Mycobacterium tuberculosis

Mycobacterium tuberculosis (also **Koch's bacillus'** - **KB'**) is a medium-long, sometimes granular acid-resistant rod. It is an obligate pathogenic mycobacteria and is the causative agent of tuberculosis. It is closely related to *Mycobacterium bovis*.

Characteristics

Mycobacteria are **strictly aerobic**.

The **high hydrophobicity** of the bacterium makes processing by lysosomal macrophage enzymes difficult (see below).

They have a **long generation time *(20-30 hours, cf.* Mycobacterium leprae**). Therefore, **long cultivation** on so-called slanted soils is necessary for their detection. This can take 6-10 weeks.

- They are ***intracellular parasites*** and cause chronic infections,
- **do not produce toxins**.

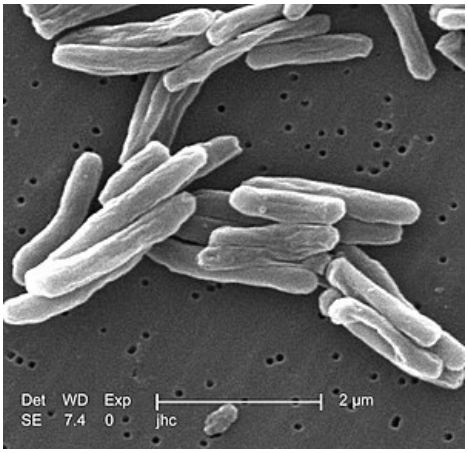
Acid resistance

Acid-resistance is poor staining with organic dyes, resistance to decolourisation by acids, bases and alcohol. It is related to **high wall lipid content** (a typical feature of mycobacteria). They are based on **mycolic acids** - the longest fatty acids in nature (C60-C90, similar but shorter in nocardia and corynebacteria).

Antigeny

Tuberculin

The Old (*Tuberculinum vetus*) was first prepared by Koch. It was obtained by concentrating liquid soil after separation of the bacterial mass. It had no therapeutic effects.



Mycobacterium tuberculosis.

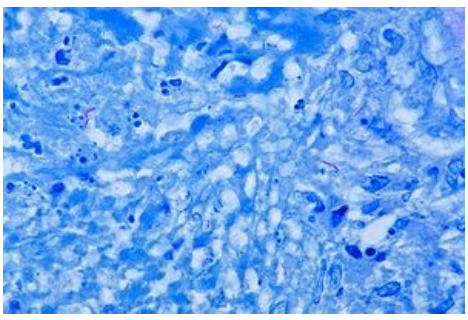
PPD

Purified Protein Derivative (PPD), purified tuberculin. It is still used for the **Mantoux test** (Charles Mantoux - French physician), i.e. detection of cellular hypersensitivity of delayed type against tuberculin:

1. intradermal injection (so that it doesn't wash away)
2. after 24-48 hours, if positive (in infected and vaccinated), an inflammatory infiltrate (pimple) is formed by T-lymphocytes and macrophages
3. induction (not erythema) is measured - positive**> 5 mm**

Cord-factor (trehalose dimycolate)

- toxic **glycolipid**, part of the cell wall
- it's a virulence factor
- alters mitochondrial membrane → **inhibition of respiration and phosphorylation**


Ziehl-Neelsen.

Other antigens

- arabinogalactan
- peptidoglycan
- complex glycolipids

Proof

Direct evidence

Directly demonstrate *Mycobacterium tuberculosis*, or its components, by the following methods:

- **direct microscopy** = basic method; *high specificity, low sensitivity*
  - *Ziehl-Neelsen* staining
    1. hot carbolfuchsin
    2. acid decolourisation (mycobacteria remain stained)

3. staining of other structures with methylene blue or malachite green
4. examination of 100 fields of view

- **fluorescence staining**
  1. hot auramine
  2. acid decolourisation
  3. fuchsin staining
  4. examination under UV - lower magnification is sufficient (25-50 fields of view)

Culture card

It has a higher sensitivity for detection in comparison to microscopy, .Gold standard for TB.

1. sample decontamination (destruction of faster growing microbes with 4% NaOH)
2. centrifugation (→ concentration)
3. inoculation on *Löwenstein-Jensen* soil (egg) / liquid soil - *Šulova*
4. reading of the result at 3, 6 and 9 weeks

- **Accelerated cultivation** in systems derived from hemoculture (indicator is CO_2); result in *5 days*

Molecular genetics

Result in 24-48 hours. PCR is used, but also non-variable bacteria are positive (proof of dead DNA), i.e. it is not possible to make a diagnosis based on PCR alone (e.g. if the clinical manifestation is'nt expressed)!

Indirect evidence

The QuantiFERON® method is available for indirect identification:

- the patient's blood is put into three tubes
- the antigen is flushed from the tube wall by shaking
- the test is not positive in vaccinated patients

There is also a risk of false negatives in immunodeficient individuals! The test scheme is as follows:

1. the tube is **empty** (negative control)
2. the tube contains **T-lymphocyte activator** (positive control) - active T-lymphocytes produce **IFN-γ** which is quantified and indicates the positivity of the reaction
3. the tube contains **specific peptides from the bacterium**

Diseases

🔎 *For more information see tuberculosis.*

Prevention

Vaccination with **BCG'** (attenuated strain of *Mycobacterium bovis*) protects against the development of *Mycobacterium tuberculosis* infection:

- induces the formation of an **artificial primary complex** at the site of injection
- develops a **nodular granuloma** after 2-4 weeks, which heals in 6-8 weeks
- those already vaccinated or infected have an accelerated reaction in as little as 24-48 hours → they may develop **necrosis** (so-called **Koch phenomenon**)
- vaccination efficacy is 40-80%
- **Chest X-ray infection screening** was previously performed , but this method is now obsolete

Treatment

Treatment of tuberculosis **takes a long time** (months). To avoid the risk of resistance, **combinations of antituberculotics** are used. In the case of monotherapy, there is a risk of developing a **fall & rise phenomenon**, i.e. selection of resistant mutants that trigger the next wave of infection.

Proportional test

The **proportional test** is used to test antibiotic susceptibility:

- bacteria are inoculated on soil *without antibiotic* and on soil with a *critical antibiotic concentration*.
- if the soil with antibiotic grows **0-1%** of colonies relative to the soil without antibiotic, the strain is susceptible

Antituberculosis are used to treat tuberculosis:

- **isoniazid** - bactericidal, highly effective - inhibits mycobacteria for 5-6 days after one dose
- **rifampicin** - bactericidal, blocks RNA polymerase, stains body fluids orange ("blood sweat")
- **pyrazinamide** - bactericidal, active in more acidic pH (suitable for intracellular environment); with rifampicin which kills even semi-dormant mycobacteria
- **streptomycin** - bactericidal aminoglycoside (binding to the 30S subunit of the ribosome), ototoxic

- **ethambutol** - bacteriostatic, inhibits synthesis of some metabolites

Links

Related articles

- Tuberculosis
- Atypical mycobacteria

External links

- Mycobacterium tuberculosis (English Wikipedia)

References

1. BENEŠ, Jiří. *Infekční lékařství.* 1. vydání. Praha : Galén, c2009. s. 277-284. ISBN 978-80-7262-644-1.

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