Ministry of Health of Ukraine
Poltava State Medical University

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Minutes № from
Head of the Department
Associate Professor _____ PhD
Borzykh O.A.

METHODOLOGICAL RECOMMENDATIONS FOR CONDUCTING AND PREPARING FOR PRACTICAL CLASSES

Academic discipline	Clinical immunology and allergology
*Module **4***	Clinical immunology and allergology
Content module	Clinical immunology and allergology
*Topic **3***	Immune inflammation and infectious diseases
Course	5
Hours	2

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Methodological recommendations for the practical training for independent work of students in preparation for the practical training and during the class were prepared by:
Assistant of the Department of Internal Medicine №3 with phthisiology Bilko V.V.

Methodological recommendations were re-approved at the meeting of the Department of Internal Medicine of Internal Medicine №3 with Phthisiology_____
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1. Relevance of the topic.

One of the main functions of the immune system is the recognition and destruction of bodies and substances that carry signs of foreign genetic information, including pathogens of infectious diseases. When developing anti-infective resistance, the body develops specific and non-specific mechanisms. Their interaction is subject to a certain temporal sequence and is characterised by the synergy of mutual reinforcement. Since the first cases of HIV and AIDS were described and the virus was identified in the early 80s of the twentieth century, the disease has become a pandemic.

The overall goal is to study the mechanisms of immune defence against infectious factors (bacteria, viruses, fungi, parasites), the peculiarities of the immune response in the acute and chronic inflammatory process of immunopathogenesis in HIV infection and immunological methods for their detection and control.

Specific objectives:

1. To study the mechanisms of immune defence in bacterial, viral, protozoan, opportunistic infections.

2. Reactions of the immune system in fungal infections and helminthiasis.

3. The importance of the immune system in the development of opportunistic and protozoal infections.

4. Immunological methods of diagnosis of infectious diseases.

5. Immune response in acute inflammatory process.

6. Dynamics of leukogram, proteinogram and immunogram in acute, recurrent and chronic inflammation.

Theoretical questions for practical training:

1. Features of immunity in bacterial infections.

2. Immune response to invasion of intracellular microorganisms.

3. Features of immunity in viral infections.

4. Features of immunity in fungal diseases.

5. Features of immunity in protozoal diseases.

6. Features of immunity in worm infections.

7. Immunological (serological) methods of studying infectious diseases.

8. Classification of immunograms in infectious inflammation.

Approximate basis of action

Immune response to invasion of extracellular microorganisms.

The immune response directed against extracellular parasitic bacteria (staphylococci, streptococci, clostridia, diphtheria, intestinal infections, etc.), as well as some major viruses (measles, poliomyelitis), has two goals: elimination of the pathogens themselves and neutralisation of their toxins. Most pathogens of extracellular bacterial infections cause the formation of specific antibodies that bind to the surface of bacteria and, in the presence of complement, cause cytotoxic reactions (bacteriolysis).

In addition, bacteria loaded with antibodies or complement are highly susceptible to phagocytosis (opsonisation). Thus, the main protective role in the immune response against bacterial infections is played by the humoral immune response, which is manifested by the synthesis of specific antibodies - immunoglobulins. B-lymphocytes, T-helper cells (CD4 T-lymphocytes) and antigen-presenting cells are involved in this response.

The formation of mechanisms of sanogenesis (recovery) in various bacterial infections underlies some of the features of immunity that arise during such diseases. For example, in bacterial infections whose pathogens produce exotoxin (diphtheria, tetanus, botulism, gas gangrene, etc.), antibodies formed in the body (antitoxins) play a leading role in the formation of immunity. The interaction of an antitoxin molecule and a toxin molecule can lead to different results: - blockade of the receptor site of the toxin molecule and, as a result, restriction of toxin fixation on the receptors of target cells; - direct neutralisation of the catalytic (enzymatic, toxic) site of the toxin molecule; - formation of an immune complex with neutralisation of the toxic, receptor and/or translocation sites (subunits) of the toxin. Such complexes are phagocytosed and utilised by the cells of the macroorganism. However, antitoxin antibodies do not block bacterial adhesion to the surface of target cells and their colonisation. As a result, artificial antitoxic immunity does not create complete

protection of the macroorganism and does not prevent bacterial adhesion to the surface of target cells, which promotes colonisation of cells and tissue, and bacterial proliferation. In cases when pathogens produce exotoxins (tetanus, diphtheria), antitoxins easily neutralise toxic substances, but in primary infection they can be synthesised too late and are unable to protect the body.

Features of immunity in viral infections

Goals of the immune response:

1) to stop virions from entering cells;

2) to destroy already infected cells to reduce the spread of the virus.

In this regard, when a virus enters the body, two types of immunological reactions develop

a) directed against the virion;

b) acting on the cell infected with the virus.

The reactions directed against the virion are predominantly humoral, and the reactions affecting the cells infected with the virus are cellular and mediated by T-lymphocytes. Interferons are a group of cytokines that increase the resistance of cells to viral infection, have an antiproliferative effect, and are also capable of regulating the immune response. There are three types of interferons: b - produced by leukocytes, c - produced by fibroblasts, and d - produced by type 1 helper T lymphocytes.

Interferon inhibits the transcription of the viral genome in the host cell and prevents the translation of viral mRNA, which reduces virulence and facilitates the completion of the process of pathogen elimination by various factors of specific immunity. There is a constant level of interferon in the intercellular space and blood, which ensures the body's natural resistance to viral infection. After a viral infection, the level of interferon increases in the intercellular space and blood within 1-3 hours. The neutralisation of the virus, which prevents its attachment to the target cell, is carried out by IgG antibodies in the extracellular fluid, IgM in the blood and secretory IgA antibodies on the surface of the mucous membranes. Immune complexes containing the virus can bind complement, which helps to neutralise the virus. When the virus spreads from cell to cell, either by contact or when the virus integrates into the genome of a susceptible cell, cellular immune reactions involving cytotoxic killer T lymphocytes take precedence.

Since viruses are intracellular parasites, the main function of protection against them is performed by cellular reactions. Specific killer T cells appear 2-3 days after infection and precede the appearance of virus-neutralising antibodies. In antiviral immunity, the destruction of cells containing viruses is carried out by both T lymphocytes and, in parallel, activated macrophages.

It should be noted that pathogens that multiply directly at the site of penetration (influenza) have a short incubation period, which can be dangerous due to a certain inertia in the development of immune reactions, especially in people with T-cell immunodeficiency, which leads to a severe course of the disease. Viral infections that spread haematogenously (poliomyelitis, measles, mumps, chickenpox) can be eliminated by humoral mechanisms, and these diseases are usually characterised by a long incubation period.

A specific antiviral immune response is triggered by infection with viruses and some protozoa (toxoplasma, listeria), when the antigen is localised in the cytoplasm of infected cells. In this case, antigen presentation is mainly performed by dendritic antigen-presenting cells. Their origin is still a controversial issue: they can differentiate either from a single progenitor cell or from a common monocyte-macrophage progenitor.

In the T-dependent immune response, B lymphocytes also act as antigen-presenting cells. B-lymphocytes bind to antigens with their antigen-recognition receptors and absorb them. In the phagosome of B lymphocytes, the antigen is digested. The peptides derived from this antigen return to the surface of B lymphocytes in association with class II histocompatibility molecules (MHC II). Here they are recognised by the T-cell receptor on the surface of the CD4+ cell. This leads to the stimulation of the CD4+ lymphocyte (helper) and the production of IL-2, IL-4 and IL-5. The resulting interleukins stimulate B-cell proliferation and differentiation, eventually transforming into an antibody-producing plasma cell. Initially, B cells produce and secrete only IgM (first 4-5 days after antigenic stimulation). Then, B cells switch synthesis from IgM to IgG and then to IgA and IgE (14-16 days, maximum 21-24 days). Thus, the T-dependent immune response induces the production of immunoglobulins of all classes.

Features of immunity in fungal diseases

The peculiarities of antifungal immunity depend on the morphological properties of fungi (cell size, shape), their antigenic composition, form and stage of mycosis. Most fungi are free-living organisms, and only a few of them can cause disease. Moreover, for a person infected with fungi to develop a disease, a

prerequisite is the presence of an immunodeficiency of polymorphonuclear leukocytes, T-lymphocytes, and complement component C3. Functional defects of leukocytes are their inability to form pseudopodia (lazy leukocyte syndrome), inability to form phagolysosomes (Chediak-Higashi syndrome), impaired ability to produce reactive oxygen species that ensure the digestion of the microbe. Deficiency in C3 also leads to a decrease in phagocyte activity. And finally, mycoses in humans most often occur with low production of T lymphocytes (T suppressors and T helper cells). The formation of immunity is associated with the restoration of the functional activity of polymorphonuclear leukocytes and increased production of T lymphocytes. Specific antibodies are formed only in some forms of deep mycoses. It is believed that they do not participate in the defence mechanisms, witnessing the immune restructuring of the body.

Peculiarities of immunity in protozoan diseases

Protozoan infections are characterised by an extraordinary diversity of antigenic composition. This is due to the intracellular localisation of pathogens, the variability of their surface antigens, the presence of antigens that are common to human cell antigens, and the immunosuppressive properties of parasites. Moreover, most of these pathogens have a rather complex life cycle mechanism, which further complicates immune defence. To this should be added the fact that the pathogens themselves have an immunosuppressive effect, as well as the fact that these pathological processes have a pronounced polyclonal mitogenic effect, which depletes the defences of the immune system without forming resistance. Protozoal diseases can produce IgM and IgG, but their specificity is extremely low due to their formation as a result of polyclonal activation of B lymphocytes and antigenic variability of parasites. Recovery occurs with the activation of T-lymphocytes (Tc, Th). Full-fledged post-infectious immunity is formed very rarely.

Features of immunity in worm infections

Worm infestations (ascariasis, trichinosis) stimulate the synthesis of IgE. An infiltrate consisting of eosinophils, basophils and mast cells is formed at the site of the pathogen's penetration. In some cases, parasitic worms manage to avoid recognition due to a layer of cross-reactive antigens with the host organism. The induction of specific immune responses during infections can lead to the

formation of immunopathological conditions (allergic, autoimmune reactions and immunological deficiencies). For example, when large amounts of antigens are suddenly released as a result of the death of microorganisms, immune complexes are formed in the sensitised body, causing autoimmune glomerulonephritis. This complicates the course of streptococcal, pneumococcal and staphylococcal infections. Toxic immune complexes can also be formed in persistent viral infections.

This is especially evident in acute hepatitis A, when hepatocyte death is manifested by typical clinical symptoms coinciding with the onset of an immune response. The appearance of antibodies in excess of antigen leads to the formation of toxic immune complexes, and the appearance of immune complexes in excess of antibodies during the destruction of infected cells leads to the elimination of the pathogen. Most worm infestations are accompanied by allergic reactions, more often immunocomplex (type III) or cellular (type IV). Such atopic reactions (type I) are found in ascariasis, urticaria and bronchial asthma. 90 Autoimmune reactions often accompany infectious diseases. A classic example is joint and endocardial damage in rheumatism, which is known to be caused by β-haemolytic streptococcus.

Immunological (serological) methods for the study of infectious diseases

Serological reactions are used in two ways:

detection of antibodies in the patient's blood serum for diagnostic purposes based on the presence of a set of known antigens. Suspensions of microorganisms inactivated by chemical or physical methods are used as antigens, or diagnostic materials representing fractions of the microorganism are used. As a rule, the results of serological diagnostics are obtained by examining paired blood sera from patients taken in the first days of the disease and at certain intervals from the onset of the disease;

determination of the genus, species and type of the microorganism or its antigens with known immune sera. Immune sera must contain antibodies in a high titer and be strictly specific. In laboratory practice, serological reactions based on the direct interaction of an antigen with an antibody (agglutination, precipitation) and indirect reactions (indirect hemagglutination reaction, complementation reaction), as well as reactions using labelled antibodies or

antigens (enzyme-linked immunosorbent assay, radioimmunoassay, fluorescent antibody method) are used.

The agglutination reaction is used in laboratory practice to identify isolated microorganisms or to detect specific antibodies in blood serum. The reaction mechanism is based on the interaction of antigenic determinant groups with active immunoglobulin centres in an electrolyte medium.

Precipitation reaction. The phenomenon of precipitation consists in the interaction of finely dispersed antigens (precipitinogens) with the corresponding antibodies (precipitins) and the formation of a precipitate. The precipitation reaction can be performed in two ways: in a liquid medium - by the type of flocculation reaction, ring precipitation or in a dense medium in agar (gel).

The complement fixation reaction (CFR) is used for the laboratory diagnosis of venereal diseases, rickettsiosis, viral infections (influenza, measles, tick-borne encephalitis, etc.) and is based on the ability of complement to bind to an antigen + antibody complex. Complement is adsorbed to the Fc-fragment of immunoglobulins G and M. The reaction proceeds in two phases. The first phase is the interaction of antigen and antibody. The test serum is used as an antibody-containing material, to which a known antigen is added. Standard complement is added to this system and incubated at 37 °C for one hour. The second phase is to detect the results of the reaction using an indicator haemolytic system (sheep erythrocytes and rabbit haemolytic serum containing haemolysins to sheep erythrocytes). The indicator system is added to the antigen + antibody + complement mixture (first phase) and incubated again at 37 °C for 30-60 minutes, after which the reaction results are evaluated. Destruction of erythrocytes occurs when complement is added to the haemolytic system.

Indirect hemagglutination reaction (IHR). The RDHA is used in two ways: with a known antigen to detect antibodies or with a known antibody to detect antigen. This reaction is specific and is used to diagnose diseases caused by bacteria and rickettsia. RNHA is performed using red blood cell diagnostics prepared by adsorption of antigens or antibodies to red blood cells, depending on the purpose of the test. In positive cases, the degree of erythrocyte agglutination is marked with a plus sign.

Haemagglutination reaction (HRA) and haemagglutination inhibition reaction (HIR). The RHA is based on the ability of red blood cells to stick together when certain antigens are adsorbed on them.

Immunofluorescence reaction (IFR). ELISA is based on the combination of antigens of bacteria, rickettsia and viruses with specific antibodies labelled with fluorescent dyes (fluorescein isothiocyanate, rhodamine, B-isothiocyanate, lysatinrodamine B-200, sulfochloride, etc.) that have reactive groups (sulfochloride, isothiocyanate, etc.). These groups combine with the free amino groups of antibody molecules, which do not lose their specific affinity for the corresponding antigen during fluorochrome treatment. The resulting antigen-antibody complexes become clearly visible structures that glow brightly under a fluorescent microscope**.** Small amounts of bacterial and viral antigens can be detected by RIF.

An enzyme-linked immunosorbent assay (ELISA) is used to detect antigens using antibodies conjugated to a labelling enzyme. After combining the antigen with the enzyme-labelled immune serum, a substrate and chromogen are added to the mixture. The substrate is broken down by the enzyme, and its degradation products cause chemical modification of the chromogen. In this case, the chromogen changes colour - the intensity of the colour is directly proportional to the number of antigen molecules and antibodies that have bound. ELISA is used to diagnose diseases caused by viral and bacterial pathogens.

Classification of immunograms in infectious inflammation

1. Neutrophilic and lymphocytic type is a classic type with a pronounced neutrophilic and lymphocytic phase. It is most commonly found in purulent-septic diseases (mouth ulcer, microbial pneumonia, etc.).

2. Neutrophilic type - at the beginning of the detailed clinical picture, the neutrophilic phase is observed as extended as possible in time, which turns into a lymphocytic phase, unexpressed and only at the stage of recovery.

3. Lymphocytic type. In this case, the neutrophilic phase is reduced to a minimum, it is weakly expressed, is detected in the prodrome, and the lymphocytic phase takes most of the time. This phase is characteristic of a number of viral infections that suppress the neutrophilic sprout of blood (measles, influenza). In this case, there are several variants of shifts in haemogram parameters.

Eosinophilia is more often detected in the early stages of the disease, characterising the presence of allergy in the pathogenesis of the disease, accompanying an increase in IgE production, as, for example, in tuberculosis and schistomatosis.

Monocytosis is a prolonged marked increase in the number of monocytes that takes over the main period of the disease. It is characteristic of pathological processes with a pronounced productive phase in the presence of hypersensitivity to tubercle bacillus, etc. Monocytopenia is characteristic of processes with an unexpressed productive phase of inflammation, as in streptococcal sore throat.

Plasmacytosis is expressed in the appearance of a significant number of plasma cells in the haemogram. This phenomenon is usually observed in acute inflammatory diseases with irritation of the lymphoid system, such as measles.

A sharp increase in ESR. Tx/Tc ratio is equal to or less than 1 (with an increased number of T-suppressors). During a number of inflammatory processes, the number of T-suppressors increases to numbers exceeding the number of T-helper cells (for example, measles). In such diseases, a decrease in the Tx/Tc ratio to values less than 1 is a very common sign that generally does not correlate with the severity of the process, and only a very sharp decrease in this ratio may indicate the severity of the disease (compare with the dynamics of this ratio in the classical course of inflammation, when any decrease in the Tx/Tc ratio to numbers less than 1 indicates a severe course of the process). The ratio of Tx/Tc is higher than the maximum value of the norm (more than 5). There are a number of pathologies in which, in contrast to the classical dynamics of the immunogram, the Tx/Tc ratio is increased throughout the inflammatory process due to a decrease in the number of T-suppressors. This is most typical for a number of autoimmune diseases, such as sarcoidosis.

Increase in the content of immunoglobulins. Some inflammatory diseases are characterised by a significant increase in the level of total immunoglobulins or their individual subclasses in the blood. An example of such a disease is viral hepatitis.

Weak immunogram response to the inflammatory process. In an immunogram that reacts poorly, there are practically no shifts in the classical blood test, and only a number of parameters newly introduced into the leukogram indicate the ongoing process. These are, first of all, a decrease in the relative number of T-lymphocytes, an increase in the level of null cells, and a decrease in the IN. Immunograms characterised by such shifts are found in diseases of all types, when their course is "erased".

Tasks to test the initial level of knowledge

1. Specify the main mechanical factors that prevent the penetration of an infectious pathogen into the body in the presence of an immunodeficiency state.

A. Intact skin.

B. Mechanical removal of the infectious agent from the body with secretions: spleen, sweat, nasal secretions, bronchial mucus.

C. None of the above answers.

2. In favour of which of the following pathological conditions is a high titer of antibodies to streptolysin O?

A. Chronic glomerulonephritis.

B. Rheumatoid arthritis.

C. Recent streptococcal infection.

D. Systemic lupus erythematosus.

3. Which of the following is a correct description of the mechanism of antiviral action of interferon?

A. Interferon forms a coating on the cell surface, thus preventing virus penetration.

B. Interferon directly destroys the virus in the extracellular environment.

C. Interferon destroys the virus that enters the cell.

D. Interferon works through the cell's genome to activate the production of antiviral proteins.

4. The rapid development of immune deficiency in AIDS is due to:

A. The defeat of CD4 cells by the immunodeficiency virus.

B. Accession of a secondary infection caused by an opportunistic pathogen.

C. Destruction of T-helper cells by the immunodeficiency virus.

D. None of the above mechanisms.

E. All of the above mechanisms.

5. Identify possible ways of spreading acquired immunodeficiency caused by an RNA-containing retrovirus.

A. Sexual transmission.

B. By parenteral transfusion of whole blood and its components.

C. Transplacental from the mother to the fetus.

D. None of the above ways.

E. All of the above ways.

6. Specify the most significant clinical signs of AIDS:

A. Pneumocystis pneumonia.

B. Dyspepsia lasting more than a month.

C. Fever lasting more than a month.

D. Weight loss of more than 10%.

E. Lymphadenopathy.

F. None of the above signs.

7. Which opportunistic pathogens are more likely to cause death in AIDS?

A. Cytomegaloviruses.

B. Epstein-Barr virus.

C. Herpes simplex virus.

D. Candida fungi.

E. Cryptococcus fungi.

F. Toxoplasmas.

G. Pneumocystis carinii.

H. None of the above pathogens.

8. The main role in protecting the newborn from infection in the first years of life belongs to:

A. IgM.

B. IgD.

C. IgG.

D. IgE.

9. Antiretroviral gamma globulin is used:

A. For the treatment of haemolytic disease of newborns associated with Rh incompatibility of the mother and fetus.

B. To prevent rhesus conflict at the time of repeated delivery to rhesus-negative mothers.

C. For the treatment of staphylococcal infection, influenza, whooping cough.

10. Which of the following structural elements of the influenza virus can induce the creation of antibodies that have a pronounced protective effect against influenza?

A. Neuraminidase.

B. Hemagglutinin.

C. Nucleic acid.

D. A membrane.

Correct answers to the questions: 1 - AB; 2 - C; 3 - D; 4 - AB; 5 - ABC; 6 - ABCDE; 7 - ABCDEFG; 8 - C; 9 - B; 10 - B.

Sources of educational information

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