CONTENTS

INTRODUCTION

The theoretical and practical significance of knowledge on the main issues of microbiology, virology and immunology determines the need to improve the quality of teaching these subjects at a medical university. The adapted English-language textbook «Microbiology, Virology, Immunology» contains up-to-date information necessary for mastering the disciplines «Microbiology, Virology» and «Immunology». The result of mastering the disciplines is the formation of the sum of knowledge, skills and abilities within the framework of the competencies necessary for the implementation of a doctor's professional activity: GPC-5 (ability to assess morphofunctional, physiological states and pathological processes in the human body to solve professional tasks), PC-3 (ability and willingness to perform a complete clinical examination of the patient, analysis and interpretation received data), UC-1 (ability to carry out a critical analysis of problem situations based on a systematic approach, develop a strategy of action).

The textbook «Microbiology, Virology, Immunology» is compiled in accordance with the approved basic professional educational program of higher education «Medical business» and the requirements of the Federal State Educational Standard of higher education in the specialty 31.05.01 «General Medicine», order No. 988 of 12.08.2020.

The textbook includes four sections: General microbiology, Special microbiology, Virology and Immunology. The chapter «General microbiology» contains information about the morphology and physiology of microorganisms, their interaction with the environment, as well as the main patterns of the infectious process. The textbook focuses on the importance of laboratory diagnostics in the practice of infectious diseases and pays special attention to the principles and methods of laboratory diagnostics. The chapter «Special microbiology» contains materials on etiology, epidemiology, pathogenesis, laboratory diagnostics, specific prevention and therapy of major infectious and microbial-inflammatory human diseases. The chapter «Virology» contains information about the features of viruses and the infectious process caused by them, as well as microbiological parameters of the main viral infections. The final chapter of the textbook «Immunology» is devoted to the structure and functioning of the immune system of the human body in normal and pathological conditions. Special attention is paid to the use of immunological reactions in the laboratory diagnosis of infectious diseases.

The textbook was prepared by a team of authors with many years of experience working with foreign students, and adapted for teaching the disciplines of «Microbiology, Virology» and «Immunology» for foreign students studying on the specialty 31.05.01 «General Medicine».

Topic 7
INFECTIONS CAUSED BY *MYCOBACTERIUM SPP.*

Actinobacteria. Human pathogenic genera mycobacterium, actinomyces, nocardia and corynebacterium belong to the phylum Actinobacteria. Mycobacterium is a genus of Gram-positive bacilli that demonstrate the staining characteristic of acid-fastness. The most important species, *Mycobacterium tuberculosis*, is the etiologic agent of tuberculosis. Tuberculosis remains a leading cause of infectious disease deaths worldwide today. A second mycobacterium, *Mycobacterium leprae*, is the causative agent of leprosy. A large number of less pathogenic species collectively referred to as «atypical mycobacteria» or «nontuberculous mycobacteria», are assuming increasing importance as disease agents in immunocompromised patients, particularly those with acquired immunodeficiency syndrome (AIDS). Actinomyces and nocardia are Gram-positive rods characterized by filamentous, tree-like branching growth, which has caused them to be confused with fungi in the past. They are opportunists that can sometimes produce indolent, slowly progressive diseases. A related genus, streptomyces, is of medical importance as a producer of many antibiotics, but it rarely causes infections.

Mycobacteria are slender bacilli that sometimes show branching filamentous forms resembling fungal mycelium (*myces* meaning fungus). They are difficult to stain but once stained, resist decolorization with dilute mineral acids and are, therefore, called acid-fast bacilli or AFB. These organisms are aerobic, non-motile, non-capsulated and non-sporing. Of particular importance are long-chain fatty acids called mycolic acids. The mycolic acids, for which the mycobacteria are named, make up more than 60% of the total cell wall mass and are distinctive for each species. Other lipid components include mycosides, sulpholipids, and lipoarabinomannan (LAM), a complex molecule extending from the plasma membrane to the surface. LAM is structurally and functionally analogous to the lipopolysaccharide of Gram-negative bacteria.

The genus includes:

1) obligate parasite of human (*Mycobacterium leprae*), reservoir — only human;
2) facultative parasites of human (*M. tuberculosis*) and animals (*M. bovis*, *M. microti*, etc.), reservoir — human and animals;
3) opportunistic or saprophytic pathogens (*M. kansasii*, *M. smegmatis*, *M. smegmatis*, *M. avium-intracellulare*, etc.), reservoir — environment and animals. ***M. tuberculosis* and *M. bovis* are typical tubercle bacilli and cause human tuberculosis.**

Classification. Mycobacterial classification has been based on a constellation of phenotypic characteristics, including nutritional and temperature requirements, growth rates, pigmentation of colonies grown in light or darkness, key biochemical tests, the cellular constellation of free fatty acids, and the range of pathogenicity in experimental animals.

***Mycobacterium tuberculosis*.** The species contains two major types, classical and South Indian type of *M. tuberculosis*. Classical type is virulent to guinea pig, but South Indian type is attenuated in this animal. South Indian type is prevalent in South India and in persons of Asian-ethnic origin living in other countries. *M. tuberculosis* is a slender, straight or slightly curved bacillus with rounded ends, occurring singly, in pairs or in small clumps. Ziehl–Neelsen staining is useful to study the morphology of this bacterium. Tubercle bacilli may also be stained with the fluorescent dyes (auramine O, rhodamine) and appear yellow luminous bacilli under the fluorescent microscope. Beaded or barred forms are frequently seen in *M. tuberculosis*. They are Gram-positive but are difficult to stain with the Gram stain due to the failure of the dye to penetrate the cell wall. *M. bovis* appears straighter, stouter and shorter with uniform staining.

Cultivation. *M. tuberculosis* is an obligate aerobe, whereas *M. bovis* is microaerophilic on primary isolation, becoming aerobic on subculture. The bacilli grow slowly (generation time 14–15 h) and colonies appear only in about two weeks and sometimes it may take up to 6–8 weeks. Optimum temperature for growth is 37 °C (range 30–40 °C). Optimum pH is 6.4 to 7.0. Tubercle bacilli can grow on a wide range of enriched culture media but Lowenstein–Jensen (L) medium is most commonly used. This medium consists of beaten eggs, asparagine, mineral salts, malachite green and glycerol or sodium pyruvate. It is solidified by heating (inspissation). It is one of the media which are solid without incorporation of agar. In this medium egg acts as a solidifying agent. Malachite green inhibits the growth of organisms other than mycobacteria and provides a color to the medium. The addition of glycerol improves the growth of human type of *M. tuberculosis*, while it is without any effect or even inhibitory to *M. bovis*.

Sodium pyruvate improves the growth of both *M. tuberculosis* and *M. bovis*. Colonies of *M. tuberculosis* are dry, rough, buff colored, raised, with a wrinkled surface. They are tenacious and not easily emulsified. In liquid media, the bacilli grow as surface pellicle due to hydrophobic properties of their cell wall. Virulent

strains tend to grow as *serpentine cords* in the citrated blood (Price' rapid test), while avirulent strains grow in a more dispersed fashion. The cord factor consists of two mycolic acids linked to a molecule of trehalose.

Resistance. 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.

Epidemiology of tuberculosis. 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. Children, patients with AIDS and other immunodeficient syndromes are the most sensitive to tuberculosis currently.

Pathogenesis of tuberculosis. Mycobacteria do not produce classic exotoxins or endotoxins. Disease processes are thought to be the result of two related host responses. The first, a delayed-type hypersensitivity (DTH) reaction to mycobacterial proteins, results in the destruction of nonactivated macrophages containing multiplying organisms. It is detected by intradermal injections of purified proteins from the mycobacteria. The second, cell-mediated immunity (CMI) activates macrophages, enabling them to destroy mycobacteria contained within their cytoplasm. The balance between these two responses determines the pathology and clinical response to a mycobacterial infection.

Koch's phenomenon. The response of a tuberculous animal to reinfection was best explained by Robert Koch. When a healthy guinea pig is inoculated subcutaneously with virulent tubercle bacilli, the puncture site heals quickly and there is no immediate visible reaction. After l0–14 days, a nodule appears at the site of injection which ulcerates and the ulcer persists till the animal dies of progressive tuberculosis. The regional lymph nodes are enlarged and caseous. On the other hand, virulent tubercle bacilli are injected in a guinea pig, which had received a prior injection of tubercle bacilli 4–6 weeks earlier, an indurated lesion appears at the site of injection in a day or two which undergoes necrosis in another day or so to form a shallow ulcer. This ulcer heals rapidly without involvement of the regional lymph nodes or tissues. This is called Koch's phenomenon. Koch's phenomenon is a combination of hypersensitivity and immunity.

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, most frequently those of the well-ventilated middle and lower lobes. Here they are engulfed by nonspecifically activated alveolar macrophages. 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. The inflammatory reaction in the seeded tissues is usually minor, and the signs and symptoms of infection are absent. However, the primary site of infection and some enlarged hilar lymph nodes can often be detected radiologically. In infants and immunocompromised adults, hematogenous dissemination of organisms may occasionally produce meningitis.

Morphologically, the resulting tubercle is a microscopic granuloma comprised of some multinucleated giant cells formed by the fusion of several macrophages (Langhans giant cells), many epithelioid cells (activated macrophages), and a surrounding collar of lymphocytes and fibroblasts. When many bacteria are present and there is a high degree of hypersensitivity, enzymes, reactive oxygen intermediates, and reactive nitrogen intermediates are released by dying macrophages and lead to necrosis of the center of the granuloma, which is termed caseous because of the cheesy, semisolid character of the gross lesion.

Primary infections are usually handled well by the host. Bacterial multiplication ceases. Most microscopic lesions heal by fibrosis, and the organisms in them slowly die. In others, especially those in well-oxygenated tissues such as the subapical areas of the lung, renal cortex and vertebral bodies, the tubercle bacilli remain viable for long periods and serve as a potential source of reactivation many months or years later if host defenses weaken.

Reactivation (secondary) 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.

Manifestations.

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. In Western countries, reactivation of previous quiescent lesions occurs most often after the age of 50 and is more common in men. 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. Recently, reactivation and progressive primary tuberculosis among younger adults have increased as a complication of AIDS.

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. Less commonly, reactivation tuberculosis can also occur in other organs, such as the kidneys, bones, lymph nodes, brain, meninges, bone marrow, and bowel. Disease at these sites ranges from a localized tumor-like granuloma (tuberculoma) to a fatal chronic meningitis. Untreated, the progressive cough, fever, and weight loss of pulmonary tuberculosis creates an internally consuming fire that usually takes 2 to 5 years to cause death. The course in AIDS and other CMI-compromised patients is more rapid.

Laboratory diagnostics. ***Homogenization of sputum and concentration of specimens.***

Petroff's method. It is a simple and widely used technique. Sputum is mixed with equal volume of 4% sodium hydroxide and is incubated at 37 °C with frequent shaking for about 30 min. It is then centrifuged at 3000 rpm for 30 min. The supernatant fluid is poured off and the deposit is neutralized by adding 8% hydrochloric acid in presence of a drop of phenol red indicator. The deposit is used for smear, culture and animal inoculation.

Other methods. Dilute acids (5% oxalic acid, 3% hydrochloric acid or 6% sulphuric acid), mucolytic agents such as N-acetyl-L-cysteine with sodium hydroxide and pancreatin are used for concentration of specimens.

Rapid tests.

1. Microscopical examination. If present in sufficient numbers, acid-fast bacilli can be detected microscopically in direct smears of clinical specimens or in smears of material concentrated for culture. 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. If a large number of smears are to be examined, fluorescent microscopy is more convenient. Smears are stained with fluorescent dyes such as auramine «O» or auramine-rhodamine and examined under ultraviolet light. The bacilli appear as bright bacilli against dark background.

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. A clean-voided male urine specimen, on the other hand, is often contaminated with *Mycobacterium smegmatis* from the prepuce, and the finding of acid-fast bacilli does not per se indicate infection. Bronchoscopy equipment and nasotracheal tubes or their lubricants are prone to contamination with free-living mycobacteria, and false conclusions have been drawn from smears of such preparations.

2. PCR. The polymerase chain reaction has been reported to be useful in the direct diagnosis of tuberculosis by a number of investigators. To date, none of these techniques are practical for routine use in the clinical laboratory.

Cultural method. Culture is a very sensitive method for detection of tubercle bacilli. It may detect only 10 to 100 bacilli per ml. Cultural confirmation of a tentative diagnosis of tuberculosis is thus essential, and the organism must be isolated for identification and susceptibility testing. Specimens from protected sites, such as cerebrospinal fluid, bone marrow, pleural fluid, and ureteric urine, can be seeded directly to culture media used for *M. tuberculosis* isolation. Those samples inevitably contaminated with normal flora, such as sputum, gastric aspirations (cultured when sputum is not available, for example, in young children), or voided urine, are treated with NaOH or sulfuric acid that kill the normal flora but allow many mycobacteria to survive because of their resistance to these agents.

The most used treatment now employs N-acetylcysteine to dissolve mucus, combined with the antibacterial effect of a weak sodium hydroxide solution. The material is concentrated by centrifugation or filtration, neutralized or washed, and inoculated onto culture media. Cultures on solid media usually take 3 weeks or longer to show visible colonies.

Specific identification of an isolated mycobacterium is essential. It may be achieved with a number of cultural and biochemical tests, but the process usually takes several weeks. 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. Specific probes are now available

commercially for detecting *M. tuberculosis* and the *Mycobacterium avium* – intracellular complex.

Susceptibility testing is important with newly diagnosed cases. When sufficient numbers of acid-fast bacilli are seen on direct smears, the treated clinical specimen can be seeded directly onto antimicrobic-containing media for susceptibility tests, thereby saving several weeks. If numbers are scanty, the initiation of tests must await primary isolation.

Biological method (inoculation of lab animal). 0.5 ml of the concentrated specimen is inoculated intramuscularly into the thigh of tuberculin-negative healthy guinea pigs. Inoculation by subcutaneous route is avoided as it causes local ulcer which may be infectious. The animals are weighted prior to inoculation and then one time a week. They are tuberculin tested after 34 weeks. There is progressive loss of weight and tuberculin test becomes positive in animals that develop tuberculosis. Animal is killed after six weeks.

Autopsy shows: caseous lesion at the site of inoculation; enlarged caseous inguinal lymph nodes. The infection may spread to other lymph nodes such as lumbar, portal, mediastinal and cervical lymph nodes; tubercles may be seen in spleen, lungs, liver or peritoneum; kidneys are unaffected. The identity of the bacteria is then confirmed by demonstration of acid-fast bacilli (AFB) from the lesions.

Serologic method includes detection of antimycobacterial antibodies in patient serum. Various methods such as enzyme linked immunosorbent assay (ELISA), radio immunoassay (RIA), latex agglutination assay has been employed. Several antigens like BCG, antigens 5 and 6, 64 kDa, antigen 60 and 32 kDa protein have been tried for detection of antibody against them. Diagnostic utility of these antibodies is equivocal. WHO has recommended that these tests should not be used for diagnosis of active tuberculosis.

Allergic method. 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). 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 (hyperergy that indicates latent or active tuberculosis), although smaller areas of induration and erythema indicate a lesser degree of sensitization to mycobacterial proteins (normergy, common among immunized against tuberculosis). 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*. 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. They lose their tuberculin hypersensitivity and become more susceptible to the disease.

The clinical value of the PPD test depends on the occurrence of primary infection in different age groups. Now, primary infection is sufficiently uncommon in much of the Western world that a negative test is frequently important in excluding tuberculosis. A positive test in infancy or childhood has significance in diagnosis and can often be used to trace a household or school source of infection. Epidemiologic surveys of tuberculin reactivity indicate trends in the incidence of infection and constitute the simplest way of monitoring the effectiveness of control measures. PPD test is used for:

1. To measure prevalence of infection in a community.
2. To diagnose active infection in children.
3. It is used as an indicator of successful BCG vaccination.

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. Second-line drugs include para-aminosalicylic acid, ethionamide, cycloserine, fluoroquinolones, kanamycin, etc.

Prophylaxis. At present, the bacillus Calmette–Guerin (BCG) vaccine (named for its originators, Calmette and Guerin) is the only available vaccine. It has been used for prophylaxis of tuberculosis in various countries since 1923; administration is usually intradermal.

Composition: as all other vaccines it contains ***known antigen***. It is a live vaccine derived originally from a strain of *M. bovis* that was attenuated by repeated subculture.

Production: *from vaccinal strain BCG M. bovis* by cultivation.

Mechanism of action: *active artificial immunity.*

Purposes (application): *for planned immunization in order to National immunizations schedule.*

The vaccine is given intradermally in a dose of 0.1 ml. BCG vaccine should be given soon after birth failing which it may be administered at any time during the first year of life. Also, BCG is used in tuberculin-negative adult subjects for recultivation. 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, and when infection rates are low, as they are now in most Western countries, this loss may offset the possible immunity produced.

Revised National Tuberculosis Control Program (RNTCP). Under RNTCR any patient with cough for 2 weeks or more is included for diagnosis of pulmonary

tuberculosis. Diagnosis is mainly based on good quality microscopy. Two sputum samples are collected from the patient. One early morning, specimen and other is collected on spot when patient visits the chest clinic. Both the sputum specimens are stained with Ziehl–Neelsen staining and observed for acid-fast bacilli (AFB). If one or both smears are positive, then patient is diagnosed as sputum positive pulmonary tuberculosis and antitubercular treatment is started.

If both sputum smears are negative for AFB, a course of antibiotic is given for 10–14 days. If cough persists after antibiotic treatment, again two sputum specimens are collected and examined for AFB by ZN staining. Antitubercular treatment is started if one or both smears are positive and it is declared as smear positive pulmonary tuberculosis.

If both smears are negative, diagnosis is done on X-ray of the chest. In case of X-ray picture suggestive of tuberculosis, patient is diagnosed as sputum negative (closed) pulmonary tuberculosis. Antitubercular treatment is started. Patient is declared not suffering from tuberculosis when there is no finding suggestive of tuberculosis on X-ray.