Doctor 021

MICROBIOLOGY

Sheet no. 11

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BACKGROUND

The mycobacteria are rod-shaped, aerobic bacteria that do not form spores.

There are three major species of this family: -

1) **Mycobacterium tuberculosis complex (MTC) a genetically related group of Mycobacterium species that can cause tuberculosis** مرض السل in **humans.**
2) **Mycobacterium leprae causes leprosy** مرض الجذام**.**
3) **Mycobacterium avium-intracellular (M avium complex** (MAI), **or MAC) and other nontuberculous mycobacteria NTM:** also known as environmental mycobacteria. They are a group of mycobacteria that don't cause neither tuberculosis nor leprosy with NO "typical granuloma.
   - ❖ **frequently infect patients with AIDS, are opportunistic pathogens in other immunocompromised persons, and occasionally cause disease in patients with normal immune systems.**

MYCOBACTERIUM TUBERCULOSIS (MTB)

Principal pathogen that causes tuberculosis in humans is MTB.

• It was not until the 19th century, when Robert Koch utilized a 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.

• Tuberculosis, consumption (consume patients, weight loss), white plaque (extreme pallor seen among patients).

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

• 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.

❖ mycobacterium bovis was the major pathogen causing tuberculosis in the past, but when pasteurization method arised it has been abolished nearly completely yet it is still used in VACCINATION –BCG vaccine- to be discussed later in this lecture.

MORPHOLOGY

• In tissue, tubercle bacilli are thin, straight rods measuring about 0.3 ~ 3 µ m.

• True tubercle bacilli are characterized by "acid fastness" (the stain which we use to identify mycobacterium is acid-fast stain (or zeihl-neelsen stain)).

That is, 95% ethyl alcohol containing 3% hydrochloric acid (acid-alcohol) quickly decolorizes all bacteria except the mycobacteria.

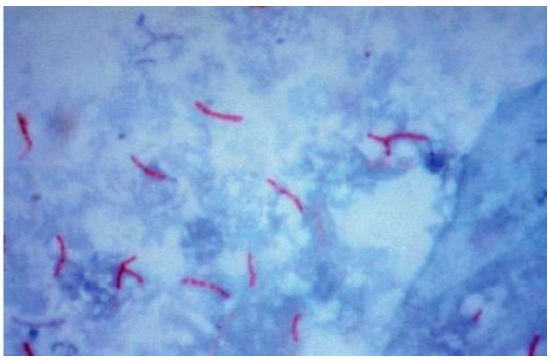
Acid-fast: acid هو الامتناع عن ادخال ال**(**that's why it doesn't get decolorized)

• Mycobacteria are obligate aerobes and derive energy from the oxidation of many simple carbon compounds.

• The growth rate is much slower than that of most bacteria. The doubling time of tubercle bacilli is about 18 hours.

- They are also resistant to sterilizing methods and resistant to desiccation. (They come after bacterial spores in terms of difficulty to sterilize)
- The main rout for transmission is through droplet nuclei and they remain infectious for a while after evaporation.
  Side note: droplet nuclei are aerosols formed from the evaporation of respiratory droplets.
- **Obligate intracellular**, obligate aerobes, non-motile, non-spore forming, non-capsulated and acid-fast bacilli.

It is taken from a patient with pulmonary tuberculosis.
Stained by **zeihl-neelsen.**



- details regarding acid-fast staining method (just understand the general idea):
  - ✓ red stain called "carbol fuchsin", is used to stain the sample which is usually taken from the sputum of the patient.
  - ✓ heat off the sample → to facilitate penetration of the stain
  - ✓ add hydrochloride acid → to wash off the stain (decolorization in other words).
  - ✓ counterstain the sample with methylene blue
    Now, acid fast bacilli will RETAIN the 1st dye (carbol fuchsin) and resist the acid treatment (they fast from the acid), so they appear red under the microscope and don't counterstain with the blue stain.

MTB CULTURE

The most specific test for diagnosing MTB is isolation through culture, but there is some problems with culturing it due to its slow dividing rate, for example it takes 4-6 weeks to start growth.

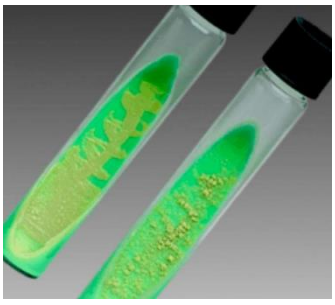
• The media for primary culture of mycobacteria should include a nonselective medium and a selective medium.

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

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

• Broth media— (e.g., Middlebrook 7H9 and 7H12) support the proliferation of small inoculate.

❖ We add Malachite green along with it, which will inhibit the growth of bacteria and flora other than mycobacteria, notice the green color in the figure aside



❖ This is a typical mycobacterium colony, its unique in a way. It's described as raised, rough and CLUMPED

MTB CELL WALL

- They live intracellular as we said, inside specialized phagocytes APC (macrophages mainly) they also inhibit phagolysosome fusion.
- They have virulence factors inside their cell wall.

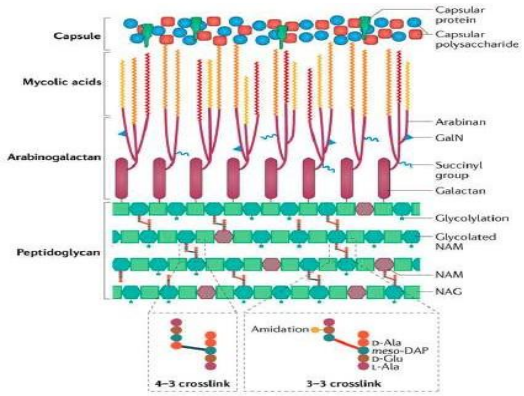
• The mycobacterial cell wall is a complex structure that is required for cell growth, resistance to antibiotics and virulence.

• It consists of an inner layer and an outer layer that surrounds the plasma membrane. The inner compartment is composed of three distinct macromolecules:

1) **peptidoglycans (PG)** N-acetyl glucosamine–N-acetyl muramic acid (NAG–NAM) cross link with each other making it impermeable.
2) **arabinogalactans (AG)**
3) **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 <u>make up 50% of the dry weight of the mycobacterial cell envelope.</u>

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

TDMs are important virulence factors. They are also responsible for the **CLUMPING morphology** of mycobacterial colonies.
*there is also a secretion system in the membrane called type 7.

EPIDEMIOLOGY

Let's talk about pathophysiology:

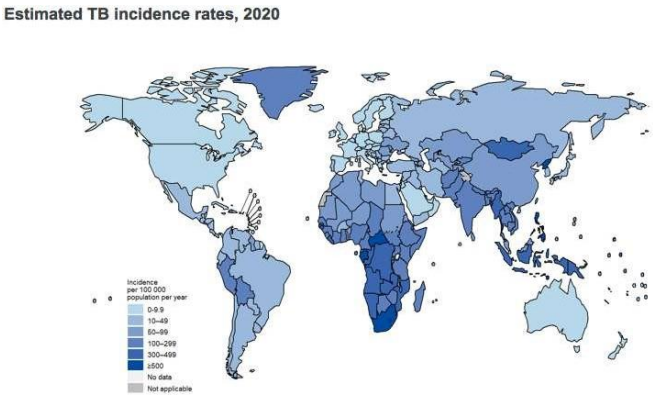
In regarding to MTB infection two TB-related conditions exist:

1) **active tuberculosis:** as soon as the individual is infected, he develops typical and classical tuberculosis disease. **If not treated properly, TB disease can be fatal.**
2) **latent TB infection (LTBI): do not feel sick, do not have any symptoms, and cannot spread TB to others (**not contagious), but will show a positive result in some tests.

• **About one third of the world's population is infected with TB bacteria (TB latency).**

• **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 worldwide.**



TUBERCULOSIS TB

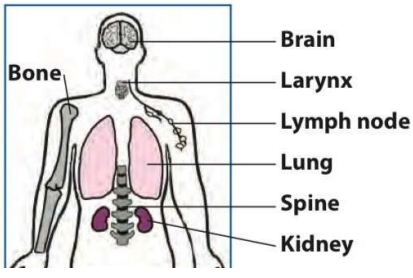
• **The primary site of TB is usually lung, from which it can get disseminated into other parts of the body. The other routes of spread can be contiguous involvement from adjacent tuberculous lymphadenopathy or primary involvement of extrapulmonary organ.**

There are two types of TB:

1) pulmonary TB, the most common one.
2) extrapulmonary TB, may start in the lungs then spread or just pass through it.

• **Spread** it can be:

1. **Lymphatic.** → from the lung to draining lymph node (can be in the liver, kidney…)
2. **hematogenous (Miliary)**→ can cause the worst type of TB (TB meningitis) it affects children.



There is also direct spreading to adjacent organs (from lungs to pleura to abdomen etc.)

The disease can have different names for example:

-TB can affect the bones = Pott disease

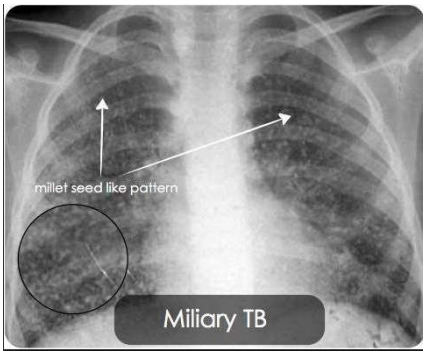
-TB can affect cervical lymph nodes = scrofula

• TB bacteria can attack any part of the body such as the pleura,L.N. ,pericardium, kidney, spine, brain and abdomen (abdominal Tuberculosis) collectively known as extrapulmonary TB.

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

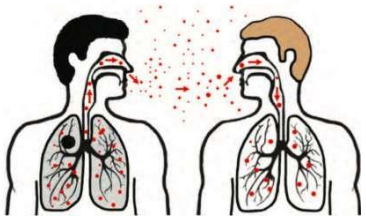
❖ Chest x-ray (Miliary)

The dots are called millet seeds (they are not confined to certain lobe they are spread in the whole lung then to other parts in the body).



TRANSMISSION

• TB is considered an airborne infectious disease although M. tuberculosis complex organisms can be spread through unpasteurized milk, direct inoculation and other means such as cough, sneeze**...**



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

This formula means that every 10 people exposed to MTB 3 of them develop latent TB and 1 will develop active TB.

PATHOGENESIS

The hallmark of the pathology of TB is granuloma formation.

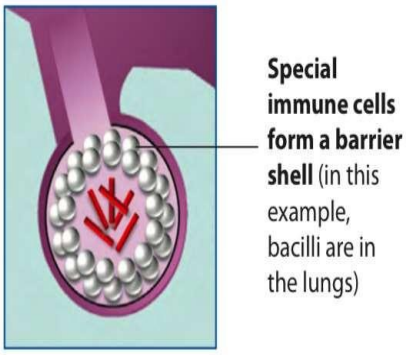
Granuloma is a state by the immune system to contain intracellular infection.

❖ usually through respiratory rout, once the MBT get inhaled, it goes actively and internalized by a receptor mediated phagocytosis into a professional phagocyte (macrophages mainly).
then the immune system can either response in the usual way or develop granuloma.

- Granuloma formation: infected macrophages recruit other macrophages, then adaptive immunity cells come there (T and B lymphocytes) and finally, surrounding the sick cell a fibrous rim is produced.

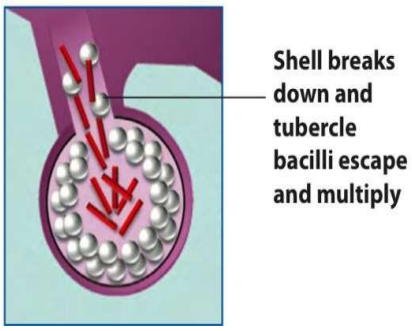
- mycobacteria don't have endotoxins or exotoxins so the damage it causes is related to the immune system (collateral damage).

• 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).

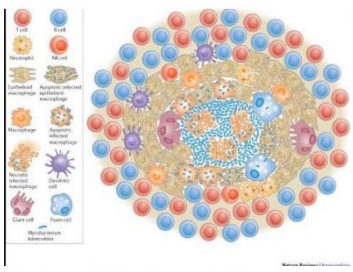
What can happen to the infection:

I. Clearance.
II. Containment.
III. chronic granuloma. (Necrosis can also happen)

after this it will spread in one of the ways we mentioned above.

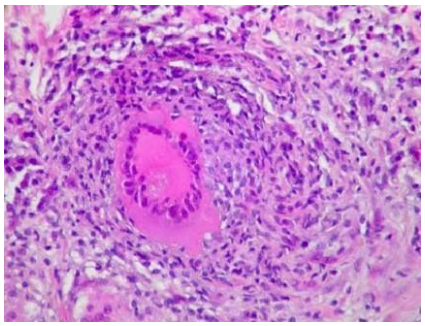
PATHOLOGY

• 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.



Edema → fluid accumulation, also the multiplication is limited

• 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.

3) a peripheral zone of fibroblasts, lymphocytes, and monocytes.

The multiplication is active. (Recruitment of many cells)

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.

we already said that when the bacteria enter the body it may:

1) primary active TB (make a disease)

• In primary infections, the involvement may be in any part of the lung <u>but is most often at the base.</u>

2) secondary reactive (it starts as latent TB (the 3 from the formula) but after a while if you get immune compromised (it may congenital or acquired like AIDs), the MBT may get reactivated

• The reactivation type is usually caused by tubercle bacilli that have survived in the primary lesion

• The reactivation type almost always <u>begins at the apex of the lung,</u> where the oxygen tension (PO2) is highest

CLINICAL MANIFESTATION

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

• 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.

- **It is one of the great imitators.** (Cause a significant weight loss)

The above chief complaints are in pulmonary TB.

If its extrapulmonary it depends on the organ involved, for example kidney TB they come with a hematuria (blood in urine), abdomen TB they come with abdominal mass and discomfort.

LABORATORY DIAGNOSTIC METHODS

If you have a high clinical suspicion you should initiate treatment; because the diagnostic methods take a long time (weeks)

❖ **Smear microscopy**

- **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 auramine (this is a more modern technique for staining).**

So here you just take a sputum sample from the patient and you use the acid fast stain for detection, but the problem is that the sensitivity is low, meaning that the results could be smear negative while the patient is actually infected. But it is useful because the patient who is smear positive is highly infectious.

❖ **Culture (the most specific)**

- **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)-this one measures the amount of radioactive metabolites that get secreted from TB- and mycobacterial growth indicator tube (MGIT).(the last two are fast, they take 2 to 3 weeks but the sensitivity and specificity are low)**
- **Culture for acid fast bacilli is the most specific test for TB and allows direct identification and determination of susceptibility of the causative**

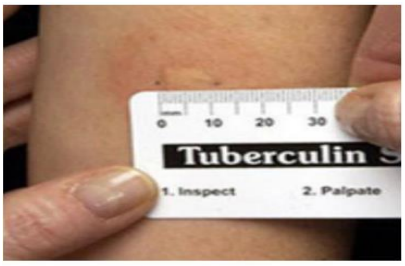
organism. But the problem is, that it takes a lot of time, if there is growth it will take 4 weeks to appear and if there isn't a growth you should wait 8 weeks to declare the result as negative.

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

This is going to be tough ☺.

Let's talk about the TSTs, first it is a measure of cell mediated immunity, simply you inject a small amount of fluid- purified protein derivative into the forearm, then the patient comes back after 48 hours, then we check for induration which is a raised, hard area or swelling. Please note it is induration and not redness.

so we measure the size of induration by a ruler



Reading the result of a TB skin test

Positive skin test: This means the person's body was infected with Mtb. Additional tests are needed to determine if the person has latent TB infection or TB disease. We also interpret numbers measured after we observed +ve results as following:

-If induration size > 15 mm-→normal healthy individual

-Induration size > 10 mm -→ intermediate risk group(homeless people or staff workers, like us ☺)

-Induration size > 5 mm-→ HIV patient [which makes sense as we don't expect patient with HIV to have large induration due to compromised immunity].

I will try to explain the results based on my habdanometer (you inject proteins to the individual and wait to see how they will react to them.so the reaction in all cases is thickening of the skin but we are looking at the size of thickening to determine the result, if the thickening is more than 15 mm then the person is normal and so on…)

Disadvantages of TST:

-You need the patient to come back after 48 hours

-Interpretation may give me FALSE POSITIVE (FP); which may arise in 2 cases that you are required to know guys:

1) patient who is immunized [he took BCG vaccine throughout his life]

2) infection with NTM (nontuberculous mycobacteria) may also give you FP
So, to overcome these problems, another technique is developed:

Interferon-gamma release assays (IGRAs) test

In this test, a blood sample is taken from the patient and distributed on different tubes that contain very specific antigens for MTB. It works by measuring the body's immune response to TB infection (based on amount of IFN-gamma released or cells that release it). --->So by that we can exclude FALSE POSITIVE results.

Note: both tests (IGRA AND TST) are used for screening purposes because they just give you an answer of (had the body faced MTB before or not?) and don't tell you whether the body faced MTB now or in the past. But normally if these tests give us POSITIVE result and the patient DOES NOT show symptoms and signs, we consider him to have {LATENT TB}.

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.

This treatment is given for about (6-12) months.

• 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).

- All four are given in intensive phase (initial 2 months)
- In slower continuation phase (remaining 4 months or more), we give the patient mainly 2 drugs -rifampin and isoniazid.
  So, it takes a long time and as a result of this is a long period of treatment many patients quit. They neglect the drug before 6 months as they feel better, and this creates many problems (no compliance with the treatment course leads to development of DRUG RESISTANT strains) such as:
  MDR-TB [multi drug resistant TB] that is resistant to isoniazid and rifampin
  EDR-TB [extensively drug resistant TB], resistant to oral drugs: isoniazid, rifampin, fluoroquinolones AND to injectable drugs: kanamycin, capreomycin and amikacin.
  Also, these drugs have side effects, for example Isoniazid is auto toxic, nephrotoxic and causes hepatitis, rifampin changes the color of body fluids to red.

to overcome this problem, a new style is used in treatment; called "DOT"☺- directly observed treatment, in which the patient is forced to come every day and take his medication in front of medical staff.

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

People with latent TB or children whose parents are diagnosed with TB are given isoniazid therapy of at least 9 months as a preventive treatment.

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(live attenuated vaccine), is the only licensed vaccine against tuberculosis (TB).

- Attenuation= weakening of the microbe by removing many of its virulence factors.
- here in Jordan we give it for neonates at the age of 1 month.
- many developed countries don't give this vaccination in its national vaccination program (NIP) anymore as the disease is much less prevalent now and the vaccine has some problems such as: -giving "false positive" results in many tests.
- additional problem with BCG vaccine is that it has different degrees of efficacy rates among people. Efficacy ranges are from 0-80 (zero means no protection even though the vaccine is given before, and 80 means 80% protection against TB). So not everyone who took it is fully protected. Then why do developing countries still give this vaccine? Most important reason is that it protects against 2 serious forms of the TB diseases: tuberculous meningitis (most importantly in babies) and miliary TB.

OTHER MYCOBACTERIA

• The nontuberculous mycobacteria (NTM)-also call MOT mycobacteria other than tuberculosis and it is also called environmental TB- is a diverse

group of organisms commonly found in the environment, and the group includes both saprophytes and human pathogens.

Note: they are not contagious

- **The NTM can be further classified into the rapid growers (grow in <7 days) and slow growers. Each group can be subdivided based on pigment production.**

So according to production of pigment they are classified to:

1) Photochromogens: produce pigmented colonies in the presence of light (carotene pigment).

2) Scotochromogen: produce pigmented colonies in the absence of light. 3) Nonchromogenic: don't produce the pigment neither in the presence nor absence of light.

- **Mycobacterium avium Complex (MAC or MAI- Mycobacterium avium intracellularly)-it is the most famous-.**
- **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.**

Another note: they are opportunistic meaning that they don't result in symptomatic disease in immunocompetent individuals they infect immunocompromised individuals.

THE NONTUBERCULOUS MYCOBACTERIA (NTM)

- **Mycobacterium kansasii(causes pulmonary disease similar to tuberculosis), Mycobacterium marinum (causes aquarium granuloma) and Mycobacterium ulcerans(causes wound infections).(they are slowly growing Photochromogens)**
- **Mycobacterium scrofulaceum. -→ scotochromogens, slow grower (remember it causes scrofula, it causes cervical lymphadenitis also MTB causes this symptom but it is more common in scrofulaceum.)**
- **Mycobacterium avium complex, or (MAI). -→nonchromogens, slow**
- **Mycobacterium fortuitum Complex, Mycobacterium chelonae-abscessus. -→ nonchromogens, fast growers. also they cause skin and soft tissue infection.**

MYCOBACTERIUM LEPRAE

- **Mycobacterium leprae is an acid-fast rod.**
- **It is impossible to grow this bacterium in vitro (they can't be grown in cultures but they can be grown in animal models).**
- **It causes the famous disease leprosy.**
- **The bacteria appear to grow better in cooler body temperatures closer to the skin surface.**

They live in the skin and in the nerves in Schwan cells of sensory nerves that's why they cause sensory loss (paresthesia)

- **Skin lesion consistent with leprosy and with definite sensory loss.**
- **The severity of the disease is dependent on the host's cell-mediated immune response to the bacilli (which live intracellular, like Mtb).**

Note: any intracellular infection depends on the cell mediated immunity. Keep this in mind☺

PATHOGENESIS

3 types of leprosy; they classified according to severity which depends on the immune system particularly cell mediated immunity.

- **Tuberculoid leprosy (TL)-the least severe-**

cell mediated immune response is apparent and strong, and there is a granuloma, so number of bacterial particles is limited. Accordingly, if we applied lepromin test -which is similar to tuberculin skin test TST-, it would give a POSITIVE result due to strong cell mediated immunity.

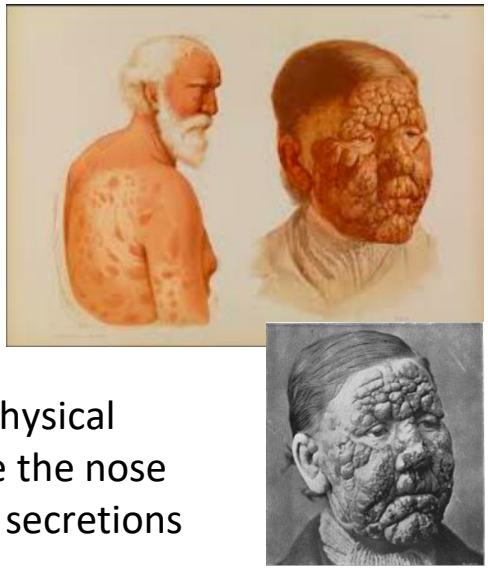
- **Lepromatous leprosy (LL)-most severe-**

cell mediated response is poor (not apparent), number of bacteria is high and lepromin test gives negative result. Why? because it depends on the reactions of cell mediated immunity and here it is poor

- **borderline lepromatous (BL)**

intermediate form between the two extremes: Tuberculoid and Lepromatous

CLINICAL MANIFESTATION



- **The onset of leprosy is insidious (very slow).**
- **The lesions involve the cooler tissue of the body, including the skin- in (skin histiocytes); causing painless skin nodules-, superficial nerves - (Schwan cells); causing sensory loss(I know that I have mentioned it before ☺), nose, pharynx, larynx, eyes, and testicles.**

Patients who are infected in the skin only require physical contact to transmit the disease but those who have the nose and pharynx involved can transmit it through nasal secretions

As you can see the patient has a lion like face.

DIAGNOSIS

- **skin or nasal mucosa or a biopsy of earlobe skin are smeared on a slide.**
- **Smears are stained by the Ziehl-Neelsen technique. Biopsy of skin or of a thickened nerve gives a typical histologic picture.**

Please remember they are not cultured.

- **No serologic tests are of value.**

Another note: all the serological tests are of no added value to intracellular infections.

TREATMENT

- **Sulfones such as dapsone are first-line therapy for both tuberculoid and lepromatous leprosy.**
- **RMP (rifampin/ rifampicin) or clofazimine generally is included in the initial treatment Regimens.**

The treatment is given for at least 2 years. And there is no vaccine.

The great Rome was not built in a day, and nor was it rushed.