VOLGOGRAD STATE MEDICAL UNIVERSITY
DEPARTMENT OF PHARMACOLOGY AND BIOINFORMATICS

Methodological recommendations for teachers for practical classes in the discipline:

"Immunobiological and genotherapeutic drugs"

Thematic block: **Immunobiological drugs**

Lesson topic:
Recombinant drugs: monoclonal antibodies. History (timeline) of the development, classification (based on murine, chimeric, humanized and human antibodies).

Faculty of Pharmacy

1. OBJECTIVES OF THE LESSON

- To teach students to analyze the effect of recombinant drugs (monoclonal antibodies) according to the totality of their pharmacological properties, mechanisms and localization of action;
- to teach students the basics of monoclonal antibody production technology;
- to teach students to evaluate the effectiveness of recombinant drug preparations (monoclonal antibodies) for therapy of oncologic, autoimmune, infectious, allergic diseases;
-to teach students to evaluate the possibilities of using recombinant drugs (monoclonal antibodies) for immunodiagnostics and assessment of immune status;
- to teach students to evaluate the advantages and disadvantages of this group of immunobiological drugs;
- to inform students about the necessity to educate the population about the effectiveness of monoclonal antibodies in various fields of medicine as a significant factor in the fight against severe, chronic diseases;

2.OBJECTIVES.

For recombinant monoclonal antibody based drugs, study:
- classification, structure and nomenclature of drugs;
- general characterization and mechanism of action;
- basic mechanisms of action and clinical targets;
- side effects of drugs;
- application in medicine, immunodiagnostics

- To study the history of development and the main stages of monoclonal antibodies production
- To study the basic terms and definitions used in the process of production of monoclonal antibodies;
- To study general requirements for storage of monoclonal antibodies

3. THE FOLLOWING PRACTICAL SKILLS AND ABILITIES WILL BE TRAINED IN THE CLASSES

- ability to classify drugs of the studied group depending on their origin and structure
- ability to identify the type of monoclonal antibody by the name of recombinant therapeutic drug and assess its immunogenicity;
- ability to analyze the possibilities of recombinant drugs (monoclonal antibodies) application
- analyze the advantages and disadvantages of monoclonal antibodies

4. ORDER OF CLASSES:

Venue: classroom of the Department of Pharmacology and Bioinformatics. Time: part 1 – 2 academic hours

Formed competencies: UK-1.1.3, UK-1.2.1, UK-1.2.2, UK-1.2.3., UK-1.3.1, UK-1.3.2., UK-6.1.1., UK-6.2.1, UK-6.2.2, UK-6.3.1, UK-6.3.2, УUK-6.3.3, УKU-6.3.4, OPK-1.1.1., OPK-1.2.1, OPK-1.2.2., OPK-1.3.1, OPK-6.1.1, OPK-6.2.1, OPK-6.3.1, PK-7.1.1, PK-7.2.1, PK-7.3.1.

4.1 Technological map of the lesson

Part	№	Lesson stage	Time
1	1	Checking the students present in the lesson, mode of the lesson, topic of the lesson.	5 minutes
	2	Checking the initial level of knowledge of students (written survey).	10 minutes
	3	Survey on the topic of the lesson.	45 minutes
	4	Independent work of students (on prescriptions with analysis of the most complex recipes (if any in the topic), analysis of errors in medical prescriptions written by students; work with synonyms).	15 minutes
	5	Checking independent work	5 minutes
	6	Summing up the lesson. Assignment for the next lesson.	5 minutes
	7	Cleaning workplaces.	5 minutes

4.2 Demonstrations

1. Demonstration of advertising brochures on this topic during a survey on the topic of the lesson.

4.3 Lesson plan

4.3.1 The lesson begins with an introductory speech from the teacher, stating the purpose of the lesson and answering students' questions.

A new class of drugs based on monoclonal antibodies (MAB), which have a great potential for targeted therapeutic action on pathogenetically significant mechanisms of disease development, has found successful application in severe, chronic diseases - oncological, autoimmune, infectious, allergic, as well as in transplantology for treatment and prevention of transplant rejection. In addition, monoclonal antibodies are widely used in diagnostics - immunohistochemistry, immuno-enzyme analysis, flow cytofluorimetry. From the mid-90s until today, more than 30 drugs with monoclonal antibodies in their composition have been approved in the global pharmaceutical market. Now most pharmaceutical companies are working on the development of new drugs and medicines based on monoclonal antibodies. There are about 300 MABs in the development stage in the world - 4 out of 10 drugs that are in the final stage of trials are MABs.

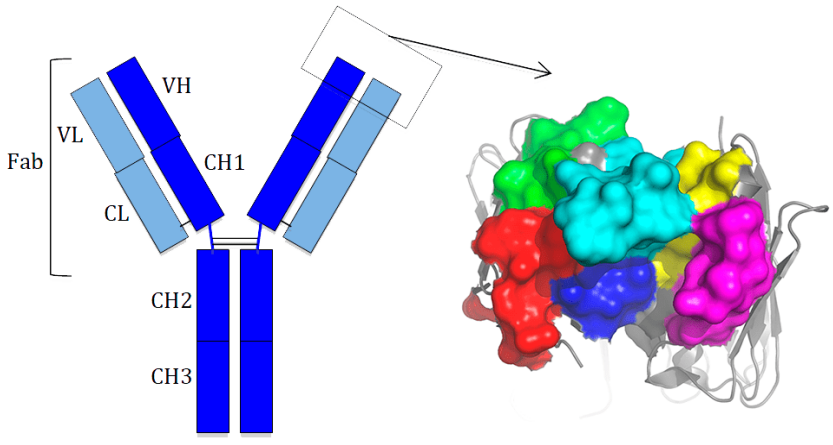
The importance of the topic in the system of training and activities of the pharmacist:
- Informing the population about the effectiveness of monoclonal antibodies in various fields of medicine and immunodiagnostics;

4.3.2 Checking the initial level of knowledge of students (written survey).

4.3.3. Analysis of theoretical material.

Plan for analysis of theoretical material

A monoclonal antibody (MAB) is an immunoglobulin with target specificity to a certain antigen produced by a stable cell line. The cells of a clone are identical and the MAB they produce have the same structure and properties.
Antibody structure ensures the ability of antibodies to recognize specific antigens (carried out by V-domains) and interact with cells of the own immune system (effector function of antibodies; C-domains are responsible for it).



Fab- fragment antigen binding - the part of the Ig molecule that binds the antigen. It consists of one constant (C) and one variable (V) domain of light and heavy chains. These domains bind the epitope of specific antigens

Distinguishing MAb from polyclonal antibodies

MAB contain the product of a single clone of plasma cells, directed to a strictly defined antigenic determinant, always having the same physical and chemical characteristics and affinity to the antigen, characterized by the **highest specificity**, **standardization and manufacturability of production.**

Polyclonal antibody drugs – a heterogeneous family of antibodies produced by different clones of plasma cells to different sites of the same antigen, contain a wide variety of antibodies that differ in their **specificity, affinity and physicochemical properties**

Polyclonal antibodies are a heterogeneous family of antibodies produced by different clones of plasma cells to different parts of the same antigen

- contain a large variety of antibodies, which differ in their specificity, affinity and physicochemical properties;

- Antibody spectrum may differ in different polyclonal preparations, because the composition of antibodies may be lost after the death of the donor animal: the organism of a new immunized animal may react differently to the antigen and give a different spectrum of polyclonal antibodies;

- cannot be used for more detailed studies, when individual antibodies binding to a specific antigenic determinant are needed.

II. History of the development and stages of the monoclonal antibodies production

Over the past 120 years, research and development of antibody-related technologies has been honored with four Nobel Prizes.

In 1901, **Emil von Behring** received the first Nobel Prize in Physiology or Medicine for the successful therapeutic application of equine hyperimmune serum containing neutralizing polyclonal antibodies against diphtheria and tetanus toxins.

Köhler and Milstein received the 1984 Nobel Prize in Physiology or Medicine for developing a **novel hybridoma technology that facilitated the isolation of MABs and their subsequent production in laboratories.**

In 2018, George P. Smith and Sir Gregory P. Winter were awarded the Nobel Prize in Chemistry for **developing a phage display of peptides and antibodies.**

In the same year, **James P. Allison and Tasuku Honze** were awarded the **2018 Nobel Prize** in Physiology or Medicine for their discoveries in cancer immunotherapy using **antibody blockade of T-cell inhibitory receptor (CTLA-4) and programmed cell death protein 1 (PD1) to enhance anti-tumor immune responses.**

In 1975, German immunologist **Georges Kohler,** who studied the genetic variability of antibodies, and British immunologist **Cesar Milstein**, who studied clones of tumor cells (plasmacytomas), created a hybrid of plasma and tumor cells producing antibodies.

Monoclonal antibodies were first produced from a hybrid cell (hybridoma) derived from:

- antibody-producing B-lymphocytes stimulated with a specific antigen
- myeloid cells (tumor cells originating from plasma cells-plasmacytoma) capable of unlimited multiplication under artificial conditions.

The hybrid cell had the immortality of a tumor cell and the ability to synthesize antibodies inherited from a normal cell.

Main stages of hybridoma production

1. Immunization of mice with the selected antigen.
At the antibody peak, the spleen was removed from the animals and the tissue was homogenized to obtain a sus-pense of B-cells, the producers of antibodies against the introduced antigen.

2. For hybridization with B-lymphocytes, **only mutant plasmacytoma (myeloma) cells were selected**, which had only the main pathway of nucleotide synthesis (from amino acids) and lacked the enzyme that provides synthesis of nucleotides through the spare pathway (from purines and pyrimidines)

3. Fusion of tumor cells with "normal" lymphocytes. Spleen cells were mixed with plasmacytoma cells in the presence of polyethylene glycol (PEG), a po-electrolyte that promotes cell membrane fusion and the formation of hybrid cells. The hybridoma retained the ability to cell division, during which the chromosomes of both nuclei intermingled and formed one common nucleus containing genes of both progenitor cells

4. Selection of hybridoma.

Metabolic selection of hybrids was based on the fact that **B-lymphocytes** can use two metabolic pathways of purine and pyrimidine nucleotide synthesis: the main (from amino acids and carbohydrate precursors) and **reserve (from hypoxanthine (purines**) or deoxythymidine (pyrimidines).

If the main metabolic pathway of nucleotide synthesis is blocked, the enzymes hypoxanthine-guanidine-phosphoribosyltransferase (G/GFRT) and thymidine kinase are activated, which ensures that nucleotides are synthesized via the reserve pathway. To separate a given hy-bridoma from other cells, a mixture of cells was placed in a selective medium (GAT medium) containing hypoxanthine, aminopterin and thymidine, in which the main pathway of nucleic acid synthesis was blocked (at the expense of toxic aminopterin), resulting in the death of mutant tumor cells that did not possess the reserve pathway of nucleic base synthesis.

B-lymphocytes capable of growing in GAT medium, being lethal, died naturally after 1-2 weeks.

Only hybridoma cells combining the properties of "deathless" tumor cells and B-lymphocytes survived in the selective medium
5. Cultivation of the produced hybridomas in order to isolate a clone producing the necessary antibodies.
Cells surviving in HAT medium were placed in plastic 96-well plates (10 hybridomas in each well). After a few days of culturing, cells containing antibodies of a given specificity were cloned (reintroduced into wells at the rate of 1 cell per 1 well). The progenitor cell gave rise to the formation of an "immortal" clone producing monoclonal antibodies.

6. Study of the produced hybridomas - determination of specificity, affinity, etc.
7. Production of monoclonal antibodies *in vivo* (by grafting clones into mice) or in vitro (by culturing clones in culture medium).
8. Purification of the resulting antibodies.
Selected hybridomas can be cultured for long periods of time to obtain large amounts of homogeneous monoclonal antibodies Affinity and ion exchange chromatography are used for purification.

III Types of monoclonal antibodies

Murine antibodies consist entirely of mouse protein, have high immunogenicity, are not effective enough in realizing the mechanism of target cell destruction, and are quickly eliminated from the body. In **1988**, British biochemist **Greg Winter** developed a method of "**humanizing**" monoclonal antibodies.

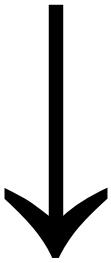
Chimeric antibodies are antibodies in which its constant domain, which has immunogenic and effector properties, is replaced with human immunoglobulina, while the variable domain, which specifically interacts with antigen, will remain murine.

Humanized antibodies are 90-95% human immunoglobulin. Only hypervariable regions responsible for binding of AT to antigen remain in them.

Recombinant human antibodies, in which the variable domains of heavy and light chains of human antibodies are combined with constant domains of human antibodies.
Human MAB have the lowest immunogenicity

IV Nomenclature of MAB drugs

The main endings of MAB drugs depending on the source of obtaining them are as follows:

Immunogenicity		**Type**	**Consistency percentage, %**		**INN ending**
			Mouse	Human	
Declining		mouse	100	0	**-omab** Muronomab
		chimeric	33	67	**-ximab** rituximab infliximab
		humanized	5-10	90-95	**-zumab** daclizumab
		human	0	100	**-umab** sarilumab

Mechanism of action of MAB

V. Mechanism of action and clinical targets of MABs
Monoclonal antibodies have high specificity to certain antigens, which allows them to be used for targeted (targeted) therapy of related diseases. The result of MAB action is:

- blocking a specific target that plays a role in disease pathogenesis, excretion or neutralization of the pathogen.

- blocking the pro-inflammatory activity of cytokines

- Inhibition of the processes of activation and interaction of immunocompetent cells

- elimination of subpopulation of immune cells involved in inflammation.

First studied and described clinical targets of MAB

- tumor necrosis factor alpha (**TNFα**) (involved in systemic autoimmune diseases)
- pro-inflammatory cytokines (**interleukins**) involved in inflammatory allergic and autoimmune reactions
- **CD20 protein** present on the surface of B-lymphocytes (in B-cell lymphoproliferative diseases)
- epidermal growth factor (**EGFR**), the increased expression of which can be observed in cancers
- human epidermal growth factor receptor 2 (**HER2**), which is overexpressed in breast cancer.

VI. Therapeutic use of MAB

- therapy of chronic diseases with a long, progressive course
- **cancer immunotherapy**

- **autoimmune diseases** (rheumatoid arthritis, systemic lupus erythematosus, psoriasis, multiple sclerosis)

- **Allergology** (for treatment of resistant, severe forms of bronchial asthma)

- **transplantology** (for prevention of transplant rejection)

- **therapy of viral, chronic inflammatory and orphan/rare diseases** (paroxysmal nocturnal hemoglobinuria, cryopyrin-associated periodic syndromes).

VII. Other applications of antibodies

The identification of human lymphocyte subpopulations by immunophenotyping is based on the detection of differentiation markers (CD-antigens) on their surface, which are unique for each subpopulation and developmental stage. Using fluorochrome-labeled monoclonal antibodies that bind to specific CD antigens, lymphocytes belonging to different subpopulations are counted to assess the cellular immune system;

Diagnostic testing - monoclonal antibodies are used in the diagnosis of various diseases to test for the presence of foreign antigens (toxins, drugs, hormones, proteins of bacteria or viruses);

ABO Monoclonal antibodies can also be used for serologic identification of blood groups. Antibodies can be isolated from human sera stimulated with blood group A or B;

Radioimmunodetection (RID) - using monoclonal antibodies, radioactive substances can be delivered to the tumor and its metastases, allowing detection of small tumor nodules by the localization of radioactivity in them;
Analysis of embryonic development - determination of fetal sex, chromosomal anomalies, single gene anomalies;

Quadromes - cells formed by the fusion of two hybrids, synthesize bifunctional antibodies with active centers to different antigens;

Immunopurification - a method of purifying individual interferons, proteins and enzymes;

VIII. Side effects of MAB

Immunogenicity
- infusion reactions, anaphylaxis, reduction of the therapeutic effect of the drug substance
- possible development of a cascade of biological reactions accompanied by release of cytokines interleukin-6, TNFα, interferon (chills, flu-like syndrome, myalgia, etc.);
- increased risks of oncologic and viral diseases, since MAB cause suppression of the activity of certain parts of the immune system;

- long-term therapy with MAB (TNFα inhibitors) may result in reactivation of latent tuberculosis process, increased risk of infections, lymphoproliferative diseases, autoimmune reactions;

- decrease in the severity of clinical effect due to neutralizing action of antibodies or formation of immune complexes.

The use of monoclonal antibodies also involves a number of peculiarities and problems that require attention and solution:
- Immunogenicity. Despite significant progress in reducing the immunogenicity of MAB, there is still a risk of developing an immune response to these drugs, especially with prolonged use. MABs can induce an immune response against themselves, which blocks their action. The formation of neutralizing antibodies was observed in 75% of patients who were administered murine antibodies, reducing the effectiveness of the treatment.

- Resistance. As with antibiotics, there is a risk of tumor cells developing resistance to MAB therapy, requiring the development of combination approaches and new drugs to overcome or minimize this phenomenon.

- Delivery. Effective delivery of MAB to target cells, especially to hard-to-reach or protected areas of the body, remains a challenge. New delivery strategies are being developed, including nanoparticles and engineered constructs.

- High cost. One of the main barriers to the widespread use of MAB is their high cost due to the complexity and high cost of production. Research is aimed at finding ways to reduce costs and simplify production processes.

4.3.4 Independent work of students:
1.According to the name of the therapeutic MAB identify the type of monoclonal antibodies, assess its immunogenicity, search and write out synonyms and indications for the use of this drug. The information is recorded in students' workbooks.

4. Work with advertising brochures for medicines on this topic.
4.3.2 Checking students' independent work.
4.3.3 Summing up the lesson. Answers to students' questions.
4.3.4 Closing remarks from the teacher.

Compiler,
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