Chapter 21

Mycobacteria (1)

Introduction

-- ***Mycobacteria*** are **aerobic, acid fast bacilli**.

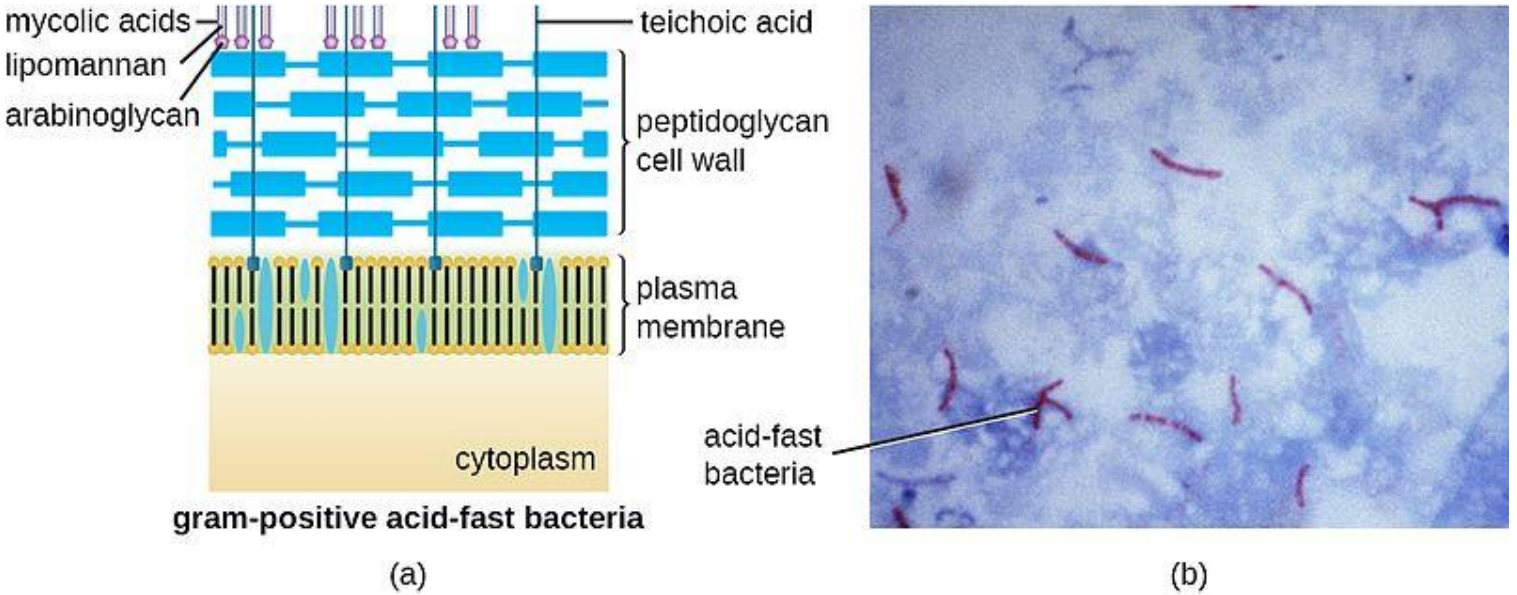
-- If a Gram stain is performed on ***Mycobacteria***, they stain **very weakly Gram-positive** or not at all (cells referred to as "ghosts").

-- Along with ***Nocardia asteroids***, they are the only bacteria which stain acid fast.

-- **Acid fast** refers to an organism's **ability to retain carbol fuchsin** stain despite subsequent treatment with an ethanol-hydrochloric acid mixture.

-- Acid fastness is **due to high lipid content (60%) of the cell wall**, esp long chain fatty acids called **mycolic acids.**

-- Popular acid-fast staining method for acid fastness is the **Ziehl-Neelsen stain**.



-- Major pathogens are ***M. tuberculosis*** and ***M. leprae***.

-- Mycobacteria such as ***M. avium-intracellulare*** complex, and ***M. kansasii*** **can cause tuberculosis like diseases** but are less frequent pathogens.

-- **Rapidly growing** mycobacteria such as ***M. chelonei*** occasionally cause human disease in **immunocompromised patients** or those in which **prosthetic devices** have been implanted.

Mycobacterium tuberculosis

-- **Tuberculosis (TB)** is the **leading cause of death in the world** from a bacterial infectious disease.

-- The disease has affected **1.8 billion people** which is equal to one-third of the entire world population.

-- **Almost 1.7 million people die of tuberculosis and 9 million new cases emerge each year.**

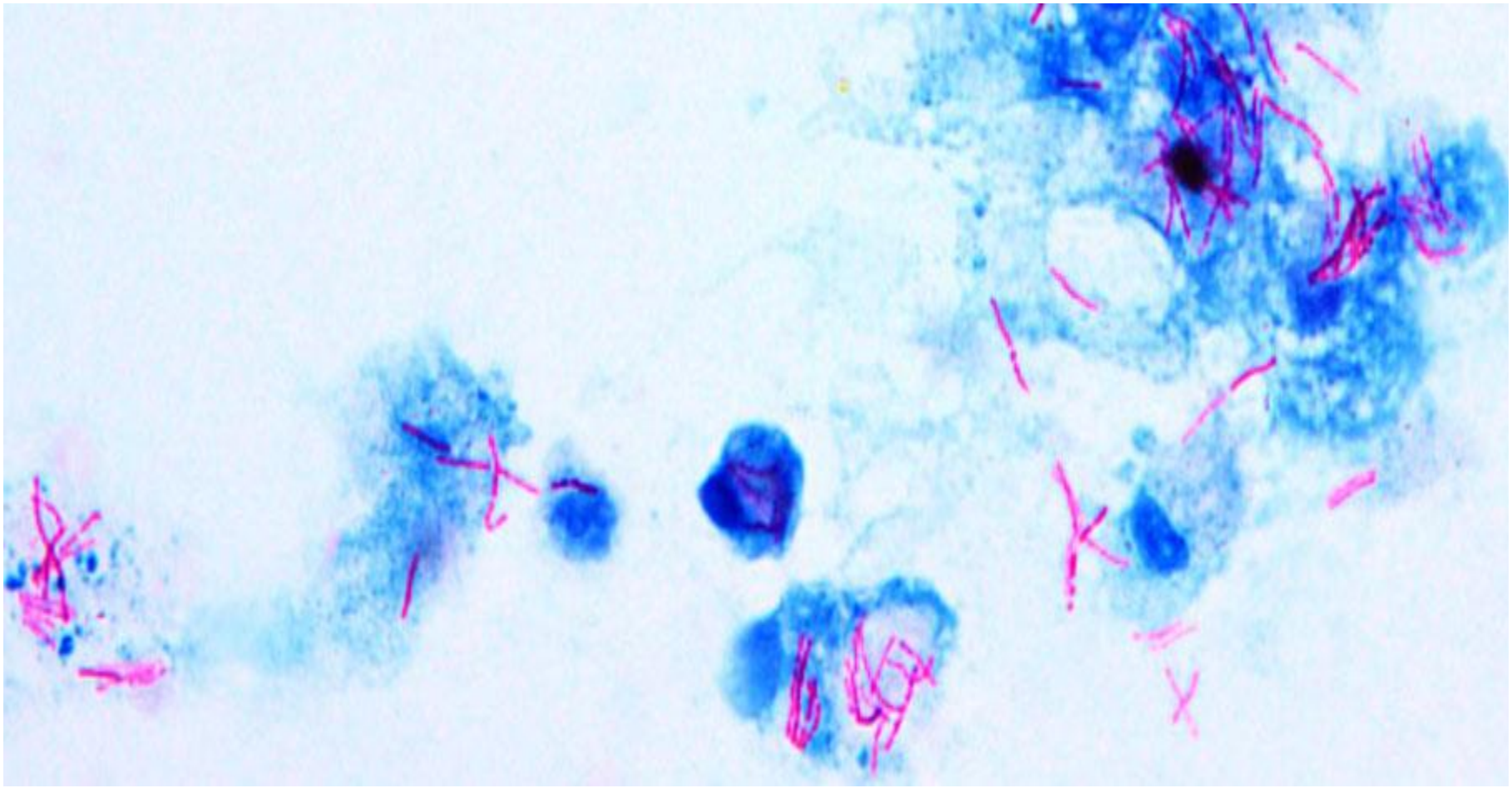
-- An estimated **500,000 people** are infected with a **multidrug-resistant** strain of ***M. tuberculosis***.

Important properties

-- ***Mycobacterium tuberculosis*** is a **fairly large non motile rod-shaped bacterium** distantly related to the ***Actinomycetes***.

-- Many **non pathogenic mycobacteria** are components of the **normal flora** of humans, found most often in dry and oily locales.

-- The rods are **2-4 um in length and 0.2-0.5 um in width**.



***Mycobacterium tuberculosis* in ZN stained sputum smear**

-- ***M. tuberculosis*** grows **very slowly** (i.e., it has a doubling time of **18 hours**).

-- For this reason cultures are not reported negative before **8 weeks**.

-- It needs **complex media**, e.g., **Lowenstein Jensen medium** that contains **egg yolk** and dyes e.g., **malachite green** which inhibit the unwanted normal flora present in sputum samples.

-- ***M. tuberculosis*** colonies are **small** and **buff colored**.

Mycobacterium tuberculosis **colonies on Lowenstein Jensen medium**

-- ***M. tuberculosis*** is an **obligate aerobe**.

-- For this reason, in the classic case of tuberculosis, MTB complexes are always found in the **well-aerated upper lobes of the lungs**.

-- The bacterium is a **facultative intracellular parasite**, usually of macrophages.

-- Chains of cells in smears made from **in vitro-grown colonies** often form distinctive **serpentine cords**.

-- This observation was first made by Robert Koch who associated **cord factor** with **virulent strains** of the bacterium.

-- ***M. tuberculosis*** also contains several proteins, which when combined with waxes elicits **delayed hypersensitivity**.

-- These proteins are the antigens in **PPD** (purified protein derivative) skin test (tuberculin skin test).

-- A lipid located in the bacterial cell wall called **phthiocerol dimycocerosate** is required for pathogenesis in the lung.

-- ***M. tuberculosis*** is **relatively resistant to acids and alkalis**.

-- **NaOH** is used to concentrate clinical specimens.

-- It **destroys unwanted bacteria, human cells** and **mucus** but not the organism.

-- ***M. tuberculosis*** is also **resistant to dehydration** and survives in dried sputum which is important in its transmission by aerosol.

-- Strains **resistant** to main antimycobacterial drug **isoniazid** (isonicotinic acid hydrazide, **INH**) as well as strains resistant to multiple antibiotics (**MDR strains**) have become a worldwide problem.

-- The resistance is due to **chromosomal mutations** as no plasmid has been detected.

-- One mutation is in a **gene for mycolic acid synthesis**, and other is in a **gene for catalase peroxidase**, an enzyme required to **activate INH** within the bacterium.

Pathogenesis

-- *M. tuberculosis* **does not produce exotoxins nor it has endotoxin in cell wall.**

-- It preferentially infect **macrophages** and other reticuloendothelial cells.

-- They survive and multiply in **phagosome**.

-- They produce a protein **ERP** (exported repetitive protein) that prevents the phagosome from fusing with lysosome.

Lesions are dependent on presence of the organism and host response.

There are two types:

-- **Exudative lesions** which consist of an **acute inflammatory response** and occur chiefly in the **lungs** at the initial site of infection.

-- **Granulomatous lesions** which consist of a **central area of giant cells** containing tubercle bacilli surrounded by a zone of epitheloid cells called **Langhan's giant cells**.

A **tubercle** is a granuloma surrounded by fibrous tissue that has undergone **central caseation necrosis**. Tubercles **heal** by **fibrosis** and **calcification**.

-- **Primary lesions** usually occur in **lower lobes** where as **reactivation lesions** usually occur in the **apices**.

-- **Reactivation lesions** also occur in other **well-oxygenated sites** such as the **kidneys, brain** and **bone**.

-- **Reactiviation** is seen primarily in **immunocompromised** or **debilitated** persons.

Spread of organism within the body occurs by **two** mechanisms:

-- A tubercle can **erode** into a bronchus, empty its caseous contents and thereby spread the organisms to other parts of lungs, to **GIT** if swallowed and to other persons if expectorated.

-- It can **disseminate via the bloodstream** to many internal organs.

-- Dissemination can occur at early stage if **CMI** fails to contain the **initial infection** or at a late stage if a person becomes **immunocompromised.**

Immunity and hypersensitivity:

-- After recovery, resistance is mediated by **CMI**. Antibodies are formed but are ineffective.

-- Mutations to **gamma interferon receptor gene** may cause defective CMI.

-- A gene called ***Nramp*** determines **natural** resistance.

-- Persons with mutated gene are at much higher risk.

-- The **NRAMP** protein is located in the membrane of the **phagosome in macrophages** and plays an important role in killing the organism within the phagosome.

Clinical findings:

-- **Fever, fatigue, night sweats,** and **weight loss** are common.

-- **Primary TB** causes **cough** and **hemoptysis**.

-- **Lymphadenitis** is the most common **extrapulmonary** manifestation.

-- **Erythema nodosum** may also be present in patients with **hyperactive CMI**.

-- **Miliary TB** is characterized by **multiple disseminated** lesions.

-- **TB meningitis** and **TB osteomyelitis** are also important.

-- **GIT TB** is characterized by abdominal pains and diarrhea with fever and weight loss. Intestinal obstruction or hemorrhage may occur.

-- In **renal TB**, dysuria, hematuria, and flank pain occurs. “Sterile pyuria” is a characteristic finding.

Transmission and Epidemiology

-- Transmission is by **respiratory aerosol** and initial site of infection is lung.

-- In the body it resides chiefly within **reticuloendothelial cells**, e.g., macrophages.

-- **Humans are the natural reservoirs.**

-- Some animals are infected but they are not a reservoir for human infection.

-- Mostly aerosols from **smear positive** (more than 10,000 bacteria per ml) people cause new infections, but almost **20%** may be infected by smear negative people.

-- ***M. bovis*** may also **cause human disease**.

-- It is found in cow's milk, which, **unless pasteurized**, can cause immune (**CMI**) response.

-- **AIDS patients** have a very high rate of **reactivation** of prior asymptomatic infection and **rapid progression** of disease.

-- In these patients, untreated disease by ***M. tuberculosis*** has a 50% mortality rate.

-- Administration of **monoclonal antibodies**, e.g., infliximab (used for treatment of rheumatic arthritis) that neutralizes TNF can activate latent TB in some cases.

Laboratory Diagnosis

-- **Acid fast staining** of sputum or other specimens is the usual initial test.

-- **Ziehl Neelsen** stain is used.

-- For **fluorescent microscopy**, auramine stain is used.

-- After **NaOH digestion and concentration** by centrifugation, the material is cultured on special media such as **Lowenstein Jensen medium** for up to **8 weeks**.

-- In **liquid BACTEC medium**, radioactive metabolites are present, and growth can be detected by the production of radioactive carbon dioxide in **about 2 weeks**.

-- Being a liquid medium, it has the advantage of **faster** growth.

-- When growth is obtained **biochemical tests** can be helpful, e.g., ***M. tuberculosis*** **produces niacin almost exclusively**, and is also **catalase positive**.

-- **Nucleic acid amplification tests** can be used for direct detection from clinical samples either by targeting ribosomal RNA or DNA.

These tests help in initiating treatment because culture results take a long time.

-- Because of common drug resistance, **sensitivity tests** should be performed but slow results are again a problem.

-- Here again, **molecular tests** can be helpful.

These target **catalase gene that mediates resistance to isoniazid** or the **RNA polymerase gene that is responsible for rifampin resistance**.

-- **Luciferase assay** which gives results in a few days can also be used.

Luciferase produces **flashes of light in presence of ATP**.

If a **drug is damaging a bacterial cell, less ATP will be produced** and thus lesser signal will be detected.

-- **Latent infection** can be diagnosed by **PPD skin test** or interferon – gamma release assay (**IGRA**).

-- **PPD skin test** has been used for long but it has problems with interpretation and patient follow up.

-- **IGRA** is available in two versions: **Quintiferon-TB** and **T-spot TB**. In both, blood cells from patient are exposed to antigens from ***M. tuberculosis***, and the **amount of interferon-gamma released** from the cells is measured.

-- Because this test is not based on Calmette-Guerin bacillus, previous **immunization with BCG is irrelevant**.

Treatment and Resistance

-- **Multidrug therapy** is used to prevent the emergence of drug resistant mutants during the **long (6-9 months)** duration of treatment.

-- **Isoniazid (INH)** is the mainstay of treatment.

-- For **pulmonary TB**, the most common combination is **INH, rifampin and pyrazinamide.**

-- **INH and rifampin** are given for 6 months but **pyrzinamide** is stopped after 2 months.

-- Alternatively, INH, rifampin, pyrazinamide and **ethambutol** are given for 2 months.

-- INH and rifampin are continued for next 2 months.

In case of **immunocompromised** (e.g., AIDS) persons, those with disseminated disease or those suspected to have INH resistance, **all four drugs** are given for **9-12 months**.

Although sputum becomes noninfectious within 2-3 weeks, **long treatment is required** because of :

1) **intracellular location** of organism

2) **presence of caseous material** that blocks the drug penetration

3) **slow growth** of organisms

4) metabolically inactive "**persisters"** within the lesion.

The **persisters** cause recurrent infections.

-- Treatment of **latent (asymptomatic) infections** consists of **INH taken 6-9 months or INH plus rifapentine for 3 months.**

-- This approach is often used in asymptomatic patients whose **PPD skin test recently converted to positive.**

-- The risk of symptomatic infection is greatest within the first 2 years infection, so **INH** is particularly indicated for these **“recent convertors”.**

-- **INH** is also used in **children exposed to patients** with symptomatic tuberculosis.

-- Patients on INH treatment, esp. those above 35 years should be observed for **drug induced hepatitis**.

-- **In case of INH resistance, rifampin can be used.**

-- **Rifampin and pyrazinamide should not be used in combination, because liver may be damaged.**

-- **MDR strains are emerging.**

-- Generally the resistance is towards **either INH or rifampin**, but some isolates are **resistant to two or more drugs**.

-- The treatment of **MDR organisms** usually involves the use of **4-5 drugs** including ciprofloxacin, amikacin, ethionamide and cycloserine.

-- **Non compliance is a major factor** in allowing resistance organisms to survive.

-- One approach to tackle this problem is **directly observed therapy (DOT)** in which healthcare workers observe the patient taking medicine.

-- **Strains resistant to INH, rifampin, a fluoroquinolone, and at least one additional drug are called extensively drug resistant (XDR) strains.**

-- Such strains emerged in 2005 in HIV-infected patients in South Africa.

Prevention

-- Incidence of TB started to decrease even before advent of drug therapy in 1940s because of **better housing and nutrition**.

-- Currently, prevention depends largely on **prompt identification** and **adequate treatment of coughing patients**.

-- **Use of masks and other respiratory isolation procedures is also important.**

-- It is also important to use **PPD skin test to detect recent converters** and to institute treatment for latent infections.

-- PPD screening should be done in close contacts of patients with active disease, low income population, alcoholics and i/v drug users, and prison inmates.

-- **Quantiferon-TB (QFT)** is a new test that requires a single blood sample and determines amount of γ-interferon by an ELISA test.

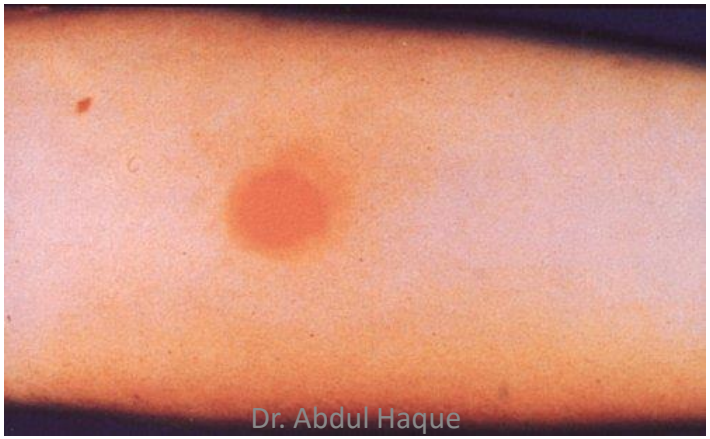
-- **BCG vaccine** provides **partial resistance to TB**.

-- It contains a strain of **live, attenuated *M. bovis*** called **Bacillus Calmette-Guerin.**

-- **It does not prevent infection but is effective in preventing appearance of clinical disease.**

-- A major problem is that its **effectiveness varies greatly**, from **0-70%.**

-- The skin test reactivity (**more than 10 mm**) should not be linked to vaccine administration unless it was done recently.



-- **BCG vaccine should not be given to immunocompromised people** because the live BCG organisms can cause disseminated disease.

-- **BCG vaccine** is also used to **treat bladder cancer**.

-- The vaccine is instilled into the bladder and serves to non-specifically stimulate cell-mediated immunity, which can inhibit the growth of carcinoma cells.

-- **Pasteurization of milk** and **destruction of infected cattle** are important in preventing intestinal tuberculosis.


Thank You!