MYCOBACTERIUM TUBERCULOSIS

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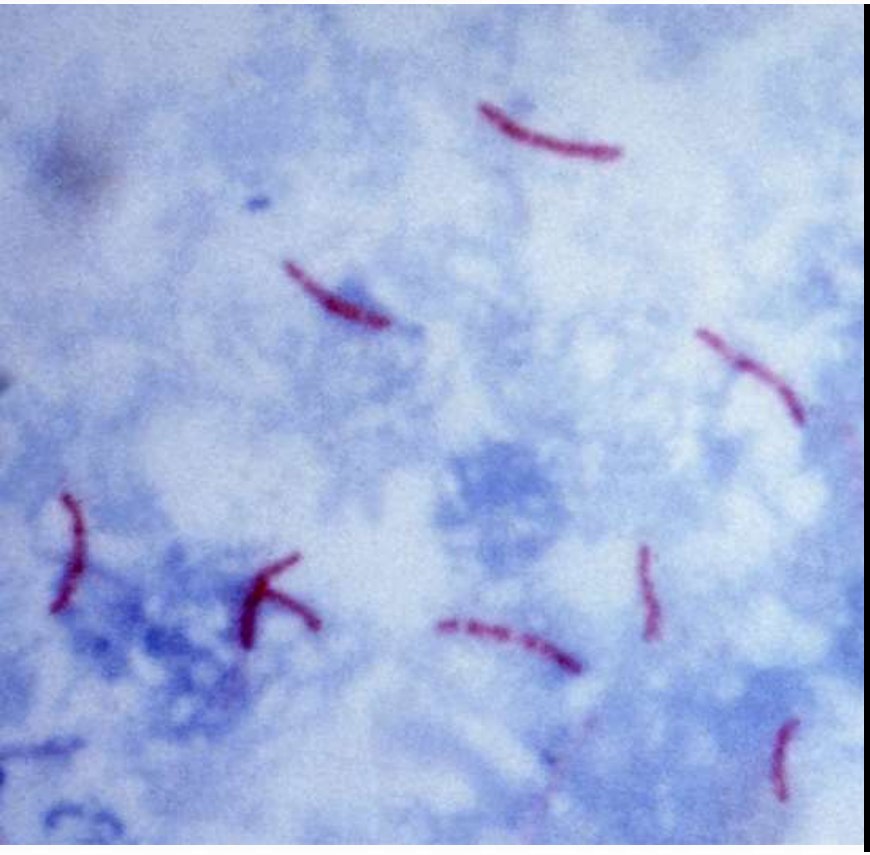
Mycobacterium tuberculosis

B A C T E R I O L O G Y

- *M. tuberculosis* is a slim, strongly acid-alcohol-fast rod. It frequently shows irregular beading in its staining, appearing as connected series of acid-fast granules. It grows at 37° C but not at room temperature, and it requires enriched or complex media for primary growth. Growth is enhanced by 5 to 10% carbon dioxide but is still very slow. The classic medium, Löwenstein-Jensen, contains homogenized egg in nutrient base with dyes to inhibit the growth of nonmycobacterial contaminants.
- The dry, rough, buff-colored colonies usually appear after 3 to 6 weeks of incubation.




Mycobacterium tuberculosis

- Due to its hydrophobic lipid surface, *M. tuberculosis* is unusually resistant to drying, to most common disinfectants, and to acids and alkalis. Tubercle bacilli are sensitive to heat, including pasteurization, and individual organisms in droplet nuclei are susceptible to inactivation by ultraviolet light.
- A purified protein derivative (PPD) of tuberculin is used for skin testing for hypersensitivity and is standardized in tuberculin units according to skin test activity.

TUBERCULOSIS

- Tuberculosis is a systemic infection manifested only by evidence of an immune response in most exposed individuals. In some infected persons, the disease either progresses or, more commonly, reactivates after an asymptomatic period (years).
- The most common reactivation form is a chronic pneumonia with fever, cough, bloody sputum, and weight loss. Spread outside of the lung also occurs and is particularly devastating when it reaches the central nervous system.

EPIDEMIOLOGY

- A recognized disease of antiquity, tuberculosis first reached epidemic proportions in the western world during the major periods of urbanization in the 18th and 19th centuries. Mortality reached 200 to 700 per 100,000 population each year, accounting for 20 to 30% of all deaths in urban centers and winning tuberculosis the appellation of the “white plague.” Morbidity was many times higher.
- The disease has had major sociologic components, flourishing with ignorance, poverty, overcrowding, and poor hygiene, particularly during the social disruptions of war and economic depression. Under these conditions, the poor are the major victims, but all sectors of society are at risk.

- The great majority of tuberculous infections are contracted by **inhalation** of droplet nuclei carrying the causative organism.
- Humans may also be infected through the gastrointestinal tract following the **ingestion** of milk from tuberculous cows (now uncommon due to pasteurization) or, rarely, through abraded **skin**.
- It has been estimated that a single cough can generate as many as 3000 infected droplet nuclei and that less than 10 bacilli may initiate a pulmonary infection in a susceptible individual. The likelihood of acquiring infection thus relates to the numbers of organisms in the sputum of an open case of the disease, the frequency and efficiency of the coughs, the closeness of contact, and the adequacy of ventilation in the contact area.

- Epidemiologic data indicate that large doses or prolonged exposure to smaller infecting doses is usually needed to initiate infection in humans. In some closed environments, such as a submarine or a crowded nursing home, a single open case of pulmonary tuberculosis can infect the majority of nonimmune individuals sharing sleeping places.
- Globally, the situation is more ominous. It is estimated that one third of the world's population is infected with *M. tuberculosis*; 30 million people have active disease, an additional 8 million develop new disease yearly, and 2 to 3 million die annually of this "captain of death."

- As a result, tuberculosis is the leading cause of death from an infectious disease worldwide. It is thought responsible for 6% of all deaths and 26% of avoidable adult deaths. Particularly concerning for the future control of tuberculosis worldwide is the marked susceptibility of patients with AIDS and the growing resistance of *M. tuberculosis* to the currently available antimicrobic agents.
- Because 40% of all new cases of tuberculosis in the United States are among foreign-born individuals, the elimination of this disease in the United States will be impossible without a substantial reduction in the global burden of tuberculosis.

PATHOGENESIS

- **Primary Infection**

  Primary tuberculosis is the response to the initial infection in an individual not previously infected and sensitized to tuberculoprotein. Inhaled droplet nuclei containing small numbers of tubercle bacilli are deposited in the peripheral respiratory alveoli. Here they are engulfed by alveolar macrophages. The ability of these cells to destroy ingested organisms depends significantly on their inherent microbicidal capacity.

- If the alveolar macrophages are unable to destroy ingested mycobacteria, they continue to multiply until the macrophage bursts. The released organisms are subsequently ingested by inactivated blood macrophages that, together with T cells, are attracted to the lung by chemotactic factors.

- The ingested mycobacteria continue to multiply intracellularly without damage to their host cell. Some of the bacterial-laden macrophages are transported through lymphatic channels to the hilar lymph nodes draining the infected site. From there, they may disseminate through blood and lymphatic systems to a number of tissues, including the liver, spleen, kidney, bone, brain, meninges, and apices or other parts of the lung.
- Morphologically, the resulting tubercle is a microscopic granuloma comprised of some multinucleated giant cells formed by the fusion of several macrophages (Langhans cells), many epithelioid cells (activated macrophages), and a surrounding collar of lymphocytes and fibroblasts.

- **Reactivation (Adult) Tuberculosis**

  Reactivation usually occurs in body areas of relatively high oxygen tension and low lymphatic drainage, most often in the apex of the lung.
- The lesions show spreading, coalescing tubercles with numerous tubercle bacilli, and large areas of caseous necrosis. Necrosis often involves the wall of a small bronchus from which the necrotic material is discharged, resulting in a pulmonary cavity and bronchial spread. Frequently, small blood vessels are also eroded.
- The chronic fever and weight loss may be mediated in part by macrophage-derived tumor necrosis factor.

- **Virulence Mechanisms**

  The basis for *M. tuberculosis* virulence is largely unknown. It produces no exotoxins. Cell wall components such as LAM have been implicated in binding to alveolar macrophages, utilizing surface fibronectin, mannose, or complement receptors (CR1, CR3). Once inside, multiple factors contribute to survival and continued multiplication. A number of genes have been identified that are linked to virulence by enhancing survival in the macrophage or by influencing the physical and chemical conditions (low pH, high lactic acid, high CO2) present in developing lesions, but their function remains unknown.

IMMUNITY

- Humans generally have a rather high innate immunity to development of disease. This was tragically illustrated in the Lübeck disaster of 1926 where infants were administered *M. tub*erculosis instead of an intended vaccine strain.
- Despite the large dose, only 76 of 249 died and most of the others developed only minor lesions. Approximately 10% of immunocompetent persons infected with *M. tuberculosis* will develop active disease any time in their life.
- There is epidemiologic and historic evidence for differences in the immunity in certain population groups and between identical and nonidentical twins.

IMMUNITY

- Lifelong immunity to coccidioidomycosis clearly develops in the vast majority of those who become infected. This immunity is associated with strong polymorphonuclear leukocyte and T lymphocyte-mediated responses to coccidioidal antigens. In most cases, a mixed inflammatory response is associated with early resolution of the infection and development of a positive delayed hypersensitivity skin test.
- Progressive disease is associated with weak or absent cellular immunity and skin test anergy. In most infected persons the infection is controlled after mild or inapparent illness. The disease progresses if cell-mediated immunity and consequent macrophage activation do not develop. Such immune deficits may be a result of disease (AIDS) or immunosuppressive therapy but may occur in persons with no other known cellular immune compromise.
- Humoral mechanisms are not known to play any role in immunity.

- Delayed-type Hypersensitivity (DTH) to tuberculoprotein and cell-mediated immunity (CMI) to *M. tuberculosis* develop 2 to 6 weeks after primary infection. The subsequent course of the infection depends on the balance between these two defensive mechanisms. DTH, through the mediation of natural killer cells, destroys the inactivated macrophages as well as the surrounding tissues, releasing still viable mycobacteria into an area of necrosis unsuitable for bacterial multiplication. CMI develops when competent T lymphocytes recognize mycobacterial antigen complexes on the surface of *M. tuberculosis*-containing macrophages.
- Acquired immunity is cell mediated but incomplete. Both helper-inducer (CD4+) and cytotoxic (CD8+) T lymphocytes are involved. Two to three weeks after infection, macrophages are activated at the site of infection by a network of pro- and anti-inflammatory cytokines and chemokines from antigen-stimulated CD4+ T lymphocytes, macrophages, and dendritic cells.

TUBERCULOSIS

CLINICAL ASPECTS

MANIFESTATIONS

- **Primary Tuberculosis**

  Primary tuberculosis is either asymptomatic or manifest only by fever and malaise. Radiographs may show infiltrates in the mid-zones of the lung and enlarged draining lymph nodes in the area around the hilum. When these lymph nodes fibrose and sometimes calcify, they produce a characteristic picture (Ghon complex) on radiograph.

- In approximately 5% of patients, the primary disease is not controlled and merges into the reactivation type of tuberculosis, or it disseminates to many organs to produce active miliary tuberculosis. The latter may result from a necrotic tubercle eroding into a small blood vessel.

- **Reactivation Tuberculosis**

  Approximately 10% of those recovering from a primary infection develop clinical disease sometime during their lifetime.

- Reactivation is associated with a period of immunosuppression precipitated by malnutrition, alcoholism, diabetes, old age, and a dramatic change in the individual's life, such as loss of a spouse. In areas in which the disease is more common, reactivation tuberculosis is more frequently seen in young adults experiencing the immunosuppression that accompanies puberty and pregnancy.
- Cough is the universal symptom. It is initially dry, but as the disease progresses sputum is produced, which even later is mixed with blood (hemoptysis). Fever, malaise, fatigue, sweating, and weight loss all progress with continuing disease.
- Radiographically, infiltrates appearing in the apices of the lung coalesce to form cavities with progressive destruction of lung tissue.

DIAGNOSIS

- Tuberculin Test

  The tuberculin skin test measures DTH to tuberculoprotein. PPD is standardized biologically against an international reference preparation, and its activity expressed in tuberculin units (TU). Most initial skin tests employ 5 TU (intermediate strength).

- When an unusually high degree of hypersensitivity or eye or skin tuberculosis is suspected, then 1 TU (first strength) or less is used initially to avoid the risk of an excessive reaction locally or at the site of a mycobacterial lesion.

- The test most commonly performed involves intradermal injection that is read 48 to 72 hours later. An area of measured induration of 10 mm or more accompanied by erythema constitutes a positive reaction, although smaller areas of induration and erythema indicate a lesser degree of sensitization to mycobacterial proteins. No induration indicates a negative reaction.
- A positive PPD test indicates that the individual has been infected at some time with *M. tuberculosis* or with a strongly cross-reacting mycobacterium of another species. It carries no implication about the activity of the infection, which may have been simply a primary complex contracted 20 years previously.

- A negative PPD test in a healthy individual indicates that he or she has not been infected with *M. tuberculosis*, is in the prehypersensitive stage of a primary infection, or has finally lost tuberculin sensitivity along with disappearance of antigen from an old primary complex.
- Patients with severe disseminated disease, those on steroid or immunosuppressive drugs, or those with certain other diseases such as AIDS and measles, may also become anergic.

Laboratory Diagnosis

If present in sufficient numbers, acid-fast bacilli can be detected microscopically in direct smears of clinical specimens. Smears are stained by the Ziehl-Neelsen procedure or one of its modifications, including the fluorescence staining method. About 65% of culture-positive sputum samples yield positive smears from concentrated specimens.

- These procedures are not specific for *M. tuberculosis* because other mycobacteria may have a similar morphology and may be etiologic agents of disease, members of the normal flora, or external contaminants. Their significance depends on the specimen.
- **Acid-fast bacilli in sputum are highly significant for mycobacterial infection.**

- Cultural confirmation of a tentative diagnosis of tuberculosis is thus essential, and the organism must be isolated for identification and susceptibility testing.
- Those samples inevitably contaminated with normal flora, such as sputum, are treated with alkali, acid, or a detergent germicide under conditions that kill the normal flora but allow many mycobacteria to survive because of their resistance to these agents. Cultures on solid media usually take 3 weeks or longer to show visible colonies.
- More rapid results can be obtained by high-resolution gas chromatographic analysis of fatty acids in mycobacterial colonies or by testing for homology between genetic probes of labeled mycobacterial DNA and ribosomal RNA extracted from the strain under test.
- Susceptibility testing is important with newly diagnosed cases.

TREATMENT

- *M .tuberculosis* is susceptible to several effective antimicrobics. Isoniazid, ethambutol, rifampin, pyrazinamide, streptomycin, and combinations of these agents constitute the primary drugs of choice for treatment of tuberculosis. All of these, except ethambutol, are bactericidal.
- Isoniazid and rifampin are active against both intra- and extracellular organisms, and pyrazinamide, a nicotinamide analog, acts at the acidic pH found within cells. Streptomycin does not penetrate into cells and is thus active only against extracellular organisms.
- *M. tuberculosis* is also susceptible to other drugs that may be used to replace those of the primary group if they are inappropriate because of resistance or drug toxicity.

- The fluoroquinolones, such as ciprofloxacin and ofloxacin, are active against *M. tuberculosis* and penetrate well into infected cells. Their role in the treatment of tuberculosis is under evaluation.
- Treatment with multiple antimicrobics to which the organism is susceptible usually renders the patient noninfectious within 1 or 2 weeks, which has shifted the care of tuberculous patients from isolation hospitals and sanatoriums to the home or the general hospital. After an initial intense phase of systemic chemotherapy, treatment is usually continued with oral antimicrobics for several months.
- Failure of chemotherapy is often associated with lack of adherence to the regimen by the patient, the presence of resistant organisms, or both.

PREVENTION

- Prophylactic chemotherapy, usually with isoniazid alone, is now used in situations in which known or suspected primary tuberculous infection poses the risk of clinical disease.
- At present, the bacillus Calmette-Guérin (BCG) vaccine is the only available vaccine. It has been used for prophylaxis of tuberculosis in various countries since 1923; administration is usually intradermal. It is a live vaccine derived originally from a strain of *M. bovis* that was attenuated by repeated subculture.
- BCG is used only in tuberculin-negative subjects. Successful vaccination leads to a minor local lesion, self-limiting multiplication of the organism locally and in draining lymphatic vessels, and development of tuberculin hypersensitivity. The latter results in loss of the PPD test as a diagnostic and epidemiologic tool.

Antimicrobics Commonly Used in Treatment of Tuberculosis

First-Line Drug	Second-Line Drug[a]
Isoniazid	*para*-Aminosalicylic acid
Ethambutol	Ethionamide
Rifampin	Cycloserine
Pyrazinamide	Fluoroquinolones
Streptomycin	Kanamycin, etc