13

Microbiology

Doctor 2018 | Medicine | JU

◉ Sheet ○ Slides

Done By

Laila Nazzal

Contributed in the Scientific Correction

Batool Bdour

Contributed in the Grammatical Correction

Laila Nazzal

Doctor

Nader Alaridah

MYCOBACTERIA CONT.

Transmission and Pathogenesis of TB

Mycobacteria are present in respiratory 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.

• These amazing bacteria begin to multiply within ***macrophages*** after it escapes killing mechanisms such as [phagosomes or lysosome, ROS, RNOS and others]. Some of the macrophages develop an enhanced ability to kill the organism, but others may be killed by the bacilli.

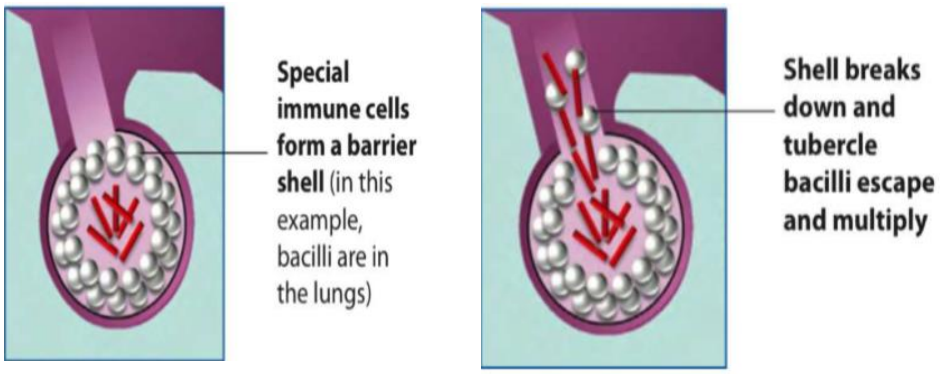
Granuloma formation
'breifly': infected macrophages recruit other macrophages, then adaptive immunity cells come there(T and B lymphocytes) and finally, surrounding the sick cell a fibrous rim is produced.

• The cells form a barrier shell, called a ***granuloma***, that keeps the bacilli contained and under control (LTBI= latent TB infection).

Granuloma is a hall mark for TB infection (intracellular infections generally)

note: less than 10 bacterial particles are enough to establish the disease

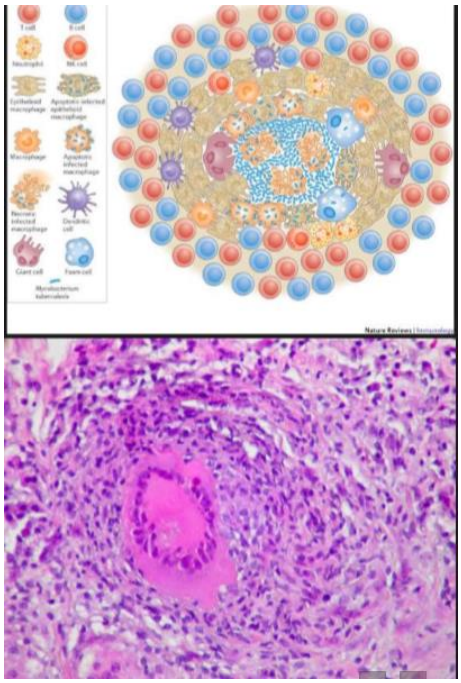
• If the immune system cannot keep the tubercle bacilli under control, the bacilli begin to multiply rapidly (active TB disease).



Pathology

this subject wasn't explained in class, but it's present in the slides. So, read what's written here..

-the following states the present understanding of inflammation occurrence in TB from a pathological (histological) perspective. There are two types that could occur concurrently:



* **Exudative type** (also called pneumonia 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. And is seen in serous cavities' infections with TB.

***Productive type**—(also called granuloma 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

Note: granuloma surrounding Mtb (but not in the case of meliary TB) is called ***Ghon focus***; which is a small area of granulomatous inflammation detected by x-ray. If ghon focus involves infection of adjacent lymphatics, it is known as **Ghons complex** (complex means involvement of draining lymph nodes.

Clinical manifestations : 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

*Tuberculosis , named also consumption disease (consumes patients with weight loss), white plaque (extreme pallor seen among patients).

*Tuberculosis is usually a **chronic disease**; it presents slowly with weight loss, low-grade fever, and if it is extrapulmonary; symptoms would be 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. ☺

Laboratory diagnostic methods

Note: Specimen is usually taken from sputum of the patient [children mostly can't give us sputum sample so we use [BAL: Broncho-alveolar lavage] procedure instead.

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 auramin.

Culture

• 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).

• **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 again remember it takes long time to give the result [about 8 weaks].

A nucleic acid amplification test (NAAT).

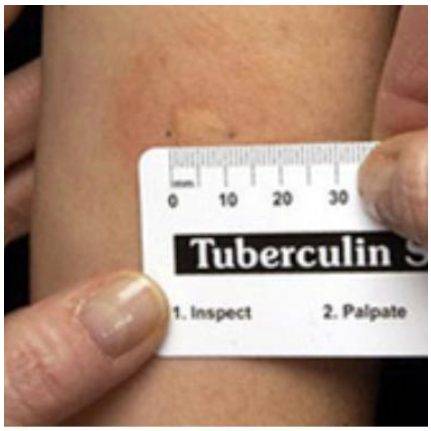
*Tests that are used in diagnosis of **latent** TB:

Tuberculin skin tests (TSTs), Interferon-gamma release assays (IGRAs) are commonly used.

**TST(also called purified protein derivatives), steps:

-The TB skin test is performed by injecting a small amount of fluid- purified protein derivative (called tuberculin) into the skin(intradermally) on the lower part of the arm

-A person given the tuberculin skin test must return back to clinic within 48 to 72 hours to look for a reaction on the arm and read the results



Reading the result of a TB skin test

-The result depends on the size of the **raised, hard area or swelling { the induration**}, so we measure the size of induration by a ruler-→

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

-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].

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 (nontuberculos 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}**

Prevention

The best way to prevent mycobacterial infections is to **diagnose** and isolate infectious cases (TB)rapidly and to administer appropriate treatment in special labs*(biosafety level 3 lab)* which is not available in Jordan of course☺ until patients are rendered noninfectious (usually 2–4 weeks after the start of proper treatment) and the disease is cured. *Also, many developed countries applies " contact racing" i.e. they look for any place or person with which the patient dealt in the previous month. They make sure that non of them has active TB.*

-Vaccine: **BCG** (Bacillus Calmette–Guérin), an attenuated vaccine derived from Mycobacterium bovis[live attenuated vaccine].

It is the only licensed vaccine against tuberculosis (TB)

*Attenuation= weakening of the microbe by removing many of its virulence factors.

Note: because it is a live attenuated pathogen, we **don't** give this vaccine to those who suffer from problems in cell mediated immunity (like AIDS patients) as its still an alive pathogen!

* here in Jordan we give it for neonates at the age of 1 month.

❖ It's given in the upper left arm and usually leaves a scar- check yours :P

Note: 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 (more details are at the end of this sheet).

-additional problem with BCG vaccine is that it has different degrees of EFFECIENCY among people. Efficiency 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 we mentioned before: tuberculous meningitis (most importantly in babies ☹) and meliary 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 months **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 of all -**rifampin and isoniazid**.

All these drugs have annoying side effects ☹. For example, **rifampin** changes the color of body fluids such as urine into [orange or red]. **Isoniazid** is autotoxic, nephrotoxic and causes sideroblastic anemia. **Pyrazinamide**, causes hyperuricemia (first step of gout). **Ethambutol** causes optic neuritis.

→Isoniazid preventive therapy **IPT,** is the recommended treatment for **LTBI** but the regimen's (the prescribed treatment's) main drawback is the **long duration of therapy.**

- As you see this is a long period of treatment [as long as this sheet ☺], so 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, flouroquinolones AND to injectable drugs: kanamycin,capreomycin and amikacin. ☐

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

OTHER MYCOBACTERIA

A) NTM [nontuberculous mycobacteria]. Also called environmental bacteria

They are classified by two criteria as following

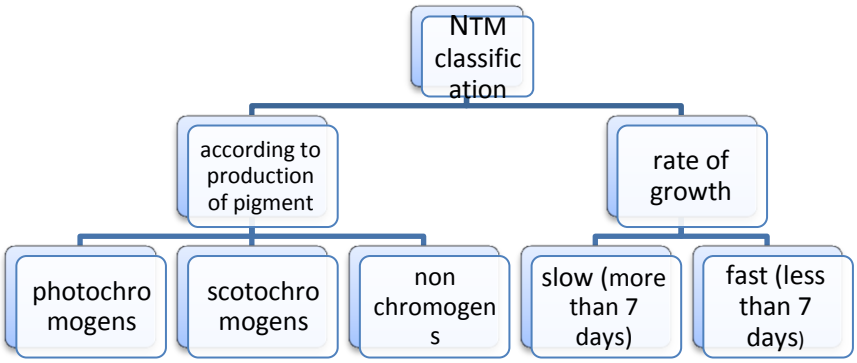
According to production of carotene pigment:

1) **Photochromogens**: produce pigmented colonies in the presence of light (carotene pigment) and non pigmented ones in the dark.
2) **Scotochromogens**: produce pigmented colonies in the presence and **absence** of light.
3) **Nonchromogenic**: don't produce the pigment neither in present nor absent of light.

According to rate of growth

1) Rapidly growing species: they form clearly visible colonies in less than 7 days

2) Slowly growing species: more than 7 days



Apply previous criteria to the following bacteria:

- *Mycobacterium* ***kansasii*** ,*Mycobacterium* ***marinum*** and *Mycobacterium* ***ulcerans*** → Photochromogenes, *slow* **{ group 1}**

- *Mycobacterium* ***scrofulaceum*** [remember it causes **scrofula**, TB of the lymph nodes: tuberculosis lymphadenitis] → scotochromogens, slow **{ group 2}**

- *Mycobacterium* ***avium intracellularae complex***, or (**MAI**) →nonchromogens, slow **{ group 3}**
- *Mycobacterium* ***fortuitum Complex*** ,*Mycobacterium* ***chelonae-abscessus*** → nonchromogens, fast { **group 4}**

B) Mycobacterium leprae: leprosy disease

Pathogenesis: 3 types of leprosy;

- **TUBERCULOID** leprosy (**TL**): 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**): cell mediated response is **poor** (not apparent) , number of bacteria is **high** and consequently; lepromin test gives **negative** result.
  - Patients of ***this type*** shed the bacteria from their **nasal secretions**, so its very dangerous and contagious (transmissible).
- **BORDERLINE** lepromatous (BL): intermediate form between the two extremes: **Tuberculoid** and **Lepromatous**

More about lepromin test:

This test is used to dermine **what type** of leprosy a person has.

It depends on the amount and the magnitude of **cell mediated immune reaction** against it. **NOT** the amount and number of organisms present, that's why it's positive in tuberculoid leprosy and negative in lepromatous leprosy.

Clinical manifestation

-The lesions involve the cooler tissue of the body(optimum temp for this bacteria is about 30 C), including the skin (skin histiocyts) **– causing painless skin nodules,** superficial nerves(Schwan cells)- **causing sensory loss**, nose, pharynx, larynx, eyes, and testicles.

The onset of leprosy is insidious.

Notice the 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; **granuloma**.

*No serologic tests are of value because it is an **intracellular** infection and the role of **humoral** immunity is **limited**

Don't forget!

****** this bacteria is NOT culturable in the lab, only in vivo******

Treatment

Treatment is given for at least 2 years. 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.

العِبرة بكمالِ النّهايات لا بنقصِ البدايات.

–ابن تيميّة.

(برعاية الفاينل)