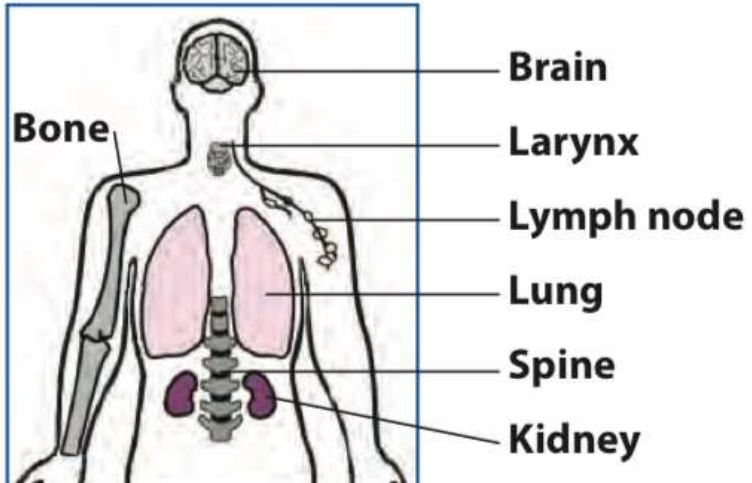
pleura + bronchi

- The primary site of TB is usually lung, from which it can get disseminated into other parts of the body. The other routes of spread can be contiguous involvement from adjacent tuberculous lymphadenopathy or primary involvement of extrapulmonary organ.
- Spread – Lymphatic vs hematogenous (Miliary).



- (10-20)% of the cases are extra pulmonary TB
- TB bacteria can attack any part of the body such as the pleura ,L.N. ,pericardium, kidney, spine, brain and + Bone abdomen (abdominal Tuberculosis) collectively known as extrapulmonary TB.

⇒ Spinal TB in the vertebral bodies ⇒ Pott's disease
⇒ Cervical lymph nodes ⇒ Scrofula



- Primary Infection(Active) and Reactivation Types of Tuberculosis.

secondary (latency → reactivation)

Transmission

⇒ by droplet nuclei or airborne aerosols that are generated when speaking, coughing.

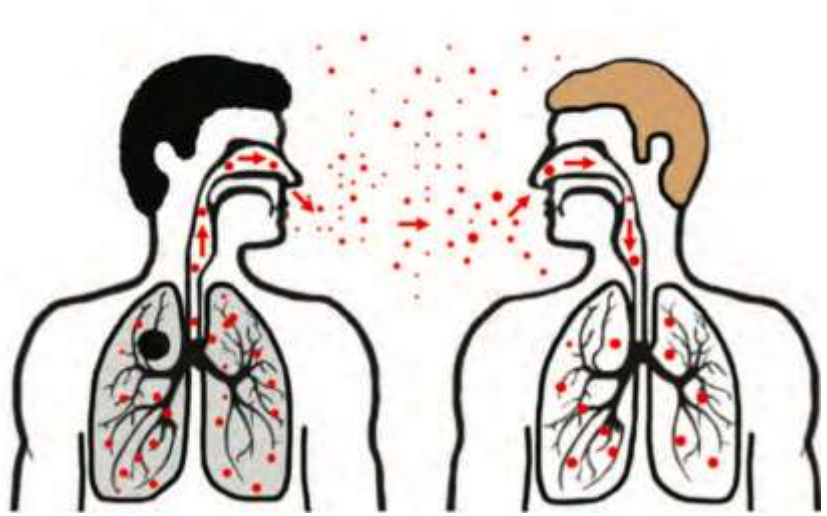
⇒ In term of resistance to steralization, TB ranks second after spore forming bacteria.

⇒ TB is resistant to desiccation (dryness) ⇒ viable and infectious in sputum for a long period.

⇒ Sensitive to UV light.

⇒ The concept of Quaratine originated from (TB) patients.
الحجر الصحي.

- TB is considered an airborne infectious disease although M. tuberculosis complex organisms can be spread through unpasteurised milk, direct inoculation and other means.



- The underlying pathophysiology of TB is the "10/3/1 formula.

is exposed to (TB) — developed (LTBI) — develope active (TB)

⇒ 6 by their innate or adaptive immune system clear the infection although many immunologists argue that steralizing immunity against TB doesn't exist.

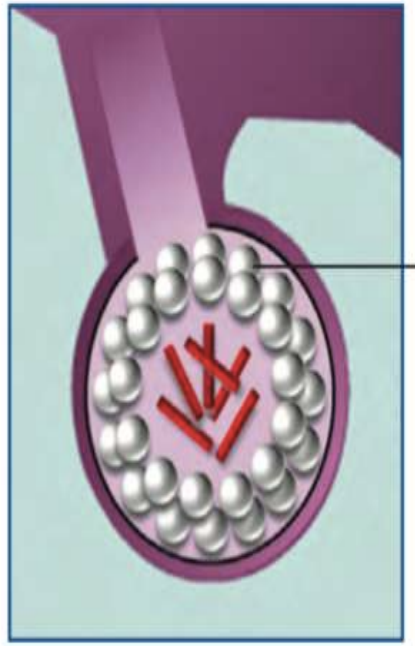
⇒ Hallmark of pathogenesis of intracellular infection (TB or others) ⇒ Granuloma formation

Pathogenesis

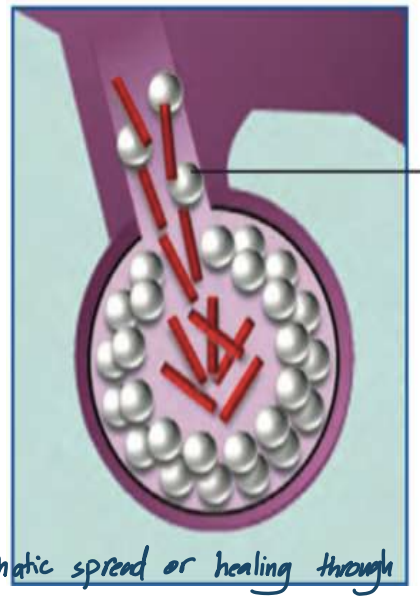
- Mycobacteria are in droplets when infected persons cough, sneeze, or speak. The droplets evaporate, leaving organisms that are small enough, when inhaled, to be deposited in alveoli
- Inside the alveoli, the host's immune system responds by release of cytokines and lymphokines that stimulate monocytes and macrophages.
- Mycobacteria begin to multiply within macrophages. Some of the macrophages develop an enhanced ability to kill the organism, but others may be killed by the bacilli.
- The cells form a barrier shell, called a granuloma, that keeps the bacilli contained and under control **(LTBI)**.
- If the immune system **cannot** keep the tubercle bacilli under control, the bacilli begin to multiply rapidly (**TB disease).**

Fibrous rims or epithelioid cells

cascated granuloma ⇒ necrosis in the centre



Special immune cells form a barrier shell (in this example, bacilli are in the lungs)



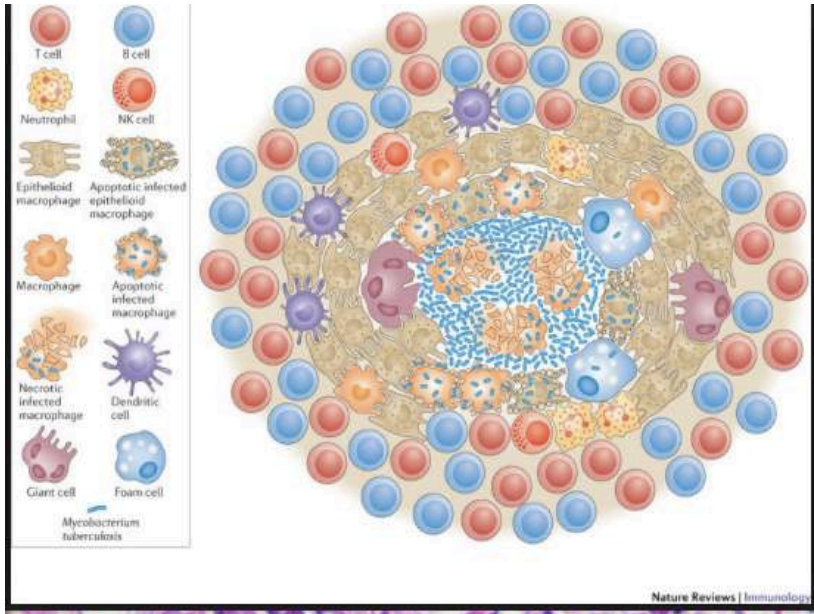
Shell breaks down and tubercle bacilli escape and multiply

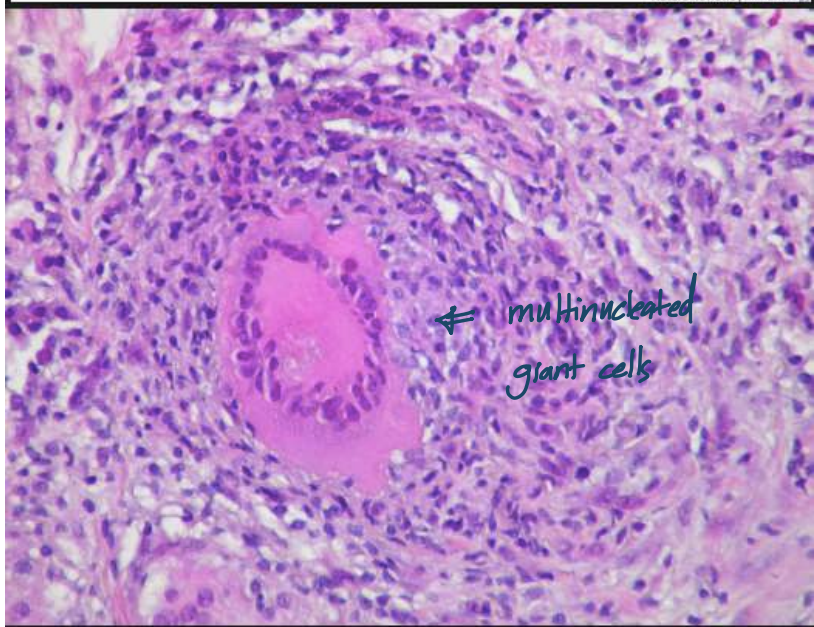
⇒ This granuloma may undergo erosion and open to other areas, like miliary TB, hematogenous spread or lymphatic spread or healing through fibrosis or calcification.

Pathology

- Exudative type—This consists of an acute inflammatory reaction with edema fluid; polymorphonuclear leukocytes; and, later, monocytes around the tubercle bacilli. This type is seen particularly in lung tissue, where it resembles bacterial pneumonia.

- Productive type—When fully developed, this lesion, a chronic granuloma, consists of three zones: (1) a central area of large, multinucleated giant cells containing tubercle bacilli; (2) a mid zone of pale epithelioid cells, often arranged radially; and (3) a peripheral zone of fibroblasts, lymphocytes, and monocytes.





Primary Infection and Reactivation
Types of Tuberculosis

- An acute exudative lesion develops and rapidly spreads to the lymphatics and regional lymph nodes. The exudative lesion in tissue often heals rapidly.
- In primary infections, the involvement may be in any part of the lung but is most often at the base.
- The reactivation type is usually caused by tubercle bacilli that have survived in the primary lesion
- The reactivation type almost always begins at the apex of the lung, where the oxygen tension (PO2) is highest. ⇒ obligate aerobe

Clinical manifestation

⇒ Patients share constitutional symptoms

- Classic clinical features associated with active pulmonary TB are coughing, weight loss/anorexia, fever, night sweats, haemoptysis* (coughing blood), dyspnea (chest pain) and malaise/fatigue.

productive (produce sputum)

⇒ differential diagnosis

shortness of breath

⇒ different from haematemesis (vomiting blood)

- Tuberculosis is usually a chronic disease; it presents slowly with weight loss, low-grade fever, and symptoms related to the organ system infected. Because of its slow course, it may be confused with cancer. Whenever you have an infection of any organ system, tuberculosis will be somewhere on your differential diagnosis list.

⇒ Extrapulmonary TB ⇒ symptoms are related to the organ affected. (Kidney ⇒ haematuria / vertebral bodies ⇒ low-back pain, scrofula ⇒ enlarged lymph n.)

- It is one of the great imitators

Laboratory diagnostic methods

⇒ Smear (-) doesn't necessarily mean negative TB

⇒ Smear (+) ⇒ more contagious

❖Smear microscopy

sputum in pulmonary TB

- Three specimens from each patient with suspected TB should be examined microscopically for Acid Fast Bacilli AFB (classically Ziehl-Neelsen) or mycobacteria can be demonstrated by yellow fluorescence after staining with auramin. Rhodamin staining

❖Culture ⇒ defent diagnosis

- Both liquid and solid mycobacterial cultures should be performed for every specimen, and recovered isolates should be according to standard criteria (Lowenstein-Jensen or Middlebrook 7H10), Radiometric broth culture (BACTEC radiometric system) and mycobacterial growth indicator tube (MGIT). ↖ rapid (2-3) weeks

⇒ negative results doesn't necessarily mean negative TB ⇒ pathogenesis lower in the RT ⇒ not exist in expectorated sputum

- Culture for acid fast bacilli is the most specific test for TB and allows direct identification and determination of susceptibility of the causative organism

+ PCR probs (from urin or sputum sample) ⇒ specific but less sensitive

❖A nucleic acid amplification test (NAAT), Tuberculin skin tests (TSTs), Interferon-gamma release assays (IGRAs) are commonly used as well. ⇒ called screening tests

Tuberculin skin test

⇒ purified protein derivative (PPD) ⇒ injected intradermally ⇒ After 48 hours ⇒ we measure the induration (thickness) ⇒ not only erythema (raised and solid)

⇒ >5mm thick ⇒ positive result for HIV (weak immunity)

⇒ >10 mm thick ⇒ positive result for patien with risk factor

⇒ >15 mm thick ⇒ positive result for the general population

⇒ may give false negative and false positive because of vaccination & NTMs

⇒ indicated whether there has been previous exposure to the bacteria

⇒ a positive result means the immune system recognizes the bacteria and its antigens

⇒ doesn't indicate whether the infection is active or latent, nor does it reveal if the person was vaccinated previously. It simply confirms exposure!

Interferon Gamma release assays

⇒ the test is performed by drawing blood from the patient, then mixing it with specific antigens in the lab.

⇒ the release of interferon gamma is measured, and based on a predetermined cutoff value of 30 IU, the result is classified as (+)/(-)

⇒ serve as an alternative to (TST), helping to avoid false positives caused by prior vaccination

⇒ it uses the antigens ESAT-6 and CFP-10 which are only related to Mycobacterium Tuberculosis, not in other Mycobacteria.

⇒ it also determines whether there has been exposure to the bacteria.

Treatment

- The course of TB treatment depends on whether the individual is in the latent or active stage, and on his or her probability of risk.

- Treatment of TB usually involves a drug cocktail, or a mixture of multiple drugs, with an intensive initial 2-month phase followed by a slower 4- to 6-month continuation phase the main anti-tuberculosis drugs used in the chemotherapy of TB are: isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and either ethambutol (EMB) or streptomycin (SM).

4 drugs ⇒ (INH) / (RIF) / (PZA) / (EMB)

2 drugs (INH, RIF)

red body fluid (red urine)

⇒ neuritis

hepatotoxicity, hyperuricemia

optic neuritis

⇒ After 3 weeks of treatment, a TB patient may show significant improvement and leave quarantine. However, if the patient does not continue the treatment, there is a risk of developing multidrug-resistant (MDR) or extensively drug-resistant (XDR) TB.

- Isoniazid preventive therapy IPT is the recommended treatment for LTBI but the regimen's main drawback is the duration of therapy

Prevention

- The best way to prevent TB is to diagnose and isolate infectious cases rapidly and to administer appropriate treatment until patients are rendered noninfectious (usually 2–4 weeks after the start of proper treatment) and the disease is cured.

- Additional strategies include BCG vaccination and treatment of persons with LTBI who are at high risk of developing active disease.

- Mycobacterium bovis Bacillus Calmette–Guérin (BCG), an attenuated vaccine derived from M. bovis, is the only licensed vaccine against tuberculosis (TB)

⇒ the first vaccine given to new born babies (their first month)
⇒ America and many European Countries stop giving this vaccine, because they believe it doesn't prevent the most frequent form of TB (pulmonary TB)
⇒ its efficacy ranges from (0-80)% ⇒ wide range
⇒ it is given in Jordan because it is believed to prevent the most sever forms of TB (meningitis & milcary TB)

OTHER MYCOBACTERIA

⇒ they are not contiguous

- The nontuberculous mycobacteria (NTM) or (MOTs) is a diverse group of organisms commonly found in the environment, and the group includes both saprophytes and human pathogens. soil and water
- The NTM can be further classified into the rapid growers (grow in <7 days) and slow growers (>7 days). Each group can be subdivided on the basis of pigment production. ⇒ presence of light (photochromogens) / ⇒ absense of light (scotochromogens) / ⇒ do not produce pigment (nonchromogens)
- Mycobacterium avium Complex (MAC or MAI) ⇒ Mycobacterium avium intracellularis
- MAC organisms infrequently cause disease in immunocompetent humans.
- MAC infection is one of the most common opportunistic infections of bacterial origin in patients with AIDS.

affect mainly immunocompromized

The nontuberculous mycobacteria (NTM)

⇒ Runyon Classification System

- Mycobacterium kansasii ,Mycobacterium marinum and Mycobacterium ulcerans. ⇒ Photochromogens ⇒ slowly growers

commonly isolated is the US ⇒ cause pulmonary-like disease

Aquarium granuloma in fish aquarium

skin & soft tissue infection

- Mycobacterium scrofulaceum. ⇒ Scotochromogen ⇒ slowly grower

lymphadenitis in young children

- Mycobacterium avium complex, or (MAI). ⇒ Non chromogen ⇒ slowly grower
  ⇒ Same as TB (nonchromogens and slowly growers)

(AIDs)

- Mycobacterium fortuitum Complex ,Mycobacterium chelonae-abscessus. ⇒ fast growers

lung, skin and soft tissue infectio

skin & soft tissue infections (abscess formation)

Mycobacterium leprae

⇒ targets skin histiocytes, Schwann cell (nerves) and endothilial cells

- Mycobacterium leprae is an acid-fast rod.
- It is impossible to grow this bacterium In vitro . cannot be cultured in the lap (whether artificial media or cell culture)
- It causes the famous disease leprosy. الجُذام ⇒ Hansen's disease
- The bacteria appear to grow better in cooler body temperatures closer to the skin surface . ⇒ lionine face (30–32)°c
- Skin lesion consistent with leprosy and with definite sensory loss.

⇒ nodules in the skin and thickness in nerves ⇒ sensory loss / Paresthesia above the lesion

- The severity of the disease is dependent on the host's cell-mediated immune response to the bacilli (which live intracellular, like Mtb).

Pathogenesis

- Lepromatous leprosy (LL) ⇒ low cell-mediated immunity host.
  ⇒ high number of bacilli inside the lesions
  ⇒ Negative Lepromin test
- borderline lepromatous (BL) ⇒ Intermediate.
- Tuberculoid leprosy (TL) ⇒ high cell-mediated immunity host.
  ⇒ high T-helper-1
  ⇒ high interferon gamma
  ⇒ limited number of bacilli inside the lesions
  ⇒ Positive Lepromin test ⇒ strong cell-mediated immune respose ⇒ strong positive reaction (type 4 hypersensitivity reactions)

Clinical manifestation

⇒ nodules in the skin and thickness in nerves

⇒ sensory loss / Paresthesia above the lesion

⇒ loss of eyebrows

- The onset of leprosy is insidious.

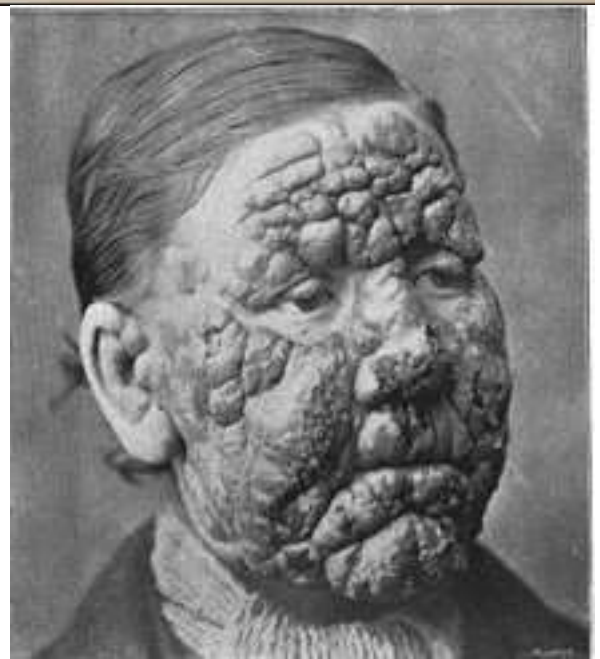
Slow development

Chronic nature

long incubation period

⇒ Transmitted by prolonged contact with the skin lesions or nasal discharge

- The lesions involve the cooler tissue of the body, including the skin, superficial nerves, nose, pharynx, larynx, eyes, and testicles.



Diagnosis

- skin or nasal mucosa or a biopsy of earlobe skin are smeared on a slide.

⇒ no culture

- Smears are stained by the Ziehl-Neelsen technique. Biopsy of skin or of a thickened nerve gives a typical histologic picture.

- No serologic tests are of value.

Treatment

- Sulfones such as dapsone are first-line therapy for both tuberculoid and lepromatous leprosy.

- RMP or clofazimine generally is included in the initial treatment Regimens.

THE END