

THE GENUS
MYCOBACTERIUM

- The mycobacteria, or acid-fast bacilli, are responsible for tuberculosis and leprosy and a number of saprophytic species occasionally cause opportunist disease.
- There are about 50 species of mycobacteria, which are divisible into two major groups, the slow and rapid growers, although the growth rate of the latter is slow relative to that of most other bacteria.
- The leprosy bacillus has never convincingly been grown in vitro.

Mycobacterium tuberculosis

- Tuberculosis is a chronic granulomatous disease affecting man, many other mammals, birds, fish and reptiles.
- Mammalian tuberculosis is caused by four very closely related species:
 - *Mycobacterium tuberculosis* (the human tubercle bacillus)
 - *Mycobacterium bovis* (the bovine tubercle bacillus)
 - *Mycobacterium microti* (the vole tubercle bacillus)
 - *Mycobacterium africanum*

- Most human tuberculosis is caused by *M. tuberculosis* but some cases are due to *M. bovis*, which is the principal cause of tuberculosis in cattle and many other mammals.
- The name *M. africanum* is given to tubercle bacilli with rather variable properties and which appear to be intermediate in form between the human and bovine types. It causes human tuberculosis and is mainly found in Equatorial Africa.
- *M. microti* is seldom, if ever, encountered nowadays.

- Tubercle bacilli are able to grow on a wide range of enriched culture media but Löwenstein-Jensen (LJ) medium is the most widely used in clinical practise. Agar-based media or broths enriched with bovine serum albumin are also used. Human *M. tuberculosis* strains produce visible growth on LJ medium in about 2 weeks, although on primary isolation from clinical material colonies may take up to 8 weeks to appear.
- Tubercle bacilli have a rather limited temperature range of growth: their optimal growth temperature is 35-37 °C but they fail to grow at 25 or 41 °C. Most other mycobacteria grow at one or other, or both, of these temperatures.

- Tubercle bacilli are non-motile, non-sporeforming, non-capsulate straight or slightly curved rods about $3 \times 0.3 \mu\text{m}$ in size.
- In sputum and other clinical specimens they may occur singly or in small clumps and in liquid cultures human tubercle bacilli often grow as twisted rope-like colonies termed serpentine cords.

- Like other mycobacteria, the tubercle bacilli are obligate aerobes but *M. bovis* grows better in conditions of reduced oxygen tension.
- Thus, when incorporated in soft agar media, *M. tuberculosis* grows on the surface while *M. bovis* grows as a band a few millimetres below the surface. This provides a useful differentiating test.
- The human tubercle bacillus also differs from the bovine type in its ability to reduce nitrates to nitrites, its production of large amounts of niacin, its sensitivity to pyrazinamide.
- Mycobacteria are acid- and alcohol-fast bacilli, which can be stained by Zielh-Neelson stain.

- *M. tuberculosis* causes tuberculosis but other species of mycobacteria also cause infection in the lungs. These are called the "atypical mycobacteria", "non-tuberculous mycobacteria" or "mycobacteria other than tuberculosis - MOTT".
- Tuberculosis is a killer and ranks as one of the most serious infectious diseases of the developing world. This has become particularly obvious in patients with AIDS.
- Tuberculosis is primarily a disease of the lungs but may spread to other sites or proceed to a generalized infection ("miliary tuberculosis").
- Infection is acquired by inhalation of *M. tuberculosis* in aerosols and dust. Airborne transmission of tuberculosis is efficient because infected people cough up enormous number of mycobacteria.

Laboratory diagnosis

- The definitive diagnosis of tuberculosis is based on the detection of acid-fast bacilli in clinical specimens by microscopy or cultural techniques.
- Numerous unsuccessful attempts have been made to develop serological tests for the disease.

Laboratory diagnosis

- A provisional diagnosis of tuberculosis is usually made by demonstrating acid-fast bacilli in stained smears of sputum or of gastric washings. For rapid staining of smears, some laboratories employ fluorescence microscopy.
- Whether the smear is positive or not, the material should be cultured since fewer organisms can be detected, the human tubercle bacilli can be differentiated from other acid-fast bacilli, and testing for anti-microbial sensitivity can be carried out. Solid culture media are preferred for primary isolation and they often contain egg yolk to promote growth. These media often contain malachite green or other antibiotics to inhibit the growth of other organisms. A positive culture usually grows in 2 to 4 weeks. A selective liquid medium with a radiolabelled carbon substrate allows automated detection of growth several days sooner than with conventional culture.

Laboratory diagnosis

- Delayed type hypersensitivity to tuberculin is highly specific for tubercle bacilli and this is the basis of the tuberculin test. A positive test reveals previous mycobacterial infection but it does not establish the presence of active disease. Reactivity appears about 1 month after infection and persists for many years.
- The tuberculin test (or Mantoux test) is a useful diagnostic test and epidemiological tool.

Anti-tuberculous agents

- The treatment of infection caused by *Mycobacterium tuberculosis* and other mycobacteria presents an enormous challenge to medicine and pharmaceutical industry.
- They grow and multiply extremely slowly and effective inhibition takes weeks or months.
- A number of anti-tuberculous agents are now available.
- Most are restricted to this use to prevent resistance emerging in other species and potentially being transferred to mycobacteria or because the toxicity of the drugs makes them unattractive for general use.

- **First-line therapy**

- Treatment regimens may vary between countries in general.
- First line (or first choice) therapy is combination of isoniazid, ethambutol and rifampicin for 6 to 9 months. Streptomycin may be added in treatment of tuberculous meningitis.

- **Second-line therapy**

- Despite the use of antibiotics in combination, the incidence of resistance among mycobacteria is increasing and a cure may not be achieved by the first-line drugs.
- Infections with mycobacteria other than *M. tuberculosis* are on the increase as opportunist infections in AIDS patients and these microorganisms tend to be innately more resistant than *M. tuberculosis*.
- Drugs such as paraminosalicylate, pyrazinamide and thiacetazone are used as second-line therapy for drug resistant *M. tuberculosis*. Their selection and administration requires specialist knowledge.

Mycobacterium bovis

- *M. bovis* causes tuberculosis in cattle and is also highly virulent for man.
- In the past man usually get infected through unpasteurised or other dairy products produced from tuberculous cows. After ingestion, the organism penetrates the mucosa of the oropharynx and intestine, giving rise to early lesions in the cervical or mesenteric lymph nodes. Subsequent dissemination from these sites principally involves the bone and joints. Infection of the vertebra has resulted in the hunchbacks of previous generations. When inhaled e.g. by dairy farmers, the organism can also cause pulmonary tuberculosis indistinguishable from that caused by *M. tuberculosis*. Bovine tuberculosis had been virtually eliminated by the pasteurisation of milk and the virtual eradication of tuberculosis in cattle.

Mycobacterium leprae

- The leprosy bacillus was first observed microscopically in leprosy tissue by Hansen in 1874, but has not yet been grown on inanimate culture media despite claims to the contrary. It has been a major limiting factor in the study of leprosy.
- The bacillus is straight or slightly curved, about the same size as the tubercle bacillus, but not as strongly acid-fast. To demonstrate the organism, the Ziehl-Neelsen staining method should be modified by the use of a weaker decolourizer than used for tubercle bacilli.

Mycobacterium leprae

- Although *M. leprae* has never been cultivated, it has long been recognised as the aetiological agent of human leprosy as it is readily demonstrated in stained smears of exudates of persons with leprosy.
- *M. leprae* is indistinguishable in morphology and staining properties from *M. tuberculosis* and leprosy has many clinical features in common with tuberculosis.
- The failure to culture the organism has hampered its investigation and made it difficult to test strains for drug sensitivity.
- The lepromin test may give a positive result in tuberculous individuals and occasionally in healthy individuals. It is mainly used in determining the prognosis of disease.

Mycobacterium leprae - pathogenesis

- *M. leprae* causes granulomatous lesions resembling those of tuberculosis, with epithelioid and giant cells but without caseation.
- The organisms are predominantly intracellular and can proliferate within macrophages, like tubercle bacilli.
- Leprosy is distinguished by its chronic slow process and by its mutilating and disfiguring lesions. These lesions may or may not be characteristic as to be diagnostic for leprosy. The organism has a predilection for skin and nerves. In the cutaneous form of the disease, large firm nodules are distributed widely and on the face they create a characteristic leonine appearance. In the neural form, segments of peripheral nerves are involved, more or less as random, leading to localised patches of anaesthesia. The loss of sensation in the fingers and toes increases the frequency of minor trauma, leading to secondary infection and mutilating injuries.

Mycobacterium leprae

- pathogenesis

- In either form of the disease, three phases may be distinguished:
 - Lepromatous (progressive) type – the lesions contain many leprae cells, which are macrophages with a characteristic foamy cytoplasm, in which acid-fast bacilli are abundant. The lepromin test is usually negative. The disease is progressive and the prognosis is poor.
 - Intermediate type – bacilli are seen in areas of necrosis but rare elsewhere, the lepromin test is positive, and the long-term outlook is fair.
 - Tuberculoid (healing phase) – the lesions contain few leprae cells and bacilli, fibrosis is prominent, and the lepromin test is usually positive.
- The organism may be widely distributed in other tissues such as liver and spleen without any ill effect. Deaths of leprosy patients are not caused by leprosy itself but by intercurrent infections with other organisms such as tuberculosis.

- Smears may be made from any ulcerated nodule on the skin. The leprosy bacilli are typically found tightly packed within macrophages, but some extracellular bacilli can also be seen.
- *Mycobacterium leprae* causes leprosy. Transmission of infection is directly related to over crowding and poor hygiene. Leprosy is not highly contagious and prolonged exposure to an infected source is necessary, it seems that children living under the same roof as an open case of leprosy are most at risk.
- *Mycobacterium leprae* cannot be grown in artificial culture media and little is known about its mechanism of pathogenicity. The organism grows better at a temperatures below 37 °C and it grows extremely slowly.
- *Mycobacterium leprae* grows intracellularly, typically within skin histiocytes and endothelial cells and the Schwann cells of peripheral nerves.

Diagnosis of leprosy

- Diagnosis is accomplished by demonstrating acid-fast bacilli in scrapings or fluid from ulcerated lesions.
- The lepromin skin test is useful in determining whether or not the patient is in the lepromatous stage and thus the prognosis.

Treatment of leprosy

- Infection caused by *M. leprae* is characterized by persistence of the microorganism in the tissues for years, necessitates very prolonged treatment to prevent relapse.
- For many years dapsone, a sulphone derivative has been used. This drug has the advantage that it is given orally and it is cheap and effective.
- However, widespread use as monotherapy has resulted in the emergence of resistance and multidrug regimens are therefore preferable. Rifampicin can be combined with dapsone. Alternatively clofazime is active against dapsone-resistant *M. leprae*, but it is expensive.

- In addition to the tubercle and leprosy bacilli there are many species of mycobacteria that normally exist as saprophytes of soil and water.
- Some of these, termed environmental or „atypical“ mycobacteria, occasionally cause opportunist disease in animal and man.

According Ernest Runyon, these mycobacteria are divided into four groups:

- I. photochromogenes - pigmentation on exposure to light
M. kansasii, M. marinum, M. simiae
 - II. scotochromogenes - pigmentation formed in the dark
M. scrofulaceum, M. gordonae, M. szulgai, M. ulcerans, M. xenopi
 - III. non-chromogenes - no pigmentation
M. avium, M. intracellulare, M. gastri, M. terrae, M. malmoense
 - IV. rapid growers
M. chelonae, M. fortuitum, M. phlei, M. smegmatis
- All strains in group I, II and III grow slowly.

Diseases due to atypical mycobacteria:

- skin lesions following traumatic inoculation of bacteria,
- localized lymphadenitis,
- tuberculosis-like pulmonary lesions,
- disseminated disease.

Mycobacterium avium complex

- The *M. avium* complex includes *M. avium* and *M. intracellulare*.
- *M. avium* causes tuberculosis in chickens, other birds, and swine. There are many documented cases caused by *M. avium*, mostly in farmers and men with silicosis.
- *M. intracellulare* is not usually pathogenic for birds or animals. *M. intracellulare* is a frequent cause of disseminated infection in patients with AIDS.
- In contrast to the more virulent *M. tuberculosis*, the identification of MAC in an isolated sputum culture does not constitute definite evidence of disease because MAC can colonise healthy persons. Because the presence of MAC in the sputum does not constitute a public health hazard and MAC pulmonary disease is not rapidly progressive, it is important to obtain evidence to establish MAC disease before embarking on a prolonged course of therapy. The diagnosis of disseminated infection can be made by the identification of MAC from a sterile site. MAC is generally resistant to the first-line antituberculous agents.

Mycobacterium scrofulaceum

- *M. scrofulaceum* is a common cause of lymphadenitis in children aged 1 to 3 years.
- Lymphadenitis usually involves a single node or a cluster of nodes in the submandibular area. Characteristically, the nodes enlarge slowly over a period of weeks. There are very few local or systemic symptoms. Untreated, the infection will usually point to the surface, rupture, form a draining sinus and eventually calcify. Infection in other tissues occurs occasionally. A very few cases resembling progressive primary tuberculosis have been encountered in children. In children, metastatic bone disease may be prominent. Colonies are usually yellow-orange even when grown in the dark (scotochromogenic). They are usually resistant to antituberculosis drugs in vitro.

Mycobacterium ulcerans

- *M. ulcerans*, found mainly in Africa and Australia, will grow only below 33 °C.
- It causes chronic deep cutaneous ulcers in man. It usually produces lesions in the cooler parts of the body.
- It has a unique drug sensitivity pattern;- resistance to INH and ethambutol and susceptibility to streptomycin and rifampin.
- Human disease responds poorly to drug treatment and extensive excision followed by skin grafting is often necessary.

Mycobacterium marinum

- *M. marinum* also grows best below 33 °C.
- It causes a tuberculosis-like disease in fish and a chronic skin lesion known as “swimming pool granuloma” in humans.
- Infection is acquired by injury of a limb around a home aquarium or marine environment and can lead to a series of ascending subcutaneous abscesses.
- *M. marinum* resembles *M. kansasii* in being photochromogenic.
- *M. marinum* varies in susceptibility to antimicrobial agents.

Mycobacterium fortuitum complex

- Most of the fast-growing disease-associated mycobacteria are members of this complex, which may be divided into two accepted species: *M. fortuitum* and *M. chelonae*.
- They abound as saprophytes in soil and water. A wide variety of clinical symptoms may be encountered. Sporadic infections have involved almost every tissue and organ systems, and outbreaks have followed cardiothoracic surgery, peritoneal dialysis, and haemodialysis. The spectrum of diseases caused by these organisms includes soft-tissue abscesses, sternal wound infections after cardiac surgery, prosthetic valve endocarditis, disseminated and localised infection in haemodialysis and peritoneal dialysis patients, pulmonary disease, traumatic wound infection, and disseminated disease often with cutaneous lesions.
- The most predictably effective therapy for infections due to rapidly growing mycobacteria is surgical removal of all involved tissues. The rapidly growing mycobacteria vary widely in their susceptibility to chemotherapeutic agents.

Mycobacterium xenopi

- *M. xenopi* was first isolated in 1959 from a South African toad.
- It had been reported to cause pulmonary infection in several parts of the world, most prominently in England.
- Contaminated hot water tanks may serve as reservoir for infection, especially in institutions that care for patients with chronic lung disease.
- Most infections resemble pulmonary tuberculosis. It is scotochromogenic and grows optimally at 42 °C.

Mycobacterium kansasii

- *M. kansasii* and *M. avium-intracellulare* account for most of the human mycobacterial disease attributable to acid-fast organisms other than tubercle bacilli.
- *M. kansasii* is photochromogenic; the overnight change of the colonies to yellow is followed by the formation of red crystals of B-carotene on exposure to light for several more days. Most strains are sensitive to rifampin and to several other drugs.
- *M. kansasii* has been identified as an agent of disease in nearly all parts of the world. It characteristically produces a chronic lung infection that closely resembles pulmonary tuberculosis. Symptoms are usually milder than tuberculosis.
- *M. kansasii* may infect extrapulmonary tissues often as a result of inoculation or haematogenous dissemination.
- *M. kansasii* is among the most predictably sensitive of the mycobacterial species.