Microbiology/tuberculosis

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

Nontuberculous (NTM) mycobacteria: opportunistic pathogens infecting immunocompromised persons.

Most common NTM is M.avium complex (MAC)>> infects AIDS patients

Mycobacterium tuberculosis complex (MTC) can cause Tuberculosis (TB) in humans and other livings, and the principle one nowadays is M.tuberculosis (MTB), but in the past it was M.bovis to commonly cause TB in humans, however milk pasteurization has significantly reduced it's rule in causing TB.

TB is also called: 1) consumption>> as it causes weight loss.

2) white plaque (extreme pallor seen among patients).

Mycobacterium is facultative intracellular; it lives inside the macrophages but in some stages of the infection it will be extracellular.

Morphology

Mycobacteria are Acid fast bacilli, why?

Because they retain the red color of the primary stain(carbolfuchsin) after the addition of HCl and 95% ethanol, so unlike other bacteria, the red color is not washed out of the cell wall of the mycobacterium after the addition of the strong acid… the acid-fast stain is called zell neelsen stain.

Culture: (the primary culture media should include selective and non selective media)

Slow growth rate; dublication time(18-24hs)>> 8 weeks for the colony to grow in the culture {note: definitive diagnosis is based on the culture, but if we suspect MTB we immediately start the treatment and isolate the patient}

1) • Semisynthetic agar media (eg, Middlebrook 7H10 and 7H11)>> the colonies are white, creamy and raised.

2) Inspissated egg media (eg, Löwenstein- Jensen), malachite green is added to inhibit the growth of other bacteria, and selectively allows the growth of Mycobacterium only.
3) Broth media (eg, Middlebrook 7H9 and 7H12)>> the culture here needs 4 weeks not 8, but it's less sensitive and less specifc than the agar culture.

The slow growth rate and the long duration of treatment of MTB are associated with it's complex cell wall that's referred to as waxy as it contains too much lipid which is the mycolic acid (50% of the dry weight of MTB). It has inner layer and an outer layer that surrounds the plasma membrane. inner compartment >>peptidoglycans (PG), arabinogalactans (AG) and mycolic acids (MA). peptidoglycan layer disaccharide N-acetyl glucosamine–N-acetyl muramic acid (NAG–NAM)

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).

Note: the mycobacterium shows clumbing growth when cultured due to presence of (cord factor/ TDM), it's also one of the factors responsible of having a dormant stage of TB in the body.

Note: cord factor and lipoarabinomannan (LAM) are considered virulence factors.

Epidemiology:

2/3 of the world's population>> latent TB (not infectious and don't show signs and symptoms of the disease).

small proportion of those infected will become sick with TB (active TB).

TB has high incidence rate in the developing countries like South Africa, also in soviet union countries.

Incidence rate in Jordan: 25-35 cases/ 100,000 individuals.

TB is the number one killer in AIDS patients (lethal combination)

Transmission: TB >> **airborne** infection. MTC organisms can be spread through **unpasteurized milk**, **direct inoculation**. MTB is in the droplets of **coughing**, **sneezing**, and **speaking**.

Airborne infection means that the MTB **survives in dryness** after the evaporation of the water in the droplet containing it.

Note: MTB is resistant to common disinfectants… to kill it; heating at high temp. is needed (basis of pasteurization) or U.V light is needed.

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

10 are exposed to MTB, 3 develop latent TB, one developpes active TB.

Tuberculosis TB

-**primary site** (in 90% of TB, most common site) of TB is the lung (**pulmonary TB**), and it's the most common site of TB in **adults**, it can disseminate to extrapulmonary site, this dissemination can be directly>> e.g through the diagphram to the abdomen.

TB bacteria can attack any part of the body:

1) the **meninges** (the **most serious TB**/ most commonly affects **children**, BCG vaccine can protect children against it)
2) lymph node>> TB lymphadenitis (Scrofula)
3) TB in spine>> Pott disease

Extrapulmonary TB can be a dissmenation from the lung, or it can be a primary site of TB.

Spread – Lymphatic vs hematogenous (Miliary).

***Miliary TB**: it's named so due to the milet seed like pattern seen in the chest x-ray of the lung>> it happens due to involvement of the whole lung by the TB after hematogenous spread of MTB back to the lung… (without the hematogenous spread the MTB granuloma is localized in a particular location in the lung).

Note: hematogenus spread is dangerous>> it increases risk of TB meningitis.

Note: MTB doesn't live in the blood>> blood culture is negative for MTB (low level of bacterimia), It's presence in the blood is transient for spreading.

• Primary Infection **(Active)** V.S secondary **(Reactivation)**Types of Tuberculosis.

The active type is seen usually in the well oxygenated areas of the lung (middle and lower lobes)

Reactivation: the patient got infected by TB, but the infection was dormant and contained, and if the immunity of the individual gets compromised, the dormant bacilli will reactivate causing TB disease (this happens in the **apex** of the lung, may be because this location has high amount of O2).

Pathogenesis: **inhalation of less than 10 mycobacteria is enough to start the infection.**

Once the bacteria is in alveoli>> internalized in macrophages by receptor mediated endocytosis, and now they are inside phagosomes. If the macrophages are activated normally they'll kill the MTB, but others are killed by the bacilli.

How does the MTB escape the destruction in macrophages?

1) inhibit the fusion of the phagosome with the lysosome.

2) inhibit the acidification of the phagolysosome.

3) leave the phagosome to be in the cytoplasm.

Then we have activation of the adaptive immune system (CD4+ cells and secretion of cytokines like IFN-gamma), still they are not able to activate the macrophages.

The hallmark of TB pathogenesis: Granuloma formation

Granuloma: infected macrophages+ other recruited macrophages>> differentiate into giant epithelioid foamy cells/ T cells / fibroblasts (will form a fibrous ring around the granuloma)

The shell of the fibrous ring and immune cells (granuloma) contains the mycobacteria>> **latent TB**.

If this shell is broken>> MTB multiply and infect other cells>> **active TB**

On **x-ray** we see the granuloma in the lung>> **Ghon focus.**

In active TB the granuloma will have a central necrosis (caziation).

Clinically (signs and symptoms):

fever, night sweats, productive cough, haemoptysis (coughing blood) , weight loss, dyspnea (chest pain) and malaise/fatigue.

TB diagnosis might be confused with cancer as it is a chronic disease that developpes slowly and causes weigth loss.

Diagnosis:

Lab Dx: 1) **Smear microscopy**>Acid Fast Bacilli

2)**Culture:** broth and solid mycobacterial cultures (the **most specific test for TB**, and it's used for **definitive diagnosis)**

mycobacterial growth indicator tube (MGIT)>> it takes 3 weeks

3)nucleic acid amplification test (NAAT).

4) we have two screening tests that till if the body was introduced with the mycobacterial antigens or not, but these are not diagnostic, as they don't tell if the infection is active or from the past.

-Tuberculin skin test (TSTs): injecting a purified mycobacterial antigen in the skin>> if the patient has antibodies against the antigen>> erythema after 48hs.

This test gives false positive if the individual has an environmental mycobacteria or was vaccinated before.

-Interferon-gamma release assays (IGRAs): a blood sample is taken from the patient, it's added to a lab tube containing MTB antigen, then the amount of released INF-gamma is measured>> baised on this we know if the patient was exposed to mycobacterium or not.

Treatment: we give a combination of antibiotics to reduce the probability of developing resistance by the MTB.

Treatment of active TB: intensive initial 2-month phase followed by **4- to 6-month continuation phase.** Drugs are: isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and either ethambutol (EMB) or streptomycin (SM).

In some countries the "dot" treatment is applied>> directly observed treatment, so the patient takes the pills Infront of the doctor.

Patients with latent TB>> prevention treatment>> isoniazide for 9 months.

Prevention: 1) **isolation of infectious cases** for about 2-4 weeks after Initiation of treatment.

2) **treatment of individuals with latent TB.**

3**) BCG vaccination- efficiency 0-70% -** >> it's given to **children** to protect them from TB meningitis, but it doesn't protect against pulmonary TB that infects adults.

Bacillus Calmette–Guérin **(BCG)**, an attenuated vaccine derived from **M. bovis**.

NonTB Mycobacteria(NTM)>> environmental mycobacteria

(opportunistic infections that cause disease in immunocompromised)

classified into the **rapid growers** (grow in<7 days), **slow growers** (grow in >7 days)

Each group can be **subdivided** on the basis of **pigment production**

Photochromogens>> produce pigment in **light.**

Scotochromogens>> produce pigment in **darkness.**

Nonchromogens >> **don't** produce pigment.

Note: **MTB** is nonchromogene.

*****The most common NTM is M. avium complex (MAC) >> infects AIDS patients**.

1-**M. kansasi** ➔ Pulmonary disease / 2-**M. marinum** ➔ Aquatic Granuloma, infects fisher men. / 3-**M. ulcerans** ➔ skin and soft tissue infection
(1, 2 ,3 are slow growing and Photochromogen)
4-**M. scrofulaceum** ➔ lymph node inflammation (slow growing and Scotochromogen) **.**

5-M. fortuitum Complex ➔ Pulmonary infection. / 6**. M. chelonae-abscessus** ➔ skin and soft tissue infection (5 and 6 are fast growing).