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METHODOLOGICAL DEVELOPMENT OF TOPIC №2

practical lesson **" Immunodiagnostics and immunotherapy in oncology"** (2 hours) in the discipline "Clinical Immunology and Allergology" for 6-th year students of specialty "Medicine"

1.Theme №2: Immunodiagnostics and immunotherapy in oncology

2. Relevance of the topic: Modern doctor needs modern knowledge of immunological bases of carcinogenesis, immunodiagnostic of tumors, immunotropic drugs for the treatment of cancer processes

2. Goals of class:

- Educational: Students should study the main immunological antitumor mechanisms; Laboratory diagnostics of cancer, immunotropic drugs that are used in oncology

- Professionally oriented: Students must know the main stages of clinical and laboratory immunological diagnostics of oncological diseases, approaches to using of immunotropic drugs

- Educational: to form a sense of responsibility for the timeliness and correctness of professional actions.

4. *Equipment* for conducting classes: Presentation for multimedia demonstration, schemes, tables, immunograms, tests, situational tasks, histological and cytological preparations

5. *Integrative Relations of the theme:*

5.1.Internal Integration: The topic of this practical lesson is related to the topics outlined in the course of general immunology: the organs of the immune system, the types of regulation of the immune response in the norm and in pathology, clinical manifestations of oncopathology, modern approaches to the using of immunotherapy

5.2. Interdisciplinary integration:

Disciplines	Knowledges	The skills
Anatomy	Organs of the immune system	To describe the structure of the immune system organs
Histology	The cells of the immune system	Microscopically distinguish normal and atopically changed cells
Medical biochemistry	Structure and function of immune proteins	To interpret levels of basic immune proteins (antitumor antibodies, tumor antigens, oncomarkers)
Genetics	Features of genetic inheritance of oncopathology,	Estimate the probabilities of genetic inheritance oncopathology

	knowledges about telomers	
Pathophysiology	Mechanisms of carcinogenesis	Intract changes in general immunological parameters of blood (leukogram, proteinogram) in conditions of oncopathology
Propedeutic therapy	Features of the examination of the organs in adults and children	Palpation, percussion of the immune system bodies, evaluation of the results of general billatory and instrumental methods of their examination
Pharmacology	Basic groups of preparations with cytotoxic, cytostatic, immunosuppressive action, immunocorrective drugs	Evaluate the results of clinical, laboratory and instrumental methods in patients with suspicion of oncopathology. Recommendations for using of immunotropic drugs
Infectious diseases	Oncoassociated viruses	Diagnostic of chronic viral infection associated with oncopathology, types of antiviral therapy
Oncology	Types of tumors	Main schemes for treating oncological diseases

6. Contents of the topic of the class:

6.1. Learning questions.

6.1.1. Tumor process and the role of the immune system in its formation
6.1.2.The tumor antigens
6.1.3.Mechanisms of immune recognition of tumor antigens
6.1.4.Effector mechanisms of the immune response against tumor cells (antiblastomy)
6.1.5. 5. Cytokines in tumor process
6.1.6. Factors of tumor immunoresistance
6.1.7. Immunological mechanisms, promoting tumor growth
6.1.8. Laboratory Immunological Diagnostic of Tumor Processes
6.1.9. Immunotherapy of tumors.

A short content of the lesson

The tumor is a pathological process, which is characterized by continuous reproduction of cells that is not subject to the regulatory influence of the body. Malignant tumors, in contrast to benign, are also characterized by aplasia (loss of signs of differentiation), invasive growth and metastatic distribution**.**

The reasons for the formation of cancer are: tobacco smoking (30%); incorrect nutrition (35%); alcohol abuse (2-3%); negative impact on the body of harmful professional factors (4-5%); infection agents (10%): hepatitis B and C viruses, human papillomas virus 16 and 18 types, Epstein-Barr virus, human herpes viruses 6 and 7 types, T-cell leukemia virus, Helicobacter pylori, etc.; ultraviolet rays (2-3%); ionizing radiation (4-5%); pollution of the atmosphere, water, food products with xenobiotics (1-2%).

Factors indicating the role of the immune system in the development of tumors: 1) spontaneous remission of the tumor or a long absence of tumor growth; 2) in children with primary immunodeficiency, the frequency of tumor formation increases 100-1000 times; 3) infiltration of tumors with lymphocytes and macrophages; 4) after surgical removal of the tumor, atypical cells in the blood are detected in each patient, but metastases are not formed in all; 5) detection of specific antibodies to tumor antigens; 6) a decrease in the activity of the immune system in the cancer patients; 7) a high frequency of tumor formation in patients with reduced activity of the immune system, for example, in patients with HIV infection / AIDS; for systemic diseases of connective tissue, lymphoma, lung cancer and bronchi, polycytemia, chronic myelo- and lympholeucemia, myeloma, etc.

Critical age periods of tumor development: children's age (period of formation of the immune system); adolescence (reorganization of hormonal regulation of the immune system); old people (period of decrease in the activity of the immune system).

Tumor antigens are pathologically changed (under the impact of physical, chemical, viral and other factors) of the human organism autoantigens, which will be divided into the following groups

antigens present on tumor and on normal unchanged cells (for example, the antigen encoded by the gene CAMEL has an epitope MLMAQEALAFL, present on tumor cells of melanoma and normal testicles, placenta, heart, skeletal tissue, pancreas);

differentiated antigens present on tumor cells and normal cells from which the tumor occurs (tumor-associated protein antigens are melanocyte antigens, prostastic antigen, tyrosinase, etc;

1) ***"Common" antigens present on tumors of several types*** (for example, the antigen encoded by the MAGE-A1 gene and is presented by the HLA-A1 molecules, has an EADPTGHSY epitope present on melanoma cells, breast tumors, pulmonary tumors);
2) ***oncofetal or tumor-embryonic antigens*** are present on embryonic tissues; normally disappear after birth in the process of cell differentiation (α-fetoprotein, cancer-embryonic antigen);

3) ***tumor-specific transplant antigens*** play a significant role in the processes of tumor rejection due to the formation of a cellular immune response; in tumors they are different when they arise under the influence of carcinogens and identical when they arise under the influence of oncoviruses;
4) ***virus-specific antigens*** **-** are characterized exclusively for virus-specific tumors (for example, oncoproteins E6 and E7 papillomavirus cervical cancer;
5) ***antigens that are associated*** with clonal reconstruction of immunoglobulin genes and are associated with an individual immunological portrait of myeloma, B cell lymphoma;
6) ***tumor-associated antigens*** **-** antigens that are specific to tumors and are present only on tumor cells (as a rule, human tumors do not have specific tumor antigens, but there are exceptions, for example, beta-catenin, changed due to point mutations presented by HLA molecules -A24 has an epitope of SYLDSGIHF, present only on cells of some types of melanoma***).***

The tumor-associated antigens include the following antigens: a) antigens with mutations (*mutated*) - antigens epitopes encoded as a result of tumor-specific mutations in oncogens or in suppressor genes, or appear as a result of increasing the expression of one or another genetic element (Mutations Gena Ras, reorganization in the BCR / ABL gene, super-relict the HER-2 / NEU gene, mutation in the P53 gene); b) *non-mutated* antigens - antigens present on tumor cells of different histogenesis (MAGE, BAGE, RAGE i NY-ESO).

Recognition of the immune system of tumor antigens occurs by such three mechanisms: with *T-lymphocytes, antibodies* and joint recognition.

1. Recognition of tumor antigens by T-lymphocytes relates to antigens presented by CD8 + -lymphocytes using the I-th class molecules of the HLA system. It is known that a decrease in tumor cells of expression or complete absence of class I class correlates with the degree of malignancy of the tumor. Also known are tumor antigens presented by CD4 + lymphocytes (T-helper) with HLA class II molecules, incl. HLA-DR molecules;
2. Recognition of tumor antigens using antibodies is carried out in relation to the following tumor antigens: 1) antigens B-cell lymphoma with CD19 receptors, CD20, CD21, CD2, CD37; 2) cancer-embryonic antigen (CEA with a CD66E receptor, which is present on tumor cells of the colon, pancreas, stomach); 3) α-fetoprotein (on tumor cells and normal liver); 4) tumor antigen CA-125 present on ovarian tumor cells, pancreas, lungs; 5) prostatospecific antigen (PSA) present both on embryonic cells and prostate tumor cells;
3. Recognition of tumor antigens T-lymphocytes in conjunction with antibodies (most often concerns the cancer-embryonic antigen (CEA).

There are four groups of factors involved in the development of the tumor: 1) antiblast immunological factors; 2) factors of tumor immuno-resistance; 3) probable factors that suppress the activity of the immune system; 4) factors that enhance tumor growth..

The basic antiblastfactors of the immune system include:

- *NK-cells* (carry out cell-mediated cytotoxicity, restrain tumor growth due to cytostasis, destroy tumor cells due to cytolysis);

- *T-cytotoxic lymphocytes* - (operate against tumors after stimulation of antibodies in the participation of HLA 1 class; activate monocytes / macrophages);
- *cytokines - IL-4, IL-5, IL-6* (stimulate B-lymphocytes in relation to the synthesis of specific antitumor antibodies); *IFN-γ* (stimulates macrophages); *chemokines* CXCL10-IPI10, CXCL9-MIG, CXCL4-PF4 (inhibit the development of vessels in tumor tissue); *TNF-α, β* (contribute to the microtrobes of vessels that feed the tumor; reduce the disposal of tumor cells of fatty acids, resulting in tumor cachexia, enhance apoptosis of tumor cells);
- *activated macrophages* (destroy tumor cells directly or indirectly through synthesis of TNF-α and IL-1) and neutrophils (performing phagocytosis through optonization IgG, C3B, NC);
- *antibody-dependent cellular cytotoxicity* (most effectively destroys tumor cells pentomer IgM, but it mainly works in the blood and its concentration in oncopathology patients decreases; monomeric IgG acts as blocking / protective antibodies - promotes tumor growth);
- *Complement-mediated cytotoxicity of antibodies* (more often activation of the complement system passes in an alternative way).

Inhibitory antitumor action of cytokines:

- 1) IL-4, IL-5, IL-6 - contribute to the synthesis of specific antitumor antibodies B-lymphocytes;
- 2) IL-2 - activates the antitumor function of macrophages; promotes differentiation and activation of T-cytotoxic lymphocytes;
- 3) IFN- γ - activates NK cells;
- 4) TNF-α, TNF-β - directly destroy tumor cells or inhibit their proliferation; chemokines IP-10, MIG and PF-4 are attracted to a tumor of neutrophils, monocytes, lymphocytes that infiltrate tumor tissue; inhibit angiogenesis in the tumor

In the cancer, there are certain changes in the implementation of the immune system that suppress its activity: 1) *a violation of the function of T-lymphocytes* - a decreasing in the expression of the CD3 antigen that is connected to the TCR receptor; 2) *increasing the activity of regulatory-suppressor T-lymphocytes* and myeloid suppressor cells (heterogeneous group of mature and immature myeloid cells, macrophages, granulocytes, dendritic cells); 3) *antiidiotypical antibodies* that may simulate their spatial structure of tumor antigens; 4) *antigen-antibody complexes* are anti-antibodizing effector cells that are destroyed by macrophages by transmitting a suppressive "signal" to B-lymphocytes; 5) T-lymphocytes regulatory-suppressor inhibit an immunological antitumor response; 6) cytokines (TGF-β, IL-10, VEGF), which are synthesized by tumor cells, have immunosuppressive properties; 7) macrophages and monocytes carry out prostaglandin-dependent suppression of the antitumor response; 8) antitumor antibodies that are not capable of activating the complement system are attached to surface tumor antigens and block access to the effector cells; 9) tumor antigens (especially those tumors that are prone to necrosis) block the activity of specific T-cytotoxic lymphocytes; 10) Soluble adhesive molecules (ICAM-1) block the interaction of lymphocytes with tumor cells.

Laboratory Immunological Diagnosis

In patients with oncopathology it is recommended to carry out the following immunological research:

• *phenotyping of lymphocytes* (CD3 +, CD4 +, CD4 + 25 +, CD8 +, CD16 + CD56 +, CD19 +); in case of suspicion of B cell lymphoma, determination of B-cell antigens must be determined;

• d*etermination of the activity of apoptosis* (CD95 +, agglutination lectin test, DNA electrophoresis, determination of the cell cycle phase of the cell using flow cytofluorimetry);

• *determination of the level of immune complexes* in serum;

• *determination of activity of phagocytosis* (phagocytic index, NST-test spontaneous and stimulated, activity of lysosomal enzymes, etc.);

• if necessary - study *of the general complementary activity of serum* and the level of individual components of complement (C1Q, C2, C3, C4);

• determination of the concentration of individual cytokines, tumor antigens: oncomarkers using an immuno-enzyme analysis;

• Immunogenetic studies (HLA antigens associated with oncoprocesses);

• Biochemical studies (hormones, enzymes, metabolites, etc.);

• Cultural studies (determination of prostaglandins of type PGE2, alpha-2-macriglobulin, C-reactive protein in supernatants of monocyte culture and lymphocytes of cancer.

Laboratory markers of malignant growth include:

1) tumor specific antigens (PSA - early period of prostate cancer development; Ca19-9 - cancer of the organs of the gastrointestinal canal, pancreas; Ca125 - breast cancer and ovarian; SA15-3 - breast cancer);

2) other;

3) cancer-embryonic antigen (CEA - marker of colon cancer, liver, pancreas, stomach, thyroid and breast);

4) hormones (β-chorionic gonadotropin - trophoblastic tumors of ovarian, uterus, testicles);

5) enzymes (lactate dehydrogenase - LDH); glycoproteins (α-fetoprotein in hepatocellular cancer); lipids; proteins (lactoferrin - increases with Hodgkin's disease, decreases with prostate cancer; CRP - increases with all tumors); metabolites;

6) Diagnosis of the immune system tumors by detecting CD antigens on immunocompetent cells: for B cell lymphoma (plasmocytoma) are characterized by the following markers - CD5 / 20/22; "Plescuous Lymphoma" - FMC 7/19, CD23 / CD 9, CD43 / CD19; leukemia - CD10 / CD19, CD38; CD10 / CD19, CD20, CD22;

Immunotropic tumor therapy is a modern direction in oncology.

Tumors, in the treatment of which can be used immunotherapy

At the first stage of treatment: melanoma; kidney cancer; Non-Hodzhkin's lymphoma; rectum cancer; ovarian cancer; gleoma; sarcoma of soft tissues.

At present, there are subsequent forms of immunotherapy tumors: 1) active (specific and non-specific), 2) adaptive (specific and non-specific), 3) passive using monoclonal antibodies.

MAB for using in the clinic

• *Rituximab (Maptera)* - anti-CD20 chimeric MABs that are recommended for recurrent and refractory in B-cell lymphomas.

• *Trastuzumab (Herceptin)* - used in breast cancer patients; The humanized MAB against protein transmembrane receptors of growth factors HER / NEU or C-ERB2, which are expressed by 25-30% tumors, is a marker of highly aggressive tumor process. The best effect is obtained by combined using with chemotherapia (taxol or doxorubicin, cyclophosphamide).

• *Adrocololomab (Panorex)* - mouse MAB to glycoprotein normal cells and adenocarcinoma cells are used in patients with colorectal cancer; particularly effective with 5-fluorouracil.

• *Ibritumomab (Zevalin)* - anti-CD20 mouse radio immunoconjugated with yttrium90 MAB, recommended for use in patients with B-cell lymphoma.

• *Tozitumomab (Bexar*) - anti-CD20 mouse radio immunoconjugated with iodine131 MAB, recommended for use in patients with B-cell lymphoma;

Promising MABs: 1) against epidermal growth receptors (EGFR); 2) against the vascular endothelial growth factor - VEGF.

6.2. Control questions.

6.2.1. Types of tumor-associated antigens.
6.2.2. Viruses and tumor-associated antigens.
6.2.3. Mechanisms of antitumor protection.
6.2.4. Immunological factors of tumor resistance.
6.2.5. Cytokines and tumor growth.
6.2.6. Basic approaches to immunotropic treatment of cancer patients.
6.2.7. Active, passive and adaptive immunotherapy of cancer patients.

Practical experience:

6.3.1. Implementation of clinical and laboratory immunological surveys in patients with oncopathology.
6.3.2. Implementation of impressions to immunotherapy of cancer patients.

6.2. *Plan and organizational structure of class (2 academic hours or 90 min.).*

Main stages of classes, their functions and content	Levels of knowledge	Methods of control and training	Methods of methodical supporting	Time in min
1. The first stage Organization of classes Educational goals Control of the primaryt level of knowledge and skills: -regulation of the immune response - the causes of violations of immune response regulation	I	Frontal poll Express-poll Test control	Tests Schemes	10
2. Basic stage Formation of professional knowledge and skills: - To describe the types of tumor antigens; - to determine the main mechanisms of antitumor immune protection; - to identify the main factors of immuno-resistance of tumors; - to form the main directions of immunotherapy of tumors	II II III, IV	Individual control tests Professional training in typical tasks ("step-2")	Tables Schemes Immunological observation cards Typical situational tasks Histological and cytological preparations, immunograms	70
Final stage -Control and correction of professional knowledge, skills	 III IV	Testing (Output Level) Individual survey Solving of non-typical situational tasks	Schemes Tests Non-typical situational tasks	10
Conducting a summary of classes. Homework for the next topic				

6.5. Conclusions:

6.5.1. Generalized knowledge of the main types of tumor antigens.

6.5.2. The main mechanisms of antitumor immune protection are formulated.
6.5.3 Formed Basic principles of immunodiagnosis of tumors
6.5.4. Classification of immunotropic drugs used in oncology
6.5.5. Immunotherapy of tumor diseases.

7. *Materials for control*
8. *Tasks for individual work on this topic*

1 /. Make a list of characteristic changes in the basic general-clinical and immunological laboratory parameters in the early and late stages of oncological diseases
2 /. Develop a table (scheme) of the main clinical manifestations of tumor processes
3 /. To form the main features for immunotropic drugs for the treatment of oncological diseases and for rehabilitation of the immune system after treatment of patients with radiation therapy and chemotherapeutic preparations.