Mycobacteria

genus *Mycobacterium*

- nonmotile,
- non-spore-forming,
- aerobic rods (0.2 to 0.6 × 1 to 10 μm in size)
- branched filaments
- The cell wall is rich in lipids – hydrophobic surface
- resistant to many disinfectants and common laboratory stains
- **acid-fast** bacteria
- divide slowly - cultures require incubation for up to 8 weeks

mycobacteria

- cause of morbidity and mortality
- 130 species
- few species or groups cause most human infections:
- *M. tuberculosis,*
- *M. leprae,*
- *M. avium* complex,
- *M. kansasii,*
- *M. fortuitum,*
- *M. chelonae,*
- *M. abscessus.*

Physiology and Structure of Mycobacteria

- Bacteria are classified in the genus *Mycobacterium* on the basis of
- (1) their acid-fastness,
- (2) the presence of **mycolic acids**

(containing 70 - 90 carbons)

Physiology and Structure of Mycobacteria – cell wall

- **lipid-rich cell wall** (e.g., acid-fastness; slow growth; resistance to detergents, common antibacterial antibiotics, and the host immune response; antigenicity).
- plasma membrane - proteins, phosphatidylinositol mannosides
- **lipoarabinomannan (LAM)-**functionally related to the O-antigenic lipopolysaccharides
- lipids, glycolipids, and peptidoglycolipids
- **(gram-positive bacteria)**

Physiology and Structure of Mycobacteria – cell wall

- Transport proteins and porins
- biologically important antigens - stimulating the patient's cellular immune response.
- Extracted and partially purified protein derivatives **(purified protein derivatives [PPDs])** - used as skin test reagents

Mycobacteria - classification

- Growth properties and colonial morphology
- *M. tuberculosis* (and closely related species in the *M. tuberculosis* complex) - slow-growing bacteria
- colonies - nonpigmented or a light tan color
- ***Mycobacterium tuberculosis* Complex**
- *M. tuberculosis*
- *M. leprae*
- *M. africanum*
- *M. bovis*
- *M. bovis* BCG (bacille Calmette-Guérin strain)

Mycobacteria - classification

- other mycobacteria - **"nontuberculous mycobacteria" (NTM)** - rate of growth and pigmentation

- The pigmented mycobacteria - **yellow carotenoids,**
  - stimulated by exposure to light (photochromogenic organisms)
  - produced in the absence of light (scotochromogenic organisms)

- The **Runyon classification** scheme of NTM :

- **slow-growing photochromogens** (*M. kansasii, M. marinum*),

- **slow-growing scotochromogens** (*M. gordonae*),

- **slow-growing nonpigmented mycobacteria** (*M. avium, M. intracellulare*),

- **rapidly growing mycobacteria** (*M. fortuitum, M. chelonae, M. abscessus*).

- pigmented or a rapidly growing *Mycobacterium* should never be mistaken for *M. tuberculosis*

Mycobacterium tuberculosis - Epidemiology

- **humans are the only natural reservoir.**
- close person-to-person contact
- inhalation of infectious aerosols
- Large particles - trapped on mucosal- removed by the ciliary action
- small particles (one to three tubercle bacilli) - reach the alveolar spaces - establish infection
- HIV-infected individuals
- drug-resistant *M. tuberculosis* strains

Mycobacterium tuberculosis
Pathogenesis and Immunity

- intracellular pathogen - lifelong infection
- Exposure - *M. tuberculosis* → respiratory airways → alveoli → → phagocytized by alveolar macrophages

M. tuberculosis

- prevents fusion of the phagosome with lysosomes - phagosome - fuse with other intracellular vesicles - phagocytized bacteria - evade macrophage killing

Mycobacterium tuberculosis Pathogenesis and Immunity

- macrophages - **interleukin-12 (IL-12)** and **tumor necrosis factor-α (TNF-α).**
  - increase localized inflammation
  - recruitment of T cells and natural killer (NK) cells
  - inducing T-cell differentiation into **TH1 cells (T-helper cells),**
    - subsequent secretion of **interferon-γ (IFN-γ)**

In the presence of IFN-γ:
- infected macrophages are activated
- increased phagosome-lysosome fusion and intracellular killing

TNF-α:
- stimulates intracellular killing

Mycobacterium tuberculosis Pathogenesis and Immunity

- **granuloma** (prevents spread of the bacteria)
- alveolar macrophages,
- epithelioid cells,
- **Langhans giant cells** (fused epithelioid cells)
- intracellular mycobacteria, necrotic mass surrounded by macrophages and CD4, CD8, and NK T cells.

- small antigenic burden - minimal tissue damage;
- many bacteria - large necrotic or caseous granulomas- encapsulated with fibrin (protects the bacteria from macrophage killing)
- bacteria remain dormant
- can be reactivated years later (old age, immunosuppressive disease, or therapy

Tuberculosis - clinical signs and symptoms

- primary disease - lower respiratory tract (**pulmonary tuberculosis**)
  - nonspecific
    malaise,
    weight loss,
    cough,
    night sweats
  - sputum : scant, or bloody and purulent
  - hemoptysis - tissue destruction (**cavitary disease**)

One or both upper lobes of the lungs are usually involved.

- Extrapulmonary tuberculosis
  - hematogenous spread of the bacilli during the initial phase of multiplication (**disseminated tuberculosis)**

Laboratory Diagnosis of Mycobacterial Disease

- **Immunodiagnosis**
- Tuberculin skin test (TST)
- Interferon-γ release assays (IGRA)
- **Microscopy**
- Ziehl-Neelsen (hot acid-fast) stain
- **Nucleic Acid-Based Tests**
- Nucleic acid amplification tests
- **Culture**
- Agar- or egg-based media
- Broth-based media
- **Identification**
- Morphologic properties
- Biochemical reactions
- Analysis of cell wall lipids
- Nucleic acid probes
- Nucleic acid sequencing

MYCOBACTERIUM TUBERCULOSIS–SAMPLE COLLECTION

- Sputum
- Laryngeal Swab
- Other Respiratory Specimen
- Gastric Lavage
- Extrapulmonary Specimens

Mycobacterium tuberculosis – microscopy (Ziehl-Neelsen)

- Drop suspension onto slide
- Air dry, heat-fix
- Flood slide with Carbol Fuchsin
- Hold a flame beneath the slide until steam appears but do not allow it to boil (3x)
- rinse with tap water
- Flood slide with 3% hydrochloric acid in isopropyl alcohol
- rinse with tap water
- Flood slide with Malahit green (Methylene Blue)
- Allow to sit 1 minute, rinse with tap water
- Blot dry
- View under oil immersion lens

Mycobacterium tuberculosis -culture

Löwenstein-Jensen medium

The usual composition applicable to *Mycobacterium tuberculosis* is:

- Malachite green (inhibits most other bacteria)
- Glycerol (enhances the growth of *Mycobacterium tuberculosis*)
- Asparagine
- Potato starch
- Coagulated eggs
- Mineral salt solution (Potassium dihydrogen phosphate, Magnesium sulfate, Sodium citrate)
- Low levels of penicillin and nalidixic acid (to inhibit growth of gram positive and gram negative bacteria)

Löwenstein-Jensen medium doesn't contain any agar, solid consistence is attained by heat coagulation of the egg albumin.

MANTOUX TUBERCULIN SKIN TEST

- **Mantoux** – in vivo detection of specific cell immunity after exposition to antigen
- Purified protein derivative (PPD) tuberculin - precipitate of species-nonspecific molecules obtained from filtrates of cultures.

- - burden of patient by antigen
- - possible immunodeficiency of patients (anergy, risk of alergic reaction)
- - memory cells after BCG vaccination,
- - exposition to antigen = activation of MC
- - interpretation,
- - booster dose of antigen,

INTERFERON-GAMMA RELEASE ASSAY (IGRA)

Interferon-Gamma Release Assays (IGRAs) are whole-blood tests that can aid in diagnosing *Mycobacterium tuberculosis* infection. They do not help differentiate latent tuberculosis infection (LTBI) from tuberculosis disease. Two IGRAs are commercially available:

- QuantiFERON®-TB Gold In-Tube test (QFT-GIT)
- T-SPOT®.*TB* test (T-Spot)

Mycobacterium tuberculosis – Treatment, Prevention, and Control

- Prolonged treatment - multiple drugs -to prevent development of drug-resistant strains
- Isoniazid (INH), ethambutol, pyrazinamide, and rifampin (2 months)
- 4 to 6 months of INH and rifampin or alternative combination drugs
- Prophylaxis for exposure - INH (6 to 9 months) or rifampin for (4 months);
- pyrazinamide and ethambutol or levofloxacin are used for 6 to 12 months after exposure to drug-resistant *M. tuberculosis*

- **Isoniazid** -prodrug, activated by a bacterial catalase-peroxidase enzyme, blocking fatty acid synthesis
- **Rifampicin -**inhibits bacterial DNA-dependent RNA synthesis
- **Ethambutol** – actively growing TB bacilli, obstruction of cell wall formation
- **Pyrazinamide** - to inhibit the enzyme fatty acid synthase(FAS)
- **Levofloxacin -** inhibits cell division. This can also affect mammalian cell replication

Antituberculotics –susceptibility testing

Proportion method

- Enables a precise estimation of the proportion of mutants resistant to a given drug.
- Several 10 fold dilutions of inoculum are planted on to both control and test media.
- At least one dilution should yield isolated countable (50-100) colonies.
- Viable count can be calculated by counting the colonies and multiplying with the dilution of the inoculum used.

✓The pure culture inoculated in the drug and drug free slopes of media

- The average number of colonies obtained for the drug containing slopes indicates the number of resistant bacilli contained in the inoculum
- Dividing the number of colonies in the drug containing slopes by that in drug free slopes gives the proportion of resistant bacilli existing in that strain

Mycobacterium tuberculosis – Prevention

- Immunoprophylaxis - Bacille Calmette-Guérin (BCG) in endemic countries

Control of disease through:

- active surveillance,
- prophylactic and therapeutic intervention,
- careful case monitoring

Mycobacterium leprae

Biology, Virulence, and Disease

- Weakly gram-positive, strongly acid-fast rods
- Lipid-rich cell wall
- Unable to be cultured on artificial media
- Disease primarily from host response to infection
- Tuberculoid (paucibacillary) and lepromatous (multibacillary) forms of leprosy

Mycobacterium leprae - **Epidemiology**

- Lepromatous form of disease, but not the tuberculoid form, is highly infectious
- Person-to-person spread by direct contact or inhalation of infectious aerosols
- Humans and armadillos - the only known resevoir

Mycobacterium leprae

- **Leprosy** (**Hansen disease**)
- incubation period is prolonged
- symptoms developing as long as 20 years after infection

- **tuberculoid leprosy** (**paucibacillary Hansen disease**)
- **lepromatous leprosy (multibacillary Hansen disease)**

Mycobacterium leprae

Diagnosis

- Microscopy is sensitive for the lepromatous form but not the tuberculoid form

- Skin testing is required to confirm tuberculoid leprosy
- Culture is not useful

Mycobacterium leprae

- leprosy test
- injection of an antigen under the skin
- injection site is labeled and examined 3 days and 28 days later
- Positive reaction:
- A) 10mm or more induration after 48hrs
- B) 5mm or above nodule after 21days

Mycobacterium leprae – Treatment, Prevention, and Control

- **Tuberculoid form** :
- rifampicin and dapsone for 6 months;
- **Lepromatous form:**
- clofazimine is added (12 months)
- **Controll:**
- prompt recognition
- treatment

Thank you

Sources:

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